RESULTS AND DISCUSSION

The present study deals with the biochemical changes in vital organs viz. liver, kidney, gills and brain of the teleost fish *Heteropneustes fossilis* under the influence of acute and chronic exposure of lead nitrate; lead nitrate + natrolite, and natrolite only. Acute and chronic changes were observed in these organs for 35 (at the interval of 7 days) and 180 days (at the interval of 30 days) respectively. After seven days interval in acute study and thirty days interval in chronic study, fish sacrificed, their tissues were removed and processed for the biochemical estimation of protein, glycogen, RNA and cholesterol in liver, kidney, gills and brain. For the study of remediation of lead toxicity, natural zeolite, natrolite was used and it has been observed that natrolite decreases lead toxicity in the fish *Heteropneustes fossilis*. The observation thus obtained have been summarized in the form of tables (No. 1 to 32) and graphs. Data were analysed statistically by using mean value, standard error, student ‘t’ test, probability and percent change.

For estimation of the proposed biochemical contents in tissue, the following methods have been applied-

1. Protein contents- Lowry method.
2. Glycogen content- Anthrone method.
3. Cholesterol content- Liebermann Burchard method
4. RNA content- Orcinol method
I. ACUTE STUDY

For acute study, 120 adult healthy, and acclimatized experimental fish have been divided into four equal groups. Group one received only natural and artificial fish meal and served as control, group two exposed to sublethal concentration of lead nitrate, group three and group four were exposed to lead nitrate + natrolite and only natrolite respectively. After 7, 14, 21, 28 and 35 days of exposure fish were sacrificed, their liver, kidney, gill and brain were removed and processed for the estimation of glycogen, protein, RNA and cholesterol contents. All experiments were run in triplicate and mean values were summarized in tables 1 to 16.

1. Protein contents:

In control fish, the range of values (minimum-maximum) protein contents (mg/g) have been observed in the liver 79.98±1.0 - 80.12±1.64 (Table 1) in kidney 67.85±1.49 - 67.94±1.50 (Table 2), in gills 62.14±1.44 - 62.22±1.44 (Table 3) and in brain 68.49±1.50 - 68.56±1.50 (Table 4). In control fish the value of protein contents increases consistently in all the tissue of experimental fish, might be due to the growing age of the fish. When experimental fish are exposed to sublethal concentration of lead nitrate, the protein contents decrease significantly in all the tissues of experimental fish (Table 1-4).

Liver

The values of protein contents in liver after exposure to lead nitrate up to 35 days (at the interval of 7 days) are shown in Table 1. Highest
decrease in protein contents was observed (29.12%) in liver at 7 days exposure and it was minimum after 35 days (21.02%). It has been observed that with the increase of exposure period the percent change in protein content reduced gradually, may be due to resistance developed in the fish against lead toxicity.

When fish of Group III exposed to lead nitrate + natrolite, the values of protein contents in liver increased significantly from 7 to 35 days compared to the fish of Group II exposed to lead nitrate. The maximum improvement in the protein content observed after 7 days (3.59%) and minimum after 35 days of exposure period (7.03%). However, when fish of Group IV exposed to natural zeolite natrolite the protein contents increased comparing their respective control.

Decrease in protein contents might be due to enzyme inhibition which play an important role in protein synthesis (Sastri and Gupta, 1978). Liver is the most important target organ for heavy metal toxicity (Holcombe et al., 1976). Patterson and Settle (1977), Hodson (1978) and Varanasi and Gmur (1978) observed that liver, kidney, gills and bones are chief lead accumulating tissue in the body. Lead is unique in its physiological toxicity and a series of responses are due to intoxication of lead (Davies et al., 1976 and Hodson et al., 1984).

According to Sprey et al. (1981) and Hodson et al. (1984) exposure of fish to lead in high concentration can elicit several biochemical responses. Lead inhibits a part of haemoglobin synthesis pathway (Spry and Wiener, 1991). Liver protein contents decreased after lead treatment
(Hoffman et al., 1985). Johnson and Damson, 1982 found in Chinese geese that lead causes increase in the size of the liver and some yellow discolouration. In birds like starlings (Sturnus vulgaris) toxicity of lead was studied by Osborn et al. (1983). He found highest concentration of lead in the liver of triethyllead-treated birds (40.2 mg/kg wet weight). Bagley and Locke (1967) analysed wild birds of many species from the eastern USA for tissue lead levels and found that mean liver residue of lead ranged from 0.5 to 3.7 mg/kg weight. Martin (1972) and Martin and Wickerson (1973) analysed starlings in the USA for lead and found residues from 0.4 to 13.3 mg/kg in 1970 and 0.12 to 6.6 in 1971.

Mckim and Benoit (1971) reported that heavy metals cause reduction in food consumption. Jain et al. (1996, 1997, 1999, 2000 and 2001) studied remediation of metal toxicity through natural and synthetic zeolites and reported that zeolites are ion exchanger and remove heavy metals from polluted water and thus reduce deleterious effects.

**Kidney**

The protein is an important component to stimulate growth in fish. In the present study, protein contents (mg/g) in the kidney of control fish have been 67.85±1.49 - 67.94±1.50 (Table 2). When fish of group II exposed to lead nitrate, the maximum decrease in protein content was observed after 7 days while it was minimum after 35 days of exposure period. When fish of Group III exposed to lead nitrate + natrolite, the protein contents increases with time rapidly in comparison to the fish of
Group II. Fish of group IV exposed to natrolite only exhibit increased protein contents compared to their respective control. The observation showed that the tested biochemical parameter was improved in natrolite added and natrolite alone group.

The present study indicate that the addition of natural zeolite natrolite to the lead media significantly reduced the lead level in water. Natrolite can bind with lead ions forming ion-exchanged natrolite complex which could readily enter into the fish body; however it appears that the complex was not accumulated in the body but eliminated through faeces, thus reducing the metal burden.

The lead and natrolite could interact in the experimental medium and the mechanism is that natrolite having extra framework ion (Na+) is easily exchangeable. The ionic radii of Na+, Al^{3+} and Si^{4+} are 0.95, 0.50 and 0.54 Å; the ionic radius of lead is 0.84 Å heavier than Na+ and therefore suitably matched to the ionic radius of Na (0.95 Å) in natrolite, and hence both ions could be easily exchanged with each other. Al^{3+} and Si^{4+} ions are framework ions and non exchangeable.

Jackim (1973) exposed mummichog (Fundulus heeroclitus and winter flounder (Pseudo-Pleuronectes americans) to an initial concentration of 10 mg lead (as lead nitrate/litre). He found 58% decrease in delta ALAD activity in kidney. Osborn et al. (1983) studied metal (lead) toxicity in starlings (Sturnus vulgaris) and found that tetramethyl lead is accumulated more in the brain, kidney and liver than triethyl lead. The highest lead levels were observed in the kidney: triethyl
lead treated birds contained 1.85 mg/kg wet weight and trimethyl lead treated birds contained 5.38 mg/kg wet weight in their kidneys.

Target tissues of heavy metals are metabolically active, therefore, metal accumulation in these tissues occur higher level in liver, kidney and gill compared to some other tissues like the muscles, where metabolic activity is relatively low (Heath, 1987; Langston 1990, Serra et al., 1993; Roesijadi and Robinson, 1994. canli et al.,1998).

According to Holcombe et al. (1976), Patterson and Settle (1977), Hodson (1978), Varanasi and Gmur (1978), and Hodson et al. (1982) accumulation of lead is maximum in liver, kidney, gills and bones. The toxic effects of lead poisoning in fish have been reviewed from time to time with reference to haematological and biological variable (Jackim, 1973; Johnson Sjobeck and Larson, 1979; Haux and Larson, 1982). Alkaline solution of cystein and glycerin treated scales of fish protects against heavy metal toxicity (Coello and Khan, 1996).

Briggs and Smith (1996) found that zeolites have the capacity to remove ammonia and other metabolites from freshwater by ion-exchange and adsorption. James et al., (1998) reported that addition of another chelating agent EDTA to Cu contaminated medium cause the formation of stable ion (Cu²⁺), exchanged EDTA complex and elimination of more amount of copper in faeces, which significantly reduced the metal burden in tissues and improved the haematological parameters in Oreochromis mossambicus. Muramota (1980) found that metal chelating compounds nitrilotriacetic acid (NTA) and EDTA reduced the metal toxicity in fish by
preventing the accumulation of metal in tissues. He also suggested that cadmium complexed to EDTA is indeed taken up, but the complex is quickly excreted through urine (Babikev and Rankin, 1975).

The present study indicates that lead uptake by fish has been lower in natrolite treated groups in comparison to the lead exposed group. It was found that natrolite caused the elimination of lead in polluted water which in turn reduced the metal burden in fish. James and Sampath (1999b) reported that addition of zeolite to cadmium polluted environment, resulted in least accumulation of metal and maximum improvement in food intake and growth in the cadmium exposed _Heteropneustes fossilis_. Gworek and Borowiak (1990) reported that application of synthetic zeolites cause the immobilization of heavy metals and recommended for clean up method. In aquaculture practices, application of zeolite is suggested in ponds before stocking or during the pond preparation (Briggs and Smith, 1996). When the concentration of ammonium ion exceeds the permissible limit to aquaculture ponds, it becomes toxic to fish life (Sampath _et al._, 1991; James _et al._, 1993). It is therefore advisable to reduce the concentration below the permissible limit by application of zeolite (James _et al._, 2003). However, doses of chelating agent like EDTA could cause deleterious effects on survival, development and growth in crustacean larvae (Simon, 1981) and zeolite is the cheapest, causes no side effects and more suitable than EDTA and NTA and hence it may be considered as one of the best chemical agents for the removal of toxic element like lead from polluted environment.
GILLS

In control fish, the protein contents in gill (62.17±1.44 mg/g) has been similar during all the exposure periods. When fish of group II were exposed to lead nitrate, the maximum decrease was observed after 7 days while it was minimum after 35 days of exposure. When fish of Group III exposed to lead nitrate+natrolite, the protein contents increases significantly in comparison to the fish of group II, exposed to lead nitrate. This indicates the important role of natrolite in remediation of lead toxicity. When fish of group IV exposed to natrolite only the value of protein contents increased in comparison to the control fish. The range of increase in protein contents observed was from 0.72% to 0.81% during the exposure period (Table 3).

The concentration of lead and other metals in the aquatic organism depend mainly on their environmental levels (Amiard et al., 1987; Heath, 1987; Bryan and Longston, 1992). In invertebrates like Shrimps respond to heavy metal exposure by producing metallothionin particularly in hepatopancreas (Dormono et al., 1990; Howard and Hacker, 1990; Dallinger, 1994). High levels of metals found in the hepatopancreas of shrimp is possibly due to binding of the metals to metallothionine, proteins. Kargin et al. (2000) observed high concentration of lead in the gills of the shrimp *Penaeus semiculatus* and *metapenacus monocerus* and reported that this is because of biding of the metals to metallothionein proteins. According to them in gill, active and passive exchangers occur
between the animal and aquatic environment. First high levels of metals accumulate in the gill tissue by absorption and adsorption (Heath, 1987).

Anderson (1978) observed the effect of lead on crayfish *Orconectes virilis* in their gills. The metal reduce the capacity of oxygen uptake through the gills. Chinnayya (1971) pointed out that lead nitrate in freshwater reduced the oxygen consumption of the shrimp *Caridina Rajadhari*. Enk and Mathis (1977) detected lead in all components of a stream with no industrial contamination. The level of lead in fish was 2.47 to 2.88 mg/kg, in snails 13.64 mg/kg and 6.83 to 12.59 mg/kg in aquatic insects.

Gilmartin and Revelante (1975) analysed anchory and sardine from the Adriatic sea, the highest lead levels were found in the gills 6.8 and 6.5 mg/kg wet weight respectively.

In fish, gills are the most important organs of respiration. Damage to gills by different heavy metals and pesticides has been observed by a number of workers (Couch, 1979; Khangarot, 1982; Pawar and Katdare, 1983 and Nilkan and Sawant, 1993). Acute toxicity studies measure the response of an organism to a biologically active substance (Alderdice, 1966) and are useful in determining water quality Jadhav (1985), Thorat (1998) and Amte and Sawant (1992) have reported similar observation in different test animals using different heavy metals. Decrease in blood lead and lead concentration in bone is controlled by the intensity of exposure versus exposure period. Heavy metals in sediments have been measured using the three step sequential extraction and acid digestion
method described by Carpeto and Purchase (2000). Pesticide absorption properties of pedal mucous of the freshwater snails *Peregra* and *Potamophrygus jenkinsii* have been demonstrated by Brereton *et al.* (1999). Metals strongly bind with sulphahydryl groups to form mercaptides, inhibit large number of enzymes containing functional thiol group (Mass Oliva, 1989 and Rajanna *et al.*, 1990). Heavy metal chiefly target on the cell membranes and thus this respiratory organ gets damaged by the accumulation of toxic metals. The removal of ammonia from contaminated aqueous solution by natural zeolite has been investigated by Aral *et al.* (1999). Jain *et al.* (1995) has already reported the effect of heavy metal toxicity and protective action of zeolite on biochemical parameters. Since zeolites are ion exchangers therefore sodium of zeolite is replaced by metal ion within their molecular sieve (Jain *et al.*, 1996, 1997, 1999, 2000, 2001).

**Brain**

In control fish, protein contents in brain have almost been similar during all the exposure periods. A slight increase with increasing age has been observed. When fish of group II exposed to lead nitrate, the value of protein content decreased at all time interval up to 35 days of treatment. The percent change after 7 and 35 days of exposure observed 15.30% and 15.28% respectively. When fish of group III exposed to lead nitrate + natrolite, the value of protein content in tissue increased in comparison to group II exposed to lead nitrate. The minimum and maximum improvement in protein contents observed 5.17% and 5.15% respectively.
The IV group of fish were exposed to only natrolite, where protein contents were higher comparing with control group (Table 4).

Holcombe et al. (1976) exposed brook trout *Salvelinus fontinalis* to lead nitrate and observed spinal deformities in it. Neurobehavioural disorders due to prenatal exposure to low level of lead have been reported in several human studies (Bellinger *et al.*, 1987, 1990; Leviton *et al.*, 1993). In immature rodents, a high uptake of lead in the brain has been demonstrated by Momcilovic and Kostial (1974) and Levesey *et al.* (1986). However, due to the high affinity of lead to casein only, low concentration of unbound lead may be present in the cytoplasm of cell and milk. Beach and Henning (1988); Palminger Hallen and Oskarsson (1995) found that in rat $^{203}$Pb was bound to milk protein casein to more than 9% in vitro and in vivo. Heavy metals including lead can affect fish olfactory system directly inducing structural and functional alterations (Rhenberg and Schreck, 1986; Beatrap, 1991; Blaxter and Ten Hallers, 1992; Julliard *et al.*, 1993; Saucier and Astic, 1995; Hansen *et al.*, 1999). The complex effect, both toxic and irritant, of even concentration of heavy metals can be synergetic with respect to fish olfactory system and that lead to fish behavioural responses (Sveccevicesov, 2001).

Natural and synthetic zeolites are ion exchanger and can remove heavy metals from polluted water. Zeolites are also used as feed additives in case of cattles, chelation therapy for metal ion toxicity has been suggested by Sharma (1995). Protective action of natural zeolite on lead toxicity with reference to biochemical content has been reported by Jain
et al. (1995). Faghhiian et al. (1999) have already reported that toxic metals can be removed from municipal waste water with the use of clinoptilolite.

2. RNA Contents

In control fish, the range of RNA contents observed in the liver 8.10±0.45-8.19±0.45, in kidney 7.10±0.43-7.19±0.43, in gills 7.15±0.44-7.21±0.44 and in brain 6.09±0.30-6.18±0.30 mg/g. A consistent slight increase in the RNA contents observed as the exposure periods proceeds due to advancing age of the fish. When fish exposed to lead nitrate, RNA content decrease. When exposed lead nitrate+natrolite and only natrolite the RNA contents in the tissues first decreased than improved respectively (Table No. 5 to 8).

Liver

In the present investigation, it has been observed that, when fish exposed to subethal concentration of Pb(NO₃)₂, the RNA contents decreased consistently from 7 to 35 days of exposure. Addition of natrolite along with lead nitrate, improved the level of RNA contents. When fish exposed to only natrolite, the RNA contents observed higher in comparison to control fish [Table No 5]. In group II fish exposed to lead nitrate, the maximum and minimum decrease in the RNA contents observed -19.75% and -19.65% respectively. In lead nitrate+natrolite exposed group of fish, improvement in RNA contents have been observed. When the fish of group IV exposed to natrolite, a slight increase in the RNA contents observed.
James et al. (1998) found level of RNA increased in the tissue of control fish *Oreochromis mossambicus* with increase of rearing time.

The increases in RNA suggest that it is involved in protein synthesis without changing the amount of DNA in the control fish. The similar result was found by Brachet, (1995) and Love, (1980). The amount of DNA in each cell nucleus is constant for a species (Love, 1980) and it is considered as an index of cell numbers contributing to unit weight of tissue, while the concentration of RNA in a cell is related to protein synthesis (Brachet, 1995) and metabolic activities of tissue (Leslie, 1955; Bulow, 1970). Therefore, RNA: DNA ratio indicates the amount of protein synthesis and could be a more sensitive tool for measuring the growth rate of fish (Bulow, 1971; Fauconneau, 1985). James et al. (1998) observed gradual increase in RNA contents with rearing time. He further observed 10 times increase in zeolite treated fish as compared to fish exposed to cadmium alone. Sastri and Gupta (1978) emphasized that overall decrease in RNA contents is probably due to enzyme inhibition which play an important role in protein synthesis.

Coello and Khan (1996) reported that alkaline solution of cystein and glycine treated scales of fish protects against heavy metal toxicity.

The lead and natrolite could interact in the experimental medium as follows; zeolite has extra framework ion (Na⁺) and framework ions Al³⁺ and Si⁴⁺ which are easily exchangeable and non-exchangeable respectively. The ionic radii of Na⁺, Al³⁺ and Si⁴⁺ are 0.95, 0.50 and 0.54Å respectively. The ionic radius of Pb is 0.84Å (Sanderson, 1960 and Huheey, 1983).
which isSuitably matched to the ionic radius of Na⁺ (0.95 Å) in zeolite, and hence both ions could be easily exchanged with each other than Al³⁺ and Si⁴⁺ ions. Briggs and Smith (1996) found that zeolite has the capacity to remove ammonia and other metabolites from EDTA in Cu contaminated medium caused the formation of stable ion (Cu⁺) exchanged EDTA complex and elimination of more amount of Cu⁴⁺ in faeces. Over doses of chelating agents like EDTA could cause deleterious effects on survival, development and growth in crustaceaus larvae (Davis et al., 1976) and survival and haematological in fish (James et al., 1998). James et al. (1998) reported that application of even the higher dose of zeolite (8g z/l) did not cause any adverse affects in Orechromis mossambicus.

**Kidney**

According to Table 6, depletion in RNA contents has been observed in the kidney of experimental fish, exposed to lead nitrate from 7 to 35 days of exposure period. The RNA contents decreased -22.67% after 7 days and -22.39% after 35 days of exposure period. When fish exposed to lead nitrate + natrolite, the RNA contents increased consistently with increasing exposure period. In case of fish exposed to only natrolite, the RNA contents improved further in comparison to control at all time interval from 7 to 35 days.

The concentration of RNA in the cell of organism is related to protein synthesis (Brachet, 1995) and metabolic activities of a tissue (Leslie, 1955; Bulow, 1970). Sumino et al. (1975) observed highest
cadmium is rapidly cleared from the blood and accumulates in the kidney and liver, which contains approximately 2/3 of the total body burden. Johanson-Sjoebeck and Larson (1979) reported the depression of activity of delta-ALAD in rainbow trout exposed to lead nitrate. The enzyme was depressed in red blood cells, spleen and renal tissue. Mass Oliva (1989) and Rajanna et al. (1990) reported that metals bind strongly with the sulphahydryl groups to form mercaptides, inhibiting large number of enzyme containing functional thiol groups as also observed with other heavy metals such as cadmium (Paier et al., 1993) and mercury (Visser et al. 1976).

Lead is similar in many aspects to calcium and may perform a competitive action on mitochondrial and neurological function.

Natural and synthetic zeolites have capacity to remove heavy metals from metal containing waste water as reported by many investigators (Sherman1978; Semmens and Seyfarth, 1978); Carmine Colella (1995); James et al. (1998); Faghihian et al. (1999) and Jain et al. (1999, 2000, 2001).

Gills

When fish exposed to sublethal concentration of lead nitrate, RNA contents in gill reduced from 7 to 35 days of exposure periods in comparison to their respective control. Fish, exposed to lead nitrate + natrolite showed increase in RNA contents. In lead nitrate exposed fish the changes in RNA contents found -21.39% and -21.22% decrease from 7 to 35 days of exposure periods. In group of fish exposed to lead nitrate
along with natrolite, the improvement in RNA contents was recorded +11.74% and 11.44% and in group IV of fish, exposed to natrolite only, a minimum of 0.42% and 0.97% maximum changes was observed [Table No. 7].

Sultana Rafia and Umadevi(1995), studied the oxygen consumption in a cat fish *Mystusgulio* exposed to heavy metals and suggested that due to CuSO$_4$ and ZnSO$_4$ of various concentrations, respiratory activities are inhibited by Cu then Zn. Accumulation of lead in the gills is higher in comparison to liver especially, when one compares accumulation levels of other non-essential metals (Tulasi *et al.*, 1992; Allen, 1994; Roesijadi and Robinson, 1994). It is well established that lead can reach drinking water through the dissolution of plumbing material (Murrett, 1990; Schock and Neff, 1988 and Subramanyam *et al.*, 1991) and that lead in water may influence blood lead levels in human (Gulson, 1994 and Gulson *et al.*, 1997 and Cosgrove *et al.*, 1989).


Reports of 1993 of ICMR (Indian Council of Medical Research) have warned that high concentration of heavy metals in air, water and soil are detrimental to all living beings.

James and Sampath (1998), Pansini and Collela (1991) reported that zeolite are ion–exchangers and are useful for the removal of heavy metals from waste water.
Brain

In control fish, RNA contents in the brain of experimental fish observed 6.14±0.37 mg/g which is almost similar during 7 to 35 days of exposure period. A slight increase observed with the increasing age of fish. When fish of group II exposed to lead nitrate, decrease in RNA content was observed in all the exposure periods. The minimum and maximum percent changes 17.24 and 16.83 were recorded after 7 and 35 days respectively. When fish of group III, exposed to lead nitrate with natrolite, the RNA content improved, compared to their respective group II fish, exposed to lead nitrate. The minimum and maximum improvement in RNA contents a fish 7 and 15 days of exposure were 17.86% and 16.73%. However, when fish exposed to only natrolite, the RNA content observed to be increased in comparison to their respective control. The range of improvements observed has been from 3.28% to 3.72% (Table 8).

Several investigators demonstrated that exposure during the prenatal period, exerts adverse effect on development and function of the nervous system (Kumar and Desiraju 1992; Salanki et al., 1993; Shao and Suzukiw, 1991). Additionally, it was reported that lead treatment modifies neuro muscular junctions (AlDhaheri et al., 1996) like other heavy metal such as cadmium (Guan et al., 1987); lead exhibited special effects on neuro transmitters release potentially causing muscle weakness (Hiratta and Kusako, 1993; Oortgiesen et al., 1993, Shao and Suzukiw, 1991; Strzynska and Rafałowska, 1994). Muscle palsy
occurred in fatigue frog muscles (Zacharova, et al., 1993) may be a result of lead effect on cholinergic nerve terminal (Silbergeld et al., 1974). Evidences presented earlier indicates that lead reduces acetyl choline at neuromuscular junction rather than a direct effect on the muscle (Kostial and Vouk, 1957).

The effect of lead on calcium phospholipid–dependent protein kinase in the brain microvessels from 6 days old rat pups have been examined by Markovac and Coldstein (1988). It has been found that micro molar concentration of lead activate this enzyme to an extent equivalent to micro molar calcium and suggested that this may be involved in the mechanism of lead cellular toxicity in capillaries which consist of endothelial cells.

For the remediation of metal toxicity, application of zeolite have been reported by James and Sampath (2003). Use of natural zeolite chabazite and phillipsite for removal of heavy metals from waste water has been reported by Carmine Colella (1995). Jain (2001) has also observed the protective role of zeolite over metal toxicity.

The values of RNA contents improved in the brain of fish exposed to natrolite indicates that natrolite may be useful in the biological system. Similar trend is followed with the improvement of protein and RNA as RNA is responsible for protein synthesis.

3. Cholesterol contents

The cholesterol contents in liver, kidney, gill and brain of experimental fish of control group observed almost similar during all the
exposure period. When fish of group II exposed to sublethal concentration of lead nitrate the cholesterol contents in all the four experimental tissue found to be increased in comparison to their respective control. The maximum increase in these tissues reported 10.79, 7.79, 7.74 and 2.04% respectively up to 35 days of exposure while the minimum increase observed after 7 days of exposure i.e. 6.63, 7.20, 6.66 and 1.79% in liver, kidney, gill and brain respectively. When fish of group III exposed to lead nitrate + natrolite, an increase in cholesterol contents has been observed. In the fish of group IV exposed to only natrolite again an increase was observed (Table 9, 10, 11 and 12).

**Liver**

In control fish, the cholesterol contents observed 6.93±0.38 almost similar during all the exposure periods. In lead nitrate exposed group of fish, the minimum increase in cholesterol contents observed was 6.63% (after 7 days) which was maximum 10.79% (after 35 days). In lead nitrate + natrolite exposed group of fish, the values of cholesterol content decrease in liver consistently from 7 to 35 days of exposure period in comparison to group II fish. The fish of group IV exposed to only natrolite, the range of values have been found almost similar to the control fish during all the exposure period (Table 9).

Kargin (1998) observed highest metal concentration in liver and gills in comparison to muscles in freshwater fish *C apoeta barroji*. These results were consistent with studies carried out on freshwater fish (Norris and Lake, 1984; Dallinger and Kantzky, 1985; Lagorburu et al.,
Medina et al. (1986) and Kalay et al. (1999) also observed high level of lead in fish. Kalay et al. (1999) studied concentrations of various heavy metals in fish tissue and observed lead level higher than the acceptable value for human consumption by various health organizations. Daily tolerable lead intake form food according to WHO/FAO committee's proposal is 7 μg Pb/Kg body weight (Merian, 1991).

In shrimps, the hepato-pancreas is the main regulatory organ and prime site for metal storage. According to Kargin et al. (2000) the high levels of metals found in the hepatopancreas of shrimp Panaeus semiculatus is possibly due to binding of the metals to metallothionine proteins. The toxicity due to lead causes neonatal mortality, morbidity and sterility in animals (Koegh, 1992). Lead impairs the enzymatic activity of delta-amino-levulinic acid dehydratase which is involved in haem synthesis (Satija and Vij, 1995).

Pansini and Colella (1989) have reported the removal of metal by zeolites. Raizada et al. (1998) studied the effects of heavy metals and the effects on eggs, frys and fingerlings of Rasbosa daniconius and observed that the fingerlings are most resistance and eggs are least resistant to metal toxicity.

**Kidney**

As evident from (Table 10), in control fish the cholesterol contents in kidney of the experimental fish recorded 5.97±0.31 (mg/g), almost similar during all the exposure periods. A slight increase observed with
increasing age. When fish of group II were exposed to lead nitrate, an increase in cholesterol content was noticed. When fish of group III exposed to lead nitrate + natrolite, cholesterol contents improved in comparison to their respective control but remain lower than their group II fish. However, the group IV fish exposed to natrolite only, indicated improvement in cholesterol content in comparison to control fish.

In lead workers cholesterol and unsaturated fatty acids were found comparatively higher (Matteo Valentino et al., 1991). Karai et al. (1982) reported that cholesterol increase in tissues is due to lead toxicity. Use of natural zeolites in remediation of metal toxicity in fish has been reported by Raizada et al., (1998) and Jain et al., (2005).

**Gills**

In control fish, the cholesterol contents observed 5.42±0.30 mg/g almost similar during all the exposure periods.

When group II fish exposed to lead nitrate, the cholesterol contents increased in comparison to control fish. The maximum and minimum percent change in cholesterol contents observed 7.74 and 6.66% respectively. When fish of group III were exposed to lead nitrate+ natrolite, improvement observed but remain lower than lead nitrate exposed fish. When IV group of fish exposed to only natrolite, a slight increase in cholesterol content observed in comparison to control group (Table 11).

Jia-xiuying et al., 2001 studied the effect of heavy metals including lead on the respiration intensity of juvenile *Misgurnus*
angwillicandidates. They found respiration intensity decreased in them. These studies indicate that lead effects the normal function of gills by inhibiting enzymatic biochemical reaction. Depuration of lead in gills in brook trout has been reported 12 weeks after transfer to normonal water (Holcombe et al.; 1976 and Strippes et al., 1990). Potential lead antagonish diethyl ditheiocarbonate (DDC) (Gale et al., 1986); l-cystein and N-acetyl-L-cystein (NAC) Llobet et al., 1985, 1986 and also EDTA has been reported by Khangarot et al. (1984). James et al. (1998), Jain et al. (1999, 2000) reported that zeolites are not toxic but beneficial to biological system.

Zeolite can absorb metallic ions by cation exchange reactions. Continuous exposure of the teleost fish Heteropneustes fossilis to sublethal concentration of lead nitrate in water for 35 days increased the cholesterol contents but decreased protein, RNA, and glycogen contents in various tissues. The presence of zeolite in the exposure water decreased all of the adverse affects (Jain et al., 2001).

Brain

As evident from table no. 12, in control fish the cholesterol contents in brain measured 3.93 ±0.26 mg/g, nearly similar during all the exposure periods. When fish of group II were treated with lead nitrate, increase in cholesterol contents observed but remain lower then their respective control group fish. When fish of group III exposed to lead nitrate + natrolite, showed increase in cholesterol contents but the amount was not higher to their group II fish exposed to lead nitrate. In
fish of IV group exposed to only natrolite, a slight improvement in cholesterol contents was recorded in comparison to control. In group II fish exposed to lead nitrate only, the minimum increase 1.79% (after 7 days) and maximum increase 2.02% was noticed (after 35 days).

Peng Peigin (1991) found that lead pollution is very serious in Zhuzhou Smeltry industrial area. Heavy metals like Pb getting into a cell stimulates it to produce a defensive stress-increasing lysosomes in the cell. Matteo Valentino et al. (1991) reported increased cholesterol in lead worker compared to non-lead workers. Lead produces an increase in cholesterol Karai et al. (1982).

Pansini and Colella (1991) and Cioffi et al. (1991) reported that chabazite and philipsite are useful in remediation of heavy metal toxicity from waste water. James and sampath (2003) reported that zeolites decreased metal uptake in animals. He found that zeolite caused the elimination of heavy metals in polluted water. Like other natural zeolite, natrolite is also a ion exchanger and remove lead from polluted water.

4. Glycogen Contents

Glycogen contents in liver, kidney, gills and brain in control fish observed almost similar during all the exposure periods. In these organs the values of glycogen contents were measured 43.84±1.20, 30.23±0.98, 22.31±0.63 and 6.51±0.40 mg/g respectively. In all above organs the glycogen contents slightly increased with the increasing exposure periods. The reason behind it may be the growing age of fish [Table No.13-16].
Liver

As evident from Table No. 13, in control fish, glycogen contents in liver observed 43.84 ±1.20 mg/g almost similar during all the exposure periods. When fish of group II exposed to lead nitrate, showed rapid decrease in glycogen content. The highest decrease in the value of glycogen content noticed after 7 days which decrease gradually and after 35 days lowest decrease was observed. When the fish of group III exposed to lead nitrate + natrolite, the glycogen contents in these organs regularly improved from 7 to 35 days in comparison to group II. The maximum increase noticed after 7 days, while it was minimum after 35 days of exposure duration. However, when the fish of IV group exposed to natrolite only, the level of glycogen contents, increased in comparison to their control group fish. During this period of exposure, the range of increase observed from 0.98% to 1.75%.

Hoffman et al. (1985) reported that liver delta-ALAD activities were inhibited by lead. Winter flounder Pseudopleuronectes americanus showed decrease of 66% in delta activity in liver (Jackim, 1973). Glycogen synthesis in the tissue take part in it (Johansson Sjoback and Larson 1979).

Shaffi (1979) examined various biochemical parameters in fresh water fish exposed to lead nitrate. He observed glycogenolysis due to lead exposure in all fish studied. Zeolites are ion exchangers. The sodium of zeolite is exchange by lead ions in their molecular sieve Jain et al. (1996). The improvement in the value of glycogen in the tissue of experimental
fish exposed to only zeolite, suggests that zeolite is not toxic rather beneficial to the biological system. Natural zeolite are also used as feed additive in case of cattles. Sharma (1995) also suggested Chelation therapy for metal ion toxicity.

Santiago et al. (1998) found mean lead level 0.57 mg/kg w/wt in the liver of deer and 2.61 mg/kg in liver wild boar. In order to preserve the health of wild and domestic animals they concluded that the lead contamination should be controlled and considered that there should be a more rigorous control of metal contamination of organs destined for human consumption. Abou-Arab (2001) studied accumulation of heavy metals content including lead in goat meat and recorded lead 0.080 mg/kg in liver.

Oti et al. (2000) studied the effect of lead toxicity on glycogen contents in the African freshwater cat fish Clarius gariapinus and Heterobranchus Longfjils and found that glycogen contents in liver decreased significantly after two weeks exposure to lead nitrate.

Al-Ekel (1994) found that there were falls in the glycogen contents of liver and muscle in freshwater carp, Cyprinus carpio as a result of lead acetate exposure.

Zeolites decrease the toxicity of metals in fishes. Zeolites can adsorb metallic ions by cation exchange reactions. Continuous exposure of the teleost fish Heteropneustes fossilis to sublethal concentration of lead nitrate in water for 35 days (Short term) decreased the glycogen contents in the liver. The presence of zeolites in the exposure water
decreased all of the adverse effects. In fish exposed to zeolite as feed additive all the parameters improved in comparison to control fish, indicating that zeolites can be used safely in biological system (Jain et al., 1997).

**Kidney**

As evident from table No 14 in control fish the value of glycogen contents remain almost similar during all exposure periods. A normal increase in glycogen contents with increasing age has been observed. When fish of group II exposed to lead nitrate, the maximum decrease observed 22.24±0.63 and 23.10±0.63 after 7 days and 35 days of exposure respectively. When fish of group III subjected to lead nitrate + natrolite, it showed increase in glycogen content in comparison to group II. However when fish of IV group exposed to natrolite only showed a slight increase in comparison to their control group.

Jackim (1973) observed that exposed mummichog *Fundulus heteroclitus* and winter flounder *Pseudopleuronectes americanus* to an initial concentration of 10 mg lead (as lead nitrate/litre) under static conditions in sea water. There were decreases of 18.5% in liver –ALAD activity in mummichogs after two weeks. Winter flounder showed decreased of 58% in delta-ALAD activity in kidney after one week. Lead accumulation in bones, scales, gills, kidney and liver has been reported by Holcombe et al. (1976), Patterson and Settle (1977), Hodson (1978) Varanasi and Gummur (1978) Bollingbers and Johanson (1979) and Hodson et al. (1982). James and Sampath (1999) reported the effect of
cadmium and the impact of the ion exchanging agent zeolite on food utilization and metal up take in cat fish *Heteropneustes fossilis*.

Santiago *et al.* (1998) studied lead accumulation in red deer and wild boar from Sierra Moreno mountains in Spain and found lead accumulation to a greater extent in the liver and kidney. Since this metal is present in all living organisms and is bioaccumulated through the food chain. Lead is present in all ecosystems of much higher levels than cadmium. He reported mean lead concentration in the kidney of deer and wild boar 0.3 mg/kg wet wt and 0.62 mg/kg respectively. The highest lead levels in deer were 2.15-mg/kg in kidney. Abou-Arab (2001) also recorded lead contents in kidney of goat upto 680 mg/kg.

In man, nephrotoxicity is most commonly seen in childhood. Lead intoxication and in moonshine plumbism, which may be more acute than chronic, lead cause tubular dysfunction, with ultra structural changes in the mitochondria, which result in amino- aciduria, glycosuric and phosphaturia, as well as gout. Despite earlier reports, there is no apparent increase in hypertension (Tsuchiya, 1977; Stokinger, 1981 and Cullen *et al.*, 1983).

When fish of group II exposed to lead nitrate, glycogen contents decreased, and increased on exposure to lead nitrate + natrolite. The IV group fish exposed to only natrolite indicated increase in glycogen contents compared to their respective control group, show that natural zeolite natrolite acts as protective agent against lead toxicity. It is a non toxic inexpensive substance and hence can be used in biological system
for removal of lead poisoning. These observations about decreasing lead toxicity through natrolite agree with those observed by Jain et al., 1996 with *Heteropneustes fossilis*.

**Gills**

The glycogen contents in gill of experimental fish observed 22.31±0. 63 mg/g almost similar during short term exposure in control fish. Exposure of group II fish to subethal concentration of lead nitrate resulted in decrease in glycogen contents. The maximum and minimum decrease observed 15.79% and 15.03% respectively after 7 days and 35 days of exposure period. When group III exposed to lead nitrate + natrolite, improvement in glycogen contents was observed in comparison to group II fish. However, when group IV fish exposed only to natrolite, the glycogen contents found to be increased in comparison to their respective control. The range of glycogen content observed from 2.42% to 2.63% during the exposure period (Table No.15).

In the present study lead nitrate has been found to inhibit glycogen contents might be due to increased glycogenolysis as reported by Shaffi (1979). Johenson sjobeck and Larson (1979) reported that lead nitrate depresses enzyme activity in tissue. Anderson (1978) maintained the crayfish *Orconectes virilis* in natural river water, with lead acetate added to concentration of 0.5, 1.0 and 2.0 mg/litre. The water was changed at regular intervals to maintain the lead concentration and at 1 day intervals, the oxygen consumption of the crayfish was measured. There was a dose-related reduction in O₂ consumption after 10 days of
exposure to lead acetate after 20,30 and 40 days of exposure there was no difference in $O_2$ consumption between control and treated crayfish; the animals had acclimatized to the lead. The crayfish was found to be compensating for the effect of the lead, which reduced the capacity for oxygen uptake through the gill by increasing the flow of water over the gill surface. These was a dose related relationship between ventilation volume and lead concentration in the water over the range 0-2-2 mg/l the ventilation volume at 2.0 mg lead acetate /litre was 19 ml/min composed with 12 ml/min for controls. Since the water in the test tank was kept saturated with oxygen the crayfish were able to restore fully their $O_2$ uptakes. Due to lead toxicity embryonic abnormalities have been reported by Ozoh (1979) suggestive of abnormal biochemical contents. 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. When fish exposed to lead nitrate along with natrolite the values of glycogen contents in gills improved significantly. In case of gills the improvement in glycogen contents has been observed to be 11.40, 11.33, 11.25, 11.02 and 11.11 percent after exposure of 7,14,21,28 and 35 days respectively. When fish were exposed to only natrolite the glycogen content further increased compared to their control groups.
Biochemical effects due to lead toxicity and protective action of zeolite has already been reported by Jain et al. (1995). Zeolites (sodium aluminium silicates) natural and synthetic are ion exchangers. The sodium of zeolite present within their molecular sieve are exchanged with lead ions Jain et al. (1996 1997). The improvement in glycogen contents in the gills of experimental fish due to exposure of lead nitrate along with natrolite suggests that natrolites zeolite is a remedial factor of lead toxicity. Jia–Xiuying et al. (2001) found respiration intensity decreased in the fish *Mugilus anguillicaudatus* due to lead exposure. The respiration intensity of the fish decreased with prolonged poisoning time and decreasing heavy metal (Pb) concentration.

**Brain**

The glycogen contents in the brain of control fish found to be 6.51±0.40 mg/g nearly similar during all the exposure periods. When fish of II group subjected to sublethal concentration of lead nitrate the glycogen contents reduced in all fish. When fish of group III exposed to sublethal concentration of lead nitrate + natrolite in same quantity, improvement in glycogen contents was noticed during all exposure periods. The fish of IV group exposed to only natrolite showed increase in glycogen contents to their respective control [Table No. 16].

Mercury also causes depletion in glycogen contents and hepatic enzymes (Shakoori et al., 1994). Jain et al. (1995) already reported biochemical effects due to lead toxicity and protective action of natural zeolites on it. The use of another natural zeolite clinoptilolite and its
sodium form for removal of heavy metals from municipal waste water has been reported by Faghihian et al. (1999). Holcombe et al. (1976) exposed three generations of brook trout *Salvelinus fontinalis* to lead nitrate in the water. All second generation trout exposed to 0.235 and 0.474 mg lead/litre, and 34% of those exposed to 0.119 mg/litre, developed spinal deformities.

II. CHRONIC STUDY

For chronic study 120 fish were divided into four equal groups. Group I served as control while group II, III and IV exposed to sublethal concentration of lead nitrate, lead nitrate+ natrolite, and natrolite only respectively. After exposure periods of 60, 90, 120, 150, and 180 days, fish sacrificed and processed for the estimation of protein, glycogen, cholesterol and RNA content, in experimental tissue liver, kidney, gill and brain. All experiments were run in triplicate and average values have been summarized in table 17 to 32.

1. Protein

Protein content in control fish during all the exposure period found almost similar. The values recorded were 81.14±1.65 mg/g in liver, 73.94±1.45 mg/g in kidney, 66.43±1.55 in gill and 68.90±1.44 mg/g in brain respectively. A slight and continuous increase in the values have been recorded due to advancing age of the fish [Table No. 17-20].

Liver

The protein contents in control fish have been recorded 81.14±1.65 mg/g during the exposure period. In fish of group II exposed to lead
nitrate, decrease in protein contents recorded during all the exposure periods from 60 to 180 days. After 60 and 180 days of exposure, the protein contents decreased 3.02% and 2.97% respectively. When fish of group III exposed to lead nitrate + natrolite, the protein contents improved consistently in comparison to group II exposed to lead nitrate. The maximum improvement was observed after 60 days and minimum after 180 days of exposure period. When fish of Group IV exposed to only natrolite, the protein contents in liver found to be improved further compared to their control. The improvement in protein contents ranged from 0.43% to 0.74% (Table 17).

Shaffi et al. (2001) reported the effect of cadmium on gills of teleost fish *Labeo rohita*. Oxygen uptake is reduced in gills followed by the liver muscles and kidney due to cadmium exposure. Protein synthesis under metallic stress reduces in tissues of *Labeo rohita* (Syversen, 1981 and Prasanta Nanda & Malan Kumar Behera, 1996).

Sastry and Gupta (1978) observed the effect of mercury on liver in *Channa punctatus*. They observed that 3 phosphatase (alkaline phosphatase, acid phosphatase and glucose-6-phosphatase) are inhibited in the liver due to mercury stress. Liver, kidney and gills are metabolically very active in fish and metal in these organs tend to accumulate in large concentrations compared with organs like muscles and gonads (Amiard et al., 1987).

Toxic metals including lead ions are bound by sulphydryl groups of proteins (Viarengo, 1985). Sulphydryl binding changes the structural and
enzymatic activities of proteins and causes detrimental effects evident at
the whole organism level (Hodson, 1978).

Niklowitz and Yeager (1973) reported that lead inhibits biological
systems and competitively interferes with zinc and copper. Ozoh (1980)
observed that lead interferes with pigmentation patterns in fishes.
According to Shakoori et al. (1994) heavy metal mercury causes depletion
in soluble protein content and hepatic enzymes in freshwater China
grass carps.

Natural and synthetic zeolites are ion exchangers. The sodium of
zeolite is replaced by lead ions in their molecular sieve (Jain et al., 1996;
1997). The increase in the protein contents in liver of experimental fish
exposed to zeolite proves that zeolite is useful to biological systems
including fishes as zeolite are also used as feed additives in case of cattle
Jain et al. (1996).

Kidney

The protein contents in the kidney observed 73.94±1.45 mg/g
nearly similar during all the exposure periods. When fish of group II
exposed to lead nitrate, the protein contents decreased drastically in
comparison to their respective control.

To observe the protective role of natrolite on lead toxicity with
reference to protein contents, the group III of fish exposed to lead nitrate
+ natrolite, the values of protein content increased in kidney consistently
from 60 to 180 days to lead nitrates. The maximum improvement of
6.71% has been observed after 60 days of exposures while it was
minimum (3.7%) after 180 days. However when fish exposed to only natrolite, the protein content found to be increased in comparison to the control fish. The range of improvement in this group was observed from 0.55% to 0.58% during the exposure period (Table No. 18).

Kidney is one of the most sensitive organs readily responding to heavy metal intoxication. Heavy metals are well known inhibitors of enzymes (Battigelli, 1960). Urease is readily inhibited by heavy metals Sumner and Somers (1953). Chang et al. (1973) demonstrated a fall in the activities of alkaline phosphatase and glucose-6-phosphatase and a slight increase in acid phosphatase in kidney. Hinton et al., (1973) also observed an initial increase and later decrease in acid phosphatase activity due to mercury intoxication. The increase in acid phosphatase activity is probably related to the increase in lysosomal activity in the induced cells occurring as part of prenecrotic changes (Deduve 1963, Novikoff, 1961). Alkaline phosphatase is a brush border enzyme present in kidney tubules. This enzyme is involved in the reabsorption of glucose from the renal tubular lumen (Hickman and Trump, 1969). According to Douglas (1997), the action of heavy metals to proteins have two main types.

(i) Action to some special groups such as imidazole group, carboxyl group and mercapto group.

(ii) Action to special structures of amino acid residues. In addition, the phosphate groups of nucleic acid can also combine with positive ions of heavy metals into chelates (Amdur et al., 1991) reported highest concentration of metallothionine protein in fish kidney.
Ram and Sathyanesan (1984) reported that heavy metals reduce the protein, lipid and cholesterol contents in various tissue of *Channa punctatus*. Wang and Horrisberger (1996) observed that the heavy metals interfere with protein synthesis. Heavy metals inhibit Na⁺/K⁺ ATPase in oocytes at micromolar concentrations. The improvement in the values of protein in the experimental tissues exposed only to natrolite suggests that natrolite is not toxic rather beneficial to the biological system. Sharma (1995) has reported chelation therapy for metal ion toxicity. There are a number of chelators which are used for remediation of heavy metal toxicity (Hammond, 1971; Chisolm, 1970, 1971; Friedhein et al., 1978; Graziano et al., 1985 and Klaavassen, 1985).

**Gill**

When fish exposed to lead nitrate, the protein contents decreased in comparison to their respective control fish in all exposure periods of time upto 180 days (table No. 19). The maximum and minimum decrease in protein contents recorded 15.44% and 15.38% respectively after 60 and 180 days of exposure periods. To observe the remediation of lead toxicity, the fish of group III were exposed to lead nitrate + natrolite, improvement in protein contents observed 5.20% in gill. The maximum and minimum improvement recorded 2.10% and 1.99% after 60 to 180 days of exposure periods. In fish of group IV exposed only to natrolite, the protein contents slightly increased in comparison to their control fish.
Watson and Beamish, 1981; Stagg and Shuttleworth, 1982; Pinkey et al., 1989; Thaker et al., 1996; Canli and Stag, 1996; Morgan et al., 1997); reported that Na, K-ATPase are active in aquatic animals is sensitive both in vitro and in vivo exposure to heavy metals. Ay et al. (1999) observed highest accumulation of copper and lead in gills of a fresh water fish Tilapia zillii. Recheirt et al. 1979; Amiard et al. 1987, Tulasi et al., 1992; Allen, 1994 also noted the same pattern of lead accumulation in different fish species. Thaker et al., (1996) reported significant inhibitions of Na, K-ATPase are in the gills of costal telescop Perioph thalamus dipses. Morgan et al. (1997) showed that silver inhibits activity of branchial Na, K-ATPase are in the rainbow trout in front Oncorhynchus mykiss.

Borah and Yadav (1996) reported decreased ALP activity in gills of Hetropneustes fossilis following dimethoate exposure. Lopez et al. (2003) also noted the same effects of pollutants on the gills of various fish species.

According to Mayzand and Conover (1988) ammonium is one of the final products following catabolism principally of amino acid that might have an alimentary or muscular origin depending on nutritional conditions. Amino acids are also used as an energy substrates and component of body structure. Besides this, amino acid can be more important than ions in the maintenance of osmotic pressure in prawns such as Paeneus setiferus (McForland and Lee, 1963; Rosas et al. 1999). Ammonium excretion was inhibited in Paeneus indicus post larvae exposed to lead (Chinni et al., 2000; 2002).
Brain

The protein contents in the brain (68.90±1.44mg/g) have been recorded nearly similarly during all the exposure periods. When fish exposed to lead nitrate, the protein content decrease in comparison to their control fish. The maximum decrease of 13.97% and the minimum decrease of 13.92% were recorded after 60 to 180 days respectively. To observe the remediation of lead toxicity through natrolite the fish of III group were exposed to lead nitrate+natrolite, an improvement in protein content observed during all exposure periods. The range of improvement in protein content in this tissue was observed from 2.01% to 1.99% from 60 to 180 days respectively. When fish of group IV exposed to only natrolite, a slight increase in protein content observed in comparison to control (Table 20).

Heavy metals can affect fish olfactory system directly increasing structural and functional alterations (Rhenberg and Schreck 1986; Beatrup 1991; Blaxter and Ten Hallers-Tjabbes, 1992; Julliard et al. 1993). Joao et al. (1995) reported the inhibitory response of ALA-D to lead in brain increased upon the pre incubation of the enzyme with metals. Lead binding proteins have been detected in brain (Duval and Fowler 1989). Novelli et al. (1999) reported that heavy metals might cause pathological changes in brain and nervous system. Lead was found to inhibit the impulse conductivity by inhibiting the activities of mono amino oxidase and acetylcholine esterase (Katti and Sathyanesan, 1986) to cause pathological changes in tissues and organs and to impair

Among the various cation exchangers, natural zeolites including natrolites are preferred due to high selectivity for heavy metals cations and low cost compared with synthetic cation exchange materials also it has been proven protective action of natural zeolite worked without any side effect (Sherman 1978; Semmens and Seyfarth 1978). Use of zeolite for removing ammonia and ammonia caused toxicity has been reported by Burgess et al. (2004).

2. RNA Contents

In control fish, the RNA content in the liver, kidney, gills and brain observed 8.37±0.45 mg/g, 7.42±0.44mg/g, 7.28±0.42 mg/g and 6.41±0.41 mg/g respectively during all the exposure periods. A consistent slight increase in the RNA contents in all tissues observed as the exposure period proceeded (Table 21-24).

Liver

The RNA contents in liver of fish exposed to lead nitrate significantly decreased after 60 to 180 days experiment period compared with the control fish. The maximum decrease of 5.16% was observed after 60 days and the minimum decrease of 4.64% was recorded after 180 days. To observe the remedial effect of natrolite on lead toxicity the fish of group III were exposed to lead nitrate + natrolite. The fish of group III showed improvement in RNA contents in comparison to their
respective group II fish. The maximum and minimum improvement observed 2.78% and 2.50% after 60 and 180 days respectively.

When fish of group IV exposed to natrolite only a slight increase in RNA content recorded in comparison to control fish. The minimum and maximum increase of RNA contents observed were 0.96% and 1.67% after 60 to 180 days exposure period (Table No. 21).

Hilderbrand et al. (1973) studied the effect of lead in rats and observed that it reduce the smooth endoplasmic reticulum in liver cells of rats. Chow and Cornish (1978), Fouts and Pohl (1971) found out that lead compounds inhibit enzyme activities in liver cells. Villareal et al. (1986) noted that lead interferes with the functioning of biological system and exerts toxic effect in organism.

Todd Hsu et al. (1998) pointed out that heavy metals may disturb the binding of upstream binding factor to the regulatory sequence of RNA genes and inhibit RNA polymerase I-associated RNA transcription. According to Tschuchya (1977), Forni et al. (1980) lead causes chromosomal aberration in animals.

Shakoori et al. (1994) observed depletion in soluble protein glycogen, RNA contents and hepatic enzymes in mercury stressed Chines grass carp Clenopharyngodon idella. Zeolite has been used for decades to decrease concentration of ammonia in municipal effluents (Mercer et al., 1970). In addition to this zeolite has been shown to remove several metals from aqueous solution (Kesraoui-Ouki et al., 1994).
Kidney

Compared with the control group, the RNA content in kidney of fish exposed to lead nitrate (Group II) was significantly decreased after 60 to 180 days experimental period. When the fish of group III were exposed to lead nitrate + natrolite, improvement in RNA content observed in all exposure periods from 60 to 180 days in comparison to group II fish. the values ranged from 7.08±0.35 (after 60 days) to 7.58±0.35 (after 180 days) of exposure, whereas when fish of group IV exposed to natrolite only, the improvement level of RNA contents enhanced continuously till 180 days of exposure period (Table 22).

According to Passow et al. (1961) the toxic effect in animals may result from the bioconcentration of metals and their consequent binding with biologically active constituents of the body such as lipids, amino acids, enzymes and proteins. In all animals, kidney is a target organ for toxicity and major site of antagonistic interaction between essential trace metals and heavy metals (Friberg and vahter 1983 and Bremmor, 1987). Mass Oliva (1989) and Rajanna et al. (1990) reported that metal binds strongly with sulphydryl group to form mercaptides, inhibiting large number of enzyme containing functional thiol group as also observed with other heavy metals (Paier et al., 1993). Shastri and Gupta (1978) emphasized that over all decrease in RNA contents is probably due to enzyme inhibition which plays an important role in protein synthesis.

The most widely used techniques for the removal of toxic metals involves the process of neutralization and metalhydroxide precipitation
(Himesh and Mahadevaswamy (1994). From industrial wastes or polluted media, chemicals can effectively remove certain toxic elements. However, there are some cheap chemicals which are also free from undesirable side effects. Natural and synthetic zeolites are such chemicals that act as an ion-exchanging agent. Reports of (Piper 1984, Boyd 1990: Briggs and Smith, 1996) document the use of zeolite in fish or shrimp ponds for the removal of ammonia. Zeolites are preferred due to their high selectivity for the removal of heavy metal cations as observed by Sherman (1978) Semmens and Sayfarth (1978).

**Gills**

When fish of group II exposed to lead nitrate, RNA content decreased in all exposure periods in comparison to their control fish. In this group, the RNA contents ranged from 6.86±0.37 to 6.95±0.37 from 60 to 180 days. In fish of group III exposed to lead nitrate + natrolite, the values of RNA contents improved towards normal. The improvement observed ranges from 6.97 ± 0.40 to 7.04 ± 0.41 after 60 and 180 days respectively. However, when fish of group IV exposed to natrolite only, the values of RNA content increased in comparison to control fish consistently up to 180 days (Table 23).

Viarengo (1985) pointed out that heavy metals ions are strongly bound by sulphhydryl group of proteins. This sulphhydryl binding changes the structure and enzymatic activities of protein and causes toxic effects in biological system (Hodson, 1978).
Mizrahi *et al.* (1989) observed inhibition of cytochrome oxidase activity in *Donax trunculus* (a bivalve mollusc) exposed to heavy metals. According to Lavie and Nevo (1986) prolonged exposure to heavy metals may result in genetic changes in the animals.

The gill of teleost fish plays an important role in regulation, gas exchange, acid base balance and nitrogenous waste excretion, which means it has a key role at the interface of fish with its environment. Because bronchial epithelium of teleost gills is a tissue where both active and passive exchange occurs between animals and its environment is also likely to be action of heavy metals.

Watson *et al.* (1981) have shown that Na, K-ATPase activity in rainbow trout *Salmo-gairdneri* is sensitive to heavy metals. Lopez *et al.* (2003) observed decreased alkaline phosphatase activity in muscles and gills of *Heteropneustes fossilis* due to various pollutant received from a suger industry. Spicer and Webor (1991) studied the effect of heavy metal on crustaceans and mollusc and stated that oxygen consumption generally decreases when these animals are acutely exposed to heavy metals.

Among the various cation exchangers zeolites and especially natural zeolites such as natrolite, chabazite and stiblite etc. are preferred due to their high selectively for heavy metal cations and low cost compared with synthetic cation exchange materials. Also it has been proved that natural zeolites worked without any side effect (Sherman, 1978, Semmens Seyfarth, 1978). Reduction of cadmium toxicity through natural zeolite has been reported by James *et al.* (1990).
Brain

When fish of group II exposed to lead nitrate, depletion in RNA contents observed ranging from 6.16±0.29 to 6.27±0.29 from 60 to 180 days of exposure period in comparison to their respective control. When the fish of group III, exposed to lead nitrate + natrolite, the values of RNA contents improved significantly. The improvement has been 6.31±0.34 after 60 days to 6.38±0.35 after 180 days of exposure. When fish of group IV exposed to natrolite only the values of RNA contents slightly increase in comparison to their control fish (Table No. 24).

Rhenberg and Schreck (1986) studied the effect of heavy metal on olfactory system in fish and reported that structural and functional alterations occurs in olfactory system due to heavy metal exposure. Amundsen et al. (1997) noted faster uptakes of metal in tissues of gonads than muscles in fish. Cory-Slechta (1990) reported that lead levels increases in brain with advancing age in rats. Joao et al. (1995) studied the effect of lead acetate during the second stage of rapid post natal brain growth on δ- amino levulinic acid dehydratase (ALA-D) activity in brain of suckling rats and observed that lead is a potent in vitro inhibitor of ALA-D but has a small effect on the enzyme in vivo. Landrigan et al. (1975) and David (1974) observed neuropsychological dysfunction in children exposed to lead. Seth et al. (1976) reported decreased blood delta-aminolevulic acid dehydratase activity in mice. Shakoori et al. (1994) reported the depletion in soluble protein, glycogen and RNA contents in mercury exposed to freshwater Chinese grass carp.
Ctenopharyngodon idella. The removal of ammonia from aqueous solution through natural zeolite has been investigated by Aral et al. (1999). Use of natural zeolite clinoptilolite in drinking water treatment has been reported by Gasparo (1983). Pansini and Colella (1991) have reported use of natural zeolite chabazite and philipsite for removal of heavy metals from waste water.

3. Cholesterol Contents

In control fish, the values of cholesterol contents observed in the liver 7.05 ±0.45; in kidney 6.12±0.41; in gill 5.49±0.35 and in brain 4.01±0.33 mg/g. The values of cholesterol contents in all experimental tissues increased after exposure of fish to lead nitrate during all exposure periods (Table No. 25-28).

Liver

In control fish, cholesterol contents are ranged from 7.01±0.42 to 7.10 ± 0.47 mg/g during exposure periods of 60 to 180 days. When fish of group II exposed to lead nitrate, the cholesterol contents increased gradually up to 180 days of exposure period and the values ranges from 7.17±0.49 to 7.27±0.52 mg/g. In the fish of Group III exposed to lead nitrate, cholesterol contents decreased consistently from 60 to 180 days of exposure in comparison to the fish of group II. When fish of group IV exposed to natrolite only the values of cholesterol contents remained unchanged (Table No.25).

Naqvi et al. (1990, 1993) reported that lead, cadmium and arsenic exposed crayfish Procambarus clarkii accumulate these metals rapidly.
Barry and Mossaman (1970) noted that human liver, stores more lead than kidney. Ortel (1995) studied the effects of heavy metals on the gypsy moth *Lymantria dispar* and found that elevated levels of heavy metals decrease the level of fat. Lindquist et al. (2001) reported the same result in the carabid beetle *Pterostichus melanarius*. Hinton et al. (1973) observed that liver damage in the form of necrosis in portal areas and formation of connective tissue septa due to heavy metals – methyl mercury poisoning. Chang et al. (1973) studied the histochemical changes in the liver and other organs of rat after chronic intoxication and observed that these organs are effected structurally and functionally by these metals.

Sastry and Gupta (1978) studied the effect of mercury on the digestive system of teleost fish *Channa punctatus* and observed that the activities of three phosphatase–alkaline phosphatase, acid phosphatase and glucose 6-phosphatase is inhibited in liver.

**Kidney**

As indicated in the table No. 26 a slight increase in cholesterol contents has been observed in the kidney of fish exposed to lead nitrate during exposure period of 60 to 180 days in comparison to control. When the fish of group III exposed to lead nitrate + natrolite the values of cholesterol content decreased in comparison to the fish of group II exposed to lead nitrate. The maximum and minimum decrease in cholesterol contents observed 1.12 % and 0.32% respectively after 60 to 180 days of exposure period. In fish of group IV exposed to natrolite only
the values of cholesterol contents observed almost similar to that of control fish (Table 26).

Cholesterol levels is increased due to lead toxicity in various tissues of animals (Revis et al., 1980; Tarugi et al., 1982; Leddo et al., 1987, Yogminas et al., 1990). Hami et al. (2006) also reported elevated cholesterol levels in animal tissues. Applications of natural zeolites in removal of heavy metal toxicity has been reported by Faghihean et al. (1999), Pansini et al. (1991) and Jain (1999).

Gills

In the present study after 60 to 180 days of exposure of lead nitrate, the values of cholesterol contents in gill observed increased and ranged from 5.60±0.42 to 5.67±0.45mg/g. The minimum and maximum increase in cholesterol contents observed 2.37 and 2.71% respectively after 60 to 180 days of exposure. When fish of group III exposed to lead nitrate + natrolite, the values improves to normal. In fish of group IV exposed to natrolite only the values of cholesterol contents remain unchanged (Table 27).

In fish and other aquatic organism gill is a primary target organ for pollutants and may be one of the fish organs to exhibit symptoms of sublethal toxicity. Torreblancha et.al., (1991) observed increase of lipids and caloric concentration with time in hepatopancreas in freshwater crayfish Procambrus clarkia exposed to cadmium.

According to Dixon and Webb (1964) heavy metals inhibit the activity of a wide range of enzyme of biological system. Brown and Nowell
(1972) working with zinc and copper, reported that copper exert an inhibitory effect on the oxygen consumption of the whole animal *Mutilus edutis*.

Kargin (1998) studied the concentration of heavy metals in tissue of the freshwater fish *Copoeta barroisi* and found the highest concentration in liver and gills. Dhanapakiam *et al.* (1998) studied the gill of adult *Channa punctatus* to effluents of industrial wastes in the Cauvery river water, revealed deformities and reported that the industrial and sewage carried by river Cauvery induce considerable chemical stress on fish populations. Reduction of food consumption in the presence of heavy metals has been reported by Mckim and Benoit (1971).

The reduction of toxic elements through zeolite in a freshwater fish *Oreochromis mossambicus* has been reported by James *et al.* (1999a).

**Brain**

In fish of group II, exposed to lead nitrate the value of cholesterol contents observed increased during 60 to 180 days of exposure period in comparison to control group. The minimum of 2.01% and 2.74% maximum increase in cholesterol contents recorded during the exposure period. When fish of group III exposed to lead nitrate + natrolite; the value of cholesterol contents improved towards normal. The value of cholesterol contents ranged from 4.02±0.38 to 4.07±0.38 mg/g after 60 to 180 days of exposure period. The minimum and maximum improvement in cholesterol content recorded 0.98% and 1.21% during the experimental period. When fish of group IV exposed to natrolite, no
significant changes recorded in the value of cholesterol content in comparison to their control fish (table 28).

Dranscher et al. (1975) studied the effect of various heavy metals on rats and reported that lead and other heavy metals cause memory loss in rats. Chandra et al. (1984) reported hypo and hyper activity in lead exposed adult rats.

According to Muldon et al. (1996) elevated blood lead concentration have detrimental effect on neuro-physiological functions. Kumar and Desiraju (1992), Salanki et al. (1993), Shao and Suszkiw (1991) pointed out that lead poisoning is a potential health hazard that affects various systems including nerves and muscles lead exposure during the prenatal period exerts adverse effect on development and function of the nervous system.


Deleterious biochemical effects due to lead in fish have been reported by Spry et al. (1981) and Hodson et al. (1984). Removal of ammonia from contaminated aqueous solution through natural zeolite has been studied by Aral et al. (1999).
4. Glycogen Content

The glycogen content in control fish have been similar during all the exposure periods in liver, kidney, gills and brain. The values observed are 46.44±1.24 mg/g in liver, 33.71±1.01 mg/g in kidney, 27.77±0.95 mg/g in gills and 8.04±0.42 mg/g in brain. A little and continuous increase in the glycogen contents observed in all experimental tissues of control fish during exposure periods, might be due to the advancing age (Table No. 29-32).

Liver

As evident from table No 29, in control fish glycogen contents in liver have been found similar during all the exposure periods of time. When fish of group II exposed to lead nitrate, the glycogen contents decreased in all exposure periods in fish. The maximum decrease recorded were 11.18% and 11.13% respectively from 60 to 180 days. The fish of group III were exposed to lead nitrate along with natrolite; an increase in glycogen content observed in all exposure periods in fish. The maximum increase recorded was 7.69% after 60 days and 7.67% minimum after 180 days of exposure period. When the fish of group IV exposed to natrolite only, a slight increase in glycogen contents observed compared to their respective control.

Duryan and Vallee (1962) observed that cadmium can replace zinc in liver alcohol dehydrogenase and thus inactivates it. Svobodova et al., (1987) observed the toxic effect of cadmium on the liver of Cyprinius carpio. Vaglio and Landriscana (1999) recorded a significant decrease in
activities of liver cytoplasm aspartic aminotransferase (AST) and alanine
aminotransferase (ALT) while a simultaneous increase of the serum
activities of these same enzymes. Almeida et al. (2002) observed
decreased activities of lactate dehydrogenase (LDH) and creative kinase
(CK), decreased glycogen content, glucose uptake in white muscle and
increased CK and LDH activities and glucose uptake in red muscle.
According to Soengas et al. (1996) toxic metal change carbohydrate
metabolism in the Atlantic salmon (Salmon salar).

Sastry and Gupta (1978) examined the effect of mercuric chloride
exposure in the activities of alkaline phosphatase, acid phosphatase
glucose-6-phosphatase in Channa punctatus. They found that mercury
inhibits the activities of phosphatases in liver. Chow and Cornish (1978)
Fouts and Pohl (1971) found in most studies that lead inhibits enzymes
activities in liver. Quyyum and Shaffi (1977), Gill and Pant (1981),
Radhakrishnaiah et al. (1992), Mary Chandravathy and Reddy (1995)
James et al. (1995) and Vutukuru (2003) observed decrease in glycogen
content of teleost fish exposed to heavy metals. The decrease in the
glycogen concentration of the tissue of fish may be due to its enhanced
utilization since glycogen forms the immediate source of energy to meet
energy demands under metallic stress.

According to Dezwaan and Zandee (1973) decrease in glycogen
content in liver might be due to the prevalence of hypoxic or anoxic
conditions which normally enhances glycogen utilization. Under hypoxic
conditions the animals derives its energy from anaerobic breakdown of
glucose which is available to cells by the increased glycogenolysis (Mary Chandravathy and Reddy (1995).

Zeolites have been shown to remove several metals from aqueous solutions (Kesraoni-ouki et al. (1994). Removal of NH₃ from contaminated aqueous solution through zeolite has been studied by Burgess et al. (2004). Jain et al. (1997) reported the protective action of zeolite over lead toxicity in fish. Natural zeolite due to their ion exchange capacity are preferred for removal of metal ions due to their high selectivity for heavy metal cations (Sherman, 1978; Semmens and Sayfarth, 1978).

Kidney

Glycogen content in the kidney of control fish have been observed nearly similar during all the exposure periods. When fish of group II exposed to lead nitrate, glycogen content decreased in comparison to control fish with advancing exposure period. However, in group III fish exposed to lead nitrate + natrolite, glycogen contents improved in comparison to the fish of group II, exposed to lead nitrate. The improvement recorded was 5.71% after 60 days while 5.59% minimum after 180 days of exposure period. When fish of group IV were exposed to only natrolite, a slight increase in glycogen contents observed in all exposure periods (Table No. 30).

Victory et al. (1981) observed that acute as well as chronic administration in dogs and rats lead to increased excretion of zinc in urine and decreased renal zinc levels. According to Vander et al., (1979)
lead has a direct inhibitory effect on several zinc containing enzymes including δ-aminolevulinic acid dehydratase.

Seth et al. (1976) observed that cadmium and lead interact with the metabolism of other essential metals like copper and Zinc in mice kidney. Fox (1974) reported that cadmium has a major influence on zinc metabolism after absorption in several tissue and also interferes with the metabolism of Mg and Cu. Battigelli (1960) observed that heavy metal and their compounds are inhibitors of enzymes. Chang et al. (1973) demonstrated a fall in the activities of alkaline phosphatase and glucose 6-phosphatase and a moderate increase in acid phosphatase in kidney. Alkaline phosphate is a brush border enzyme localized in the intestinal mucosa and kidney tubules. Hickman and Trump (1969) observed that alkaline phosphatase enzyme is involved in the reabsorption of glucose from the renal tubular lumen. Sastri and Agrawal (1979) reported that the transphosphorylation reactions and absorption of glucose are adversely effected by Hg treatment in kidney of a teleost fish Channa punctatus. They also reported that acid phosphatase, alkaline phosphatase, glucose 6-phosphatase and urease enzymes activities are inhibited in kidney of Heteropeustes fossilis after chronic exposure to lead nitrate. According to Passow et al., (1961) and Blackwood et al. (1965) the inhibition of enzyme activities by heavy metals may be due to the direct binding of the metal with enzyme protein are the toxic effects produced by them in the tissue.
Zeolite has been used for decades to decrease concentration of ammonia in municipal effluents. Mercer et al. (1970) and in freshwater toxicity identification evaluation (Durhan et al., 1993; Beser et al., 1998 and Burgess et al., 2003 and 2004).

**Gills**

In fish of control group the glycogen content in the gills remain nearly similar (27.77±0.95 mg/g) during all the exposure periods. A slight and continuous increase in glycogen contents observed with advancing period from 60 to 180 days. When fish of group II exposed to lead nitrate the glycogen contents decreased in fish of all exposure periods in comparison to their control fish. The values observed from 24.42±0.65 to 24.54±0.67 mg/g. The maximum decrease of glycogen contents observed 11.90% after 60 days of exposure and minimum of 11.79% after 180 days. When fish of group III exposed to lead nitrate along with natrolite to determine the removal of lead toxicity, the glycogen contents increased in comparison to their group II fish in all exposure periods. The increased values observed are 26.68±0.85 to 26.78±0.87 mg/g in this group. The maximum and minimum increase observed 9.25% and 9.12% from 60 to 180 days respectively. In fish of group IV exposed to only natrolite, the glycogen contents improved slightly in comparison to their control fish (Table No. 31).

According to Wong et al. (1975) complexation of metals by co-ordinal linkage with appropriate organic molecules in biological tissues is an important process involved in metal accumulation by aquatic
organism. Respiratory system of fish differ from all other system because damage to gills has immediate impacts on the rest of the fish body (Satchell, 1984). Gupta (1988), Radhakrishnaiah (1988) and Ray et al. (1990) observed accumulation of metals of gills in Channa punctatus (Bloch.), Labeo rohita (Hamilton) and Heteropneustes fossilis (Bloch.) respectively. The adverse effects of oxygen consumption after exposure to heavy metals in fish have been widely studied and much work has focused on the dysfunction of gills due to cytological or structural alteration. Skidomore (1970) stated that exposure of rainbow trout Salmo gairdneri to Zn affects gills and can alter its structure including destruction of gill’s epithelium as well as concentration of blood space causing death through the breakdown of several gills functions. Inhibition of alkaline phosphatase activity in muscle and gills of Heteropneustes fossilis was reported following dimethoate exposure by Borah and Yadav (1996). Shaffi and Choudhary (1979) reported that glycogenesis increases due to heavy metal toxicity because of more energy is required to minimize the deleterious effects. Glycogen synthesis in tissue is hampered due to the inhibition of enzyme activity by toxic metals.

Role of zeolite has been studied by Jain et al. (1996). Use of zeolite for removing ammonia caused toxicity has been reported by Burgess et al. (2004). Zeolite is a naturally occurring hydrated aluminosilicate mineral and is composed of symmetrically stocked aluminum and silica tetrahedron (Kesraoni-ouki et al., 1994; Ruzie et al., 2000). The negative
charge allows for the absorption of certain positively charged ions. In aqueous solution, the negative charge is generally neutralized by Na\(^+\). However Pb is preferentially absorbed to the zeolite matrix.

**Brain**

The glycogen contents in the brain of control fish observed 8.04±0.42 mg/g, nearly similar in fish of all exposure periods. When fish of group II exposed to lead nitrate, the glycogen contents decreased in tissue of all exposure periods in comparison to their control fish. The values of glycogen contents observed from 6.99±0.38 to 7.03 ±0.33 mg/g from 60 to 180 days of exposure respectively. However, in fish of group III exposed to lead nitrate along with natrolite, the glycogen contents improved in comparison to their group II fish. When fish of last group exposed to only natrolite, the glycogen contents further improved compared to their control fish in all exposure periods (Table No. 32).

Choi (1989) reported that the developing central nervous system of foetus and neonates and particularly, the cerebellum is one of the main targets of mercury toxicity in mice cerebellar granule cells which are characterized as glutamatergic neurons (i.e. they synthesize, store and release glutamate Glu) and aspartate (ASP) upon stimulation are sensitive to methyl mercury (Clarkson, 1987; Fonnum and Lock 2000). Needleman and Bellinger (1990); Beck (1992) stated that the central nervous system may be effected by lead levels which are lower than the lowest serum lead levels at which damage has been reported.
According to Davies and Everhart (1973) low concentration of lead in water cause spinal curvature of rainbow trout \( (Salmo gairdener) \). Holcombe \textit{et al.} (1976) also observed similar effects on long term exposure of brook trout \( Salvelinus fontinalis \). Zaccaroni \textit{et al.} (2003) observed that lead increases with age in liver and brain. Dranscher \textit{et al.} (1975) reported that in adult rats exposure of lead result in to loss of memory. Studies with lead intoxication in rodents have shown either hyper activity or hypo-activity or no change in spontaneous motor activity (Goldberg and Silbergeld, 1974; Sobotka and Cook, 1974; Michaelson \textit{et al.}, 1974; Modak and Satovinoha, 1979). Koegh (1992) stated that the toxicity due to lead causes neurological, immunological effect, carcinogenesis and sterility.

Lead causes glycogenolysis in the brain of fish exposed to lead nitrate. Shaffi (1979), Sastry and Gupta (1978) found marked inhibition in enzyme activities of intestine in \textit{Channa punctatus} exposed to lead nitrate.

Zeolites act as an ion exchanging agent. Many authors have studied the role of zeolite in reducing toxic metabolites, absorption of gases like \( \text{CO}_2 \), \( \text{SO}_2 \) and \( \text{H}_2\text{S} \) in aquaculture ponds (Chaberlain, \textit{et al.}, 1978; Briggs and Smith, 1996), and mineral nutrition in fish and shrimp (Battes \textit{et al.}, 1981). Use of zeolites in removal of metal toxicity in java tilapia \textit{Oreochromis mossambicus} (Petors.) has been reported by James \textit{et al.} (2003).