SUMMARY
AND
CONCLUSION
INTRODUCTION

A pollutant is nothing but a misplaced resource. This is very true in the case of heavy metals. Heavy metals are the indicators of human progress and pillars of all major civilization, past or present. Without metals we would not be able to farm, travel, compute, communicate or perform any other task is a manner a developed society is expected to perform. Nor can we have any major industry without the support of the metals. Metals are also the environmental pollutants of major concern, because, like most organic pollutants, the metals are not biodegradable or perishable.

Heavy metals can reach any where, they are in the air we breathe, the food we eat and the water we drink. Most soils contain most of the metals known to us. Sea water also contains most of the known metals. Some metals are also present in freshwater.

Toxic metals are added in aquatic system from industrial processes, domestic sewage discharge, street dust, land run off and fossil fuel burning. Among the industries with the highest emission of heavy metals are the mining industry, metallurgical industry, chemical industry, leather industry, sugar industry, distilleries, battery industry and thermal power plants.

Mercury is a metal which is liquid at normal temperature and pressures. It forms salts in two ionic states mercury (I) and mercury (II). Mercury (II) or Mercury salts are much more common than Mercury (I)
salts. Mercury also forms organometallic compounds, some of which have found industrial and agricultural use. "Organometallic" is used here to indicate a covalently-bonded compound, and does not include mercury bond to proteins nor salts formed with organic acids. These organometallic compounds are stable, through some are readily broken down by living organisms, while others are not readily biodegraded. Elemental mercury gives rise to a vapour which dissolves only slightly in water. High concentrations of the metal are still present in sediments associated with the industrial applications of mercury. Some mercury compounds have been used in agriculture, principally as fungicides. Mercury, a heavy metal, is used in a number of industrial applications and products. It is a highly mobile element and cannot be broken down into harmless components. When it combines with carbon, it forms organic mercury compounds such as methyl mercury, which is the most common form of mercury found in the environment. This methyl mercury passes into the air, soil and the food chain, mostly through aquatic animals. It can then become a considerable health risk.

Mercury pollution in water is widespread in India. This is a very serious problem and urgent steps need to be taken to completely ban or severely restrict the usage of mercury. The coastal area of India is significantly polluted with mercury and high levels of mercury is being detected in Indian fish, both saline and freshwater. The Minamata disaster was caused by consuming mercury-tained fish. To prevent such a tragedy from happening in India, people who eat fish need to be made
aware of mercury contamination and its implications. Inorganic mercury is toxic to fish at low concentration. Organic mercury compounds are more toxic, toxicity is affected by temperature, salinity, dissolved oxygen, and water hardness. A wide variety of physiological and biochemical abnormalities have been reported after exposure of fish to sublethal concentration of mercury. Reproduction is also adversely affected by mercury.

The present study has been planned at assessing the biochemical changes occurring in various tissues i.e. brain, liver, kidney and gills of teleost fish *Heteropneustes fossilis* due to mercury toxicity and remediation of mercury toxicity by the use natural zeolite, natrolite.

**MATERIALS AND METHODS**

**Experimental animal**

Healthy living specimens of teleost fish *Heteropneustes fossilis* (Bloch.) were collected from local fishery. The average length and weight were 13.5 cm and 10.5 gm respectively. Fishes were kept in aquaria in laboratory (25±8°C) and fed regularly with commercial fish food. Fishes were acclimatized to laboratory conditions for 3 weeks, prior to experimentation.

**Mercuric chloride (HgCl₂)**

Mercuric chloride solution has been used in the present study. Prior to experimentation LC50 value for HgCl₂ was determined for 96 hours. Water of aquarium was replaced and a fresh dose of mercuric chloride sprinkled into the water every alternate day.
Zeolite

The zeolites are synthetic and naturally occurring colourless crystals. These are aluminosilicate minerals that contain alkali and alkaline earth metals such as sodium, potassium and calcium as well as water molecules within their structural framework. The later is relatively porous enclosing inter connected cavities in which the metal cations (positively charged atoms) and water molecules residue. This gives the zeolites the cation exchange and reversible dehydration properties for which they are noted.

The zeolites are used to separate molecular mixtures on the basis of the size and shape of molecular compounds.

Natural zeolite natrolite and its ion exchange properties

Natrolite consists of silicon and aluminium tetrahedra link to from chains. The repeating unit of each chain is five tetrahedra that have a dimension of about 6.6 angstroms. Chains are linked laterally by shared apical oxygen atoms; the lower SiO₄ tetrahedron of one chain are linked to the upper AlO₄ tetrahedra of neighbouring chains. The fibrous nature of natrolite is explained by the relatively few bonds linking chains laterally as compared with the bonds within the chain. Natrolite has channels with a minimum free diamter of 2.08Å, parallel to the axis and a system of intersecting channels with a minimum free diamter of 2.60Å between neighbouring chains. Sodium atoms and water molecules lie within the channels. Each sodium is surrounded by six oxygens and each water molecule is close to two sodium atoms.
EXPERIMENTAL PROCEDURE

Acute study

For acute study, after acclimatization, 120 fishes were divided into four equal groups (each of 30 fishes). Group I receiving only fish meal, served as control while group II, III and IV exposed sublethal concentration of mercuric chloride, mercuric chloride + zeolite and zeolite Natrolite only respectively. Fishes were sacrificed after 7, 14, 21, 28 and 35 days of exposure and their tissues liver, kidney, gills and brain were removed and processed for biochemical study. Observations of the study have been summarized in the form of tables and graphs.

Chronic study

For chronic study, after acclimatization, 120 fishes were divided into four equal groups (each group of 30 fishes). Group I received only fish meal and served as control while group II, III and IV received sublethal concentration of mercuric chloride, mercuric chloride + natural zeolite natrolite and only zeolite, natrolite respectively. Fishes were sacrificed after 60, 90, 120, 150 and 180 days of exposure, their tissues liver, kidney, gills and brain were removed and processed for biochemical study. Observations of the study have been given in the form of tables and graphs.

Biochemical study

In each set of experiment, biochemical estimation for protein, RNA, glucose and cholesterol in Liver, kidney, gills and brain etc. have been performed by applying following methods-
a. Protein estimation: Lowry method.

b. RNA estimation: Orcinol method.


**Statistical analysis**

Experimental data have been processed for mean, standard error and student 't' test so as to see whether the data is significant statistically.

**RESULTS AND DISCUSSION**

The present study deals with the biochemical changes in liver, kidney, gills and brain of fish *Heteropneustes fossilis* due to acute and chronic exposure of mercuric chloride, mercuric chloride + natrolite and natrolite only and the observations have been mentioned in the form of tables and graphs.

In acute and chronic study, protein, RNA and glycogen contents in liver, kidney, gills and brain of control fish have been observed similar to that of initial control fishes. A slight increase with age has been noted. Observations of the study indicates that protein, RNA and glycogen contents in liver, kidney, gills and brain decrease significantly due to mercuric chloride in acute and chronic study. When fishes exposed to mercuric chloride + zeolite, the values improved in all the experimental tissues. Exposure of fish to zeolites, values of these parameters improved further towards normal indicate enzyme inhibition caused due to mercuric chloride and protective action of zeolite over the toxicity of
mercury, due to their ion exchange property as also reported by many scientists.

Cholesterol contents in liver, kidney and gills of control fishes were similar to that of initial control fish in case of both acute and chronic study. In acute (35 days) and chronic (180 days) exposure of mercuric chloride, significant increase in cholesterol contents of liver, kidney and gills have been observed. When fishes exposed to HgCl₂+zeolite, the cholesterol contents turns to normal. Exposure of fishes to natural zeolite natrolite cause no effects on the cholesterol contents. Increase in cholesterol contents along with decrease in soluble protein RNA and glycogen contents indicates the involvement of carbon skeleton of amino acids and acetyl CoA in to cholesterol biosynthesis.

CONCLUSION

The results of the present investigation on the toxicity of mercury in the liver, kidney, gills and brain with reference to protein, RNA, glycogen and cholesterol contents are supported by many workers of the research field. The findings related with the remediation of mercury toxicity are entirely new however, zeolites have not yet been used extensively to remove heavy metals from water, despite their documented affinity towards metals and the evident advantage of ion exchange with respect to methods based on precipitation. During the present study, biochemical changes in fish tissues due to short and long term exposure
to mercury and the usefulness of zeolite to adsorb mercury in water and to decrease its adverse effects to fish have been investigated. It would have been more useful for the present study, if mercury contents in the experimental tissue measured to see whether natural zeolite natrolite play positive role for the removal of mercury from the tissues thus, reduce the deleterious effects. Analysis of heavy metal contents in the tissues before and after the exposure of fish to zeolite may be studied in future. Since natural zeolites are used as feed additive to increase the protein contents and ultimately the body weight, they may be very useful as feed additive importance to animals of humans.