Chapter - 6

Summary and Recommendations
Environmental problems have always existed throughout human history but widespread recognition has come, understandably, only belatedly, after many years of steadily accumulating pollution. The transformation of environmental quality from a free to scarce good is, paradoxically, the result of man's success in achieving economic plenty. This economic growth accompanied by pollution and urbanization had deleterious effect on quality of our water resources through the addition of sheer quantity of effluents generated by high levels of industrial production, mal-agropractices, and unchecked domestic activities. The quantity of varied effluents added has overcome the natural, self cleansing powers of the waters and the waste disposal function of this environmental medium has become incompatible with its use for other purposes.

Addition of industrial wastes to the aquatic ecosystem is a world wide problem and is more acute in industrialized countries where millions of tone of pollutants are discharged into the rivers and streams and also directly into the sea. Aquatic life is seriously affected and threatened out of existence. In turn, it affects human life through consumption of fish and other edible aquatic animals. Also pollutants of this category are innumerable and so well known to our specialists that there is hardly any need to list them here.
Heavy metals are one of the most common forms of anthropogenic pollutants in the environment. The sources of heavy metal pollution are mainly geological weathering, mining effluents, industrial effluents, domestic wastes and storm water run-off. Among the toxic heavy metals, copper and zinc were selected for the present study. These enter the environment as contaminants from mining and metallurgy, chemical industries, scrap metal treatment, electroplating and super phosphate fertilizers.

In Rohtak district (Haryana) metals are widely used in industries and small quantities are discharged into surface fresh water. Copper is used as an algaecide in aquaculture and small quantity of this chemical reaches the surface water through agricultural run-off. These wastes in aquatic bodies pose a threat to aquatic fauna. When present together, they may interact with each other and produce synergistic, antagonistic or simple additive effects.

Fish is consumed in large quantity nowadays. Any type of stress caused by pollutants and other human activities will adversely affect fish population, indirectly affecting the human health. Therefore, in the present study the individual as well as combined toxic effects of copper and zinc on the physiology of the fish has been examined in details. Further, the effect on protein bands was noticed by SDS-PAGE.
Chapter 6: Summary

The working model selected for the present study is the fresh water teleost and Indian major carp *Catla catla*. It was selected because being one of the Indian major carps, it is widely used in aquaculture practices as a fast growing fish and having high nutritive value.

As some phytoplankton species, aquatic macrophytes and algae accumulate heavy metals from ambient water, consumption of such species can lead to biomagnification and accumulation of heavy metals in fish tissues. It is important to search for such type of plant/algae/phytoplankton species which are not consumed by fish and which are efficient bioaccumulators of pollutants.

As water bodies consist of various types of pollutants like heavy metals and other chemicals, it is very essential to test for the interaction between these heavy metals in order to predict their effects such as synergistic, additive and antagonistic.

The present study aims at abatement of water pollution by searching for suitable phytoremediation measures and adsorbents to decrease the concentration of water pollutants so that fish culture in water bodies is not adversely affected by water pollution.
Estimation of Environmental Levels of Heavy Metals

Located in North Western part of India, Haryana has made adequate progress in agriculture and allied fields. Pisciculture is also catching momentum and becoming popular in Haryana. Every year water resources covered by fishery department are constantly increasing. In order to examine whether these rural ponds are suited for pisciculture, the present limnological study was undertaken so as to evaluate the different physicochemical factors that affect the fish yield.

Twenty-village ponds were selected in different directions within 20-km radius from university campus to study the physicochemical characterization of water in which selected fish *Catla catla* were being cultured. This fish is a phytoplankton feeder. It has got a high nutritive value and largely consumed by local people. The limnological study of pond water was carried from June 2002 to May 2004. Each month water samples were collected from the sampling sites between 8.30 to 9.30 A.M. The pH, E.C., salinity, hardness, chloride ions (Cl⁻), calcium ions (Ca⁺⁺), calcium and magnesium hardness and dissolved oxygen (DO) were quantitatively estimated in the laboratory.
pH of pond water ranged between 7.50-8.25±0.19 which is suitable for fish growth and most suitable for pond culture. Absence of free \( \text{CO}_2 \) during most of the observation period is highly favorable for fish growth.

Electrical conductivity is an important measure of total solids. High value of residue yields high electrical conductivity, which indicates positive correlation with total dissolved solids. High level of dissolved oxygen \( (7-9\pm1.29 \text{ mg/l}) \) was observed during sampling period, which is very favorable for fish growth. High total alkalinity values \( (832.0\pm30.89 \text{ mg/l}) \) were conducive to high yield of fish. In spite of the above favorable factors other parameters exceeded the limit prescribed by CIFE for fish culture ultimately leading to the pollution of pond water. Copper in water and soil samples of all the ponds exceeded the safe limit, while zinc was found within the safe limits in most of the ponds. Iron, nickel and lead were also found in pond water. Nickel and lead were in traces, while iron was in high concentration. Thus, soil and water samples are widely contaminated with copper, zinc and iron due to application of super phosphate fertilizers and disposal of sewage water and industrial wastes into nearby fishponds.
Bioassay Studies

Toxicity tests were conducted to determine the LC₅₀ value for 96 hrs of these toxicants. Acute toxicity test was used to determine the concentration of a toxicant that produces a specific adverse effect on a specified percentage of test organisms in a short span of time. The mortality data were statistically analyzed by the probit method of Finney (1971) to compute LC₅₀ value of fry, fingerling and adult. The median lethal concentration of Copper for Catla catla fry, fingerling and adult was 0.037 mg/l, 0.12 mg/l, 0.32 mg/l and for zinc; 0.15 mg/l, 4.2 mg/l and 9.62 mg/l respectively. To study chronic effects of copper and zinc, sublethal concentrations were determined. 1/10 concentration of 96 hrs LC₅₀ values was selected as the sublethal concentration for individual toxic effects. For combined toxic effect of copper and zinc, 1/20 concentration of LC₅₀ (96 hrs) were selected.

Survival and Growth

Survival and growth of the fish after exposing the fish to different sub-lethal concentrations of copper and zinc in individual as well as different combinations was studied from July 2002 to June 2004. Results showed that no mortality occurred during exposure period. The growth of the fish in exposed groups was reduced as compared to control and maximum reduction was noticed in-group D i.e. 1/10 of LC₅₀ of 96 hrs of copper and zinc both. The length- weight
relationship was studied by formula $W = AL^b$. Where $W$ = weight of the fish $L$ = length and $A$ = constant.

**Uptake and Tissue Distribution of Toxicants**

In the present study, uptake of copper and zinc by liver, muscle, kidney and gills of *Catla catla* has been studied after 15, 30 and 60 days exposure to different sub-lethal concentrations of copper and zinc. Minimum quantity of both the metals accumulated in muscles. The order of accumulation was liver > kidney > gills > muscles in copper exposed group, while the order was liver > gills > kidney > muscle in zinc exposed group. The probable reason for maximum accumulation in liver may be that liver is the main detoxifying organ. In combination the two heavy metals showed antagonistic effect. Presence of one metal decreased the uptake of the other metal.

**Effect of Copper, Zinc and their Combination on Enzyme Activities**

In order to investigate the individual as well as combined toxic effects of copper and zinc on the activities of different enzymes and to correlate the accumulation with physiological and biochemical changes, alteration in the activities of enzymes involved in glycolytic pathway such as glucose-6-phosphatase, fructose-1, 6-
diphosphatase and lactate dehydrogenase: the enzymes of Kreb's cycle namely succinate dehydrogenase and glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase involved in purine and protein metabolism were estimated in muscle, liver and kidney of *Catla catla* after 15, 30 and 60 days exposure. Exposure of *Catla catla* to individual as well as combination of copper and zinc produced marked alteration in the activity of different enzymes, which varied with the tissues. On chronic exposure for 15, 30 and 60 days to copper, zinc and their combination, increase in the activity of LDH in muscle, liver and kidney indicates increased rate of glycolysis or anaerobic metabolism in the fish. This increase in activity may be due to the copper and zinc induced damage to gill epithelium, which can reduce the oxygen uptake and bring about hypoxia in organs of vital importance.

Decrease in the activity of SDH in liver of copper exposed group after 15, 30 and 60 days and increase in zinc exposed group after 15, 30 and 60 days is due to the maximum accumulation of copper in liver of the fish. Decreased activity of this enzyme in copper exposed group is due to oxygen stress, energy crisis and mitochondrial disturbance. Further, it also indicates that pyruvate is not metabolized aerobically in copper exposed group. In the present study, increase in SDH activity by copper exposure is due to prevalence of oxidative metabolism.
The activities of fructose-1,6-diphosphatase and glucose-6-phosphatase increased significantly in liver after 15 days exposure while, the activities of these enzymes decreased after 30 and 60 days exposure. Decrease in the activity of these enzymes after 30 and 60 days is evidence in favor of impairment of glycolysis, while increase in the activity of these two enzymes after 15 days indicates favoring of glycolysis. Impaired gluconeogenesis and glycogenolysis is indicated by the reduced activity of glucose-6-phosphatase. The elevation in liver glucose-6-phosphatase is possibly due to synthesis of glucose through gluconeogenesis in Copper exposed group (B). Decrease in protein levels in tissues can be attributed to the increase in GOT and GPT activities. This pattern of change shows that protein was increasingly metabolized.

In the present study, it has been found that in combination of metals, the impact of Copper and Zinc in-group D i.e. 1/10 of LC₅₀ of both was more pronounced than their individual effect.

**Effect of Copper and Zinc on Biochemical Components**

It is well known that when encountered by various stressful conditions, fish respond by inducing certain biochemical changes. The results obtained in the present study showed that exposure to copper, zinc and their different combinations led to a significant
variation in biochemical composition of the fish. Decrease in protein content noted in different tissues may be related to hydrolysis and oxidation of protein through TCA cycle to meet the increased energy demand caused by heavy metal stress. The depletion in carbohydrate content may be attributed to enhanced glycogenolysis. The decrease in lipid could be due to increased utilization of lipids by fish under stress. Thus, change in biochemical parameters and disturbed carbohydrate metabolism in liver, muscle and kidney positively confirm the toxic manifestation of copper and zinc on the fish.

**Phytoremediation to Water Pollution**

Adsorption of metal ions from the ambient water by *Lemna minor* and *Azolla pinnata* in wet and dry conditions have been studied. In the present study, it was observed that uptake of copper and zinc was higher with wet biomass of both the plants in comparison to dry. In case of dry biomass the removal of metal takes place by plain sedimentation and absorption while with wet biomass the plants adsorb the metal in addition to the sedimentation and absorption. This may be due to the large supply of oxygen to rhizosphoric zone through foliar parts, which both the cases was by groundnut husk, which may be due to the porous
facilitates oxidation of metals and penetration of membrane. The observed increase in percentage removal of copper and zinc with increase in biomass of macrophytes may be due to the availability of increased number of binding sites for the complex at ion of copper and zinc ions resulting in increase in the rate of biosorption. However, very slow or no removal beyond an optimum dose may be attributed to the binding of almost all the ions to the biosorbent and establishment of equilibrium between adsorbate and biosorbent at the existing operating conditions.

**Use of Adsorbent in Water Pollution Control**

The present investigation deals with the copper and zinc removal from synthetic solution with the help of different kinds of agricultural wastes as adsorbents namely wheat husk, rice husk, groundnut husk, sugarcane bagasse and sugarcane leaves. Maximum adsorption in nature of groundnut husk. In case of sugarcane bagasse and sugarcane leaves, maximum adsorption took place with sugarcane bagasse as compared to sugarcane leaves, although both are the parts of the same plant. This was due to the fact that bagasse is a part of stem and in stem food storing cells are present which store the food for further use in the form of starch, while the function of leaves is mainly to form food and transfer to other parts of the plant. Thus, adsorption rate of leaves was less as compared to stem.
The phenomenon of increase in percentage removal of copper and zinc with increase in quantity of agricultural wastes up to certain level and beyond that more or less constant removal may be explained as, with the increase in adsorbent dose, more and more binding sites became available for the complexation of copper and zinc ions and this increased the rate of adsorption. However, very slow increase in removal beyond an optimum dose may be attributed to the binding of almost all the ions to the adsorbents and establishment of equilibrium between adsorbate and biosorbent at the existing operating conditions. In combination of agricultural wastes with each other, the maximum adsorption occurred in a combination of groundnut husk with sugarcane bagasse. In the present study, it has been noticed that the adsorption time was decreased (from 96 hrs to 36 hrs) after combining the agricultural wastes with activated charcoal. This was due to the fact that by the addition of activated carbon, total number of binding sites for the metal ions was increased and more metal ions got bounded on the adsorbate surface in little time.

**Gel Electrophoresis**

The electrophoretic patterns of protein bands exhibited by fishes were monitored and recorded throughout the exposure period to sublethal concentration of copper, zinc and their different
combinations. Alterations in protein bands after specific duration of exposure (15, 30 and 60 days) were studied. In liver of control fish eleven bands and in muscle ten bands appeared. After the treatments of 15, 30 and 60 days in liver two new band (19.20, 22.63 KDa) and in muscle (32.40, 37.03 and 28.26 KDa) band newly formed. The number of bands missed in liver (73.99, 83.71, 62.25, 67.03, 49.84, 15.24, 22.62, 26.89, 16.41, 44.06 and 16.14 KDa) while in case of muscle (83.71, 43.06, 47.45, 27.53, 18.12, 57.01, 22.07, 57.80 and 15.62 KDa) estimated qualitatively by SDS-PAGE. There was a gradual decrease in the protein bands due to degeneration of natural protein. Toward the end of exposure period, additional bands were noticed when compared with control, which was correlated with surface bioaccumulation and the total energy dissipation of fish simultaneously.

**The salient points arising from the present study includes:**

1. Water in most of the ponds was polluted as the level of majority of the selected physico-chemical parameters was above the permissible limits.
2. Pond water and soil contained copper and zinc in slightly high concentration in some of the selected ponds.
3. Copper and Zinc were toxic to fish. Acute toxicity tests
reveal that copper is more toxic than zinc.

4. The metal-metal interaction in case of bioaccumulation was antagonistic, while in case of toxicity both the metals showed synergistic as well as additive effect.

5. The level of total protein, carbohydrate and lipids decreased in all exposure groups.

6. The activity of LDH, SDH, GOT and GPT increased, while the activity of G-6-phosphatase and Fructose-1, 6-diphosphatase decreased after exposure to the two metals.

7. The Electrophoretic pattern of muscle and liver proteins showed gradual decrease in protein bands when exposed to copper, zinc and their combinations, which was correlated with surface bioaccumulation and the total energy dissipation of fish simultaneously.

8. Different natural wastes can be used in wastewater treatment process for the removal of metal ions.

9. Adsorption of metal ions on different natural wastes had different removal rates. Groundnut had the highest removal rate, while, wheat husk had the slowest removal rate.

10. Addition of activated charcoal with natural waste decreased the time for removal of metal ions (36hrs).

11 In case of phytoremediation Lemna minor gave better results for metal removal than Azolla pinnata.
Recommendation

The fresh water fish *Catla catla* is one of the promising candidate species of aquaculture and main fish of the polyculture system considering its wide demand and consequent market value. Consumers are lured by the quality like soft flesh, pleasant taste, high nutritive value and therapeutic value of this fish. This fish, one of Indian major carps gained wide popularity among the fish farmers for its faster growth and high fecundity value. It is very sensitive to water pollution.

Haryana stands third in fish production throughout the world. There are a large number of fresh water ponds in Haryana but presently they are not exploited from fishing point of view. If these ponds are exploited for fish culture, fish production can be further enhanced. In the present study it has been found that some physico-chemical parameters exceeded the limit prescribed by CIFE, Mumbai for fish culture. But these factors can be controlled by some treatment. It has been found that copper and zinc are toxic to fish. They produce alterations up to biochemical levels and ultimately reduce the nutritive value of the fish. An attempt therefore was made to search for phytoremediation measures to control heavy metal pollution. For this
purpose Lemna and Azolla were selected. It has been found that Lemna is a better adsorbent as compared to Azolla. Moreover, in Haryana large number and huge quantity of agricultural wastes are available. They can be used as cheap and good adsorbents for the removal of pollutants.

Five types of adsorbents were tested namely rice husk, wheat husk, ground husk, sugarcane leaves and sugarcane bagasse. These wastes are tried by combining with each other as well as mixing with activated charcoal. From the present study it has been found with combination of activated charcoal time for removal of adsorbents decreased as compared to individual adsorbents. Maximum adsorption was with groundnut husk and minimum by rice husk. Thus, farmers can use these adsorbents for the purification of pond water. Farmers use these ponds for fish culture first of all put these wastes in ponds and than discard these wastes after that fish culture can be done profitably.

1) The salinity of most of the ponds was found to be very high, which proves deleterious for fish growth. It can be reduced by mixing shallow tube well water or canal water, which is available at that site.

2) The nutritive value of fish is reduced after exposure to various metals. Therefore it can be prevented/reduced by phytoremediation measures. For this purpose, Lemna either fresh or dry can be wrapped in muslin cloth in bundles and introduced into the pond. After 48 hrs
these bundle can be collected and disposed off. It is recommended that 1g/l of fresh and dry *Lemna* be introduced and collected. After 72 hrs and 96 hrs respectively.

3) On the basis of availability of agricultural wastes groundnut husk can be used as good adsorbent. Its efficiency for removal of heavy metals can be further enhanced by crushing it into smaller pieces so as to increase surface area and mixed with activated carbon. The specified quantity which should be added is 1g/l activated charcoal + 0.7g/l groundnut husk for 16 hrs.