Chapter - 2

Review of Literature
REVIEW OF LITERATURE

Physico-chemical Characteristics of Water

The need for ecobiological studies of fresh water is much in demand than ever before because the fresh water ecosystem is most indiscriminately exploited as the most convenient and cheapest way of water disposal system. Chacko and Krishnamoorthy (1954) studied the ecology of three fish ponds in Madras. Das and Srivastava (1959) studied the physico-chemical characteristics of a fresh water pond in Uttar Pradesh. They noted inverse relationship between the abundance of phytoplankton and zooplankton. Sreenivasan et al. (1964) made some observations on the limnology of Bhavanisagar reservoir in Madras emphasizing mainly the hydrological parameters. George (1965) made comparative study of the ecology of five fish tanks in Delhi giving seasonal abundance of the major groups of phyto and zooplankton and variations in the physico-chemical characteristics of water. Sehgal (1967) made observation on physico-chemical characteristics of water and noted well-marked fluctuations in temperature, transparency, pH, D.O. and free carbon dioxide. Saha et al. (1971) selected a perennial fresh water pond in Cuttak, Orissa for studies related with physico-chemical characteristics and biological conditions of the pond. These authors gave a detailed account of diurnal variations in phytoplankton.
and zooplankton and physico-chemical characteristics of water. Sharma (1975) made a study of the ecology of a typical urban pond in Ambala (Haryana) in order to explore the effect of certain physico-chemical characteristics and biological factors on the relative abundance of plankton. He concluded that low temperature and low turbidity were more conducive to the development of plankton in general and pH was found to be the controlling factor for abundance of certain rotifers. Zutshi and Vora (1976) studied the limnology of Dal Lake in Srinagar, Kashmir and observed that when carbon dioxide and hardness change, the pH of water also changed. Khan and Siddique (1977) studied seasonal changes in the limnology of a perennial fishpond in Aligarh and made a brief report on the seasonal distribution of main groups of phytoplankton and zooplankton in the pond.

Mandal and Karim (1978) studied the limnology of a fresh water pond at Bhagalpur. They observed a direct correlation between free CO₂ and bicarbonate alkalinity. Singh and Sahai (1978) studied the seasonal fluctuation in zooplankton population in relation to certain physico-chemical factors for a period of one year at Jalwania pond, Gorakhpur. These authors reported that higher bicarbonate in water gave higher production. Silicates were recorded relatively in somewhat higher proportions during winter season and lower proportions during rainy and summer season. The average value did
not rise beyond 4.6 mg/liter. This observation was made by Goldman and Horne (1983).

Trivedy and Goel (1984) described certain chemical and biological methods for testing water pollution. Trivedy et al., (1985) studied the interrelationship between few physico-chemical characteristics of a fresh water pond in Kolhapur. Nitrates and D.O. showed an inverse relationship with temperature. Pragatheeswaran et al., (1986) studied the distribution of iron, cadmium, manganese zinc, mercury and organic carbon in sediments collected North of Visa Khapatnam. Noor-ul-Haque et al., (1988) studied physico-chemical parameters of a tropical pond in Aligarh and found eighteen species of rotifers. It was also observed that rotifers population was enhanced with increasing load of sludge.

Wasay and Jain (1988) measured the concentration of heavy metals in river Yamuna and treated effluents of Delhi sewage disposal plant. Saikia et al., (1988) studied the water and bed sediments of river Ganga from a stretch of 480 K.M. extending from Badrinath to Narora and reported that Mn, Fe, Cu and Zn were below the toxic limits indicating the water to be unpolluted.

More and Gajjar (1990) made a hydrobiological study of Palan pond in Valsad, Gujrat. They found that rotifers dominated indicating congenial condition of the reservoir. Higher number of rotifers was found during the summer months. This may be due to
higher concentration of nutrients like phosphate and nitrate found in Palan pond, Valsad. Adholia and Upkar (1991) studied the phytoplankton community in relation to limno-chemistry of Mansarover Reservoir, Bhopal. They found that phosphate in natural water occurs in very small quantities and it is an important nutrient in the maintenance of the fertility of reservoir. During the investigation period minimum \(90.06 \text{ mg/l}\) phosphate content was recorded in the months of November and December and maximum \(0.17 \text{ mg/l}\) was observed in the month of October, through the phosphate content in the reservoir was recorded low throughout the study.

Chona (1991) reported higher value of B.O.D., nitrates phosphates and chloride in water samples of Halomajra pond (Chandigarh), which showed that the pond water was polluted. Verghese et al., (1992) studied a pond polluted by domestic waste and noted that total hardness was lesser in rainy season than in winter. High contents of sulphate and chloride revealed pollution of pond water by human waste. Kumar and Paul (1994) studied diurnal fluctuation in some physico-chemical parameters of fishpond at Dumka, Bihar. They observed that amplitude of variation in carbonate alkalinity was not more than \(85 \text{ mg/l}\). Hazarika and Dutta (1994) examined the limnology of two fresh water ponds of Guwahati. The study revealed some variations in
physico-chemical characters and the pond showed ecological diversity. Paka and Rao (1995) studied the interrelationship between different physico-chemical factors of a pond and noted that all the parameters like pH, temperature, carbonates, bicarbonates, chlorides, dissolved oxygen and organic matter were present in low concentration. pH and carbonates varied directly whereas pH and bicarbonate showed an inverse relationship. Dissolved oxygen and organic matter had a negative correlation, which might be due to the utilization of oxygen for the oxidation of organic matter. Kataria et al., (1995) studied heavy metal water pollution of Pipariya Township in Madhya Pradesh. Contamination of surface water has been attributed to industrial wastes containing copper and zinc. Alam et al., (1995) studied the limnology of four lentic fresh water bodies at Aligarh infested by varying dominant biota. All the four ponds were alkaline with high pH values, transparency D.O. and free CO₂.

Tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in increased flux of metallic substances in aquatic environment. Industrial wastes constitute the major source of various kinds of metal pollution in natural water. Sukul and Chaudhuri (1997) reported that in Kalyani (West Bengal) the pond water was highly contaminated while potable water was least contaminated. Gupta (1997) studied coastal water pollution in Tamilnadu. Various parameters studied include temperature,
salinity D.O., pH, turbidity, ammonia nitrogen, nitrate nitrogen, inorganic phosphate, total phosphorus, Cd, Hg, Pb, Fe, Ni and petroleum hydrocarbons in land, coastal and offshore locations. Water quality also affected fish farming and agriculture. Large quantities of heavy metals enter water bodies through industrial effluents and render these water bodies unsafe for fish and man. Large deposits of zinc, silver, lead and cobalt were noted by Mulachiro and Darve (1997) in the reservoir Bari near Udaipur (Rajasthan). Further, Kurshid et al., (1998) noted that the concentration of trace elements around industrial belt was higher than the Vembanad Lake, which may be attributed to steady discharge of effluents in floor regions. Saha and Mandal (1998) studied the heavy metal pollution in a fresh water fishpond in West Bengal. Copper, iron and manganese were analyzed in the pond water. The concentration of zinc, iron and manganese in the water was below the toxic limit whereas the concentration of copper was above the acceptable limit. Vasu et al., (1998) detected the presence of high levels of zinc, Copper, manganese and iron in soil around an industrial area in Kochi (Kerala). Ciba et al., (1998) studied the occurrence of metal in composted municipal waste and their removal. Tiwari (1999) studied the physico-chemical conditions of the upper lake water of Bhopal (Madhya Pradesh). Sivakumar et al., (2000) studied the physico-chemical characteristics of water source
of Ooty, South India. Mobilization of potentially toxic elements (Copper, lead, nickel, chromium and cadmium) in soil and plants of fields irrigated with mixed industrial effluents has been studied by Kishu et al., (2000). Jindal and Bhavleen (2000) studied the effect of heavy metals toxicity on the productivity of a fresh water pond at village Khuda Lahora (Chandigarh). Higher concentrations of cadmium and nickel were recorded in comparison to other heavy metals. Chitdeshwari et al., (2002) while studying fractionation and characterization of sewage sludge observed high concentration of heavy metals (zinc, iron, manganese, copper, cadmium, chromium, nickel).


Recently, Ebenezer (2003) studied heavy metal concentrations (copper, zinc, lead, cadmium) in a rural pond (Marthandeswar) and urban pond (Udayarpathy) of Tamilnadu. The level of heavy metals, except copper was below the permissible levels as per various standards. Maity et al., (2003) estimated the levels and occurrence of some metals like iron, zinc, lead, nickel, cobalt, chromium, cadmium, arsenic and mercury in the water of river Hooghly.
(Kolkata). Average levels of metals followed the order, Zn > Pb > Cr > Cd > Ni > Co > As > Hg. Hoo et al., (2003) measured the concentration of cadmium and lead in the water of Labu-river system in Malaysia. Results obtained showed that concentration of cadmium and lead in the river exceed the levels permitted for drinking water by WHO; water from this system was, therefore, not suitable to be used as potable water. The order of toxicity to primary productivity was Hg > Cu > Cd > Zn > Cr.

**Effect of Heavy Metals on Fish**

The discharge of heavy metals into aquatic environment causes serious eco-biological problems due to toxic properties and has adverse effects on aquatic fauna and flora. Heavy metals and numerous other chemicals released by industrial operations are toxic to animals and may cause sublethal pathology of the liver, kidney, muscle, gills, physiological system, nervous system and death in both aquatic and terrestrial vertebrates (Wilbur, 1969). Gupta and Rajbanshi (1981) observed abnormal behavior of *Mystus bleekeri* exposed to copper for 96 hr. Exposure of fish to heavy metals often produces behavioral alterations (Saxena and Parashari, 1983; Abbasi and Soni, 1986; Agarwal, 1991; Sharma and Sharma, 1995; Dhanapakiam et al., 1998; Khangarot and Prakash, 2002).
The effects of different heavy metals on fish have been extensively studied by various workers (Larsson et al., 1984; James et al., 1992; Chandravathy and Reddy, 1996; Shukla and Sastry, 1998; Sinha et al., 2001; Lafuente et al., 2001; Desai et al., 2002; Khangarot and Prakash 2002; Pillai et al., 2003; Levesque et al., 2003; Vosyliene et al., 2003; Haffor and Ai- Ayed, 2003; Rai and Srivastava, 2003 and Kar, 2004). Yet, consensus is generally lacking on several aspects of physiological alterations. It is known that very small quantities of these metals are essential for normal developmental, metabolic and physiological activities of fish (Vallee, 1988; and Singh and Bhati, 1991). If the concentration of metals exceeds the permissible limit or the limit of tolerance, then these metals cause harmful effects on fish either directly or indirectly (Pamila et al., 1991; James et al., 1992; Reddy et al., 1998; Dinodia et al., 2002 and Fatma et al., 2003). Florence et al., (1992) have shown that fishes have effective defense mechanism against ingested metals, but evolution has not equipped them with mechanisms to tolerate free metal ions contained in water. According to Playle et al., (1993) heavy metal toxicity can be reduced by reducing the concentration of available free metal ions. In general, waterborne metals are most toxic to fish in soft water containing low concentrations of dissolved organic matter which influences copper, calcium and cadmium binding to gills of Rainbow trout (Hollis et al., 1997).
Biochemical Components

Sastry and Gupta (1978) studied the enzyme alterations in the digestive system of Heteropneustes fossilis induced by lead nitrate. Bhaskaran (1980), Manoharan and Subbaiah (1982) have reported that the depletion in protein level could be due to diversification of energy to meet the impending energy demands when an animal is under toxic stress. Shigeaki Muramoto (1981) reported variations in the concentration of calcium, phosphorus, magnesium and cadmium in the bodies of deformed and normal fish exposed for long period to water containing low concentration of copper. Sastry and Subhadra (1982) reported elevation in the activity of LDH and SDH in liver and muscle of Heteropneustes fossilis exposed to sub-lethal concentration of cadmium for 15 and 30 days. As pollution may induce certain biochemical changes in fish earlier to the manifestation of drastic cellular and systemic dysfunction, appropriate biochemical parameters could be used effectively as sensitive indicators (Aldridge, 1983). The chronic toxic effects of chromium on the carbohydrate metabolism of a teleost fish Channa punctatus were examined after 60 and 120 days by Sastry and Sunita (1983). The depletion of tissue protein may reflect a prior increased cost of homeostasis, tissue repair and detoxification under stress (Neff, 1985). Sastry and Subhadra (1985) reported decrease in
the activity of acid phosphatase, alkaline phosphatase, hexokinase, xanthine oxidase and glutamate dehydrogenase in liver of Heteropneustes fossilis exposed to cadmium (0.26 ppm) for 15, 30 and 60 days.

Roch and McCarter (1986) studied survival and activity of hepatic metallothionein in developing rainbow trout exposed to cadmium, copper and zinc. Carpene et al. (1987) studied the cadmium metallothionein and metal-enzyme interaction in gold fish Carassius auratus. Chandra et al., (1988) reported stimulation of GPT and GOT activities in kidney and brain of Notopterus notopterus after 96 hr exposure to cadmium. Variety of pollutants has proved to alter the protein metabolism in fish (Palanichamy et al., 1989). Sastry and Shukla (1989) reported decrease in the activity of G-6-pase and increase in the activity of LDH, SDH and hexokinase in liver and muscle of Channa punctatus chronically exposed to cadmium for 15 and 30 days. Dimitrova and Tishinova (1989) have studied the effect of cadmium on some biochemical processes in Cyprinus carpio.


Lauren and McDonald (1994) exposed juvenile trout to 35 μg Cu/l for 28 days. Sodium uptake was inhibited and whole body sodium concentration reduced. Sastry and Sachdeva (1994) investigated the effect of chronic exposure to sublethal concentration of cadmium (1.6 mg/l) and copper (0.3 mg/l) individually and in combination on the blood composition of a fish *Channa punctatus*.

Evaluation of liver protein due to stress under 2,4-D intoxication was carried out by Singh and Bhatti (1994). Somnath (1991), observed decreased level of protein content in liver, brain and muscle tissue of *Labeo rohita* exposed to tannic acid. Decrease in protein bands in muscle and liver may be due to anaerobic conditions produced by the toxic stress (Tazeen et al., 1996).
Kasthuri and Chandran (1997) observed decreased level of protein content in the tissues of *Mystus gulio* exposed to sublethal concentration of lead. Alterations in biochemical profile have been reported in fish exposed to various heavy metals (Desai et al., 2002; Dinodia et al., 2002; Kothari et al., 2003 and Masud et al., 2003). Radhakrishanan et al., (1992) examined the effect of lethal and sublethal concentration of copper on glycolysis in liver and muscle of Labeo rohita. A decline in biochemical composition of muscles was reported in Heteropneustes fossilis after exposure to a single sub-lethal concentration (6 mg/l) of lead nitrate for 120 days by Singhal (1994). Kasthuri and Chandran (1997) observed decrease in total protein, glycogen, and cholesterol in several tissues (gills, skin, muscles, liver, kidney and gonads) of *Mystus gulio* after exposure to a single dose of lead (10% of LC50 value) for 3 weeks. Shukla and Sastry (1998) observed a decreasing trend in total protein and glycogen content of the liver and muscle of *Channa punctatus* after a very short exposure (96 hr) to cadmium. Effect of heavy metals on some metabolically important enzymes of *Oreochromis mossambicus*, was studied by Rema and Philip (1999). Sinha et al., (2001) reported decreased levels of protein, carbohydrate and lipids in liver, muscle and ovary of *Garra mullya* after exposure to cadmium for 4 months.
Mukherjee and Sinha (1993) recorded the hematological and biochemical responses induced in *Labeo rohita* due to long term exposure to cadmium to a sublethal concentration of 20 mg/l of CdCl₂. Marked decrease in glycogen content of liver and muscle was reported. Cyril Arun Kumar et al., (1993) worked on nickel and lead and found that these two metals induced changes in carbohydrate, protein and lipid content in liver and muscle of *Cyprinus carpio*. The effect of exposure to sublethal concentration of lead nitrate on glycogen and lactic acid levels in liver, muscle and blood of *Heteropneustes fossilis* has been studied by Singal (1995). Das and Kaviraj (1994) studied the individual and interactive effects of cadmium, potassium permanganate, cobalt chloride and vitamin B complex on protein level of common carp, *Cyprinus carpio*. Jha and Jha (1995) conducted a study on alteration induced by chronic exposure of the fish *Anabus testudineus* to a sublethal concentration of nickel chloride on the profile of glycogen, total protein of gonad and liver. Stouthart (1995) studied the effect of water pH on chromium toxicity to early life stages of *Cyprinus carpio*. Vincent et al., (1995) reported biochemical response of the Indian major carp *Catla catla* to chromium toxicity. Shah et al., (1996) studied the effect of vanadium on Na+, K+ ATPase, and super oxide dismutase activity and protein concentration in the liver and brain tissue of the fish, *Clarius batarachus*. Vincent et al., (1996) reported that cadmium
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causes anemia in the riverine major carp *Catla catla*. The effect of sublethal concentrations of tannery effluents on the survival and feeding energetics of *Cyprinus carpio* was worked out by Rajan et al., (1996). Virk and Dhawan (1997) reported deleterious effect of nickel and chromium on flesh quality of *Cyprinus carpio*. The protein content of flesh decreased significantly following exposure to safe and sublethal concentration of both the metals during all reproductive phases.

Very little work has been done on the combined toxic effect of copper and zinc on biochemical parameters of fish. Sastry et al., (1997) investigated the toxic effect of cadmium and copper, and their combination on enzymological and biochemical parameters in *Channa punctatus*.

nickel on the protein metabolism in liver, muscle, intestine, brain, gills and blood of *Heteropneustes fossilis*. Khan et al., (2001) while studying cadmium toxicity on glycogen level from body parts and whole body of marine gastropod, *Babylonia spirata* found decrease in glycogen content in body parts as well as whole body. Shukla et al., (2002) studied nutritive value of fresh water fish *Channa punctatus* exposed to cadmium individually and in combination with other metals. Desai et al., (2002) while studying toxic effect of nickel on some biochemical parameters of fresh water fish *Channa punctatus* noticed decrease in liver protein content and liver glycogen.

Desai et al., (2002) noted decrease in liver proteins and liver ascorbic acid in *Channa punctatus* and by Shukla et al., (2002) after exposing the fish to cadmium for 60 days. Prasad (2002) studied the effect of copper and zinc on the gills of *Channa punctatus*. High quantities of zinc are known to alter biochemical activity (Srivastava et al., 2002). Protein content was significantly decreased in liver and muscle of *Cyprinus carpio* under stress (Dhanapackiam et al., 2000). Gradual decrease in the liver and brain protein levels due to the stress of nickel in *Channa punctatus* was observed by Desai et al., (2002). Dinodia et al. (2002) observed marked reduction in residual protein levels in *Labeo rohita, Cirrhinus mrigala* and *Cyprinus carpio* due to cadmium toxicity. Srivastava et al., (2002) recorded changes
in protein biochemistry in the liver and muscle of *Channa punctatus* produced by zinc toxicity.

From the above reports it is evident that biochemical parameters are easy targets of any change in copper and zinc concentrations of water bodies.

**Uptake and Tissue Distribution of Toxicants**

Bioaccumulation can only be assessed when the rate of uptake, storage and elimination process utilized by xenobiotics are addressed. The fate and transport of zinc and copper in aquatic environment is important in environmental quality control and regulation. Though both copper and zinc are considered to be essential trace elements of the human body and fish, they become toxic at higher concentration. Stable and non-biodegradable, heavy metals remain in the ecosystem and pass on to the living tissues in increasing concentrations through biomagnification. Apart from posing a public health hazard for the fish consumer, heavy metal accumulation in the tissues of fishes and other organisms causes pathological disorders such as necrosis of the liver, damage of nephrons in kidney, hematological aberrations, decline in growth rate and fecundity (Rama, 1978).
Zinc also reduces cadmium uptake through the gills by entering the epithelial cell by facilitated diffusion (Spry and Wood, 1989). Viarengo (1989) has studied the mechanisms for the internal deposition of cadmium with emphasis on sequestration by the metal binding protein metallothionein and granules in membrane-bound vesicles.

Free metal ions are the bioavailable chemicals species, which determine metal toxicity to fish in the liver, kidney and brain. Metal cations, including copper and cadmium bind to negative sites on fish gills (Pageenkopf, 1983; Reid and Mc Donald, 1991 and Morel and Hering, 1993). Kuroshima (1992) studied accumulation of cadmium in the mummichog, Fundulus hetroclitus adapted to various salinities. Sastry and Shukla (1993) studied uptake and tissue distribution of cadmium in Channa punctatus. Yakood et al., (1994) reported concentration of trace metals in the gills of fish from Arabian Gulf. Levels of chromium, copper, nickel and lead were investigated in the gills of fish from potentially impacted areas along the Western side of the Arabian Gulf after the 1991 oil spill. Karal et al., (1995) studied the uptake and tissue distribution of dietary and aqueous cadmium by Cyprinus carpio. Pelgrom et al (1995) reported some effects of simultaneous presence of copper and cadmium on uptake of metals. Deb and Santra (1997) studied the bioaccumulation of metals in fishes of sewage fed ecosystem.
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Environmental stress is an inescapable component of the life of fish, compounded by the effects of adverse environmental conditions, including pollutants and land or water project developments in an area (Wedemeyer et al., 1984). The amount of heavy metal accumulation in various tissues could be traced in ppb levels (Tanner, 1992; Livingstone et al., 1994; High et al., 1995; Claudio et al., 2000; Mette et al., 2000 and Narong and Houk, 2000). Friberg and Mottel (1989) experimented fish exposed to heavy metals and found out that the total mercury in the brain was very high. According to most workers (Dallinger et al., 1987; Gupta and Rajbanshi, 1988 and Gupta and Sharma, 1994) the probable reason for the death of certain aquatic organisms was attributed to unequal accumulation of metallic ions in various tissues and their translocation within the body from one tissue to another. Accumulation of heavy metals has been reported in various tissues of fish body by Radhakrishnaiah et al (1992), Norrgren et al., (1991), Gupta and Sharma (1994), Balasubramaniam et al., (1997), Saxena (2002), Aditya et al., (2002), Borana and Qureshi (2002), and Pillai.
et al., (2003). Radhakrishnaiah et al., (1992) reported accumulation of copper in various organs of the fresh water carp *Labeo rohita*, when the fish were exposed to lethal and sublethal concentration of copper for 2 days and 30 days respectively. Degree of accumulation was recorded in the order of gills>brain>muscle>liver under lethal concentration and gill>liver>muscle>brain under sublethal concentration. Mazon and Fernandes (1999) examined differential accumulation of copper in fish tissues after acute and sublethal exposure. Moson et al., (2000) studied the bioaccumulation of cadmium in the fresh water invertebrates and fish. Sinha et al., (2002) studied bioaccumulation of heavy metals in different organs of some common edible freshwater fishes of a river in Jamshedpur. In the gills, liver, kidney, muscle and intestine of *Cyprinus carpio*, and some other species higher concentration of heavy metals were found.

**Bioassay Studies**

Measurement of toxicity of any chemical substance by LC<sub>50</sub> determination was first suggested by Trevan (1927) and this method has been accepted by many investigators (Bliss, 1935; Miller and Tainter, 1944; Duodorff, 1951). Henderson et al., (1960) and several others have formed a core to this field of research. Toxicity studies
should not be highly standardized due to variation in the environmental conditions (Warren, 1971). In a large-scale study of the toxicity of cadmium to the mummichog Fundulus heteroclitus, Voyer and Heltsher (1984) showed a distinct effect of dissolved oxygen concentration on the toxicity of copper to the same species of fish in freshwater. Committee on methods for toxicity test with aquatic organisms, U.S. Environment Protection Agency (1975) studied the methods for acute toxicity tests with fish, microinvertebrates and amphibians. Different LC$_{50}$ values are reported, which could be explained on the basis of a number of factors such as supplies of animal (Russell and Overstreet, 1987), conditions of bioassay (Chambers and Yarbrough, 1974) and weight of animal (Abel, 1990). Evaluation of toxicity of a test chemical is a sensitive phenomenon, which can be influenced by several factors like temperature (Murphy and Murphy, 1974; Reinert et al., 1974; Hertzberg et al., 1980; Sprague, 1985; Keller et al., 1988), hardness of the water (Pickering et al., 1962) nutritional state of the animal (Pal and Kushwant, 1981), salinity of water (Hertzberg et al. 1980), operation (Hertzel et al. 1976), duration of exposure (Jacob et al., 1982), physiological factors (Gupta, 1978), synergistic effects (Lowerenz et al., 1980) and flow of water (Burke and Ferguson, 1969).
USEPA, 1975 (United states Environmental Protection agency) outlined short-term methods for estimating the chronic toxicity of effluents and receiving water to fresh water organisms. USEPA (1987) also pointed out the role of acute toxicity bioassay in the remedial action process at hazardous waste sites.

Toxicity tests with fish were first reported by Penny and Adams (1893). Acute toxicity test is to determine the concentration of a test material or the level of an agent that produces a deleterious effect on a group of test organisms during a short-term exposure under controlled conditions. Acute toxicity tests constitute only one of the many tools available to the aquatic toxicologist but they are the basic means of producing a quick, relatively inexpensive and reproducible estimate of the toxic effects of a test material (Wood et al., 1968; Jensen and Jernelov, 1969; Duffs, 1980 and Spacie and Hamelink, 1985). Although toxicity tests in aquatic organisms can be conducted by administrating the material directly by injection or incorporating it into food, most tests are conducted by exposing groups of organisms into several treatments in which different concentrations of material are mixed in water. According to Parrish (1985) the most common acute toxicity test is the acute lethality test. A toxicant is an agent that can produce an adverse response or effect in a biological system, seriously damaging its structure or function or producing death. Acute toxicity tests are the corner
stones in the assessment of biological effects of any chemical or toxicant, pollutant on the organisms (Rand and Pertocelli, 1985).


The acute toxicity (96 hr) of mercury and lead studied in Guppy and Zebra fish proved that mercury was more toxic than lead in Guppy (Gallo et al., 1995). Haniffa et al., (1995) studied the synergistic effect of copper and zinc salts on *Oreochromis mossambicus*. Gupta and Chakrabarti (1993) studied the toxicity of zinc to fresh water teleosts, *Notopterus notopterus* and *Puntius javanicus*. Sexena et al., (1993) conducted experimental studies on toxicity of cadmium and zinc to *Hetroptistes fossilis*. Muley et al., (1996) studied sodium fluoride induced toxicity to the freshwater fish *Tilapia mossambica*. Ganesh et al., (2000) have reported acute
toxicity of copper to three life stages of common carp. Ghosh and Mukopadahay (2000) tried *Rita rita* as test species for toxicity bioassay of some commonly used industrial metals (copper, zinc, chromium, cadmium and lead). Vincent et al., (2002) studied toxicity and impact of cadmium on food utilization of India major carp *Catla catla*. They found that *Catla catla* under stress of cadmium exhibited depletion in food utilization.

**Electrophoresis of Protein in Tissues**

Changes in the protein pattern of different tissues in relation to the impact of pollutants have been studied. Depletion of tissue proteins in fish *Oncorhynchus kisutch* and *Channa punctatus* was observed when exposed to heavy metals, (Sakaguchi and Hamaguchi, 1975 and Shakkori et al., 1976). In *Tilapia mossambica*, Subharani et al., (1983) noticed a rapid decrease in soluble protein when the fish was exposed to acid medium. The electrophoretic study of protein fractions in the muscle of *Tilapia mossambica* irradiated and treated with sodium arsenate and mercuric chloride revealed striking differences in number, mobility and density of bands in muscle proteins compared to the control indicating that there was considerable inactivation of genetic loci for protein synthesis (Manna and Mukherjee, 1986). Srinivas et al (1987) examined heat-induced
expression of proteins in tissues from adult and embryonic liver in rats by SDS-PAGE profiling. A comparative electrophoretic study on the tissue proteins of some catfishes was carried out by Hussain and Siddiqui (1974). Krishnaja and Rege (1977) have made electrophoretic study on the genetics of two species of Indian carps and their fertile hybrids. Basu et al., (1981) have studied the egg proteins in *Notopterus notopterus* and *Mystus vittatus*. Electrophoretic investigation on protein variation in *Channa punctatus* and *Channa marulius* was carried out by Dhar and Chatterjee (1984). Karyotypic and electrophoretic estimates of selected enzymes were used as tools for determining the genetic characteristics of various fish species (Chatterjee and Dhar, 1985). Myosin content from red and white muscles of marine fish species such as saithe and haddock were analyzed electrophoretically by Martinez et al. (1990).

Changes in SDS-PAGE profile of the vitelline envelop before and after fertilization in the urodele, *Cynops pyrrhogaster* was defined by Adachi and Onitake (1990). Loir et al. (1990) described the main seminal plasma proteins using chromatographic and electrophoretic methods, and found that many proteins are antigenically related to blood serum proteins. Shakoori et al. (1992) studied the pattern of blood serum proteins of *Cirrhinus mrigala* exposed to sub-lethal dose of Terbon by SDS-PAGE.
Ojima (1990) studied the characteristics of chromosomes and electrophoretic pattern of muscle protein and lactate dehydrogenase in fish *Carassius auratus*. Studies on blood serum protein on *Channa punctatus* and *Channa striatus* was assessed by electrophoretic techniques (Sahai, 1990). The electrophoretic spectra of muscle myogens in *Hypothalamichthys molitrix, Aristichthys nobilis* and their hybrids were investigated by Tsekov et al., (1990).

Shakoori et al., (1992) studied the pattern of blood serum proteins of *Cirrhinus mrigala* by SDS-PAGE exposed to sublethal doses of Terbon (ethofenprox). Banerji and Lall, (1994) used polyacrylamide gel electrophoresis (PAGE) technique to monitor the effect of sublethal dose of CdCl₂, PbNO₃ and mixture of CdCl₂ and PbNO₃ on the protein and lactate dehydrogenase binding patterns of the heart, liver and ovarian tissue of *Channa punctatus*. Purified copper and zinc super oxide dismutase (SOD) gave a single band with molecular mass of 17.8 kDa under reducing SDS-PAGE conditions (Osatomi et al., 2001). Identification of different shellfish species using SDS-PAGE electrophoresis and densitometric scanning of soluble protein patterns were performed by Kitts et al., (1997). Martinez (1999) has analyzed raw samples of heat-processed fish by SDS-PAGE and Urea-IEF for quality monitoring. Passamonti et al., (2000) identified raw and cooked clam of *veneridae* family by SDS-PAGE total protein patterning.

**Phytoremediation**

*Lemna minor* is a floating hydrophyte commonly called as duckweed. Egbert and Rose (2000) studied the efficiency of *Lemna minor* in the treatment of synthetic wastewater and sewage water. The observed increase in percentage removal of copper with increase in biomass of macrophytes may be due to the availability of increased number of binding sites for the complexion of copper 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 (Bai and Abraham, 2001; Rai and Kumar, 1999). Presence of large
accumulators of metals (Sinha et al., 1994). Chandra et al., (1997) studied the removal of copper from water by *Bacopa monnieri*. The ability of *Ulothrix zonata* to remove copper from aqueous solutions was investigated by Nuhoglu et al., (2002). Nicholas et al., (2003) examined the ability of microspores species and Lemna minor to remove soluble lead and nickel under various laboratory conditions. Studies on heavy metals removal from waste water and wastewater treatment by *Lemna minor* was studied by Sastry et al., (2000).

**Adsorbants**

Several workers have reported the potential use of agricultural products as substrates for the removal of metal ions from aqueous solutions (Freidman et al., 1973; Randall et al., 1978; Roberts and Rowland, 1973; Kumar and Dara, 1981; Okieimen et al., 1985, 86, 88; Okieimen and Okundaye, 1989). These studies demonstrated that considerable amount of metal ions can be removed from aqueous solution by cellulosic materials which are relatively abundant and cheaper. Marshell and Champagne (1995) evaluated soybean and cottonseed hulls, rice straw and sugarcane bagasse as metal ion adsorbents in aqueous solutions. Periasamy et al., (1991) studied chromium removal by activated groundnut husk carbon. Further Sujatha and Kumar (1995) studied the removal of chromium by activated carbon. Ranjana et al., (1994) studied
adsorption and recovery of zinc (II) ions by fly ash in aqueous media, while Eromosele and Otitolaye (1994) studied binding of iron, zinc and lead ions from aqueous solution by Shea butter seed husk (Butyrospermum parkil). Rampure and Patil (1995,96) reported the use of Dhaoda bark for scavenging cadmium ions from the industrial wastewater and use of Palsa bark substrate for the recovery of copper, lead, zinc and nickel from waste water. The most widely used techniques for the removal of dissolved heavy metals involve the process of neutralization and metal hydroxide precipitation. The shortcoming of this technique is that all the methyl hydroxides exhibit some stability in addition to specific nature of several metals (Rouse, 1976). Initial metal hydroxide precipitates are amorphous and gelatinous in nature, thus solid liquid separation step is very difficult (Venbakm et al., 1992). Huang (1977) investigated the role of possible metallo-organic species coordination in the removal of heavy metals. The sorption of copper (II) by soil is generally enhanced by foreign species such as phosphates, organic acid or color forming compounds. The metal ions have tremendous binding capacity for many types of naturally occurring organic ligands such as humic and fulvic acids (Iversion and Brickman, 1978).

Other adsorbents used are rice husk, composite adsorbent and fly ash (Haribabu et al., 1992). Singh et al (1991) studied the
removal of zinc (II) from water by adsorption on china clay. Huang et al., (1988) studied the adsorption of zinc (II) on the hydrous aluminosilicates in the presence of EDTA. Kaur et al., (1992) developed a method for the removal of trace metals such as lead and copper present in synthetic as well as in waste water samples by adsorption on bottom ash at different pH ranges. Salim and Qashoia (1994) reported removal of lead from polluted water using decaying leaves.

Salim (1988) studied removal of nickel from water using decaying leaves. pH and type of leaves were the concerned factors for adsorption. Bhattacharya and Sharma (1993) reported that fly ash could be used as a scavenger for mercury and other heavy metal pollutants. Victoria et al., (2000) reported that phytosorbent prepared from sunflower seed husk prevents mercuric chloride accumulation in kidney and muscle of adult rabbits. Later on Chang et al., (2003) studied the removal of copper from water by activated carbon. Kadirvelu et al., (2000) used activated carbon cloths to sorb heavy metals from aqueous solution and suggested that the sorption mechanism involved ion exchange, especially on the carboxylic group, and precipitation. Activated carbon generated from the walnut shell was found to sorb copper according to the Freundlich isotherm (Kim et al., 2001). Jia et al., (2002) used activated carbon made from pecan shells to sorb metal ion solutes. Higher
electronegativities and stability constants generally corresponded to a higher adsorption level of metal ions onto the activated carbon (Dastgheib et al., 2002). Goyal et al., (2001) determined the sorption of copper on to activated carbon as type I of the BET classification that represents a rapid sorption in the early stage followed by a slow sorption reaching a plateau maximum. It was observed that oxidation of activated carbon could increase its sorption capacity for copper due to the transformation of the C=C bond into carbon oxygen acidic surface groups (Goyal et al., 2001). Roberts and Rowland, (1973) reported removal of mercury from aqueous solution by nitrogen containing chemically modified cotton. Kannan (1991) reported that the percentage removal of nickel depends upon the contact time and dose of adsorbents. The adsorption of lead on bituminous coal has been studied by Rawat and Singh (1993) under different conditions of concentration, pH and temperature. They reported that the adsorption of lead (II) increased with rise in temperature, high concentration and alkaline pH. Khawas and Dara (1994) studied pre-concentration and determination of trace metals using modified Techtona grandis.