CHAPTER-1

INTRODUCTION
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Man has been changing his environment almost from the beginning of prehistoric civilization. Human being is always impatient to get on with changing the world either by physical or chemical means or by destroying the natural ecological balance of his planet. The main culprits can be attributed to the ever increasing demands of human needs along with ever-increasing population explosion, specially in the last two and present century. Further, mans’ industrial efforts without proper planning and safety measures with indiscriminate dumping of innumerable pollutants into air, water and soil, over exploitation of natural resources without suitable planning for conservative measures and judicious utilization has brought serious undesirable consequences to this planet. Today we are risking much more serious changes in this biosphere than anything noted so far in the recent past. With reckless abandon human being dumps his half million forms into biosphere not knowing whether or not one of these chemicals or some combination of them might be deadly, toxic for one of the steps in the Nitrogen Cycle and so cause the extinction of life on earth (Cole, 1969). Depletion of Ozone layer in atmosphere due to the release of Chloroflouro-carbon compounds into air is another steps toward the extinction of life on earth. Thus human being is a rational creature who goes on changing his environment without understanding the possible effects, and at the same time argue that it is necessary to keep the destructive process expanding each year. In the last half century mans’ impact on the earth has grown to the point where there is a real possibility that he can destroy its ability to support life. Ironically, what now popularly known as “progress” begins to look very much like the path to extinction through the eyes of ecologist. As far as water pollution is concerned, aquatic bodies are contaminated through six major

According to Podany (1985) these pollutants can be assigned to agricultural as 45% to 50%, industrial as 40%, urbanization as 5% and rest is supplied from other sources. In India polluted water has become a direct cause of 42% human and animal diseases (Chandrapal, 1999).

People living in industrial areas are more susceptible to a large number of different chemicals, which are potentially inducers, or inhibitors of xenobiotic mechanism. The exposure of pollutants can be either short and cause acute effects or long term exposures, which through delayed consequences but permanent effects. As per the estimation about one hundred thousand chemicals are used in everyday life and this number goes on increasing every day (Duffus and Kello, 1993).

Since the fall of last century there is increasing use of heavy metals and their compounds in industries and agriculture, which in turn lead to sharp increase in the level of unwanted metals in the environment (Nriagn and Pacyna, 1988). Metals and their compounds are now indispensable to the safety and economy of most nations and have been key factors in the liberation of modern civilization from hunger, diseases and discomfort (Nriagn, 1988). Metals are of particular interest as far as anthropogenic pollution is concerned. Because they are natural substances, non-biodegradable and when extracted in pure form. Some of them are needed as a micro-nutrients in very low quantity in organisms, whereas in high concentration they exhibit toxic effect. The incidence of metal pollution in environment is mainly due to the anthropogenic sources of metal mobilization. Everything that man injects into the biosphere-inorganic, organic and
biological may finally reach natural waters (Swarup et al., 1992). The ichthyofauna and other organisms have been declining or disappearing in water environment due to the metal poisoning (Brown et al., 1970). The biological effect of heavy metals in aquatic environment is adverse mainly due to the complex nature (Stebbing and Fandino, 1983). The metallic compounds take the route directly into aquatic weeds or in microorganisms and in animals either directly or through food chains. Once they enter into animal body they get stored or blocked into sequestering organs like liver, kidney and muscle. These metals induce the formation of detoxifying protein substance Metallothionen in the affected organs, which prevents the toxic effect of heavy metals to a great extent (Olsson and Haux, 1996; Roch and McCarter, 1984). However, the overloading of metallic substances in the organs and tissues can cross the threshold of its protective action and prevent the natural detoxifying mechanism in animals.

Fishes are often the first link in aquatic food chain to terrestrial organisms. Thus the metal concentration of various fish species in relation to metal contents of water is very essential to analyse (Wilson and McMohan, 1981; Canten and Stoof, 1982). The increased level of heavy metals in water bodies may be caused by liquid effluent discharge and leaching of metal bearing minerals apart from atmospheric fall out (Vanloon, 1977). This is further intensified with rapid increase in industries with substandard technology and effluent treatment, run off from streets, highways and land cleaning. Further, the toxicity depends more on the interaction with its constituents and the physico-chemical nature of water. The solubility and toxicity of metals depends mainly on pH, hardness, temperature, alkalinity and dissolved gases of water body. Hence, the physico-chemical parameters of water medium play a crucial role on the toxicity in presence of heavy metals.
(Metelev, et al., 1971, Bengeri and Patil, 1986; Kallanagoudar and Patil, 1997). The sensitivity of aquatic animals to toxic metallic compounds differs with variation in species and genera. Among the same species the toxicity also depends on the size and age (Pickering and Henderson, 1966; Zitco and Carson, 1977; Patwardhan and Gaikwad, 1991; Biswas and Kaviraj, 2002) and sex of the organisms (Mudgall and Patil, 1984; Patil et al., 1995; Perkinsa et al., 1997; Lindquist and Block, 1998; Lewis et al., 1999). Thus it is of grave concern to safeguard these aquatic organisms from extinction by protecting against metallic poisons either by preventing the release of heavy metals and their compounds into the aquatic body or by treating the effluents prior to getting contact with the threatened organisms.

Generally it has been observed that Nature takes care of the pollution menace to some level by detoxifying the pollutants either through physical process or by chemical process. Natural substances like humic and fulvic acid (present in soil humus), amino, amino-hydroxy, thioester, carboxylate and phosphate are some of the natural products found in soil organic matters that play an important role of chelating agents. Apart from that, natural polymers (Alginate acid, chitin and chitosan) also act as chelating substances with metals and prevent the flow of metallic compounds into aquatic bodies (Sanghi, 2000). Even powdered tree bark of Bheria (Rampure and Patil, 1997), pine bark (Asheh and Duvntak, 1997) and poultry litter (Kaviraj and Ghosal, 1997) are employed to reduce the toxicity of metals through sorption mechanism in water. However, the quantities of natural organic compounds are not sufficiently available and the actions are not rapid to curb the increasing heavy metal toxicity in aquatic bodies. Therefore, the search of synthetic chelating agents with strong affinity to heavy metals and rapid mobilization has been
brought into existence. Some synthetic chelating organic compounds like EDTA, DTPD, NTA, sodium citrate and various amino acids have been found to be ideal chemicals to form a strong metal-ligand complex in the metal polluted water and these synthetic products counteract to prevent the metal related toxicity in organisms (Sillanpaa, 1997; and Sanghi, 2000). Under practical circumstances these chelating agents are always in metal complexes rather than in uncomplexed form (Sillanpaa, et al., 2001).

Fishes are usually chosen for the investigations as they are comparatively the best understood organisms in the aquatic environment as indicators of pollution, easily available in water bodies among the higher class of vertebrates. Aside from that, some are also employed to control malaria by consuming mosquito larvae and pupae in pond and swamps. The fishes under consideration for study are of Poeciliid family, *Gambusia affinis* (Baird and Girard) and *Poecilia reticulata* (Peters). Both are small larvivorous fishes found in pond, pool, swamps and rivulet (WHO, 1982; Singaravelu, et al., 1997).

Among the heavy metals cadmium is a non-essential and highly toxic with long biological half life when enters into the body of organisms (Cherians, 1980). This metal is now encountered in numerous occupational and environmental circumstances directly or indirectly and enters the food chain and food webs, which in turn poses an increasing threat to the aquatic life. The toxicity of cadmium and its compounds varies according to various factors like temperature, pH, DO, hardness and alkalinity of the medium (Singh and Yadava, 1987; Inzaa, et al., 1998).

The objectives of the present study help to comprehend some of the basic mechanisms of metal toxicity and controlling of metal toxicity with the help of chelating agents in fishes. These can be outlined in the following manner:-
1. Lethal toxicity of metal on *Gambusia affinis* (B&G) and *Poecilia reticulata* (P). It deals with the comparative account of lethal concentrations of cadmium in male, female and fries of various ages and action of chelating agents against cadmium toxicity on the bases of mortality, potency and mode of action.

2. Evaluation of respiratory and excretory metabolic rates in adult *Gambusia affinis* and *Poecilia reticulata* exposed to both lethal and sublethal concentration of cadmium and cadmium with chelating agents.

3. Rate of bioaccumulation of cadmium due to the lethal and sublethal exposure to cadmium and cadmium with chelating agents.

4. Behavioral response of fishes by means of preference and avoidance reactions through the exposure to lethal and sublethal cadmium, chelating agents and sublethal cadmium with chelating agents.

Discussions of the results of above studies are elaborated in the final chapter.

The present experiments are mainly based on Bioassay Test. Bioassay tests are one of the most important parts of toxicity test in water pollution evaluation because physical and chemical tests alone are not sufficient to assess the toxic effects of chemicals on aquatic life (APHA, 1998). Analytical determinations, relatively expensive, do not yield an absolute value of toxic potential, because synergism and antagonism among the components may occur. Thus, bioassays can be considered as a compliment to the analytical determinations employed in assessing ecotoxicology (Sillanpaa, 1997).
Cadmium is among the modern toxic heavy metals. It was discovered an element in 1817 and its industrial usage were very minor at that time. But currently the use of this element is at the peak level by forming an industrial metal with numerous applications. Cadmium belongs to second member of Group II b triad (Zn, Cd and Hg) in the Periodic Table Classification of elements. The most usual valency of cadmium is 2+, being stable state in the natural environment. Physically it is soft, silver white, ductile with a faint blue tinge and electropositive metal. It loses its luster in moist air and readily attacked by most acids. Its Atomic wt.=112.40, Sp.Gr.=8.642, M.P. =32°C and B.P. =762+ 2°C. Metal itself is insoluble in water. Cadmium is mostly found in association with zinc (Zn) in carbonate and sulfide ore. It is also derived from the smelting of lead (Pb) and copper (Cu) ores. The present production of cadmium and its compounds is many folds as compared to the past. The total production during 1911 to 1920 was \(<0.01\times10^3\) metric tons. But it increased to \(1.5\times10^5\) tons during 1971 to 1980 (Nriague, 1979; Moore and Ramamoorthy, 1984). The world wide annual release of this metal from natural sources was about \(8.43\times10^5\) Kg (Nriague, 1979).

Main use of cadmium is for electroplating to provide bright appearance and resistance to corrosion. The next largest use and byproducts is for Cd-Ni bearing alloys. Cadmium is also widely used as pigments in plastic industries, ceramics, paints and coatings (Moore and Ramamoorthy, 1984). Apart from that cadmium is also used for plastic stabilizers in the production of Polyvenyl chloride plastics (PVC), Ni-Cd based rechargeable batteries and cells used in house appliances, small industries, etc. Cadmium is also used in electronic and electric materials like TV tubes, fluorescent lamps, X-ray screen, Cathode ray tubes, switches, etc. (Moore and Ramamoorthy, 1984; Stokinger,
1981). Cd(NO₃)₂ is used in reactors of nuclear fission plant (Stokinger, 1981). One of the 
major sources of non-occupational source is cigarette smoke inhalation (Stokinger, 
1981). The content of cadmium per packet of 20 cigarettes is between 10 to 18 µg 
(Kudesia, 1990). Cadmium is unique among non-essential metals because of its long 
biological half life, slow excretion and delayed action on kidneys (Cherian, 1980).

Effects on Fishes:

The effect of cadmium in fish and aquatic organisms is very versatile in nature. 
Cadmium exerts its toxic influences in several ways. Numerous studies have been 
reviewed and highlighted the toxic potential of cadmium on fish (Friberg, et al., 1974; 
Hiatt and Huff, 1975; Nriagu, 1979; Stokinger, 1981; Moore and Ramamoorthy, 1984; 
Kaviraj and Das, 1994). There are several reports on cadmium toxicity that brings out the 
various alterations in fishes including physical or structural damages, biochemical 
changes and physiological disturbances. Cadmium intoxication leads to skeletal 
deformity in fishes (Nakamura, 1974; Bengtsson, et al., 1975; Muramoto, 1981; 
Vijayaram, et al., 1990). Prolonged exposure to cadmium lead to abnormal bone 
metabolism leading to loss of Ca and Mg in fish *cyprinus carpio* (Muramoto, 1981). 
Sublethal chronic cadmium treated to *Anabas testudineus* (bloch) for 120 days showed 
the skeletal deformity with loss of Ca, P and Mg in vertebrae (Bandyopadhyay, et al., 
1998) and skeletal shortening (osteosclerosis) in rainbow trout, *Salmo gairdneri* 
(Buhringer, et al., 1990). There are several reports on the impact of cadmium on various 
hematological parameters in various fishes. Increased in blood hemocrit, hemoglobin 
erythrocyte ATP, cardiac and ventilatory rates were reported in rainbow trout, *Salmo*
gairdneri exposed to 6.4 μg/l of Cd for 178 days (Majewski and Gales, 1981). Gill and Pant (1986) observed that cadmium caused nuclear anomalies like clumping of chromatin material and increased in inter-chromtic spaces in erythrocyte nuclei in Puntius conchonius. Cadmium induced decrease in hemocrit, hemoglobin and total erythrocyte count (TEC) in fish Sarotherodon mossambicus was observed by Ruparelia, et al. (1987). However, in Tilapia aurea, cadmium was found to reduce the haemocrit value but produced no effect on hemoglobin percentage (Papoutsoglou and Abel, 1988). Sublethal level of cadmium induced decreases WBC number in which neutrophil, eosinophil and basophil number increased in goldfish, Carassius auratus (Murad and Houston, 1988). Treatment of 100 ppm of cadmium caused the reduction of haemoglobin percentage in fish, Channa punctatus (Kumari, et al., 1989). But the drastic decline in TEC and haemoglobin reflecting the anaemic state was reported in major carp Catla catla with the treatment of cadmium (Vincent, et al., 1996). Cadmium caused oxidative stress and tissue damage accompanied with decreased Hb and Ht value in 24 hr exposed fish Cyprinus carpio (Zikic, et al., 1997).

There are several reports on change in biochemical parameters by cadmium intoxication in fishes. Larsson, et al., (1981); Larsson and Haux (1982) reported the case of serum hypocalcemia in flounders Platichthys flesus after exposing to sublethal level of Cd. Hypocalcemia was also reported in rainbow trout (Salmo gairdneri) after intoxication of cadmium (Roch and Maly, 1979). Increase of blood glucose level in rainbow trout (Salmo gairdneri) and flounders Platichthys flesus l. with decrease in muscle glycogen content when treated with sublethal level of cadmium for 8 days suggesting an imbalance in metabolism in the endocrine control of the carbohydrate metabolism was reported.
In Heteropneustes fossilis treated with cadmium, liver lactic acid content decreased after 18 days and again elevated after 30 days of exposure (Sastry and Subhadra, 1983). Cadmium induced reduction in levels of lipid and cholesterol in brain, ovary and testes but increased them in liver of Clarias batrachus (Katti and Sathyanesan, 1984). Himly, et al., (1985) observed the increase of protein level in liver and gill of Mugil cephalus treated with cadmium. But protein content and RNA significantly decreased in Channa punctatus with concomitant increase in free amino acid content in liver, kidney and muscle tissues after exposure to 5.0mg/l of cadmium for 7 days (Jana and Bandyopadhyaya, 1987). Bhattacharya, et al., (1987) reported the elevation of blood glucose level and depression in the liver glycogen content in Channa punctatus with the exposure to cadmium. According to Soengas, et al., (1996), cadmium induced increase in liver protein, plasma glucose and plasma lactate with concomitant decreased liver glycogen in Atlantic salmon (Salmo solar) treated with cadmium for only 8 hours. Cadmium induced changes in endocrine and carbohydrate metabolism in fishes were reported with significant increased in cortisol level in fish rainbow trout Oncorhynchus mykiss under cadmium stress (Oriz, et al., 1996). Hontelaa, et al., (1996) reported the increase in the level of plasma cortisol, plasma T₄ levels, plasma glucose and decreased liver glycogen in fish Oncorhynchus mykiss juvenile exposed for one week to cadmium. Gradual decrease in protein content in plasma, liver and muscle with concomitant increase in the concentration of cadmium in body following sublethal cadmium intoxication for 30 days in major Indian carp Labio rohita was reported by Sinha (1997).
Histopathological disorders in fishes are very common effects of cadmium intoxication. Tubular lesions in kidney were reported in *Leiostomus xanthurus* (Hawkins, *et al.*, 1980) and in fathead minnow, *Pimephales promelas* (Stromberg, *et al.*, 1983) following exposure to cadmium. Renal impairments were also reported in *Puntius conchonius* (Gills, *et al.*, 1988) with tubular epithelial cells degeneration and obliteration of lumen by cadmium treatment. Saxena (1981) observed neoplasia in kidney with necrosed tubule, degeneration of interstitial tissue and shrinkage of glomeruli in *Channa punctatus* treated with cadmium. Damages in liver was also reported in various fishes following treatment of cadmium. Hepatic necrosis and karyomegaly are observed in the liver of cadmium treated English sole, (*Parophrys vetulius*) by Larson and Haux (1982). Sastry and Gupta (1979) reported hepato-cellular necrosis in *Heteropneustes fossilis* treated with cadmium. Lethal concentration of cadmium exposed to *Lepidocephalichthys guntea* and *Puntius arulius* showed necrosis, vacuolization of hepatic parenchyma and formation of multinucleated giant cell in liver (Shivraj, 1990), whereas, sublethal treatment for 30 days caused severe cord disarray, destruction of hepatocytes and accumulation of pigmented cells in *L. guntea* and break down of cell boundaries and necrosis in centro-lobular area in *P. arulius* (Shivraj, 1990).

One of the most prevalent histopathological effects of cadmium is lesion in gill tissue of fishes due to its direct contact with cadmium compounds dissolved in water medium. Necrosis and sloughing of the mucosa of gills was apparent in mummichog (*Fundulus heteroclitus*) treated with cadmium for short hours (Voyer, *et al.* 1975). Fusion of gill lamellae was reported in *Barbus ticto* with short term treatment of cadmium (Wagh, *et al.*, 1985). Karlsson-Norrgren, *et al.*, (1985) reported the increase in volume of
the non-tissue spaces and curling of the secondary lamellar epithelium and degeneration of chloride cells after exposure to 10μg/l Cd to Zabrafish (*Brachydanio rerio*) and in gills in rainbow trout (*Salmo gairdneri*). Proliferation of Chloride cells in gills increased the uptake of cadmium but decreased Zn uptake in gills of *Oncorhynchus mykiss* when exposed to $^{109}$Cd and $^{65}$Zn for 12 hour (Zia and McDonald, 1994). Exposure of common carp *Cyprinus carpio* to sublethal (2-5mg/l) cadmium produced hypertrophy of secondary gill epithelium after 90 days (Kaviraj and Das, 1994). Alazemi, *et al.*, (1996) documented the damage of gills with lamellar aneurism and thick layer of mucus accumulation in the gills of freshwater fish *Gnathonemus petersii* with the treatment of 10mg/l cadmium for 6 hours. Freshwater fish, *Macropsobryan uruguayanae* treated with sublethal cadmium for 30 and 60 days resulted into hyperplasia of Chloride cells, primary and secondary lamellar epithelium, shortening of secondary lamellae, epithelial necrosis and mucinous metaplasia (Randi, *et al.*, 1996). Gills, *et al.*, (1988) observed severe disruption of gill lamellae, necrosis, accumulation of cellular debris and wilting of the pillar cell system and fusion of secondary lamellae in *Puntius conchonius* following cadmium treatment. Damage in primary and secondary lamellae was also reported in *Cyprinus carpio* fingerlings following treatment of chronic cadmium (Dhanapakiam, *et al.*, 1998). Manoj and Ragothaman, (1999) reported severe changes in the structure of gills with impairment of basement membrane, degeneration of gill lamella, cyst formation, increased inter lamellar spaces and sloughing of epithelial lining following treatment with cadmium to edible fish *Boleophthalmus dussumieri*. On the basis of works by Wong and Wong, (2000) the sublethal treatment of cadmium for 7 days caused the reduction of Ca$^{2+}$ transport capacity per unit Chloride cells in gill of Tilapia (*Oreochromis mossambicus*).
suggesting Chloride cells being the primary target of cadmium which subsequently lead to fish hypocalcemia.

Many of the studies have been done by numerous workers on the acute toxicity of cadmium as a basis of their researches. According to Moore and Ramamoorthy, (1984), the acute 96h LC50 values vary from 0.09 to 105 mg/l for freshwater and 8 to 85 mg/l for estuarine fishes. Acute toxicological study was conducted in the beginning of 20th century by Thomas (1915) and reported that a concentration of 6 mg/l Cd was fatal to *Fundulus heteroclitus* within 36 hour. Ellis (1937) found that 0.0165 mg Cd/l killed the goldfish within 8.5 to 18 hours in distilled water. The acute toxicity and LC50 for adult and larvae were found influenced by the hardness, temperature and salinity of the test medium (Moore and Ramamoorthy, 1984). The 96h LC50 of cadmium to different species of warm water fishes was found to be 0.63 to 1.05 mg/l in soft water and 72.60 to 73.50 mg/l in hard water for fathead minnows; 2.84 mg/l and 16.00mg/l in soft water and hard water respectively for green sunfish (Pickering and Henderson, 1966). The 48h LC50 of Cd for adult rainbow trout increased from 0.09 to 3.70 mg/l as the hardness increased from 20 to 320 mg/l (Calamari, et al., 1982). In experiments with mummichog (*Fundulus heteroclitus*) the 192h LC50 at 5°/oo (salinity) was 15 mg/l and at 35°/oo it was 28 mg/l (Eisler, 1971). Acute toxicity of cadmium and zinc to flagfish (*Jordanella floridae*) was reported and 96h TLm values for cadmium and zinc were 2500 µg and 1500 µg/l respectively (Spehar, 1976). Bellaravere and Gorbi (1981) observed that 96h LC50 of cadmium for zebrafish (*Brachidionio rerio*) was 4.35 mg/l. In case of *Channa punctatus* the 96h LC50 of cadmium was found to be 19.70 mg/l (Saxena and Parashari, 1983). But on the basis of report by Sastry, et al., (1997) the 96hr LC50 of cadmium for *Channa*
punctatus was 16.2 mg/l. In Danio malabaricus and Puntius ticto the value of 96h LC50 of cadmium was reported to be 25.00 and 32.00 mg/l (Saxena et al., 1982). Shivraj and Patil (1988a & 1988b) reported that 96h LC50 values of cadmium to Lepidocephalichthys guntea and Puntius arulius were 55mg/l and 39 mg/l respectively. Wagh, et al., (1985) found that 96h LC50 value of cadmium sulphate (Cd SO4) for Barbus ticto (Ham.) was 0.3348 ppm. Victor, et al., (1986) conducted a sublethal tests of CdCl2 to Lepidocephalichthys thermalis for its impact on reproduction and observed that the 96h LC50 value of cadmium was 1.303 mg/l. In case of African catfish Clarias gariepinus the 96h LC50 of cadmium was computed to be 12.0mg/l (Alkhahem, 1995). Sekine, et al., (1997) studied the LT50 (Lethal Time) in Killifish. The LT50 of killifish in distilled water containing 1.05M. CdSO4 was 31 hours and in the river water containing similar concentration of CdSO4 was 327 hours. Kanagaraj, et al., (1998) studied the sublethal effects of cadmium nitrate, Cd(NO3)2 in freshwater fish Catla catla, and observed that 24h LC50 of cadmium was 3.7 ppm. On the basis of study by Danapakiam, et al., (1998), the 96h LC50 value of Cd in common carp (Cyprinus carpio) was 3.5 mg/l.

Rate of oxygen consumption is considered as a reflection of the total metabolism and the metabolic state of the fish (Fry, 1971). Wagh, et al., (1985) reported the disturbance of O2 consumption in short-term treatment of cadmium to Barbus ticto (Ham.). Rate of O2 consumption was found to decrease in Lepidocephalichthys guntea and Puntius arulius following sublethal treatment of cadmium (Shivraj and Patil, 1985 & 1988a; Shivraj, et al., 1989). The similar result was observed with lethal cadmium exposure to Puntius arulius (Shivraj and Patil 1988b). In Channa punctatus O2 uptake was reduced in the whole body by 12% and in gill 23% after exposure to cadmium for 96 hour. However, in
long term (120 days) exposure to cadmium the O₂ consumption in tissues were reduced, with decreased in gill (70%) followed by muscle, liver and kidney (Sastry and Shukla, 1994). In sublethal cadmium treatment to Cyprinus carpio there was an increase in O₂ uptake on 10, 20, and 30 days of exposure (Dhanapakian, et al., 1998). High level of cadmium exposed to fish Cyprinus Carpio also resulted into decreased rate of oxygen consumption (Sarkar, 1999).

Bioaccumulation of metals in organisms has also been studied extensively by various workers. The studies in various species of fishes have drawn the attentions that bioaccumulation of cadmium and other heavy metals in the tissues are inconsistent in distribution. McIntosh and Bishop (1976) analysed a lake receiving effluents from electroplating industry where bluegill fishes were subjected to 2.0 µg/l of Cd. They found that Cd accumulation was highest in liver (3.52µg/g) followed by gill (0.11µg/g) and muscle (0.08µg/g). Brown and Chow (1977) reported the accumulation of cadmium in 15 fish species from Toronto and Baie du Dore Harbour and found that, the accumulation was highest in the kidneys followed by liver and muscle. Thomas, et al., (1983) exposed Rainbow trout to 9µg/l in water for 12 to 36 weeks and found that cadmium accumulation was highest in kidney (65%) followed by liver (20-30%) and gills (5-15%). According to Kraal, et al., (1995), the distribution of cadmium in organs of Cyprinus carpio varies with the route of toxic material. Food contaminated with Cd leads to the accumulation in the following order: gut > gill > kidney > liver > muscle. Kumar and Mathur (1996) reported that the major site of cadmium accumulation in teleost fish Colisa fasciatus was in gill and liver. But least accumulation was observed in muscle. Vigh, et al., (1996) collected grass carp (Clenopharyngodon idella) from lake Balaton
(Hungary) and from a fish pond farm nearby. On the analyses of metals, they reported that metal concentrations (Cd, Zn, Pb and Hg) in most cases were highest in kidney or in liver, and lowest in muscle or gut. In *Oreochromis aureus* exposed to 0.1 mg/l Cd for 24 hour or one week the highest accumulation was observed in intestine and the lowest in caudal muscle (Allen, 1995a). Schultz, *et al.*, (1996) reported that most of the cadmium was accumulated by liver and kidney within 24 hour, with little change occurring after 335 days in Channel catfish (*Ictalurus punctatus*) administered with 4.0μg/kg of $^{109}$CdCl$_2$ via a dorsal aortic cannula. The study by Cinier, *et al.*, (1997) on carp (*Cyprinus carpio*) tissues in long term exposure of cadmium showed that at the end of 140 days accumulation of Cd was in the order of: kidney (250 mg/kg) > liver (91 mg/kg) > muscle (9 mg/kg) dry wt. body weight. According to Melgar, *et al.*, (1997), trout (*Oncorhynchus mykiss*) exposed to sublethal oral doses of CdCl$_2$ (0.01 to 0.50μg/ml) for 3 weeks showed that bioconcentration order of kidney > liver > gills. Farag, *et al.*, (1994) reported that metal accumulation in tissues of juvenile rainbow trout treated for 21 days was higher in gill and kidney with water borne exposure and in case of food borne exposure it is higher in stomach and pyloric cecae. According to Allen (1995b), *Oreochromis aureus* exposed to cadmium alone at 0.05 and 0.1 mg/l, and mixtures of 0.05 mg/l cadmium with 0.05 mg/l Hg or Pb (0.05 and 0.50 mg/l) through fish food and observed that the cadmium was consistently accumulated by kidney. This bioconcentration was not effected by Hg or Pb. Glynn, (1996) reported that the intercellular distribution of cadmium in zebrafish (*Brachydanio rerio*) altered as the concentration increased due to the saturation of Cd-binding capacity of metallothionein. The cadmium influx increased drastically in a non-linear fashion at the higher level of exposure. On the basis of studies by Shivraj (1990),
the bioaccumulation of cadmium in *Lepidocephalychlthys guntea* at the end of 96h exposure of 25mg/l was in the order of: Gill (44μg/g) > muscle > kidney > brain > intestine > liver (14μg/g). But at the exposure of 40mg/l the accumulation of Cd was in the following order: Gills (29.66μg/g) > kidney > intestine > liver > brain > muscle (13μg/g). In case of sublethal exposure of 5.5 and 11mg/l Cd in *Lepidocephalychlthys guntea* at the end of 30 days, the maximum accumulation was found in gill (37.47μg/g and 39.92 μg/g) and minimum in intestine (11.74μg/g and 7.11 μg/g) respectively.

Warner, *et al.*, (1966), at a landmark Symposium at the Institute for Terrestrial Ecology commented that: - 1) The behavior (or activities) of an organism represent the final integrated results of a diversity of biochemical and physiological processes. Thus a single behavioral parameter is generally more comprehensive than a physiological and biochemical parameter. 2) Behavioral patterns are highly sensitive to changes in the steady state of an organism and are one of the key values for its use in exploring sublethal toxicity. 3) Behavioral measurements can be made without direct physical harm to the organism.

A critical review on the effects of heavy metals on fish behavior has been made by Atchinson, *et al.*, (1987) and the authors commented that the change in certain fish behavior, especially cough rate and avoidance reactions are the sensitive indicators of sublethal exposure to metals and also easier to carry out the study than life cycle tests. According to Hidaka and Tutsukawa (1985), the fish avoidance test is well established in the laboratory as a means to showing effects well below the lethal range. Giatina and Garton (1983) compiled the literatures regarding the studies on preference-avoidance response of fishes on aquatic contaminants. They quoted that the use of behavioral end
points in environmental hazard and impact assessment has become a major field of study in recent years. The study helps to find concentrations effective causing avoidance response “thresh-hold concentration” from the control instead of proceeding to chronic sublethal test and its effect on physiology and biochemical parameter in long run. The authors further suggested that avoidance/selection responses should be used in addition to (or in place of) measures of lethality when setting water-quality criteria or when estimating a potential environmental impact.

According to Beitinger and Freeman (1983), behavioral responses comprises the first “line of defense” since the organism come into contact within a second after and an adverse chemical encountered. Fishes are highly mobile animal, capable of sustaining swimming speeds over 10 body length per second (Bainbridge, 1958). They also possess acute chemical discrimination abilities (Hasler and Wisby, 1949). These organisms may behaviorally avoid the chemical and, lessen the expected negative impact of the chemical. However, some times they are lured by the chemical toward it, even though the chemical is toxic in nature (Giatina and Garton, 1983). Thus according to Beitinger and Freeman (1983), chemicals may be detected directly by specialized nerve cells of fish or indirectly, by effecting changes in the internal state such as metabolic rate (which requires more time for detection). Therefore, on the nature of chemical the fishes either avoid it or get lured to it.

Of numerous avoidance / preference studies on fish to different heavy metals, the cadmium is one of the least studied chemicals in this field. (Giatina and Garton, 1983). McNicol, et al., (1996) reported that lake whitefish (Coregonus clupeaformis) when subjected to the avoidance test showed that both the individual and shoaling responded to
the cadmium by avoiding the region when Cd was released and spent more time in clean water side. Individuals in shoals responded to Cd more strongly and at lower concentrations than did solitary. This suggested that social influence of shoaling enhanced a fish's ability to response to the preference of toxicants.

Some of the behavioral changes due to cadmium treatment include alterations in the normal spawning behavior (courting, aggressive territoriality, and female acceptance of males) of flagfish during chronic exposure (Spehar, et al., 1978). Benoit, et al., (1976) observed the inability of brook trout to spawn at 3.4μg/l Cd (Sullivan, et al., 1978).

Cadmium also exerts its toxic effects on reproductive system and development of fishes. Some of the serious effects are impairment of reproductive hormones and testicular injury in male brook trout (Sangalang and O’Hulloran, 1972,1973; and Sangalang and Freeman, 1974); and reduction in ovarian activity, vitellogenic oocytes and increased number of attretic follicles in catfish Clarias batrachus (Saksena and Agarwal, 1986). Impairment of vitellogenesis and degenerative lesions in the ovary of burrowing teleost, lepidocephalichthys thermalis following the treatment of cadmium was also reported (Victor, et al., 1986). Rosenthal and Sperling (1974) reported the reduction of incubation time, decreased in percentage of viable hatched eggs and smaller in larvae size by incubating the eggs of herring Clupea harengus in cadmium contained water. Cadmium also caused considerable reduction of egg-laying capability in adult crayfish (crustacean) Procambarus clarkii and decreased in number of hatched eggs (Naqvi and Howell, 1993). Long term exposure (90 weeks) of cadmium to rainbow trout Oncorhynchus mykiss and brown trout Salmo trutta L. prevented the egg development into fry stage and delayed oogenesis in female fishes (Brown, et al., 1994). Long term
exposure of cadmium also decreased body weight gain with negative % of specific growth rate (SGR) in goldfish Carassius auratus L. (Moza, et al., 1995). Cadmium when tested on Cyprinus carpio showed genotoxic effect with a formation of micronuclei in the erythrocytes in sublethal concentration for 21 days treatment (Thiruvalluvan, et al., 1997).

Enzyme activities are also altered in the fishes treated with cadmium. In catfish Heteropneutes fossilis treatment with cadmium for 15 to 60 days inhibited liver, ovary and gill acid phosphatase (ACP) but increased it level in kidney and intestine. On the other hand, there was a decrease of Alkaline phosphatase (ALP) level in liver, kidney and intestine with concomitant elevation in ovary and muscle (Sastry and Subhadra, 1985).

**Effects on Experimental Mammals:**

Acute toxicity of cadmium has been studied extensively in experimental mammals. Cadmium induces hepatic and renal toxicity, gastro-intestinal tissue damages and testicular necrosis in mice (Anderson, 1989a &b), nephrotoxicity and hypertension in rats (Lall, et al., 1996). Cadmium was also found to be embryotoxic in early rat embryos with degeneration of blastocysts (Fein, 1997), embryocidal and teratogenic with development malformations in golden hamster (Ferm and Carpenter, 1969). Cadmium also disturbed the pituitary hormone release with abnormal secretion of LH and FSH, which in turn caused reduction in testes weight and its accessory reproductive organs, decreased sperm population and necrosis of testes in male rat, blocked ovulation, prolonged estrus cycle and fetal re-absorption in female rat and hamster (Cooper, et al., 1986). The cadmium induced decreased ovarian follicle number and size in rabbit ovary was also reported
(Massanyi, Uhin and Valent, 1997). According to Gupta, et al., (1993), cadmium caused neurotoxic effect through altering the neurochemical secretion in brain of developing rat. Bone lesions with osteoporotic symptoms were found in rats fed with CdCl₂ with diet (Stokinger, 1981).

It is now an established concept that cadmium induce metallothionein (MT) synthesis in organs and has overall protective effect by reducing the cellular toxicity of cadmium (Stokinger, 1981; Roch and McCarty, 1984; Kanaga, et al., 1996 and Smet, et al., 2001). But according to review substantiated by Miettiner and Krenkel (1973), this protective effect of MT, however, is somewhat paradoxical. Because, binding of cadmium to MT no doubt does protect against testicular necrosis, but at the same MT-bound cadmium injected in mice produces severe necrosis of renal tubular lining cells in kidney that does not occur with injection of non-protein bound cadmium salts. In a similar observation, extensive renal tubular necrosis in rats following the treatment of chronic cadmium bound metallothionein of equine was reported (Holt, et al., 1985).

**Effect on Human being:**

According to the review substantiated by Stokinger (1981), the most prevalent chronic toxicity of cadmium through industrial emission was found to be renal tubular dysfunction with proteinuria, as an earliest symptom. The same cases were reported by Shaikh, et al., (1987) and Cai, et al., (1998). The disease also leads to aminouria, aciduria, glucosuria, renal stones and decrease in urine concentration capacity. Chronic inhalation of cadmium oxide dust causes pulmonary emphysema and anemia in industrial workers (Stokinger, 1981). Skeletal related diseases are reported with Cadmium
exposure. The Japanese "itai-itai" disease is attributed to the high intake of cadmium wastes (Tohyama, et al., 1982a and Miettiner and Krenkel, 1973). The symptoms of severe back pain and difficulty in walking have also been reported in storage battery workers exposed to cadmium oxide dust. The reports on reduced in testosterone levels in workers was revealed in the review by Stokinger, (1981). There are several reports on the relationship between cancer incidence and exposure to cadmium (Moore and Ramamoorthy, 1984). Kjellstrom et al., (1979) reported that number of death due to prostate cancer was significantly higher than expected among workers in cadmium alloy factory. The gastrointestinal diseases were also reported in man due to the ingestion of CdCl₂ The cases of hemorrhagic necrosis of gastrointestinal, focal hepatic necrosis, slight pancreatic hemorrhage and gastroenteritis were reported in man at high dose (Andersen, 1989). According to Bernier et al., (1996), ingestion of fish contaminated with Cd, Hg and Pb can adversely effect the immune system in man and increase the susceptible risk to infection, auto-immune disease and allergic manifestation.

**Ethylene diamine tetraacetic acid (EDTA):**

EDTA (Ethylene diamine tetraacetec acid), an aminocarboxylic acid is a widely known chelating agent. The compound is also commercially available in various forms like Na₂EDTA, FeNaEDTA, CaEDTA, etc. EDTA and its analogue compounds are used as processing aids in many industries which deal with metals, textiles, leather, rubber, pharmaceutical, polymer production, pulp and paper production and agricultural (fertilizer), cleaning agents in radiochemical laboratories, waste water treatments and
ground waters near radioactive waste disposal sites (Helz and Horzempa, 1981; Sillanpaa, 1997).

Molecular formulae of EDTA = C₁₀H₁₆O₉N₂

Molecular structure of EDTA is as follows:

```
  HOOC
  |    |
  CH₂-CH₂-N
  |    |    |    |
  HOOC  COOH
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Molecular wt = 292.243

The EDTA and its metallic compounds are biodegradable under favorable conditions in natural water, soil and sediments in a long term, but the compound can undergo rapid chemical and photolytic degradation for decomposition (Sillanpaa, 1997).

The existing toxicological effect of EDTA shows that it is relatively nontoxic by acute exposure to many animals. On the basis of report by Whittaker, et al., (1993), the US Food and Drug Administration (FDA) has approved the use of Na₂EDTA and CaNa₂EDTA for direct addition to food after evaluating the observations of the compounds as non-carcinogenic, non-genotoxic and non-teratogenic in experimental animals, and also found extremely low response on human skin sensitization. Apart from that there was no significant difference in weight gain, food efficiency, organ weighs in the treated rats when compared to controls. According to the reports released by Joint-FAO/WHO (1966, 1974), there was no evidence of histopathological abnormality in the EDTA treated rats and further Organization determined and fixed the ADI (Acceptable
Daily Intake) for CaNa$_2$EDTA intake to 2.5mg/b.wt/day or 150mg/person/day (Joint FAO/WHO, 1966; 1974). The concentration needed to cause mortality is high in rat and mice. The LD$_{50}$ of CaEDTA in mice by i.p. (intraperitoneal) administration is 17.4 mmol/Kg.b.wt and in rat it is 28 mmol/Kg.b.wt by oral treatment. But LD$_{50}$ of EDTA in rat by i.p. is 1.38 mol/Kg.b.wt (Andersen, 1989b). According to Shrivastava et al., (1986), 14 day LD$_{50}$ for rat by i.p. administration is 513mg/kg.b.wt. In fish bluegill (Lepomis macrochirus) 96hr.LC$_{50}$ of EDTA was found to be 159mg/l (Batchelder, et al., (1980).

EDTA was first used in the 1940s for the treatment of heavy metal poisoning after understanding the chelating property and strength. And currently it is effectively used in emergency treatment of hypercalcemia and the control of ventricular arrhythmias associated with digitalis toxicity (Sanghi, 2000). Chelation therapy particularly with CaNa$_2$EDTA is often used therapeutically to reduce the body burden of lead (Flora et al., (1995).

The protective role of EDTA against heavy metal toxicity has been evaluated in many organisms. In mammals a detailed review was substantiated by Andersen, (1989a) with special reference to mice treated with cadmium, and subsequently recovered from toxicity by the treatment of EDTA and its related compounds. The report further summarized that oral administration of cadmium when complex with EDTA did not cause any tissue damage when compared with the tissue damage in experimental animals treated with cadmium alone (Andersen, 1989a). Llobet, et al., (1988) tested 16 chelating agents in mice for relative efficacy for antidote, and reported that EDTA, DTPA and CPTA appeared to be the most effective chemicals in offsetting acute zinc intoxication.
Singh and Yadava (1987) reported that growth retardation due to cadmium toxicity in *Cynabacterium Anacystis nidulans* was reversed following treatment with EDTA. The toxicity of Cd, Cu and Hg were reduced noticeably following treatment with EDTA and DTPA in *Photobacterium phosphoreum* (Sillanpaa and Oikari, 1996).

In plant, the uptake of lead (Pb) in presence of EDTA in vegetable crop Okra (*Abelmoschus esculatus*) was found far lesser than in Okra cultivated in Lead contained sewage-irrigated soil (Denduluri, 1993). The addition of EDTA or its related chelating agents partially reversed the inhibitory effect of Hg on the growth of plant *Lemma paucisostata* (Singh and Jaiswal, 1997).

Ameliorative action of EDTA against metallic intoxication in invertebrates is assessed with rate of mortality and on the basis of bioaccumulation of toxic metals in the body. Castille and Lawrence (1981) observed the decrease in toxicity of Cd, Cu and Mn by EDTA in shrimp nauplii (*Penaeus stylirostris*). The cadmium bioconcentration was found significantly reduced in the body of American oyster *Crassostrea virginica* following mixture treatment of EDTA and cadmium when compared to oyster treated with only cadmium (Hung, 1982). Chelation of cadmium with EDTA reduced the uptake and body burden of cadmium in marine clam *Macona bathica* (McLeese and Ray, 1984) and in freshwater clam *Anodonta anatina* (Holwerda, *et al.*, 1998) Sorvari and Sillanpaa (1996) found that chelation by EDTA decreased the toxicity of metals (Mn, Fe, Cu, Zn Cd) except Hg in *Daphnia magna*.

In case of fishes the mortality due to metallic toxicity was found decreased with treatment of EDTA. Rosenthal and Sperling (1974) observed that cadmium complex with EDTA or displaced by Zn increased the percentage of viable hatch considerably in
herring fish *Clupea harengus* eggs and no cadmium was accumulated during incubation. In *Lebistes reticulatus* mortality due to acute copper toxicity was found decreased following the treatment of EDTA (Khangrot, 1981). Muromoto (1980a) demonstrated the ameliorative effects of EDTA against cadmium toxicity in fish *Cyprinus carpio* with decrease of cadmium concentration in various organs in comparison with the metal-alone ones for 14-days treatment. Addition of EDTA resulted in the decreased accumulation of treated metals (Cd, Zn, Pb, Cu) in tissues of viscera and gills for 48 hr exposure in *Cyprinus carpio* (Muramoto, 1980b). EDTA also decreased the mortality rate of *Cyprinus carpio* caused by cadmium in 100-days treatment (Muramoto, 1981). According to Part and Wikmark (1984) complexing agent EDTA reduced the cadmium transfer through gills and protected the fish rainbow trout (*Salmo gairdneri*) from its toxicity. Kargin (1996) reported that EDTA increased elimination of cadmium from the tissues of cadmium contaminated fish *Tilapia zilli* when compared with that of clean freshwater post-treated fishes. Exposure of EDTA considerably reduced the cadmium induced gill damage in *Cyprinus carpio* (Kaviraj and Das, 1994). The uptake and bioaccumulation of cadmium and copper was also found decreased in presence of EDTA in *Cyprinus carpio* for 4 days treatment (Xiaorong, et al., 1997).

However, in some instances EDTA synergised the toxic effect of metals. In plant *Lemma paucicotsata* and Duckweed *Lamna gibba* EDTA failed to antagonize the toxicity of cadmium (Nasu, et al., 1983; Polar and Kucukuzzer, 1986). George and Coombs (1977) found that EDTA increased the tissue content of cadmium and iron considerably in mussel (*Mytilus edulis*). Sillanpaa and Oikari (1996) observed that EDTA increased
the toxicity of iron in *Photobacterium phosphoreum* (through microtox bioassay test) and also the toxicity of mercury (Hg) in *Daphnia magna* (Sorvari and Sillanpaa, 1996).

**Nitrilotriacetic acid (NTA):**

Nitrilotriacetic acid (NTA) is an aminotricarboxylic acid and has high capacity to sequester metal ions. It is available commercially in the highly pure form. The acid form of NTA was first synthesised by Heinzt in 1862 and its properties and chemistry was later determined in 1865 (Heinzt, 1865). The present primary industrial use of NTA is in the treatment of boiler water to prevent buildup of mineral scales; textile industries to sequester trace metals for the prevention of uneven fabric dyeing. It is also used in the industries of pulp and paper, metal industries and detergent manufactures (Anderson, et al., 1985). Molecular formula of NTA is N(CH$_2$COO)$_3$ and it is also available commercially in form of Na$_3$NTA-H$_2$O.

Molecular structure on NTA is as follows:

```
\[
\begin{array}{c}
\text{CH}_2\text{COO}^- \\
\text{OOCH}_2\text{C-N} \\
\text{CH}_2\text{COO}^- \\
\end{array}
\]
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Both free acid (NTA) and trisodium monohydrated Nitrilotriacetic acid (Na$_3$NTA-H$_2$O) are white crystalline materials.

Molecular wt. of NTA=191 and Molecular wt. of Na$_3$NTA-H$_2$O=272

NTA and its analogue compounds are highly biodegradable under natural condition by bacteria (Wong, et al., 1973). Numerous laboratories and field studies have demonstrated that biodegradation is the key mechanism by which NTA is removed from the
environment. It can also undergo photodegradation and chemical degradation under certain conditions (Anderson, et al., 1985). The use of NTA may not constitute an environmental problem, as it can be biodegraded in activated sludge process and presumably natural water decreases at low temperature (Moore and Ramamoorthy, 1984). The biodegradation of Cd-NTA chelates required only 60 days (Chau and Shiomi, 1972). NTA has an economic advantage over EDTA that on a weight basis, loss of it is needed to complex cations in 1:1 mole ratio and further the new material form which is manufactured are less expensive than needed for EDTA (Sanghi, 2000).

The toxic effect of NTA was critically discussed in the reviews by Anderson, et al., (1985) and Andersen, (1989a). The reports revealed that sodium form of NTA used in consumer product is only moderately toxic during acute oral exposure. The review disclosed that NTA as doses relevant to human exposure is not teratogenic either alone or in combination with heavy metals, does not cause adverse effects in bone function or composition, is non-genotoxic, and is not metabolized during passage through mammalian systems. Among many mammal tested human showed least absorption. Sub-chronic and chronic exposure studies of NTA on man and rodents showed that kidney is the primary target organ and the most susceptible to tumorigenesis in the urinary tract. The development of tumor can take place only when Zn and Ca distribution between urinary tract tissue and urine that accompany renal clearance and urinary excretion of NTA is considerably high in dose (Clayson and Cooper, 1970). But human exposure to NTA, to be expected through consumer products is $10^5$ to $10^6$ less than the lowest dose that alters Zn and Ca distribution in rodents. Hence, NTA does not establish a health risk to man as a result of its commercial use (Anderson, et al., 1985). However, in high dose
of NTA (>2.5mM) it can induce chromosome abnormalities in human lymphocytes only when the level of NTA exceeded that of the divalent cations (m⁻²) in the medium (Bora, 1975).

In rats oral LD₅₀ of Na₃NTA is 1.90g/kg and time of mortality was less than one day (Anderson, et al., 1985). According to the review by Andersen, (1989) the LD₅₀ of NTA in mice through i.p. (intraperitonal) is 1.70 mmol/kg.b.wt and in rats LD₅₀ through oral treatment is 34 mmol/kg.b.wt.

In fish Fathead minnow (Pimephales promelas) 96hr LC₅₀ is 114mg/l (Arthur, et al., 1974). In rainbow trout (Salmo gairdneri) 96hr LC₅₀ is 98mg/l (Macek and Sturm, 1973). In Guppy (poecilia reticulata) 96hr LC₅₀ is between 560 to 1000mg/l (Canton and Sloof, 1982). Macek and Sturm (1973) observed and found no histological changes in gill tissue of bluegill (Lepomis macrochirus) continuously exposed to 179mg/l of NTA for 28 days. In another report, Hamilton (1977) exposed Rainbow trout to 400mg/l NTA for 100days and observed no histological changes in gills, liver, kidney and spleen of treated fishes when compared with untreated controls. According to Maki (1979), the concentration of NTA up to 180mg/l tested did not produce significant changes in the ventilation frequency rate (Respiratory activity) in bluegill (Lapomis machrochirus), which in turn was an indication of free from stressful condition under NTA exposure. In one life cycle test (One generation test), NTA exposure to fathead minnow (Pimephales promelas) indicated No Observed Effect Concentration (NOEC) greater than 54mg/l (Arthur, et al., 1974).

In aquatic invertebrate Amphipod, Gammarus 96hr LC₅₀ was found to be 98mg/l (Arthur, et al., 1974). Flannagan (1971) evaluated the toxicity of NTA to a selected
aquatic invertebrates and reported the 96hr survival of Scuds (*Amphipod*), snail (*Gastropod*), Midge (*Chironomidae*), Caddisfly (*Trichoptera*), Mayfly (*Ephemeroptera*) and Dragonfly (*Odanata*) were not more than or equal to 500mg/l in all these invertebrates. In water flea (*Daphnia magna*) 96hr LC50 was recorded between 560 and 1000mg/l (Canton and Sloof, 1982). In chronic toxicity test of NTA, the result of one life cycle test exposure in Amphipod (*Gammarus Pseudomnaeus*) was found to be 19mg/l (Arthur, *et al.*, 1974). But in water flea *Daphnia magna* NOEC for one generation exposure to NTA was 100mg/l (Cantoon and Sloof, 1982).

Protective action of NTA against the toxicity of some heavy metals has been observed in various organisms. According to Anderson, *et al.*, (1985) the metal toxicity can be controlled in organisms only when molar concentration of NTA exceeds that of metals.

In plants increased yield of corn was reported when Zn-NTA chelate was added to Zn deficiency soils (Wallace and Romney, 1962). The improved yields of rice was grown in Mn and Fe deficiency soils and found improved growth of tomatoes at 85ppm NTA treatment (Matsuda, 1968a; 1968b). Denduluri (1993) reported that the accumulation of lead (Pb) in Okra plant (*Abelmoschus esculatus*) was reduced following the treatment to NTA. The reduction of Nitrate reductase activity chlorophyll content and protein in leaves of Okra due to lead (Pb) toxicity were reversed by the treatment of NTA (Denduluri, 1993).

In vertebrates (rodents) the action of NTA against metallic toxicity was also reported. NTA was found to be one of the most effective in low doses in preventing mortality due to lethal dose of Manganese chloride and Potassium chromate (*K2CrO4*) intoxication in mice (Tandon and Khandelwal, 1982; Tandon and Srivastava, 1985).
There are several reports that reveal about the reversal of metallic toxicity with help of NTA treatment in aquatic invertebrates. Biesinger and Arthur, (1974) observed the protective role of NTA against chronic copper (Cu) and zinc (Zn) toxicity in *Daphnia magna*. However, the toxicity of iron (Fe) was not altered. According to Kimerle (1974), the equimolar concentration of NTA reduced the toxicity of cadmium in *Daphnia magna*. The reduction of cadmium uptake in presence of NTA in *Daphnia magna* was also reported by Poldoski (1979). The similar progressive result by the treatment of NTA was observed in cadmium infested grass shrimp *Palaemonetes pupio* (Sunda, *et al*., 1978).

Bioaccumulation of cadmium was found decreased in the body of American oyster (*Crassostrea virginica*) exposed to NTA with cadmium when compared with oyster treated with only cadmium (Hung,1982). The ameliorative impact of NTA on metallic toxicity in fishes was also reported by some authors. Sprague (1968) reported that NTA could be used to curb the mortality of fish Brook trout (*Salvelinus fontinalis*) from copper and zinc toxicity. Shaw and Brown (1974) also observed the protective role of NTA against copper toxicity in rainbow trout. NTA exposure has shown to reduce bioaccumulation of metals in fish body by the formation of NTA-metal complex in water medium. Muramoto, (1980b) observed the decreased accumulation of toxic metals (Cd, Cu, Zn and Pb) in tissues of viscera and gills of *Cyprinus carpio* through the treatment of NTA for 48hr. On the basis of report by Barica, *et al*., (1973), 5mg/l of NTA concentration prevented the accumulation of metals (Fe, Mn, Pb, Zn, Sr and Hg) in rainbow trout after 8-weeks exposure. Eisler, *et al*., (1972) reported that NTA decreased the toxicity of mercury in marine fish Mummichog (*Fundulus heteroclitus*).
There are few cases in which NTA enhanced the metallic toxicity synergistically. NTA increased the acute toxicity of injected (imp.) cadmium in mice (Andersen, et al., 1982; Engstrom and Nordbergs, 1978; Andersen, 1989b). The subcutaneous administered complexes of cadmium with NTA were rapidly deposited in kidneys at about twice the concentrations after administration of the same dose of unchelated cadmium (Engstrom, 1981).

Cadmium was chosen as the challenge metal due to its widespread pollution with high toxicity to any of the studied organisms and high toxicological potentiality with a long biological half-life. The study was also undertaken because of contradictory results concerning the impacts on lethal toxicity, metabolic activities and bioaccumulation of cadmium in various organs studied. The chelating agents (Na$_2$EDTA and NTA) were selected for the present experiments due to their broad action and binding capacity with metals in sequestering the metals in aquatic as well as in terrestrial organisms. Further, these chelating agents are ubiquitous and commercially available for various purposes in industries. But the studies on the potentiality of their ameliorative action in detoxifying the cadmium contamination are very meager, especially in Poeciliid fishes.