

Heavy metal contamination in the fishes of Inland wetlands of India

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ENVIRONMENTAL SCIENCE

by

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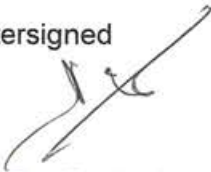
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August 2007


CERTIFICATE

This is to certify that the thesis entitled "HEAVY METAL CONTAMINATION IN THE FISHES OF INLAND WETLANDS OF INDIA" submitted to the Bharathiar University, in Partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy in ENVIRONMENTAL SCIENCE is a record of original research work done by Mr. R. JAYAKUMAR during the period April 2002 to July 2007 of his research in the Department of ECOTOXICOLOGY at SÁLIM ALI CENTRE FOR ORNITHOLOGY AND NATURAL HISTORY, ANAIKATTY, COIMBATORE – 641 108 under my supervision and the thesis has not formed the basis for the award of any Degree/Diploma/Associateship/Fellowship or any other similar title of any candidate of any university.

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DECLARATION

I, R. JAYAKUMAR hereby declare that the thesis, entitled "HEAVY METAL CONTAMINATION IN THE FISHES OF INLAND WETLANDS OF INDIA" submitted to the Bharathiar University, in Partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy in ENVIRONMENTAL SCIENCE is a record of original research work done by me during April 2002 to July 2007 under the Supervision and guidance of Dr. S. MURALIDHARAN, Department of ECOTOXICOLOGY at SÁLIM ALI CENTRE FOR ORNITHOLOGY AND NATURAL HISTORY, ANAIKATTY, COIMBATORE – 641 108 and it not formed the basis for the award of any Degree/Diploma/Associateship/Fellowship or any other similar title of any candidate of any university


Signature of the Candidate

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1. INTRODUCTION

Metals are one of the indispensable components in all the domestic and industrial activities. Indeed quite a large number of metals in small amounts are essential to man, plant and animal life. These include, iron, manganese, copper, nickel, zinc, cobalt, molybdenum and vanadium which are considered to be micronutrients. If any of these metals are eliminated from our diet we would begin suffering from metal deficient diseases. Metals such as lead, cadmium, arsenic and mercury are not required even in smaller concentrations and they exert toxicity when their levels cross a limit. Virtually even essential metals turn toxic if exposure level is high (Abassi *et.al*, 1998).

Thus, the dividing line between essentiality and toxicity is quite delicate to define. Precisely we can construe that although metals are essential for normal homeostatic functioning of any organism they turn virtually toxic at higher concentrations (Laws, 1981).

The episodes, which brought the attention of the world towards heavy metal pollution in the most poignant and dramatic manner, are the Minamata Bay Disease due to mercury and itai- itai disease due to cadmium in human beings in Japan (Abassi *et.al*, 1998). Heavy metals may remain dormant for a long time and then surface when the environmental conditions are conducive. Symptoms of heavy metal toxicity are often similar to the symptoms of other common ailments. This makes it all the more difficult to distinguish the causative factor. The effects get recognized at alarming proportions only when no counter effective measures could be taken. It is also deplorable to note that the impact can also be passed on to one's progenies.

On a global scale, it has been noted that the discharge of heavy metals into the environment is progressively increasing at an alarming rate (Depledge *et.al*, 1994) through a wide variety of sources ranging from industrial discharge to natural weathering process. Thus, heavy metals are blacklisted as core pollutants due to their persistence and bioaccumulative property (Rosenthal & Alderice, 1976; Carvalho *et.al*, 1999). Their deleterious effects get sequenced in organisms at higher trophic

levels through the food chain. Especially in aquatic ecosystems, the effects of metals get more pronounced owing to various abiotic and biotic factors.

Aquatic organisms can acquire trace elements from food, suspended particles or directly from the water. The ability of aquatic animals to absorb heavy metals and the degree of toxicity is influenced by chemical form of the metal, the presence of other metals, pH, temperature and dissolved oxygen levels (Vinogradov, 1953).

Of the various aquatic organisms, fishes are proven as excellent indicators of heavy metal contamination, because they clearly illustrate a relationship with the heavy metal contamination and to its assessment end point (Suter, 1993). Further, fish can be found virtually everywhere in the aquatic environment and they play a major ecological role in the aquatic food webs because of their function as a carrier of energy from lower to higher trophic levels (Beyer, 1996). Moreover the understanding of toxicant uptake, behaviour and responses in fish may, therefore have a high ecological relevance. Despite their limitations, such as a relatively high mobility, fish are generally considered to be the most feasible organisms for pollution monitoring, especially heavy metal contamination in aquatic ecosystems.

In recent years, heavy metal contamination in fishes has received greater concern (Riedel *et.al*, 2002). A wide range of metals and metallic components found in the marine environment can get deposited in aquatic organisms through bioconcentration, bioaccumulation and the food chain process, and eventually threaten the health of humans through food chain (Eromosele *et.al*, 1995; Austin, 1999; Chen, 2002).

Bioaccumulation patterns of metals in fish tissue can be utilized as effective indicators of environmental contamination (Atchinson *et. al*, 1977; Larsson *et. al*, 1985). Accumulated metals may eventually reach toxic levels even if the exposure may be extremely low. Further, even though no toxic effect occurs in the organism accumulating the metal, an effect may appear at a higher trophic level because of the ingestion of the accumulating organism as foodstuff (Kneip and Lauer, 1973). As a result, fishes become the medium most routinely used to monitor the levels of toxic metals and their trends in any aquatic system.

Heavy metals are found to exhibit a broad spectrum of toxicity in fishes. The heavy metals in general interfere with the protein synthesis (Syversen, 1981) and further, under stress conditions, the dietary protein consumed by fish is not stored in the body tissues (Baskaran and Palanichamy, 1990). In fishes metals such as cadmium, zinc, copper and mercury accumulate more in liver due to high concentrations of metallothionein (Wu *et.al*, 1999; Langston *et.al*, 2002; DeBoeck *et.al*, 2003). Metallothioneins are low molecular cysteine-rich heat stable proteins induced by metals. They are associated with the metabolism and detoxification of metals. Further, they also regulate the intracellular homeostatic condition of essential metals through specific molecular interactions (Roesjadi, 1993).

An early indicator of the biological effect of heavy metals is represented by the metallothionein (Mt) level in the tissue of the species which provides a suitable monitoring procedure in order to assess the biological availability and impact of heavy metals in the aquatic environment (George and Olsson, 1994; Lionetto *et. al*, 2001).

Glutathione, yet another potential candidate involved in detoxification of endogenous and exogenous compounds, is a predominant thiol compound found both in prokaryotes and eukaryotes. It is involved primarily in maintaining the cell membrane integrity, oxidation-reduction balance and scavenging free oxy radicals. Studies done have observed Glutathione (GSH), to provide first line of defense against metal toxicity especially cadmium toxicity before induction of metallothionein synthesis occurs (Zarogian and Norwood, 2002). Thus, it plays an important role in early cellular protection responses to metals and their levels decline only after a toxic response (Bell and Cowey, 1990). Thus these biochemical parameters serve as biomarkers and to provide an early warning signal to metal toxicity.

It is well known that heavy metals measured in tissues of aquatic animals can also reflect the past exposures. Tissue concentration of heavy metals can also be a reasonable measurement for public health standards and in the point of view of animals' health. Over a few decades there has been growing interest to determine heavy metals in the aquatic environment and public food supplies particularly fish

(Kalay *et.al*, 1999). Today, presence of heavy metals in the human environment has been of global concern due to known hazards (WHO/FAO, 1972; Nauen, 1983). The main way of human body fortification of metals is through food chain depending on the kind and quantity of the consumed food according to dietary habits (Spivey, 1972). In many developed countries limits of metal concentrations in fish and other foods have been set in order to safeguard public health (Nauen, 1983). In India no such standards are available, however, standards have been levied on water quality with particular reference to heavy metals (Abbasi *et.al*, 1998).

Fresh water fishes are preferred food to the inhabitants of many tropical countries. In a country like India, the intake of meat and milk is low, and fishes have special importance as a supplement to ill-balanced cereal diets. It is estimated that about 10 million tons of fishes are required annually to meet the present day demand of fish protein in India against an annual production of only 3.5 million tons (Shukla and Upadhyay, 1998).

In India, inland water with potential of fish culture is approximately 7.5 million hectares, which accounts for 2.34% of the total area of the country. The total water spread area in India is about 4.5 million hectares. Inland aquaculture resources cover about three million hectares. These include about 0.72 million hectares of natural lakes and two million hectares of constructed reservoirs. Many reservoirs remain either unused or not properly used for fish culture due to lack of proper scientific know-how. However, in recent years the Central Inland Fisheries Research Institute has revolutionized fish culture in India and a net production of 5,000 kg/hectare/year has already been achieved (Shukla and Upadhyay, 1998).

Despite such progressive development in fishery sector in India, scientific investigations pertaining to heavy metal contamination in fishes are far from desired. As the public demand for fishes increases day by day, it becomes highly essential and important to know as to how safe they are for consumption with reference to contaminants.

The information available on metals in fishes, are largely from controlled laboratory experiments (Vincent and Ambrose, 1994; Aida *et.al*, 1994; Nile *et.al*, 1994; Kanagaraj, 1996; Sivakumari, 1997; Rani, 1999). However, some information are available from the natural systems also (Ayyadurai *et.al*, 1994; Pandey *et.al*, 1995; Sadhukhan *et.al*, 1996; Mwachire and Durve, 1997) but their suitability for public consumption inadequately documented. The bioaccumulation pattern of heavy metals with respect to specific sites are available (Muralidharan, 1995; Suresh Babu, 1997; Sultana and Rao, 1998; Muralidharan *et.al*, 2002; Selvam, 2002) on fishes that are of less commercial value. Nevertheless, some information is also available on the suitability of certain commercially potential fishes for human consumption (Muralidharan, 1995; Jayakumar, 2001; Mathew, 2004; Vijayan *et.al*, 2005).

Wetlands besides serving as excellent stocking grounds for many commercial as well as indigenous fish species, they support agricultural activities and meet a variety of domestic requirements of the local community. Further, they also act as excellent ground water recharging source. Today, wetlands are converted into solid waste dumping yards and to drain industrial and domestic effluents. It is very painful to note that many of the wetlands are presently at the brim of extinction due to a variety of threats. The percentage of wetland loss in the country is alarming as high as 80% in states like Rajasthan (Vijayan *et.al*, 2004).

The current study attempts to document heavy metal contamination in fishes from representative wetlands spread across the country. It is also to be noted that this study is a part of a large participatory exercise which documented the ecological status of 655 wetlands spread across the country. Totally 66 species of fishes were collected from 174 wetlands and analyzed for heavy metals such as copper, lead, zinc, cadmium and chromium. To have uniformity and comparison only 19 species collected from 90 wetlands from 10 states are discussed in detail. Further, investigations were continued on prioritized wetlands for a year (2002-2003) in Tamil Nadu. Biomarkers of metal contamination, namely Metallothioneins (Mt) and metallothionein like proteins and Glutathione were quantified in a few species of fishes collected from select wetlands in Tamil Nadu. Biomarkers are expected to reveal the heavy metal stress in fishes and cast an early warning signal. In the perspective of

heavy metal contamination, fishes were evaluated for their suitability for human consumption.

1.1 OBJECTIVES

Document the magnitude of heavy metal contamination in the wetlands of India, using fish as an indicator. Quantify biomarkers, namely metallothionein (Mt) and metallothionein like proteins, and glutathione in the fishes of select wetlands of Tamil Nadu and try relate them with metal contamination. Evaluate the suitability of fishes for public consumption in the perspective of metal contamination.

2. LITERATURE REVIEW

By definition, heavy metals are mostly transition metals with densities greater than 5 g/cm³. It includes essential elements such as copper, iron and zinc as well as toxic metals such as lead, cadmium, chromium, mercury and arsenic. Although the trace metals such as copper, iron and zinc are required in small quantities for both plant and animal life, excessive amounts may produce serious consequences. It can even reduce species diversity and hence it assumes great ecological significance (Austin, 1999; Matta *et.al*, 1999).

Among the innumerable contaminants, heavy metals are some of the most toxic, persistent and widespread contaminants in aquatic systems (Carvalho *et.al*, 1999). The impact of metals was not realized until the Mina Mata disease and Itai-Itai disease which claimed thousands of victims in Japan owing to mercury and cadmium contamination respectively. Unfortunately the impacts still linger at genetic level and are recognized at population level (Abbasi *et.al*, 1998).

It is well known that heavy metals measured in tissues of aquatic animals can reflect past exposures (Kalay *et.al*, 1999). Thus, heavy metals are studied in aquatic animals owing to their bioaccumulative nature and sequestering the effect to the heir of the food chain.

During the last couple of decades, considerable progress has been made in research in the field of trace elements. From a public health point of view, it is important to assure that the levels of all essential trace elements are adequate in the average, daily diet of the general public. At the same time, the ideal diet should not contain more than the permitted levels of toxic heavy metals. It is thus essential to monitor periodically the levels of elements in the daily diets to assess the adequacy of the intake levels of essential trace element as well as toxic heavy metals. Barring occupational exposure, the food chain remains the major pathway through which the trace elements enter the human body (Abdulla *et.al*, 1996).

Today, trace metal contamination is common in foodstuffs and are detrimental to the exposed population than those of the more common pollutants (Lucas *et.al*, 1970; Harrison and Laxen, 1984). The human intake of trace metals from the contaminated foodstuff varies depending upon the dietary habit, tolerance, immunity and other social and cultural factors (Louenkari and Salminen, 1986).

Various food commodities, namely vegetables, meat, beef and alcoholic beverages have been analyzed for heavy metal constituents and the dietary intake of trace elements through these materials studied by many investigators (Golow, 1993; Schumacher *et.al*, 1993; Srikanth *et.al*, 1995; Rahlenbeck *et.al*, 1999). Although the above mentioned food commodities have been studied for heavy metal concentration, fishes have been prioritized owing to their relative abundance, their higher tropic level and capacity to accumulate. In addition, epidemiological studies have shown that populations, which consume large amounts of fish and marine mammals, have low incidence of heart disease due to the presence of omega-3 fatty acids.

Aquatic organisms, namely fish acquire substantial quantities of trace elements from water, suspended particles and sediments (Chen, 2002). Most chemicals are taken up into the organism by passive diffusion or active uptake through the semi-permeable membrane of gill and gut epithelia (Campbell, 1995). Effectiveness of metal uptake from the sources may differ in relation to ecological needs and metabolism of animals and also contamination gradients of water, food and sediments as well as other factors such as salinity, temperature and interacting agents (Norey *et.al*, 1990; Langston, 1990; Roesijadii and Robinson, 1994; Bervoets and Blust, 2003). Uptake of metals from food are also affected by various factors such as, methylation, oxidation state, temperature, redox potential of environment and interactions among metals (Rand *et.al*, 1995; Mason *et.al*, 2000). Thus, the metal levels bioaccumulated in fish tissue are the result of total environmental exposure (Barron, 1995). Thus, variations in the metal levels in fishes might be related to a number of factors such as age, size of animals, feeding habits, low dissolved oxygen concentrations, fluctuating temperature, elevated sediment metal loads, salinity, bioavailability of chemicals in

food and water, physiochemical parameters of aquatic environment (Kargin *et.al*, 2001; Eastwood and Couture, 2002).

The key factor in metal pollution however is the biochemical availability of the metal to the organism. Metals may be stored as metalloproteins with protein moiety showing some specificity of metal binding. Metal binding with the protein moiety in a cell is a complex phenomenon. Metals may act as Lewis acids or bases accepting or donating an electron to bind or may have specific affinity to a particular protein functional group (Cd, Hg and Pb prefer to bind to sulph-hydryl group). In addition, cadmium also prefers phosphate (R-PO⁻) and carboxyl (R- COO⁻) groups and mercury prefers amino (R-NH⁻) groups or is stored in compartments of all cells (Goering, 1993). In fishes metals such as Cd, Zn, Cu and Hg accumulate more in the liver due to high concentrations of metallothioneins.

Metallothionein, a low molecular weight protein, whose apoprotein, thionein is induced by exposure to cadmium, copper, mercury and zinc which plays an important role in the detoxification mechanism. It provides protective role against the toxic effects of these metals by sequestering and thus reducing the amount of free metal ions (Bouquegnear *et.al*, 1975; Cherian and Goyer, 1978).

Binding tendencies of protein often reflect in part the affinity of metal ions for amino acids. Each metal ion has characteristic affinities for the O, N & S sites in the ligands to which they bind predominantly (Sillen and Martell, 1971). The sequestering of metal contaminants by metallothionein and subsequent toxic effects in fishes and other animals have been described by the "spill-over" hypothesis, which states that metallothionein is saturated by metals, excess metal ions spill over into the other cellular compartments and cause pathological lesion (Olsson and Haux, 1985). Vincent and Ambrose (1994) observed increased uptake of cadmium and chromium in kidney in addition to liver in *Catla catla* which may be attributed to the increased synthesis of metallothioneins and their storage as a constituent of liver and kidney cytoplasm (Bremner and Davies, 1975). The presence of many metals may affect the fish in a toxic or non-toxic manner. Lopez and Lee (1977) and Black *et.al*, (1982) reported that though copper concentration in Torch lake Michigan was high, other

substances found in the lake may be complexing with copper to form relatively non-toxic compounds. Dissolved humic substances, moderate alkalinity and neutral pH, found in the lake would allow some degree of complexation which increases with increasing alkalinity, pH and total hardness (Pagenkopf *et.al*, 1974). Complexation may partly account for the relatively good hatching rates of Perch despite elevated copper concentrations found in Torch lake water (Ellenberger *et.al*, 1994). But mixtures of salts of metals, especially copper and zinc were observed to produce an additive toxicity in the Rainbow Trout and Atlantic Salmon.

It may be difficult to generalize the uptake and accumulation of metals in aquatic organisms because of species differences in trace metal concentrations in fish tissues (Wiener and Giesy, 1979). The physiological mechanisms involved may also protect the organism at much higher levels, making interpretation of effects somewhat difficult. Background levels are not easily defined because of wide dissemination by wind and water (Mason *et.al*, 1988).

Accumulation of heavy metals in fishes is influenced by water quality parameters such as hardness and acidity. Moreover, the life cycle of an organism is also an important consideration in examining the effects of heavy metals, as the concentration of heavy metals in body tissues vary with age or size of the organism (Atchinson *et.al*, 1977; Chernoff and Dooley, 1979).

Fishes are sensitive to metal contamination in water and may significantly damage certain physiological and biochemical process when they enter the organs of these animals (Namcsok *et.al*, 1987). Chronic exposure of fish to sub lethal trace metal levels causes disturbed ion regulation, reduced growth and swimming speed (Hollis *et.al*, 1999; Alsop *et.al*, 1999).

Several authors have described the hematological alterations, biochemical changes and hampering of locomotion in fishes like Rainbow Trout and Salmon at a concentration of 0.012 ppm of lead (Hodson *et.al*, 1978; Shrivastava and Mishra, 1979). Chromium has been found to cause alterations in biochemical processes and

immune responses. Kunhert *et.al*, (1976) reported significant inhibition in activity of enzyme Na/K ATP ase, which is involved in osmoregulation in the kidney of *Salmo gairdneri* after exposure at 2.5 ppm Cr (VI) for 48 hours. Singh and Singh (1979) found that 0.003 ppm of cadmium inhibiting the oxygen consumption of *Mystus vittatus* by 50% during an exposure of 12 hours. Although the current levels are alarming, the physiological mechanisms, species differences, physico-chemical properties of the surviving water, availability and absorption of metal are also to be admitted while considering the ill effects. If the exposure is continuous it can be anticipated that certain physiological damages such as decline of proximal principles, hampered locomotion, disturbances in osmoregulation could happen.

Copper and its compounds are ubiquitous in the environment and are frequently found in surface water. Copper ion precipitate gill secretions, causing death by asphyxiation. Similarly it is the same in the case of iron. Zinc is an abundant element and constitutes approximately 0.04g/Kg of the earth's crust. Its occurrence in sewage is expected because of its expensive use in making household appliances and by leaching from galvanized pipes (Pandey *et. al*, 1995).

Copper and zinc are essential metals and homeostatic control mechanisms that are not yet fully elucidated are believed to adjust their tissue concentration through the regulation of uptake and excretion (Grosell *et.al*, 1997; McGeer *et.al*, 2000).

In a study conducted in Hoogly estuary, India during 1977-1981, 127.8 ppm of copper and 20 ppm of chromium were recorded in various tissues of fish. Although a high concentration of zinc (218.5 ppm) was observed in the muscle tissue of *Mastacembelus pancalus*, it indicates frequent exposure to increased concentration of the metal. Although muscle is not a suitable body part to determine the extent of heavy metal contamination reflected by the low concentration of the metal in majority of the samples, the levels determine its fitness for human consumption (Kaviraj, 1989).

Ayyadurai *et.al*, (1994) investigated the heavy metals such as copper, iron, manganese, zinc and mercury present in water and Fin Fish, *Oreochromis*

mossambicus during 1990-91 at three locations in River Cauvery, South India. The accumulation of these metals was maximum in liver as compared to other organs of the fish. The mean concentrations of these metals in the muscle were 1.28, 6.3, 0.86, and 6.36 mg/kg for copper, iron, manganese and zinc respectively while that of mercury was 0.065mg/kg which is below the stipulated toxic limit.

In a study on metal concentration in dried fish from Nigerian markets, the average lead concentration (6.70mg/kg dry wt) was high in comparison to the British standard of 5 mg/kg in dried fish (Nauen, 1983) while lower to the WHO limit of 8mg/Kg (Okoye, 1994).

Muralidharan (1995) studied heavy metal contamination in 16 species of fishes collected from Keoladeo National Park, Bharatpur, Rajasthan, India and found the contamination levels to be significantly varied among various types of herbivorous, carnivorous and omnivorous fishes. The average load of cadmium in the fishes was 0.18 ppm. Both copper and lead had a high level of 0.74 ppm. Zinc with 7.19 ppm was the highest in concentration of all the metals. Further the variation in contamination level ($P < 0.05$) among species was reported to be dependent on the contamination level of the water, sediment and invertebrates and also the duration of stay of the fishes in the contaminated water.

Pandey *et.al*, (1995) studied the heavy metal (Cu, Fe, Zn, Cr) accumulation in fish samples of sewage fed ponds in Rahara. Among the metals studied, Fe showed higher concentration, while Cr showed lower concentrations. Copper concentration in fish was about 0.02mg/g and found to be the lowest. The concentration of Zn was less and the accumulation of chromium in all the samples was very low which might be due to its low solubility and availability in water. The average value of chromium was 0.01 mg/g in fish samples. The values in all the samples were far below the standard limits of ISI for drinking water and do not pose any serious threats to the consumer or the fish themselves.

Mwachire and Durve (1997) analyzed the concentration of heavy metals in water and their accumulation in different organs (kidney, liver, heart, ovary, muscles, skin,

vertebral bones, brain, intestine and gills) of the fish, *Cirrhinus mrigala*, an endemic species of the Lake Bari near Udaipur. Copper was found to be the maximum (0.58mg/g) in liver and minimum (0.1mg/g) in brain, intestine and gills. Zinc was found to range between 0.061 and 0.082mg/L in water. Cadmium concentration was maximum (0.073mg/g) and minimum (0.023mg/g) in the brain. The concentration of lead was 0.96mg/g in vertebral bones and below detectable level in brain and intestine. Zinc was high in bones and as the bones are rarely consumed, zinc getting into humans through food chain appears remote. Cadmium concentration found was not above the critical level and hence harmless for human consumption. Lead was found to be in low levels. Thus the study concluded that the fishes and water appear to be safe for consumption.

Suresh Babu (1997) observed highest copper concentration (3.63ppm) in the fishes of Pykara and zinc (78.67 ppm) in fishes of Emerald, while the lead contamination was found to be low in all the reservoir bred fishes. As fishes from the reservoir are regularly sold to the public and many piscivorous birds too depend on them, the study recommended an intensive work to see whether the levels were safe for human consumption.

Jayakumar (2001) has outlined a clear picture on the levels of heavy metals in the fishes of commercial importance and their suitability for human consumption. Of all the 12 species of commercially important fishes, *Rasbora daniconius* recorded the maximum load of metals (copper 0.68 ppm; lead 1.34 ppm; cadmium 0.28 ppm; chromium 0.29 ppm and zinc 27.99 ppm) followed by *Puntius dorsalis* (copper 0.9 ppm; lead 0.95 ppm; cadmium 0.28 ppm; chromium 0.37 ppm; and zinc 22.28 ppm). It was also suggested that these levels could be considered as background concentrations. Moreover the study reported the levels to be not harmful to human beings as per the statutory limits prescribed by WHO/FAO.

Assessment of heavy metal concentration in grass carp (*Ctenopharyngodon idella*) by Misra *et al*, (2002) revealed that lead (0.6 to 1.45 ppm), copper (0.37 to 1.02 ppm), chromium (0.46 to 1.18 ppm), cadmium (0.35 to 0.324 ppm) and zinc (0.8 to 1.17 ppm) concentrations measured in muscle of the fish are found to be low to cause any

adverse effects to fishes. Further, the levels were within prescribed limits and found to be safe for human consumption.

Selvam (2002) documented quantitatively the levels of a few heavy metals in select species namely *Channa punctatus*, *Channa striatus*, *Heteropneustes fossilis*, *Labeo rohita* and *Cirrhinus mrigala* from Keoladeo National Park, Bharatpur, Rajasthan, India, which was a follow-up investigation of the study executed during the late 1980's. Of all species *Labeo rohita* (Cu 0.70 ppm; Pb 0.74 ppm; Zn 8 ppm and Cd 0.08 ppm) and *Channa striatus* (Cu 0.34 ppm; Pb 0.73 ppm. Zn 6.64 ppm and Cd 0.04 ppm) recorded the maximum metal load. It is reported that there are no major differences in contamination between then and 2002.

Pyle *et.al*, (2005), in their study on the Yellow Perch collected from various lakes in the Sudbury area found that muscle Zn and Cu concentrations ranged from 23.5 to 276.9 ppm and 2.9 to 14.1ppm respectively). Metals such as copper and zinc are known to have an effect and also to be accumulated by aquatic biota such as fish and crustaceans (Zou and Bu, 1994) and aquatic plants. Although aquatic plants, algae and invertebrate, generally seem to be more resistant to copper than fish (Alabaster and Lloyd, 1980) they all contribute to the diet of the species sampled. Large amount of silt are also taken in with the food sources by species. Silt particles play an important role in the transport and availability of metals as they are adsorbed onto the silt particles (Heath, 1987). Most metals are furthermore known to become more bioavailable and have increased toxicity with decreasing pH (Shaw and Brown, 1973). The pH in the stomach of *Oreochromis mossambicus* decreases significantly (from above 6 to as low as 2.9) after feeding commences (Deacon 1988). This reduction in pH could thus result in metals becoming more bioavailable from the food sources and silt in the stomach. The metals could therefore be taken up via the intestine, causing increased metal levels.

Papagiannis *et.al*, (2002) in their study on heavy metals in Lake Pamvotis (NW. Greece) ecosystem found Cu and Zn levels in muscle tissue of fishes were significantly different between species and being essential elements and the species differences for these probably reflect the range of requirement of tolerance, rather

than humanly derived exposure. Moreover, species-dependent differences may be attributed to the life history pattern that influences their exposure to metals (including trophic levels and geographic distribution of life stages). Dietary habits may have this impact on species differences in metals concentrations. Species such as *Rutilus rubilis* and *Cyprinus carpio* are zooplanktonivorous and detritivorous respectively and they had increased Zn concentrations. Thus this study illustrates strong positive relationship between Zn concentrations in zooplanktonic fractions and their tissue (Timmermans *et.al*, 1992).

The growing concern over the heavy metal contaminants in fishes have resulted in the formulation of guideline values for heavy metal intake through fishes prescribed by various statutory agencies. The Joint Expert Committee on Food Additives, WHO/FAO (1993) allows a daily intake of 0.21mg of lead and 0.06 mg of cadmium for a 60-Kg adult. Further, 3 to 32 mg of copper and 15-22 mg of zinc was considered as the allowable daily intake (WHO, 1993). European Commission (1997) has set the permissible levels of lead (0.5µg/g) and cadmium (0.05µg/g) in fish muscle. As per the International and FDA criterion (CAC, 1984) the permissible levels of copper, lead, zinc and cadmium in the edible part of the fish were 15µg/g, 0.5µg/g, 60µg/g and 0.5µg/g respectively.

Thus, fish become a crucial component to be studied as they are found to be the major victims of heavy metals in an aquatic ecosystem. Further, the demand for better dietary protein source, increasing population and availability of best quality protein at affordable cost in fishes necessitates studying elemental contaminants in them, besides studying the nutritional composition as it is expected to throw light on the impact of metals in the fishes themselves as well consumers. So far no attempt has been made to document the metal levels in fishes of Inland wetlands in a comprehensive manner in the entire country in a single time frame. The current study assumes significance since India is bestowed with excellent freshwater aquatic resources, especially with wetlands. The emerging industrial fronts in India have to an extent challenged many ecosystems particularly the freshwater aquatic ecosystems. So, the underlying magnitude of contamination in freshwater aquatic ecosystems, particularly wetlands, are to be necessarily outlined so that besides generating a

baseline information an early warning on metal contamination through fishes to humans can be addressed and also the risk of heavy metal uptake through fishes across the country can be identified.

3. MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

The job of collection of fish samples from almost the entire country would not have been possible but for the cooperation of the state-level coordinators. The procedure for collecting, packing and forwarding fish samples to the laboratory at Sálim Ali Centre for Ornithology and Natural History was explained in the coordinators meet at Delhi, as well as at the state-level workshops where all the key resource persons were present. It is needless to mention that the samples have to reach the laboratory at SACON before they turn unfit for chemical analysis. Hence, a kit was developed for the transportation of samples. The kit comprises rectangular thermocol carton, polythene bags, tag, rubber band, sealing tape, fastening rope and instructions for packing and forwardance to the laboratory. Prior to sampling, the gel packs were freezed at -20°C over night. The fishes were collected using standard gear such as gill net, cast net depending on the species and habitat. After collection of fishes, the morphometric details (total length and body weight) were recorded in the data sheet already provided and the samples were wrapped in clean polythene covers and sandwiched between the frozen gel packs in the thermocol box. The boxes were well sealed and air lifted to the laboratory at SACON.

Eight species of commonly occurring fishes, namely *Cyprinus carpio*, *Labeo rohita*, *Anabas testudineus*, *Oreochromis mossambicus*, *Clarias batrachus*, *Heteropneustes fossilis*, *Cirrhinus mrigala* and *Channa orientalis* were chosen for the study, based on their abundance, distribution and trophic status. Desired size and weight range were also specified. However, these specifications could not be adhered to, because of the practical difficulties, and the fishes were received irrespective of size and altogether the following nineteen species were considered for the study. During the year 2001-02, a total of 889 fishes comprising 19 species, namely *Anabas testudineus*, *Anguilla bengalensis*, *Anguilla bicolor bicolor*, *Catla catla*, *Channa maurilius*, *Channa orientalis*, *Channa punctatus*, *Channa striatus*, *Cirrhinus mrigala*, *Clarias batrachus*, *Cyprinus carpio*, *Heteropneustes fossilis*, *Labeo rohita*, *Mystus vittatus*, *Notopterus notopterus*, *Ompok bimaculatus*, *Puntius dorsalis*, *Puntius sophore* and *Oreochromis*

mossambicus (Plate.1) were collected from 90 wetlands across 10 states in the country (Table.1).

Table.1 Number of fishes, wetland and species recorded in each state.

State	Fishes	Wetland
Andhra Pradesh	101	9
Assam	72	6
Bihar	69	9
Gujarat	128	11
Karnataka	150	12
Madhya Pradesh	39	6
Maharashtra	43	5
Tamil Nadu	155	17
Uttar Pradesh	27	4
West Bengal	105	11
Total	889	90

Samples on arrival were transferred to deep freezers till processing. It may be noted that states, namely Bihar, Madhya Pradesh and Uttar Pradesh have been considered undivided. However, references have been made to new states wherever necessary.

3.2 SAMPLE DISSECTION

The selection of tissue from the fishes to investigate metal contamination is closely linked to the objectives of the study. The study aimed at assessing the level of contamination of Indian wetlands using fish as a tool. Although the vital organs such as kidney, gill and liver are prone to accumulate more, muscle tissues were chosen as it forms the major edible portion of the fish. Moreover, muscle tissue is usually analyzed when the objective is to determine the potential effect of heavy metals on human health (Robert and Rod, 1999). Since the present study aims at evaluating the suitability of fishes for human consumption, the selection of muscle tissue for metal analysis is adequately justified.

The fish samples were taken out from the deep freezer, thawed, well cleaned in running tap water to remove any external dirt. The scales were sloughed off and muscle tissue was dissected between the pectoral fin and vent of the fish, minced into smaller pieces and a subsample was taken from the homogenate. About 1 to 1.5g of the sample was weighed using a top loading electronic balance Mettler AE420, transferred to clean specimen vials and stored at -20°C till the process of digestion. In our later investigations on select wetlands, vital organs such as liver, kidney and gills were also included for metal analysis as well for biomarker estimation.

3.3 SAMPLE DIGESTION

Although there are various techniques such as aqua regia heating, nitric acid digestion, wet ashing and dry ashing available to digest the biological and non-biological components, microwave assisted digestion has been proved to be the most efficient (Tam and Yao, 1999). This system deserves merit over other conventional systems such as hot plate as it minimizes the requirement of mineral acids / organic solvents whereby the impurities being added is avoided. Volatilization of metals and short duration of the digestion process are the other notable advantages.

The samples were digested in Microwave Digestion System (Milestone Model 1200) using 10 ml of HNO_3 (69%) for 10 min, 1 ml of HClO_4 (70%) for 5 min and 5 ml of H_2O_2 (30%) for 10 min at 250 W power settings. The digested solutions were made up to 25 ml and stored in well-cleaned polythene vials in refrigerator till the time of analysis.

3.4 SAMPLE ANALYSIS

The digested samples were analysed for metals, namely copper, lead, zinc cadmium and chromium in a double beam Atomic Absorption Spectrophotometer (Perkin Elmer, Model 3300) fitted with an impact bead. While the detection limit for copper, chromium and cadmium was 0.002 ppm, the same for lead and zinc were 0.02 ppm and 0.009 ppm respectively. Atomic absorption spectrometry standards for all the metals manufactured by SRL, India were used for calibration and recovery studies.

High purity acids and all quartz double distilled water were used for the analysis. Recovery rates for copper, lead, zinc, cadmium and chromium were 86, 85, 90, 88 and 95 per cent respectively.

3.5 SUITABILITY FOR HUMAN CONSUMPTION

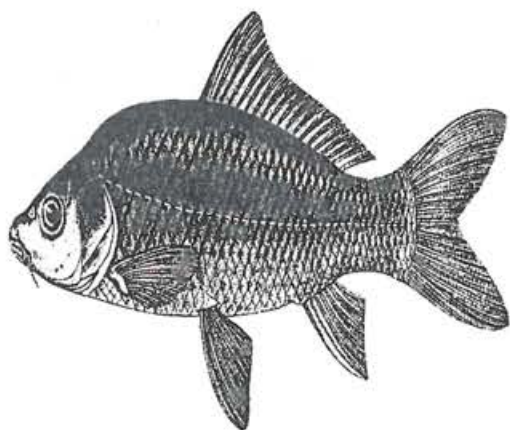
The annual per capita consumption of fish in India is said to be 5.32 Kg and the daily per capita intake is about 14.56g (MoA, 2000). Bearing this factor in mind, assuming that a person consumes, 250g of fish per week, the average daily intake of metals through fishes could be calculated as follows:

$$ADI = \frac{\text{Average concentration of the metal in the tissue} \times 250g}{7}$$

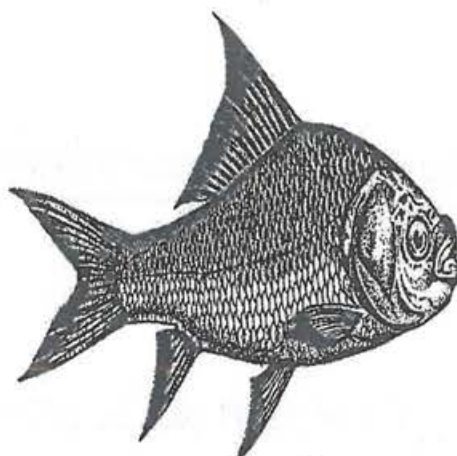
3.6 STATISTICAL ANALYSIS

The data were compiled to test the statistical significance among the species and wetland. As the data were not normally distributed, non-parametric test, namely Kruskal Wallis test was employed to test the variation in metal contamination among species and wetlands. To test the relationship between contamination and size of the fish, Karl Pearson correlation was used. The level of significance was $P < 0.05$. All the statistical tests were performed in SPSS statistical software, Version.10.

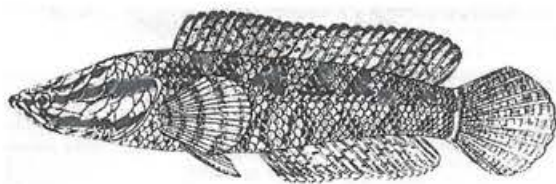
Plate 1. List of species studied for metal contamination



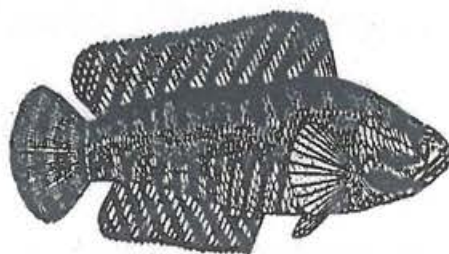
Cyprinus carpio



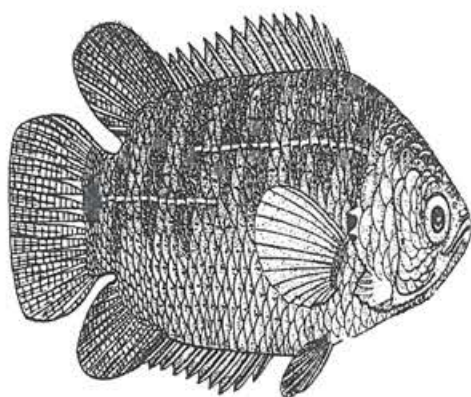
Catla catla



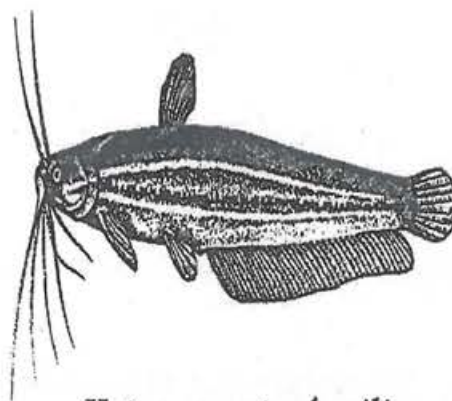
Channa punctatus



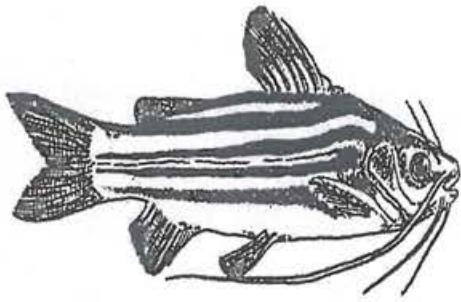
Channa striatus



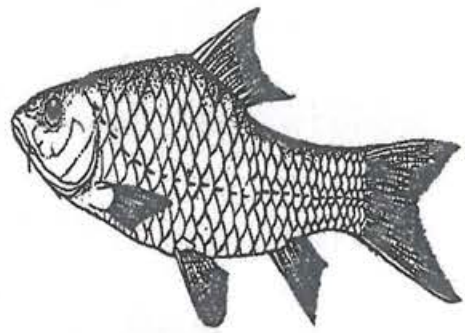
Anabas testudineus



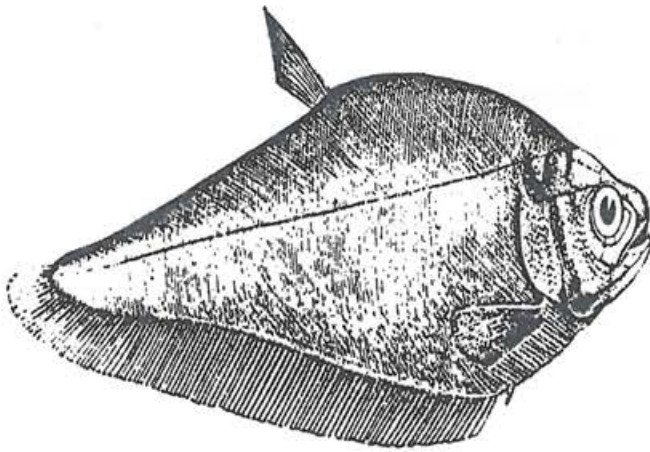
Heteropneustes fossilis



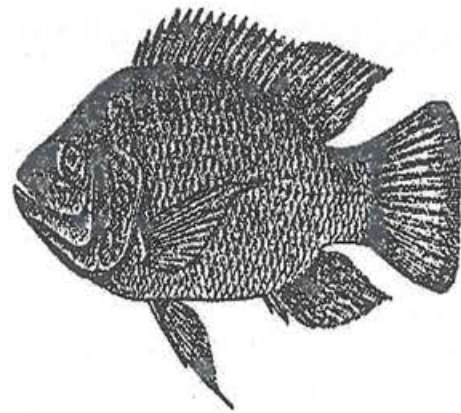
Mystus vittatus



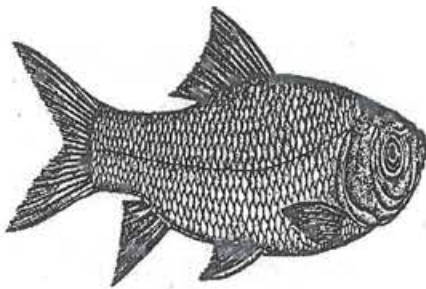
Puntius dorsalis



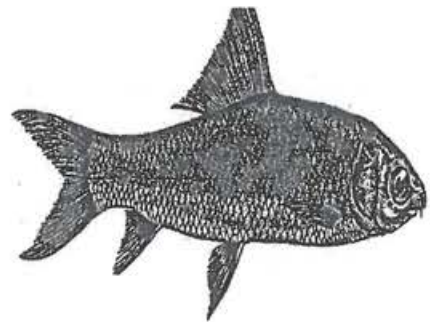
Notopterus notopterus



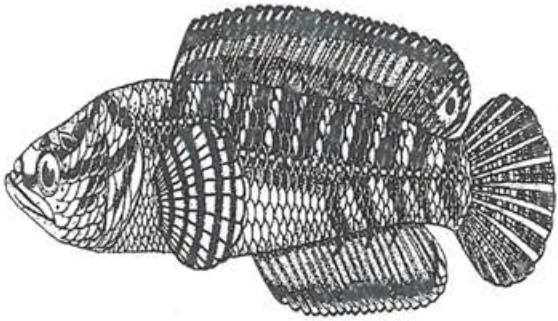
Tilapia mossambica



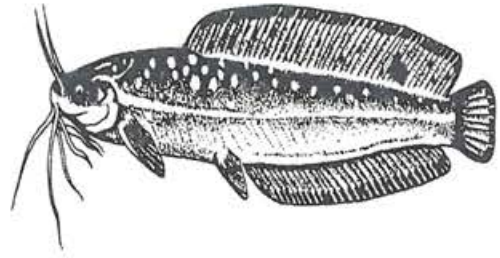
Labeo rohita



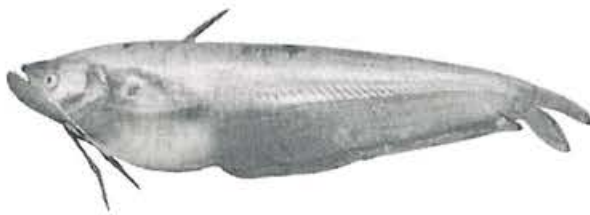
Cirrhinus mrigala



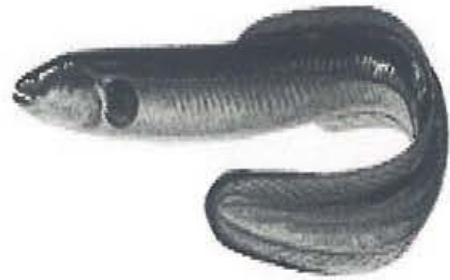
Channa orientalis



Clarias batrachus



Ompok bimaculatus



Anguilla bicolor bicolor



Puntius sophore

HEAVY METAL CONTAMINATION IN THE FISHES OF ANDHRA PRADESH WETLANDS

4.1 INTRODUCTION

Andhra Pradesh, the rice bowl of South India, has good wetland wealth including one of the most magnificent wetlands in the country, the Kolleru. The state is broadly divided into three regions, namely the Circars or Coastal districts, Rayalaseema close to the coastal districts and the Telengana region close to Maharashtra's Marathwada region. The broad physiographic divisions made using remote sensing data of the state are: Deccan plateau, hill ranges and, coastal plains. The Eastern Ghats in Andhra Pradesh are much less in elevation than the Western Ghats, and are also discontinuous. The hills between the Godhavari and Krishna rivers in the coastal areas, for a distance of about 150 km are scattered. To the south of River Krishna, the Ghats have an elevation from 300 to 1,500m.

The climate of Andhra Pradesh presents a transition from tropical to sub-tropical monsoon climate of semi-arid to arid in Telengana to Rayalaseema regions and humid to sub-humid in the coastal region. The average rainfall of the state is 830 mm, which varies considerably from 690 mm in Rayalaseema to 860 mm in Telengana and 950 in coastal region. Temperature varies from 22 to 42°C. Andhra Pradesh has 1,493 wetlands of the size 56.25 ha and above, covering an area of 3,666.60 sq km, as per the satellite imageries of 1992-93 SAC (1998). Towards documenting the contamination status, nine wetlands were chosen (Map.1).

4.2 STUDY AREA

4.2.1 Chinna Tumbalam Tank

Chinna Tumbalam Tank is situated between 75° 10' N and 14° 19'E in the district Kurnool and is about 20 kms from Adoni. The water spread area ranges from 0.703 to 1.16 sq km. The irrigation department of the state government maintains this tank and fishing and irrigation activities are seen. This seasonally cultivated wetland is

also fed by Tungabhadra high level canal and has no change in the fish yield during the past five to six years. This tank also caters to the domestic requirements of the surrounding population (app.1500). Paddy, Jowar and Groundnut are the commonly cultivated crops in the nearby agricultural lands. DAP, NPK and super phosphate are the fertilizers applied to increase the yield and the notable pesticides sprayed are endrin, endosulfan, ekalux and Rallis W66D1SD6. The tank is free from any industrial discharges.

4.2.2 Draksha Rama (Kotipalli)

This is located in the East Godhavari District between 17°25' E and 77°45' N and having maximum water spread area of 445 ha. This actually forms a small stretch of Godhavari River. Seawater intrusion is a common phenomenon from February to early August. However, the water from August to February appears almost fresh and due to dam construction across the river has constricted many of its tributaries thereby resulting in the considerable decline of the water flow. Additionally, sand mining is yet another anthropogenic disturbance noticed in this area. Paddy is the major crop cultivated here.

4.2.3 Jankampet

This unprotected wetland has 3 to 4.05 sqkm of water spread area and lies between 18°43'E and 78°04'N. It is situated in the Nizamabad district. This wetland furnishes the domestic requirement of approximately 5000 people. Recreational activities such as boating are maintained by the tourism department of the state government. Due to failure of monsoon, during the past 5-6 years a decline of 30% in the fish yield is reported. Paddy and Sugarcane are cultivated in the nearby areas. Application of fertilizers and pesticides are a common practice.

4.2.4 Jataprole

State irrigation Department of the state government presently maintains this unprotected wetland which is located between 15°62'E and 78°14'N. Approximately,

1750 people depend on this wetland for their basic needs. *Labeo rohita* and *Catla catla* are the most common species of fish found in this wetland. Crops such as paddy, groundnut and chilly are cultivated in the vicinity of this wetland. There is no cultivation inside the wetland. There is also no industrial discharge into this water body. This wetland provides favourable habitat for waterfowl. Poaching of waders is reported to be very common.

4.2.5 Kazipet tank

This tank located at Warrangal district between 16°19'E and 81°03' N, and has a water spread area of 30 ha receives urban sewage and surrounded by agricultural fields. No change in fish yield has been observed during past 5-6 years. The lake drains several parts of Warangal town in addition to other areas. The point load contributions to the lake waters are generally through uncontrolled sewage from areas without proper individual household disposal facilities. The town without proper stormwater drainage network during the post-monsoon carries lot of point loads from the open sewers. Also flooding of storm drains is due to disposal of solid wastes in the drains during the premonsoon season contributes considerable loads to the lake.

4.2.6 Kolleru

This wetland is located in the Eluru taluk of West Godhavari District between 81°20'N and 16°45'E and its water spread area ranges between 13.5 and 900 sq km. Prawn and fish culture is prominent in this wetland. This wetland receives industrial effluents. Agricultural activity seen at the vicinity employs a wide varieties of fertilizers such as Nuvan, superphosphate, Urea, Zinc potassium and DAPS. Surrounding this lake, there are about 122 villages. Sewage inflow from the towns of Eluru, Gudivada and even Vijayawada and industrial effluents, runoff containing pesticides and fertilisers from the Krishna-Godavari delta region contaminate the lake. Eleven major industries release about 7.2 million litres of effluents into the lake every day. An Andhra Pradesh Pollution Control Board report states that more than 17,000 tonnes of fertiliser wash enters the lake annually. Studies have shown the presence of organic pollutants in

lake sediment and in the fast-growing weeds. The sewage and discharge from factories have also affected the growth of aquatic organisms that the fish consume.

4.2.7 Mandyal tank

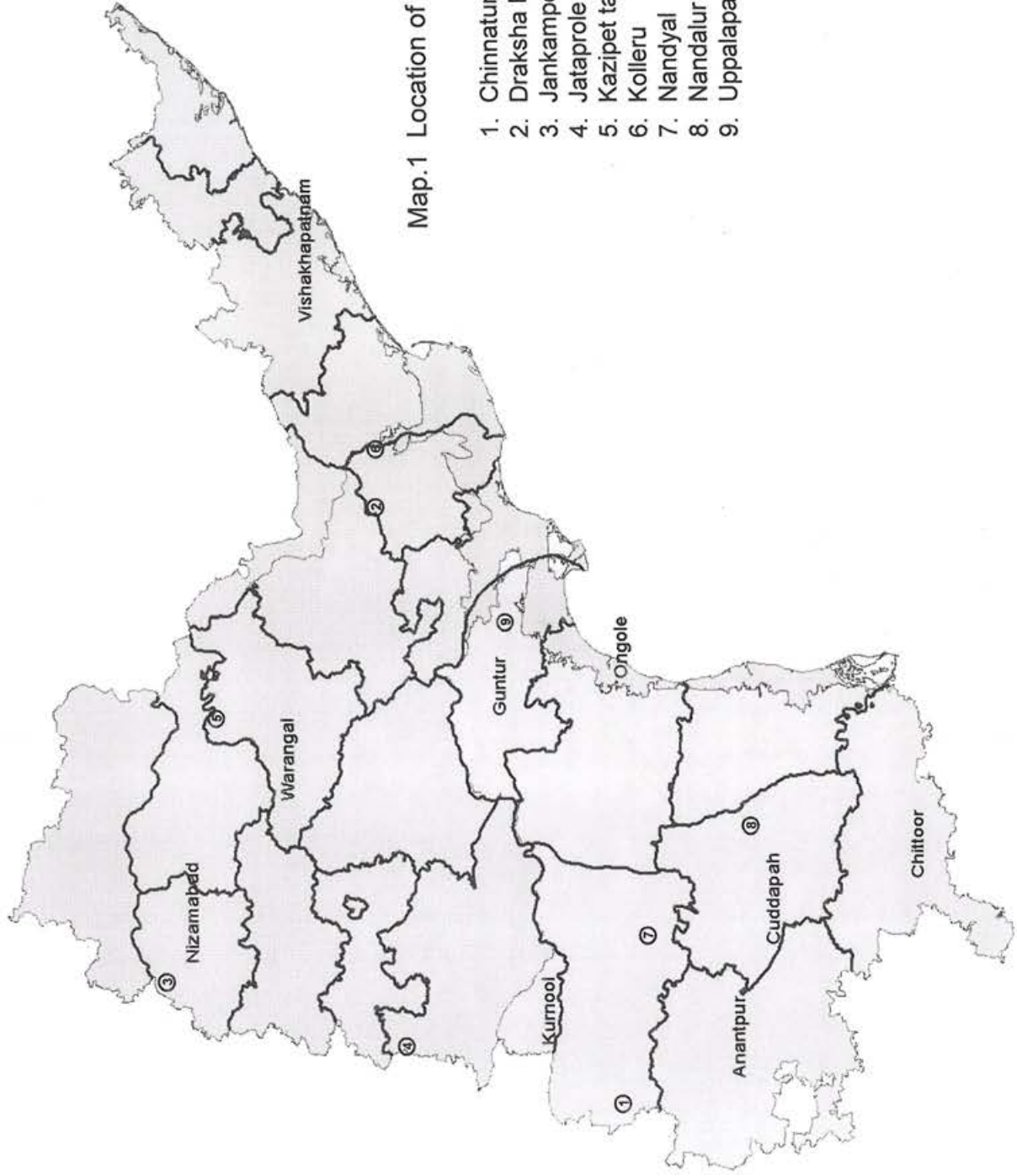
It is located in the Kurnool district between 78°26'N and 15°30'E. Municipal Corporation, Kurnool distributes water from this lake for drinking purpose to about 1,50,000 people. Having a wide water spread area of 1.05 to 1.48 sq km this lake offers favorable fishing grounds. The municipal corporation has established recreational facilities such as boating and park on the bank. This seasonally cultivated wetland does not receive any industrial or domestic effluent. Agricultural activities are also seen in its vicinity. Paddy, Banana and Turmeric are the crops cultivated. Fertilizers such as urea and potash are reported to be applied.

4.2.8 Nandalur tank

It is located in Cuddapah district between 79°05' N and 14°15' E and has a water spread area of 1 sq km. During the past three years, the tank suffered severe drought and it was inundated only during the heavy downpours in 2001. Local fishermen do harvest common carps and catfishes. Suspicion over the leakage of alloy industrial effluent into this tank prevails among the local community. Paddy is the prominent crop and endosulfan is widely applied in the nearby agricultural lands. Murate of Potash is the common fertilizer used.

4.2.9 Uppalapadu

Uppalapadu is an important bird area specified under A1, A4i, A4iii IBA categories, located in Guntur district between 15°54' E and 79°55' E. The area is about 15 ha. This tank is maintained by local village panchayat. Reduction of the tank area, draining of the tank in summer to harvest fish, death of nesting trees in and around the tank due to excessive deposition of birds, anthropogenic disturbances, opposition from local populace due to pollution of water by bird excrement, leading to skin



Map.1 Location of select wetlands studied in Andhra Pradesh.

1. Chinnatumbalam
2. Draksha Rama
3. Jankampet
4. Jataprole
5. Kazipet tank
6. Kolleru
7. Nandyal
8. Nandalur
9. Uppalapadu

disease are some of the notable threats to this wetland. Paddy and cotton are the common agricultural crops seen nearby.

4.3 RESULTS

A total of one hundred and one fishes collected from nine select wetlands (Map.1) in Andhra Pradesh (Table 2) comprising 11 species were analyzed for heavy metal contamination.

Table 2. List of wetlands examined for heavy metal contamination

S.No.	Name of the wetland	No. of fishes collected
1	Chinna Tumbalam Tank	14
2	Draksha Rama	9
3	Jankam Pet	8
4	Jataprole	9
5	Kazipet Tank	9
6	Kolleru	24
7	Mandhyal Tank	11
8	Nandalur Tank	12
9	Uppalapadu	6
Total number of fishes collected		101

4.3.1 Variation in heavy metal contamination among select wetlands of Andhra Pradesh

i. Lead

On an average, the lead contamination was found to be the maximum in Kolleru of West Godhavari district (6.01 ± 0.24 ppm) and Kazipet (6.01 ± 0.30 ppm) of Warangal district followed by Nandalur tank (5.88 ± 0.29 ppm) of Cuddapah district (Fig.1.a). Draksha Rama of East Godhavari district recorded the minimum concentrations of

lead (1.46 ± 0.47 ppm). Lead contamination varied significantly among the wetlands ($P < 0.01$) (Table.3).

ii. Zinc

This metal was detected in appreciable levels in the fishes of almost all the wetlands. It ranged from below detection limits (BDL) at Uppalapad of Guntur district to a maximum of 94.88 ppm at Draksha Rama of East Godhavari district. On an average, Draksha Rama of East Godhavari District recorded the maximum concentrations (23.57 ± 9.94 ppm) and was thrice higher than Jataprole (8.69 ± 2.93 ppm) of Mahboobnagar which recorded the minimum concentration (Fig.1.b). Nearly 76% of the wetlands showed zinc concentrations to measure between 10 and 20 ppm and the concentrations varied significantly ($P < 0.05$) (Table.3).

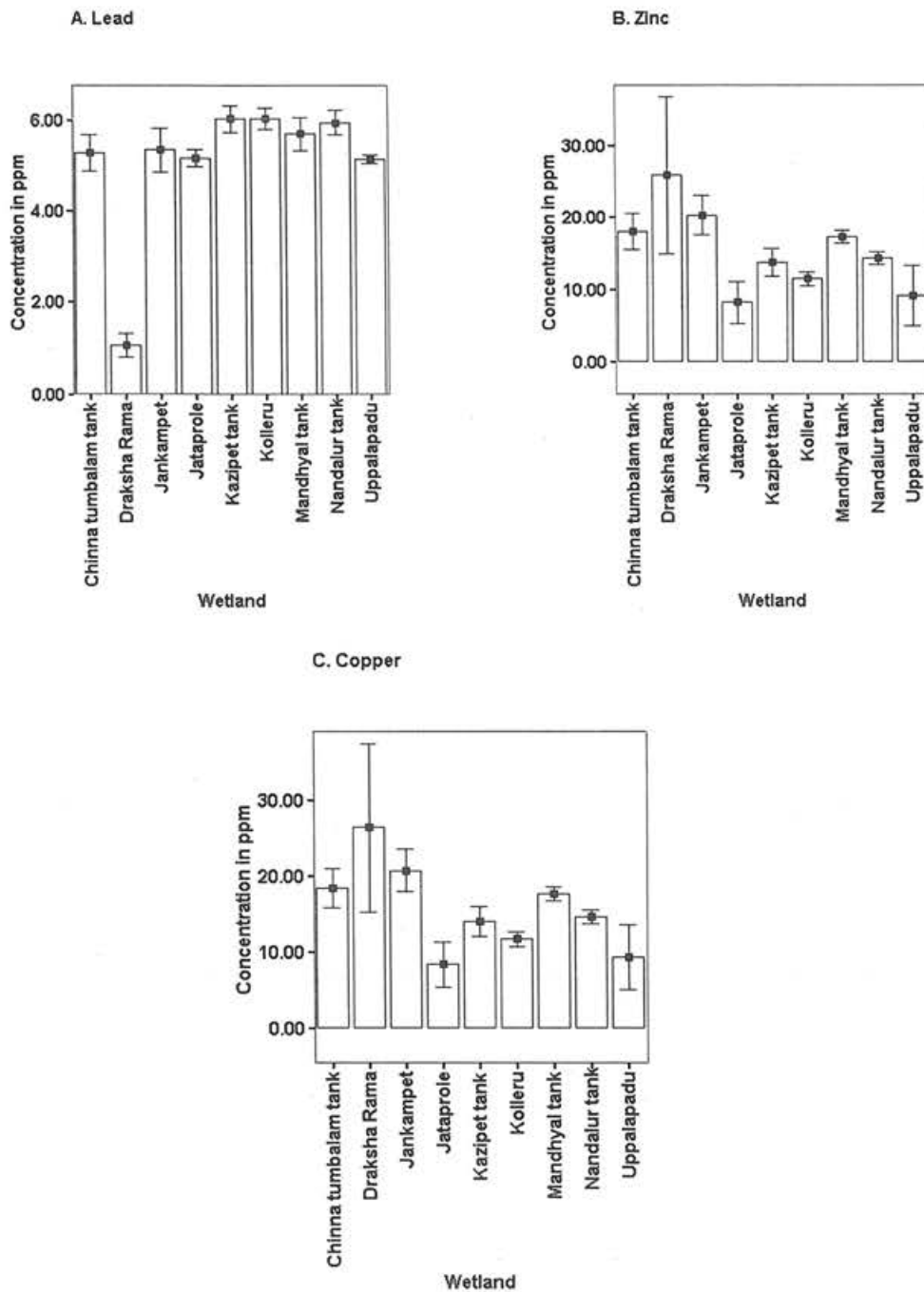
iii. Copper

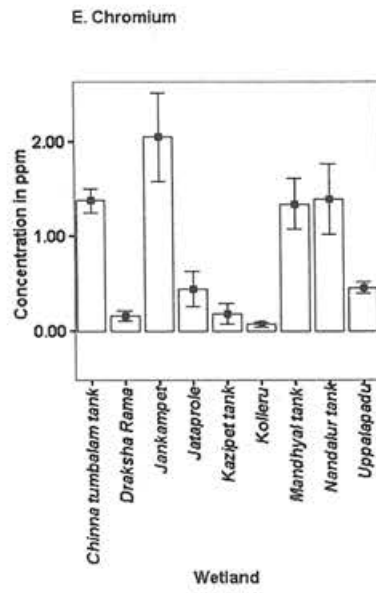
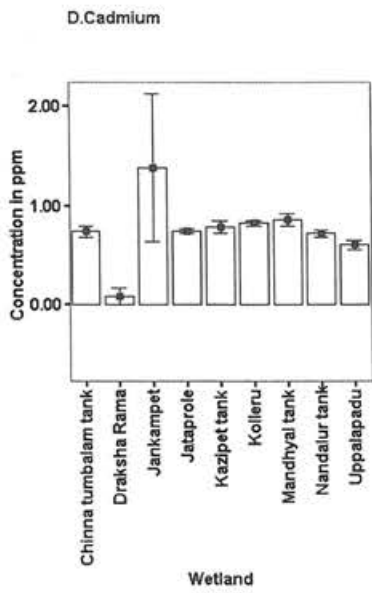
Fishes of Draksha Rama of East Godhavari District measured the highest concentration of copper (23.98 ± 10.12 ppm) on an average, followed by Jankampet (19.97 ± 3.09 ppm) of Nizamabad District, and Jataprole (8.84 ± 2.99 ppm) of Mahboobnagar recorded the lowest (Fig.1.c). Significant variation in copper contamination among wetlands ($P < 0.05$) could be observed (Table.3)

iv. Cadmium

On an average, fishes of Chinnatumbalam Tank of Kurnool district gauged the highest concentrations of cadmium (1.16 ± 0.12 ppm) followed by Mandhyal of Kurnool district (0.87 ± 0.07 ppm). Least concentrations were observed in Draksha Rama (0.13 ± 0.09 ppm) of East Godhavari district. No significant variation in cadmium contamination among wetlands could be perceived ($P > 0.05$) (Table.3).

Fig.1 Levels of heavy metal contamination (ppm) among select wetlands of Andhra Pradesh (Mean \pm SE).





heavy

Table 3. Variation in metal contamination in the fishes of Andhra Pradesh wetlands (Kruskal Wallis Test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	32.661	13.09	13.09	6.051	28.917
df	5	5	5	5	5
Asymp. Sig.	0.000*	0.023**	0.023**	0.301	0.000*

*P<0.01; **P<0.05

v. Chromium

Chromium was the highest (1.83 ± 0.49 ppm) in the fishes of Jankampet of Nizamabad district. Near equal concentrations were noticed in Chinnatumbalam Tank (1.41 ± 0.11 ppm) and Mandhyal tank (1.46 ± 0.26 ppm) of Kurnool district and Nandalur Tank (1.44 ± 0.37 ppm) of Cuddapah district (Fig.1.e). Least concentration of chromium was observed at Kolleru (0.05 ± 0.02 ppm) of West Godhavari District. Significant variations in metal contamination could be observed ($P < 0.01$) (Table.3).

4.3.2 Variation in ^{heavy} metal contamination among various species of fishes in the wetlands of Andhra Pradesh

As mentioned earlier in the chapter data pertaining, to eleven species (Table.4) are examined statistically.

Table 4. List of species examined in detail for metal contamination ^{? Area?}

S.No.	Name of the species	Number of individuals collected
1	<i>Anabas testudineus</i>	8
2	<i>Catla catla</i>	14
3	<i>Channa orientalis</i>	6
4	<i>Channa striatus</i>	8
5	<i>Cirrhinus mrigala</i>	6
6	<i>Clarias batrachus</i>	9
7	<i>Cyprinus carpio</i>	10
8	<i>Heteropneustes fossilis</i>	8
9	<i>Labeo rohita</i>	25
10	<i>Mystus vittatus</i>	4
11	<i>Oreochromis mossambicus</i>	9
Total number of individuals collected		101

i. Lead

On an average, *Channa striatus* recorded the maximum concentrations of lead (6.36 ± 0.30 ppm) which is six-folds higher than the minimum level recorded in *Mystus vittatus* (0.81 ± 0.37 ppm) (Fig.2.a). Lead was detected appreciably in all the species and it varied significantly ($P < 0.01$) (Table.5).

ii. Zinc

The average concentration ranged between 10 and 15 ppm in about 50% of the species studied. *Cirrhinus mrigala* recorded the maximum (31.48 ± 14.05 ppm) followed by *Anabas testudineus* (20.85 ± 2.93 ppm). *Mystus vittatus* (7.62 ± 2.40 ppm) recorded the least concentration (Fig.2.b). The magnitude of contamination did not vary significantly among the species ($P > 0.05$) (Table.5).

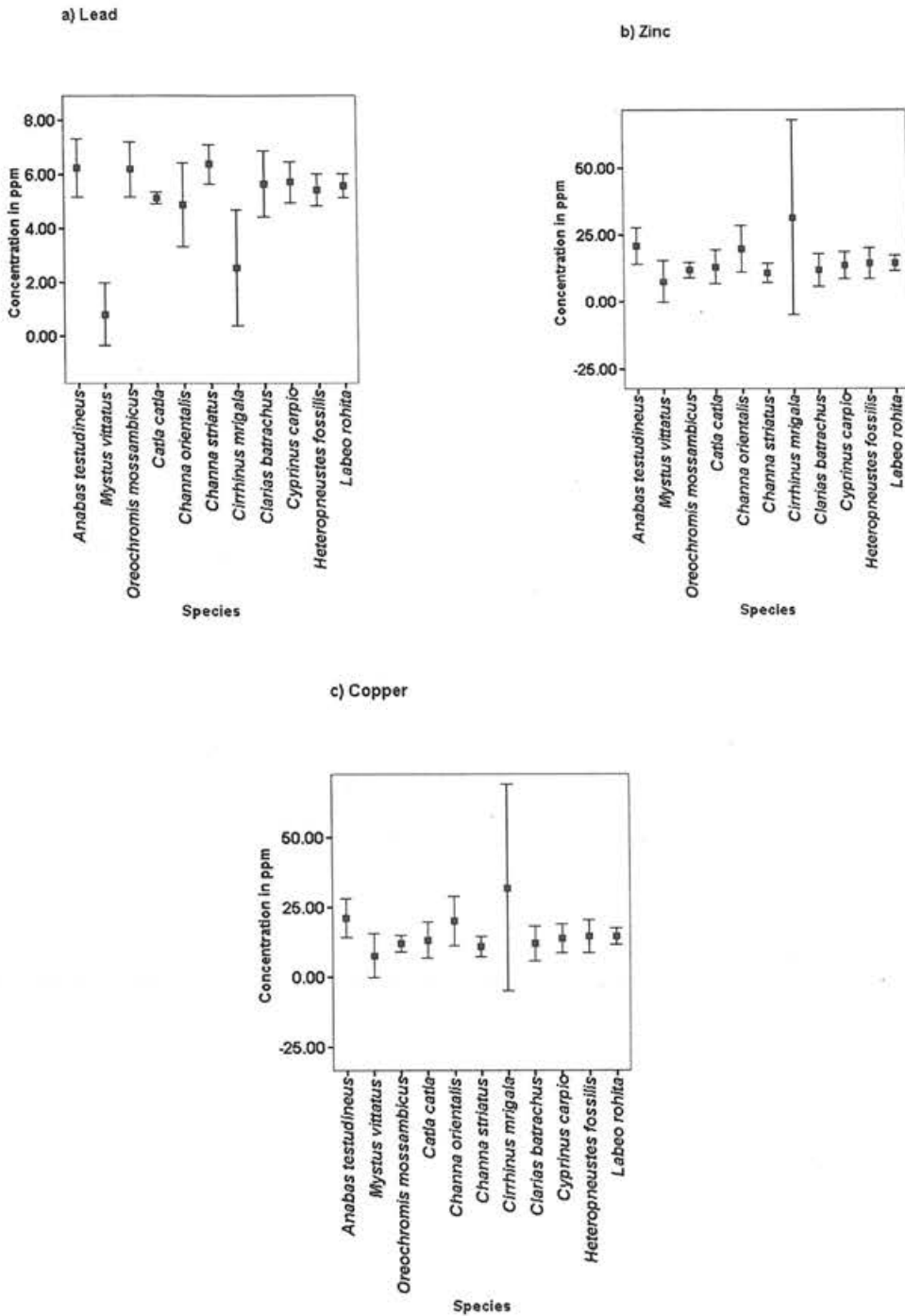
iii. Copper

Cirrhinus mrigala recorded the maximum concentration of copper (32.04 ± 14.30 ppm) followed by *Anabas testudineus* (21.22 ± 2.98 ppm), while *Mystus vittatus* recorded the least (7.76 ± 2.45 ppm) (Fig.2.c). *Heteropneustes fossilis* (14.66 ± 2.45 ppm) and *Labeo rohita* (14.63 ± 1.41 ppm) recorded near equal concentrations. Similarly *Clarias batrachus* (12.04 ± 2.70 ppm) and *Oreochromis mossambicus* (12.02 ± 1.31 ppm) measured near equal concentrations. No significant variation could be seen among the species ($P > 0.05$) (Table.5).

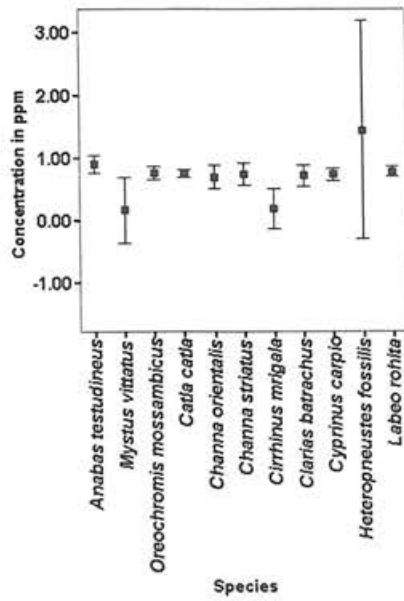
iv. Cadmium

Nearly 70% of the species studied showed an average concentration ranging between 0.70 and 0.80 ppm. *Heteropneustes fossilis* recorded the maximum concentration (1.44 ± 0.73 ppm) while *Mystus vittatus* recorded the minimum (0.16 ± 0.01 ppm) (Fig.2.d). Significant variation in contamination could be observed among species ($P < 0.01$) (Table.5).

Fig. 2 Variation in metal contamination (ppm) among various species of fishes in the wetlands of Andhra Pradesh (mean \pm SE)



d) Cadmium



e) Chromium

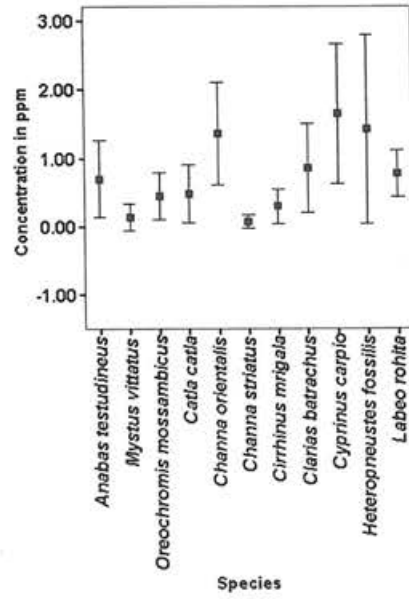


Table. 5 Variation in metal contamination among various species of fishes in Andhra Pradesh Wetlands (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	31.425	17.512	17.512	27.400	23.858
df	10	10	10	10	10
Asymp. Sig.	0.000*	0.064	0.064	0.002*	0.008**

*P<0.01; **P<0.05

v. Chromium

Cyprinus carpio recorded the maximum concentration (1.65 ± 0.45 ppm) followed by *Heteropneustes fossilis* (1.42 ± 0.59 ppm) while *Channa striatus* recorded the minimum (0.07 ± 0.04 ppm) (Fig.2.e). Significant variation could be observed among species ($P < 0.05$) (Table.5).

4.4 DISCUSSION

4.4.1 Variation in heavy metal contamination among select wetlands of Andhra Pradesh

Of all the wetlands, fishes of Draksha Rama of East Godhavari District recorded the maximum concentrations of zinc and copper. Further, the concentrations varied significantly for all the metals except cadmium ($P < 0.05$). Jankampet of Nizamabad district recorded the maximum concentrations of cadmium and chromium while Kolleru of East Godhavari district recorded the maximum for lead. Sewage inflow from the towns of Eluru, Gudivada and even Vijayawada and industrial effluents, pesticides and fertilisers from the Krishna-Godavari delta region contaminate the lake. The industrial discharge to an extent of 7.2 million litres/day and the 17,000 tonnes of fertilizer wash that enter the lake (Andhra Pradesh Pollution Control Board, 1993) annually, explain the elevated level of copper, zinc, lead in the fishes. Studies have shown the presence of organic pollutants in lake sediment and in the fast-growing weeds (Down to Earth, 1993). The sewage and discharge from factories have also affected the growth of water-borne organisms that the fish consume. The pattern perhaps reflects the contamination level in the food and water. ([www.rainwaterharvesting.org /Crisis/Kolleru_etc](http://www.rainwaterharvesting.org/Crisis/Kolleru_etc)).

Jankampet wetland of Nizamabad district besides supporting agricultural activity, also meets the domestic requirement of approximately 5000 people living nearby. Further, as this lake invites tourism, contamination through anthropogenic sources has

to be primarily accounted. Recreational activities, such as boating is expected to disperse lead through the diesel exhaust.

Draksha Rama of East Godhavari district, an equally contaminated wetland as that of Jankampet do receive sea water during February to early August which could be a probable source of contamination. Although no data on effluent discharge or agricultural activities is available, sand mining and sea water intrusion could be responsible for the increased levels of contamination in Jankamapet. Physicochemical and geochemical studies conducted on estuarine water bodies by various authors (Padma and Periakali, 1999; Nayak *et.al*, 2004; Nayak and Behera, 2004) reported that sea water influx and efflux to waterzones do influence the contamination load of the waterbodies.

4.4.2 Variation in metal contamination among various species of fishes in the wetlands of Andhra Pradesh

Cirrhinus mrigala, a subsurface dweller recorded the maximum concentrations of copper and zinc and minimum concentrations of lead significantly ($P < 0.05$). *Channa striatus*, a sediment feeder recorded higher concentrations of lead and biotransformation of contaminants through sediments could be related to their high levels. Effectiveness of metal uptake from the sources may differ in relation to ecological needs and metabolism of animals and also contamination gradients of water, food and sediments as well as other factors such as salinity, temperature and interacting agents (Norey *et.al*, 1990; Langston, 1990; Roesijadii and Robinson, 1994; Bervoets and Blust, 2003). Uptake of metals from food are also affected by various factors such as, methylation, oxidation state, temperature, redox potential of environment and interactions among metals (Rand *et.al*, 1995; Mason *et.al*, 2000). Thus, the metal levels bioaccumulated in fish tissue are the result of total environmental exposure (Barron, 1995).

In a study conducted in Hoogly estuary, India during 1977-1981, 127.8 ppm of copper and 20 ppm of chromium were recorded in various tissues of a fish species, namely *Mastacembelus pancalus*. High concentrations of zinc (218.5 ppm) were observed in

the muscle tissue of *Mastacembelus pancalus*, which indicate frequent exposure to increased concentrations of the metal. (Kaviraj, 1989).

Sultana and Rao (1998) documented the bioaccumulation patterns of Zn, Cu, Pb and Cd in different organs of Grey mullet *Mugil cephalus*, a detritus feeder living in contaminated waters of the Vishakapatnam harbor, India. They observed high zinc (157.33 ± 15.82 ppm) and copper (25.17 ± 5.13 ppm) levels in the liver tissue. Further, high concentrations of lead (16.98 ± 1.3 ppm) in gill and cadmium (2.79 ± 0.51 ppm) in kidney were reported. Zinc and copper were found in greater concentrations as they form essential elements for the normal growth, reproduction and longevity of the animals. Copper and zinc are essential metals and homeostatic control mechanisms that are not yet fully elucidated are believed to adjust their tissue concentration through the regulation of uptake and excretion (Grosell *et.al*, 1997; McGeer *et.al*, 2000). Hence it may not be toxic. The presence of many metals may affect the fish in a toxic or non-toxic manner. Lopez and Lee (1977) and Black *et.al*, (1982) reported that though copper concentration in Torch lake Michigan was high, other substances found in the lake may be complexing with copper to form relatively non-toxic compounds. Dissolved humic substances, moderate alkalinity and neutral pH, found in the lake would allow some degree of complexation which increases with increasing alkalinity, pH and total hardness (Pagenkopf *et.al*, 1974). Complexation may partly account for the relatively good hatching rates of Perch despite elevated copper concentrations found in Torch lake water (Ellenberger *et.al*, 1994). But mixtures of salts of metals, especially copper and zinc were observed to produce an additive toxicity in the Rainbow Trout and Atlantic Salmon. Although, the levels documented in the present study are comparable with the above cited study, species and organ specific difference limit the interpretation.

It may be difficult to generalize the uptake and accumulation of metals in aquatic organisms because of species specific differences (Wiener and Giesy, 1979). The physiological mechanisms involved may also protect the organism at much higher levels, making interpretation of effects somewhat difficult. Background levels are not easily defined because of wide dissemination by wind and water (Mason *et.al*, 1988)



The levels recorded currently are comparatively higher than the levels recorded elsewhere in Indian context. Muralidharan (1995) recorded a highest concentration of 1.7 ppm of lead in *Channa striatus* collected from Ajanbund, Bharatpur, Rajasthan, India. Jayakumar (2001) reported 2.1 ppm of lead in the same species collected from a wetland in Coimbatore, Tamil Nadu. Selvam (2002) documented quantitatively the levels of a few heavy metals in select species, namely *Channa punctatus*, *Channa striatus*, *Heteropneustes fossilis*, *Labeo rohita* and *Cirrhinus mrigala* from Keoladeo National Park, Bharatpur, Rajasthan, India, which was a follow-up investigation of the study executed during the late 1980's. Of all species *Labeo rohita* (Cu 0.70 ppm; Pb 0.74 ppm; Zn 8 ppm and Cd 0.08 ppm) and *Channa striatus* (Cu 0.34 ppm; Pb 0.73 ppm; Zn 6.64 ppm and Cd 0.04 ppm) recorded the maximum metal load. It is reported that there are no major differences in contamination between then and 2002. Hence the levels recorded in the present study are to be viewed with concern.

4.5 Suitability of fishes for human consumption in Andhra Pradesh

Dietary intake of metals by man through consumption of fishes was calculated for all the species and wetlands (Table 6, 7) as described in Chapter III.

Table. 6 Average daily dietary intake of metals through consumption of fishes (mg) - Species wise

Name of the Species	Lead	Zinc	Copper	Cadmium	Chromium
<i>Anabas testudineus</i>	0.22*	0.74	0.76	0.03	0.02
<i>Catla catla</i>	0.18	0.46	0.47	0.03	0.02
<i>Channa orientalis</i>	0.17	0.71	0.72	0.02	0.05
<i>Channa striatus</i>	0.23*	0.39	0.39	0.03	0.00
<i>Cirrhinus mrigala</i>	0.09	1.12	1.14	0.01	0.01
<i>Clarias batrachus</i>	0.20	0.42	0.43	0.03	0.03
<i>Cyprinus carpio</i>	0.20	0.49	0.50	0.03	0.06
<i>Heteropneustes fossilis</i>	0.19	0.51	0.52	0.05	0.05
<i>Labeo rohita</i>	0.20	0.51	0.52	0.03	0.03
<i>Mystus vittatus</i>	0.03	0.27	0.28	0.01	0.00
<i>Oreochromis mossambicus</i>	0.22*	0.42	0.43	0.03	0.02

Table.7 Average daily dietary intake of metals through consumption of fishes (mg) - Wetland wise

Name of the Wetland	Lead	Zinc	Copper	Cadmium	Chromium
Chinna tumbalam tank	0.19	0.66	0.67	0.04	0.05
Draksha Rama	0.05	0.84	0.86	0.00	0.01
Jankam Pet	0.19	0.70	0.71	0.02	0.07
Jataprole	0.19	0.31	0.32	0.03	0.01
Kazipet tank	0.21*	0.49	0.50	0.03	0.01
Kolleru	0.21*	0.42	0.43	0.03	0.00
Mandhyal tank	0.21*	0.61	0.63	0.03	0.05
Nandalur tank	0.21*	0.49	0.50	0.03	0.05
Uppalapadu	0.19	0.39	0.40	0.02	0.02
WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption	0.50	10-15	1.30	0.05	0.4
WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limit

The dietary intake level of heavy metal through the consumption of various species of fishes shows that the lead input is higher through consumption of *Channa striatus* (0.23 mg), *Oreochromis mossambicus* (0.22 mg) and *Anabas testudineus* (0.22 mg). The maximum dietary input of zinc (1.12 mg) and copper (1.14 mg) through the consumption of *Cirrhinus mrigala* and lower input was through *Mystus vittatus* (Zn- 0.27; Cu- 0.28 mg). The calculated range of cadmium and chromium varied between 0.01 and 0.05 mg respectively.

Of the various wetlands, fishes of Kazipet tank, Kolleru, Mandhyal tank and Nandalur tank showed invariably high level of lead (0.21 mg) than the other wetlands. The input of zinc (0.84 mg) and copper (0.86 mg) appeared to be high through consumption of fishes from Draksha Rama and minimum through Jataprole 0.31 and 0.32 mg respectively. The input of cadmium ranged from 0.02 to 0.04 mg and chromium from 0.01 to 0.05 mg/day/person.

The input of lead through consumption of fish species such as *Anabas testudineus*, *Channa striatus* and *Oreochromis mossambicus* was found to be higher than the

allowable daily dietary levels prescribed by WHO/FAO (1993) for a 60 kg adult. Similarly, the fishes of Kazipet Tank, Kolleru, Mandhyal tank and Nandalur Tank showed almost equal or higher than the tolerable lead input permitted for human consumption by various statutory agencies. Hence, if the consumption rate increases beyond 250 g/week, it may inflict serious physiological perturbations. Wetlands such as Jankampet and Draksharama require continuous monitoring as it can track the changes in the contamination pattern and caution the public. According to WHO/FAO the tolerable daily intake of copper, lead, zinc, cadmium and chromium is 1.3, 0.50, 10-15, 0.05 and 0.4mg/day/person respectively through food and water. Further according to JECFA of WHO/FAO (WHO, 1989; 1993) the allowable limit of lead through fishes is 0.21 mg for a 60-Kg adult. It is observed that species, namely *Channa striatus* and *Oreochromis mossambicus* are the commonly occurring species in the wetlands studied and consumption of these species may make the consumer vulnerable to lead contamination.

HEAVY METAL CONTAMINATION IN THE FISHES OF ASSAM WETLANDS

5.1 INTRODUCTION

Assam, the sentinel of the east is a very fascinating and diverse state. It is surrounded on all sides by predominantly hilly or mountain tracts. The entire area of Assam is divided into three major parts – the Brahmaputra valley (or the Assam valley), Barak valley (Cachar plains) and the hills and plateau (Karbi Anglong and Barail range). The Assam valley built mostly by the aggradations of Brahmaputra and its tributaries, is a flat terrain having very little slope from its northeast corner at Sadiya to Dhubri in the west. The valley is fairly wide in upper Assam, with an average width of 80-100 kms; but is narrow with an average width of 55 km in the middle. According to the Directorate of Fisheries of Assam (1997-98) there are 1,196 beels in Assam, of which 430 are registered while the remaining 766 are unregistered. The areas covered by registered and unregistered beels are 60,250.24 and 40,603.37 hectares respectively.

Assam has humid tropical climate. Average temperatures ranges from 7°C in winter to 36°C in summer. It gets a rainfall between 1780 mm and 3050 mm from June to September. To document the metal contamination six, wetlands were examined (Map.2)

5.2 STUDY AREA

5.2.1 Botha Beel

It is located in Kamrup district between 26°10' E and 91°45'N. It has a minimum water spread area of 3.20 Km². This seasonally cultivated wetland experienced a 40% decline in fish harvest over the last 5-6 years. The abundance of species such as *Labeo* sp., *Channa striatus*, *Wallago* seems to be on the decline. Water scarcity, siltation and effluent from a local spinning mill are identified as the reasons for the decline. This wetland also supports agricultural activity nearby. Application of pesticides such as gammexane and Dimucron on crops such as paddy and bajra are recorded.

5.2.2 Diplai Beel

It is located between 26°17' E and 90°19'11" N of Kokrajhar district. It has a minimum water spread area of 0.367 Km². A 30% decrease in fish yield particularly of species, namely *Anabas testudineus*, *Clarias batrachus* and *Notopterus chitala* has been observed. The probable reasons put forth for the decline include excessive fishing, use of mist net, clearance of forest vegetation in the surrounding areas, soil erosion of surrounding hillock, suspected cases of fish poisoning, agricultural pollutants and disease in fishes. There is no record on discharge of industrial effluents into this wetland. Crops such as paddy, jute, mustard and tapioca using a variety of fertilizers such as urea, superphosphate and potash have been reported near the wetland. Folidol, gamexene and thiodan were the pesticides applied to control the crop pests.

5.2.3 Misamari Beel

It falls between 27° 26' 25" E and 94° 25' N in the district Jorhat. It has a water spread area of 6 to 20 Km². This wetland is perennial and it supports the agricultural activities in the vicinity. No information on effluent discharge, fertilizers use or pesticide application is available. Further this wetland receives conservation inputs from a local NGO, named Ketekee.

5.2.4 Monoha Beel

This wetland is located in Morigaon district and falls between 26° 14' E and 92° 12' 30" N. It has a water spread area of 0.71 to 0.85 Km². Paddy, mustard and wheat are grown around the Beel. No information on the pollution details is available in the wetland.

5.2.5 Son Beel

This seasonally cultivated wetland is located in Hailakandi district (24° 41' E & 92° 34' N). It has a water spread area of 2 to 4.2 Km². This wetland supports paddy



Map.2 Location of select wetlands studied in Assam.

1. Botha Beel
2. Diplai Beel
3. Misamari Beel
4. Monoha Beel
5. Son Beel
6. Vereki Beel

cultivation. Although there exists no information on the effluent discharged into this wetland, it is reported to have suffered in fish yield (approximately 30%) during the last 5-6 years.

5.2.6 Vereki Beel

It is situated in the Kamalabarj taluk of Jorhat district (26° 45' E and 94° 13' N). This seasonally cultivated wetland serves as an excellent habitat for many species of water fowl mainly goose and storks. Extensive fishing in this wetland has resulted in the decline of one bottom dweller namely *Clarias batrachus*. Agricultural activity is noticed in the vicinity where crops such as paddy are cultivated.

5.3 RESULTS

Totally, 72 fishes (Table 8) comprising five species were collected from six wetlands and analyzed for metals, namely Pb, Zn, Cu, Cd and Cr.

Table.8 List of select wetlands examined for metal contamination

S.No.	Name of the wetland	No. of fishes collected
1	Botha Beel	8
2	Diplai Beel	16
3	Misamari Beel	12
4	Monoha Beel	12
5	Son Beel	13
6	Vereki Beel	11
Total no. of fishes collected		72

5.3.1 Variation in heavy metal contamination among select wetlands of Assam

i. Lead

Lead contamination was recorded in the fishes of all wetlands and the levels ranged between 2.34 and 10.9 ppm. On an average, lead concentration was the maximum in the fishes of Misamari Beel (8.33 ± 0.39 ppm) of Jorhat District while minimum in Monoha Beel (4.05 ± 0.40 ppm) of Morigaon district (Fig.3.a). The variation in magnitude of contamination was significant ($P < 0.05$) (Table.9) among the wetlands.

ii. Zinc

Of all the wetlands on an average, fishes of Son Beel of Karimganj district had the maximum concentrations of zinc (27.83 ± 4.27 ppm) while Botha Beel recorded the minimum (13.29 ± 0.94 ppm) (Fig.3.b). Nearly fishes of 63% of the wetlands had zinc concentrations between 15 and 30 ppm. Significant variation in the level of contamination could be perceived ($P < 0.05$) (Table.9).

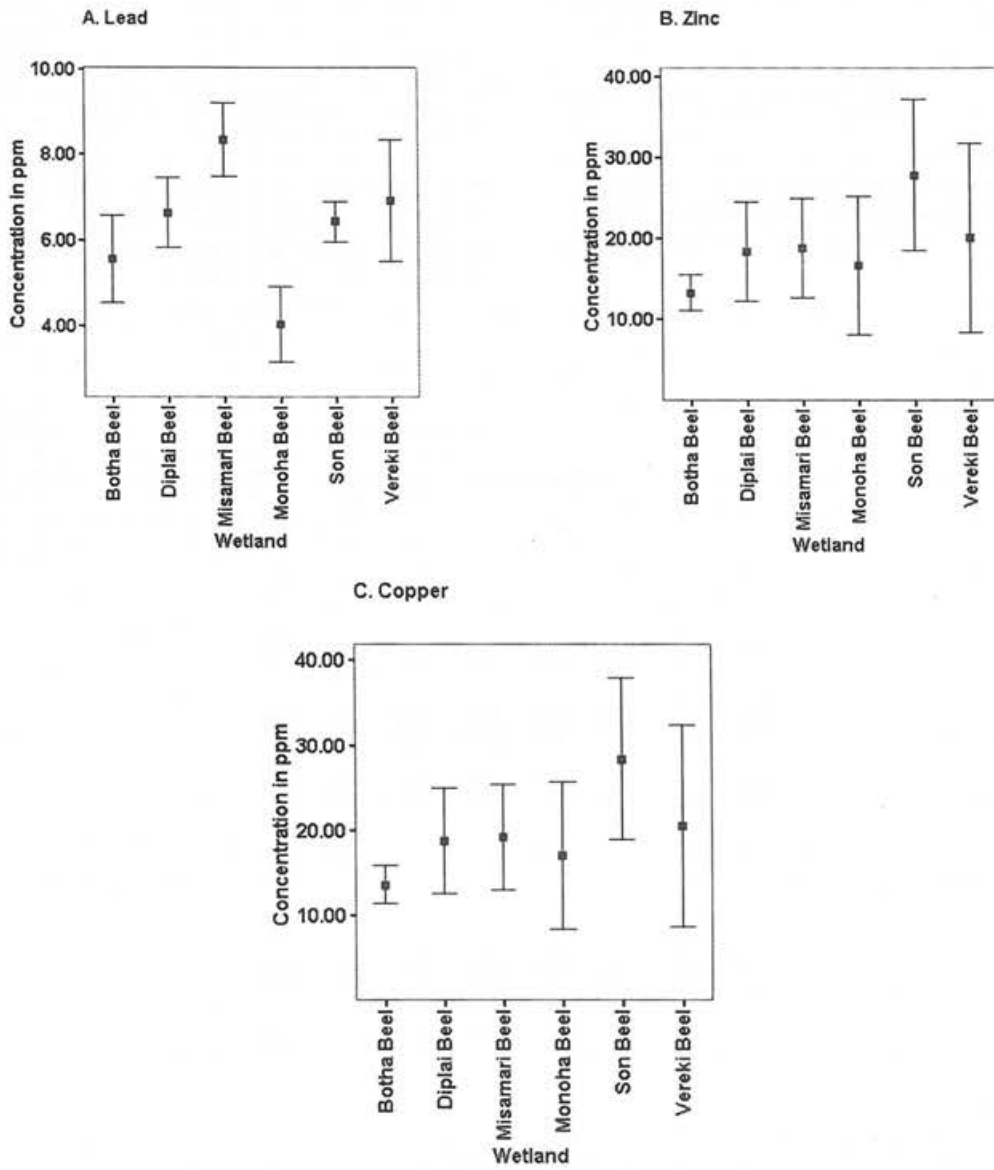
iii. Copper

Alike zinc levels, copper concentrations were also appreciably detected in the fishes of all the wetlands with the maximum being observed in Son Beel of Karimganj district (28.32 ± 4.35 ppm) and minimum in Botha Beel of Darrang district (13.52 ± 0.96 ppm) (Fig.3.c). Significant variations in contamination level could be noticed among the wetlands ($P < 0.05$) (Table.9).

iv. Cadmium

Fishes of Son Beel of Karimganj district recorded the highest concentrations of cadmium (0.87 ± 0.07 ppm) while Botha Beel (0.65 ± 0.04 ppm) of Darrang district recorded the minimum (Fig.3.d). Fishes of Misamari Beel (0.75 ± 0.06 ppm) and Diplai Beel of Dhubri district (0.75 ± 0.05 ppm) measured near equal concentrations. No significant variation in contamination could be observed ($P > 0.05$) (Table.9).

Fig. 3 Levels of heavy metal contamination (ppm) among select wetlands of Assam (Mean±S.E.)



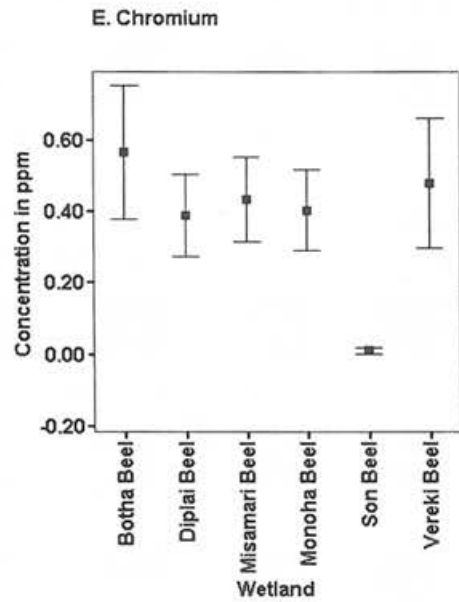
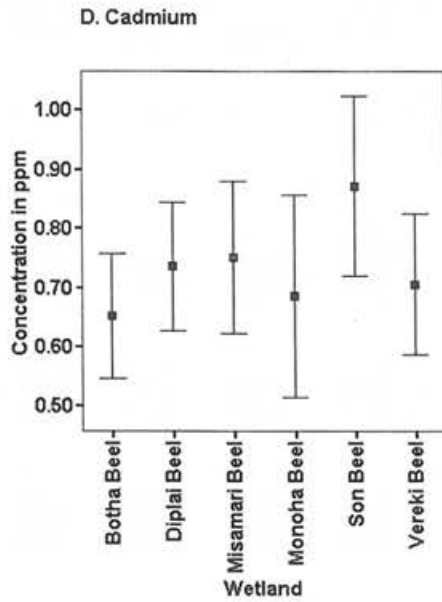


Table. 9 Variation in metal contamination in the fishes of Assam Wetlands (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	32.661	13.090	13.090	6.017	28.917
df	5	5	5	5	5
Asymp. Sig.	0.000*	0.023**	0.023**	0.305	0.000*

*P<0.01; **P<0.05

v. Chromium

Detectable concentrations of chromium were observed in the fishes of all the wetlands. Monoha Beel of Morgaon district measured the highest concentration (0.90 ± 0.52 ppm) and Son Beel of Karimganj district the lowest (0.04 ± 0.03 ppm). Misamari Beel (0.43 ± 0.05 ppm) and Vereki Beel (0.48 ± 0.08 ppm) of Jorhat district recorded near equal concentrations (Fig.3.e). No significant variation in the contamination level could be seen ($P > 0.05$) (Table.9) among the fishes of six wetlands studied.

5.3.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Assam

Variation in heavy metal contamination among five species of fishes totaling seventy two (Table.10) was compiled.

Table.10 List of species examined in detail for heavy metal contamination

S.No.	Name of the Species	No. of individuals
1	<i>Anabas testudineus</i>	20
2	<i>Channa orientalis</i>	8
3	<i>Clarias batrachus</i>	12
4	<i>Cyprinus carpio</i>	8
5	<i>Heteropneustes fossilis</i>	24
Total no. of fishes collected		72

i. Lead

Lead concentrations were detectable in all the species of fishes and on an average *Channa orientalis* recorded the maximum (7.78 ± 0.58 ppm) and *Clarias batrachus* the minimum (5.27 ± 0.66 ppm) (Fig.4.a). The variation in lead contamination was significant among the species ($P < 0.05$) (Table.11).

ii. Zinc

Although zinc concentrations ranged between BDL and 71.13 ppm in *Anabas testudineus*, the highest average concentration was observed in *Cyprinus carpio* (38.63 ± 5.85) and the lowest in *Heteropneustes fossilis* (14.45 ± 1.41 ppm) (Fig.4.b). Thus, the former species had 2.7 times higher load of zinc than the latter. Significant variation in zinc contamination could be noticed ($P < 0.05$) (Table.11)

iii. Copper

Alike zinc concentration, on an average *Cyprinus carpio* (39.32 ± 5.96 ppm) had the highest concentration followed by *Anabas testudineus* (20.30 ± 2.63 ppm). *Heteropneustes fossilis* (14.71 ± 1.43 ppm) recorded the least level of zinc (Fig.4.c). Significant variations in the contamination level could be established ($P < 0.05$) (Table.11).

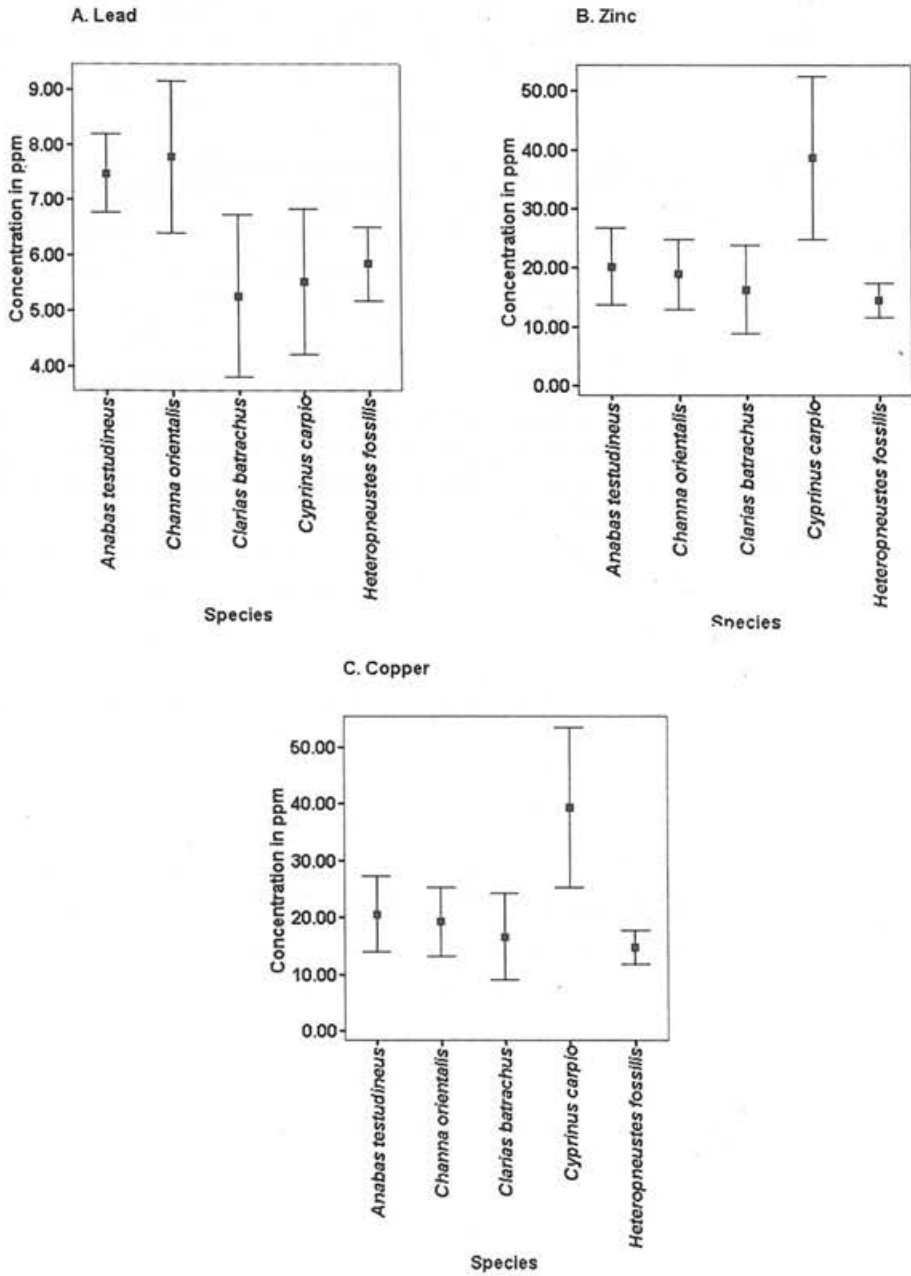
iv. Cadmium

The concentrations observed in all the species of fishes were around 0.80 ppm (Fig.4.d). In the level of contamination, all the species did not have any significant variation ($P > 0.05$) (Table.11).

v. Chromium

Among all the species, *Heteropneustes fossilis* recorded the maximum concentration (0.65 ± 0.26 ppm) of chromium while *Cyprinus carpio* (0.28 ± 0.08 ppm) the minimum. Near equal concentrations were observed in *Anabas testudineus* (0.39 ± 0.05 ppm) and *Channa orientalis* (0.36 ± 0.06 ppm) (Fig.4.e). Significant variation in contamination levels could be observed ($P < 0.05$) (Table.11).

Fig. 4 Variation in heavy metal contamination (ppm) among various species of fishes in the wetlands of Assam (Mean \pm S.E).



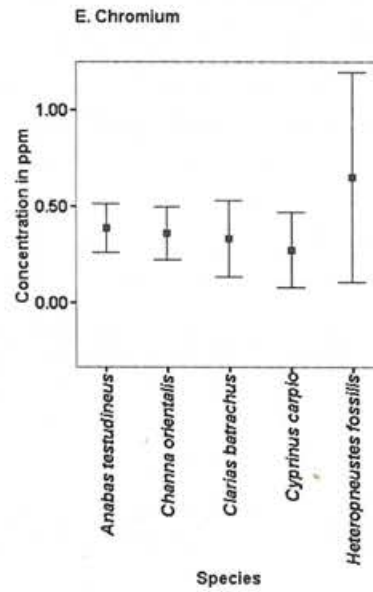


Table. 11 Variation in metal contamination among various species of fishes in Assam wetlands (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	19.526	20.132	20.132	.543	4.039
df	4	4	4	4	4
Asymp. Sig.	0.001*	0.000*	0.000*	0.969	0.401

*P<0.01

5.4 DISCUSSION

5.4.1 Variation in heavy metal contamination among select wetlands of Assam

Of all the wetlands, Son Beel of Karimganj district recorded the maximum concentrations of zinc, copper and cadmium. Lead and chromium were the maximum at Misamari Beel of Jorhat district and Monoha Beel of Marigaon district respectively. It is also interesting to note that two wetlands, namely Misamari Beel and Vereki Beel of Jorhat district recorded near equal concentrations of cadmium and chromium. This may perhaps indicate the level of exposure to be the same. Further, there was also no significant variation ($P > 0.05$) in cadmium contamination among all the wetlands. Many of these seasonally cultivated wetlands also support agricultural activity at the vicinity and wetlands such as Son Beel and Diplai Beel have suffered a reduction in the fish harvest during the past 5 – 6 years owing to excessive fishing, clearance of forest vegetation in the surrounding areas, soil erosion of surrounding hillocks and fish poisoning. Reduction in catch may also indicate deterioration in the overall health of the wetland. Although, there exists no information on effluent (domestic or industrial) discharge into many of these wetlands, agricultural leachate, dumping of solid waste could be the plausible reasons for the present level of contamination. However, this requires further investigation.

Although sources for lead contamination in the fishes could not be clearly defined, the overall global increase in lead levels through vehicular emission could be one of the reasons (Wang, 2002). Further Assam's economy is based on agriculture and oil. Assam produces more than half of India's petroleum. Hence, there are chances of lead contamination through these refinery activities, which may require further studies.

Fishes of wetlands, namely Son Beel, Misamari Beel, Diplai Beel and Vereki Beel require continuous monitoring and more data on the contamination levels have to be generated. Vereki Beel serves as an excellent habitat for aquatic birds such as Barheaded Goose. As the levels of cadmium and chromium are fairly high it is advisable to have a monitoring plan in place.

5.4.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Assam

Among the five species, *Heteropneustes fossilis* recorded the minimum concentrations of zinc, copper and cadmium, and maximum concentrations of chromium. *Channa orientalis* and *Cyprinus carpio* had the highest concentrations of lead and zinc respectively.

Biotransformation of contaminants through sediments appears to be a potential factor for high concentration of metals recorded in *Channa orientalis*, an detritus feeder. Although not much information is available on the metal contamination in *Channa orientalis* there exists some information on the related species, namely *Channa striatus*. While Muralidharan (1995) reported 1.7 ppm of lead in *Channa striatus* collected from Ajanbund, Bharatpur, India, Jayakumar (2001) recorded 2.1 ppm of lead in the same species collected from the wetlands of Coimbatore, Tamil Nadu, India. Selvam (2002) documented quantitatively the levels of a few heavy metals in select species namely *Channa punctatus*, *Channa striatus*, *Heteropneustes fossilis*, *Labeo rohita* and *Cirrhinus mrigala* from Keoladeo National Park, Bharatpur, Rajasthan, India, which was a follow-up investigation of the study executed during the late 1980's. Of all species *Labeo rohita* (Cu 0.70 ppm; Pb 0.74 ppm; Zn 8 ppm and Cd 0.08 ppm) and *Channa striatus* (Cu 0.34 ppm; Pb 0.73 ppm. Zn 6.64 ppm and Cd 0.04 ppm) recorded the maximum metal load. It is reported that there are no major differences in contamination between then and 2002. These levels are lower than the levels reported in the present study in *Channa orientalis*. Hence, the implications of the levels reported in the present study have to be assessed.

High levels of copper and zinc have been observed in most of the species of fishes. Copper and zinc are essential metals and homeostatic control mechanisms that are not yet fully elucidated are believed to adjust their tissue concentration through the regulation of uptake and excretion (Grosell *et.al*, 1997; McGeer *et.al*, 2000). Pyle *et.al*, (2005), in their study on the Yellow Perch collected from various lakes in the Sudbury area found that muscle Zn and Cu concentrations ranged from 23.5 to 276.9 ppm and 2.9 to 14.1ppm respectively. Metals such as copper and zinc are known to

have an effect and also to be accumulated by aquatic biota such as fish and crustaceans (Zou and Bu, 1994) and aquatic plants. Although aquatic plants, algae and invertebrate, generally seem to be more resistant to copper than fish (Alabaster and Lloyd, 1980) they all contribute to the diet of the species sampled.

The fishes in the present investigation had varying levels of cadmium and chromium. As studies elsewhere have shown such variations in the metal levels in fishes might be related to a number of factors such as age, size of animals, feeding habits, low dissolved oxygen concentrations, fluctuating temperature, elevated sediment metal loads, salinity, bioavailability of chemicals in food and water, physiochemical parameters of aquatic environment (Kargin *et.al*, 2001 ; Eastwood and Couture, 2002).

Assessment of heavy metal concentration in grass carp (*Ctenopharyngodon idella*) by Misra *et.al*, (2002) revealed that lead (0.6 to 1.45 ppm), copper (0.37 to 1.02 ppm), chromium (0.46 to 1.18 ppm), cadmium (0.35 to 0.824 ppm) and zinc (0.8 to 1.17 ppm) concentrations measured in muscle of the fish are found to be low to cause any adverse effects to fishes. Further, the levels were within prescribed limits and found to be safe for human consumption. The present levels do appear to be non-toxic.

The key factor in metal pollution however is the biochemical availability of the metal to the organism. Metals may be stored as metalloproteins with protein moiety showing some specificity of metal binding. Metal binding with the protein moiety in a cell is a complex phenomenon. Vincent and Ambrose (1994) observed increased uptake of cadmium and chromium in kidney in addition to liver in *Catla catla* which may be attributed to the increased synthesis of metallothioneins and their storage as a constituent of liver and kidney cytoplasm (Bremner and Davies, 1975). Thus, it can be logical to presume that bioavailability of metals such as cadmium and chromium should be reduced by the metalloproteins.

5.5 Suitability of fishes for human consumption in Assam

The daily dietary intake of heavy metals through consumption of fishes based on species and wetland wise were calculated (Table.12) as described in chapter III.

Table.12 Average daily dietary intake of metals through consumption of fishes (mg) - species wise

Wetlands	Lead	Zinc	Copper	Cadmium	Chromium
<i>Anabas testudineus</i>	0.27*	0.72	0.74	0.03	0.01
<i>Channa orientalis</i>	0.28*	0.68	0.69	0.03	0.01
<i>Clarias batrachus</i>	0.19	0.58	0.59	0.03	0.01
<i>Cyprinus carpio</i>	0.20*	1.38	1.40*	0.03	0.01
<i>Heteropneustes fossilis</i>	0.21*	0.52	0.53	0.03	0.02

Table.13 Average daily dietary intake of metals through consumption of fishes: wetland wise

Wetlands	Lead	Zinc	Copper	Cadmium	Chromium
Botha Beel	0.20*	0.47	0.48	0.02	0.02
Diplai Beel	0.24*	0.66	0.67	0.03	0.01
Misamari Beel	0.30*	0.67	0.36	0.03	0.02
Monoha Beel	0.14	0.59	0.60	0.02	0.03
Son Beel	0.23*	0.99	1.01	0.03	0.00
Vereki Beel	0.25*	0.72	0.73	0.03	0.02
WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption (mg)	0.50	10-15	1.30	0.05	0.4
WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limits.

The calculated dietary intake of lead through the consumption of fishes was above 0.2 mg except *Clarias batrachus* (0.19 mg), while zinc ranged between 0.52 and 1.38 mg. The maximum intake of 1.40 mg of copper was calculated to be through the consumption of *Cyprinus carpio* and minimum of 0.53 mg through *Heteropneustes fossilis*. The calculated input of cadmium and chromium were 0.03 and 0.01 mg respectively through all the species.

When all fishes in a wetland were considered together irrespective of species, and the input calculated ranged between 0.14 and 0.30 mg/day/person (Table.12). The maximum calculated value of zinc was 0.99 mg though the consumption of fishes of Son Beel wetland and minimum was through the fishes of Botha Beel wetland. The intake of copper ranged from 0.36 mg in Misamari Beel to 1.01 mg in Son Beel. The daily dietary intake concentration for cadmium and chromium were from 0.02 to 0.03 and 0.01 to 0.03 mg respectively (Table. 13).

Increased awareness among the public on the levels of contaminants in food commodities and their impacts on health warrants calculation of daily intake of contaminants through consumption of fish. Moreover, fishes occupy a significant portion in non-vegetarian human diet. The results reveal that the calculated dietary lead intake through consumption of all fishes irrespective of species from all the wetlands is quite high compared to the levels prescribed by Joint Expert Committee on Food and Agriculture (JECFA) of United Nations (UN) and WHO (1989, 1993) which admit only up to 0.21 mg/day for a 60 Kg adult. As referred to elsewhere in this report, increased and regular consumption may pose serious problems. Among the five species of fishes calculated for dietary intake, it is noted that the calculated dietary intake of copper through consumption of *Cyprinus carpio* is higher than the tolerable daily intake proposed by WHO/FAO (1972) (Table.13). According to WHO/FAO (1972) the tolerable daily intake of copper, lead, zinc, cadmium and chromium is 1.3mg, 0.50mg, 10-15mg, 0.05mg and 0.4mg respectively through food and water. The levels of cadmium and chromium appear to be safe for human consumption. However, if the per capita consumption increases, even the present levels may pose problems.

HEAVY METAL CONTAMINATION IN THE FISHES OF BIHAR WETLANDS

6.1 INTRODUCTION

Bihar, a storehouse of vast mineral wealth and a land of wetlands and waterfowl, is flanked by West Bengal, Orissa, Madhya Pradesh and Uttar Pradesh and stretches up to the foothills of Himalayas in the north and is completely landlocked. The river Ganga cuts the state into two unequal halves, flowing from west to east. The southern half is almost double in size of the northern part. Physiographically, north Bihar is almost entirely a level tract while south Bihar is hilly and undulating. South Bihar is again divided into two parts consisting of alluvial land along the bank of Ganga and the Chotanagpur plateau. Chotanagpur plateau largely comprises highlands, hill and valleys. This region is also noted for waterfalls on some rivers like Suvarnarekha (Hundru falls). The major rivers in north Bihar are the Ganga, Kosi, Gandak, and in south Bihar they are the Punpun, Phalgu, Sone, Sakri, Kiul, Damodar, Barker and Suvarnarekha. Most of the rivers flow into the Ganga.

The state generally has three seasons, hot season from March to May, the rainy season from June to September and the cold season from November to February. The average rainfall varies from about 1,000 mm to 1,500 mm.

The Space Application Centre has estimated that the wetlands of 56.25 ha and above cover a total of 1,776.8 sq km in Bihar; 1.02% of the total geographical area. The total number of wetlands in the size of 56.25 ha and above in Bihar was estimated at 759.

In Bihar totally nine wetlands (Map.3) have been examined for metal contamination.

6.2 STUDY AREA

6.2.1 Anarag dam

This wetland is located in Garhwa district of Jharkand ($23^{\circ}59'$ E & $83^{\circ}47'$ N) and has a water spread area of 35 sq km. This wetland appears to be an excellent source of revenue through fishing (Rs. 3 lakhs/ annum). Fry of *Labeo rohita* and *Catla catla* are regularly introduced into this wetland. Agricultural activities are observed in the vicinity

where crops such as wheat, maize, barley and grams are cultivated. Endosulphan is one of the commonly used pesticides in this area. Potash, urea and DAP are the fertilizers used to enhance the yield in the agricultural fields.

6.2.2 Baghar Beel

It is located in Katihar district of Bihar ($23^{\circ}40'E$ and $84^{\circ}33'N$). Its water spread area shrunk to 25 ha in summer from about 400 ha during the monsoon. This seasonally cultivated wetland is subjected to large scale fishing operations. Wheat, maize and rice are the crops cultivated in the vicinity. Fertilizers such as urea, DAP, super phosphate and calcium are used extensively. Out break of disease in fishes has been reported in this wetland.

6.2.3 Dimna Lake

It is located in west Singbhum district of Jharkhand between $22^{\circ} 49' 30'' E$ and $85^{\circ} 57' 30'' N$. It has a minimum water spread area of 2 sq km. Boating activities are noticed in this water body and it mainly supports irrigation. Wheat, maize and rice are the crops cultivated at the vicinity. Fertilizers such as urea, DAP, super phosphate and calcium are the major agricultural inputs.

6.2.4 Hazaribag Lake

This is located in Hazaribag district of Jharkhand. It has a minimum water spread area of 5 sq km. This small lake appears to have no change in fish yield over the years. Application of organophosphates on crops such as paddy is reported. Further there is no information on the pollution details in this wetland.

6.2.5 Maithon Dam

It is located in Dhanbad district of Jharkhand. The area is approximately 15 sq.km. About 25% decline in fish harvest over the last 5-6 years has been recorded. Although seasonal cultivation in this wetland is less, it supports agricultural activities

closeby where paddy, wheat and vegetables are the major crops cultivated. BHC and malathion are the common pesticides applied.

6.2.6 Malay Dam

This 25 sq. km wetland is in Palamau district of Jharkhand ($24^{\circ} 02' E$ & $84^{\circ} 04' N$). Fishing in this wetland is the major activity. Paddy, wheat and vegetables are the major crops cultivated in the immediate catchment.

6.2.7 Panchet Dam

It is located in Dhanbad district of Jharkhand. It has a minimum water spread area of 15 sq km. About 40% decline in fish harvest over the last 5-6 years has been recorded. Although there is very little cultivation in this wetland, it irrigates in nearby crops such as paddy, wheat and vegetables area. BHC and malathion are some of the common pesticides used.

6.2.8 Sukhaldhari Dam

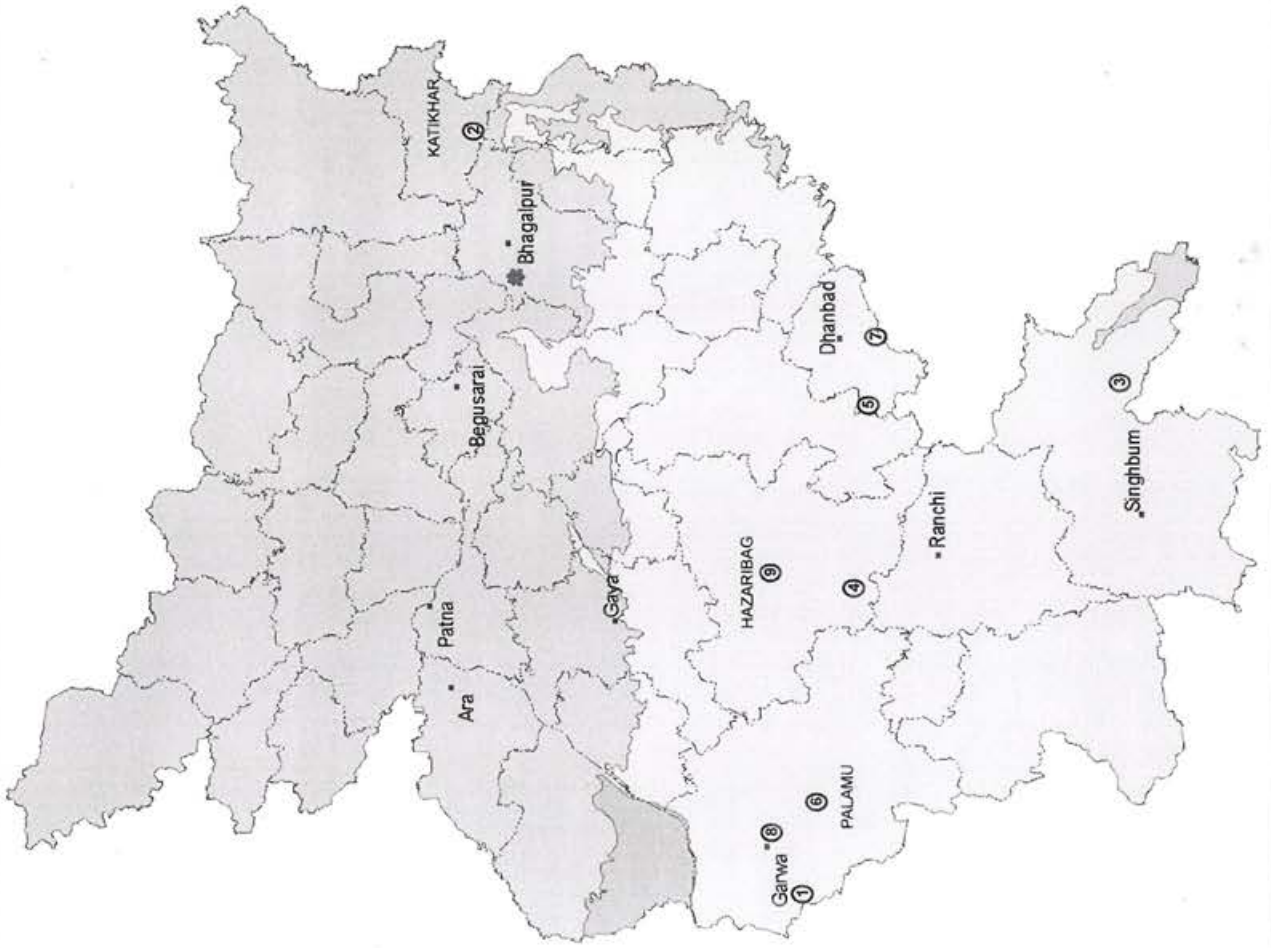
It is located in Gharwa district of Jharkhand ($24^{\circ}11'E$ & $83^{\circ}53'N$). The extent of this wetland is about 15 sq km (minimum). Fishing is currently prohibited and the water is supplied for drinking purposes. This wetland does receive effluents from limestone and dolomite mines. Further, this wetland also supports agricultural activity nearby where crops such as wheat, maize, barley, gram and vegetables are grown. Application of fertilizers such as potash, urea, DAP and pesticides, namely rogar and endosulphan are reported in the crop lands.

6.2.9 Tilaya Dam

Tilaya Dam spreads over an area of approximately 5 sq km. It is located in the Hazaribag district of Jharkhand. This wetland appears to have witnessed no change in the fish yield over the last 5-6years. This supports agricultural activities nearby. Crops such as paddy and maize were cultivated. BHC and folidol are the pesticides sprayed on these crops.

Map.3 Location of select wetlands studied in Bihar and Jharkand.

1. Anarag Dam
2. Baghar Beel
3. Dimna Lake
4. Hazaribagh Lake
5. Maithon Dam
6. Malay Dam
7. Panchet Dam
8. Sukhaldhari Dam
9. Tilaya Dam



6.3 RESULTS

Contamination status of 69 fishes comprising 10 species from 9 wetlands in Bihar was studied (Table 14).

Table.14 List of wetlands examined for heavy metal contamination

S.No.	Name of the Wetland	No. of individuals collected
1	Anarag Dam	4
2	Baghar Beel	4
3	Dimna Dam	8
4	Hazaribag Lake	9
5	Maithon Dam	12
6	Malay Dam	6
7	Panchet Dam	9
8	Sukhaldari Dam	4
9	Tilaya Dam	8
Total no. of fishes collected		69

6.3.1 Variation in heavy metal contamination select wetlands of Bihar

i. Lead

Baghar Beel of Kathihar District recorded the maximum concentrations of lead (7.85 ± 0.96 ppm) followed by Sukhaldari Dam (7.77 ± 1.20 ppm) in the Gharwa district. Dimna Dam of Singbhum district (4.33 ± 0.42 ppm) had the least concentration. Hazaribag Lake, Malay Dam and Tilaya Dam of Jharkhand had an average concentration of 5 ppm (Fig.5.a). The variation in contamination was significant among the wetlands ($P < 0.05$) (Table.15).

ii. Zinc

The maximum concentration of zinc was observed in Anarag Dam in Jharkand district (38.94 ± 5.79 ppm) followed by Sukhaldhari Dam (35.85 ± 9.89 ppm) in the same district (Fig.5.b). Minimum levels were found in Panchet Dam of Dhanbad district (14.44 ± 1.58 ppm) and the variation in contamination appeared significant ($P < 0.01$) (Table.15).

iii. Copper

Nearly 98.5% of the fishes measured detectable levels of copper. Least concentrations were seen in the fishes collected from Panchet Dam of Dhanbad district (14.61 ± 1.97 ppm) and these levels were three folds lower than the levels recorded in Anarag Dam of Jharkand District (39.63 ± 5.89 ppm) which measured the maximum (Fig.5.c). Significant variation in copper contamination could be observed ($P < 0.01$) (Table.15)

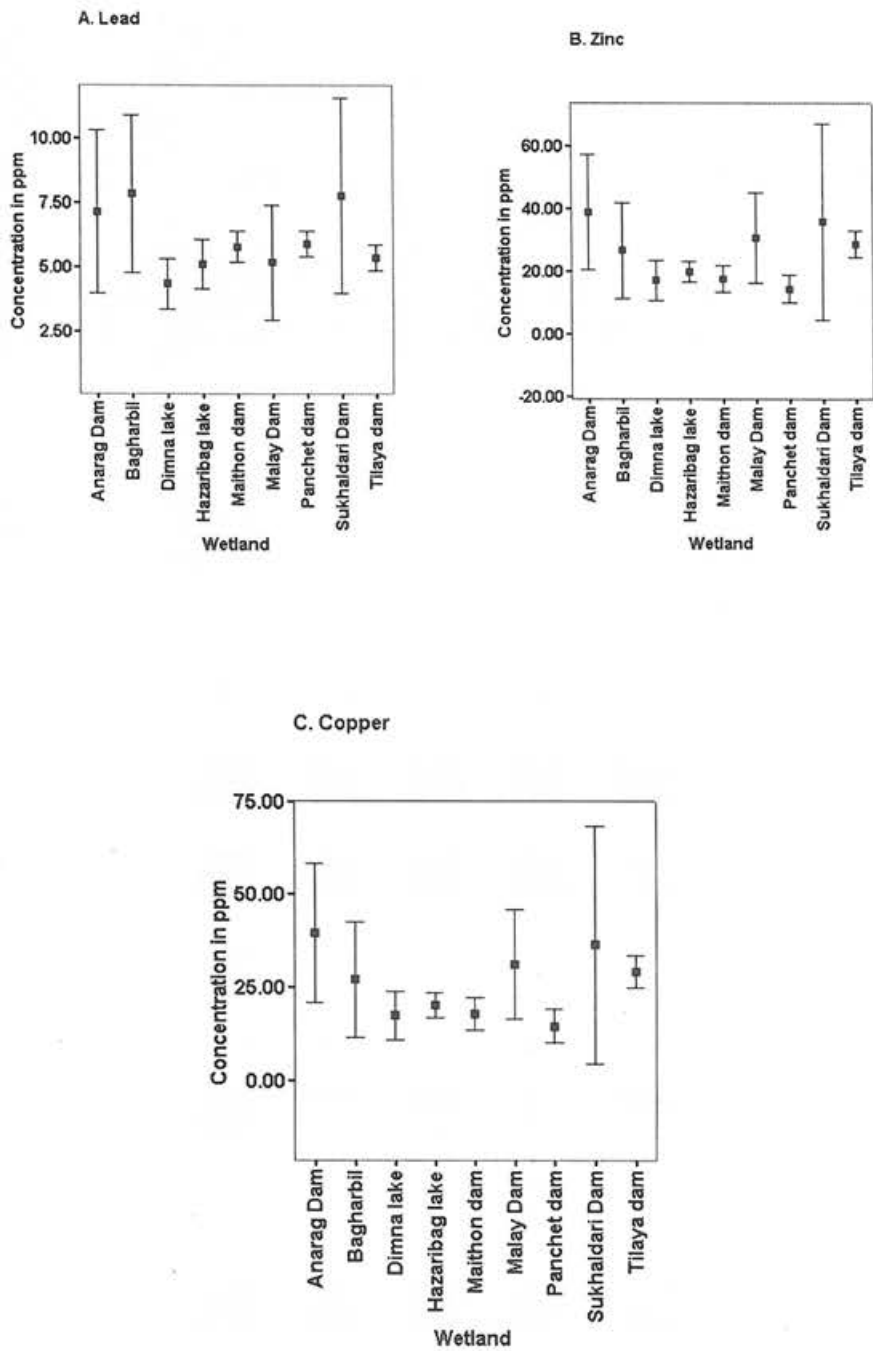
iv. Cadmium

Cadmium levels were detected in 82.4% of the fishes with no significant variation ($P > 0.05$) (Table.15). The maximum level was in Sukhaldari Dam of Jharkand District (0.92 ± 0.14 ppm) followed by Anarag Dam of the same district (0.92 ± 0.22 ppm). The minimum concentration of cadmium was seen in Maithon Dam of Dhanbad district (0.59 ± 0.03 ppm) (Fig.5.d).

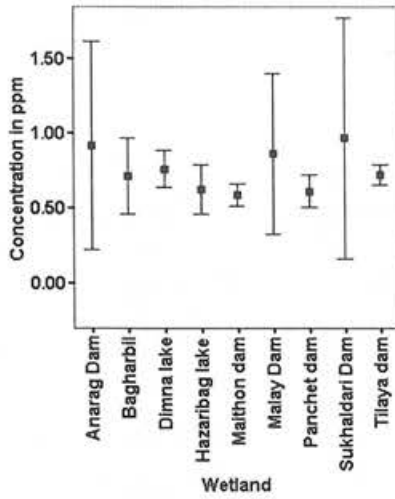
v. Chromium

Malay Dam in Jharkand District (0.20 ± 0.08 ppm) recorded the minimum concentrations while Maithon Dam (0.68 ± 0.07 ppm) in the same district recorded the maximum (Fig.5.e). Metal contamination among the wetlands significantly varied ($P < 0.01$) (Table.15).

Fig.5 Levels of heavy metal contamination (ppm) among select wetlands of Bihar (Mean±S.E)



D. Cadmium



E. Chromium

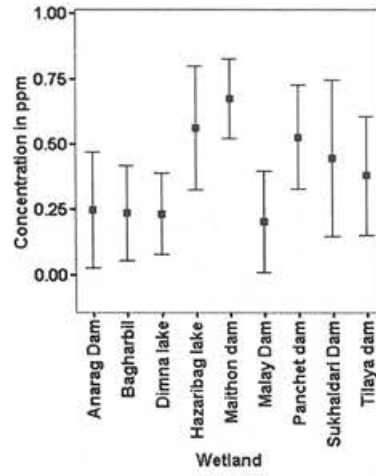


Table. 15 Variation in heavy metal contamination among select wetlands of Bihar (Kurskal Walis test)

	Pb	Zn	Cu	Cd	Cr
Chi-Square	16.804	19.598	19.598	7.483	24.371
df	6	6	6	6	6
Asymp. Sig.	0.010*	0.003**	0.003*	0.278	0.000**

* P<0.05; ** P<0.01

6.3.2 Variation in heavy metal contamination among select species of fishes in the wetlands of Bihar

Altogether seventy five individuals comprising ten species collected from select wetlands are presented (Table.16)

Table. 16 List of species examined in detail for metal contamination in Bihar

S.No.	Name of the species	No. of individuals
1	<i>Anabas testudineus</i>	7
2	<i>Channa orientalis</i>	4
3	<i>Channa striatus</i>	8
4	<i>Clarias batrachus</i>	8
5	<i>Heteropneustes fossilis</i>	4
6	<i>Labeo rohita</i>	9
7	<i>Mystus vittatus</i>	8
8	<i>Notopterus notopterus</i>	6
9	<i>Puntius dorsalis</i>	8
10	<i>Oreochromis mossambicus</i>	8
Total no. of fishes collected		69

i. Lead

Almost all the species measured detectable levels of lead with the maximum in *Channa orientalis* (6.60 ± 1.03 ppm) and minimum in *Channa striatus* (3.90 ± 0.45 ppm) (Fig.6.a). Although, the levels ranged between 5 and 6 ppm in all other species no significant variation in contamination could be recorded ($P > 0.05$) (Table.17).

ii. Zinc

About 98.5% of the samples recorded measurable levels of zinc. *Heteropneustes fossilis* recorded the lowest concentration (14.07 ± 0.23 ppm) which was three-fold lower than the levels recorded in *Puntius dorsalis* (39.06 ± 3.04 ppm) (Fig.6.b).

Significant variation in zinc contamination could be established among all the species ($P < 0.01$) (Table.17).

iii. Copper

The magnitude of copper contamination significantly varied among the ten species of fishes ($P < 0.01$). Copper levels ranged between BDL and 65.43 ppm. On an average, similar to zinc levels, *Puntius dorsalis* (39.75 ± 3.10 ppm) recorded the highest level of copper which was three-fold greater than the levels recorded in *Heteropneustes fossilis* (14.31 ± 0.85 ppm) (Fig.6.c) (Table.17).

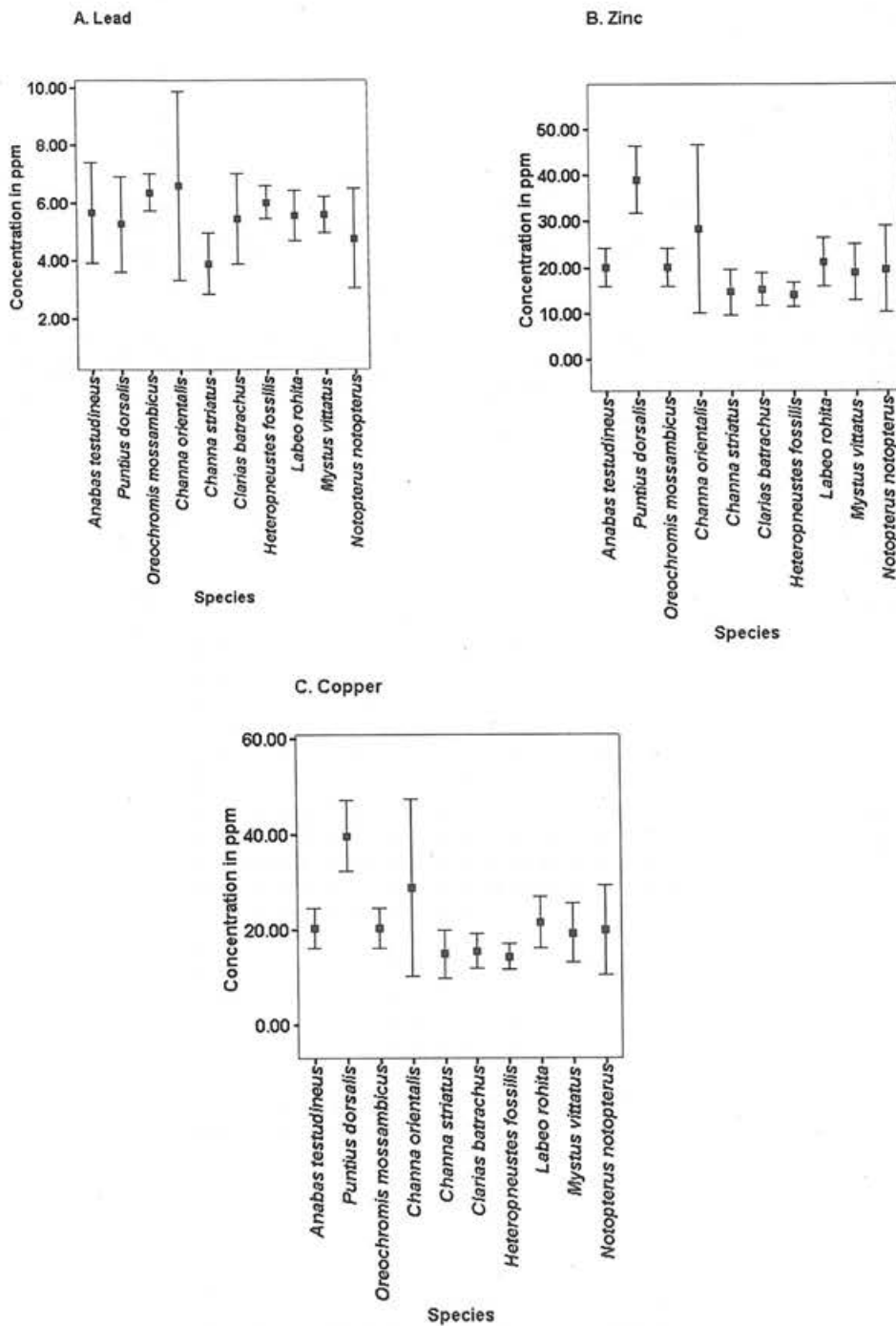
iv. Cadmium

About 82.4% of the fishes recorded detectable levels of cadmium with the values ranging between 0.45 ± 0.13 ppm and 0.84 ± 0.12 ppm. *Puntius dorsalis* (0.84 ± 0.12 ppm) measured the maximum Cd while *Channa striatus* (0.45 ± 0.13 ppm) the minimum levels (Fig.6.d). No significant variation in Cd contamination ($P > 0.05$) could be observed (Table.17)

v. Chromium

On an average, *Clarias batrachus* (0.60 ± 0.13 ppm) had the highest concentration and *Anabas testudineus* (0.20 ± 0.06 ppm) the lowest, which is three folds lower than the highest value (Fig.6.e). The variation in contaminant level among the species included in the study was not significant ($P > 0.05$) (Table.17)

Fig.6 Variation in heavy metal contamination (ppm) among various species of fishes in the wetlands of Bihar (Mean±S.E)



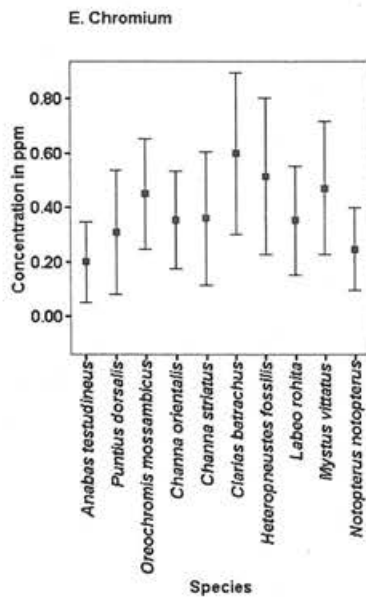
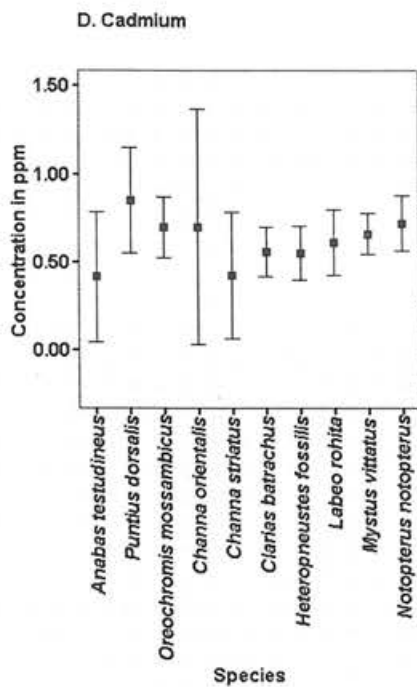


Table.17 Variation in heavy metal contamination among various species of fishes in the wetlands of Bihar (Kruskal Wallis test)

	Pb	Zn	Cu	Cd	Cr
Chi-Square	15.042	31.808	31.808	12.896	9.264
df	9	9	9	9	9
Asymp. Sig.	0.090	0.000**	0.000**	0.167	0.413

** P<0.01

6.4 DISCUSSION

6.4.1 Variation in heavy metal contamination select wetlands of Bihar

Among the select wetlands, Anarag Dam in Jharkand district recorded the maximum load of copper and zinc followed by Sukhaldhari Dam which had the highest levels of cadmium. While lead levels were high in Baghar Beel, chromium concentrations were high in Maithon Dam of Jharkand District. Significant variation in contamination levels ($P < 0.05$) could be attributed to contamination source and magnitude of contamination.

From the available information on the land use pattern around the wetlands, it can be inferred that these wetlands support agricultural activities at their close proximity. It is quite evident that considerable amount of chemical pesticides and fertilizers are used and the runoff contributes to the contamination level in these wetlands. The information available on the fish yield also showed declining trend over a period of time which requires further monitoring.

Anarag Dam acts as a potential reservoir to stock fishes and propagates them commercially. Thus, besides generating huge revenue (Rs.3 lakhs/annum) it also supplies water to the agricultural activities nearby. However, no information exists on effluent discharges into this wetland (Anarag Dam), while the effluents from Limestone and Dolomite mines are suspected to be contributing to the high concentrations of lead and zinc in the fishes of Sukhaldari dam.

Further high lead level recorded in Baghar Beel is a matter of concern, as large scale fishing operations are carried out here. An unidentified disease is also reported in this wetland fishes which needs further investigation. Thus wetlands, namely Anarag dam, Baghar Beel and Maithon Dam require further investigations and monitoring.

6.4.2 Variation in heavy metal contamination among select species of fishes in the wetlands of Bihar

Among the species, *Puntius dorsalis* recorded the maximum concentrations of zinc, copper and cadmium. The level recorded by this species appears to be three-fold greater than the concentrations measured in *Heteropneustes fossilis*. *Channa punctatus* recorded the highest load of lead and lowest load of chromium while *Puntius sophore* measured the maximum concentrations of chromium.

The average lead level in *Channa punctatus* (8.47 ± 1.53 ppm) in the wetlands of Bihar is higher than the levels recorded elsewhere. Muralidharan (1995) reported 1.18 ppm of lead in *Channa punctatus* collected from Numaish Pond in Bharatpur, Rajasthan, India. Further, the levels of copper (0.9 ppm), zinc (13.3 ppm), cadmium (0.3 ppm) and chromium (1 ppm) observed in *Puntius sophore* collected from the same pond were comparably lower than the levels documented in the present study.

The present levels of lead are of concern as the levels are comparably high with the levels documented elsewhere. Hence, long-term exposure may lead to deleterious effects to fishes and their consumers. Fishes are sensitive to metal contamination in water and may significantly damage certain physiological and biochemical processes when they enter the organs of these animals (Namcsok *et.al*, 1987). Chronic exposure of fish to sub lethal trace metal levels causes disturbed ion regulation, reduced growth and swimming speed (Hollis *et.al*, 1999; Alsop *et.al*, 1999). Several authors have described the hematological alterations, biochemical changes and hampering of locomotion in fishes like Rainbow Trout and Salmon at a concentration of 0.012 ppm of lead (Hodson *et.al*, 1978; Shrivastava and Mishra, 1979).

Sastry and Shukla (1993) reported that *Channa punctatus* to accumulate the least in their muscle tissues when exposed to 11.2 ppm of cadmium for 30 days. Further, they interpreted that the levels could be influenced by various factors. Metallothionein was proposed as an important factor. Metallothionein, a low molecular weight protein, whose apoprotein, thionein is induced by exposure to cadmium, copper, mercury and zinc which plays an important role in the detoxification mechanism. It provides

protective role against the toxic effects of these metals by sequestering and thus reducing the amount of free metal ions (Bouquegnear *et.al*, 1975; Cherian and Goyer, 1978).

Species, namely *Channa punctatus* is a bottom dwelling and an omnivorous fish. Hence, contamination through varied food sources has to be accounted. Further, sediment biotransformation of contaminants are also to be admitted (Kotze *et.al*, 1999). This species consumes large amount of silt along with the food materials. Silt particles play an important role in the transport and availability of metals as they are adsorbed onto the silt particles (Heath, 1987). Most metals are furthermore known to become more bioavailable and have increased toxicity with decreasing pH (Shaw and Brown, 1973). The pH in the stomach of fish decreases significantly (from above 6 to as low as 2.9) after feeding commences. This reduction in pH could thus result in metals becoming more bioavailable from the food sources and silt in the stomach. The metals could therefore be taken up via the intestine, causing increased metal levels. Thus, species specific differences, dietary habit and habitat variations could have contributed to the significant variation in metal contamination among species ($P < 0.05$).

6.5 Suitability of fishes for human consumption in Bihar

Dietary intake of metals by man through consumption of fishes was calculated for all the species and wetlands (Table 18, 19) as described in Chapter III.

Table.18 Average daily dietary intake of metals through consumption of fishes (mg) - species

Name of the Species	Lead	Zinc	Copper	Cadmium	Chromium
<i>Anabas testudineus</i>	0.21*	0.85	0.08	0.02	0.01
<i>Channa orientalis</i>	0.24*	1.01	0.32	0.02	0.01
<i>Channa punctatus</i>	0.30*	0.63	0.08	0.03	0.01
<i>Channa striatus</i>	0.14	0.49	0.01	0.02	0.02
<i>Clarias batrachus</i>	0.19	0.55	0.16	0.02	0.02
<i>Heteropneustes fossilis</i>	0.23*	0.42	0.04	0.02	0.02
<i>Labeo rohita</i>	0.21*	0.81	0.06	0.02	0.01
<i>Mystus vittatus</i>	0.20	0.68	0.05	0.02	0.02
<i>Puntius dorsalis</i>	0.19	1.40	0.11	0.03	0.02
<i>Puntius sophore</i>	0.21*	1.19	0.17	0.02	0.03
<i>Oreochromis mossambicus</i>	0.24*	0.71	0.06	0.02	0.02

Table.19 Average daily dietary intake of metals through consumption of fishes (mg)- wetland

Name of the Wetland	Lead	Zinc	Copper	Cadmium	Chromium
Anarag Dam	0.26*	1.39	1.42*	0.03	0.01
Baghar Beel	0.28*	0.95	0.97	0.03	0.01
Dimna Lake	0.15	0.61	0.62	0.03	0.01
Hazaribag Lake	0.18	0.71	0.73	0.02	0.02
Maithon Dam	0.21*	0.63	0.64	0.02	0.02
Malay Dam	0.18	1.10	1.12	0.03	0.01
Panchet Dam	0.21*	0.51	0.52	0.02	0.02
Sukaldari Dam	0.28*	1.28	1.30*	0.03	0.02
Tilaya Dam	0.19	1.03	1.05	0.03	0.01
WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption	0.50	10-15	1.30	0.05	0.4
WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limit

The calculated dietary intake of lead through the consumption of various species of fishes in Bihar clearly reveals the wide range of exposure of heavy metals to human beings. Of the various species studied, the input of lead appeared to be high through the consumption of *Channa punctatus*, *Channa orientalis*, *Oreochromis mossambicus* and *Heteropneustes fossilis* with 0.30, 0.24, 0.24, 0.23 mg/day/person respectively. The minimum input was through the consumption of *Channa striatus* (0.14 mg). The maximum input of zinc and copper was found through *Channa orientalis* followed by *Puntius dorsalis* and *Puntius sophore*, while it was minimum through the consumption of *Heteropneustes fossilis*. The input level of cadmium and chromium through consumption of various species of fishes is almost the same with the concentration ranging from 0.02 to 0.03 and 0.01 to 0.03 mg/day/person respectively.

When all the fishes irrespective of species in a wetland were pooled together to assess suitability for consumption, the input of lead through the consumption of fishes from Baghar Beel (0.28 mg) and Sukaldari Dam (0.28 mg) appeared to be high followed by Anarag Dam (0.26 mg), Maithon Dam (0.21 mg) and Panchet Dam (0.21 mg). The maximum input of zinc and copper through consumption of Anarag Dam fishes was calculated to be 1.39 and 1.42 mg respectively. The input of cadmium through consumption of fishes from all the wetlands was around 0.02-0.03 mg/day/person, while chromium input ranged between 0.01 and 0.02 mg.

When all the species irrespective of wetlands were considered separately, the intake of lead through consumption of species, namely *Channa orientalis*, *Channa punctatus*, *Heteropneustes fossilis* and *Oreochromis mossambicus* from many wetlands exceeded the permissible input prescribed for human consumption by Joint Expert Committee on Food and Agriculture (JECFA) of United Nations (UN) and WHO (1989, 1993) which admit only up to 0.21 mg/day for a 60 Kg adult. When the lead input through consumption of fishes from various wetlands is considered, the fishes of Baghar Beel, Sukaldari Dam and Anarag Dam are not suitable for human consumption. Similarly, copper input through Anarag Dam and Tilaya Dam exceeded the permissible limits prescribed by various agencies. The daily dietary intake concentration of zinc, copper, cadmium and chromium were compared with the tolerable dietary intake described by WHO/FAO (1972) for human consumption.

According to WHO/FAO (1972) the tolerable daily intake of zinc, copper, cadmium and chromium is 10-15, 1.30, 0.05, 0.4 mg respectively through food and water. Although the calculated levels of metals except lead and copper appeared to be safe for human consumption, continued exposure may create problem.

HEAVY METAL CONTAMINATION IN THE FISHES OF GUJARAT WETLANDS

7.1 INTRODUCTION

Gujarat, known for its 'Flamingo' in the Great Rana of Kutch and for the multitude of migratory waterfowl and cranes, is one of the dream lands of bird – lovers. Situated on the west coast of India, Gujarat consists mostly of plain interspersed with low hills or small mountains extending from Rajasthan, Madhya Pradesh and Maharashtra. Aravalli ranges from the Pavagadh region, near Baroda and merges with the Vindhyas. This state has mainly three geographical regions, namely the mainland, the peninsular region of Saurashtra and the Kutch. The last one has a large extend of desert, known as Rann of Kutch where the Flamingoes breed in thousands. The mainland on the whole is a plain of alluvial soil. This state has a long coastline of over 1,600 km.

Gujarat has an intensely hot or cold climate. The maximum temperature varies from 36.7 to 43.3°C in summer and the minimum between 2 and 18.3°C from November to February. There is much variation in the rainfall in the state from an average of 330 mm in Kutch and parts of Saurashtra to 1,520 mm in the southern parts with Dangs recording the highest, 1,900 mm.

The Space Application centre reported 393 wetlands of 56.25 ha and above, covering a total area of 2,092.02 sq km (SAC, 1998).The present study was carried out in eleven wetlands in the state (Map.4).

7.2 STUDY AREA

7.2.1 Ajwa Lake

It is located in Vadodara district between 22° 24' 30" E and 73° 25' 30" N. The maximum water spread area is reported to be 14.2 sq km. Cotton and maize are the major crops cultivated in the vicinity. As fishing is not allowed by the municipal

corporation there exists no data in the fish yield. No information is available on the pollution details.

7.2.2 Dandiwada Dam

It is located in Banaskantha district. Besides supporting the agricultural activities nearby, it also act as a good fishing ground. Apart from surface runoff, no industrial effluent input is reported in this wetland.

7.2.3 Deo dam

It is located in Panchmahal district between 22° 23' E and 73° 33' N. The water spread area is estimated at 16.2 sq km. This seasonally cultivated wetland also irrigates agricultural fields closeby. Maize is the common crop cultivated around.

7.2.4 Dharoi Dam

It is located in Mehsana district between 24° 00' E and 72° 52' N and has a maximum water spread area of 199 sq km. This dam is a major source of drinking water for Ahmedabad city and several other towns nearby. Wheat is grown in the nearby agricultural land with the help of water from this wetland.

7.2.5 Kanewal

It is located in Anand district between 22° 20' E and 72° 33' N and has a maximum water spread area of 6.25 sq km. This wetland supports the agricultural activities nearby where crops such as paddy, wheat, chickpea and bajra are grown. Carbofuran and phorate are the commonly sprayed pesticides.

7.2.6 Meshwo dam

It is located in Sabarkanth district at 23°41'4"N and 73°23'55"E having a catchment area of 259 Km². It supports agricultural activities nearby and is a potential fishing ground. Species such as *Catla catla*, *Channa punctatus* are stocked in this wetland.

The water quality parameters of this lake show that it appears free of any effluent discharge (www.gec.gov.in/envis/SoER_Table_htm/WatQuaRes.).

7.2.7 Nalsarovar bird sanctuary

It is located in Surendranagar, Ahmedabad between 22°47'00"N and 72°02'00"E. It is spread over an area of 12,082 ha and is one of the largest shallow freshwater lakes in India. It has been proposed as a Ramsar site. The Sanctuary lies in the semi-arid districts of Ahmedabad and Surendranagar in North Gujarat. The Lake has an elliptical basin with a gentle slope. It is very shallow with a maximum depth of 3 m. Commercial fishing is prohibited in this wetland. This wetland appears to be free from any effluent discharge. Further this wetland support the agricultural activities closeby where crops such as paddy, wheat, cotton are grown. Pesticides such as carbofuran is applied in limited quantum. There is no evidence of mortality of fish or bird due to the use agrochemicals.

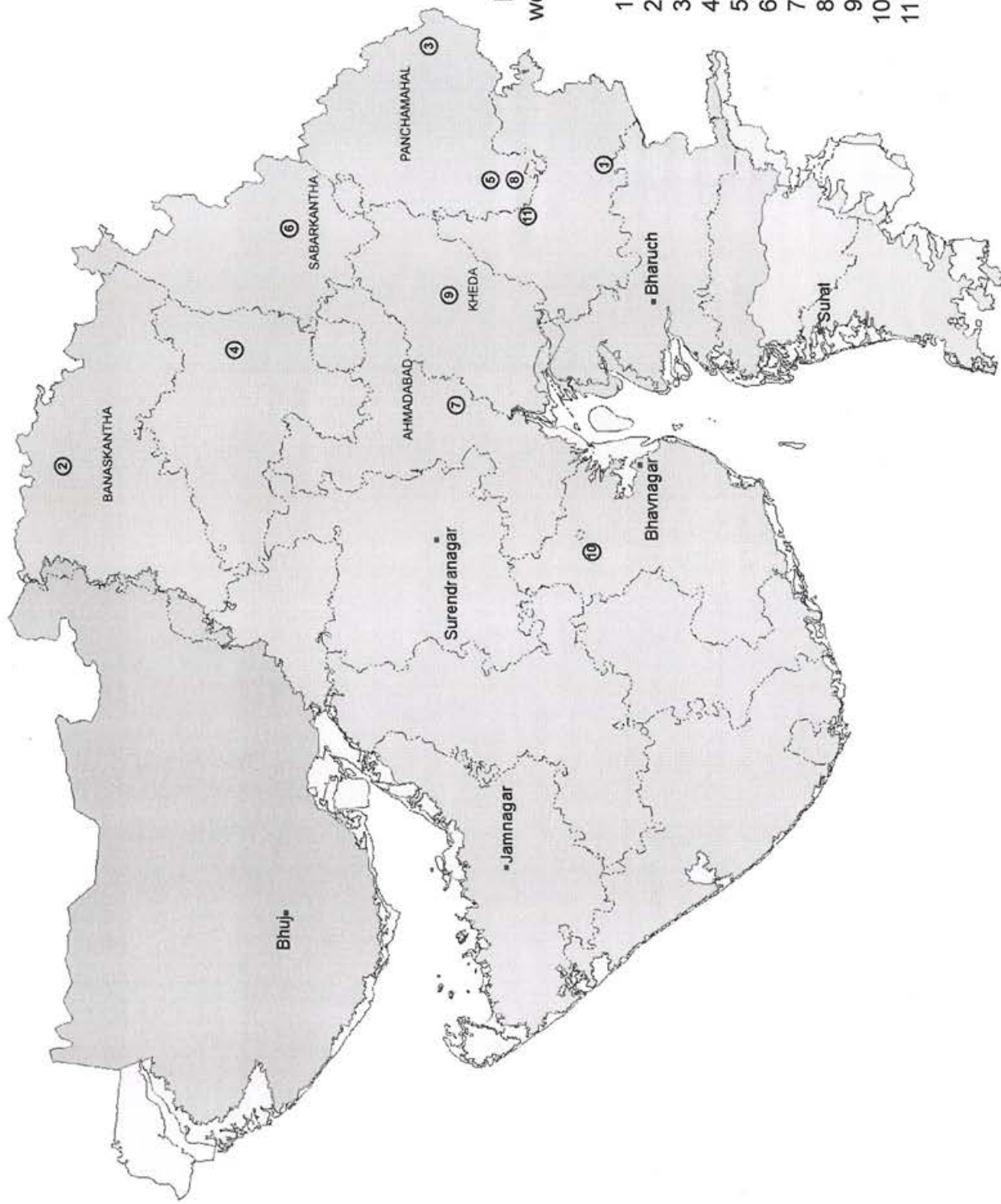
7.2.8 Pariaj

It is located in Anand between 22°33'30" E and 72°38'30" N and has a maximum water spread area of 7 sq km. This wetland supports the agricultural activity nearby where crops such as paddy and wheat are cultivated. Carbofuran is the commonly sprayed pesticide.

7.2.9 Saiyant

The area of the wetland is 2.68 sq km and is located in Kheda district between 22°49' E and 73°06' N. Although no quantitative information on the fish yield is available fluctuations are reported due to water availability. Although no information on the pollution details are available, Carbofuran is the widely applied pesticide to control pest in the agricultural lands. Urea and sulfate are the commonly used fertilizer to grow tobacco, paddy and wheat in the agricultural lands nearby.

Map.4 Location of select wetlands studied in Gujarat



1. Ajwa Lake
2. Dantiwada Dam
3. Deo Dam
4. Dharoi Dam
5. Kanewal
6. Meshwo Dam
7. Nalsarovar Bird Sanctuary
8. Pariej
9. Saiyant
10. Sukhbhadar Dam
11. Vadhwana

7.2.10 Sukhbhadar Dam

It is located in Bhavnagar district between 22°21' E and 70°33' N and has a maximum water spread area of 10.45 sq km. This seasonally cultivated wetland supports the agricultural activity in its vicinity where crops such as sorghum and wheat are grown. Fertilizer such as urea is used in large quantity.

7.2.11 Vadhavana

It is located in Vadodara district between 22°24' E and 73°25' N and has a maximum water spread area of 5.67 sq km. Fishing is carried in this wetland and water is used to irrigate the agricultural crops such as wheat and maize grown nearby. No information on pollution details are available.

7.3 RESULTS

A total of 128 fishes collected from 11 wetlands in this state were examined for heavy metal contamination (Table.20)

Table. 20 List of wetlands examined in detail for metal contamination

S.No.	Name of the Wetland	No. of fishes collected
1	Ajwa Lake	12
2	Dantiwada Dam	10
3	Deo Dam	12
4	Dharoi Reservoir	12
5	Kanewal	12
6	Meshwo Dam	6
7	Nalsarovar Bird Sanctuary	16
8	Pariaj	12
9	Saiyant	12
10	Sukhbhadhar Dam	5
11	Vadhavana	17
Total no. of fishes collected		128

7.3.1 Variation in heavy metal contamination among select wetlands of Gujarat

i. Lead

The average lead concentrations in many wetlands were between 5 and 6 ppm. Among wetlands, Deo Dam of Panchmahal District recorded the maximum concentration of lead (7.91 ± 0.23 ppm) followed by Ajwa Lake in Vadodra district (6.94 ± 0.34 ppm). Meshwo Dam in Sabarkanth district recorded the minimum concentration (4.90 ± 0.34 ppm) which was almost twice less than Deo Dam (Fig.7.a). Significant variation in contamination could be perceived among the wetlands ($P < 0.01$) (Table.21).

ii. Zinc

Zinc concentration in the wetland fishes ranged between below detection limit (BDL) and 80.99 ppm. Almost 97% of fishes recorded detectable levels of zinc. Fishes of Dharoi reservoir in Mehsana district recorded the maximum (31.94 ± 5.83 ppm) on an average. Kanewal of Anand district had the lowest level of zinc (6.62 ± 2.95 ppm) (Fig.7.b). Significant variation in contamination could be observed among the wetlands ($P < 0.01$) (Table.21).

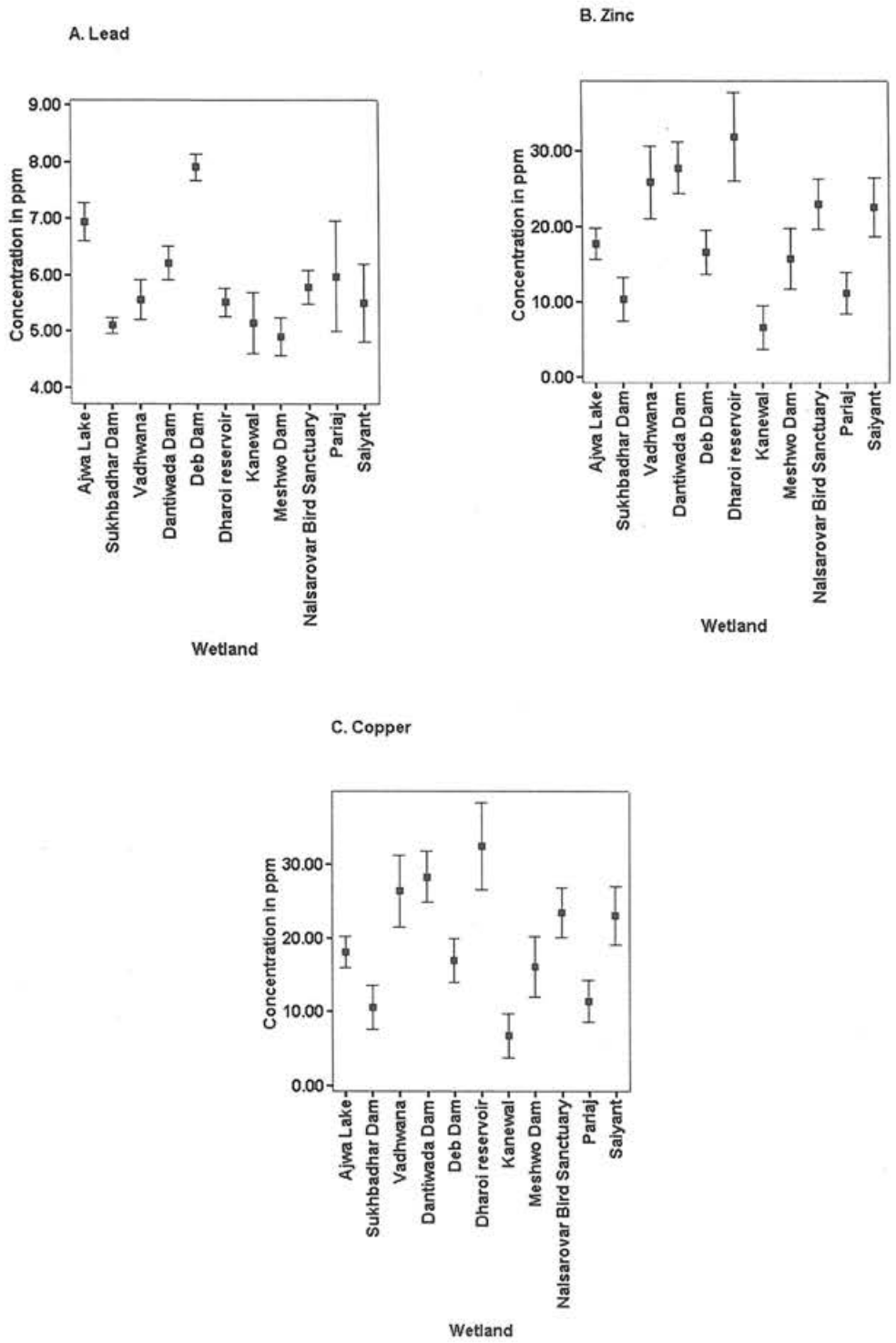
iii. Copper

About 2.7% of the samples recorded below detectable levels of copper. The remaining samples had significantly varying levels ($P < 0.01$) (Table.21). The maximum copper level was observed in the fishes of Dharoi reservoir (32.51 ± 5.94 ppm) of Sabarkanth district. Kanewal (6.74 ± 3.00 ppm) in Anand district recorded the lowest concentration (Fig.7.c).

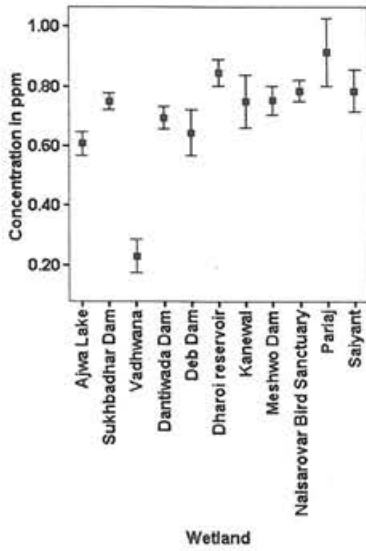
iv. Cadmium

All the wetlands recorded less than 1 ppm of cadmium. While 94.6% of the samples had appreciable levels of cadmium, maximum concentration was recorded in the

Fig. 7 Levels of heavy metal contamination (ppm) among select wetlands of Gujarat (Mean \pm S.E)



D. Cadmium



E. Chromium

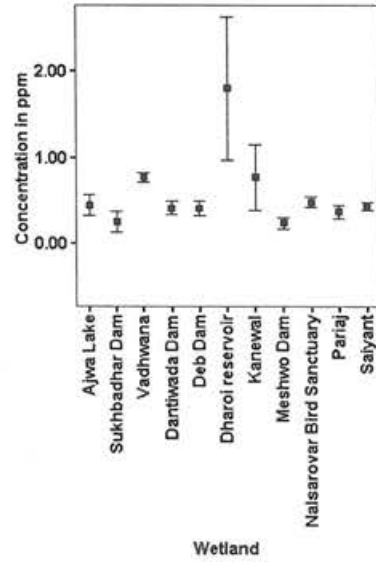


Table.21 Variation in heavy metal contamination among select wetlands of Gujarat (Kurskal Walis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	34.277	31.308	31.308	20.717	5.090
df	8	8	8	8	8
Asymp. Sig.	0.000*	0.000*	0.000*	0.008*	0.748

*P<0.01

fishes of Pariaj (0.91 ± 0.11 ppm) in Anand. The minimum levels were recorded in Vadhavana (0.24 ± 0.03 ppm) of Baroda (Fig.7.d). Significant variations in cadmium concentration among the wetlands were observed ($P < 0.01$) (Table.21).

v. Chromium

On an average, fishes of Dharoi reservoir (1.80 ± 0.83 ppm) in Sabarkanth district showed the highest levels of chromium while Sukhbadhar Dam (0.25 ± 0.12 ppm) in Bhavanagar district the lowest (Fig.7.e). Alike, cadmium concentration, the levels of chromium were also below 1 ppm in all the wetlands. No significant variation in contamination could be observed among the wetlands ($P > 0.05$) (Table.21).

7.3.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Gujarat

The list of species of fishes examined for heavy metal contamination (Table.22) is tabulated below:

Table.22 List of species examined for heavy metal contamination.

S. No.	Name of the species	No. of individuals collected
1	<i>Catla catla</i>	4
2	<i>Channa punctatus</i>	18
3	<i>Cirrhinus mrigala</i>	25
4	<i>Heteropneustes fossilis</i>	11
6	<i>Labeo rohita</i>	21
7	<i>Notopterus notopterus</i>	7
8	<i>Ompok bimaculatus</i>	8
9	<i>Puntius dorsalis</i>	10
10	<i>Puntius sophore</i>	16
11	<i>Oreochromis mossambicus</i>	8
Total no. of fishes collected		128

i. Lead

On an average, the lead level ranged between 4 and 7 ppm. The species, which ranked the highest in lead contamination, was *Oreochromis mossambicus* (7.31 ± 0.64 ppm) while the lowest accumulation was noticed in *Catla catla* (4.55 ± 0.32 ppm) (Fig.8.a). While 97 % of fishes had detectable levels of lead, the variation among the species was significant ($P < 0.01$) (Table.23).

ii. Zinc

Zinc concentration was the highest in *Ompok bimaculatus* (36.15 ± 8.20 ppm) and the least in *Catla catla* (13.25 ± 1.80 ppm) (Fig.8.b). Significant variation in contamination could be perceived ($P < 0.01$) among the species (Table.23).

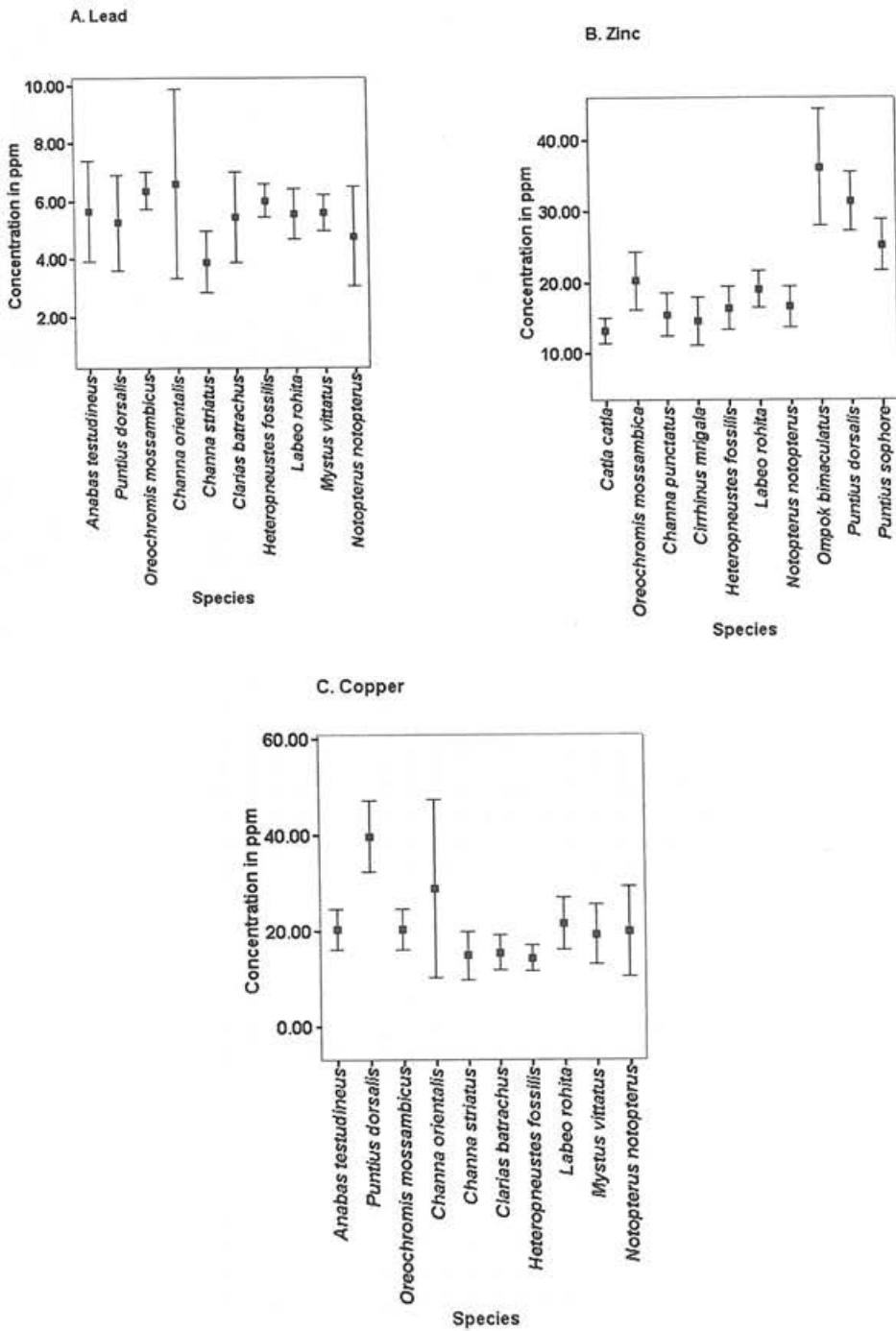
iii. Copper

Alike zinc levels, *Ompok bimaculatus* (36.79 ± 8.35 ppm) recorded the highest level of copper while *Catla catla* (13.48 ± 1.84 ppm) had the least (Fig.8.c). On an average, detectable levels of copper were found in 97.3% of the samples. Except *Ompok bimaculatus*, *Puntius dorsalis* and *Puntius sophore*, the copper values were detected in the range of 15-20 ppm. Significant variation in contamination could be established ($P < 0.01$) (Table.23).

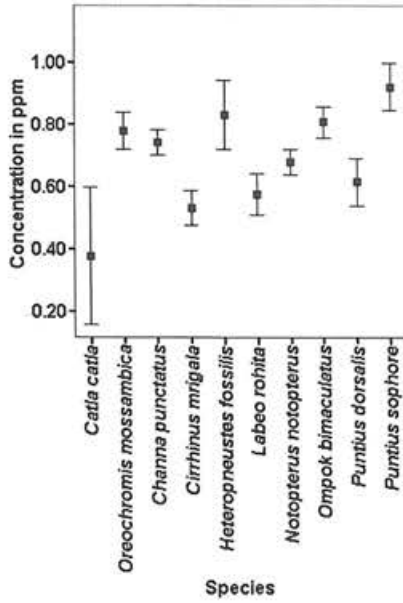
iv. Cadmium

Maximum level of cadmium was recorded in *Puntius sophore* (0.92 ± 0.07 ppm) followed by *Heteropneustes fossilis* (0.83 ± 0.11 ppm). Almost 95% of the samples measured appreciable levels of cadmium. *Catla catla* was found to accumulate the least level of cadmium (0.38 ± 0.22 ppm) (Fig.8.d) and the variation in accumulation among the species was significant ($P < 0.01$) (Table.23).

Fig.8 Levels of heavy metal contamination (ppm) among various species of fishes in the wetlands of Gujarat (Mean±S.E)



D. Cadmium



E. Chromium

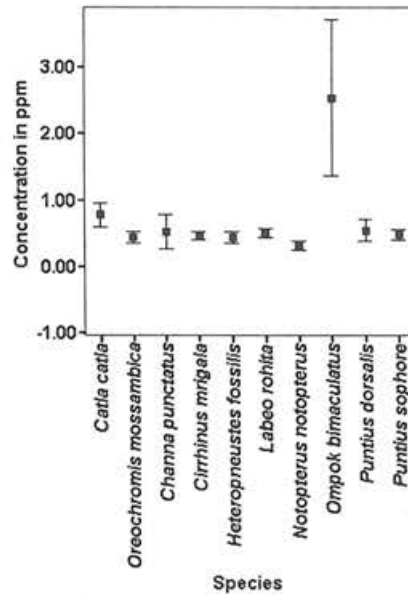


Table.23 Variation in metal contamination among various species of fishes in the wetlands of Gujarat (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	22.637	25.141	25.141	25.163	10.444
df	9	9	9	9	9
Asymp. Sig.	0.007*	0.003*	0.003*	0.003*	0.316

*P<0.01

v. Chromium

Ompok bimaculatus recorded the highest level (2.54 ± 1.18 ppm) of chromium and all the other species had levels less than 1 ppm. *Notopterus notopterus* recorded the lowest level of chromium (0.32 ± 0.06 ppm) (Fig.8.e). About 88% of the samples recorded appreciable levels of chromium. No significant variation in the metal accumulation ($P > 0.05$) (Table.23) was noticed.

7.4 DISCUSSION

7.4.1 Variation in heavy metal contamination among select wetlands of Gujarat

Lead, cadmium and chromium levels were found to be the highest in Deo Dam, Pariaj Dam and Dharoi Reservoir respectively, while zinc and copper concentrations were found to be the maximum in Vadhwana Reservoir.

It is learnt that many of the wetlands receive agricultural runoff and a few among them turn into agricultural fields once water is drained for irrigating crops cultivated nearby. Besides domestic sewerage, industrial operations also seem to contribute to contamination in many of the wetlands.

Further, the significant variation in metal contamination among the wetlands ($P < 0.05$) could be related to their contamination sources. Gujarat has seen considerable growth over the last couple of decades in agricultural and industrial sectors. According to a recent report (Vijayan *et.al*, 2004), the total number of registered industry in this state is 14,087. It is needless to mention that the unscientific growth in the above-referred sector ultimately leads to contamination of the aquatic environment.

On the whole in Gujarat wetlands, namely Deo Dam, Pariaj Dam, Dharoi Reservoir and Vadhavana Dam require continuous monitoring so that the temporal variations, if any can be documented and necessary correction measures adopted.

7.4.2 Variation in heavy metal contamination among select species of fishes in the wetlands of Gujarat

Among the species *Oreochromis mossambicus* had the highest load of lead while *Ompok bimaculatus* had the lowest concentration of zinc, copper and chromium. *Puntius sophore* recorded the maximum concentration of cadmium.

Ayyadurai *et.al*, (1994) investigated the heavy metals such as copper, iron, manganese, zinc and mercury present in water and Fin Fish, *Oreochromis mossambicus* during 1990-91 at three locations in River Cauvery, South India. The accumulation of these metals was maximum in liver as compared to other organs of the fish. The mean concentrations of copper, iron, manganese and zinc in the muscle were 1.28, 6.3, 0.86 and 6.36 mg/kg respectively while that of mercury was 0.065mg/kg which is below the reported toxic limit. The levels mentioned in the above stated study were comparably low with the present documented values.

The significant differences in contamination among the species are explicable in terms of their feeding habits. For example, *Oreochromis mossambicus*, an omnivorous fish recorded high concentration of lead than *Catla catla*, a plankton feeder. Moreover, *Catla catla* lives at the surface and *Oreochromis mossambicus* at the bottom. Hence, the contamination level in the food items would influence the contamination level in the fishes (Sastry and Shukla, 1994; Kotze *et.al*, 1999; Bervoets and Blust, 2003).

The levels recorded in the fishes of Gujarat are comparatively higher than the levels recorded elsewhere. For instance, the concentrations of copper (0.7 ppm), zinc (9 ppm) and chromium (3.13 ppm) reported in *Ompok bimaculatus* from Keoladeo National Park, Bharatpur, Rajasthan, India (Muralidharan, 1995) were found to be very low with the present values except chromium.

Further, the lead level (2.77 ppm) recorded in *Oreochromis mossambicus* collected from Kurichi Tank, Coimbatore, Tamil Nadu, India (Jayakumar, 2001) was found to be 2.5 times less than the level of lead documented in the present study.

Similarly, the level of cadmium (0.96 ± 0.56 ppm) recorded in *Puntius sophore* collected from Hoogly estuary, India (Kaviraj, 1989), were comparatively lower than the present levels. Assessment of heavy metal concentration in grass carp (*Ctenopharyngodon idella*) by Misra *et al*, (2002) revealed that lead (0.6 to 1.45 ppm), copper (0.37 to 1.02 ppm), chromium (0.46 to 1.18 ppm), cadmium (0.35 to 0.324 ppm) and zinc (0.8 to 1.17 ppm) concentrations measured in muscle of the fish are found to be low to cause any adverse effects to fishes. Further, the levels were within prescribed limits and found to be safe for human consumption.

High concentrations of copper and zinc have been observed in most of the species studied. It is needless to mention that chemical and physical processes at the sediment-water interface can potentially recycle copper and other metals back into the water column, thereby serve as a constant source of metal contamination in lakes (Belzile and Morris, 1995). Levels of copper and zinc recorded in the present investigation were comparable with the levels reported by Pyle *et.al*, (2005). In their study on the Yellow Perch collected from various lakes in the Sudbury area found that muscle Zn and Cu concentrations ranged from 23.5 to 276.9 ppm and 2.9 to 14.1 ppm respectively.

Papagiannis *et.al*, (2002) found Cu and Zn levels in muscle tissue to be significantly different between species. Being essential elements the differences probably reflect the range of requirement or tolerance, rather than humanly derived exposure. Moreover, species-dependent differences may be attributed to the life history pattern that influences their exposure to metals (including trophic levels and geographic distribution of life stages). Dietary habits may have this impact on species differences in metals concentrations. There are studies illustrating strong positive relationship between Zn concentrations in zooplanktonic fractions and fish (Timmermans *et.al*, 1992).

Thus, the contamination levels in fishes of Gujarat are comparably higher than the levels recorded elsewhere in India and abroad and hence, to be viewed with concern.

7.5 Suitability of fishes for human consumption in Gujarat

Dietary intake of metals by man through consumption of fishes was calculated for all the species and wetlands (Table 24, 25) as described in Chapter III.

Table.24 Average daily dietary intake of metals through consumption of fishes (mg) - Species wise

Species	Lead	Zinc	Copper	Cadmium	Chromium
<i>Catla catla</i>	0.16	0.47	0.48	0.01	0.03
<i>Channa punctatus</i>	0.22*	0.55	0.56	0.03	0.02
<i>Cirrhinus mrigala</i>	0.19	0.52	0.53	0.02	0.02
<i>Heteropneustes fossilis</i>	0.26*	0.58	0.59	0.03	0.02
<i>Labeo rohita</i>	0.18	0.68	0.69	0.02	0.02
<i>Notopterus notopterus</i>	0.22*	0.59	0.60	0.02	0.01
<i>Ompok bimaculatus</i>	0.22*	1.29	1.31*	0.03	0.09
<i>Puntius dorsalis</i>	0.21	1.12	1.14	0.02	0.02
<i>Puntius sophore</i>	0.22*	0.90	0.92	0.03	0.02
<i>Oreochromis mossambicus</i>	0.26*	0.72	0.74	0.03	0.02

The daily dietary intake of lead through the consumption of fishes, namely *Oreochromis mossambicus* (0.26 mg), *Heteropneustes fossilis* (0.26 mg) was found to be higher than other species. The lead input through consumption of *Channa punctatus*, *Notopterus notopterus*, *Ompok bimaculatus* and *Puntius sophore* was found to be the same (0.22 mg). The calculated input of zinc and copper appeared to be high through *Ompok bimaculatus* and *Puntius sophore*. The calculated dietary input of cadmium and chromium ranged from 0.01 to 0.03 mg and 0.01 to 0.09 mg respectively (Table. 24).

Table.25 Average daily dietary intake of metals through consumption of fishes (mg) - wetland wise

Wetlands	Lead	Zinc	Copper	Cadmium	Chromium
Ajwa Lake	0.25*	0.63	0.64	0.02	0.02
Dantiwada Dam	0.22*	0.99	1.01	0.02	0.01
Deb Dam	0.28*	0.59	0.61	0.02	0.01
Dharoi reservoir	0.20	1.14	1.16	0.03	0.06
Kanewal	0.18	0.24	0.24	0.03	0.03
Meshwo Dam	0.17	0.57	0.58	0.03	0.01
Nalsarovar Sanctuary	0.21*	0.82	0.83	0.03	0.02
Pariaj	0.21*	0.40	0.41	0.03	0.01
Saiyant	0.20	0.81	0.82	0.03	0.02
Sukhbadhar Dam	0.18	0.37	0.38	0.03	0.01
Wadhwana	0.20	0.92	0.94	0.01	0.03
WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption (mg)	0.50	10-15	1.30	0.05	0.4
WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limit

Of the various wetland fishes studied, the input of lead through the consumption of fishes from Deo Dam was found to be the maximum (0.28 mg/day/person) and minimum through Meshwo Dam (0.17 mg). The zinc and copper input was maximum in Dharoi reservoir and minimum in Kanewal. The cadmium and chromium input ranged from 0.01 to 0.03 mg and 0.01 to 0.06 mg respectively. The Dharoi Reservoir fishes appeared to be highest source of copper and zinc input. The input of lead, zinc, copper, cadmium and chromium was 0.20, 1.14, 1.16, 0.03 and 0.06 mg/day/person respectively (Table.25).

The calculated level of metal input through the consumption of wetland fishes was compared with the maximum tolerable dietary intake prescribed for human consumption by various statutory agencies. The lead input through the consumption of many of the study species exceeded the limits. The daily dietary intake of lead through fishes such as *Channa punctatus*, *Heteropneustes fossilis*, *Notopterus notopterus*, *Ompok bimaculatus*, *Puntius sophore* and *Oreochromis mossambicus* was found to be higher than the levels prescribed by WHO/FAO (0.21 mg per 60 kg Adult). Increased and regular consumption of these fishes will certainly inflict serious damages to kidney and liver of humans. Thus, it can be concluded that about 50% of the species examined for metal contamination appear unsafe for public consumption.

HEAVY METAL CONTAMINATION IN THE FISHES OF KARNATAKA WETLANDS

8.1 INTRODUCTION

Karnataka is a land of unparalleled natural beauty and bounty. It is situated on the western edge of the Deccan plateau with Maharashtra and Goa to its north, Kerala and Tamil Nadu to its south, Andhra Pradesh to its east and the Arabian sea to its west. Based on the soil characteristics, elevation and rainfall pattern, the state can be classified into the following ten agro-climatic zones; according to the Dept. of Agriculture, UAS, Karnataka: North east transition zone, North east dry zone, Northern dry zone, Central dry zone, Eastern dry zone, Southern dry zone, Southern transition zone, Northern transition zone, Hilly zone and Coastal zone.

This state exhibits sub-regional climatic variation within the tropical monsoonal zone. The average rainfall in the mainland is 2000-2540 mm while parts of the Kanara coast accounts for 7,620 mm and the Maiden North remains the driest with below 700 mm. Average temperature varies from 15.1 to 33.9°C.

About 1.19% (2,288.16 sq.km) of the land area in Karnataka is covered by inland wetlands of the size above 56.25 ha. The total number of inland wetlands was estimated at 622 in the entire state (SAC, 1998). The metal contamination was reported for twelve select wetlands (Map.5).

8.2 STUDY AREA

8.2.1 Bannur Heggere

It is located in the district Mandhya between 12°20'E and 76°53'N with a water spread area of 33 sq Km. This is a perennial lake and irrigates crops such as rice and sugarcane in the nearby area. Fishing is noticed in this wetland.

8.2.2 Heche Tank

This wetland has a water spread area of 70 acres and is located in the district Shimoga between 14°50'E and 74°22'N. The wetland area is approximated at 119.92 sq.km. About 25% decline in the overall fish yield has been observed due to fish disease. Although this wetland appears free from any industrial discharge it supports agricultural activities closeby where crops such as paddy, ginger, groundnut and cotton are grown. Rogor, kitagen, monocrotopos, copper sulphate and metacid were the commonly sprayed pesticides. Fertilizers such as urea, DAP, sulfates, MOP are reported to be applied to enrich the growth of crops. Eutrophication is a serious problem observed in this tank.

8.2.3 Karigala Kere

It is located in the district Mysore between 12°17'E and 76°14'35"N and have a water spread of 4 sq.Km. This seasonally cultivated wetland is reported to have gained approximately 25% increase in the fish yield over the years. Further, the wetland appears to be free from any industrial effluent discharge. Although this seasonally cultivated wetland is not utilized for any agricultural activity, cultivation of crops is observed in the nearby.

8.2.4 Cauvery River Stretch, Srirangapatna

This is located in the district Mandhya between 12°29'E and 76°11'N. No information is available on the fish yield. This wetland do receive effluent discharges but the quantum is not known. Agricultural activities are noticed in the closeby areas. Crops such as rice and sugarcane are grown predominantly. Pesticides such as rogor and pyridine are sprayed to control pests.

8.2.5 Krishnarajsagar Reservoir

It is located in the district Mysore between 12°18'18"E and 76°33'N with water spread area of 125 sq.Km. The 12,500 ha Krishnarajsagar reservoir was formed by

impounding the Kaveri river at Kannambadi in 1924. It is located at the confluence of three rivers, the Kaveri and its tributaries, the Hemavathi and the Lakshmanathirtha. It was one of the earliest large dams constructed in India and at present irrigates 0.4 million hectares of India in Mysore and Mandya districts of southern Karnataka. Large marshes form at the points where the rivers enter the reservoir. They come into existence every year as the reservoir fills up by September, and are used by thousands of migrant wader birds on passage. The marshes and mudflats expand as water is withdrawn from the reservoir for irrigation and they are at peak in December-February. Fields of millet, sorghum and vegetables are cultivated around the reservoir. The floodplain marshes are used in summer to cultivate vegetables. During monsoon, water lilies are cultivated for their edible roots. This seasonally cultivated wetland appears to be free from any industrial effluent discharge and shows an increase in fish yield (10%). Agricultural activities are observed at the vicinity where crops such as rice, green chilies and greens were grown. The marshes around the reservoir are used to grow vegetables in summer and this result in the rampant use of pesticides that permeate the food chain at various points.

8.2.6 Mandhakhalli Kere

It is located in the district Mysore (12°05'E and 76°43'N). Sewage inflow to the wetland is observed. This wetland is subjected to seasonal cultivation and crops such as rice are grown. Rogar and pyridine are the commonly applied pesticides.

8.2.7 Marchalli Kere

It is located in the district Mandhya (12°56'E and 76°50'N). An increase in the fish yield has been observed over the last 5-6 years. This wetland appears to be free from any industrial or domestic effluent discharge. Although this wetland is not seasonally cultivated, agricultural activities are observed closeby where crops such as rice are cultivated. Rogar and pyridine are the commonly sprayed pesticides and there are no incidences of fish or bird mortality in this wetland.

8.2.8 Ravanduru Kere

It is located in Chamarajanagar district between 12°06'E and 77°04'N. About 50% decline in the fish yield over the last 5-6 years has been recorded. Silting could be cited as the probable reason for the decline. This seasonally cultivated wetland appears to be free from any effluent discharge. Pyridine and rogar are the commonly sprayed pesticides.

8.2.9 Tumkur Ammanikere

It is located in the district Tumkur having a water spread area of 288 ha. Water is used for irrigation and it supports the nearby agricultural lands. Fishing is carried where fishes such as *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* are commercially exploited.

8.2.10 Salagaon Tank

It is located in Uttarakannada district between 14°55'E and 75°03'N. The water-spread area is 19 ha. A considerable decline in the fish harvest over the last 5-6 years has been noticed. The decline is attributed to epizootic ulcerative syndrome (EUS), mainly in species which are not commercially propagated, over harvest and fishing during breeding season. Enquiries with fisherman put the reduction percentage close to 60%. The notable species which suffered EUS are *Channa maurilius*, *Channa punctatus*, *Wallago attu*, *Mystus singhala* and *Aorichthys aor*. It is further reported that these species are not found in this wetland. Paddy, cotton, arecanut, coconut and mango are some of the crops cultivated nearby. Endosulphan, monocrotopos and nuvacron are some of the pesticides commonly applied.

8.2.11 Samunder Talab

It is located in district Raichur between 16°21'E and 77°18'N. The area of the wetland is approximately 124.85 sq.Km. This seasonally cultivated wetland is free from any industrial effluent discharges. Agricultural activities involving cultivation of crops such

as paddy, jowar and sunflower are observed in the vicinity. Endrin, endosulphan, ekalux are some of the notable pesticides sprayed in the nearby agricultural land. DAP and Urea are used as fertilizers to enrich the growth of crops. There are no instances of fish or bird mortality in this wetland.

8.2.12 Tailur Tank

It is located at Mysore district having a water spread area of 2.5 sq.km between 12°19'40"E and 77°05'56"N. This wetland appears free from any effluent discharge. Agricultural activities around the wetland are limited. Pyridine is found to be the widely applied pesticide. No incidence of fish or bird mortality is reported in this wetland.

8.3 RESULTS

Totally 150 fishes comprising thirteen species (Table.26) were collected from twelve wetlands in Karnataka and analyzed for metals, namely Pb, Zn, Cu, Cd and Cr.

Table.26 List of select wetlands examined in detail for metal contamination

S.No.	Name of the wetland	No. of fishes collected
1	Bannur Heggere	15
2	Heche	6
3	Karigala Kere	14
4	Cauvery River Stretch	13
5	Krishnaraj Sagar Reservoir	12
6	Mandhakhalli Kere	10
7	Marchalli Kere	10
8	Tumkur Ammanikere	26
9	Ravandur Kere	12
10	Salagaon Village Tank	6
11	Samunder Talab	11
12	Tailur Kere	15
Total no. of fishes collected		150

8.3.1 Variation in heavy metal contamination among select wetlands of Karnataka

i. Lead

Among the wetlands studied, fishes of Heche wetland (5.50 ± 0.23 ppm) in Shimoga district recorded the maximum concentration of lead. Near equal concentrations of lead were recorded in Salagaon Village Tank (5.32 ± 0.25 ppm) of Uttarakannada district, Madhakhalli (5.31 ± 0.26 ppm) of Mysore district, Ammanikere of Tumkur

(5.29 ± 0.23 ppm) and minimum was recorded in Marchalli Kere (3.96 ± 0.13 ppm) of Mandhya district. (Fig.9.a). Although, the level of lead was similar in the fishes of a five wetlands, the variation on the whole was significant among the wetlands ($P < 0.05$) (Table.27).

ii. Zinc

Samunder Talab of Raichur (17.47 ± 1.95 ppm) recorded the higher concentrations of zinc followed by Cauvery river stretch at Srirangapatna of Mandya district (15.34 ± 3.30 ppm). The lowest was measured in Mandhakhalli Kere (4.86 ± 1.27 ppm) of Mysore (Fig.9.b). Significant variation in zinc contamination among wetlands could be well established ($P < 0.01$) (Table.27).

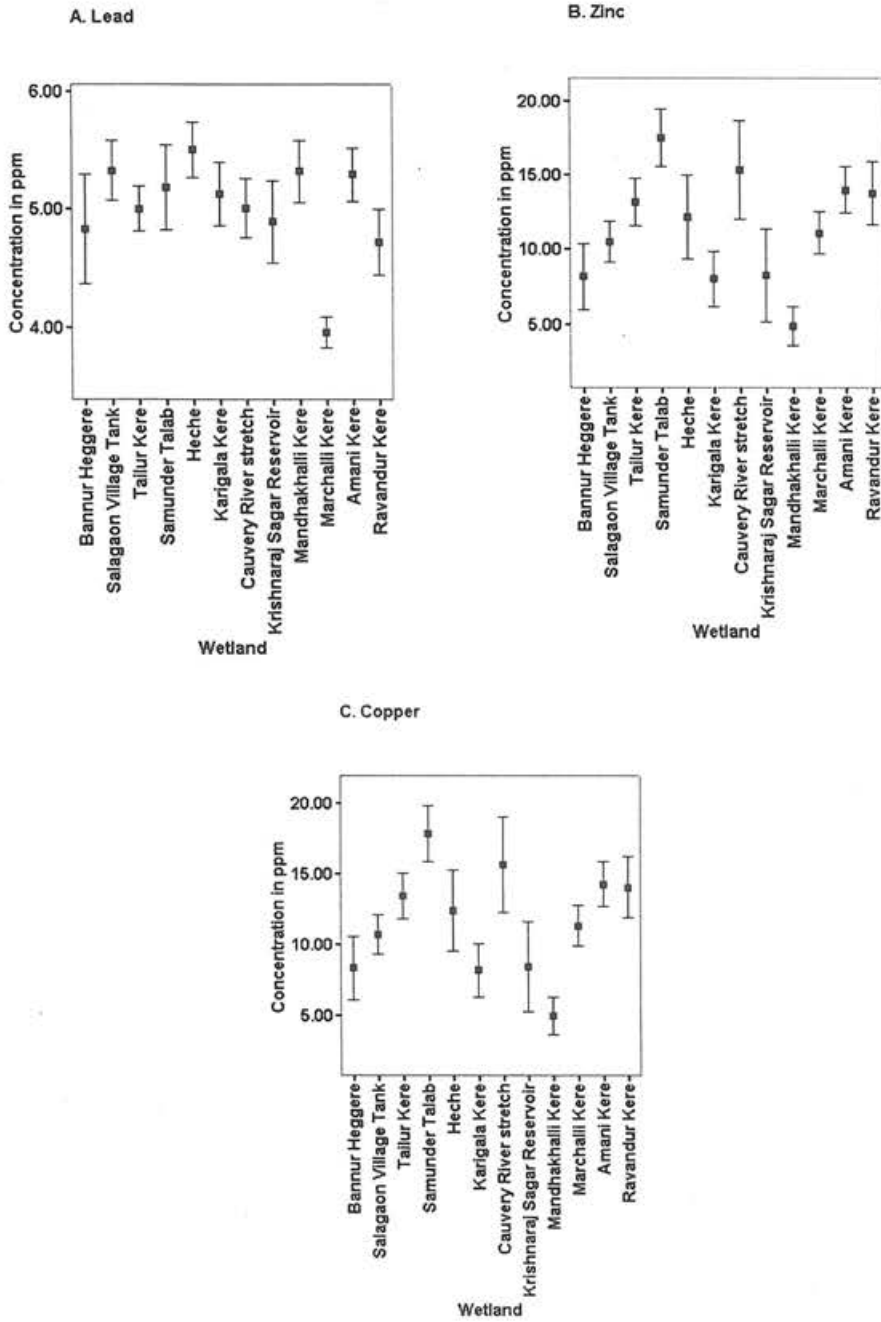
iii. Copper

On an average, alike zinc levels, fishes of Samunder Talab (17.78 ± 1.99 ppm) of Raichur district had the highest concentrations followed by Cauvery River stretch (15.61 ± 3.36 ppm) at Srirangapatna of Mandhya. Minimum concentrations were recorded in Mandhakhalli Kere of Mysore (4.94 ± 1.29 ppm) (Fig.9.c). The copper levels ranged between 8 and 15 ppm among the other wetlands thus establishing a significant variation in copper contamination ($P < 0.01$) among wetlands (Table.27).

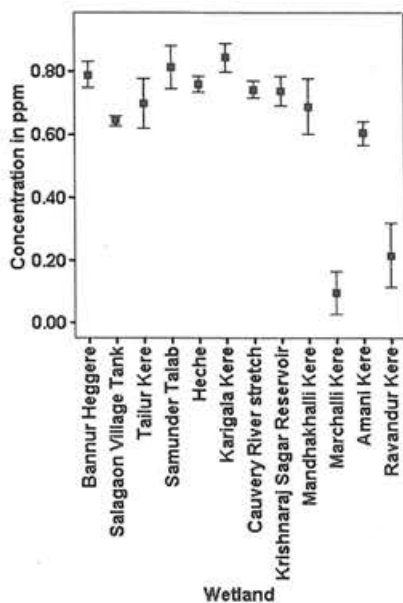
iv. Cadmium

The cadmium level was around 0.8 ppm in fishes of all the wetlands except in Marchalli Kere of Mandya (0.10 ± 0.09 ppm) and Ravandur Kere (0.25 ± 0.03 ppm) of Mysore district (Fig.9.d). The variation in cadmium contamination among the wetlands appeared to be significant ($P < 0.01$) (Table. 27).

Fig. 9 Levels of heavy metal contamination (ppm) among select wetlands of Karnataka (Mean±S.E)



D. Cadmium



E. Chromium

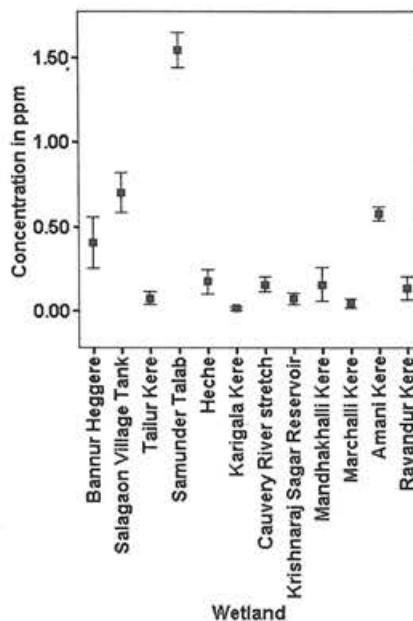


Table.27 Variation in heavy metal contamination among select wetlands of Karnataka (Kurskal Walis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	25.410	29.718	29.718	54.243	96.358
df	11	11	11	11	11
Asymp. Sig.	0.008*	0.002**	0.002**	0.000**	0.000**

* P<0.05; **P<0.01

v. Chromium

Low levels of chromium were detected in the fishes of Karigala Kere wetland (0.01 ± 0.00 ppm) of Mysore, Marchalli Kere (0.04 ± 0.01 ppm) of Mandya, Krishnaraj Sagar Reservoir (0.07 ± 0.02 ppm) of Mysore and Ammani Kere of Tailur (0.08 ± 0.02 ppm) district. Samunder Talab of Raichur recorded the maximum levels (1.54 ± 0.34 ppm) (Fig.9.e). Variation in contamination levels appear to be significant ($P < 0.01$) (Table.27).

8.3.2 Variation in heavy metal contamination among various species in the wetlands of Karnataka

Variation in the levels of contamination was viewed in thirteen species of fishes (Table.28) totaling 150 individuals.

Table.28 List of species examined for heavy metal contamination

S.No.	Name of the Species	No. of individuals collected
1	<i>Anguilla bicolor bicolor</i>	23
2	<i>Catla catla</i>	11
3	<i>Channa maurilius</i>	6
4	<i>Channa orientalis</i>	3
5	<i>Channa punctatus</i>	3
6	<i>Channa striatus</i>	11
7	<i>Cirrhinus mrigala</i>	7
8	<i>Clarias batrachus</i>	8
9	<i>Cyprinus carpio</i>	11
10	<i>Heteropneustes fossilis</i>	8
11	<i>Labeo rohita</i>	16
12	<i>Ompok bimaculatus</i>	4
13	<i>Oreochromis mossambicus</i>	40
Total no. of fishes collected		150

i. Lead

About 98% of the fishes analyzed, detected appreciable concentrations of lead. Maximum concentration was observed in *Channa punctatus* (5.85 ± 1.16 ppm) followed by *Channa striatus* (5.79 ± 0.26 ppm) (Fig.10.a). Although the average levels recorded in other species were around 5 ppm, significant variation in contamination could be established ($P < 0.05$) (Table.29).

ii. Zinc

The highest level of zinc was observed in *Channa striatus* (17.78 ± 9.05 ppm) followed by *Labeo rohita* (17.43 ± 2.40 ppm). Lowest concentration was recorded in *Ompok bimaculatus* (1.00 ± 0.22 ppm) (Fig.10.b). All the species significantly differed ($P < 0.01$) among themselves in zinc contamination (Table.29).

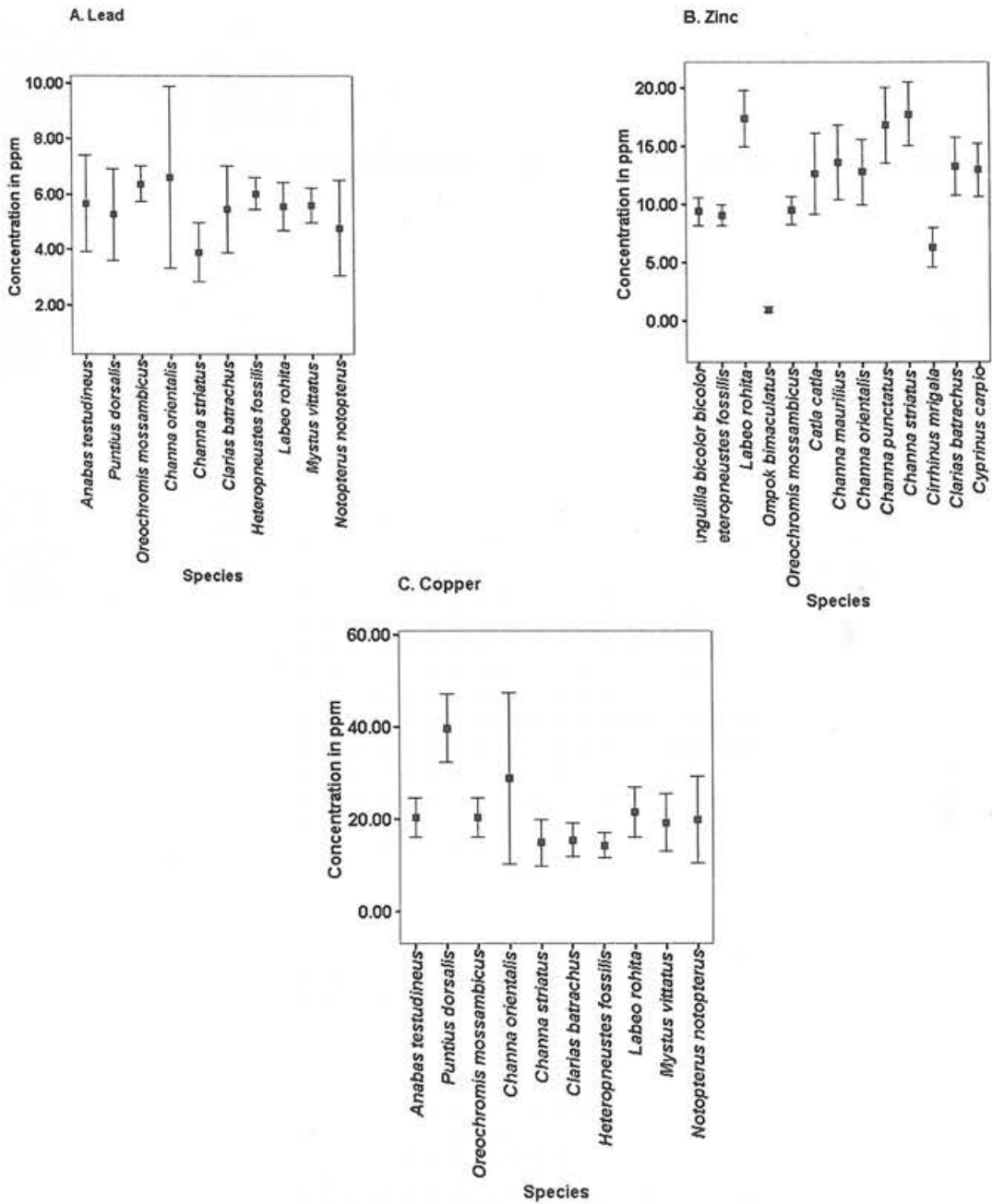
iii. Copper

Alike zinc levels *Ompok bimaculatus* recorded the least level of copper (1.02 ± 0.22 ppm) which was 18 folds lower than the levels recorded by *Channa striatus* (18.10 ± 2.78 ppm) (Fig.10.c). Significant variations in contamination among the species could be well established ($P < 0.01$) (Table.29).

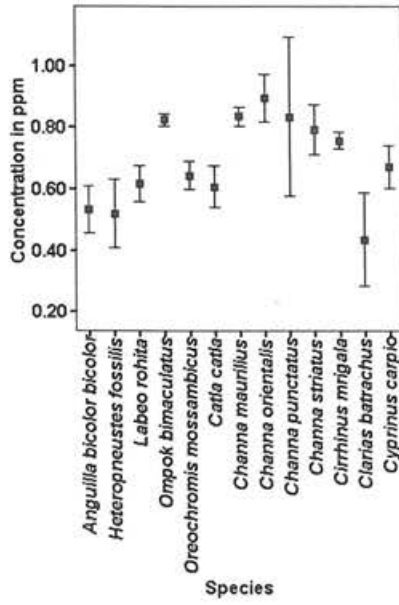
iv. Cadmium

On an average, *Channa orientalis* (0.89 ± 0.08 ppm) recorded the highest level followed by *Channa maurilius* (0.84 ± 0.03 ppm) and *Channa punctatus* (0.83 ± 0.25 ppm) (Fig.10.d). While the level of cadmium in the other species was between 0.4 and 0.7 ppm, on the whole the level of cadmium among species was significantly different ($P < 0.05$) (Table.29).

Fig.9 Levels of heavy metal contamination (ppm) among various species of fishes in the wetlands of Karnataka (Mean±S.E)



D. Cadmium



E. Chromium

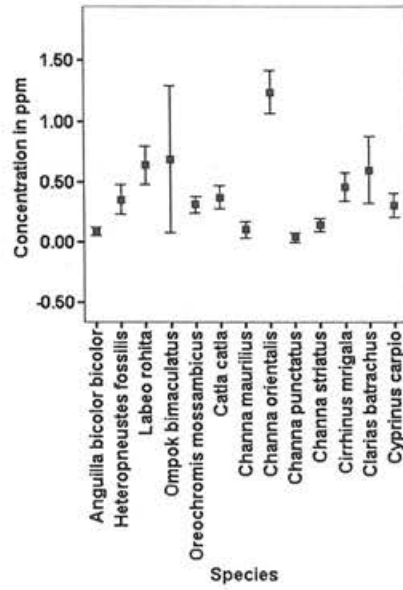


Table.29 Variation in heavy metal contamination among various species of fishes in the wetlands of Karnataka (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	23.539	31.813	31.813	24.247	32.980
df	12	12	12	12	12
Asymp. Sig.	0.023*	0.001**	0.001**	0.019*	0.001**

*P<0.05;** P<0.01

v. Chromium

About 31% of the samples analyzed showed detectable concentrations of chromium. Among these significant variations in contamination levels could be obtained ($P < 0.01$) (Table.29). *Ompok bimaculatus* (0.67 ± 0.59 ppm) recorded the highest concentrations followed by *Labeo rohita* (0.64 ± 0.16 ppm) while *Channa punctatus* (0.04 ± 0.03 ppm) the least (Fig.12.e).

8.4 DISCUSSION

8.4.1 Variation in heavy metal contamination among select wetlands of Karnataka

Lead concentrations were found to be the highest in Heche while the other metals were in Samunder Talab. Significant variation in metal contamination among the wetlands could be well established ($P < 0.05$).

One of the ecological services rendered by the wetlands is to support the agricultural activities in the nearby area. In return, the wetland receives agricultural leachate containing appreciable quantum of fertilizers and pesticides.

None of the wetlands studied presently in Karnataka escapes from the receipt of agricultural drain. In fact, many wetlands act as a convenient sink for discharges leading to eutrophication and decreased fish yield owing to infectious disease (Vijayan *et.al*, 2004).

In the present study, Heche wetland of Shimoga district suffered from Eutrophication and the fishes of this wetland were reported to suffer from undefined infectious disease to an extent of wiping out certain species. Further, Epizootic Ulcerative Syndrome disease is prevalent in this wetland. As a result, the fish yield has declined to a considerable extent (40%).

Of all the wetlands in Karnataka, Heche and Samunder Talab require further monitoring for effective conservation management.

8.4.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Karnataka

Most of the *Channa sp.*, had the maximum concentrations of all the metals. The levels recorded in these species are comparably higher than the levels recorded elsewhere in Indian context. Although there are many studies on the metal contamination in fishes in India (Muralidharan, 1995; Pandey *et.al*, 1995; Sadhukhan *et.al*, 1996; Mwachire and Durve 1997; Sultana and Rao, 1998; Jayakumar, 2001; Misra *et.al*, 2002) and abroad (Kotze *et. al*, 1999; Al-Yousuf and El-Shahawi, 1999; Kalay *et.al*, 1999; Al-Saleh and Shinwari, 2002), it is very difficult to compare the magnitude of contamination. It is largely because the species referred are different and so also the level of contamination and sources.

Surface dwellers such as *Cyprinus carpio* and *Catla catla*, subsurface dwellers, namely *Labeo rohita* had levels of all metals below detection limits when compared with *Channa sp.* which is a bottom dweller. Significant variation in their metal levels could be related to their feeding habits ($P < 0.05$). Further the accumulation of metal toxicants from the aqueous environment by fishes depend upon the availability and persistence of the contaminants in water (George and Olsson, 1994), the duration and level of exposure (Muralidharan, 1995), dissolved humic substances, alkalinity, pH and hardness of the water (Langston, 1990; Roesijadi and Robinson, 1994; Kotze *et. al*, 1999), species differences (Kotze *et. al*, 1999), and physiological mechanisms involved (Al-Yousuf and El-Shahawi, 1999; Kalay *et.al*, 1999).

Further, in eutrophicated wetlands such as Heche, metal contaminants normally get adsorbed to the roots of hydrophytes and the algal blooms lock these contaminants in their cellular matrix. Thus, it is logical to postulate that fishes such as *Channa species*, which feed on the dead algal mats, should have accumulated the maximum.

8.5 Suitability of fishes for human consumption in Karnataka

Dietary intake of metals by man through consumption of fishes was calculated for all the species and wetlands (Table.30, 31)

Table.30 Average daily dietary intake of metals through consumption of fishes (mg) - species wise

S. No.	Species	Lead	Zinc	Copper	Cadmium	Chromium
1	<i>Anguilla bicolor bicolor</i>	0.17	0.34	0.34	0.02	0.00
2	<i>Catla catla</i>	0.19	0.45	0.46	0.02	0.01
3	<i>Channa maurilus</i>	0.18	0.49	0.50	0.03	0.00
4	<i>Channa orientalis</i>	0.18	0.46	0.47	0.03	0.04
5	<i>Channa punctatus</i>	0.21*	0.60	0.61	0.03	0.00
6	<i>Channa striatus</i>	0.21*	0.64	0.65	0.03	0.00
7	<i>Cirrhinus mrigala</i>	0.21*	0.23	0.23	0.03	0.02
8	<i>Clarias batrachus</i>	0.16	0.48	0.48	0.02	0.02
9	<i>Cyprinus carpio</i>	0.19	0.46	0.47	0.02	0.01
10	<i>Heteropneustes fossilis</i>	0.17	0.32	0.33	0.02	0.01
11	<i>Labeo rohita</i>	0.16	0.62	0.63	0.02	0.02
12	<i>Ompok bimaculatus</i>	0.17	0.04	0.04	0.02	0.02
13	<i>Oreochromis mossambicus</i>	0.18	0.34	0.35	0.02	0.01

The maximum input of lead (0.21 mg) was calculated to be through consumption of *Channa punctatus*, *Channa striatus* and *Cirrhinus mrigala* while the minimum input was through *Labeo rohita* and *Clarias batrachus*. (0.16 mg). The maximum calculated input of zinc (0.65 mg) and copper (0.65 mg) was found to be through consumption of *Channa striatus*. The calculated input of cadmium was found to be almost the same in all the species analyzed while the chromium input ranged between 0.01 and 0.04 mg/day/person.

Table.31 Average daily dietary intake of metals through the wetland fishes (mg) -wetland wise

Wetlands	Lead	Zinc	Copper	Cadmium	Chromium
Bannur Heggere	0.17	0.29	0.30	0.03	0.01
Heche	0.20	0.43	0.44	0.03	0.01
Karigala Kere	0.18	0.29	0.29	0.03	0.00
Cauvery River Stretch	0.18	0.55	0.56	0.03	0.01
Krishnaraj Sagar Reservoir	0.17	0.30	0.30	0.03	0.00
Mandhakhalli Kere	0.19	0.17	0.18	0.02	0.01
Marchalli Kere	0.14	0.40	0.40	0.00	0.00
Ammani Kere	0.19	0.50	0.51	0.02	0.00
Ravandur Kere	0.17	0.49	0.50	0.01	0.00
Salagaon Village Tank	0.19	0.38	0.38	0.02	0.02
Samunder Talab	0.18	0.62	0.64	0.03	0.05
Tailur Kere	0.18	0.47	0.48	0.02	0.00
WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption	0.50	10-15	1.30	0.05	0.4
WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limit

Of all the thirteen wetlands studied, lead input through the consumption of fishes from Heche (0.20 mg) was found to be the highest and Marchalli Kere (0.14 mg) the lowest. The maximum input of zinc was found to be higher in Samunder Talab (0.62 mg) followed by Cauvery River Stretch (0.55 mg) and Ammani Kere (0.50 mg), while minimum through Mandhakhalli Kere (0.17 mg). Copper input appeared to be almost the same in most of the wetlands studied with input ranging between 0.18 and 0.64 mg. The input of cadmium and chromium were comparatively lower than the other metals.

The calculated input values were compared with the guidelines stipulated by statutory agencies for human safety. The results (Table. 31) revealed that the calculated dietary lead intake through consumption of *Channa punctatus*, *Channa striatus* and *Cirrhinus mrigala* appeared to be equal to the lead level prescribed by Joint Expert Committee on Food and Agriculture (JECFA) of United Nations (UN) and WHO (1989, 1993) which admit only up to 0.21 mg/day for a 60 Kg adult. As referred to elsewhere

in this report, increased and regular consumption may pose serious problems. Although, the calculated dietary input of metals through these wetlands fishes appeared to be safe, the fishes of Cauvery river stretch, Ammanikere and Samunder Talab show comparatively higher input than the others. According to WHO/FAO (1972) the tolerable daily intake of copper, lead, zinc, cadmium and chromium is 1.3 mg, 0.50 mg, 10-15 mg, 0.05 mg and 0.4 mg respectively through food and water. However, if the per capita consumption increases, even the present levels may pose problems.

HEAVY METAL CONTAMINATION IN THE FISHES OF MAHARASHTRA WETLANDS

9.1 INTRODUCTION

Maharashtra, literally meaning the great state, with its rich biodiversity is a huge irregular triangle with its base facing the Arabian Sea. Physiographically the state may be divided into four natural divisions – the coastal strip (the Konkan), the Sahyadri or the Western Ghats, the Deccan plateau and the forests of North Maharashtra. The Konkan consisting of undulating coastal low lands is 720 km long and 30-80 km broad. North Konkan has vast stretch of hinterlands. The Western Ghats run almost parallel to the sea coast. The average height of the Sahyadri is 1200 m.

Maharashtra receives its rainfall mainly from southwest monsoon, and it varies considerably in the coastal region (2000 mm) scanty rains in rain shadow areas in the central part (500 mm) to moderate rains in eastern parts (1,000 mm) of the state. The average temperature varies from 20.1 to 33.3°C.

About 0.93% of the land area in Maharashtra is covered by wetlands of the size 56.25 ha and above. The total number of wetlands was estimated at 609, which cover an area of 2,849.42 sq.km in the entire state (Vijayan *et.al*, 2004). In the present investigation, five wetland have been selected towards documenting the heavy metal contamination (Map.7).

9.2 STUDY AREA

9.2.1. Bhigwan

It is located in the district Pune between 18°19'E and 74°46'N and the water spread area is about 300 ha. It offers excellent habitat for a wide variety of flora and fauna. It harbors 31 species of fishes. It is understood that local fishermen are concerned about declining fish population due to industrial pollution. Further,

over harvesting of fish from this area could also be responsible for decreasing fish abundance to a great extent. Mostly agricultural land surrounds the wetland. The major crops grown in the area are sugarcane, wheat, jowar and vegetables. This wetland has been used for grazing, fishing, irrigation and social forestry operations and drinking/wallowing for cattle. Incidents of heavy fish mortality were reported in the past. Agricultural runoff in addition to industrial effluents brings in contamination to the wetland.

9.2.2. Ambazari Lake

It is located in the district Nagpur between 21°00'E and 79°00'N and has a water spread area of 15 sq km. About 40% decline in fish harvest has been recorded over the last 5-6 years. The wetland do receive industrial effluents through varied sources. This wetland is also subjected to seasonal cultivation where crops such as rice is grown in the vicinity.

9.2.3 Pimpalwadi

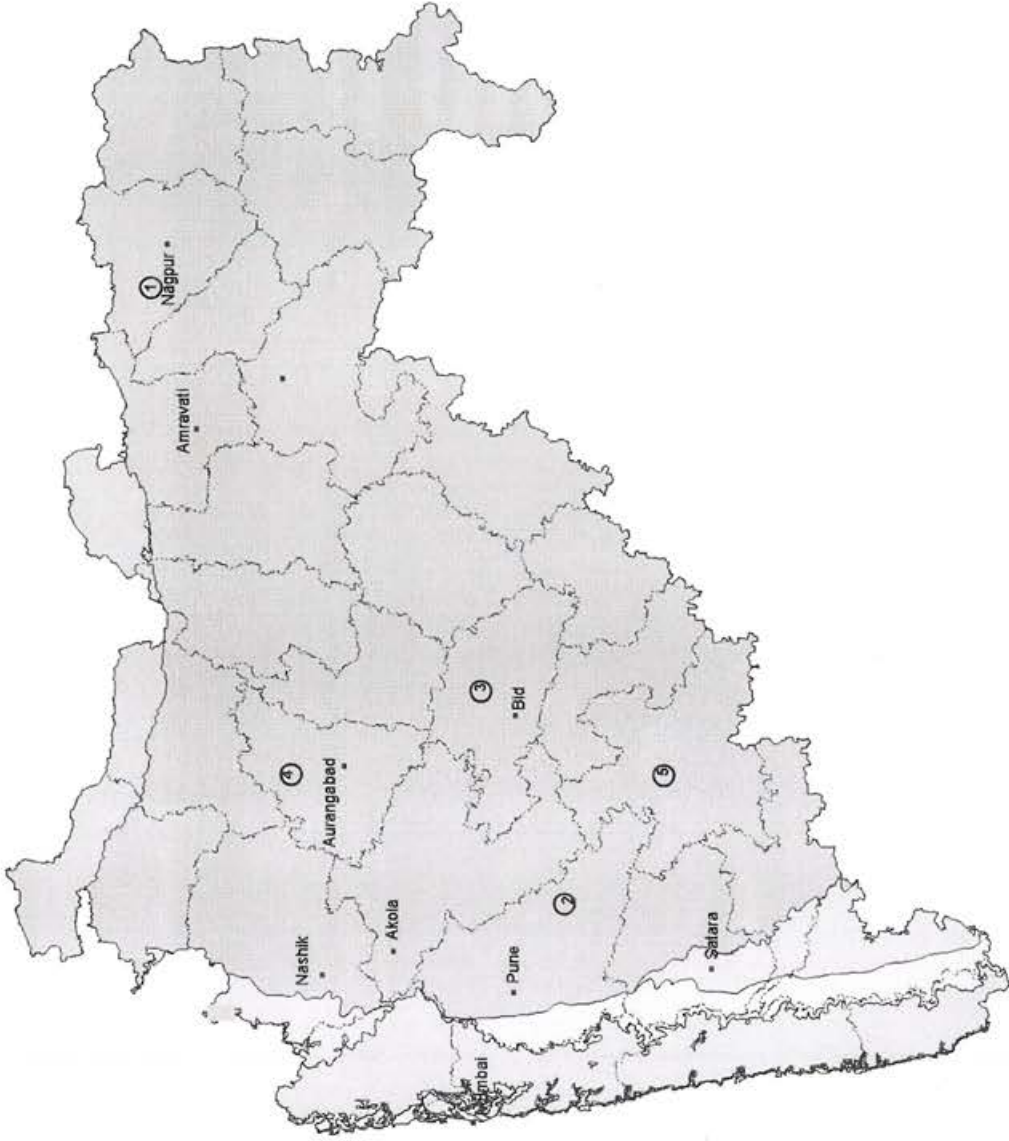
It is located in the district Beed between 19°03'E and 75°57' N and has a water spread area of 25 sq km. This wetland does not receive any effluent discharge. No agricultural activities are witnessed in the surroundings.

9.2.4 Jaikwadi

It is located in the district Aurangabad between 19°31'30"E and 75°13'50"N. The area is approximately 34.105 ha. This is a man-made reservoir that was created after the construction of a dam in 1975 on the upper reaches of River Godavari. In the absence of natural depressions and hilly terrain, this dam has been constructed on almost flat land, because of which the impounded water spread is large, approximately 55 km long and 27 km wide. This shallow water spread, with a receding water line is very attractive to a large number of waterfowl and waders. Fishing is the biggest problem as far as the sanctuary is concern. According to local fishermen, fish harvest has declined by about 20% over the

Map.6 Location of select wetlands studied in Maharashtra

1. Ambazari Lake
2. Bhigvan
3. Pimpalevadi
4. Jaikwadi
5. Kambal talav



last few years mainly due to no inputs of fish seeds and less rainfall. There are 85 village settlements surrounding the wetland with a population of approximately two lakhs. The dam water is used for irrigation in Ahmednagar and Aurangabad districts. There is an industrial area located nearby where industries such as sugar, paper mill, poultry and pharmaceuticals are located. Major alcohol and beer industries are also located here.

9.2.5 Kambal Talav

It is located at the district Sholapur between 17°45'E and 75°30'N. The area is approximately 1.07 ha. Due to increased effluent discharges from dyeing industry and domestic sectors an 80% reduction in fishes has been reported over the years. Further, dumping of solid waste is also noticed in this wetland. No agricultural practices are witnessed around the lake. Activities such as washing of clothes, utensils, vehicles and cattle are carried out by the slum dwellers. They also use it for sanitary purposes.

9.3 RESULTS

Altogether forty three fishes comprising five species were collected from five wetlands (Map.6) and analyzed for heavy metal contamination (Table.32).

Table.32 List of select wetlands examined for heavy metal contamination

S.No.	Wetland	No. of fishes collected
1	Ambazare Lake	8
2	Bhigwan	6
3	Pimpalwadi	10
4	Jeyakwadi	15
5	Kambal Talav	4
Total no. of fishes collected		43

9.3.1 Variation in heavy metal contamination among select wetlands of Maharashtra

i. Lead

The lead levels were around 5 ppm in all the fishes of the other wetlands except Kambal Talav (6.18 ± 2.16 ppm) (Fig.11.a). No significant variation in the contamination levels could be observed ($P > 0.05$) (Table.33).

ii. Zinc

Zinc concentrations were detected in almost all the samples. While Kambal Talav (18.08 ± 3.87 ppm) of Sholapur district recorded the maximum concentration, Pimapalwadi (10.89 ± 2.44 ppm) of Aurangabad showed the minimum levels (Fig.11.b). No significant variation in contamination could be established among the wetlands ($P > 0.05$) (Table.33).

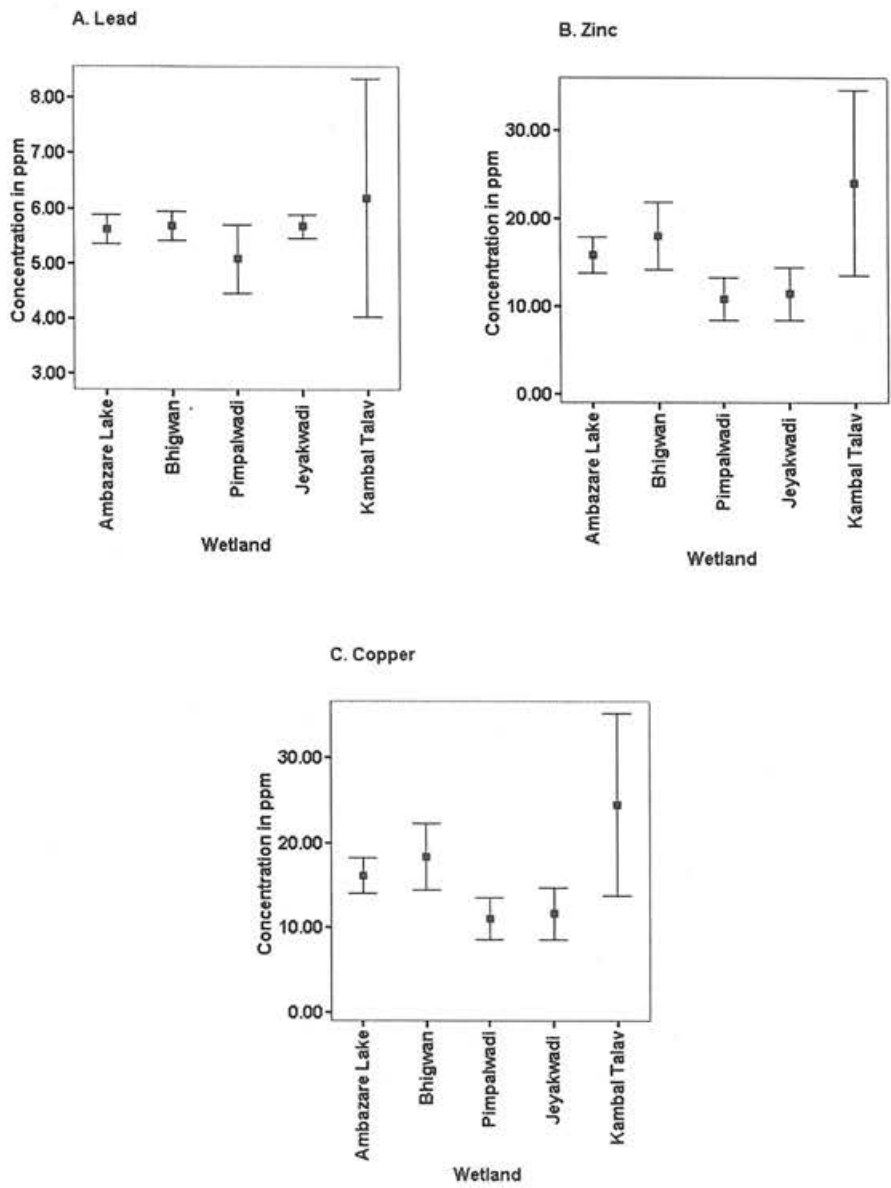
iii. Copper

Alike zinc, levels measured maximum in Kambal Talav (24.51 ± 10.69 ppm) of Sholapur district. Fishes of Pimpalwadi (11.08 ± 2.43 ppm) had the least levels (Fig.11.c). Further, the variation in contamination among the wetlands were also found to be not significant ($P > 0.05$) (Table.33).

iv. Cadmium

On an average, the maximum cadmium levels were recorded in Ambazare Lake (0.89 ± 0.13 ppm) of Nagpur district while the fishes of other wetlands were around 6 ppm (Fig.11.d). Significant variation in heavy metal contamination ($P < 0.05$) could be observed (Table.33).

Fig.11 Levels of heavy metal contamination (ppm) among select wetlands of Maharashtra (Mean±S.E)



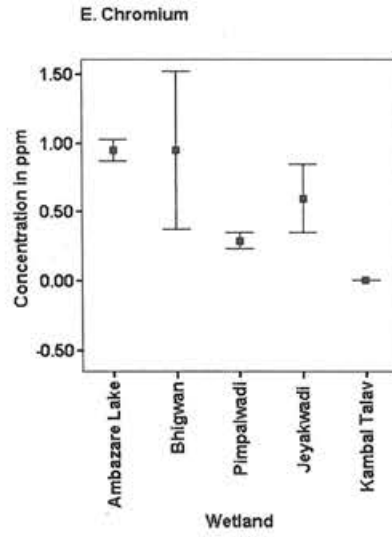
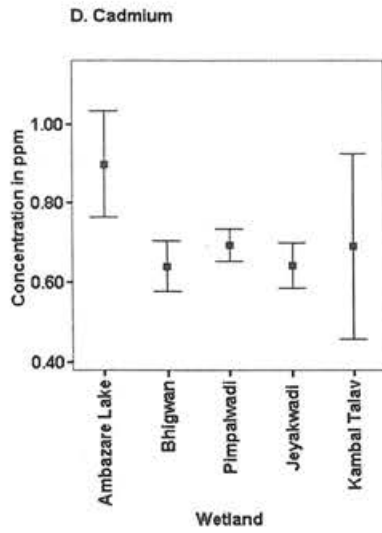


Table.33 Variation in heavy metal contamination among select wetlands of Maharashtra (Kurskal Walis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	2.571	5.930	5.930	9.863	19.775
df	4	4	4	4	4
Asymp. Sig.	0.632	0.204	0.204	0.043*	0.001**

*P<0.05; **P<0.01

v. Chromium

The average chromium levels in the fishes of wetlands ranged between BDL and 0.95 ± 0.008 ppm in Kambal Talav and Ambazare Lake respectively (Fig.11.e). Significant variation in metal contamination levels could be observed ($P < 0.01$) (Table.33).

9.3.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Maharashtra

Altogether forty three individuals comprising eight species were examined for heavy metal contamination (Table. 34).

Table.34 List of species examined for heavy metal contamination

S.No.	Species	No. of individuals
1	<i>Anguilla bengalensis</i>	5
2	<i>Channa punctatus</i>	5
3	<i>Channa striatus</i>	4
4	<i>Cyprinus carpio</i>	5
5	<i>Labeo rohita</i>	6
6	<i>Mystus vittatus</i>	7
7	<i>Notopterus notopterus</i>	6
8	<i>Oreochromis mossambicus</i>	5
Total no. of fishes collected		43

i. Lead

Among the species, *Oreochromis mossambicus* recorded the maximum concentrations of lead (6.62 ± 0.94 ppm) while *Labeo rohita* the minimum concentrations (4.65 ± 0.94 ppm) (Fig.12.a). No significant variation in lead contamination could be identified ($P > 0.05$) among the wetland fishes (Table.35).

ii. Zinc

On an average, zinc levels were found highest in *Oreochromis mossambicus* (24.95 ± 7.94 ppm) followed by *Notopterus notopterus* (21.94 ± 1.64 ppm) while *Labeo rohita* the lowest (6.74 ± 4.28 ppm) (Fig.12.b). No significant variation in metal contamination could be observed among the species ($P > 0.05$) (Table.35).

iii. Copper

Alike zinc levels *Oreochromis mossambicus* (24.95 ± 7.94 ppm) and *Labeo rohita* (6.86 ± 4.36 ppm) had the highest and lowest copper levels respectively. (Fig.12.c). No significant variation could be observed among the fishes studied ($P > 0.05$) (Table.35).

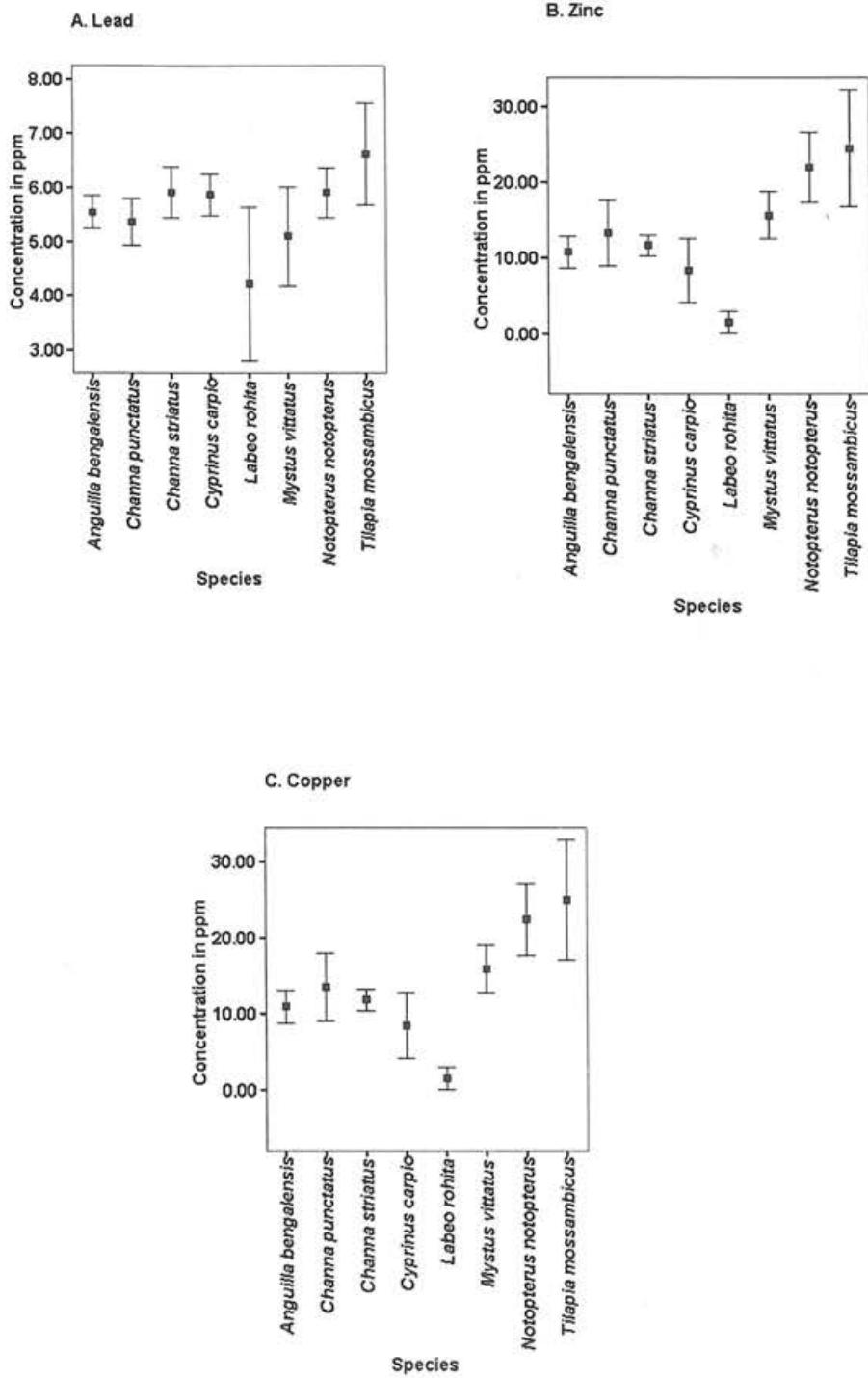
iv. Cadmium

On an average, most of the species except *Cyprinus carpio* (0.55 ± 0.16 ppm) had an average level of 0.8 ppm of cadmium (Fig.12.e). No significant variation in contamination could be observed among the species ($P > 0.05$) (Table.35).

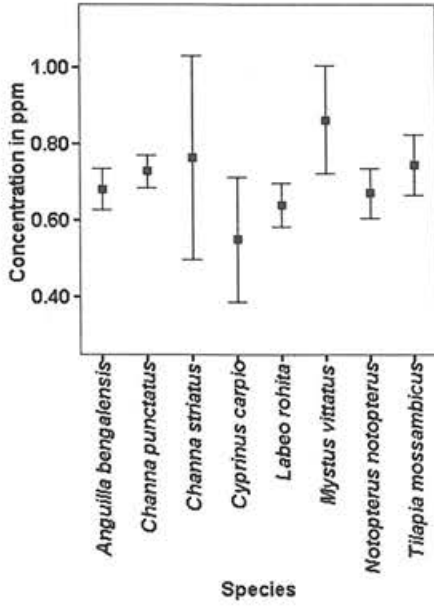
v. Chromium

Of the 97% samples measuring detectable levels of chromium, least levels were reported in *Cyprinus carpio* (0.08 ± 0.05 ppm) while the highest concentrations were found in *Labeo rohita* (1.49 ± 0.73 ppm) followed by *Channa striatus* (1.02 ± 0.14 ppm) (Fig.12.e). Significant variation in contamination could be observed among species ($P < 0.05$) (Table.35).

Fig.12 Variation in metal contamination (ppm) among various species of fishes in the wetlands of Maharashtra (Mean±S.E)



D. Cadmium



E. Chromium

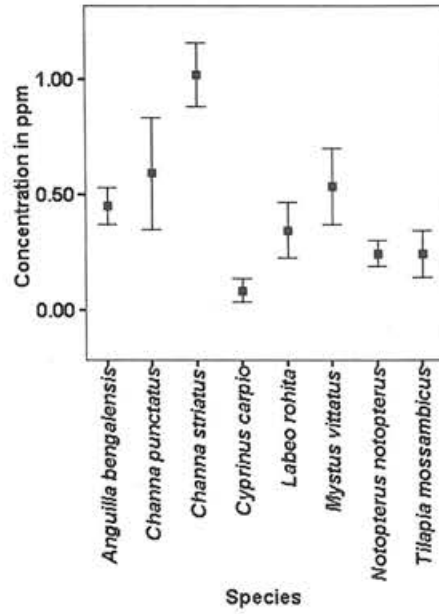


Table.35 Variation in heavy metal contamination among various species of fishes in the wetlands of Maharashtra (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	2.754	12.421	12.421	9.877	17.580
df	7	7	7	7	7
Asymp. Sig.	0.907	0.088	0.088	0.196	0.014*

*P<0.05

9.4 DISCUSSION

9.4.1 Variation in heavy metal contamination among select wetlands of Maharashtra

In all the studied wetlands, lead concentration was around 5 ppm obviously with no significant variation. This shows most of the wetlands suffer with an uniform level of contamination. From the pollution details, it has been inferred that wetlands such as Ambazare Lake receives industrial effluent discharge. Although no data is available on the quantum of effluent discharged, these wetlands are used for solid waste disposal and activities such as washing of clothes, utensils, vehicles and cattle by the surrounding locality.

It is interesting to note that zinc and copper concentrations in all the wetlands are near equal which could be related to the metals' synergistic relationship (Streit, 1992) and hence it is not surprising to note the absence of significant variation in contamination among wetlands.

Ambazare Lake had the maximum levels of cadmium and chromium and the variation was significant among the wetlands. Most of these wetlands witness dumping of solid waste and it had even resulted in 30 to 40% reduction in fish harvest according to the local fisherman.

It is important to note that metals do bear a complex interaction with various abiotic and biotic compartments of a wetland ecosystem and thus understanding and predicting the significance of metal concentrations remain as a great challenge (Rainbow, 1993; Wang, 2002).

9.4.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Maharashtra

Among the species, *Oreochromis mossambicus* recorded the maximum concentration of lead, zinc and copper. *Labeo rohita* recorded the highest concentrations of chromium. Significant variation could be found only in chromium which makes us to infer that fishes have a varied degree of exposure to metal contamination. Most of the species had near equal concentrations of cadmium. Accumulation of heavy metals in fishes is influenced by water quality parameters such as hardness and acidity. Moreover, the life cycle of an organism is also an important consideration in examining the effects of heavy metals, as the concentration of heavy metals in body tissues vary with age or size of the organism (Atchinson *et.al*, 1977; Chernoff and Dooley, 1979).

Jayakumar (2001) recorded 2.7 ppm of lead concentration in *Oreochromis mossambicus* collected from Kurichi tank, Coimbatore, India and the levels are treated as background concentrations. Lin *et.al*, (2005) documented the trace elements in farm cultured *Oreochromis mossambicus* in Southern Taiwan. On an average the lead, copper and zinc concentrations were 0.19, 1.34 and 10.79 ppm respectively, and the levels were considered as safe for public consumption. However, the present study shows high levels when compared with the above study and are anticipated to have an impact which needs to be addressed.

Species differences in metal accumulation could be ascribed to differences in feeding habits and behavior of the species. *Oreochromis mossambicus* feeds primarily on plant material and detritus. Juveniles take many small crustaceans but mainly feed on algae, especially unicellular diatoms. Larger specimens feed on filamentous green algae and adults also ingest aquatic insects, crustaceans, earth worms, small fish as well as bottom sludge rich in organic matter. Species such as *Channa punctatus* and *Channa striatus* are omnivorous scavengers and predators of other fish and are mainly confined to the bottom (especially when muddy). Metals such as copper and zinc are known to have an effect and also to be accumulated by aquatic biota such as fish and crustaceans (Zou and Bu,



1994) and aquatic plants. Although aquatic plants, algae and invertebrate, generally seem to be more resistant to copper than fish (Alabaster and Lloyd, 1980) they all contribute to the diet of the species sampled. Large amount of silt are also taken in with the food sources by species such as *Channa punctatus*. Silt particles play an important role in the transport and availability of metals as they are adsorbed onto the silt particles (Heath, 1987). Most metals are furthermore known to become more bioavailable and have increased toxicity with decreasing pH (Shaw and Brown, 1973). The pH in the stomach of *Oreochromis mossambicus* decreases significantly (from above 6 to as low as 2.9) after feeding commences. This reduction in pH could thus result in metals becoming more bioavailable from the food sources and silt in the stomach. The metals could therefore be taken up via the intestine, causing increased metal levels in the fish that could result in bioaccumulation (Kotze *et.al*, 1999). Thus in species namely *Oreochromis mossambicus* and *Channa punctatus* the metals are likely to be more bioavailable and could produce an impact to the fish.

9.5 Suitability of fishes for human consumption in Maharashtra

Dietary intake of metals by man through consumption of fishes was calculated for all the species and wetlands (Table 36, 37) as described in Chapter III.

Table. 36 Average daily dietary intake of metals through consumption of fishes (mg) - Species wise

Species	Lead	Zinc	Copper	Cadmium	Chromium
<i>Anguilla bengalensis</i>	0.20	0.38	0.39	0.02	0.02
<i>Channa punctatus</i>	0.19	0.47	0.48	0.03	0.02
<i>Channa striatus</i>	0.21*	0.41	0.42	0.03	0.04
<i>Cyprinus carpio</i>	0.21*	0.30	0.30	0.02	0.01
<i>Labeo rohita</i>	0.17	0.24	0.24	0.02	0.05
<i>Mystus vittatus</i>	0.18	0.56	0.57	0.03	0.02
<i>Notopterus notopterus</i>	0.21*	0.78	0.80	0.02	0.01
<i>Oreochromis mossambicus</i>	0.24*	0.88	0.89	0.03	0.01

Table.37 Average daily dietary intake of metals through consumption of fishes (mg) - wetland wise

Wetlands	Lead	Zinc	Copper	Cadmium	Chromium
Ambazari Lake	0.20	0.56	0.57	0.03	0.03
Bhigwan	0.20	0.65	0.66	0.02	0.03
Pimpalwadi	0.18	0.39	0.40	0.02	0.01
Jeyakwadi	0.20	0.41	0.42	0.02	0.02
Kambal Talav	0.22*	0.86	0.88	0.01	0.00
WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption	0.50	10-15	1.30	0.05	0.4
WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limit

The maximum input of 0.24 mg of lead was calculated through consumption of *Oreochromis mossambicus*. The calculated input of lead through consumption of *Channa striatus*, *Cyprinus carpio* and *Notopterus notopterus* are more or less the same (0.21 mg/day/person). The calculated level of zinc and copper ranged from 0.24 to 0.88 mg and 0.24 to 0.89 mg/day/person respectively. The calculated input of cadmium through consumption of all the species appeared to be more or

less the same (0.02 mg). The chromium concentration ranged from 0.01 in *Oreochromis mossambicus* to 0.29 mg in *Labeo rohita*. Of the five wetlands, the maximum of 0.22 mg of lead was calculated through the consumption of fishes from Kambal Talav and minimum through the fishes from Pimpalwadi (0.18 mg). The input of zinc and copper ranged from 0.39 to 0.86 mg and 0.40 to 0.88 mg respectively. Cadmium and chromium input ranged between 0.01 and 0.03 mg.

The results reveal that the intake of lead through consumption of fishes from all the wetlands is more or less equal compared to the concentration prescribed for human consumption by Joint Expert Committee on Food and Agriculture (JECFA) of United Nations (UN) and WHO (1989, 1993) which admit only up to 0.21 mg/day for a 60 Kg adult. Among the eight species of fishes calculated, input of lead through consumption of *Oreochromis mossambicus* is 0.22 mg/day/person. As reported elsewhere in this report, increased and regular consumption of fishes from the studied wetlands may pose problems. The daily dietary intake concentration of zinc, copper, cadmium and chromium were compared with the tolerable dietary intake described by WHO/FAO (1972) for human consumption. According to WHO/FAO (1972) the tolerable daily intake of zinc, copper, cadmium and chromium is 10-15, 1.30, 0.05, 0.4 mg respectively through consumption of food and water. Although the calculated levels of metals except lead appeared to be safe for human consumption continued exposure may create problem.

HEAVY METAL CONTAMINATION IN THE FISHES OF MADHYA PRADESH WETLANDS

10.1 INTRODUCTION

Madhya Pradesh, the southern most bastions of the Swamp Deer and wild buffalo, consists largely of a plateau, and has gorgeous mountain ranges, meandering rivers and large stretches of forest. The state can be divided into the following three major physiographic regions: (1) the central plateau-situated in between the Narmada–Son valley and the Vindhayan hills, (2) the Satpura ranges – in between the Narmada and the Tapti rivers the region extends east to west and parallel to the Vindhyan range, and (3) the eastern plateau - the region extends over the eastern districts of Madhya Pradesh.

The average rainfall in different region of the state ranges from 450 to 900 mm. The climate is extreme in the north, temperate and breezy in the plateau and generally hot and humid in the eastern and southern plains. The average temperature varies from 9.8 to 40.2°C. Six select wetlands (Map.7) were examined in detail for metal contamination.

10.2 STUDY AREA

10.2.1 Barna Reservoir

It is located at Raisen district between 23°04'60"E and 78°07'00"N. The area is approximated at 7,690 ha. It is located beside the national highway NH-12, 85 Km from Bhopal, the state capital. It is one of the most important water bodies of Raisen district. The reservoir has dense forest on three sides, and shares its perimeter with two sanctuaries as its bund lies inside Singhori wildlife sanctuary while the backwaters lie inside the Raatapani wildlife sanctuary. Barna Reservoir, apart from being a perennial water source to the animals of the sanctuaries, provides refuge to thousands of migratory birds during winter. It is mainly used for fishing and irrigation, and was created by damming the Barna river under the Narmada Valley Project. The reservoir looks like an amoeba when seen from above, with large numbers of bays

and meandering inland channels, surrounded on both sides by forest or agricultural land. These extensive belts of shallow water are ideal for water fowl and waders. The forest around Barna is mixed dry deciduous type. Most forest is open and subjected to heavy biotic pressure. Apart from natural vegetation, the reservoir shares its boundary with miles of agricultural land. As agricultural fields border the reservoir, the danger of pesticides reaching water, and accumulating in the different trophic levels, is very high. No study has been conducted on this aspect. Apart from this, changeover from traditional crops to cultivation of cash crops threatens the presence of many birds including the Sarus Crane. Poaching and illegal fishing in the reservoir are persistent threats.

10.2.2 Gandhisagar Reservoir

Gandhisagar is the second largest reservoir (36,862 ha) (in area) in the country, next only to Hirakud in Orissa. It is located in Neemuch, Mandsaur district between 24°36'03"E and 75°40'41"N. It is formed by an impoundment on the River Chambal. The Chambal, at the dam site, is fed by the catchment areas from the Vindhya ranges to the south and Aravalli to the northeast, covering a drainage area of 23,025 sq.km. The maximum length and width of the reservoir are 68 and 26 km respectively, while the Sanctuary is 36,700 ha in the area. Gandhisagar Dam is one among the four dams of the Integrated Chambal Development Programme. Shared by Mandsaur and Neemuch districts, the Gandhisagar reservoir and adjoining Gandhisagar Wildlife Sanctuary are about 130 km from Mandsaur. Until a few decades ago, these forests were dense and full of wildlife. Unfortunately, due to over-exploitation and unrestricted hunting, the area has become depleted both in flora and fauna. As the reservoir is under the control of the irrigation and fisheries departments, it is used for fishing. Over-fishing by small mesh size nets is detrimental for fish-dependent birds. Pesticide run-off from the surrounding agricultural fields is another danger, which has not been studied properly. Hunting of waterfowl continues near villages, but the large-scale hunting of earlier days has stopped.

10.2.3 Halali Reservoir

Halali reservoir (23°30'00" E, 77°30'00" N) is located 25 km from Bhopal, the capital of Madhya Pradesh. The reservoir was created in 1973, when an earthen dam was constructed over the River Halali. Two more rivers, Chamari and Ferozi, feed this huge reservoir. Mainly used for irrigation and fishes, the reservoir attracts a large number of birds during winter. It has vast shallow stretches of water on its western shores. Most of the birds are seen towards the shallow end. Towards the bund side, the forest attracts many terrestrial birds.

10.2.4 Gohad Talab

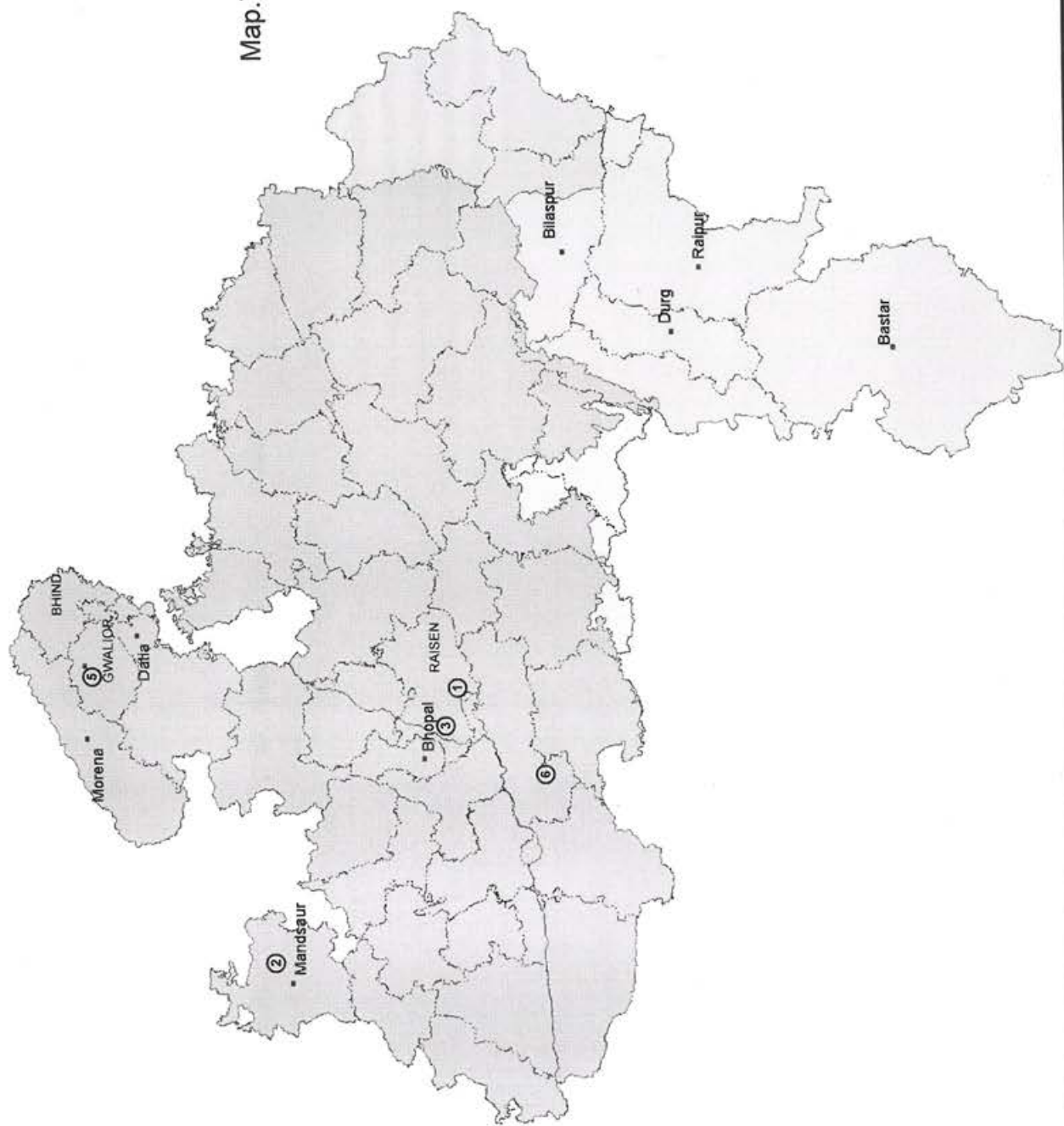
Gohad Talab is located at Bhind district between 26°30'00" E and 78°26'30" N. The approximate area is found to be 95 acres. This wetland serves as a good fish yielding zone particularly for species like *Labeo rohita*. The harvest has increased over the last 5-6 years. This wetland receives industrial effluent discharge from the Malumpur industrial area. This seasonally cultivated wetland also support the nearby agricultural activity where crops such as wheat, rice and vegetables were grown. Fertilizers such as Urea, NPK were applied.

10.2.5 Tighra Reservoir

Tighra Reservoir is located near Gwalior between 26°13'00" E and 78°00'00" N having a water spread area of 182 ha. This wetland besides supporting the nearby agricultural activity also serves as good fishing ground where no change has been observed in the fish yield over the last 5-6 years. Wheat, mustard, pea and ground nut are the few crops grown nearby. Urea, Phosphate and NPK are the chemicals used to raise the crops.

Map.7 Location of select wetlands studied
in Madhya Pradesh

1. Barna Reservoir
2. Gandhisagar Reservoir
3. Halali Reservoir
4. Gohad Talab
5. Tighra Reservoir
6. Tawa Reservoir



10.2.6 Tawa Reservoir

Tawa Reservoir is located in Hoshangabad district between 22°48'00"N and 77°48'00". The Tawa River is a tributary of the Narmada River in central India. The Tawa is the Narmada's longest tributary 172 km. It rises in the Satpura Range of Betul and Chindwara districts, flowing north and west to join the Narmada at the village of Bandra Bhan in Hoshangabad district. This wetland supports the agricultural and fishing activities. Wheat is the common crop cultivated around.

10.3 RESULTS

To document the magnitude of contamination status, 39 fishes comprising eight species from seven wetlands (Table.38) were analyzed.

Table.38 List of select wetlands examined for metal contamination

S.No.	Wetland	No.of fishes
1	Barna reservoir	13
2	Gandhisagar Dam	6
3	Gohad talab	5
4	Halali reservoir	3
5	Tawa reservoir	4
6	Tighra	8
Total no. of fishes collected		39

10.3.1 Variation in metal contamination among select wetlands of Madhya Pradesh

i. Lead

On an average, among the wetlands Tawa reservoir (8.02 ± 0.88 ppm) of Hoshangabad district recorded the highest concentration of lead. Fishes collected from Gandhisagar Dam (3.79 ± 0.29 ppm) of Mandsur district recorded the lowest concentration (Fig.13.a). Significant variation in contamination levels could be perceived ($P < 0.05$) (Table.39).

ii. Zinc

Zinc concentrations was detected in all the wetlands with the maximum being recorded in Barna reservoir (8.70 ± 2.58 ppm) of Raisen district followed by Gandhasagar Dam (16.16 ± 2.15 ppm) (Fig.13.b). Halali reservoir (7.38 ± 1.64 ppm) of Bhopal district measured the least levels. Variation in contamination levels among the wetlands appear ($P < 0.05$) significant (Table.39).

iii. Copper

Alike zinc concentrations Barna reservoir recorded the maximum concentrations of copper (19.03 ± 2.62 ppm) followed by Gandhisagar Dam (16.47 ± 2.19 ppm). Least levels were observed in Halali reservoir of Bhopal (8.25 ± 0.29 ppm) (Fig.13.c). Significant variation could be noticed ($P < 0.05$) among wetlands (Table.39).

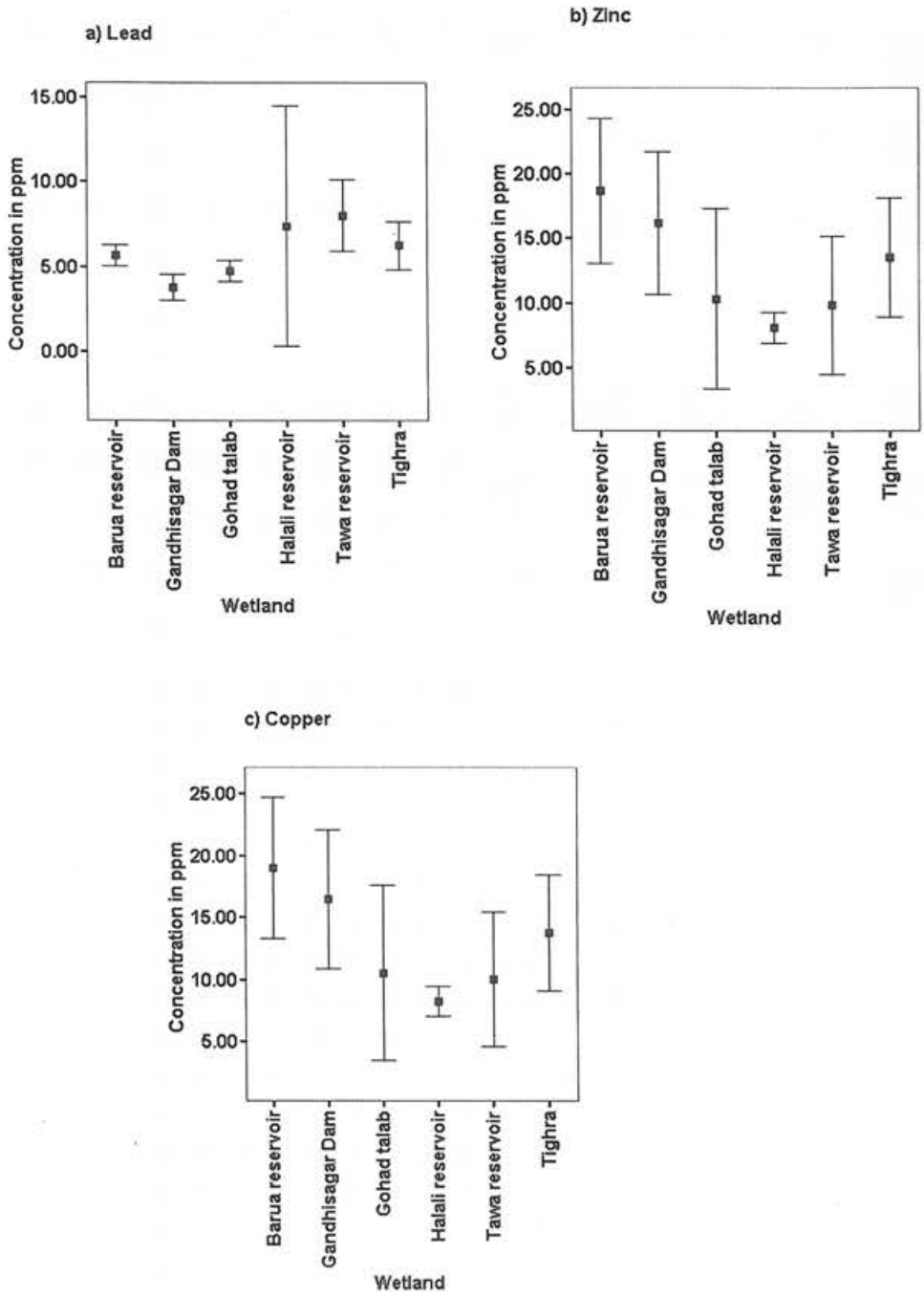
iv. Cadmium

The average cadmium levels were found to be around 0.65 ppm in all the wetlands. Barna reservoir (0.81 ± 0.03 ppm) of Raisen district and Tawa reservoir (0.45 ± 0.12 ppm) of Hoshangabad district recorded the highest and lowest levels respectively (Fig.13.d). Variation in contamination among the wetlands were significant ($P < 0.05$) (Table.39).

v. Chromium

Fishes of Gandhisagar Dam (2.83 ± 0.67 ppm) recorded the maximum concentrations of chromium followed by Gohad Talab (1.95 ± 0.16 ppm) of Bhind district. Near equal concentrations were observed in Barna reservoir (0.91 ± 0.16 ppm) of Raisen and Tighra of Gwalior (0.95 ± 0.24 ppm). Least levels were documented in Halali reservoir (0.55 ± 0.12) of Bhopal (Fig.13.e). Significant variation among the fishes of wetlands could be observed ($P < 0.01$) (Table.39).

Fig. 13 Levels of heavy metal contamination (ppm) among select wetlands of Madhya Pradesh (Mean \pm SE).



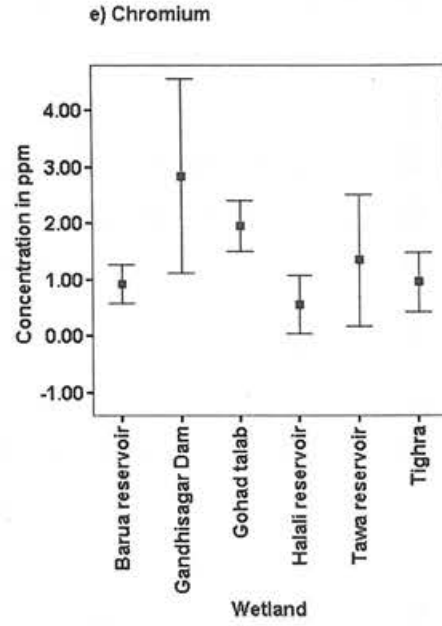
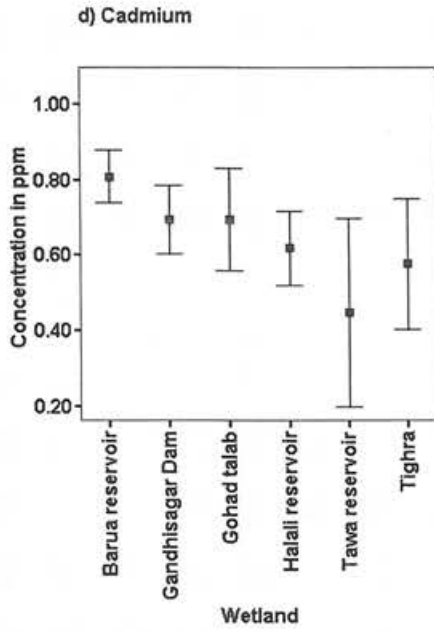


Table 39 Variation in heavy metal contamination among select wetlands of Madhya Pradesh (Kruskal Wallis Test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square Value	13.777	16.155	16.155	14.626	17.594
df	5	5	5	5	5
Asymp. Sig.	0.017*	0.006*	0.006*	0.012*	0.004**

*P<0.05; **P<0.01

10.3.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Madhya Pradesh

A sum of 39 individual fishes comprising eight species were quantified for heavy metal contamination (Table.40).

Table. 40 List of species of fishes examined in detail for heavy metal contamination

S.No.	Species	No. of Individuals
1	<i>Catla catla</i>	4
2	<i>Channa striatus</i>	3
3	<i>Cirrhinus mrigala</i>	7
4	<i>Cyprinus carpio</i>	3
5	<i>Labeo rohita</i>	5
6	<i>Notopterus notopterus</i>	8
7	<i>Ompok bimaculatus</i>	6
8	<i>Oreochromis mossambicus</i>	3
Total no. of fishes collected		39

i. Lead

On an average, among the species, *Cyprinus carpio* (9.01 ± 0.24 ppm) ranked high in concentrating lead followed by *Cirrhinus mrigala* (6.71 ± 0.62 ppm). Least levels were recorded in *Channa striatus* (3.35 ± 0.47 ppm) (Fig.14.a). Significant variation could be found among species ($P < 0.05$) (Table.41).

ii. Zinc

Zinc concentrations were detected in all the samples analyzed. *Cirrhinus mrigala* (16.52 ± 2.03 ppm), *Notopterus notopterus* (16.17 ± 2.03 ppm) and *Ompok bimaculatus* (16.30 ± 2.90 ppm) measured near equal higher levels. Least levels were observed in *Cyprinus carpio* (6.24 ± 1.07 ppm) (Fig.14.b). No significant variation could be perceived ($P > 0.05$) (Table.41).

iii. Copper

Alike, zinc values, *Cirrhinus mrigala* (16.81 ± 3.22 ppm), *Notopterus notopterus* (16.45 ± 2.06 ppm) and *Ompok bimaculatus* (16.58 ± 2.95 ppm) measured near equal higher levels. *Cyprinus carpio* measured the least concentrations (6.35 ± 1.09 ppm) (Fig.14.c). No significant variation in contamination could be perceived ($P < 0.05$) (Table.41).

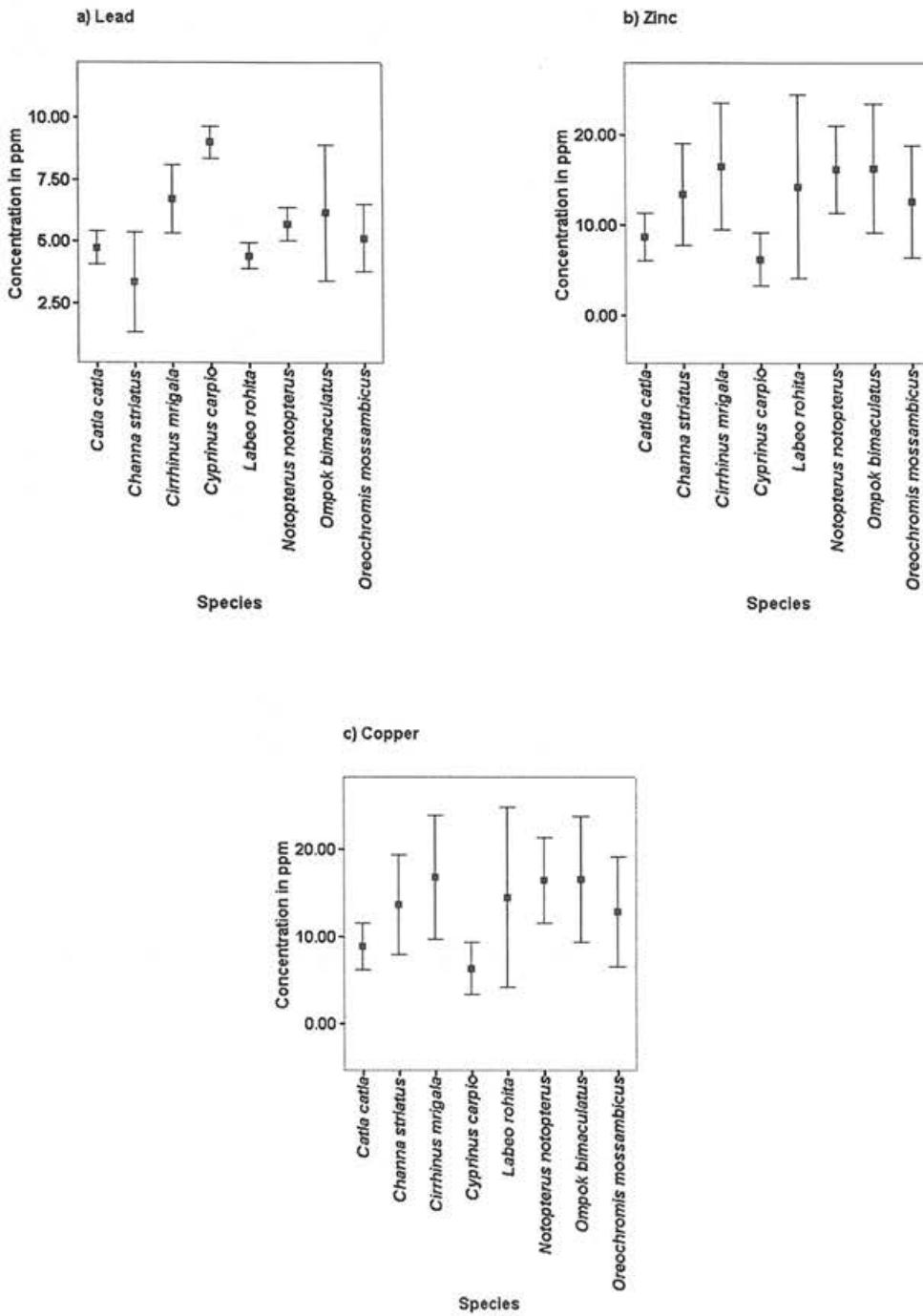
iv. Cadmium

The average levels were around 0.65 ppm in all the species with significant variation in contamination levels ($P < 0.05$) (Table.41). However, maximum concentration were measured in *Notopterus notopterus* (0.80 ± 0.13 ppm) while *Cyprinus carpio* (0.25 ± 0.02 ppm) the minimum (Fig.14.d).

v. Chromium

Highest concentrations were documented in *Cyprinus carpio* (2.42 ± 0.71 ppm) followed by *Labeo rohita* (2.05 ± 0.30 ppm). Least levels were quantified in *Notopterus notopterus* (0.65 ± 0.13 ppm) (Fig.14.e). Significant variation in contamination among the species could be noticed ($P < 0.05$) (Table.41).

Fig. 14 Levels of heavy metal contamination (ppm) among various species of fishes in the wetlands of Madhya Pradesh (Mean \pm SE).



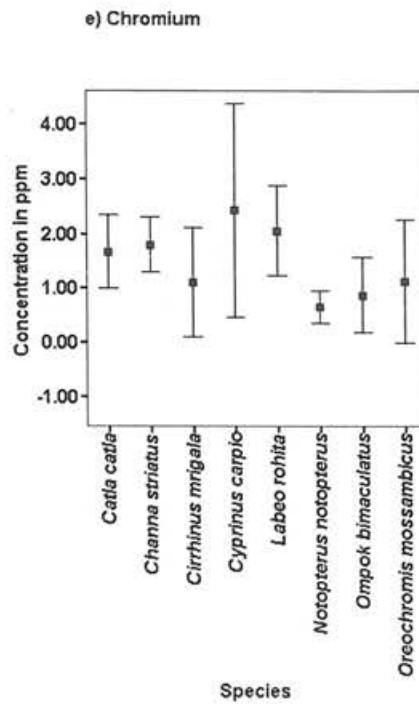
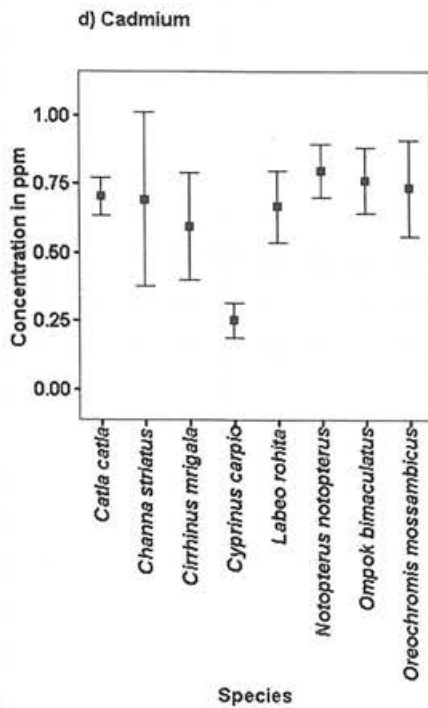


Table. 41 Variation in heavy metal contamination among various species of fishes in the wetlands of Madhya Pradesh (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square Value	18.536	13.450	13.450	17.452	20.448
df	7	7	7	7	7
Asymp. Sig.	0.010*	0.062	0.062	0.015*	0.005*

*P<0.05

10.4 DISCUSSION

10.4.1 Variation in metal contamination among select wetlands of Madhya Pradesh

It is understood that Bura talab of Raisen district had the maximum load of zinc, copper and cadmium while Gandhisagar Dam of Mandsur district and Tawa reservoir of Hoshangabad recorded the highest chromium and lead concentrations with significant variation in heavy metal contamination among the wetlands.

Copper can be found as a trace metal in nearly all waters. However, anthropogenic sources such as pollution from industry, agriculture and mining, may produce environmental concentrations that cause toxic effects in aquatic animals (Erdem and Kargin, 1992; Cogun *et.al*, 2003). Cadmium finds place among the most toxic metals in the aquatic system, and capable of exerting problem because it is highly toxic with a long biological half-life and its toxicity is also cumulative at least in invertebrate and fish (Heath, 1987). The levels of cadmium, copper and zinc are high with the earlier reported levels and its toxicity needs to be addressed.

Misra *et.al*, (2002) in their study on heavy metal assessment in Grass Carp from Upper Lake, Bhopal reported lead (1.6 ppm), copper (1 ppm), chromium (1.6 ppm), cadmium (1 ppm) and zinc (2 ppm) in the fishes. However, as the reported levels were in ppm dry weight, the comparison with the present study becomes difficult. Further in their study, they claimed that the rapid growth of the town and human settlements around the lake to have direct impact on the water quality of the lake. On account of anthropogenic activities, inflow of untreated sewage and agricultural wastes, the quality of lake water deteriorated to a considerable extent. The washing of clothes, bathing and idol immersion activities are other contributing factors towards the pollution of the lake.

In the present study many of the wetlands are seasonal in function and it is needless to mention of their utility in supporting the cultivable wetlands, which generally grow a variety of crops using fertilizers and pesticides. Although there exist no information on

the effluent discharges to Barna talab wetland the presumption of leach out from the agricultural lands cannot be dismissed. Gandhisagar Dam has suffered a drastic decline upto 60% in the fish yield during the last 5-6 years owing to the drain of industrial effluents and agricultural leachate. Further Gohad talab also receives effluent discharges from the industrial area at Malanpur, which accounts for recording high concentrations of metals. Thus, significant variation in contamination among the wetlands ($P < 0.05$) could be attributed to sources. Although, agricultural and industrial factors are the main sources of elevated levels of contaminants in many of the problem wetlands further surveys are needed to identify the source precisely so that action plan could be worked out for conservations.

10.4.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Madhya Pradesh

Cyprinus carpio recorded the maximum concentrations of lead and chromium, while *Cirrhinus mrigala* showed the highest levels of copper and zinc. Cadmium concentrations were measured high in *Notopterus notopterus*. Significant variation in contamination among species could be perceived for all the metals except copper and zinc which could be ascribed to their ecological needs. *Cyprinus carpio* and *Cirrhinus mrigala* belong to Cyprinidae family and they are the most voracious feeders of aquatic weeds/macrophytes and often accumulates the available heavy metals.

Aquatic organisms, namely fish acquire substantial quantities of trace elements from water, suspended particles and sediments (Chen, 2002). Most chemicals are taken up into the organism by passive diffusion or active uptake through the semi-permeable membrane of gill and gut epithelia (Campbell, 1995). Effectiveness of metal uptake from the sources may differ in relation to ecological needs and metabolism of animals and also contamination gradients of water, food and sediments as well as other factors such as salinity, temperature and interacting agents (Norey *et.al.*, 1990; Langston, 1990; Roesijadii and Robinson, 1994; Bervoets and Blust, 2003).

Papagiannis *et.al.*, (2002) in their study of heavy metals in Lake Pamvotis (N.W. Greece) Ecosystem found 4.53, 11.09 and 0.081 ppb of copper, zinc and lead in

muscle tissue of *Cyprinus carpio* respectively. In the present study supported by statistical analysis (Kruskal-Wallis) copper and zinc levels in muscle tissue did not vary significantly. However, Papagiannis *et.al*, (2002) found Cu and Zn levels in muscle tissue to vary significantly between species. This probably reflect the range of requirement of tolerance, rather than humanly derived exposure. Moreover, species-dependent differences may be attributed to the life history pattern that influences their exposure to metals (including trophic levels and geographic distribution of life stages). Dietary habits may have this impact on species differences in metals concentrations. There are studies illustrating strong positive relationship between Zn concentrations in zooplanktonic fractions and fish (Timmermans *et.al*, 1992). Species such as *Cyprinus carpio*, *Cirrhinus mrigala* and *Notopterus notopterus* are planktivorous and detritivorous and thus, they are expected to have increased zinc concentrations.

Pandey *et.al*, (1995) studied the heavy metal (Cu, Fe, Zn, Cr) accumulation in fish samples of sewage fed ponds of Rahara. Among the metals studied, Fe showed higher concentration, while Cr was low. Copper concentration in fish was about 0.02mg/g and found to be the lowest. The concentration of Zn was less and the accumulation of chromium in all the samples was very low which might be due to its low solubility and availability in water. The average value of chromium was 0.01 mg/g in fish samples. The levels recorded in the present study are comparably low with the levels mentioned in the above study.

There occurs no earlier information on metal contamination to any of the species mentioned above. The relative paucity of toxicological information leaves no doubt that the levels recorded here are either toxic or can be treated as baseline concentrations.

10.5 Suitability of fishes for human consumption in Madhya Pradesh

Dietary intake of metals by man through consumption of fishes was calculated for all the species and wetlands (Table 42, 43) as described in Chapter III.

Table. 42 Average daily dietary intake of metals through consumption of fishes (mg) - Species wise

Species	Lead	Zinc	Copper	Cadmium	Chromium
<i>Catla catla</i>	0.17	0.31	0.32	0.03	0.06
<i>Channa striatus</i>	0.12	0.48	0.49	0.02	0.06
<i>Cirrhinus mrigala</i>	0.24	0.59	0.60	0.02	0.04
<i>Cyprinus carpio</i>	0.32*	0.22	0.23	0.01	0.09
<i>Labeo rohita</i>	0.16	0.51	0.52	0.02	0.07
<i>Notopterus notopterus</i>	0.20	0.58	0.59	0.03	0.02
<i>Ompok bimaculatus</i>	0.22*	0.58	0.59	0.03	0.03
<i>Oreochromis mossambicus</i>	0.18	0.45	0.46	0.03	0.04

Table.43 Average daily dietary intake of metals through consumption of fishes (mg) - Wetland wise

Wetlands	Lead	Zinc	Copper	Cadmium	Chromium
Barua reservoir	0.20	0.60	0.68	0.02	0.03
Gandhisagar Dam	0.14	0.58	0.23	0.02	0.10
Gohad talab	0.17	0.37	0.38	0.02	0.07
Halali reservoir	0.26*	0.29	0.29	0.02	0.02
Tawa reservoir	0.29*	0.35	0.36	0.02	0.05
Tighra	0.22*	0.48	0.49	0.02	0.03
WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption	0.50	10-15	1.30	0.05	0.4
WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limits

Of the six species of fishes calculated for daily dietary intake of metals, *Cyprinus carpio* accounted for the maximum input of lead (0.32 mg) followed by *Cirrhinus mrigala* (0.24 mg) and *Ompok bimaculatus* (0.22 mg) while the calculated input of lead was the minimum through the consumption of *Channa striatus* (0.12 mg). The input of

zinc appeared to be higher through the consumption of *Cirrhinus mrigala* (0.59 mg) and it is lower through *Catla catla* (0.31 mg). The calculated input of copper through consumption of Madhya Pradesh fishes ranged between 0.23 and 0.60 mg while the cadmium input ranged between 0.01 and 0.03 mg/day/person. The chromium input ranged between 0.02 mg in *Notopterus notopterus* and 0.09 mg in *Cyprinus carpio*.

When all the fishes in a wetland were pooled together, the input of lead through the consumption of fishes from Tawa reservoir appeared to be high (0.29 mg) followed by Halali reservoir (0.26 mg) and Tighra (0.22 mg). The maximum input of zinc (0.60 mg) and copper (0.68 mg) were calculated through the consumption of fishes from Barua reservoir. The input of cadmium appeared to be the same (0.02 mg) from all the wetlands studied except Tighra (0.56 mg).

The results (Table.42) reveal that the calculated dietary lead intake through consumption of *Cirrhinus mrigala*, *Cyprinus carpio* and *Ompok bimaculatus* is quite high compared to the levels prescribed by Joint Expert Committee on Food and Agriculture (JECFA) of United Nations (UN) and WHO (1989, 1993) which admit only up to 0.21 mg/day for a 60 Kg adult. As referred to elsewhere in this report, increased and regular consumption may pose serious problems. When all the species were pooled together, the dietary intake of lead through consumption of fishes from Halali reservoir, Tawa reservoir and Tighra showed higher than the tolerable daily intake of lead proposed by WHO/FAO (1972) for human consumption (Table 43). According to WHO/FAO (1972) the tolerable daily intake of copper, lead, zinc, cadmium and chromium is 1.3mg, 0.50mg, 10-15mg, 0.05mg and 0.4mg respectively through food and water. The levels of cadmium and chromium appear to be safe for human consumption. However, if the per capita consumption increases, even the present levels may pose problems.

HEAVY METAL CONTAMINATION IN FISHES OF UTTAR PRADESH WETLANDS

11.1 INTRODUCTION

The undivided Uttar Pradesh popularly known about as the rainbow land is gifted with two of the mightiest rivers, the Yamuna and the Ganga which flood the lives and lure the men from time immemorial. The state can be divided into three distinct regions: a) the Himalayan region in the north b) the Gangetic plains in center and, c) Vindhya hills and plateau in the south. Out of its total length of 2,252 km, the river Ganga has as much as 1,450 km long run in UP. The other major river Yamuna meets the river Ganga at Prayag. The other rivers are the Ramganga, Gomati, Ghagra, Gandak and the tributaries of Yamuna such as Chambal, Betwa, Sind and Ken. Most of the rivers join the Ganga. The rivers are perennial, snow fed and emerges from the Himalayans except the river Gomati. They flow from west to east.

The average annual rainfall is above 1,200 mm. The state has a tropical climate except for Himalayan region, which has a temperate climate. The temperature, varies from 7.9 to 39.3°C. Heavy metal contamination status of four wetlands has been documented using fish as an indicator (Map.8).

11.2 STUDY AREA

11.2.1 Katra

It is located in the district Farukhabad between 26°56'30"E and 79°29'N. About 30% decline in the fish yield has been observed over the last 5-6 years in this wetland. Mortality of fishes is observed due to drought. *Labeo sp*, *Heteropneustes fossilis* and *Channa punctatus* are some of the species which suffered a decline in their production. The water spread area is 526.30 ha. Forest department protects this wetland from fishing and hunting. Approximately 50,000 people rely on this wetland for various activities such as fishing and irrigation. Agricultural activities are witnessed in the surroundings where crops such as sugarcane, wheat, rice and mustard are

grown using fertilizers such as urea and phosphate. Endosulfan, BHC and DDT are the commonly sprayed organochlorine pesticides.

11.2.2 Sherpur

It is located in the district Meerut and is 14 Kms from Mawan towards of Meerut between 28°55'E and 78°02'N. Although this wetland appears free from any effluent discharge about 50% decline in the overall fish yield has been observed. The water spread ranges from 8 to 10 sq km. Reed gathering, agriculture, fish culture and irrigation are some of the major uses of this wetland. Approximately 1500 people depend on this wetland for the above-mentioned activities. Agricultural activities in the surrounding areas get water from this wetland. Cultivation of crop also happens in this wetland. Sugar cane, rice and wheat are some of the crops grown. BHC and folidol are some of the pesticides sprayed in the field. Urea, DAP, Calcium and Potassium are some of the fertilizers applied to enrich the growth of the crops.

11.2.3 Haripura Dam

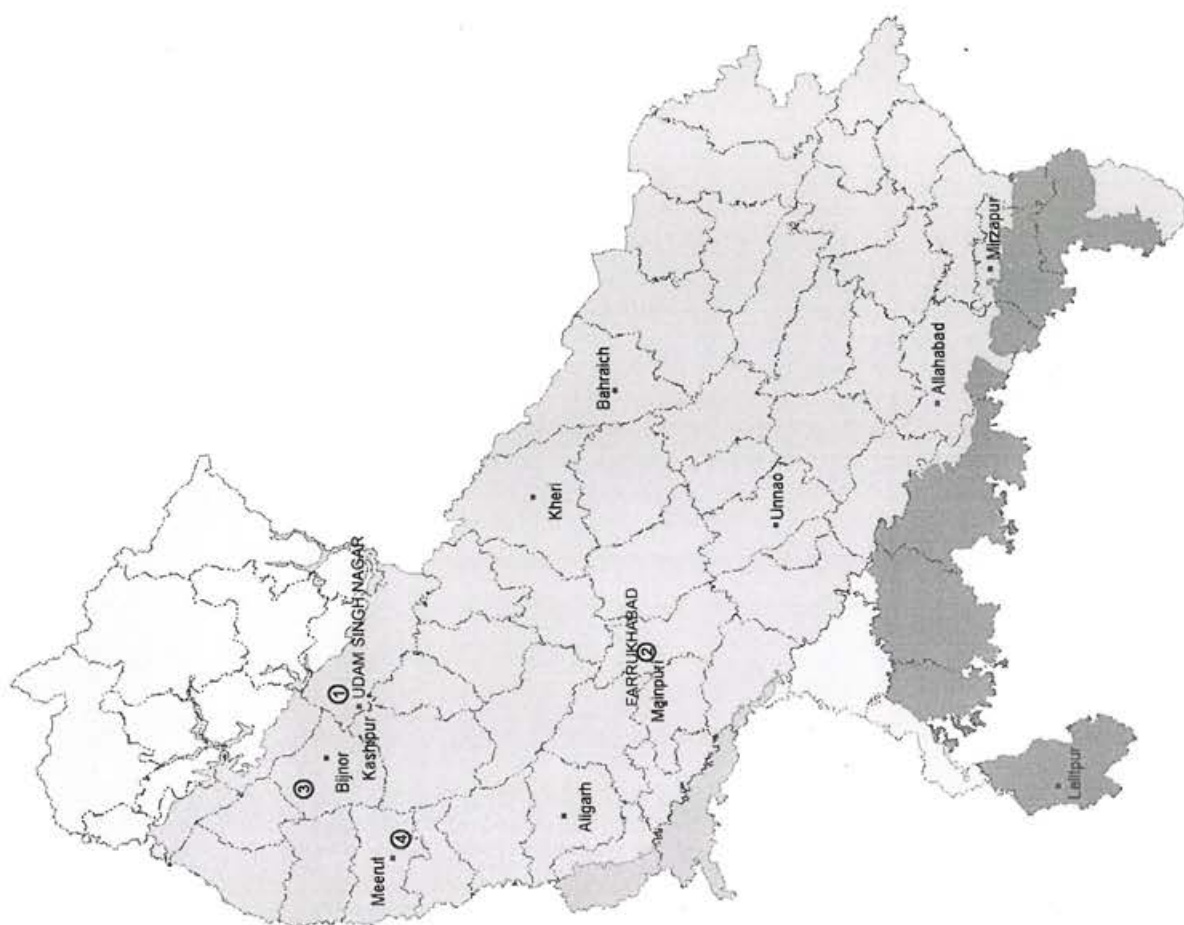
It is located close to Bajpur (13 kms) town of the district Udham Singh Nagar between 29°13'30'E and 79°29'N. The water spread area ranges between 24 and 40 sq km. This wetland enjoys a full protection from the local administration. There are five villages around this wetland and they depend on this wetland primarily for fishing. Although this wetland appears free from contamination, local fishermen complain about declining of fishes such as *Catla* sp. and *Labeo* sp. Wheat is the major crop cultivated in the nearby agricultural land. NPK, Urea, Endosulfan are some of the chemicals used for cultivation.

11.2.4 Pilee Dam

It is located in the district Bijnor between 29°33'E and 78°23'N. Jaspur is the nearest town. The catchment area is about 60 sq km. This wetland is under the custody of the irrigation department which prohibits fishing. About 20,000 to 25,000 individuals from the surrounding 13 villages use this wetland for grazing, agriculture, fuel wood

Map.8 Location of select wetlands studied in Uttar Pradesh.

1. Haripura Dam
2. Katra
3. Pilee Dam
4. Sheerpur



and irrigation. No information is available on the trend in the fish yield. The contamination by industrial effluent discharge is noticed in this wetland. This seasonally cultivated wetland has agricultural activities in the surrounding areas as well. Crops such as sugarcane, wheat, rice and black bean are grown. Fertilizers such as Urea, NPK and Potash are used. There are no incidences of fish or bird mortality reported in the wetland.

11.3 RESULTS

A sum of twenty seven fishes comprising five species collected from four wetlands was analyzed for heavy metal contamination (Table.44).

Table .44 List of select wetlands examined for heavy metal contamination

S.No.	Name of wetland	No. of fishes collected
1	Haripura Dam	3
2	Katra	6
3	Pilee Dam	4
4	Sherpur	14
Total no. of fishes collected		27

11.3.1 Variation in heavy metal contamination among select wetlands of Uttar Pradesh

On an average, among the wetlands, Katra of Farrukhabad recorded maximum concentration of lead (7.51 ± 0.44 ppm), zinc (22.37 ± 3.21 ppm), copper (22.76 ± 3.26 ppm) and cadmium (0.80 ± 0.08 ppm). Chromium concentrations were highest in the fishes of Pilee Dam of Bijnor (1.07 ± 0.3 ppm). It is interesting to note that zinc and copper concentrations were near equal in all the studied wetlands. Sherpur of Meerut showed equal concentrations of cadmium (0.71 ± 0.03 ppm) and chromium (0.71 ± 0.08 ppm) (Table.45). Significant variation could be observed for lead and chromium levels ($P < 0.05$) (Table.46).

Table. 45 Levels of heavy metal contamination (ppm) among select wetlands of Uttar Pradesh (Mean \pm SE)

S.No.	Wetlands	Lead	Zinc	Copper	Cadmium	Chromium
1	Haripura Dam (N=6)	4.99 \pm 0.25	9.92 \pm 2.98	10.09 \pm 3.03	0.77 \pm 0.03	0.44 \pm 0.06
2	Katra (N=6)	7.51 \pm 0.44	22.37 \pm 3.21	22.76 \pm 3.26	0.80 \pm 0.08	0.32 \pm 0.10
3	Pilee Dam (N=6)	5.63 \pm 0.71	14.83 \pm 5.27	15.09 \pm 5.37	0.69 \pm 0.09	1.07 \pm 0.34
4	Sherpur (N=17)	4.75 \pm 0.16	12.79 \pm 1.65	13.01 \pm 1.68	0.71 \pm 0.03	0.71 \pm 0.08

Table.46 Variation in heavy metal contamination among select wetlands of Uttar Pradesh (Kruskal Wallis Test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	12.905	6.117	6.117	3.527	10.174
df	3	3	3	3	3
Asymp. Sig.	0.005*	0.106	0.106	0.317	0.017*

*P<0.01

11.3.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Uttar Pradesh

Altogether twenty seven individuals comprising five species were analyzed (Table.47).

Table.47 List of fishes examined in detail for heavy metal contamination

S.No.	Species	No. of individuals
1.	<i>Channa orientalis</i>	3
2.	<i>Cirrhinus mrigala</i>	7
3.	<i>Heteropneustes fossilis</i>	6
4.	<i>Labeo rohita</i>	6
5.	<i>Oreochromis mossambicus</i>	5
Total no. of fishes collected		27

Among species on an average, *Cirrhinus mrigala* had the maximum accumulation of lead (6.33 ± 0.48 ppm), zinc (16.60 ± 2.97 ppm), copper (16.89 ± 3.02 ppm) and cadmium (0.84 ± 0.04 ppm) while *Channa orientalis* had the highest levels of chromium (0.89 ± 0.44 ppm) (Table.48). Most of the species had an uniform and near equal concentrations of zinc, copper, cadmium and chromium. As a result, no significant variation in heavy metal contamination among species could be observed ($P > 0.05$) (Table.49).

11.4 Discussion

11.4.1 Variation in heavy metal contamination among select wetlands of Uttar Pradesh

On an average, among the wetlands Katra of Farukkabad district had the maximum burden of lead, copper, zinc and cadmium while chromium was more in Pilee Dam of Bijnor district. As the concentrations of zinc, copper and chromium were near equal in many wetlands, no significant variation could be seen. Although there exists no information on the effluent discharge to any of the wetlands it can be presumed that these wetlands experience a similar and continuous exposure to metal contamination. As no earlier studies on these wetlands exist adequate comparison of the data and

Table. 48 Levels of heavy metal contamination (ppm) among various species of fishes in the wetlands of Uttar Pradesh (Mean±S.E.)

S.No.	Species	Lead	Zinc	Copper	Cadmium	Chromium
1	<i>Channa orientalis</i> (N=5)	5.71±0.50	15.88±4.43	16.16±4.51	0.64±0.07	0.89±0.44
2	<i>Cirrhinus mrigala</i> (N=9)	6.33±0.48	16.60±2.97	16.89±3.02	0.84±0.04	0.47±0.09
3	<i>Heteropneustes fossilis</i> (N=7)	5.16±0.46	11.85±2.77	12.06±2.82	0.74±0.03	0.50±0.09
4	<i>Labeo rohita</i> (N=9)	4.97±0.49	15.07±3.66	15.34±3.73	0.70±0.05	0.72±0.15
5	<i>Oreochromis mossambicus</i> (N=5)	4.63±0.46	10.54±3.23	10.73±3.29	0.67±0.07	0.85±0.05

Table.49 Variation in heavy metal contamination among various species of fishes in the wetlands of Uttar Pradesh (Kruskal Wallis Test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	8.191	4.901	4.901	8.702	6.979
df	4	4	4	4	4
Asymp. Sig.	0.085	0.298	0.298	0.069	0.137

interpretation become difficult. To best of our knowledge we did not find any contaminant study on these specific wetlands. Further, when compared with the data available elsewhere, the sediment quality, water physico-chemical characteristics in the study wetlands needs to be adequately justified for the absence or presence of metal concentrations.

11.4.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Uttar Pradesh

Among the species, *Cirrhinus mrigala* recorded the maximum concentrations of all metals except chromium. *Channa orientalis* recorded the highest levels of chromium. No significant variation could be observed among the species. This shows that fishes are having a uniform exposure to metal contaminants. Copper (3.82 ppm) and zinc (6.14 ppm) levels recorded in *Cirrhinus mrigala* collected from Amaravathy reservoir of Coimbatore district (Jayakumar, 2001) are seven times lower than the levels recorded in the present study.

Suresh Babu (1997) observed highest copper concentration (3.63ppm) in the fishes of Pykara and zinc (78.67 ppm) in fishes of Emerald, while the lead contamination was found to be low in all the reservoir bred fishes. The levels recorded in the present study are comparably higher. *Cirrhinus mrigala*, a subsurface plankton feeder should have accumulated more metals through its food. Chen *et.al*, (2000) analyzed concentrations of Hg, Zn, Cd, As and Pb from 20 lakes in contaminated to pristine water sheds in the NE, USA. They provided field evidence for the accumulation and biomagnification of Hg and Zn from small plankton (45 to 202 μm) to macro zooplankton (>202 μm) and from macro zooplankton to fish in these lake systems. Concentrations of Hg and Zn in the zooplankton were predictive of Hg and Zn levels in the fish.

Complex food webs may attenuate the transfer of metals to the higher trophic levels, and the high variability of metal concentrations in fish observed among different systems may be partly explained by the structural features of plankton webs. This is mainly caused by the complexity of metal-handling strategies in different groups of aquatic organisms (Stemberger and Chen, 1998).

On the contrary a higher concentration factor at higher trophic levels may result from a higher ability to accumulate a metal from the aqueous phase compared to lower trophic levels, instead of solely the uptake of a metal from the prey organism (Wang, 2002), Amiard – Triquet *et.al*, (1993) suggested that in many cases metal concentrations in animals are not related to the trophic levels in the food chain, but are dependent on the physiological characteristics of the species or populations as well as the biological role of each element. Thus, as these fishes are higher on the trophic level the exposure to the fish through food (plankton) is justified.

Generally, metals may be stored as metalloproteins with protein moiety showing some specificity of metal binding. Metal binding with the protein moiety in a cell is a complex phenomenon. Metals may act as Lewis acids or bases accepting or donating an electron to bind or may have specific affinity to a particular protein functional group (Cd, Hg and Pb prefer to bind to sulph-hydryl group). In addition, cadmium also prefers phosphate (R-PO-) and carboxyl (R-COO-) groups and mercury prefers amino (R-NH-) groups or is stored in compartments of all cells (Goering, 1993). In fishes metals such as Cd, Zn, Cu and Hg accumulate more in the liver due to high concentrations of metallothioneins (Bouquegnear *et.al*, 1975; Cherian and Goyer, 1978).

11.5 Suitability of fishes for human consumption in Uttar Pradesh

The daily dietary intake of heavy metals through consumption of five species were calculated based on fishes and wetlands (Table.50,51) as described in chapter III.

Table. 50 Average daily dietary intake of metals through consumption of fishes (mg) - species wise

S. No.	Species	Lead	Zinc	Copper	Cadmium	Chromium
1	<i>Channa orientalis</i>	0.20	0.57	0.58	0.02	0.03
2	<i>Cirrhinus mrigala</i>	0.23*	0.59	0.60	0.03	0.02
3	<i>Heteropneustes fossilis</i>	0.18	0.42	0.43	0.03	0.02
4	<i>Labeo rohita</i>	0.18	0.54	0.55	0.03	0.03
5	<i>Oreochromis mossambicus</i>	0.17	0.38	0.38	0.02	0.03

Table.51 Average daily dietary intake of metals through the wetland fishes (mg) - wetland wise

S. No.	Wetlands	Lead	Zinc	Copper	Cadmium	Chromium
1	Haripura Dam	0.18	0.35	0.36	0.03	0.02
2	Katra	0.27*	0.80	0.81	0.03	0.01
3	Pilee Dam	0.20	0.53	0.54	0.02	0.04
4	Sherpur	0.17	0.46	0.46	0.03	0.03
	WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption	0.50	10-15	1.30	0.05	0.4
	WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limits

The maximum input of lead was calculated through consumption of *Cirrhinus mrigala* (0.23 mg) and *Channa orientalis* (0.20 mg), while minimum through *Oreochromis mossambicus* (0.17 mg). The dietary intake of zinc and copper appeared to be almost equal in all the fishes studied with the concentration ranging from 0.38 to 0.42 mg and 0.38 to 0.60 mg respectively. The maximum input of cadmium and chromium were 0.02 and 0.03 mg respectively. Among the various wetlands, the consumption of

fishes from Katra showed the maximum input of 0.27 mg of lead, while the minimum input was (0.17 mg) through Sherpur. The calculated maximum input of zinc, copper, cadmium and chromium were 0.80, 0.81, 0.03, and 0.04 mg/day/person respectively

The calculated values were compared with the guidelines stipulated by statutory agencies for human safety. The calculated dietary lead intake through consumption of fishes are almost equal to the levels prescribed by Joint Expert Committee on Food and Agriculture (JECFA) of United Nations (UN) and WHO (1989, 1993) which admit only up to 0.21 mg/day for a 60 Kg adult. As referred to elsewhere in this report, increased and regular consumption may pose serious problems. Among the four wetland fishes calculated for dietary intake, it is noted that the calculated dietary intake of all metals except lead appeared to be safe for human consumption. According to WHO/FAO (1972) the tolerable daily intake of copper, lead, zinc, cadmium and chromium is 1.3 mg, 0.50 mg, 10-15 mg, 0.05 mg and 0.4 mg respectively through food and water. However, if the per capita consumption increases, even the present levels may pose problems.

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HEAVY METAL CONTAMINATION IN THE FISHES OF WEST BENGAL WETLANDS

12.1 INTRODUCTION

West Bengal is unique as it is the only state in India to stretch from the sea shore to the loftiest peaks of Himalayas. The hilly terrains with temperate and tropical forests, the enormous deltaic plains, vast stretches of sea coast, tidal creeks, swamps and the mangroves, including the extensive Sundarbans make the state rich in biodiversity. The state has four major geographical regions: the Chotanagpur plateau in its north-western parts, the Himalaya mountain, the lower Gangetic plain, and the costal belt.

An intricate network of rivers, streams and rivulets criss-cross the entire state. Rivers in the southern west Bengal contribute in the formation of the immense network of the deltaic region of the Sunderbans. Physiographically, the state of West Bengal can be differentiated into five broad divisions, namely the northern mountain, the western plateau, the great plain, the sunderban deltas and, the sand dunes of Kanthi coast. The climate of the state is tropical, hot and humid. Average temperature varies from 15 to 45°C. Rainfall varies from 1,000 mm in the south western region to 3,000 mm in the northern region. Monsoon brings most of the rain in the state. The only data available on wetlands are those generated during 1992-'93 by SAC (1998). Although there are 358 reservoirs, the total area covered by them is only 227 sq km. The total number of wetlands of the size 2.25 ha and above are 6,049; and it covers 1,438.56sq km. Eleven significant wetlands (Map.9) were considered for assessing the metal contamination.

12.2 STUDY AREA

12.2.1 Domohini

It is located in the district Jalpaiguri between 26°25'E and 88°33'N, and is 20 kms east of Jalpaiguri town. The water spread area ranges from 1.25 sq km to 3.00 sq km. Presently local village body protects this wetland and hunting is prohibited. Approximately 8,000 to 10,000 people depend on this wetland. Use of pesticides and

fertilizers has decreased the fish yield up to 50%. Agricultural activities are witnessed nearby where crops such as paddy, vegetables and fruits like cucumber and watermelon are grown. Thiodine and rogar are the commonly sprayed pesticides while urea, superphosphate and murate of potash are the fertilizers employed to enhance the crop yield.

12.2.2 Bara sagar

It is located in the district Malda between 24°57'E and 88°02'N. It is reported that there has been approximately 10% decline in the catch of *Channa orientalis* owing to fungal infection during the past 5-6 years. No information is available on neither the effluent discharge nor on seasonal cultivation. Agricultural activities are observed in the wetland vicinity. Mango is grown in the nearby orchards. Rogor is the commonly sprayed pesticide.

12.2.3 Durgapur Barrage

It is located in the district Bardhaman between 25°32'E and 88°08'N. Anthropogenic pressures such as over fishing, pollution and use of nylon net of fine mesh size are cited as the probable reasons for the 50% decrease in fish yield. No agricultural activities are observed at the vicinity.

12.2.4 Fulbari Mahananda Barrage

It is located in the district Jalpaiguri between 26°40'E and 88°25'N. The overall trend in the fish yield for the last 5-6 years appears to be decreasing; upto 30% in some species. Recruitment of fishes such as *Labeo rohita* and *Anabas testudineus* has declined sharply. On the other hand, yield of fishes, namely *Clarias batrachus* and *Heteropneustes fossilis* has increased.

Large quantum of effluents from a local distillery mixes with the river Mahananda which is the main source of water supply to the wetland. Further, cattle ranches and sewage of the city flows to the Mahananda river. Additionally large amounts of

agricultural runoff mix with the barrage water. This seasonally cultivated wetland also support agricultural activities nearby where crops such as paddy and vegetables like cabbage, cauliflower and brinjal are grown. Rogor, furadon and gammexane are some of the pesticides commonly applied on the crops. Fertilizers such as urea, superphosphate, potash and DAP are extensively applied to improve the yield of the crop. It is reported that in this wetland massive use of pesticides has resulted in mortality of fishes during 2001.

12.2.5 Gajoldaba Barrage

It is located in the district Jalpaiguri between 26°48'E and 88°36'N. Due to contamination of water, a heavy decline in the overall fish harvest has been observed. Although this wetland is not subjected to seasonal cultivation, agricultural activities are observed nearby. Crops such as paddy and vegetables are grown. Theodine appears to be the commonly applied pesticide.

12.2.6 Karnail Singh Park

It is located in the district Bardhaman between 23°52'E and 86°54'N. Although this wetland receives industrial effluents such as mineral oil from loco work spills along with sewerage and mosquito repellent oil, a minor increment (10%) in the overall fish yield is reported. This wetland is not seasonally cultivated and it does not support any agricultural activity nearby.

12.2.7 Kumari Dam

It is located in the district Puruliya between 23°20'E and 86°25'N. This wetland appears to be free from any pollution. Although this wetland is not seasonally cultivated agricultural activities are observed nearby where crops such as paddy is grown. Democron and folidol are the commonly applied pesticides.

12.2.8 Nayabandh

It is located in the district Malda between 24°55'E and 88°22'N. A 10% decline in recruitment of *Channa orientalis* was reported and it was attributed to fungal disease. On the other hand, the population of *Heteropneustes fossilis* was found to increase. Although this seasonally cultivated wetland is free from any effluent discharge agricultural activities are seen close by where hybrid varieties of rice and mustard are cultivated. Thiodon and butachlor are the commonly sprayed pesticides. DAP is the commonly applied fertilizer.

12.2.9 Palta water works

It is located in the district North 24 Parganas between 24°47'E and 88°22'N. Since this is the filtration centre for Calcutta city fishing is not allowed. As a result no change in the overall trend in the fish yield has been reported. This area appears to be free from pollution and not seasonally cultivated. No agricultural activity could be witnessed in the surroundings.

12.2.10 Rasik Beel

It is located in the district Koch-Bihar between 26°25'40"E and 89°44'10"N having a water spread area of 25 sq km. This wetland is primarily used for irrigation. Wheat is the major crop cultivated in the surroundings. Fishing is noticed where species, namely *Cirrhinus mrigala*, *Heteropneustes fossilis*, *Channa punctatus* are grown.

12.2.11 Shaheb Bandh

It is located in the district Puruliya between 23°22'12"E and 86°19'15" N. This is a filtration centre for the Puruliya water supply and fishing is not allowed. Hence, no change in the overall trend in the fish harvest has been noticed. This receives occasional drainage of oil effluents from a nearby garage. No agricultural activities are reported nearby.

Map. 9 Location of select wetlands studied in West Bengal

1. Bara Sagardighi
2. Domohini
3. Durgapur Barrage
4. Fulbari-Mahananda Barrage
5. Gajoldoba Barrage
6. Karnail Singh Park
7. Kumari Dam
8. Nayabandh
9. Palta Water Works
10. Rasik Beel
11. Shaheb Bandh



12.3 RESULTS

Altogether 105 fishes (Table.52) comprising eight species were collected from eleven wetlands and analyzed for metal contamination.

Table.52 List of select wetlands examined in detail for metal contamination

S.No.	Wetland	No. of fishes collected
1	Bara Sagardighi	12
2	Domohini	12
3	Durgapur Barrage	10
4	Fulbari-Mahananda Barrage	7
5	Gajoldoba Barrage	5
6	Karnail Singh Park	16
7	Kumari Dam	9
8	Naya bandh	11
9	Palta water works	4
10	Rasik beel	10
11	Shaheb bandh	6
Total no. of fishes collected		105

12.3.1 Variation in heavy metal contamination among select wetlands of West Bengal

i. Lead

On an average, Naya Bundh (6.47 ± 0.31 ppm) of Malda district recorded the maximum concentration followed by Shaheb Bandh (6.16 ± 0.78 ppm) of Purulia and Durgapur Barrage (6.12 ± 0.64 ppm) of Bardhaman district (Fig.15.a). Near equal levels were observed in Fulbari-Mahananda barrage (4.72 ± 0.39 ppm) of Jalpaiguri and Palta water works (4.66 ± 1.34 ppm) of North 24 Parganas district. Most of the studied fishes of the wetlands had an average level of 4 to 6 ppm of lead. Significant variation ($P < 0.05$) could be observed among the fishes of wetlands (Table. 53).

ii. Zinc

Zinc levels were detected in all the wetlands with the highest being recorded in Palta water works (20.21 ± 9.85 ppm) followed by Barasagardighi (18.92 ± 2.29 ppm) of Malda and Karnail Singh Park (5.12 ± 0.85 ppm) of North 24 Parganas the lowest (Fig.15.b). Near equal concentrations were observed in Durgapur barrage (15.76 ± 1.95 ppm) of Barddhaman and Gajoldoba (15.46 ± 3.17 ppm) of Jalpaiguri, and Rasik beel (14.02 ± 2.03 ppm) of Koch-Behar and Shaheb bandh (14.13 ± 1.63 ppm) of Purulia. Significant differences in zinc levels exist among the fishes of the wetlands ($P < 0.01$) (Table.53).

iii. Copper

Alike zinc levels, Palta water works (20.56 ± 5.01 ppm) recorded the highest while Karnail Singh Park (5.20 ± 0.87 ppm) the lowest (Fig.15.c). It is important to note that all the wetlands had near equal levels of copper and zinc and the variation in contamination among the wetlands was significant ($P < 0.01$) (Table.53).

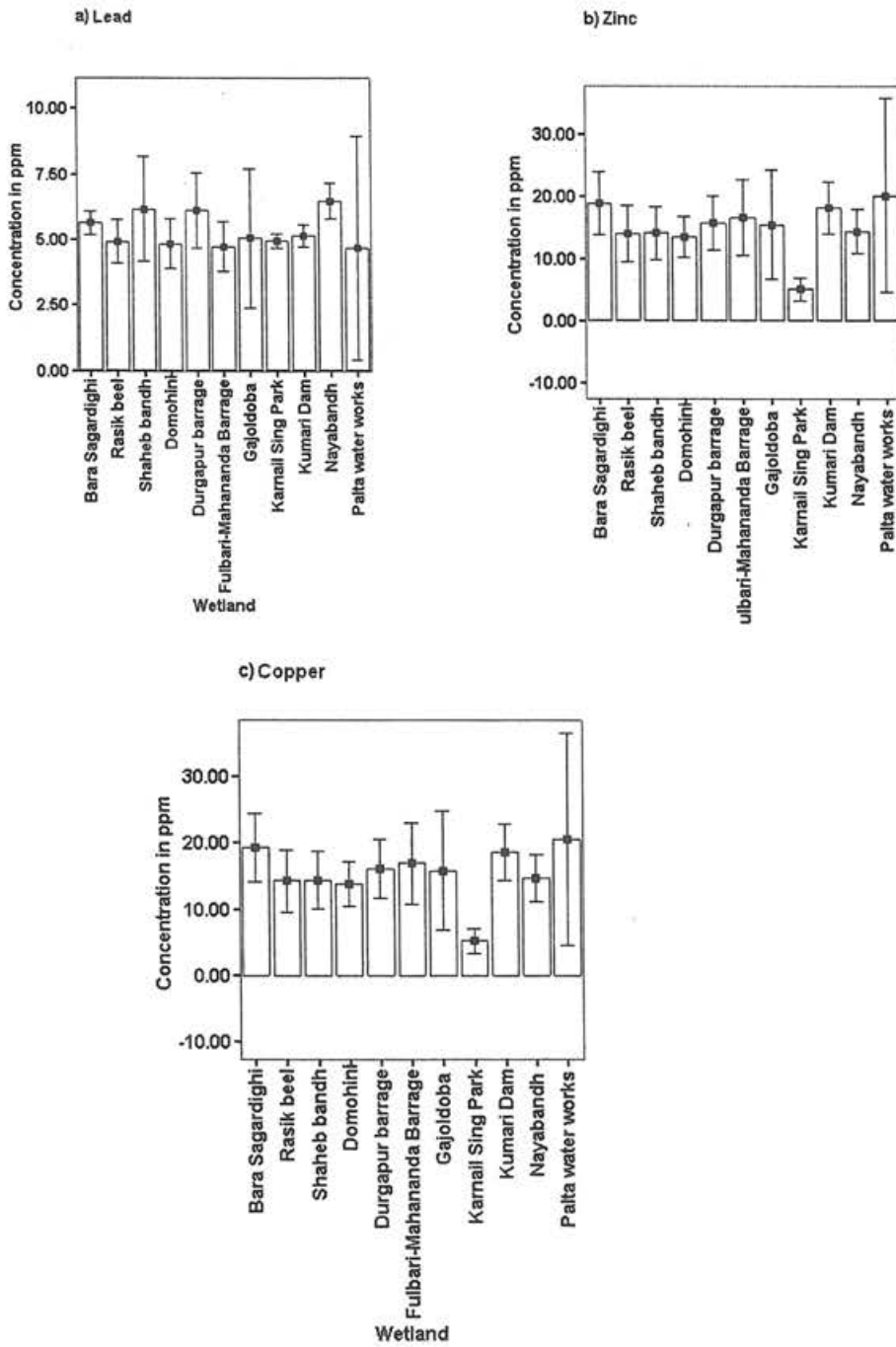
iv. Cadmium

The average cadmium concentration was around 0.7 ppm in all the wetlands, except Shaheb bandh (0.92 ± 0.11 ppm) of Purulia district (Fig.15.d) which recorded the highest. Significant variation in contamination could be perceived ($P < 0.05$) among the fishes of the wetlands (Table.53).

v. Chromium

Durgapur barrage (1.69 ± 0.18 ppm) of Barddhaman recorded the highest concentration followed by Fulbari-Mahananda barrage (1.57 ± 0.26 ppm) of Darjeeling district (Fig.15.e). Least levels were recorded in Karnail Singh Park (0.41 ± 0.09 ppm). Variation in contamination levels were found to be significant ($P < 0.01$) (Table.53).

Fig.15 Levels of heavy metal contamination (ppm) among select wetlands of West Bengal (Mean \pm SE)



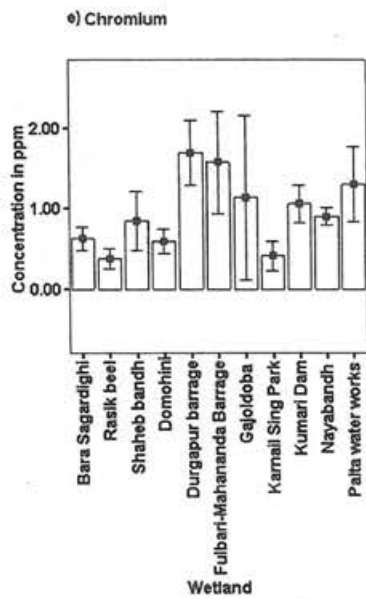
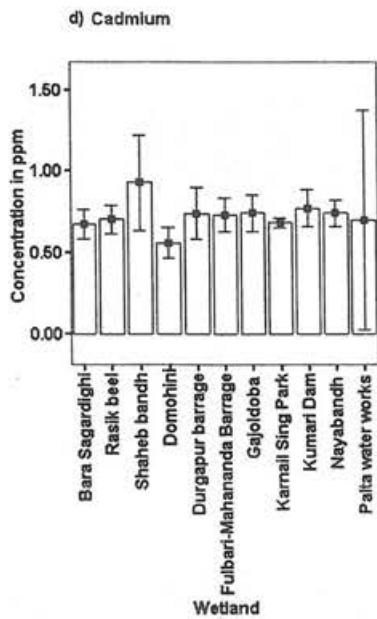


Table.53 Variation in metal contamination among select wetlands of West Bengal (Kruskal Wallis Test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	23.734	37.309	37.309	20.456	56.796
df	10	10	10	10	10
Asymp. Sig.	.008	.000	.000	.025	.000

12.3.2 Variation in metal contamination among various species of fishes in the wetlands of West Bengal

Only 105 individuals comprising eight species were examined in detail for heavy metal contamination (Table.54).

Table 54. List of species examined in detail for heavy metal contamination in West Bengal

S.No.	Fish	No. of Individuals collected
1	<i>Anabas testudineus</i>	14
2	<i>Catla catla</i>	6
3	<i>Channa orientalis</i>	16
4	<i>Channa punctatus</i>	4
5	<i>Cirrhinus mrigala</i>	21
6	<i>Clarias batrachus</i>	8
7	<i>Heteropneustes fossilis</i>	20
8	<i>Labeo rohita</i>	16
Total no. of fishes collected		105

i. Lead

Almost all the samples analyzed showed near equal concentrations of lead (Fig.15.a) with significant variation in metal contamination among the species ($P < 0.05$) (Table.53). The average level of lead contamination was around 5 ppm.

ii. Zinc

Least levels were observed in *Channa punctatus* (7.10 ± 2.18 ppm) while *Catla catla* (23.97 ± 1.73 ppm) had the maximum concentration (Fig.15.b). Significant variation in metal contamination ($P < 0.01$) could be observed (Table.53).

iii. Copper

Alike zinc levels, copper also accumulated highest in *Catla catla* (24.39 ± 1.76 ppm) and least in *Channa punctatus* (7.23 ± 2.21 ppm) (Fig.15.c) with significant variation ($P < 0.01$) in metal contamination (Table.53). It is also important to note that there is similar concentration of copper and zinc in many of the wetlands.

iv. Cadmium

Except for *Catla catla* (0.62 ± 0.03 ppm) which recorded the minimum levels of cadmium, the remaining species recorded an average range between 0.65 to 0.75 ppm. Maximum concentrations were reported in *Cirrhinus mrigala* (0.75 ± 0.05 ppm) (Fig.15.d). No significant variation ($P > 0.05$) in contamination could be found among the species (Table.53).

v. Chromium

Chromium levels were recorded maximum in *Labeo rohita* (1.12 ± 0.13 ppm) followed by *Channa orientalis* (1.02 ± 0.18 ppm) and minimum in *Channa punctatus* (0.46 ± 0.36 ppm) (Fig.15.e). The chromium concentration ranged between 0.02 and 2.38 ppm among the species studied. Significant variation ($P < 0.05$) in contamination could be established among the species.

12.4 DISCUSSION

12.4.1 Variation in metal contamination among select wetlands of West Bengal

Among the wetlands, Nayabundh, Palta water works, Shaheb Bandh and Durgapur barrage were reported to be suffering from heavy metal contamination. The significant variation in metal contamination could be due to the differences in contaminant sources. Further a heavy decline in fish yield is reported in many of the wetlands and the probable reason cited is unscientific method of fishing and use of toxic agents such as pesticides. Agricultural leachate is also an addendum for the decline.

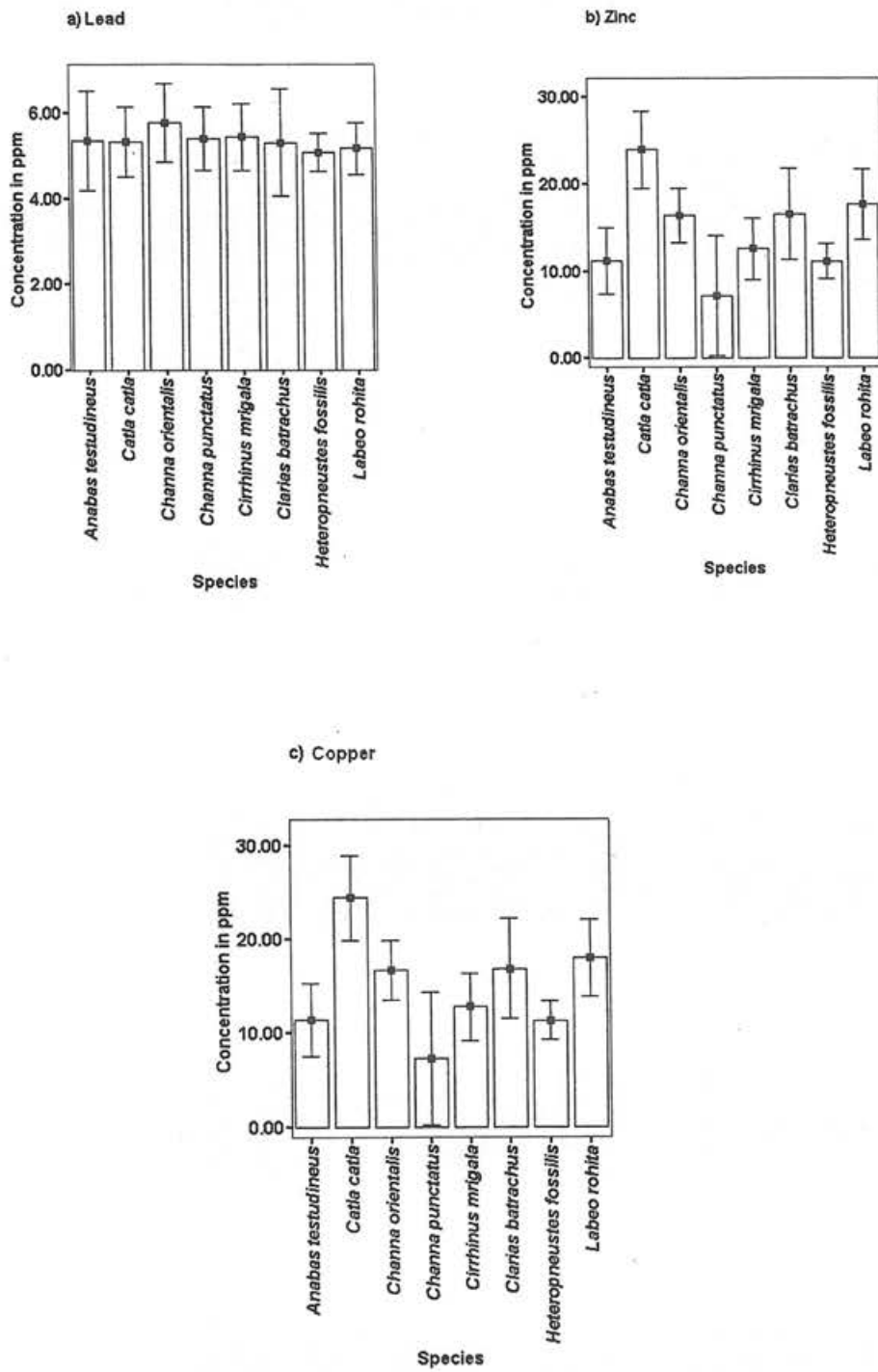
Wetland, namely Fulbari-Mahananda Barrage receives large quantity of effluent from a local distillery along with domestic sewage discharge. Vijayamohan and Nair (2000) studied the impact of titanium dioxide factory effluent on the biochemical composition of two fresh water fishes namely *Oreochromis mossambicus* and *Etroplus maculatus*. They could observe clear changes in glycogen and protein in the effluent treated fishes. It was also inferred that these changes would naturally affect the nutritive value of the fishes and the biochemical parameters reflected the changes in the normal activities of various functional systems. Although we have no studies to comment on the impact of distillery effluent on these particular species, it can be anticipated that distillery effluent containing rich organic and inorganic matter could only harm.

12.4.2 Variation in metal contamination among various species of fishes in the wetlands of West Bengal

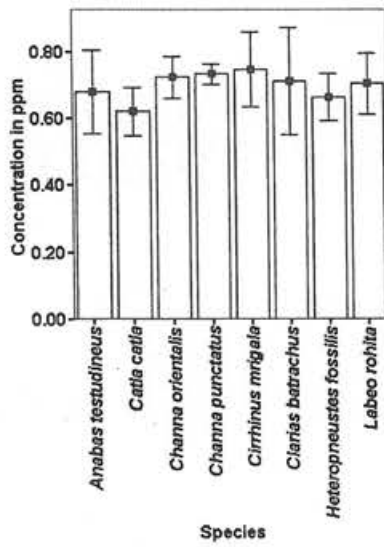
Among the species, *Channa orientalis*, *Labeo rohita* and *Cirrhinus mrigala* recorded the highest concentration of lead, cadmium and chromium respectively, while *Catla catla* had the highest levels of copper and zinc. Significant variation among the species could be established for all metals except cadmium.

The levels recorded by these fishes are comparably high with the levels documented elsewhere. Jayakumar (2001) has reported 0.5 ppm of cadmium and 0.1 ppm of chromium in *Labeo rohita* and *Cirrhinus mrigala* respectively collected from select wetlands of Coimbatore, Tamil Nadu, India. Further, the levels of copper (0.9 ppm) and zinc (6.4 ppm) documented in *Catla catla* collected from Coimbatore, Tamil Nadu, India also appeared lower than the present levels. Papagiannis *et.al*, (2002) in their study of heavy metals in Lake Pamvotis (N.W. Greece) Ecosystem found 4.53, 11.09 and 0.081 ppb of copper, zinc and lead respectively, in muscle tissue of *Cyprinus carpio*. Zn was the metal that reached the highest concentrations in muscle and the levels are comparable with levels reported by Usero *et.al*, (2003) in the fishes from salt marshes on the southern Atlantic coast of Spain. The levels were between 6.05 and 9.31 ppm. A high level of Zn in comparison to other elements is typical of fish muscle (Moiseenko and Kudryavtseva, 2001).

Fig.16 Levels of heavy metal contamination (ppm) among various species of fishes in the wetlands of West Bengal (Mean \pm SE)



d) Cadmium



e) Chromium

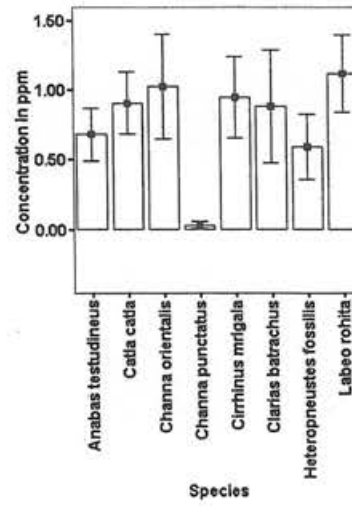


Table.55 Variation in metal contamination among various species of fishes in the wetlands of West Bengal (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	4.815	28.253	28.253	8.559	16.225
df	7	7	7	7	7
Asymp. Sig.	.683	.000	.000	.286	.023

Copper and its compounds are ubiquitous in the environment and are frequently found in surface water. Copper ion precipitate gill secretions, causing death by asphyxiation. Zinc is an abundant element and constitutes approximately 0.04 g/Kg of the earth's crust. Its occurrence in sewage is expected because of its extensive use in making household appliances and by leaching from galvanized pipes (Pandey *et.al*, 1995). Thus, it is not surprising to find high concentrations of metals like copper and zinc getting in the water bodies and their subsequent accumulation in the fishes.

The variation in contamination among the species reported in the study could be due to their ecological needs, feeding habits, species specific characteristics and the bioavailability of the metal (Mason *et.al*, 2000). Elevated zinc levels in these species could be attributed to the plankton feeding and occasional bottom feeding habit. Essential elements such as Zn are a component of phytoplankton and uptake of these pollutants from water column by these organisms plays an important role in transferring the trace elements along the successive levels in the trophic chain to higher organisms (Szefer, 1991). Sediments act as repositories for a large number of environmental contaminants, thus it is comprehensible that benthic animals may accumulate more toxic compounds given their close contact with sediment particles and interstitial water for extended periods of life cycle (Transpurger and Drews, 1996). This could possibly explain the higher concentration of Zn, Pb, Cd and Cr in fishes.

Thus the levels reported in the present study are comparatively higher and it is needless to mention that the dietary habits, species specific differences could have attributed to the significant variation observed among species.

Several authors have described the hematological alterations, biochemical changes and hampering of locomotion in fishes like Rainbow Trout and Salmon at a concentration of 0.012 ppm of lead (Hodson *et.al*, 1978; Shrivastava and Mishra, 1979). Chromium has been found to cause alterations in biochemical processes and immune responses. Kunhert *et.al*, (1976) reported significant inhibition in activity of enzyme Na/K ATP ase, which is involved in osmoregulation in the kidney of *Salmo gairdneri* after exposure at 2.5 ppm Cr (VI) for 48 hours. Singh and Singh (1979) found 0.003 ppm of cadmium to inhibit the oxygen consumption of *Mystus vittatus* by

50% during an exposure of 12 hours. Although the current levels are not alarming, the physiological mechanisms, species differences, physico-chemical properties of the surviving water, availability and absorption of metal are also to be admitted while considering the ill effects. If the exposure is continuous it can be anticipated that certain physiological damages such as decline of proximal principles, hampered locomotion, disturbances in osmoregulation could happen (Shrivastava and Mishra, 1979; Abbasi *et.al*, 1998).

12.5 Suitability of fishes for human consumption in West Bengal

Dietary intake of metals by man through consumption of fishes was calculated for all the species and wetlands (Table 56, 57) as described in Chapter III.

Table.56. Average daily dietary intake of metals through consumption of __ fishes (mg) - Species wise

Name of the Species	Lead	Zinc	Copper	Cadmium	Chromium
<i>Anabas testudineus</i>	0.19	0.40	0.41	0.02	0.02
<i>Catla catla</i>	0.19	0.86	0.87	0.02	0.03
<i>Channa orientalis</i>	0.21*	0.58	0.59	0.03	0.04
<i>Channa punctatus</i>	0.19	0.25	0.26	0.03	0.02
<i>Cirrhinus mrigala</i>	0.19	0.45	0.46	0.03	0.03
<i>Clarias batrachus</i>	0.19	0.59	0.60	0.03	0.03
<i>Heteropneustes fossilis</i>	0.18	0.40	0.40	0.02	0.02
<i>Labeo rohita</i>	0.18	0.63	0.64	0.03	0.04

The calculated dietary intake of lead through the consumption of fishes was almost equal in all the five species of fishes studied. The maximum lead input (0.21 mg) was calculated to be through consumption of *Channa orientalis* and minimum (0.18 mg) through *Labeo rohita* and *Heteropneustes fossilis*. The maximum calculated input of zinc and copper were 0.86 and 0.87 mg respectively. The minimum input were through the consumption of *Catla catla* (0.40 mg) and *Anabas testudineus* (0.41 mg) respectively. The calculated levels of cadmium and chromium ranged from 0.02 to 0.03 and 0.02 to 0.04 mg/day/person respectively among the species.

Table. 57 Average daily dietary intake of metals through consumption of fishes (mg) - Wetland wise

Name of the wetland	Lead	Zinc	Copper	Cadmium	Chromium
Bara Sagardighi	0.20	0.68	0.69	0.02	0.02
Domohini	0.17	0.48	0.49	0.02	0.02
Durgapur barrage	0.22*	0.56	0.57	0.03	0.06
Fulbari-Mahananda barrage	0.17	0.59	0.60	0.03	0.06
Gajoldoba	0.18	0.55	0.56	0.03	0.04
Karnail Singh Park	0.18	0.18	0.19	0.02	0.01
Kumari Dam	0.18	0.58	0.59	0.03	0.04
Nayabandh	0.23*	0.51	0.52	0.03	0.03
Palta water works	0.17	0.72	0.73	0.02	0.05
Rasik beel	0.18	0.50	0.51	0.02	0.01
Shaheb bandh	0.22*	0.50	0.51	0.03	0.03
WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption (mg)	0.50	10-15	1.30	0.05	0.4
WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limit

Among the 12 wetlands, high level of lead input was calculated to be through the consumption of fishes from Nayabandh (0.23 mg), Shaheb bandh (0.22 mg) and Durgapur Barrage (0.22 mg). While lead input was minimum (0.17 mg) in Domohini and Fulbari-Mahananda barrage and Palta water works. Zinc and copper input ranged between 0.48 and 0.72 mg, and 0.49 and 0.73 mg respectively, while cadmium and chromium ranged between 0.02-0.03 mg and 0.01-0.06 mg respectively.

The results reveal that the intake of lead through consumption of fishes from all the wetlands is more or less equal compared to the concentration prescribed for human consumption by Joint Expert Committee on Food and Agriculture (JECFA) of United Nations (UN) and WHO (1989, 1993) which admit only up to 0.21 mg/day for a 60 Kg adult. Among the eight species of fishes, calculated dietary intake of lead through consumption of *Channa orientalis* is 0.21 mg/day/person. Among the various wetlands, the fishes of Durgapur barrage, Nayabandh and Shaheb Bandh exceeded

the lead concentration prescribed for human consumption. As reported elsewhere in this report, increased and regular consumption of fishes from the referred wetlands may pose problems. The daily dietary tolerable intake described by WHO/FAO for zinc (10-15 mg), copper (1.30 mg), cadmium (0.05 mg) and chromium (0.4 mg) for human consumption through consumption of food and water was compared. Although the calculated levels of metals except lead appeared to be safe for human consumption continued exposure may create problem.

HEAVY METAL CONTAMINATION IN THE FISHES OF TAMIL NADU WETLANDS

13.1 INTRODUCTION

Tamil Nadu, endowed with bountiful and benign nature, has tradition of preserving ponds and tanks, dating back to the 4th and 5th centuries. With its long coast lines, alluvial plains, and hill chain of the Western Ghats, the state offers a wide variety of habitats. Situated on the southeast of the Indian peninsula, Tamil Nadu can be divided physiographically into (1) the eastern coastal plain and (2) the hilly region along the north and west. The coastal plain is further divided into (a) the Coromandel plain in the north (b) the alluvial plain of the cauvery delta and (c) the dry southern plain. Along the whole length of the western part, at distance from the sea varying from 80 to 150 km, runs the range of Western Ghats, a steep and rugged landmass averaging 1,220 m above the sea level and rising up to 2,440 m at the highest point.

The state has a number of rivers, all flowing from west to east, the Western Ghats to the Bay of Bengal. The rivers are entirely rain fed and short in length. The main river of the state is Cauvery which flows across Tamil Nadu cutting the state into two halves. Some of the other major rivers are Amaravathi, Bhavani, Tamaraparani and Vaigai.

The climate of Tamil Nadu is of tropical type, summers are not too hot and winters not too cold. The temperature varies from 21.5 to 37.7°C. Rain fall is fairly widespread throughout the year. The state gets full benefit of the north-east monsoon, which brings rainfall in October and November, and some time in December also. The south west monsoon also brings rain during June to September. The average annual rain fall varies from 670 to 1, 200 mm.

Studies by SAC (1998) show that wetland area of the state was 1.24% of the total area in 1991. Seventeen select wetlands were examined in detail for metal contamination (Map.10). It may be noted that Tamil Nadu has the highest number of wetlands included for the current study.

13.2 STUDY AREA

13.2.1 Avalpoondarai

It is located in the district Periyar between 11°15'E and 77°35'N. It is a satellite lake of Vellode Lake and is about 25 km from the Vellode Lake. Its water-spread area is approximately 2 times greater than the Vellode bird sanctuary. The local villages commercially exploit this lake. As a result the overall trend in the fish yield has considerably decreased and the overflow and gushing of lower Bhavani water into the lake during monsoon washes away the fingerlings that had been sown. This is cited as one of the probable reasons for the decline in the abundance of many species of fishes particularly *Catla catla*, *Labeo rohita*, and *Labeo calbasu*. Domestic sewage intrusion and agricultural discharge are also noticed in the lake, which are the other reasons for the declining trend in the fish harvest and also for the grayish black coloration of the water. Turmeric is the crop much cultivated in the nearby agricultural plots and pesticides like Nuvacron, Kumooan are much commonly applied.

13.2.2 Chembarampakkam

It is located at the district Chengalpattu between 12°59'E and 80°14'N having a water spread area of 25 ha. A 30% decline in fishes such as *Labeo rohita*, *Catla catla* and *Oreochromis mossambicus* are reported due to monsoon failure. This seasonally cultivated wetland receives effluents from a brewery industry. An incidence of heavy fish mortality was recorded in the past and effluent discharge from the brewery was blamed for the same. This seasonally cultivated wetland supports agricultural activities closeby where rice is found to be the major crop. DAP is the fertilizer commonly applied in the agricultural lands.

13.2.3 Alwarkurichi

It is located in the district Tirunelveli and has approximately 100 acres water spread area. This wetland supports agricultural activities nearby where crops

such as paddy and sugarcane are grown. This wetland also receives industrial discharge.

13.2.4 Gundur Big Tank

It is located in the district Trichirapalli between 10°08'E and 78°07'N. It has a water spread area of 368 acres. Water is mainly used for irrigation. Fishing, grazing, fuel wood collection, agriculture and religious use (temple) are some of the major uses of the wetland. *Oreochromis mossambicus* and *Heteropneustes fossilis* are exploited commercially. About 5 to 10% decrease in fish yield is reported over the last 5-6 years. This wetland is suspected to receive industrial effluent discharge. Rice is the commonly cultivated crop in the surrounding. Urea and DAP are the fertilizers employed to enhance the crop yield.

13.2.5 Koothapar Big Tank

It is located in the district Tiruchirapalli between 10°07'E and 78°06'N having a water spread area of 368 acres. About 5-10% decrease in fish yield has been reported over the last 5-6 years. This wetland witnesses agricultural activities nearby where rice is cultivated as a major crop. Ekalux and endrin are the pesticides commonly sprayed. This wetland is also suspected to receive industrial effluents.

13.2.6 Kunnathur

It is located in the district Madurai between 09°57'E and 78°14'N and has a water spread area of 1066 acres. Agricultural activities are seen closer to this seasonally cultivated wetland where paddy is grown. It is needless to mention about the use of pesticides and fertilizers for agriculture and their impact.

13.2.7 Vandiyur Tank

It is located in the district Madurai between 09°53'E and 78°12'N. The water spread area is about 687 ha. This wetland appears to be contaminated with municipal sewage. As a result, fishes such as *Channa orientalis* and *Channa*

striatus develop diseases characterized by ulcer on the skin and it also gets spread to the other species. This seasonally cultivated wetland supports agricultural activities minimally. Paddy is grown in the surroundings. Urea is found to be the commonly applied fertilizer to enhance the growth. Heavy drainage flow from the city, dumping of wastage in the tank, anthropogenic pressure and fishing by explosives are the notable problems posing threat to the tank. Further as the tank is close to Madurai city, there is heavy traffic on the roads surrounding the Vandiyur tank which could also create pollution.

13.2.8 Mappedu Periya Eri

It is located in the district Thiruvellur between 13°04'E and 79°53'N. The water spread area ranges from 10.117 ha to 115.07 ha. Irrigation department protects this seasonally cultivated wetland which supports the agricultural activities nearby. Paddy is the commonest crop seen around. DAP and Urea are the fertilizers used. This wetland appears free from any industrial effluent discharges. Fishing in this wetland is regularly practiced and fishes are affected by an unknown disease locally known as fix pox. As a result, decline in the fish yield is reported.

13.2.9 Pallikaranai Marsh

It is located in the district Chengalpattu between 12°59'E and 80°14'N and the area is about 50 sq km. Pallikaranai is a vast marshland complex, which is currently under progressive deterioration due to anthropogenic pressures. This marsh experiences solid waste dumping and industrial and domestic effluent discharge, leading to deterioration of the ecosystem nevertheless the fish resources are being exploited.

13.2.10 RS Mangalam

It is located in the district Ramanathapuram between 09°22'E and 78°52'N. It has a water spread area of 8.93 sq km. About 25% decline in the fish yield has been observed during the last 5-6 years. Although this seasonally cultivated wetland is

free from any industrial discharge, it supports the agricultural activities closely where crops such as paddy is grown.

13.2.11 Vembanur wetland complex

It is spread over an area of approximately 40 sq km in the district Kanniyakumari between 08°11'E and 77°22'N. This wetland supports agricultural activities nearby and effluent discharge into this wetland is suspected. Fishing is carried out in this wetland where species such as *Catla catla*, *Heteropneustes fossilis* and *Channa punctatus* are grown.

13.2.12 Singanallur Tank

This tank is located in the district Coimbatore between 10°58'E and 77°02'N and the total water spread area is 1.15 Km². The capacity of this tank is 1 million m³. The lake is highly affected by eutrophication and almost filled up with *Eichhornia*. Siltation has reduced the depth of the tank quite substantially. Although *Eichhornia* is blessed for its ability to retain nutrients and other chemicals from water and sediments, it is considered as an undesirable weed for wetlands. Singanallur receives supply from Noyyal river through Singanallur ancient channel and also receives the entire drainage from the residential area at its vicinity and from Sangnanur pallam, a jungle stream. The municipal corporation undertook a project to establish recreational boating facilities in the lake but it has been terminated due to heavy aquatic weed encroachment. However, this lake has leased to local fishermen for fishing and it serves as a potential source of fish supply to Coimbatore.

13.2.13 Sulur Tank

It is located in the district Coimbatore between 11°02'E and 77°07'N and the total water spread area is 1.25 sq km. About 75% decline in the fish yield in all commercial species stocked has been observed. Mortality of fishes has been observed during the year 2001 which could be due to overcrowding and decrease

in dissolved oxygen. Dumping of domestic sewage is also observed. Seasonal cultivation in this wetland is observed. Moreover, it supports agricultural activities closeby where crops such as sugarcane and maize are grown. Nuvacron is the notable pesticide applied in this area.

13.2.14 Theroor wetland complex

It is located in the district Kanyakumari between 08°09'E and 77°27'N. The extent of this wetland is about 35 sq km. This wetland supports agricultural activity nearby where paddy is found to be the commonest crop. Fertilizers such as DAP and Urea are applied to enhance the growth. Fishing is common and intrusion of domestic sewage into this wetland is suspected.

13.2.15 Vaduvor

The Vaduvor Lake Bird sanctuary is located at the Thanjavur district of Tamil Nadu. It is 24 km from Thanjavur and 15 km from Mannargudi. The lake has an average depth of 2.5 m, and receives water from the northeast monsoon and Vennaru River. The lake irrigates about 1,356 acres of agricultural land. Nayvasal and Vaduvor village are situated around the sanctuary. The road of Mannargudi borders one side of the lake. The other side is protected by huge bunds. Migratory birds start to arrive by October and stay till February to March. This fresh water lake is rich in aquatic flora, submerged, floating and emergent vegetation. *Ipomoea carnia* is seen in many parts of the lake. There are thickets of *Prosopis chilensis*. The Forest Department has planted *Acacia nilotica* in the lake environs. Indiscriminate fishing, over grazing, siltation, encroachment, invasion by exotic species and pollution are some of the threats. Like most other lakes of Tamilnadu, Vaduvor is silting up. It needs dredging to increase the depth to store more water. The soil containing bird guano is rich in minerals, and is collected by the villagers. This provides some degree of protection to the birds. However, removal of guano-rich soil should be regulated with the cooperation of the villagers, so that the birds are least disturbed.

Ipomoea carnia a weed in this wetland provides habitats to some birds and fish. And finally, there is a larger issue of the use of harmful pesticides in the agricultural fields, which enter the lake with the run-off from the fields. Regular monitoring of the water quality, through the local college/university could be taken up. Vaduvor Bird Sanctuary is a good site for research on fresh water ecosystem, fish, birds, impact of pesticides and economic benefits to man.

13.2.16 Suchindaram complex

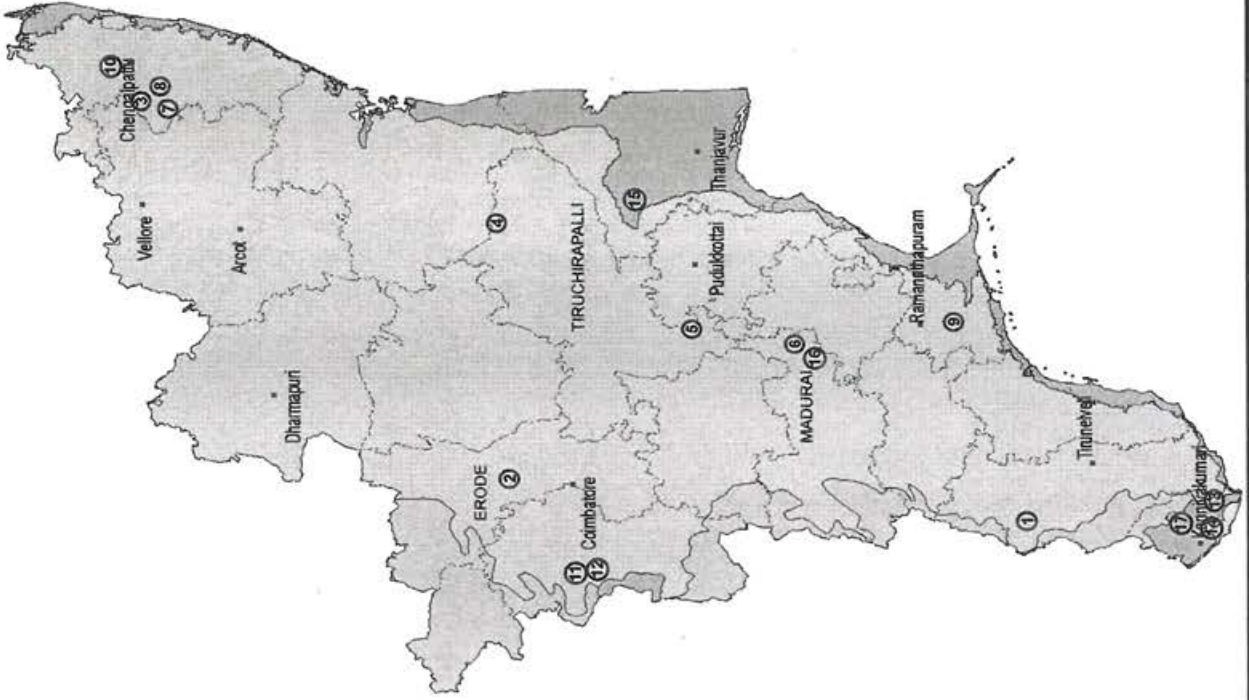
It is located in the district Kanyakumari between 08°09'E and 77°27'05"N and has a water spread area of 50 ha. This wetland besides supporting agricultural activities also serves as good fishing ground. Domestic sewage intrusion to the wetland is observed. Paddy is the commonest crop grown in the surrounding.

13.2.17 Sathyamoorthy Sagar Reservoir

It is located in the district Thiruvallur between 13°11'N and 79°51'E. The water spread area ranges between 1.00 and 34.08 sq km. The Chennai Metropolitan water supply and sewerage board holds the authority on this reservoir presently. Water stored here is supplied to the Chennai city mainly for drinking purpose. Although this reservoir is free from any industrial effluent discharges decrease in fish yield is reported. Paddy is the crop cultivated in the nearby agricultural lands.

Map. 10 Location of select wetlands studied in
Tamil Nadu

1. Alwarkurichi
2. Avalpoondarai
3. Chembarampakkam
4. Gundur Big Tank
5. Koothapar Big Tank
6. Kunnathur
7. Mappedu
8. Pallikaranai
9. RS Mangalam
10. Sathyamoorthysagar Reservoir
11. Singanailur Tank
12. Suchindaram Tank
13. Sular Tank
14. Theroor
15. Vaduvor
16. Vandiyur
17. Vembanur



13.3 RESULTS

A sum of 155 individuals belonging to 11 species collected from 17 wetlands in Tamil Nadu (Table.58) and analyzed for metal contamination.

Table.58 List of select wetlands examined for metal contamination

S.No.	Name of the wetland	No. of fishes collected
1	Alwarkurichi	7
2	Avalpoondarai	13
3	Chembarampakkam Reservoir	7
4	Gundur big Tank	6
5	Koothapar big Tank	11
6	Kunnathur	11
7	Mappedu	8
8	Pallikaranai	10
9	R.S. Mangalam	12
10	Sathyamoorthy Sagar Reservoir	4
11	Singanallur Tank	7
12	Suchindaram	8
13	Sulur Tank	9
14	Theroor complex	8
15	Vaduvor	12
16	Vandiyur Tank	14
17	Vembanur	8
Total no. of fishes collected		155

13.3.1 Variation in heavy metal contamination among wetlands of Tamil Nadu

i. Lead

Lead levels were appreciable among all the wetlands with the maximum being recorded in Singanallur tank (8.60 ± 0.41 ppm) followed by Sulur tank (7.61 ± 0.28

(2.87 ± 0.29 ppm) of Tirunelveli district (Table.59). The average lead levels were 3 and 6 ppm in most of the wetlands. Significant variation in contamination levels could be established ($P < 0.01$) in the fishes among the wetlands (Table.60).

ii. Zinc

Although zinc levels ranged between below detectable levels (BDL) and 60.67 ppm, significant variation ($P < 0.01$) (Table.60) could be perceived among the fishes of wetlands. Vadavoor (29.49 ± 3.96 ppm) of Kanyakumari district recorded the highest levels followed by Kunnathur (23.07 ± 4.09 ppm) of Madurai district. Singanallur (5.44 ± 0.89 ppm) of Coimbatore district measured the least levels (Table.59).

iii. Copper

Alike zinc levels on an average, least concentration was observed in Singanallur (5.53 ± 0.89 ppm) while Vaduvor (30.01 ± 4.03 ppm) of Kanyakumari district the highest (Table.59). Except, Pallikaranai of Kancheepuram district and Sular of Coimbatore district, the other wetlands showed levels ranged between 10 and 30 ppm. Significant variation in metal contamination could be perceived among the wetlands ($P < 0.01$) (Table.60).

iv. Cadmium

On an average, the cadmium levels are reported to be within 1 ppm in all the wetlands except Alwarkurichi (1.53 ± 0.91 ppm) of Tirunelveli district which measured the maximum concentrations. Least levels were observed in Suchindaram (0.54 ± 0.02 ppm) of Kanyakumari district (Table.59). Significant variation in metal contamination could be observed among the fishes of wetlands ($P < 0.01$) (Table.60).

Table.62 Levels of heavy metal contamination among various species of fishes in the wetlands of Tamil Nadu (Mean±S.E)

Species	Lead	Zinc	Copper	Cadmium	Chromium
<i>Anabas testudineus</i>	3.54 ± 1.14	12.60 ± 7.43	12.82 ± 7.59	0.76 ± 0.09	0.06 ± 0.02
<i>Channa orientalis</i>	4.94 ± 0.09	13.42 ± 1.18	13.66 ± 1.20	0.71 ± 0.03	0.69 ± 0.17
<i>Channa striatus</i>	5.43 ± 0.21	14.70 ± 1.28	14.96 ± 1.31	0.77 ± 0.03	0.53 ± 0.15
<i>Cyprinus carpio</i>	5.12 ± 1.05	20.32 ± 6.88	20.68 ± 7.01	0.80 ± 0.04	0.86 ± 0.28
<i>Heteropneustes fossilis</i>	3.87 ± 0.48	11.49 ± 4.20	11.70 ± 4.27	2.21 ± 1.59	0.40 ± 0.08
<i>Labeo rohita</i>	5.97 ± 0.56	14.03 ± 2.82	14.28 ± 2.87	0.76 ± 0.08	1.05 ± 0.26
<i>Mystus vittatus</i>	5.44 ± 0.35	22.30 ± 4.00	22.69 ± 4.21	0.76 ± 0.21	0.67 ± 0.55
<i>Notopterus notopterus</i>	5.15 ± 0.51	25.20 ± 6.56	25.65 ± 6.67	0.63 ± 0.17	0.00 ± 0.00
<i>Puntius dorsalis</i>	7.45 ± 0.46	36.19 ± 4.71	36.83 ± 4.79	0.96 ± 0.03	0.23 ± 0.13
<i>Oreochromis mossambicus</i>	6.10 ± 0.21	11.19 ± 1.25	11.38 ± 1.27	0.77 ± 0.02	0.54 ± 0.08
<i>Channa punctatus</i>	5.62 ± 0.39	13.39 ± 1.13	13.63 ± 1.15	0.73 ± 0.05	0.54 ± 0.13

Table.63 Variation in heavy metal contamination among various species of fishes in the wetlands of Tamil Nadu (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	24.327	36.438	36.438	8.216	26.357
df	10	10	10	10	10
Asymp. Sig.	0.007*	0.000**	0.000**	0.608	0.003**

*P<0.05; **P<0.01

v. Chromium

Alike cadmium levels, the chromium concentrations were also within 1 ppm in all the wetlands except in Vandiyur tank (1.61 ± 0.13 ppm) of Madurai district, which recorded the maximum (Table.59). BDL was reported in all the fishes of Vaduvor of Kanyakumari district. Significant variation in contamination levels could be noticed among the wetlands ($P < 0.01$) (Table.60).

13.3.2 Variation in heavy metal contamination among various species of fishes in the wetlands of Tamil Nadu

Variation in heavy metal contamination among eleven species (Table. 61) totaling one hundred and fifty five were compiled.

Table.61 List of species examined in detail for heavy metal contamination

S.No.	Name of the species	No. of individuals collected
1	<i>Anabas testudineus</i>	6
2	<i>Channa orientalis</i>	4
3	<i>Channa punctatus</i>	21
4	<i>Channa striatus</i>	23
5	<i>Cyprinus carpio</i>	6
6	<i>Heteropneustes fossilis</i>	4
7	<i>Labeo rohita</i>	7
8	<i>Mystus vittatus</i>	21
9	<i>Notopterus notopterus</i>	3
10	<i>Oreochromis mossambicus</i>	56
11	<i>Puntius dorsalis</i>	4
Total no. of fishes collected		155

i. Lead

Among the species, on an average, maximum lead contamination was documented in *Puntius dorsalis* (7.45 ± 0.46 ppm) which was two folds higher than the contamination levels reported in *Anabas testudineus* (3.54 ± 1.14 ppm) (Table.62). Significant variation in contamination levels could be established among the species ($P < 0.05$) (Table.63)

ii. Zinc

Among the 93.5% samples recording measurable levels, maximum metal accumulation was observed in *Puntius dorsalis* (36.19 ± 4.71 ppm) while *Oreochromis mossambicus* (11.19 ± 1.26 ppm) recorded the minimum (Table.62) with significant differences in metal contamination ($P < 0.01$) (Table.63).

iv. Copper

Alike zinc concentrations, *Puntius dorsalis* had the maximum metal burden (36.83 ± 4.80 ppm) and *Oreochromis mossambicus* (11.38 ± 1.28 ppm) the minimum (Table.62). Significant variation in metal contamination could be well established among the species ($P < 0.01$) (Table.63). Most of the species had near equal levels of zinc and copper.

iv. Cadmium

Cadmium was detected in 98% of the samples analyzed. On an average, *Heteropneustes fossilis* (2.21 ± 1.60 ppm) ranked high among other species in accumulating metal concentration. *Notopeterus notopterus* (0.63 ± 0.17 ppm) recorded the minimum levels (Table.62) which were twice less than *Heteropneustes fossilis*. Most of the other species had levels less than 1 ppm. No significant variation in cadmium contamination among the species could be observed ($P > 0.05$) (Table.63).

Table.59 Levels of heavy metal contamination (ppm) among select wetlands of Tamil Nadu (Mean \pm S.E)

Wetlands	Lead	Zinc	Copper	Cadmium	Chromium
Alwarkurichi	2.87 \pm 0.29	14.86 \pm 1.81	15.12 \pm 1.84	1.53 \pm 0.91	0.32 \pm 0.07
Aval Poondarai	6.57 \pm 0.04	12.68 \pm 1.38	12.90 \pm 1.40	0.96 \pm 0.03	0.37 \pm 0.18
Chembarbakam Reservoir	4.44 \pm 0.54	21.68 \pm 4.11	22.07 \pm 4.80	0.76 \pm 0.13	0.27 \pm 0.19
Gundur big Tank	7.29 \pm 0.48	15.74 \pm 3.06	16.02 \pm 3.12	0.80 \pm 0.13	0.64 \pm 0.14
Koothapar big Tank	5.87 \pm 0.38	11.98 \pm 3.60	12.19 \pm 3.67	0.66 \pm 0.06	0.33 \pm 0.05
Kunnathur	6.65 \pm 0.24	23.07 \pm 4.09	23.48 \pm 4.16	0.82 \pm 0.02	1.16 \pm 0.19
Mappedu	5.47 \pm 0.26	11.10 \pm 3.60	11.30 \pm 1.27	0.65 \pm 0.04	0.55 \pm 0.08
Palikarnai	3.63 \pm 0.81	6.42 \pm 4.09	6.54 \pm 1.95	0.74 \pm 0.05	0.25 \pm 0.10
RS Mangalam	5.01 \pm 0.15	17.06 \pm 1.24	17.36 \pm 2.61	0.72 \pm 0.02	0.71 \pm 0.07
SathyamoorthySagar Reservoir	5.07 \pm 0.08	11.48 \pm 1.91	11.68 \pm 2.13	0.70 \pm 0.05	0.71 \pm 0.10
Singanallur	8.60 \pm 0.41	5.44 \pm 2.57	5.53 \pm 0.89	0.54 \pm 0.02	0.51 \pm 0.14
Suchindaram	4.79 \pm 0.16	13.37 \pm 2.31	13.61 \pm 2.35	0.54 \pm 0.01	0.03 \pm 0.02
Sulur tank	7.61 \pm 0.27	6.26 \pm 2.38	6.38 \pm 2.42	0.81 \pm 0.06	0.45 \pm 0.06
Theroor complex	4.87 \pm 0.16	17.82 \pm 6.44	18.14 \pm 6.55	0.85 \pm 0.02	0.69 \pm 0.41
Vaduvoor	6.25 \pm 0.26	29.49 \pm 3.95	30.01 \pm 4.02	0.88 \pm 0.05	0.00 \pm 0.00
Vandiyur Tank	5.32 \pm 0.09	14.15 \pm 1.55	14.40 \pm 1.57	0.79 \pm 0.01	1.63 \pm 0.13
Vembanur	4.87 \pm 0.17	16.20 \pm 3.60	16.49 \pm 3.66	0.89 \pm 0.03	0.26 \pm 0.08

Table.60 Variation in heavy metal contamination among select wetlands of Tamil Nadu wetlands (Kruskal Wallis test)

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	102.508	64.792	64.792	61.855	104.471
df	16	16	16	16	16
Asymp. Sig.	0.000*	0.000*	0.000*	0.000*	0.000*

*P<0.01

v. Chromium

About 27% of the samples analyzed showed below detectable levels. The concentration recorded in all the species were within 1 ppm except *Labeo rohita* (1.05 ± 0.26 ppm) which recorded the highest level while *Notopterus notopterus* showed BDL (Table.62). The differences in the levels recorded were found to be significant ($P < 0.05$) (Table.63).

13.4 DISCUSSION

13.4.1 Variation in metal contamination among select wetlands of Tamil Nadu

Among the wetlands, Ariyakulam, Alwarkurichi and Vandiyur recorded the highest concentrations of lead, cadmium and chromium while Vaduvor recorded the maximum concentration of zinc and copper.

Most of these wetlands are seasonally cultivated and reported to have suffered a minimum of 30% decline in fish harvest during the past five years. They also irrigate agricultural lands nearby. Wetlands such as Pallikaranai, Vandiyur, Singanallur, and Avalpoondarai do suffer from domestic sewage discharge which account for their elevated metal concentrations. However no data exists on the quantum of effluent discharged into these wetlands. Further the overall global increase in metals such as lead and cadmium through solid waste disposal and vehicular emission could also be appreciable. Thus the significant variation in contamination ($P < 0.05$) among the wetlands is attributed to their varied sources.

Comparing with the earlier contamination data in fishes collected from Singanallur of Coimbatore (Cu – 0.55 ppm; Pb – 1.13 ppm; Cd – 0.37 ppm; Cr – 0.26 ppm and Zn – 9.79 ppm) (Jayakumar, 2001), there is an increase in metal concentration. Especially, the current lead levels appear 10 folds greater than the earlier reported values. As the Singanallur Lake is subjected to recreational activity presently, there

are chances of increased input of metal contamination through the exhausts of motorboats and sewage discharge from the nearby colonies.

It is also important to note that the wetlands such as Suchindaram, Theroor, Vembanoor, Pallikaranai and Chembarampakkam are equally contaminated as that of the other wetlands mentioned above. Their overall metal burden appears high and they require further investigations.

13.4.2 Variation in metal contamination among various species of fishes in the wetlands of Tamil Nadu

Among the species, *Heteropneustes fossilis* recorded maximum concentration of cadmium. *Puntius dorsalis* measured the highest levels of lead, zinc and copper while *Labeo rohita* measured the maximum chromium concentration. Significant variation in zinc, copper and chromium concentrations ($P < 0.05$) among species could be related to a number of factors such as species specific differences, feeding habits, bioavailability of chemicals in food and water and the physico-chemical parameters of the aquatic environment (Taylor *et.al*, 2000; Eastwood and Courture, 2002; Cogun *et.al*, 2005 ; Pyle *et.al*, 2005).

The presence of many metals may affect the fish in a toxic or non-toxic manner. It may be difficult to generalize the uptake and accumulation of metals in aquatic organisms because of species differences in trace metal concentrations in fish tissues (Wiener and Giesy, 1979). The physiological mechanisms involved may also protect the organism at much higher levels, making interpretation of effects somewhat difficult. Background levels are not easily defined because of wide dissemination by wind and water (Mason *et.al*, 1988).

Accumulation of heavy metals in fishes is influenced by water quality parameters such as hardness and acidity. Moreover, the life cycle of an organism is also an important consideration in examining the effects of heavy metals, as the

concentration of heavy metals in body tissues vary with age or size of the organism (Atchinson *et.al*, 1977; Chernoff and Dooley, 1979).

Fishes are sensitive to metal contamination in water and may significantly damage certain physiological and biochemical process when they enter the organs of these animals (Namcsok *et.al*, 1987). Chronic exposure of fish to sub lethal trace metal levels causes disturbed ion regulation, reduced growth and swimming speed (Hollis *et.al*, 1999; Alsop *et.al*, 1999).

The values recorded in *Heteropneustes fossilis* for lead and cadmium are indicative of physiological disturbances to the fish (Abbasi *et.al*, 1998; Misra *et.al*, 2002). *Heteropneustes fossilis* is a bottom dweller and an omnivorous feeder and hence the higher levels could be plausibly through biotransformation from food and sediments. Mercy (1999) studied the metal concentration in the sediments of Singanallur tank and observed high levels of zinc (72.5 ppm) and lead (4 ppm). Thus the possibility of biotransformation of metals from the sediments can be admitted for the high concentration of metals in the fishes collected from the tank.

Several authors have described the hematological alterations, biochemical changes and hampering of locomotion in fishes like Rainbow Trout and Salmon at a concentration of 0.012 ppm of lead (Hodson *et.al*, 1978; Shrivastava and Mishra, 1979). Chromium has been found to cause alterations in biochemical processes and immune responses. Kunhert *et.al*, (1976) reported significant inhibition in activity of enzyme Na/K ATP ase, which is involved in osmoregulation in the kidney of *Salmo gairdneri* after exposure at 2.5 ppm Cr (VI) for 48 hours. Singh and Singh (1979) found 0.003 ppm of cadmium inhibiting the oxygen consumption in *Mystus vittatus* by 50% during an exposure for 12 hours. Although the current levels are alarming, the physiological mechanisms, species differences, physico-chemical properties of the surviving water, availability and absorption of metal are also to be admitted while considering the ill effects. If the exposure is continuous it can be anticipated that certain physiological damages such as decline

of proximal principles, hampered locomotion, disturbances in osmoregulation could happen.

13.5 Suitability of fishes for human consumption in Tamil Nadu

Dietary intake of metals by man through consumption of fishes was calculated for all the species and wetlands (Table 64, 65) as described in Chapter III.

Table.64 Average daily dietary intake of metals through consumption of fishes (mg) - Species wise

Name of the species	Lead	Zinc	Copper	Cadmium	Chromium
<i>Anabas testudineus</i>	0.13	0.45	0.46	0.03	0.00
<i>Channa orientalis</i>	0.18	0.48	0.49	0.03	0.02
<i>Channa striatus</i>	0.19	0.53	0.53	0.03	0.02
<i>Cyprinus carpio</i>	0.18	0.73	0.74	0.03	0.03
<i>Heteropneustes fossilis</i>	0.14	0.41	0.42	0.08	0.01
<i>Labeo rohita</i>	0.21	0.50	0.51	0.03	0.04
<i>Mystus vittatus</i>	0.19	0.80	0.81	0.03	0.02
<i>Notopterus notopterus</i>	0.18	0.90	0.92	0.02	0.00
<i>Puntius dorsalis</i>	0.27*	1.29	1.32	0.03	0.01
<i>Oreochromis mossambicus</i>	0.22*	0.40	0.41	0.03	0.02
<i>Channa punctatus</i>	0.20	0.48	0.49	0.03	0.02

Among the 13 species of fishes calculated for dietary input of metals, the lead input through consumption of *Puntius dorsalis*, *Oreochromis mossambicus* and *Channa punctatus* appeared to be high with a concentration of 0.27, 0.22 and 0.20 mg respectively. The maximum calculated concentrations of zinc and copper through the consumption of *Puntius dorsalis* were 1.29 and 1.32 mg/day/person respectively, while it was minimum through *Oreochromis mossambicus* 0.40 and 0.14 mg/day/person. Cadmium and chromium ranged between 0.02 and 0.08 mg and between 0.01 and 0.04 mg/day/person respectively (Table. 64).

Table.65 Average daily dietary intake of metals through consumption of fishes (mg) - Wetland wise

Wetland	Lead	Zinc	Copper	Cadmium	Chromium
Alwarkurichi	0.10	0.53	0.54	0.05	0.01
Aval Poondarai	0.23*	0.45	0.46	0.03	0.01
Chembarmbakam Reservoir	0.16	0.77	0.79	0.03	0.01
Gundur Big Tank	0.26*	0.56	0.57	0.03	0.02
Koothapar Big Tank	0.21	0.43	0.44	0.02	0.01
Kunnathur	0.24*	0.82	0.84	0.03	0.04
Mappedu	0.20	0.40	0.40	0.02	0.02
Pallikarnai	0.13	0.23	0.23	0.03	0.01
RS Mangalam	0.18	0.61	0.62	0.03	0.03
Sathyamoorthy Sagar Reservoir	0.18	0.41	0.42	0.02	0.03
Singanallur	0.31*	0.19	0.20	0.02	0.02
Suchindaram	0.17	0.48	0.49	0.02	0.00
Sulur tank	0.27*	0.22	0.23	0.03	0.02
Theroor complex	0.17	0.64	0.65	0.03	0.02
Vaduvloor	0.22*	1.05	1.07	0.03	0.00
Vandiyur tank	0.19	0.51	0.51	0.03	0.06
Vembanur	0.17	0.58	0.59	0.03	0.01
WHO/FAO (1972) Tolerable daily dietary intake limits for human consumption	0.50	10-15	1.30	0.05	0.40
WHO/FAO (1989, 1993)	0.21	-	-	-	-

* Above tolerable limit

Of the various wetland fishes studied suitability for human consumption, the input of lead through the consumption of fishes from Aval Poondarai, Gundur big tank, Kunnathur, Singanallur, Sulur Tank and Vaduvloor was high when compared with other wetlands of Tamil Nadu. The calculated lead input among the wetlands ranged between 0.13mg in Pallikarani and 0.38 mg in Singanallur of Coimbatore district. The calculated input of zinc and copper through the consumption of fishes of Vaduvloor were 1.05 and 1.07 mg/day/person respectively (Table.65). The dietary intake of cadmium and chromium appeared to be the same through the consumption of fishes from all the wetlands.

An analysis of the daily dietary intake of metals by humans through consumption of fish shows that the levels of all the metals, except lead, are within the limits

suggested by various statutory agencies. The calculated input of lead through *Puntius dorsalis* and *Oreochromis mossambicus* exceeds the tolerable dietary intake concentration prescribed for human consumption by Joint Expert Committee on Food and Agriculture (JECFA) of United Nations (UN) and WHO (1989, 1993) which admit only upto 0.21 mg/day for a 60 Kg adult. Among the eight species of fishes studied, input of copper through consumption of *Puntius dorsalis* exceeded the maximum tolerable limits of 1.30 mg/day/person. The daily dietary intake concentration of zinc, copper, cadmium and chromium were compared with the tolerable dietary intake described by WHO/FAO (1972) for human consumption. According to WHO/FAO (1972) the tolerable daily intake of zinc, copper, cadmium and chromium is 10-15, 1.30, 0.05, 0.4 mg respectively through consumption of food and water. It is to be noted that these species are stocked in many of the wetlands for commercial purpose. Regular consumption of these fishes may create physiological complications in the consumers. Further, the recorded levels of lead can create physiological disturbances to the fishes themselves.

13.6 Dietary habits and variation in heavy metal contamination in fishes collected from select wetlands of India

All the 19 species included in the present study were grouped into four major categories based on their feeding habits, namely planktivorous, carnivorous, omnivorous and detritivorous. Accordingly the study had 33 planktivorous, 348 carnivorous, 264 omnivorous and 259 detritivorous fishes.

Carnivorous (5.70 ± 0.10 ppm) and omnivorous (5.69 ± 0.11 ppm) fishes had near equal levels of lead. Zinc (18.61 ± 0.65 ppm) and copper (18.94 ± 0.66 ppm) concentrations were found to be the maximum in carnivorous fishes. Lowest level of cadmium was observed in planktivorous fishes (0.63 ± 0.03 ppm) while omnivorous (0.75 ± 0.04 ppm) fishes had the highest concentrations. Detritivorous fishes (0.71 ± 0.05 ppm) had the maximum chromium concentrations followed by planktivorous fishes (0.70 ± 0.09 ppm) (Table.66). The variation in contamination among the four feeding categories of fishes was significant ($P < 0.05$) (Table.67).

Interestingly, lead and cadmium concentrations were found low in detritus feeders which are generally expected to accumulate more metals through sediment biotransformation (Mason *et.al*, 2000 and Mountouris *et.al*, 2002). But according to Gray *et.al*, (1992) some metals are easily eliminated by organism and they do not accumulate in aquatic food chain.

Planktivores, which had 33 individuals in the present study, recorded higher concentration of chromium. Generally, the small surface area and large binding sites of plankton facilitate in concentrating the contaminants. Metal uptake through food is the major route of metal concentration in fishes. Further, passive uptake by diffusion through body surfaces including gills, with elimination rates decreasing with increased body size (Leblanc, 1995; McKay & Fraser, 2000) is also to be accounted.

In the current study omnivorous fishes also had higher concentration of lead which could be attributed to their diet. Carnivorous fishes prey on a variety of aquatic organisms and so they are expected to accumulate more metals in a significant manner.

13.7 Heavy metal contamination in fishes and their relation to size and weight

It may be noted that the size and body weight of the organisms are known to influence tissue metal concentrations (Amiard-Triquet and Amiard, 1998; Canpolat and Calta, 2003). Karl Pearson correlation was used to test the relation between the morphometry of the fishes and the contamination level.

Significant negative correlation was observed between morphometry and all the metals except chromium. The copper and zinc concentrations declined with decrease in length and weight. It is well understood that copper and zinc are essential nutrients required for the growth of the fishes.

Table.68 Relationship (Correlation coefficient 'r') between heavy metal contamination in fishes and their weight and length.

Morphometry	Lead	Zinc	Copper	Cadmium	Chromium
Length	-0.146**	-0.211**	-0.211**	-0.118**	-0.057
Weight	-0.118**	-0.173**	-0.173**	-0.112**	-0.021

** Correlation is significant at the 0.01 level (2-tailed)

Similar to the present study, Krishnamurthy and Nair (1999) had observed 95% significant positive relationship between the levels of Cu, Zn and Pb with body length and weight among the fishes collected from Bombay and Thane basin creeks. Thus, it is clear, metals such as Cu and Zn are essential metals and their decline in concentrations has negative impact on the growth of the fishes. Further, Pb and Cd are toxic metals, which might have a negative impact on the morphometry of the fishes. It is noteworthy that the accumulation of metals is influenced by various factors such as uptake, elimination and also metabolism of the fish. Further, species-specific characteristics also have a strong influence in metal accumulation (Campbell, 1995).

Threat to wetlands



Sewage intrusion

Agricultural runoff, a major source of wetland contamination



Industrial Effluents

Dumping of toxic wastes from hospitals



A few of the wetlands studied



**Dharoi Dam -
Gujarat**

**Pallikaranai
Wetland
Marsh –
Tamil Nadu**



**Tumkur
Ammanikere -
Andhra Pradesh**

BIOMARKERS OF HEAVY METAL CONTAMINATION IN FISHES OF SELECT WETLANDS IN TAMIL NADU

14.1 INTRODUCTION

It is needless to mention that a comprehensive chemical analysis would establish only the presence of contaminants without revealing how bioavailable or active they are within an organism. Whereas biomarkers through biochemical responses will not only signify exposure, but also help predict future harm. Biomarker is generally used in a broad sense to include almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical or biological (WHO, 1993).

When risk assessment for animals has to be conducted, collection of detailed information on the contaminant's potential threat and effect is often costly and time-consuming. Therefore 'endpoints or biomarkers' were developed to screen specific contaminants (Burger *et.al*, 2000). A biomarker is defined as a change in a biological response (ranging from molecular through cellular and physiological responses to behavioral changes) which can be related to exposure to or toxic effects of environmental chemicals (Peakall, 1994). Thus, it provides a valuable insight on the exposure, and helps to initiate a rapid assessment of the impact of environmental contaminants on animals from a large geographical area.

The indisputable concept of the effect of environmental contaminants preceded by sub lethal changes at the molecular and cellular level, prompts us to propose use of biomarkers in evaluating the ill effects in fishes. Biomarkers provide an integrated, comprehensive and early warning signal on the biological availability of a contaminant. The dualistic meaning of 'early warning' by a biomarker is first it sheds a ray of warning signal of effects that may develop with time at higher levels of biological organization (e.g., populations and ecosystems). Second, it may indicate the detection of a response at concentrations below those causing irreversible effects (Gester and Brummelen, 1996). Thus, biomarkers can be well employed as tools in

retrospective risk assessment. Further, biomarkers in combination with community measurements help us to determine the 'real-time status of organism health.

Deleterious effects of metals may not become apparent until changes occur at the population or ecosystem level, a point at which it may become too late to take effective corrective measures. A concept of Ecotoxicology is that these effects are preceded by sub-lethal changes at the molecular and cellular level. Detection of such subtle markers can serve as early indication of contaminant bioavailability and impact, thus providing valuable information regarding the extent, the possible consequences of prolonged or continued exposure (Lionetto *et.al*, 2001; Langston *et.al*, 2002). An early indicator of the biological effect of heavy metals is represented by the metallothionein level in the tissues of the indicator species which provides a suitable monitoring procedure in order to assess the biological availability and impact of heavy metals in the aquatic environment (George and Olsson, 1994). Metallothioneins are involved in metal cell homeostasis with possible biological functions in storage, transport or compartmentalization of essential metals (Diguilio *et.al*, 1995). Although, hepatic levels of heavy metals could potentially reflect environmental exposure, they provide only partial information and do not show the global effect of exposure on aquatic organisms (Livingstone, 1993). Hence the metallothionein that provides an integrated response to metal exposure in fishes assumes greater significance.

Environmental exposure of fish to heavy metals has been shown to primarily result in renal and hepatic accumulation of metal bound to metallothionein. Therefore, liver and kidney were selected as target tissues for metallothionein determination (Filipovic and Raspor, 2003). Further, fish gills are multifunctional organs involved in ion transport, gas exchange, acid-base regulation and waste excretion. Studies have shown that metals enter gills from the ambient environment and induce metallothionein synthesis in the branchial epithelium (Dang *et.al*, 1999).

Glutathione, yet another potential candidate involved in detoxification of endogenous and exogenous compounds, is a predominant thiol compound found both in prokaryotes and eukaryotes. It is involved primarily in maintaining the cell membrane integrity, oxidation-reduction balance and scavenging free oxy radicals. Studies done

have observed Glutathione (GSH), to provide first line of defense against metal toxicity especially cadmium toxicity before induction of metallothionein synthesis occurs (Zarogian and Norwood, 2002). Thus, it plays an important role in early cellular protection responses to metals and their levels get decreased only after a toxic response (Bell and Cowey, 1990).

In the present study, an attempt has been made to quantify the biomarkers of heavy metal contamination, namely Metallothionein-like proteins and Glutathione in organs of fishes, namely gill, liver and kidney, and their relation to metal contamination.

14.2 LITERATURE REVIEW

Biomarkers are defined as a biological response to a chemical or chemicals that gives a measure of exposure and sometimes also of toxic effect (Peakall and Walker, 1994). Although the history of their usage in the pollution research is more than 35 years, the limitations and misunderstandings did not encourage the scientific community to practice extensively. Today, improved and refined methodologies are handy to study biomarkers pragmatically. Biomarkers are potential surrogate measures of chemical contaminants and it is in this context that they have been most widely used of late (Livingstone and Goldfarb, 1998; Handy and Depledge, 1999; Adams *et.al*, 2001; Wells *et.al*, 2001).

For example, rather than continuously monitoring trace metal concentrations in sediment, water or biota, the concentration of the metal binding protein metallothionein can be measured in tissues (Pedersen *et.al*, 1997 ; Hylland *et.al*, 1998). Similarly, instead of using tissue concentrations of organophosphorous pesticides in exposure assessments the extent of cholinesterase inhibition in brain tissue or blood samples of exposed organisms can be determined to provide an extend of exposure (Sturm *et.al*, 1999; Fulton and Key, 2001; Galloway *et.al*, 2002).

In both these examples, it is evident that the specific metal or pesticide to which the organism is exposed cannot be ascertained from the biomarker response, but it may indicate the involvement of environmental contaminants which is worthy of further

investigation. Thus biomarkers are currently about a weight of evidence approach to show that organism has been exposed to contaminants and/or that exposure is associated with deterioration in health (including reductions in growth and reproductive output that are potentially of great ecological significance) (Handy *et.al*, 2003).

Thus, it is lucid that estimation of biomarkers has the following advantages. Biochemical responses induced may indicate the presence of a biologically available contaminant rather than a biologically inert form. Study employing a battery of biomarkers may reveal the presence of contaminants that were not suspected initially. The responses are often long persisting after a transient exposure to a contaminant that has then degraded and is no longer detectable thus detecting the intermittent pollution events that routine chemical monitoring may miss. Importantly biomarker analyses are in many cases, much easier to perform and are considerably less expensive than a wide range of chemical analysis. In India, study on biomarkers such as metallothionein, glutathione (GSH), Glutathione- S- transferase (GST), Mixed Function Oxygenase enzyme system (MFO) such as Cytochrome P450, EROD, BROD and MROD assay and vitellogenin are limited on fishes. However, detailed researches done elsewhere are compiled here.

Metallothioneins are cysteine- rich heat stable proteins. They bind metals through metal-thiolate bonds. It has been identified in all the major classes of vertebrates (Stegman *et.al*, 1992). They also bind to other I B and II B elements. They have an additional role in reducing the toxicity of other metals such as Ag, Cd & Hg in contaminated environments (Livingstone, 1993). An attractive idea that has been receiving recent support is that MT might function as a chaperon for the synthesis of metalloproteins, it could serve as reservoir of essential metals while preventing metal toxicity and yet donates the metal to apometalloenzymes as they are synthesized afterwards (Palmiter,1998). Thus, induction of protein metallothionein has been considered as one of the potential, powerful and specific biochemical response at sub cellular level to metal contamination (Roesijadi, 1994; Wu *et.al*, 1999; Langston *et.al*, 2002 ; DeBoeck *et.al*, 2003).

The relationship between metal levels in the environment and MT concentrations in animal tissues has led to their use for monitoring the biological effects of metal exposure (Hylland *et.al*, 1992; Rotchell *et.al*, 2001). Expression of MT's has also found to be induced by steroid hormones, interferons and certain chemicals, hepatotoxic solvent and stresses (Kagi and Schaffer, 1988; Wu *et.al*, 1999). Moreover MTs have been indicated to be an efficient scavenger for free 'OH'. The intracellular concentration of MT in a large number of species so far investigated is largely variable and influenced by a number of factors such as heavy metal ions, inflammatory agents, cytokines, stress producing conditions, hormones and catecholamines (Andrews, 1990).

The levels of metallothionein have been documented in several marine and freshwater species of fishes, including salmoniformes (Rainbow trout), pleuronectiformes (Flounder and Plaice), cypriniformes (Stone loach and Gold fish) and Gadiformes (Cod) (Albergoni *et.al*, 2000; Dang *et.al*, 2001). Albergoni *et.al*, (2000) in his study observed a correlation between heavy metals and MT levels in the tissues of two Antarctic teleosts *Trematomus bernacchii* and the ice fish *Chinodraco hamatus* collected during the 10th Italian Antarctic Expedition. Identification of a metal binding protein similar to MT from *C.hamatus* was also reported. Significant linear correlations between metals and MT concentrations were found only in liver and gills. Correlations between MTs and Cd in liver and gills of both species indicate that Cd is linked to the protein.

It is worthy to note mercury or cadmium is found to be powerful inducers of MTs. Consequently, the occurrence of a metal and organ-specific isoform could be used as a tool for the assessment of an uncharacterized metallic pollution. Geret and Cosson (2002), observed Cd concentration of 7.74 ± 0.78 ppm in gills, 7.54 ± 0.67 ppm in digestive gland and 1.57 ± 0.12 ppm in mantle of *Mytilus edulis* after four days of exposure. A significant increase in total proteins and MTs was observed ($P < 0.05$) from control to the exposed fishes. It is also reported that copper produced more copper bound metallothionein like proteins (Riveros *et.al*, 2003).

Environmental exposure of fish to heavy metals has been shown to primarily result in renal and hepatic accumulation of metal bound to metallothionein. Therefore, liver and kidney were selected as target tissue for metallothionein determination (Filipovic and Raspor, 2003).

Hollis *et.al*, (2001) recorded a value of $8.09 \pm 1.66 \mu\text{g/g}$ of MT in liver of Juvenile Rainbow Trout at the initial day before chronic sub lethal exposure to $3 \mu\text{g/L}$ of cadmium and the levels increased two folds after 30 days. There are also reports showing very high levels of MT ($61.39 \pm 7.85 \mu\text{g/g}$) in fishes collected from uncontaminated sites (Lionetto *et.al*, 2001).

Many studies have observed increased MT synthesis when the metal ion concentration increases (Wu *et.al*, 2000; Hollis *et.al*, 2001). This could be because upon chronic intake of contaminated food results in stress in hepatic tissues to synthesize more MT. It is essential to mention that cadmium has a higher affinity than Zn for most MT's; it is likely that Zn would be displaced from MT-binding sites. It is possible that MT expression and tissue metal concentration may not always be correlated, especially following chronic environmental exposure to a variety of heavy metals (Burger *et.al*, 2000).

Increase in MT is observed during spawning period of fish as it is associated with MT induction by reproductive steroids (Olsson *et.al*, 1995). Moreover, a specific isoform of MT exists in each tissue and for each metal (Geret and Cosson, 2002).

Filipovic and Raspor (2003) observed 1.56 ± 0.1 ppm of MT in *Liza aurata* collected from eastern Adriatic Sea. Further the renal levels of metallothionein in fishes collected from uncontaminated (61.39 ± 7.85 ppm) and naturalistic (92.93 ± 15.48 ppm) sites (Lionetto *et.al*, 2001) indicate that variation could be related with species differences. Further, species reproductive condition, age and diet also account for the variation in MT content in fishes. Of the many factors, body weight of the organism is considered to be an important factor to explain variations of MT levels; when the weight was doubled, MT concentrations increased two-fold (Mouneyrac *et.al*, 1999). However, body weight can only explain certain percentage of variations leaving other

factors intervene, such as metabolic rate independent of metals either essential or toxic. Importantly, as MT levels are linked with total protein metabolism, an increase in metal content does not necessarily mean a corresponding increase in MT concentration. It may be hypothesized that organisms can cope, increasing the turnover of MT rather than its global concentration (Geffard *et.al*, 2001).

It is also important to know that, a biomarker such as MT needs to be validated in terms of baseline concentrations so that contaminant-induced stress may be distinguished from natural variability. Albeit basal levels of metallothionein in fish species vary with time, reproductive state, ambient temperature and season, the benefits of increased sensitivity and more readily measured responses to metals may outweigh these features. From a physiological point of view, clarifications of their biological roles remain challenging which has led to the development of large number of methods for their quantification. The metal ions in all MTs studied are organized in one or two metal thiolate clusters with thiolate ligands acting both as a terminal and bridging. The metal affinity for the binding sites follows the general order found for inorganic thiolates i.e., Hg II >> Ag I > Cu I > Cd II > Zn II (Dabrio *et. al*, 2002).

A number of methods employing electro analytical techniques UV-VIS spectrophotometry, Metal Saturation Assay or Immunological methods such as Enzyme-Linked Immuno Sorbent Assay (ELISA) and Radioimmunoassay (RIA) were successfully applied. The calorimetric properties of different reagents in combination with mercaptans are the basis for spectrophotometric determinations. The heterogeneity of the nature of the matrices under investigation, such as tissues, biological fluids or cell culture require strategies which involve different approaches either the adaptation of an existing methodology or the development of new protocols.

The estimation of MT is largely influenced by the methodology followed. For example the range of MT was 2.21-2.284 mg MT/g through polarographic methods while it was 0.081-0.167 mg MT /g through spectrophotometric assay. The result of spectrophotometric assay was 30 times lower in comparison with the level of polarographic determinations. The reason behind is polarographic method assesses

the overall MT content irrespective of a degree of sulphydryl- metal saturation, in opposite to spectrophotometric methods based upon the metal free thiolic content. (Ivankovic *et.al*, 2003).

Despite the large number of current methods, the number of non-intercalibrated protocols used to quantify the concentration of MT in organisms is staggering. This fact makes it difficult or impossible for inter-comparison of results obtained by different research teams.

Glutathione, yet another potential candidate involved in detoxification of endogenous and exogenous compounds, is a predominant thiol compound found both in prokaryotes and eukaryotes. It is involved primarily in maintaining the cell membrane integrity, oxidation-reduction balance and scavenging free oxy radicals. Studies done have observed Glutathione (GSH), provides first line of defense against metal toxicity especially cadmium toxicity before induction of metallothionein synthesis occurs (Zarogian and Norwood, 2002). Thus, it plays an important role in early cellular protection responses to metals and their levels get decreased only after a toxic response (Bell and Cowey, 1990).

One effect of heavy metals is related to their capacity for catalyzing oxidative reactions, leading to the production of reactive oxygen species (ROS) (Harris, 1992; Sies, 1993). Cellular components are susceptible to being attacked by ROS resulting in damage to proteins, DNA and lipids and ultimately in loss of their functions (Yu, 1994). Despite the potential danger of ROS, cells contain a number of antioxidant defenses to maintain a low steady state of ROS. Biochemical mechanisms involved in the cellular detoxification are particularly relevant in the understanding of deleterious effects of several metals or other environmental pollutants (Doyotte *et.al*, 1997). Antioxidant systems include enzymes such as superoxide dismutases, glutathione peroxidases, catalase and glutathione S-transferases, which are located within different cellular compartments. Glutathione has been proposed to protect cells from metal-induced oxidative damage by scavenging oxyradicals and by participating in detoxification reactions catalyzed by glutathione peroxidases (Mason and Jenkins,

1996). Given that glutathione plays such an important role in metal detoxification, factors that deplete cellular stores of glutathione may potentiate toxicity. Thus if glutathione depletion can potentiate toxicity in aquatic organisms, it may be useful as a risk factor for predicting stress in ecological risk assessments. Further, there are also studies in bivalves showing that metal detoxification is primarily mediated by biconjugation reactions involving glutathione and MT (Connors and Ringwood, 2000).

Reports (Hirata *et.al*, 1999) are available which documented a range of 1.2nMol/g to 255.6nMol/g in fish and shellfish widely consumed in Japan. Species specific differences constraint the comparison of the reported levels with the present recorded levels.

Glutathione, is found in higher concentration in the kidney and has been directly or indirectly implicated in the maintenance of normal kidney function (Rana *et.al*, 2002). Studies on inter organ relationship in the turnover of glutathione have established that the kidney is a major site of glutathione degradation to the constituent amino acids. Thus, it is inferred that both uptake and efflux can occur in the kidney. Further, gills are also said to have physiological mechanisms such as uptake and excretion in fishes.

Although a wide range of contaminants were documented in fishes (Sarkar, 1992; Muralidharan, 1995; Jayakumar, 2001) there occurs very limited or no information to evaluate the health status of fishes through biochemical responses. However, some data on metallothionein and metallothionein-like proteins and GST levels are available on select marine and freshwater fishes in India (Sindhu, 2006; Jayakumar and Muralidharan, 2006 ; Jayakumar *et.al*, 2007).

Vijayamohan *et.al*, (2000) studied the impact of titanium dioxide factory effluent on the biochemical composition of two fishes namely, *Oreochromis mossambicus* and *Etroplus maculatus*. They could observe clear changes in glycogen and protein in the effluent treated fishes. It was also inferred that these changes would naturally affect the nutritive values of the fishes and the biochemical parameters reflected the changes in the normal activities of various functional systems.

It has been reported that lead treatment would inhibit the binding of phenyl alanyl and lysyl tRNA to ribosome leading to protein depletion (Frankas, 1975). According to Dhar and Banerjee (1903), the incorporation of amino acids into proteins may also be suppressed by the metal exposure. Lead nitrate was found to decrease the protein content in the gill and brain of *Anabas* fishes (Chandravathy and Reddy, 1994). The reduced proteins may also suggest increased proteolysis processes which might have increased by stress caused by metal exposure. The reduction in protein level was reasoned out as a result of diversification energy to meet the impending energy requirements when the fish is under toxic stress (Chandra and Marlia, 1999). It was also inferred that shift in protein metabolism was due to increased metabolism of protein which could be related with increase in adrenocortical hormone that stimulates proteolysis.

Several authors (Virk, 1999; Maruthi & Rao, 2000; Ramalingam *et. al*, 2000; Jayanthi, 2001) have critically documented the effect of heavy metals on proteins and they observed clear decline in total protein concentration when the levels of heavy metals went up.

The exhaustive compilation of literature cogitates to infer that the relationship between metal exposure and biomarkers should be understood and hence the present study does attempt to quantify the biomarker levels and relate with the metal exposure.

14.3 MATERIALS AND METHODS

Standard methods were employed to collect fishes as explained in Chapter III. Altogether 42 individuals comprising five species, namely *Channa punctatus*, *Oreochromis mossambicus*, *Glossogobius giuris*, *Catla catla* and *Cirrhinus mrigala* were collected from five wetlands, namely Chembarampakkam and Pallikaranai in Kancheepuram district and Suchindaram, Theroor and Vembanoor in Nagercoil district of Tamil Nadu. After collection, the fishes were packed in pre-cleaned polythene covers and transported in ice to the laboratory. The fish samples were immediately dissected out for organs such as gill, liver and kidney. About 1 to 1.5 g of tissue was weighed with the aid of a top loading electronic balance, Mettler AE420

for metal estimation while 0.1 to 0.5 g of tissue was used for biomarker quantification. The samples were transferred to pre-cleaned and acid rinsed specimen vials and stored at -20° C for metal contamination analysis and -80 ° C freezer for biomarker analysis. The tissue samples were digested using specific mineral acids as referred in Chapter III.

14.3.1 BIOMARKERS

14.3.1.1 METALLOTHIONEIN LIKE PROTEINS

The procedure for Metallothionein and Metallothionein like proteins estimation was as described by Liebrich *et.al*, (1995) and Wu *et.al*, (1999) with minor modifications. About 0.1- 0.5 g of the tissue was homogenized with 4 volumes of homogenization buffer (5mM mercaptoethanol in Tris- Hcl, pH 8.6) and vortexed in SPINIX mixer for 5- 10 minutes. The homogenized solution was centrifuged in an ultra centrifuge (Beckman Coulter, Optima - LE80 K) employing a near vertical rotor (Type 90 Ti) at 15000 g for 40 minutes at 4° C. The cytosolic supernatant was heated at 65° C on a water bath for 10 minutes to denature the high Dalton proteins. The heated supernatant was cooled for about 5 minutes and again centrifuged for an hour at 40000g. After centrifugation, the cytosolic supernatant was loaded on to a Sephadex G-75 column (8cm X 2.5cm). The column was eluted with 0.02% sodium azide. The flow rate was adjusted to 0.7ml/min. The eluant contain SH based proteins and the absorbance was read at 254nm, using Perkin Elmer Bio Lambda 35 UV/VIS spectrophotometer. Rabbit metallothionein standards purchased from Sigma chemical Co. USA was used as reference at concentrations of 500,600,700 and 800ng. The results were expressed in µg of metallothionein/g of tissue. All the procedural steps were executed at 4° C.

14.3.1.2 GLUTATHIONE (GSH) Reduced

GSH was measured by its reaction (Ellman's reaction) with DTNB [5 5'- Dithio-bis(2-Nitrobenzoic acid)] to give a compound that absorbs at 417 nm. About 0.5g of the tissue (liver/gill/kidney) was homogenized with 1-3ml of 0.2M sodium phosphate buffer

at pH 8. 125 μ l of 25% Trichloro acetic acid (TCA) was added to 0.5 mL of tissue homogenate and the tubes were cooled in ice for 5 minutes and the mixture was further diluted with 0.6mL of 5% TCA and centrifuged at 1000 rpm for 10 minutes. 0.1 to 0.2 ml of the supernatant was taken for the estimation of GSH. The volume of the aliquot was made upto 1 mL with 0.2 M sodium phosphate buffer (pH 8.0). 2.0 ml of freshly prepared DTNB solution (0.6mM in 0.2 M phosphate buffer, pH 8.0) was added to the tubes and the intensity of the yellow colour that formed was read at 412 nm in Perkin Elmer Bio Lambda 35 UV spectrophotometer after 10 minutes. A standard curve of GSH was prepared using concentrations ranging from 50 to 100 nmoles of GSH in 5% TCA. The results are expressed as mean \pm S.E. of n mol GSH/g wet weight of the tissue.

14.3.2 STATISTICAL ANALYSIS

Non Parametric test, namely Kruskal Wallis test was employed to check out the degree of variation in metal contamination, Metallothionein like proteins and Glutathione among the tissues. Karl Pearson Correlation was attempted to illustrate the relationship between metal contamination and biomarkers. The significance level was considered at $P < 0.05$. All tests were performed using SPSS statistical Software Version.10.

14.4 RESULTS

A total of 71 tissues comprising gill (29), liver (21) and kidney (21) were dissected out from five species and analyzed for heavy metal contamination.

14.4.1 Accumulation of metals and their variation among various tissues in fishes collected from wetlands of Tamil Nadu

Data on the levels of metals in different tissues were compiled to understand the variation in accumulation among organs. Here, species specific variation was not calculated. Gill had the highest concentration of lead (1.78 ± 0.12 ppm), zinc

(43.84±8.77 ppm) and cadmium (0.30±0.02 ppm) while liver had the highest levels of copper (15.91±2.45 ppm) and chromium (1.29±0.35 ppm) (Table.69). Interestingly, the lead was found to be below detection limit in kidney. Significant variation in accumulation was observed only for lead, copper and cadmium ($P<0.01$) (Table.70) among the organs.

Table.69 Accumulation of metals in various tissues in fishes collected from wetlands (Mean ± S.E.) of Tamil Nadu

S.No.	Organ	Pb	Zn	Cu	Cd	Cr
1	Gill (n=29)	1.78±0.12	43.84±8.77	1.36±0.07	0.30±0.02	0.59±0.05
2	Kidney (n=21)	BDL	35.12±5.02	4.11±0.98	0.16±0.03	1.10±0.31
3	Liver (n=21)	0.33±0.09	41.83±7.04	15.91±2.45	0.15±0.05	1.29±0.35

Table.70 Kruskal Wallis test showing the variation in metal contamination among the organs of fishes studied.

	Lead	Zinc	Copper	Cadmium	Chromium
Chi-Square	53.675	.478	42.427	17.728	.455
df	2	2	2	2	2
Asymp. Sig.	0.000*	0.788	0.000*	0.000*	0.797

* $P<0.01$

14.4.2 Variation in biomarker among select species of fishes collected from wetlands of Tamil Nadu

i. Metallothionein like proteins

A total of 63 tissues comprising gill, liver and kidney collected from five species, namely *Catla catla*, *Channa punctatus*, *Cirrhinus mrigala*, *Labeo rohita* and *Oreochromis mossambicus* were quantified for metallothionein and metallothionein like proteins. Gill-MT concentrations were found maximum in *Labeo rohita* ($18.38 \pm 2.17 \mu\text{g/g}$) followed by *Cirrhinus mrigala* ($17.61 \pm 1.69 \mu\text{g/g}$) and the least in *Channa punctatus* ($6.44 \pm 1.71 \mu\text{g/g}$) which showed the highest renal levels ($32.89 \pm 10.92 \mu\text{g/g}$). Renal MT-like protein concentration was the highest in *Channa punctatus* ($24.29 \pm 6.95 \mu\text{g/g}$) twice higher than the lowest which was recorded in *Oreochromis mossambicus* ($16.05 \pm 6.61 \mu\text{g/g}$). *Channa punctatus* had the maximum hepatic level ($24.29 \pm 6.95 \mu\text{g/g}$) while *Oreochromis mossambicus* ($6.51 \pm 2.60 \mu\text{g/g}$) (Table.71). No significant variation in MT levels with respect to species could be observed ($F^2 = 4.517$; $df = 4$; $P > 0.05$).

Table.71 Metallothionein and Mt-like proteins in select species of fishes collected from select wetlands of Tamil Nadu.

S.No.	Species	Organ		
		Gill	Kidney	Liver
1	<i>Catla catla</i> (n=4)	14.74 ± 1.22	25.42 ± 1.82	11.24 ± 2.12
2	<i>Channa punctatus</i> (n=6)	6.44 ± 1.71	32.89 ± 10.92	24.29 ± 6.95
3	<i>Cirrhinus mrigala</i> (n=4)	17.61 ± 1.69	6.90 ± 1.87	13.66 ± 4.38
4	<i>Labeo rohita</i> (n = 4)	18.34 ± 2.17	11.29 ± 5.02	8.21 ± 1.80
5	<i>Oreochromis mossambicus</i> (n = 3)	8.86 ± 2.00	16.05 ± 6.61	6.51 ± 2.60

ii. Glutathione

Hepatic levels of glutathione were found at appreciable concentrations in all fishes with the maximum in *Cirrhinus mrigala* (3.52 ± 2.60 nMol/g) and the minimum in *Channa punctatus* (0.41 ± 0.04 nMol/g) (Table.72). Renal levels of glutathione were found to be the highest in *Oreochromis mossambicus* (3.45 ± 2.27 nMol/g) and the lowest in *Channa punctatus* (1.28 ± 0.40 nMol/g) (Table.72). The levels of glutathione are found low in the gills of all the species. Interestingly, *Oreochromis mossambicus*, which recorded high renal levels of glutathione, had shown minimum levels in gills (0.44 ± 0.01 nMol/g) (Table.72). No significant variation in GSH levels among species could be observed ($X^2 = 7.373$; $df = 4$; $P > 0.05$).

Table.72 Glutathione levels in select species of fishes collected from select wetlands of Tamil Nadu.

S.No.	Species	Organ		
		Gill	Kidney	Liver
1	<i>Catla catla</i> (n=4)	0.48 ± 0.08	0.95 ± 0.18	0.78 ± 0.11
2	<i>Channa punctatus</i> (n=6)	0.58 ± 0.09	1.28 ± 0.40	0.41 ± 0.11
3	<i>Cirrhinus mrigala</i> (n=4)	0.49 ± 0.05	1.03 ± 0.16	3.52 ± 2.60
4	<i>Labeo rohita</i> (n = 4)	0.78 ± 0.17	1.16 ± 0.29	1.96 ± 0.56
5	<i>Oreochromis mossambicus</i> (n = 3)	0.44 ± 0.17	3.45 ± 2.27	1.85 ± 0.02

14.4.3 Correlation between metal contamination and select biomarkers

Table.73 Correlation between Metallothionein and Mt-like proteins and metal contamination among select species of fishes

Species	Organ	Pb	Zn	Cu	Cd	Cr
<i>Catla catla</i>	Gill	-0.784	-0.166	-0.501	-0.835	-0.044
	Kidney	-	-0.389	0.543	-0.272	-0.928
	Liver	-0.288	-0.909	-0.887	-0.286	0.860
<i>Channa punctatus</i>	Gill	0.208	-0.469	0.193	-0.542	-0.141
	Kidney	-	0.564	-0.032	0.893*	0.813*
	Liver	-0.199	-0.728	-0.318	0.938**	0.983**
<i>Cirrhinus mrigala</i>	Gill	0.539	0.968*	0.935	0.217	0.446
	Kidney	-	-0.427	-0.962*	0.181	-0.441
	Liver	-0.879	0.862	0.760	0.517	0.192
<i>Labeo rohita</i>	Gill	0.465	-0.511	-0.192	-0.196	0.683
	Kidney	-	0.840	0.466	0.545	0.867
	Liver	-	0.963*	0.629	0.297	-0.188
<i>Oreochromis mossambicus</i>	Gill	0.614	0.934	0.698	-0.045	0.553
	Kidney	-	0.992	0.892*	0.995	0.845
	Liver	-	-0.521	-0.307	0.835	0.900

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

- cannot be computed as the lead values were below detection limit.

Significant strong positive correlation could be observed between the renal and hepatic levels with cadmium and chromium in *Channa punctatus*. In *Cirrhinus mrigala* significant positive and negative correlation could be noticed between gill-Zn and renal-Cu levels respectively while in *Oreochromis mossambicus* a significant positive correlation was observed between renal-Cu levels (Table.73). Although correlation was observed among the metal levels in the other species they are not significant. When the correlation was attempted irrespective of species and organs strong

significant positive correlation was obtained between Mt-like proteins and cadmium ($r=0.437$; $P<0.01$) and chromium ($r=0.455$; $P<0.001$).

Table.74 Correlation between Glutathione (GSH reduced) and metal contamination among select species of fishes

Species	Organ	Pb	Zn	Cu	Cd	Cr
<i>Catla catla</i>	Gill	-0.419	-0.280	-0.969*	-0.813	-0.843
	Kidney	-	0.526	-0.772	-0.921	-0.745
	Liver	-0.890	0.766	0.666	-	-0.024
<i>Channa punctatus</i>	Gill	-0.056	-0.250	0.485	-0.387	-0.467
	Kidney	-	-	-	-	-
	Liver	0.867	-0.715	-0.640	0.896	0.531
<i>Cirrhinus mrigala</i>	Gill	0.316	-0.524	-0.498	-0.423	-0.160
	Kidney	-	-0.163	0.526	-0.983*	0.632
	Liver	0.086	-0.319	-0.376	-0.695	0.215
<i>Labeo rohita</i>	Gill	0.396	-0.106	-.757	0.188	0.097
	Kidney	-	0.488	-0.450	0.369	-0.105
	Liver	0.239	-0.022	0.412	-0.960	0.545
<i>Oreochromis mossambicus</i>	Gill	0.911	0.993	0.284	0.425	0.877
	Kidney	-	0.996	-0.059	0.896*	0.979
	Liver	-	-	-	-	-

* Correlation is significant at the 0.05 level (2-tailed)

- cannot be computed as there was no samples.

Significant negative correlation was established between renal-Cd levels in *Cirrhinus mrigala* while a significant positive correlation was noticed between renal-Cd levels in *Oreochromis mossambicus*. No significant correlation could be observed among the other species (Table.74). Similar to the observation noted for Mt-like proteins and metals strong significant positive relationship could be found for reduced GSH and cadmium ($r=0.369$; $P<0.05$) and chromium ($r=0.455$; $P<0.05$) when the data was pooled irrespective of species and organs.

14.4.5 Assessment of the health status of fishes through metals: MT-metal binding ratio

The study also viewed whether the total metal levels (Cd+Zn+Cu) exceeded MT binding capacity, as metals (especially essential ones) can be also stored or incorporated into proteins and enzymes that are not associated with MT. Hence, the ratio of actual metal to theoretical maximum metal-MT was calculated for gills, liver and kidney using the following equation:

$$\text{Metal: Metal-MT} = \text{Tissue Zn}/(\text{MT} \times 7) + \text{Tissue Cd}/(\text{MT} \times 7) + \text{Tissue Cu}/(\text{MT} \times 12)$$

Where "Tissue Zn" is Zn accumulation in gills, liver or kidney (nmol Zn/g wet wt), "Tissue Cd" is Cd accumulation in gills, liver or kidney (nmol Cd/g wet wt), "Tissue Cu" is Cu accumulation in gills, liver or kidney (nmol Cu/g wet wt), "MT" is measured MT levels in gill, liver or kidney (nmol MT/g; using a molecular weight of 6,000g/mol for MT). The MT value is multiplied by seven for Zn and Cd because 1 mole of MT binds 7 moles of divalent metal. The MT value is multiplied by 12 for Cu because 10-12 moles of Cu can bind to MT in its Cu (I) oxidative state. Theoretically, if the actual metal to theoretical maximum metal-MT value is less than one, potentially all of the metals (i.e., Cd or Cd+Zn+Cu) could be bound by MT.

The ratio of actual Cd to theoretical maximum Cd-MT did not exceed '1' in all the organs of all the species examined. Theoretically, if the ratio stays less than 1.0, potentially all of the metals could be bound to MT. Thus, there was a clear induction of MT in all tissues, adequate enough to bind all Cd.

14.5 DISCUSSION

14.5.1 Accumulation of elemental contaminants and its variation among various tissues in fishes collected from wetlands of Tamil Nadu

The levels of Zn, Cd and Cu reported in the present study are comparable with the levels reported elsewhere. Pyle *et.al*, (2005) in their study observed that in metal contaminated lakes the liver copper concentrations ranged between 6.9 and 87.8 ppm. The Pb and Zn concentrations to be 41.3 and 17.9 ppm respectively. Generally, in metal-contaminated lakes, liver Cu concentrations in fishes are regulated below 50ppm dry weight. However, once the threshold is exceeded, Cu homeostatic control mechanisms become overloaded and liver Cu concentrations increase. Further, increased Cu ligands in liver could result in decreased muscle Cu concentrations. It is therefore possible that lower muscle Cu may be related to the increased deposition of Cu in the liver. Zn levels were found to be high in all the organs and Zn uptake is dependent on many other ions present in water and particularly dependent on Ca^{2+} ions (Spry and Wood, 1989). Once taken up, Zn typically accumulates in gill and muscle tissues (McGeer, 2000).

It has been well established that variations in water chemistry can alter metal toxicity by affecting the amount of metal available to bind with the fish gills (Bury *et.al*, 1999; MacDonald *et.al*, 2002). Characterizing the binding affinities of various natural ligands in surface waters, as well as understanding how the gills of freshwater fishes interacts with cations present in the water, furthers the understanding of how metals exert their toxic effects and how these effects are related to water chemistry. Essentially, the fish gills through a negatively charged ligand could bind metals because of their tendency to carry a net negative charge at environmental pH and further the gills have the potential to strip metals from weaker ligands. The gills of freshwater fish are the primary target for Cu, Pb and Cd toxicity, where an inhibition of ion transport mechanisms takes place, impairing the fish's ability to maintain proper ionic balance and eventually causing death (Hollis *et.al*, 1999).

Thus, the significant differences in the levels of accumulation in the different organs of a fish can be primarily attributed to the differences in the physiological role of each organ. Regulatory ability, behavior and feeding habits are the other factors that could influence the accumulation in different organs. The metal concentration in the liver (not in direct contact with the metal in the water) which plays a major role in detoxification as well as storage, would therefore differ from the concentration detected in the gills (in direct contact with the metals in the water) which play a role in the uptake and excretion of the metals.

14.5.2 Variation in biomarker among select species of fishes collected from Tamil Nadu Wetlands

i. Metallothionein like proteins

Hollis *et.al*, (2001) reported $8.09 \pm 1.66 \mu\text{g/g}$ of MT in Juvenile Rainbow Trout at the initial day before chronic sublethal exposure to $3 \mu\text{g/L}$ of cadmium and the levels increased two folds after 30 days. The present levels are comparable with levels recorded in Juvenile Rainbow Trout chronically exposed to cadmium. There are also reports showing very high levels of Mt ($61.39 \pm 7.85 \mu\text{g/g}$) in fishes collected from naturalistic uncontaminated sites (Lionetto *et.al*, 2001). Surprisingly, no significant correlation could be established between the MT and metal levels ($P > 0.05$) in gills of most of the fishes except *Cirrhinus mrigala*.

The factor that might be contributing for poor correlation between MT and Cd, Zn or Cu levels in the gills is the possibility that MT binding particularly Hg may be involved (Rotchell *et.al*, 2001). Further, increase of MT is observed during spawning period of fish as this is associated with MT induction by reproductive steroids and also with increase in Zn that occurs in females during sexual maturation (Olsson *et.al*, 1995). Expression of MT's has also found to be induced by steroid hormones, interferon and certain chemicals, hepatotoxic solvent and stresses (Wu *et.al*, 1999).

Although the current Mt levels in gills indicate sublethal chronic exposure to metals, there exists a complex relationship between metallothionein expression and the tissue

distribution of heavy metals, which requires further investigation. In the light of our recent increased understanding of this phenomenon, it is possible that MT expression and tissue metal concentration may not always be correlated, especially following chronic environmental exposure to a variety of heavy metals (Burger *et.al*, 2000). Thus, an extensive study to further characterize the relationship between metal concentrations and metallothionein expression in specific tissues is needed.

On an average, *Channa punctatus* recorded the maximum MT levels in liver ($24.29 \pm 6.95 \mu\text{g/g}$) while *Oreochromis mossambicus*, the minimum ($6.51 \pm 2.60 \mu\text{g/g}$). There are reports showing MT levels in the range of 150-200 $\mu\text{g/g}$ in Eel collected from polluted site (Linde *et.al*, 2001). Further, a strong significant positive correlation between MT and cadmium concentrations in liver of *Channa punctatus* could be noticed. This leads us to infer that increase in metal ions increases MT synthesis as observed in a few other studies (Wu *et.al*, 2000; Hollis *et.al*, 2001). Chronic intake of contaminated food could result in stress in hepatic tissues to synthesize more MT. It is essential to mention that cadmium has a higher affinity than Zn for most MT's, it is likely that Zn would be the displaced metal from MT-binding sites. The levels recorded in the present study provide us adequate scope for further investigation on MT and MT like proteins and its interaction with heavy metals in tissues such as liver. Moreover, a specific isoform of MT exists in each tissue and for each metal (Geret and Cosson, 2002) which also requires further investigation.

Renal levels of MT in fishes such as *Channa punctatus* ($32.89 \pm 10.92 \mu\text{g/g}$) and *Catla catla* ($24.29 \pm 6.95 \mu\text{g/g}$) were comparably high with the levels recorded in other species. Although the present levels are comparably low with the renal levels ($1.56 \pm 0.21 \mu\text{g/g}$) recorded in *Liza aurata* collected from Eastern Adriatic Sea (Filipovic and Raspor, 2003) species specific differences could justify the variation. As cadmium is sequestered and filtered in kidney, it is not surprising to find a significant positive correlation between cadmium and MT levels in *Channa punctatus* as proved elsewhere (Barjaktarovic *et.al*, 2002). There are also studies reporting varying levels of MT content in fishes collected from uncontaminated ($61.39 \pm 7.85 \mu\text{g/g}$) and naturalistic ($92.93 \pm 15.48 \mu\text{g/g}$) sites respectively. From the literature it has been inferred that MT concentrations in a large number of species so far investigated is

largely variable and depends on a large number of factors such as heavy metal ions, inflammatory agents, cytokines, stress producing conditions, hormones and catecholamines (Andrew, 1990; Carginale *et.al*, 2000). Further species, reproductive condition, age and diet also accounts for the variation in MT content in fishes. Of the many factors, body weight of the organism is considered to be an important factor to explain variations of MT levels; when the weight was doubled, MT concentrations increased two-fold (Mouneyrac *et.al*, 1999). However, body weight can only explain certain percentage of variations leaving other factors intervene such as metabolic rates, independent of metals either essential or toxic. Importantly as MT levels are linked with total protein metabolism, an increase in metal content does not necessarily mean a corresponding increase in MT concentration. It may be hypothesized that organisms can cope, increasing turnover of MT rather than its global concentration (Geffard *et.al*, 2001).

ii. Glutathione (GSH reduced)

Hepatic levels of glutathione recorded in the present study are to be viewed with concern as liver supplies kidney and intestine with certain constituents for glutathione resynthesis. As described elsewhere in Oysters, exposure to high concentrations of metals deplete the glutathione reserves (Connors and Ringwood, 2000). Hirata *et.al*, (1999) reported glutathione in the range of 1.2 to 255.6 nMol/g in fish and shell fish which are widely consumed in Japan. Comparison becomes difficult due to species specific variations. However, our results support the hypothesis that low hepatic glutathione levels may predispose the fishes to toxic effects of metals. Especially, *Channa punctatus*, which besides recording high levels of lead and cadmium, also had high metal: theoretical metal-MT ratio where the bioavailability of metals was more and they are anticipated to deplete the glutathione levels.

Glutathione, is found in higher concentration in the kidney and has been directly or indirectly implicated in the maintenance of normal kidney function (Rana *et.al*, 2002). Studies on inter organ relationship in the turnover of glutathione have established that the kidney is a major site of glutathione degradation to the constituent amino acids. Thus, it is inferred that both uptake and efflux can occur in the kidney. Further, gills

are also said to have physiological mechanisms such as uptake and excretion in fishes. The low levels in gills may indicate that epithelial branchial cell reserves of glutathione should have depleted which requires further validation.

It has to be noted that glutathione is not only involved in the detoxification of heavy metals but also other xenobiotics which is not included in the current study. However, a strong significant positive correlation in renal glutathione levels with cadmium in *Oreochromis mossambicus* predisposes our notion that metal contamination is a predominant factor in depleting glutathione levels.

14.5.3 Assessment of the health status of fishes through metals: MT-metal binding ratio

When the ratio of actual metal to theoretical maximum metal-MT was calculated, (Cd+Cu+Zn) accumulation in the tissues generally surpassed '1'. Obviously, this reflects the fact that Cu and Zn are essential micronutrients, associated with a vast number of enzymes and other proteins. Only a relatively small fraction could have been bound by endogenous MT levels. It is also worrying to consider that as the ratio levels far exceeded the theoretical value, chances of bioavailability of copper and zinc appears high. As these metals do have synergistic and antagonistic relationships with other metals such as lead, cadmium and chromium, the harmful metals can very well displace these metals or become freely available to induce irreversible damages to fishes and to humans through progressive accumulation via food chain.

Exposure to metals such as Cd or Cu have been reported to decrease the rate of respiration (Radhakrishnaiah *et.al*, 1992; Sastry *et.al*, 1997) possibly by interfering with the oxidative metabolic pathway (Casalino *et.al*, 2000) or through the impairment of mitochondrial function (Marr *et.al*, 1996). Anaerobic activities can also be affected by metal exposure. For example, Cu exposure has been shown to increase the glycolytic activity in fish (Radhakrishnaiah *et.al*, 1992).

Prior studies on fish MT as a bioindicator of aquatic metal pollution in the field situation are a few and not comparable. Further, when comparisons are made with

laboratory observations, the ecological realism is not fully highlighted. Further, there are a variety of intercalibrated protocols to quantify the MT in organisms, which make the comparison difficult.

This study also proposes *Channa punctatus* as an indicator of metal contamination. The levels of metals are high in this omnivorous species which had increased MT and depleted glutathione levels. A strong positive correlation with metal levels and the biomarkers signify their importance to consider this particular species. Further, the actual metal to theoretical maximum metal-MT of total metal (Cu+Cd+Zn) ratio is very high, we find this species to be under metal stress and has freely available metal ions that can exert toxicity on progressive accumulation. As this species is an air breathing bottom dweller it consumes a wide variety of prey along with silt. As sediment is the final sink of heavy metals this could also reflect the long-term exposure. When compared to any of the other species, it is a prolific breeder, matures in the first year and shows rapid development. Hence, the risk of metal contamination through this fish to humans could also be anticipated. During our earlier studies on heavy metal contamination in freshwater fishes of commercial importance in Coimbatore, we could observe considerable levels of heavy metals in this species. Further, levels of metals in this species collected from even a National park (Keoladeo National Park, Bharatpur) (Muralidharan, 1995) were more. It is further noted that *Channa punctatus* is present in many of the wetlands (upto 70%) spread across the country. Hence *Channa punctatus* could be used as an indicator of heavy metal contamination in wetland ecosystems.

SUMMARY AND CONCLUSION

Heavy metals are one of the most toxic, persistent and widespread group of contaminants in aquatic systems. Their effects may often occur and remain unrecognized at the individual level but slowly, over a period of time, show up at population level.

It is well known that heavy metals measured in tissues of aquatic organisms can reflect past exposures. They can also be a reasonable measurement for public health standards and in the point of view of animals' health. Over a few decades globally there has been growing interest to determine levels of heavy metals in the aquatic environment and public food supplies, particularly fish due to their known hazards.

In India heavy metals although have not created any major problems, varying magnitudes of heavy metal contamination do exist in various biotic and abiotic components. Information on heavy metal contamination in fishes lay scattered and many of them do illustrate the toxic effects at laboratory conditions. Further information on the suitability of fishes for human consumption, in the perspective of metal contamination, is limited.

Comprehensive information on heavy metal contamination in fishes all over the country from freshwater wetland ecosystems are not available. Wetlands besides serving as excellent stocking grounds for many species of commercial importance, they also support agricultural activities and meet the requirements of the local community. Further, they also act as excellent ground water recharging source. Today, many wetlands are converted into solid waste dumping yards and to drain industrial and domestic effluents. It is very painful to note that many of the wetlands are presently at the brim of extinction.

The current study has attempted to document the heavy metal contamination status of select wetlands spread across the country using fish as an indicator. Altogether 889 fishes comprising 19 species were collected from 90 wetlands spread across 10 states in the country and examined for heavy metal contamination. Further,

biomarkers of metal contamination, namely Metallothionein and Glutathione were also quantified in a few species of fishes collected from select wetlands in Tamil Nadu. Biomarkers are expected to indicate the stress in fishes and serve as an early warning signal. Suitability of fishes for human consumption was evaluated as it will help assess the impact on human beings in long run.

Samples of fishes were brought to the laboratory over frozen gel packs by air or the next fastest mode. Muscle tissue was analyzed for heavy metals, namely copper, lead, zinc, cadmium and chromium using Atomic Absorption Spectrophotometer. Microwave Digestion system was used for sample digestion.

Of all the metals analyzed, chromium appeared to be a less prevalent contaminant. While 95% of the fishes had detectable levels of Cu, Cd, Pb and Zn, Cr was detected only in 17.3% of samples.

Among the wetlands, lead contamination was found to be the highest in Misamari Beel (8.33 ± 0.39 ppm) of Jorhat district, Assam. Lowest levels was recorded in Draksha Rama (1.46 ± 0.47 ppm) of East Godhavari district, Andhra Pradesh.

Anarag dam in Jharkhand district, Bihar had the maximum concentration of zinc (38.94 ± 5.79 ppm) and copper (39.63 ± 5.89 ppm) followed by Sukhaldhari Dam in the same state (Zn - 35.85 ± 9.89 ppm; Cu - 36.48 ± 10.06 ppm). Lowest levels were recorded in Mandhakhalli Kere of Mysore district, Karnataka (Zn - 4.86 ± 1.27 ; Cu - 4.94 ± 1.29 ppm).

Cadmium concentration was found to be the maximum in Alwarkurichi (1.10 ± 0.46 ppm) of Tirunelveli district, Tamil Nadu while Marchalli Kere of Mandya, Karnataka had the minimum level (0.10 ± 0.09 ppm). Chromium (2.51 ± 0.43 ppm) was found to be the maximum in Gandhisagar Dam (2.83 ± 0.67 ppm) of Mandsur district, Madhya Pradesh while below detectable levels were found in Kambal Talav of Maharashtra and Vaduvloor of Tamil Nadu. However, Karigala Kere of Karnataka (0.01 ± 0.00 ppm) recorded the minimum value.

When the wetlands were ranked to look at the overall burden of metals, Anarag Dam, Sukhaldhari Dam, Bhaghar Beel and Malay Dam of Bihar, Misamari Beel and Son Beel of Assam, Dharoi Reservoir of Gujarat appeared to be more contaminated while Marchalli Kere and Draksha Rama of Karnataka and Andhra Pradesh respectively were least contaminated.

Significant variation in contamination ($P < 0.05$) could be observed for lead, zinc and copper among all the wetlands studied. Although cadmium and chromium varied significantly, the same could be noticed only among a few wetlands. The variation in contamination could be attributed to the variation in contamination sources. The significant variation in contamination could be attributed to the level of contaminants in the water and ultimately the source.

It is obvious that many of the wetlands receive industrial and domestic effluents. Further, as they support agricultural activities nearby, there are plausible chances for leachates and run off from the agricultural fields to find their way into the nearby wetlands. As the sampling was carried out early summer (2002), concentration of contaminants in the fishes collected from these wetlands could also be accounted. It is also to be noted that, rainfall in the whole country was comparatively less during the year 2002-2003.

Among the species, *Cyprinus carpio* collected from Madhya Pradesh recorded the highest concentration of lead (9.01 ± 0.24 ppm) followed by *Channa orientalis* (7.70 ± 0.58 ppm) from Assam. *Puntius dorsalis* recorded the maximum concentrations of zinc (39.06 ± 3.04 ppm) and copper (39.75 ± 3.10 ppm) while the minimum levels was reported in *Ompok bimaculatus* (Zn – 1.00 ± 0.22 ppm; Cu – 1.02 ± 0.22 ppm) of Karnataka. *Heteropneustes fossilis* of Tamil Nadu recorded the highest levels of cadmium (2.21 ± 1.60 ppm). Least levels of cadmium (0.16 ± 0.01 ppm) and lead (0.81 ± 0.37 ppm) were documented in *Mystus vittatus* of Andhra Pradesh. Chromium concentration was the maximum in *Ompok bimaculatus* (2.54 ± 1.18 ppm) of Gujarat. While below detectable level was reported in *Notopterus notopterus* of Tamil Nadu, *Channa punctatus* (0.04 ± 0.03 ppm) of Karnataka had the lowest level.

Species, namely *Puntius sophore*, *Puntius dorsalis*, *Channa orientalis*, *Heteropneustes fossilis*, *Cyprinus carpio* and *Oreochromis mossambicus* had the maximum metal burden while *Catla catla* and *Labeo rohita* had the least level.

The significant variation in contamination ($P < 0.05$) noticed among these species could be related to a number of factors. *Cyprinus carpio* a subsurface dweller and a voracious detritivorous feeder could have accumulated lead through its feeding habit and habitat. *Heteropneustes fossilis* a bottom dweller and an omnivorous feeder could have accumulated higher levels plausibly through biotransformation from food and sediments. Further its air breathing adaptation enables it to exist in almost any kind of water. Other factors such as species specific differences, bioavailability of chemicals in food and water and the physico-chemical parameters of the aquatic environment. Carnivorous fishes had significantly higher concentrations ($P < 0.05$) of copper, zinc and cadmium than planktivorous. Further, lead contamination was higher in omnivorous fishes, which could be attributed to their varied diet. Carnivorous fishes prey on a variety of aquatic organisms and so they are expected to accumulate more metals in a significant manner.

The level of Cd recorded in *Heteropneustes fossilis* is indicative of physiological disturbances to it as per the information available in the literature. Further, the metal levels recorded in many of the species of fishes studied appeared to be higher than the levels recorded elsewhere in India. In a few cases there exists no information to compare.

Although the current levels of metals such as lead and cadmium are alarming, the physiological mechanisms, species differences, physico-chemical properties of the ambient water, availability and absorption of metal are also to be admitted while considering the ill effects. If the exposure is continuous that certain physiological damages such as decline of proximal principles, hampered locomotion, disturbances in osmoregulation could happen.

When suitability of fishes was evaluated for human consumption by presuming that a person consumes 250g/week, the lead input was found to be high through consumption of many of the species. Species such as *Channa punctatus*, *Oreochromis mossambicus*, *Heteropneustes fossilis*, *Cyprinus carpio* and *Labeo rohita* irrespective of their site of collection contributed substantially. It is also noteworthy that these species are stocked in many of these wetlands and are commercially propagated. Long-term consumption of these fishes are likely to create serious physiological disturbances to humans. Unfortunately many of the symptoms may be similar to common ailments but may have deadly impact.

Biomarkers, namely Metallothionein and Glutathione were also quantified in the fishes of Tamil Nadu wetland as they are expected to serve as an early warning signal.

The study also identified certain wetlands in each state based on their contamination status to conduct further investigations. Owing to time constraints, investigations have been carried out only in Tamil Nadu in select wetlands such as Theroor, Suchindaram, Vembanoor, Chembarampakkam and Pallikaranai. Fishes, namely *Channa punctatus*, *Oreochromis mossambicus*, *Catla catla* and *Cirrhinus mrigala* were considered for the study. Biomarkers, namely Metallothionein and Glutathione were also quantified using standard protocols in the fishes of Tamil Nadu wetlands.

The general pattern of metal concentrations in different tissues was liver>kidney>gill>muscle complying with organs' physiological role in handling metal contamination.

The results support the view that elevated metal levels induces the Metallothionein and reduce Glutathione levels. Further, the ratio of actual metal to theoretical maximum metal-MT was calculated. Cumulative accumulation of Cd, Cu and Zn in the tissues generally surpassed '1' which indicates the bioavailability of these metals to make the physiology of the fishes vulnerable to metal.

The study comprehensively documented the levels of heavy metals in the wetlands of the country using fish as an indicator. It is well known but often not kept in memory that wetlands are the ecological barometers of an area. The services rendered by these dynamic and fragile wetlands are innumerable. Right from acting as a natural reservoir to store water and furnishing the domestic needs of humans, it provides an excellent habitat for fishes, birds and other flora and fauna. It is very clear from the study that the health of many of the wetlands is critical and requires serious and stringent conservation measures.

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