SUMMARY
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Due to rapid industrialization and urbanization, large amounts of heavy metals and their compounds are continuously releasing the reverine system of India in general and of the industrial areas province in particular. With the extensive growth of industrial development the use of metals have grown enormously. Metals play an important part in modern societies and have historically been linked with industrial development and improved living standards. Society can draw on metal resources from Earth’s crust as well as from metal discarded after use in the economy.

Chapter – I deals with the introduction of the problem taken up for investigation, due to load of heavy metal in environment the aquatic as well as terrestrial animal get affected from it. The fish *Labeo rohita* and *Clarias batrachus* are very common fish inhabiting ponds and river such as Betwa and Pahuj River passing through district Jhansi. These fish form a major bulk of the edible variety of the fish locally. Hence in the present study the effect of heavy metals, cadmium, mercury and nickel has been studied as cadmium chloride, mercuric chloride and nickel sulphate.

Chapter – II deals with the historical account of the research work, which has been undertaken by different workers on the effect of different metals and other pollutants on fish.

Chapter – III, deals with the experimental programme which has been discussed, wherein material and methods used for the present investigations have been given. Three heavy metals cadmium, mercury and nickel have been used in the form of cadmium chloride, mercuric chloride and nickel sulphate. Two fresh water edible varieties of fish, *Clarias batrachus and Labeo rohita* have been used to determine the toxicity of these metallic pollutants. In these experiments *Clarias batrachus* is treated with cadmium chloride and nickel sulphate while
*Labeo rohita* is subjected to mercuric chloride. Observations were made for acute toxicity, causing mortalities among the fish used for test. Median lethal concentration values for different exposure duration, 24 hrs, 48 hrs, 72 hrs, and 96 hrs were ascertained. Accumulation of these metals in vital organs was also observed. The method for conducting these experiments followed EIFAC (1983)\(^{50}\) and APHA (1985)\(^{12}\). Lethal threshold concentration for each metal and fish were recorded. Histological changes were studied. The change in weight and length due the effect of metal were studied.

Chapter - IV describes the following results observed in the experiment and Chapter V deal with discussion in details. For *Labeo rohita* the LC50 value was recorded on probit analysis is 680 mg/l, 533 mg/l, 516 mg/l and 428 mg/l for 24 hrs, 48 hrs, 72 hrs and 96 hrs. The lethal threshold concentration which is the minimum concentration of the metal, sufficient enough to cause first mortality within 96 hrs, was observed to be 50 mg/l and the minimum concentration at which 100 % mortality was recorded in 30 days exposure was 100 mg/l. Lethal threshold concentrating of cadmium chloride for fish *Clarias batrachus* found to be 0.5 mg/l in which the first mortality was recorded in 48 hrs. Median lethal concentration for cadmium sulphate were recorded as 4.6 mg/l, 2.85 mg/l, 2.62 mg/l and 2.31 mg/l for 24 hrs, 48 hrs, 72 hrs and 96 hrs durations. 6.0 mg/l concentration was found to be highly toxic which caused 100 % mortality in 48 hrs.

Nickel toxicity was observed in fish *Clarias batrachus*. Lethal threshold value of nickel is recorded as 1.0 mg/l in which first mortality was recorded in 96 hrs. LC50 value were calculated to be 11.36 mg/l, 8.8 mg/l, 7.25 mg/l and 6.6 mg/l for 24 hrs, 48 hrs, 72 hrs, and 96 hrs. At 12.0-mg/l concentrations, all the fish were found to be dead in 96 hrs.

Studies on the growth in length and weight of the fish were made. The maximum growth in length of *Labeo rohita* subjected to mercuric chloride was recorded to be 3.07 percent in 200-mg/l concentrations while
minimum growth in length is 2.85 Percent was observed in 300-mg/l concentrations. In weight the maximum growth was observed in 50-mg/l mercuric chloride while loss of weight by 0.87 percent was recorded in 300-mg/l concentrations. In the studies in *Clarias batrachus* subjected to cadmium chloride, the fish showed 12.5 and 6.25 percent growth in length in 0.5 mg/l and 1.0 mg/l concentration while in other concentration the growth was not observed. In weight the maximum growth of 5.862 percent was observed in 0.5-mg/l concentrations. A loss in weight was registered in 2.0 and 2.5 mg/l concentration.

The effect of nickel sulphate was studied on *Clarias batrachus* where maximum increase in length was found to be 11.76 percent in 1.0 mg/l and maximum gain in weight was recorded to be 0.291 percent in 1.0 mg/l nickel sulphate. While loss of weight, which was measured by 0.140 percent and 0.099 percent in 4.0 and 6.0 mg/l concentration. The histopathological studies were made to observed the effect of these metals on different organ of fish; these histopathological changes were observed in gill, liver and kidney of the fish. Due to the effect of metals, these organs showed lesions and different degrees of degenerative changes depending upon the dose i.e. concentration of the metals and the duration of the exposure. In gill due the effect of these metals the first symptom which are come is that the secretion of the mucous, degenerative changes in gills include, vacuolar degeneration of the cytoplasm of the epithelial cells, necrosis, haemorrhage, hyperplastic and hypertrophy. Histopahtological change occur in liver is degenerative of cytoplasm hepatocytes, necrosis, hyperemia, haemorrhage and engorgement of blood cell in the blood vessel. Tublonecrosis, degenerative changes in the epithelial cell of the tubule, haemorrhage and oedematos conditions of Bowman's capsule were the histopathological changes seen in the kidney of the fish. Histopathological changes is seen
more prominent in the fresh water fish "Labeo rohita" due to the toxic effect of mercuric chloride as compare to fresh water fish Clarias batrachus due to the toxic effect of cadmium chloride and due to the nickel sulphate the least effect is observed.

In gill, liver and kidney the accumulation of metals (cadmium, mercury and nickel) was also studied. The accumulation in gills, liver and kidney has been found to increase with concentration of the metal used and the duration of the exposure. In gill the minimum accumulation of cadmium is occur in 48 hrs is 3.6 percent in 0.5 mg/l concentration of cadmium chloride while maximum concentration is 12.8 percent was found to accumulate in 5 days. In liver the minimum accumulation of is 1.2 percent in same concentration of 0.5 mg/l. and maximum accumulation is 4.25 percent in 5.0 mg/l concentration of cadmium chloride. In kidney the minimum accumulation is 0.4 percent and maximum 2.3 percent of cadmium accumulations was observed.

Accumulation of 3.1 percent of mercury has occurred in 24 hrs in 50-mg/l concentration of mercuric chloride. Same as 5.2, 5.6, 5.7 and 6.0 percent of accumulation has occurred in gill at 24 hrs in 100 mg/l, 200 mg/l, 400mg/l and 500-mg/l concentration of mercuric chloride. Maximum accumulation of mercury are 25.0, 28.4, 31.0, 30.2 and 29.1 percent in 30, 30, 25, 20 and 20 days respectively. 1.3 percent and 10.4 percent of mercury were accumulate in liver in 50 mg/l concentration of mercuric chloride in 24 hrs, and in 100 mg/l concentration in 24 hrs the value of accumulation were found to be is 3.4 and 11.7 percent. 13.2 percent is maximum accumulation of mercury in liver were found to be in 400 mg/l concentration of mercuric chloride in 20 days. In kidney the minimum accumulation of mercury is found to be 1.1 percent in the duration of 24 hrs and maximum accumulating is 7.6 percent in the duration of 20 days in 50 mg/l and 400-mg/l concentrations respectively.
In gill for nickel, minimum accumulation noted is 1.2 percent in 24 hrs in 1.0 mg/l concentration of nickel sulphate and maximum accumulation is 16.5 percent in 8.0-mg/l concentration of nickel sulphate in 30 days. For the liver the value of minimum accumulation is 0.5 percent in 1.0 mg/l in 24 hrs and maximum accumulation is 4.5 percent in 8.0 mg/l in 30 days of duration. In kidney the accumulation is not be deductive in least concentration and duration. In 6.0-mg/l concentration of nickel sulphate the accumulation of nickel was found to be is 0.2 percent in 24 hrs and this is the minimum accumulation of nickel in kidney while the maximum accumulation of nickel is 2.6 percent in 8.0 percent in 30 days.

By present investigation it is clearly indicated by the observation and the results that of these three metals taken to study the effect on the fish, mercury is highly, cadmium is less and nickel is least toxic for the fish.
Introduction

Fish have great significance in the life of mankind, being an important natural source of protein and providing certain other useful products as well as economic sustenance to many nations. The gradual erosion of commercial fish stocks due to over-exploitation and alteration of the habitat is one reason why the science fish biology came into existence (Royce, 1972). It is a well known fact that the knowledge on fish biology particularly on morphometry, length-weight relationship, condition factor, reproduction, food and feeding habit, etc. is of utmost important not only to fill up the lacuna of our present day academic knowledge but also in the utility of the knowledge in increasing the technological efficiencies of the fishery entrepreneurs for evolving judicious pisciculture management. For developing fishery, it is necessary to understand their population dynamics how fast they grow and reproduce, the size and age at which they spawn; their mortality rates and its causes, on what they prey upon along with other biological processes. There are many isolated disciplines in fish biology, of which the study of morphology is inseparably related to study of the mode of life of the organism. It fact, the size and shape are fundamental to the analysis of variation in living organisms (Grant and Spain, 1977) and morphological variations even in the same species most often related to the varied environmental factors.

In India, even though industrialization has not reached the level attained in the developed countries, pollution of aquatic habitats seems to be an inevitable problem. More toxic compounds are being increasingly detected in aquatic ecosystems. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated Khare, S., S. Singh (2002) Industrial discharges containing toxic and hazardous substances, including heavy metals, Gbem, T. T., J. K. Balogun, F. A. Lawal, P. A. Annune (2001); Woodling, J. D., S. F. Brinkman, B. J. Horn (2001)., contribute tremendously to the pollution of aquatic ecosystems.

According to Satyanarayanan, D., I. M. Rao, B. R. Prashada Reddy
The presence of heavy metals on the east coast of India deserves special mention as it almost forms a repository for industrial effluents and city sewages. Among the various heavy metal pollutants, cadmium merits special attention due to its potential hazards to aquatic biota Mayer, W., M. Kretschmer, A. Hoffmann, G. Harish (1991)\textsuperscript{111}, Barber, D., M. S. Sharma (1998)\textsuperscript{18}, as well as to human beings Groten, J. P., P. J. Van Blanderen (1994)\textsuperscript{68}, Vanderpool, A., G. Reeves (2001)\textsuperscript{195}. This heavy metal is a common aquatic pollutant and is known to be highly toxic to most organisms, even at small concentrations in natural waters Lovert et al., (1972)\textsuperscript{110}. In general, cadmium is a biologically non-essential, non-biodegradable, persistent type of heavy metal and its compounds are known to have high toxic potentials. Further, continuous, low-level cadmium exposure may have a gross biological impact comparable to that of recurring exposures of much greater intensity. In fresh water fish, cadmium uptake is taking place mainly through three routes namely, gills. On the other hand, the metal retention capacity of the fish is dependent on the metal assimilation and excretion capacities of the fish concerned Rao, L. M., R. Patnaik (1999)\textsuperscript{153}. According to Ferard et al. (1983)\textsuperscript{54}, aquatic organisms take up heavy metals and concentrate them to amounts considerably higher than those found in the environment. Therefore, it is important to find the pathways of accumulation of heavy metals and their affinity to different tissues, especially in fishes. In this context, the present investigation has been designed to study the pattern of bioaccumulation of cadmium in the gills, liver and kidneys of the culturable catfish Clarias batrachus (Bloch.) exposed to sublethal concentrations of cadmium chloride. C. batrachus is highly valued as a table fish throughout the Indian subcontinent and is preferred for culture even in muddy and shallow waters where other culturable fishes may not thrive well. Obula Reddy KP et al. (1999)\textsuperscript{131}. work on ambient ammonia effects on certain biochemical constituents have been studied in fry of Cyprinus carpio. Fry which are 30 days old were exposed to 2.3 ppm of liquor ammonia for 7,
14, 21 days. There was increment in total protein content in 7 days of ammonia exposed-fries while a decrement was observed in 14 and 21 days exposed fries. The possible reasons for these changes are discussed.

Pollutants affecting the natural environment include certain chemical elements released into ecosystems as a result of multifaceted activity of humans. Their presence changes individual development of both plants and animals. Biological effects of disturbed chemical homeostasis appear in the environment much earlier, before symptoms and biochemical changes appear in individual organisms. The fish are directly associated with water; they are an important component of human diet. For humans, they can be a source of xenobiotics that adversely affect human life functions Mudzki and Szkoda 1996. Sobecka (2001) proved disorders in the iron level in the organs and tissues of wells catfish, Silurus glanis L. caused by nickel. Sharma MS et all. (2000)¹⁷³ work on water temperature variations are found to influence sensivity of fresh water, zooplankton to heavy metal toxicants. Static short term bioassay exposing representative fresh water zooplankters to different concentrations of zinc, lead and cadmium revealed that sensitivity of Daphnia to metal and thermal stress was higher than cyclops and cypris. The most resistant planktonic animal against varying metal concentrations and different water temperatures was observed to be cypris. Prakash Ram et al. (1999)¹⁴⁰ says that it is essential to monitor the various health effecting parameters in the ground water before it is used for continuos long period by the people. It is observed that some trace elements viz. copper, manganese, molybdenun, lead, zinc, nickel and iron has been observed in the shallow ground water aquifer, which needs proper monitoring, awareness and removal before its use for drinking purposes. Ramudu K et al (2000)¹⁴⁷ study on adoptive changes in respiratory movements of an air-breathing fish, Anabas testudineus exposed to sublethal concentrations 1.9, 4.75 and 9.5 ppm for 21 days were studied. Significant increase (P<0.001) in surfacing
behaviour were observed in 1.9, 4.75 and 9.5 ppm monocrotophos treated fishes compared to control. Opercular movements decreased (P<0.001) in all three sublethal concentrations compared to control.

Large amounts of heavy metals and their compounds are continuously releasing the riverine system of India in general and of the industrial areas province in particular. These points towards desperate need for assessing the problem and to develop methods for alleviating the ill effects of pollutants like lead and nickel because polluted water can cause paralysis, meningitis, cancer, sterility, schistosomiasis, poliomyelitis and filariasis in animals. Therefore, the present study was planned to assess the metals, viz. lead and nickel toxicities in fish and water of river Betwa. Chemical substances, including heavy metals, introduced into aquatic ecosystem can disturb the homeostasis of a habitat. The aim of this study was to assess the effects of cadmium compounds on common carp, *C.batrachus* L. and to follow the toxicodynamics of cadmium elimination from intoxicated fish once they were transferred to a clean ambience. Fish is a dependable source of animal protein in developing countries like India. Large-scale mortality occurs among the fresh water fishes often due to environmental stress followed by pathogenic attacks and parasitic afflictions. Sreedevi P et al. (1992)\(^{196}\) told nickel concentration, increased significantly in the gill, kidney, liver, brain and white muscle of the freshwater fish, *Cyprinus carpio*, and in the ctenidium, hepatopancreas, mantle, adductor muscle and foot of the freshwater mussel, *Lamellidens marginalis*, at 1, 2, 3 and 4 days on exposure to lethal and at 1, 5, 10 and 15 days on exposure to sublethal concentrations of nickel. Zaman Najmuz et al. (1999)\(^{202}\). Study LC50 dose for 24 hours and 48 hours duration of cythion on *Clarias batrachus* was determined by static bio-assay method. The quantity of cythion required was 17.16 mg/l and 15.6 mg/l for the two duration. The safe concentration was 5.63-mg/l. Sarkar SK (1999)\(^{163}\) study on The fish *Cyprinus carpio*, when exposed to mixture of copper sulphate
and cadmium sulphate at different ratios (1:1, 2:2, 3:3, 4:4, 5:5) exhibited less oxygen consumption than individual metals. At 0.320 and 0.317 mg/l of copper sulphate and cadmium sulphate, the oxygen consumption of fish decreased by 9 and 12% of the control respectively. Sarvana Bhavan P et al (1999) study on Juveniles of Macrobrachium malcolmsonii were exposed to a median lethal concentration (96 hr LC50 : 12,589 mg/L) of dichlorvos for a duration of 96 hr. Sampling was performed on the gills, hepatopancreas and muscle of the prawns at 24, 48, 72 and 96 hr. Decline in concentrations of total glycogen, protein and lipid were noted in the test prawns in comparison to controls. The activity of acetylcholinesterase and alkaline phosphatase were found to be lower in the test prawns in comparison to controls. Selvarajan V.R. et al (1992) work on The biochemical markers such as DNA, RNA and protein have been analysed to study their quinalphos toxicity in different tissues such as brain, liver, muscle and gill of fish Oreochromis mossambicus. The fish were exposed to LC50 concentration of quinalphos and analysed DNA, RNA and protein at the end of 24, 48, 72 and 96h. Results revealed heterogenous trend of DNA, RNA and protein. The significant alterations of the biochemical constituents in various tissues indicated the toxicity of the pesticide. US.Sinha et al (1999) work on LC50 dose for 24 hours and 48 hours duration of cythion on Clarias batrachus was determined by static bio-assay method. The quantity of cythion required was 17.16 mg/l and 15.6 mg/l for the two duration. The safe concentration was 5.63 mg/l.

1.1 Background:

Due to rapid industrialization and urbanization, large amounts of heavy metals and their compounds are continuously releasing the riverine system of India in general and of the industrial areas province in particular. These points towards desperate need for assessing the problem and to develop methods for alleviating the ill-effects of pollutants like lead and nickel because polluted water can cause paralysis, meningitis, cancer,
sterility, schistosomiasis, poliomyelitis and filariasis in animals. Therefore, the present study was planned to assess the metals, viz. cadmium, mercury and nickel toxicities in fish and water of river Betwa and Pahuj. Chemical substances, including heavy metals, introduced into aquatic ecosystem can disturb the homeostasis of a habitat. The aim of this study was to assess the effects of cadmium compounds on common carp, *Cyprinus carpio* L. and to follow the toxicodynamics of cadmium elimination from intoxicated fish once they were transferred to a clean ambiance. Banerjee V et al. (1998)\(^{15}\). The changes in blood parameters due to lethal and sublethal exposures of mercury and zinc on *Heteropneustes* fossils were reported. There was no change in erythrocyte shape, size and surface areas of erythrocyte and its nucleus. Erythropenia associated with hypochromasia, increase in ESR, leucocytosis increase in large lymphocytes, thrombocytosis and hypercoagulability of blood were observed. Sesha Srinivas V et al. (1999)\(^{172}\), attempt is made to assess the effect of 96h LC 50 concentration of hexavalent chromium (39.40 mg/l) on the oxygen consumption of the widely cultured freshwater fish, *La measles* and the metal is found to be a potential respiratory inhibitor.

1.2 Metals in the Environment:

Metals play an important part in modern societies and have historically linked with industrial development and improved living standards. Society can draw on metal resources from Earth's crust as well as from metal discarded after use in the economy. Industrial society values metals for their many useful properties. Their strength makes them the preferred material to provide structure, as girders for buildings, rails for trains, chassis for automobiles, and containers for liquids. Metals are also uniquely suited to conduct heat (heat exchangers) and electricity (wires), functions that are indispensable to industrial economies. Finally, metals
and their compounds are used for their chemical properties as catalysts for chemical reactions, additives to glass, electrodes in batteries, and many other applications. The basic and unique properties of metals, including the ability to work them into complex shapes (i.e., ductility), insure that long term demand for metals will certainly grow.

In the heavy metals and their compounds that are harming the aquatic life, the important ones are mercury, lead, chromium, cadmium, silver, nickel, zinc, copper and iron. Excess heavy metals are often introduced in to aquatic ecosystem as by product of industrial and acid mine drainage residues. Blaise and Costan (1987) have analyzed and assessed the toxicity of 300 effluent samples collected from 160 different industrial sites including 6 industrial sectors: mining, textile, food, pulp and paper, chemical and refinery. Environmental pollution by heavy metals was instantly recognized with Takeuchi et al. (1962), where several thousands of people suffered mercury poisoning by consuming the fish caught in Mimamata Bay, which was recipient of mercury released from a vinyl chloride plant between 1953-1960. The available literature deals with the toxicity of various metals and other pollutant to some fresh water fish. Since the massive outbreak of mercury poisoning in Minimata Bay of Japan, mercury has been recognized and reported by Kastuki et al (1957), Takeuchi et al (1962), as one of that most hazardous environment pollutant. AB Gupta, et al. (1997) determine the LC 50 values at 24, 48, 72 and 96 hr for the Indian catfish Heteropneustes fossilis ranged between 29. 50 and 17. 00 mg/l for Metasystox; 146. 00 and 124. 50, mg/l for Glyphosate; 1. 110 and 0. 740 mg/l for Karathane; and 0. 034 and 0. 026 mg/l for Decis.
1.2.1 Morphology of *Labeo rohita*:

*Labeo* is a large essential tropical genus of Carps distributed in tropical Africa and East India. About two dozens of species are known from India, the most common being *Labeo rohita* (rohu) and *Labeo calbasu* which occur almost throughout India, Pakistan and Bangla Desh. Rohu is commonly found in river, lakes and estuaries. It prefers cleans water and respirates by means of gills. It is chiefly hervivorous and a bottom feeder eating algae and aquatic plants. Body is spindle shaped. Colour is grayish or blackish on back and silvery white or pale on the two sides and belly. A full grown individual measures 1 meter in length and **20 to 25 kg in weight**. The weight body is divisible in to head, trunk and tail.

1.2.2 Morphology of *Clarias batrachus*:

The **walking catfish** (*Clarias batrachus*), also known as the *magur* or *pladuk dam*, is a species of airbreathing catfish with the ability to "walk" out of the water and across land. Its "walk" is more like a sort of wriggling motion with snakelike movements, as well as using its pectoral fins as "legs". This fish normally lives in slow-moving and often stagnant waters in ponds, swamps, streams and rivers (Mekong and Chao Phraya basins), flooded rice paddies or temporary pools which may dry up. When this happens, its "walking" skill comes in handy for moving to other sources of water.
Walking catfishes are around 25 cm (a foot or so) in length and has an elongated body shape. This catfish has long-based dorsal and anal fins as well as several pairs of sensory barbells. The skin is scaleless but covered with mucus, which protects the fish when it is out of water.

The walking catfish is a native of Southeastern Asia including eastern India, Sri Lanka, Bangladesh, Burma, Indonesia, Singapore, and Borneo. It was probably introduced into the Philippines. The catfish is a tropical animal and prefers a water temperature in the range of 10 - 28°C.

In the United States it is a no indigenous invasive species, which is no established in Florida and reported from California, Connecticut, Georgia, Massachusetts, and Nevada.