CHAPTER VI
BIOACCUMULATION AND BACTERIAL REMEDIATION OF FRESHWATER FISH
CYPRINUS CARPIO (COMMON CARP) AFTER EXPOSING THEM TO
HEAVY METALS

6.1. INTRODUCTION

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru, 2005; Dirilgen, 2001; Voegborlo et al., 1999; Canli et al., 1998). The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities (Watanabe et al., 2005). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and diversity of aquatic forms. Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants. Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems. The toxic effects of heavy metals have been reviewed by bioaccumulation (Witeska, 2004). The organisms developed a protective defense against the deleterious effects of essential and non-essential heavy metals and other Xenobiotics that produce degenerative changes like oxidative stress in the body. Cichlidae species are the most popular and highly economic fish (Lokeshwari and Chandrappa, 2007). In the present research, *Cyprinus carpio* (Common carp) was selected due to its adaptation and mainly present in this study area.

6.1.1. SYSTEMIC POSITION OF FISH CYPRINUS CARPIO

<table>
<thead>
<tr>
<th>Group</th>
<th>Vertabrate</th>
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</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Chordata</td>
</tr>
</tbody>
</table>
Cyprinus carpio is generally called as common carp. It is native to the rivers of India. It has been introduced in almost all the countries of the world and subjected to hybridization. This has resulted in different strains and they are called as common carp. In India, it is considered as one of the important species in poly culture. Common carp naturally breeds in confined waters. It breeds throughout the year with two peak breeding periods, one from mid January to March and the other during July and August in the plains of India. It attains sexual maturity within six months of hatching and in the up land lakes it takes about a year to mature.

Heavy metals undergo metabolic activation that provokes a cellular change in the fish. Tissue lesions and apoptosis arise from bioaccumulation, infections, diseases and parasites stimulate necrotic alterations in the fish with an inflammatory defensive reaction (Roganovic –
Zafirova et al., 2003). It is imperative that histological biomarkers are the indicators of pollutants in the overall health of the entire population in the ecosystem (Velkova – Jordanoska and Kostoski, 2005).

*Cyprinus carpio* is the major fish in aquatic farms of sivakasi. So that the present study was conducted to estimate the uptake and accumulation of heavy metals (Cd, Ni, Pb, Zn and Cu) in various tissues like gills, liver, kidney and muscles of fingerlings of common carp (*Cyprinus carpio*).

### 6.2. REVIEW OF LITERATURE

Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (More et al., 2003). Aquatic organisms have the ability to accumulate heavy metals from various sources including sediments, soil erosion and runoff, air depositions of dust and aerosol, and discharges of waste water (Goodwin et al., 2003 and Labonne et al., 2001). Therefore, accumulation of heavymetals in aquatic organisms can pose a long lasting effect on biogeochemical cycling in the ecosphere.

Hayat et al., 2007 was reported growth rate of major carps mostly affect due to the impact of heavymetals. Fish constitutes an important and cheap source of animal protein to human beings and a large number of people depend on fish and fishing activities for their livelihood. Increasing human influences through heavy metal pollution have however led to the depletion of fish resources and substantial reduction in the nutritive values (Srivastava, 2005). The danger of these heavy metals is their persistent nature as they remain in the biota for long period of time when they are released into the environment (Yoon et al., 2008 and Bolormaa et al., 2006). As a result of these heavy metals pollution several endemic fish species have become threatened. Fishes come into contact with multiple metal contaminants in the aquatic
environment and biomagnifies the pollutants. These pollutants built up in the food chain are responsible for adverse effects and death in the aquatic organisms (Farkas et al., 2002). Fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bio indicators of environmental pollution (Matos et al., 2007; Dewez et al., 2005 and Dautremepuits et al., 2004).

Pollutants enter the fish through five main routes: via food or non food particles, gills, oral consumption of water and skin (Saxena et al., 2008). On absorption of the pollutant is carried in blood stream to either a storage point or to the liver for transportation. Pollutants transformed in the liver may be stored there or excreted in bile or transported to other excretory organs such as gills, skin or kidneys for elimination or stored in fat which is an extra hepatic tissue (Olojo et al., 2005).

Olurin et al., 2006 and Gbem et al., 2001, carried out in fishes shown that heavy metals may have toxic effects, altering physiological activities and biochemical parameters both in tissues and blood showed that the concentration of metals was a function of fish species and accumulate more in some fish tissues than others. Since the toxic effects of metals have been recognized, heavy metal levels in the tissues of aquatic animals are occasionally monitored to ensure that the level do not constitute health hazards to consumers.

The environmental factors affect the uptake and accumulation of metals in fish. According to Kock et al., 1998, the level of cadmium and lead in the liver and kidney of Salvelinus alpinus indicated higher uptake rates of metals in summer when water temperature was higher. The data obtained by Carvalho et al., 2006 indicate that the rate of uptake and elimination of cadmium by Noemacheilus barbatulus increased with water temperature, and also suggested stronger effect of temperature on metal absorption than on elimination.
Hoyle et al., 2007 and Witeska, (2004), in fish, concentrations of most metals (except for mercury) are usually inversely related to the age and size. Measurements of bioaccumulation of iron, manganese, zinc, copper, nickel and lead by *Pseudocrenilabrus philander* from metal-polluted water revealed that there was an inverse relationship between metal concentrations and body mass of fish.

Increase in body mercury level with fish age and size is probably related to the affinity of this metal to the muscle tissue (Voigt, 2004, Munn and Short, 1997 and Goldstein et al., 1996). The data obtained by many authors indicate that metals show different affinity to various organs. The major part of total body loads accumulated at different concentrations of metals in the water, and at various exposure times are found in liver, kidney and gills. Some authors observed considerable concentrations of metals in the digestive tract of fish from natural water bodies (Giguere et al., 2004, Kock et al., 1998 and Al-Mohanna, 1994). According to Yamazaki et al., 1996, cadmium is accumulated primarily in the kidney and liver, but it may reach high concentrations also in the gill, digestive tract and spleen.

Lead deposits in various organs: liver, kidneys and spleen, but also digestive tract and gills. Liver accumulates high concentrations of metals, irrespectively of the uptake route (Shah, 2006). The liver is considered a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment. That is especially true for copper and cadmium. Metal levels in the liver rapidly increase during exposure, and remain high for a long time of depuration, when other organs are already cleared. Metal concentrations in the kidneys rise slower than in liver, and usually reach slightly lower values, except for such metals as cadmium and zinc that show very high affinity to kidneys,
Accumulation of metals in various organs of fish may cause structural lesions and functional disturbances (for a review see Jezierska and Witeska, 2001). Adverse effects of metals on fish are related not only to material accumulation, but also to cumulative toxic effect. Exceeding certain values of metal concentration in fish results in lethal disturbances. In most cases, fish from metal-contaminated water are safe for human consumption due to low metal accumulation (except for mercury) in the muscle tissue. However, such fish may constitute a potential risk for predatory fishes, birds and mammals feeding on contaminated fish.

The freshwater fish, common carp (*Cyprinus carpio* L.) is of great commercial importance because it is the most common fish widely consumed worldwide. Therefore, it can be a good model to study the responses to various environmental contaminations. The investigation of histological changes in organs of fish is an accurate way to assess the effects of xenobiotics compounds in experimental studies. Hence, this study was undertaken to examine the effect of different heavy metals at sublethal concentrations on histology of gills, liver, kidney and flesh of common carp (*Cyprinus carpio* L.).

### 6.3. MATERIALS AND METHODS

#### 6.3.1. HEAVYMETAL INDUCTION

Fishes were exposed to the different concentrations 2.5, 5, 10, 15 and 20 ppm of heavymetal (Cd, Ni, Pb, Zn and Cu) solution were prepared by diluting of a stock solution with freshwater. The concentration of heavymetal mixture solution caused 50% mortality in fish for 96 h was taken as the LC$_{50}$ value, calculated by Finney’s Probit Analysis (in SPSS, V. 16). During the toxicity test, the fishes were fed with commercial feed. The numbers of dead fish were counted daily and removed immediately from the aquaria. The degree of fish susceptibility to each heavy metal was determined as toxicity factor (TF). This factor is calculated by the ratio
of LC\textsubscript{50} of a metal in fish \textit{C. carpio} (Velma and Vutkuru, 2009). Based on the LC\textsubscript{50} value a sublethal concentration of mixed heavy metal was selected and the fishes were exposed to that concentration for a period of 6 weeks.

6.3.2. EXPERIMENTAL DESIGN

The fish \textit{Cyprinus carpio} (Common carp) measuring 10-13 cm were purchased from a commercial fish seed hatchery in manimuthar. All Fishes were acclimatized in 30 liters of water in plastic dubs for 6 weeks period. In the present study fifteen plastic dubs with freshwater include five were control without heavy metals and MRB 14, five dubs were treatments with heavymetal alone and another five with heavymetals and MRB strain \textit{Bacillus cereus} in the form probiotic feed. 10 fishes were maintained in each dub. The concentrations of mixed heavymetals (Cd, Ni, Pb, Zn and Cu) in the water of experimental tanks were adjusted to nominal values as 5 ppm. Control and heavymetal exposed fish dubs were fed with commercial fish feed.

6.3.3. BIOACCUMILATION FACTOR

The bioaccumulation of all five heavy metals (HM) in different samples of fish in the same tank was quantified with a bio-accumulation factor (BAF), defined as the ratio of the concentration of a specific heavy metal in the organism to the concentration of the metal in the tank water (Hassan \textit{et al.}, 2011). The BAF was calculated as follows:

\[
BAF = \frac{\text{Concentration of HM in dry fish muscle (µg kg}^{-1})}{\text{Concentration of HM in tank water (µg Kg}^{-1})}
\]

6.3.4. FISH SAMPLE
Fish, *Cyprinus carpio*, from each group were dissected to separate organs (gills, liver, kidney and muscle) as per given methods by FAO (Shah SL, Altindag A (2005). After the 6 weeks of experimental period, gills, liver, kidney and muscle of fish were dissected and tissue samples were separately oven-dried to constant weight at 105 ± 20°C and were each ground to powder. The powdered samples were digested according to Sreedevi *et al.*, (1992). 1 g of each sample was digested using 1:5:1 mixture of 70% perchloric acid, concentrated nitric acid and concentrated sulphuric acid at 80 ± 5°C in a fume chamber, until colorless liquid was obtained. Each digested sample was made up to 20 ml with de-ionized water and analyzed for heavy metals in Atomic Absorption Spectrophotometer (APHA, 1989). Values of heavy metals were recorded in ppm.

6.3.5. MICROSCOPICAL EXAMINATION

The fish were starved for experimentation, after that experimental period tissue samples were dissected at least five sampled pieces of gills, liver, kidney and muscle were collected per fish (making a representative sample of the entire organ). A gill arch of the right side of each fish, liver, kidney and muscle samples were collected and fixed in 10% formalin for 24h, dehydrated in graded ethanol concentrations and embedded in paraffin wax. Sagittal sections (5µm of thickness) were cut and mounted on glass slides. Sections were deparaffinized in xylene, hydrated in ethanol and stained with hematoxylin – eosin (HE) method and approximately 2 – 4 sections of each individual fish were analyzed by light microscope.

6.3.6. MEASURE THE FISH GROWTH AFTER THE EXPERIMENT

Growth of the fish *Cyprinus carpio* (Common carp) was measured after the 6 weeks of experimental period. Fishes were collected from experimental dubs and growth was measured by
use of commercially available centimeter scale and weight was taken using electronic weighing balance.

6.4. RESULT AND DISCUSSION

6.4.1. HEAVYMETAL INDUCTION OF FISHES FOR LC$_{50}$

The 96 hour LC$_{50}$ values of heavymetal solution for *C. carpio* were shown in table 13. The sublethal concentrations (LC$_{50}$ - 96 h) of metal solution in common carp at four durations (24, 48, 72 and 96 hrs). It is evident that the required concentrations of the examined heavy metals to reach lethal doses in common carp. Time-response to mortality of fish resulting based on the different concentrations of heavymetal about 2.5, 5, 10, 15 and 20 ppm (each five heavymetal have equal concentration) are presented in Tables 11. It indicates LC$_{100}$ values for the common carp (*C. carpio*) were at 15 and 20 ppm of heavymetal solution (after 48 h) respectively. The data clearly indicate that considerably more densities of the heavy metals are response to mortality of the fish common carp. The sublethal concentrations were observed in fish, 5 ppm of combined metal solution (Cd, Ni, Pb, Zn and Cu) containing 1 ppm of each metal ion (1/5$^{th}$ of LC$_{50}$/ 96 hours) concentration found very less mortality. Further LC$_{50}$ concentration of heavymetal about 5ppm was maintained in the respective experimental tanks throughout the 6 weeks experimental period of fishes. The fishes were allowed to live throughout the experiment respectively.

Similar reports were observed in Shuhaimi *et al.*, 2010; Hung *et al.*, 2003 and Canli and Atli, 2003. They observed LC$_{50}$ values of different fresh water fishes to exposed toxic metals in various concentrations. Their findings are confirmed the mortality of fish related to toxicity factor and exposure time.

6.4.2. HEAVYMETAL ACCUMILATION IN *CYPRINUS CARPIO*
The bio concentration of said heavymetals in the tissues of gills, liver, kidney and muscles were reported in table 14. The uptake and accumulation of heavymetals in various tissues in fingerlings of common carp (Cyprinus carpio) after exposed them for a period of 6 weeks were tabulated in table 14. Regarding the result, accumulation of heavymetals (Cd, Ni, Pb, Zn and Cu) in the gills were found to be 0.790, 1.043, 1.883, 0.400 and 0.850 ppm in heavymetal exposed where as bacterial treated fishes the accumulation level of heavymetals were 0. 230, 0.343, 0.552, 0.120 and 0.385 ppm. Accumulation of heavymetals in liver (0.863, 0.973, 1.693, 0.350 and 1.230 ppm) and MRB 14 treated fishes comparatively less accumulation of metals was noticed in liver (0.274, 0.195, 0.323, 0.177 and 0.276 ppm).

The absorption and accumulation of heavymetals (Cd, Ni, Pb, Zn and Cu) in the kidney of experimental fish was (0.943, 0.790, 1.166, 0.420 and 1.380 ppm) whereas bacterial treated fishes less amount of metal accumulation was found (0.544, 0.265, 0.586, 0.185 and 0.242ppm) Accumulation of heavymetals in muscle (0.883, 0.633, 0.646, 0.260 and 0.738) and MRB treated fishes comparatively less accumulation of metals in muscle (0.176, 0.205, 0.245, 0.095 and 0.168). The heavymetal induction comparatively 50 % less in bacterial treated fishes. It shows the probiotic role of bacteria in fish intestine can uptake the heavymetals for their metabolism.

The similar reports were observed by Eromosele et al., 1995, Gupta and Dua, 2002 in Grass carp when exposed to various heavymetals and treated with Bacillus sp. Metabolically fish can regulate metal concentration to certain extent where after bioaccumulation will occur in various tissues (Heath, 1995). The ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal that can be taken up by that specific tissue. Furthermore, physiological differences and the position of each tissue in fish can also influence the bioaccumulation of a particular metal (Kotze, 1997). The uptake of metal ions was higher in
tissues which are directly exposed to the water containing metal ions and lower in tissues which were not or less exposed to metal present in contaminated water (Javid et al., 2007 and Zhou and Wong, 2000). In aquatic ecosystem, the bacteria can inhibit the metal concentration in polluted water by their metabolic activity, due to the microbial uptake of heavymetals; the fishes were significantly less accumulation in their tissues and better growth. Decreased level of accumulation of metals noticed in the various tissues of the fish which were exposed to mixed metals in combination with bacterial treated then that of fishes exposed to mixed metal alone. The reason may be due to the effective remediation of metals by bacteria by way of their metabolic activity. Similar results were reported by Ahmed and Bibi, 2010; Rauf et al. 2009 and Korai1 et al., 2008.

6.4.3. MICROSCOPICAL OBSERVATION OF TISSUES

The morphology of gill in common carp (Cyprinus carpio L) maintained as control exhibits normal histological features in which lamellae are lined by squamous epithelium composed of non differentiated cells (Plate 16). The gill of fishes exposed to heavy metal shows epithelial lesions. The filament region constitutes edema with intense lamellar vasodilatation. The gills exhibit a stratified fusion of pigments in numerous spaces. The gills of MRB 14 treated fishes shows recovery in gill structure when compare to fishes exposed to heavymetals alone.

The liver of fish maintained as control exhibit a normal structure with no abnormalities in the hepatocytes. It exhibits a homogenous cytoplasm around the centrally located spherical nucleus (Plate. 17). The hepatic tissues of fishes exposed to heavymetal exhibit a marked cytoplasmic vacuolization and internal bleeding in the liver tissues whereas in the liver of fishes a mild damage of hapatocytes and no bleeding are found to observe fishes treated with MRB 14.
The kidney tissues of fish maintained as control exhibit an normal pattern with no abnormal changes in the cells (Plate. 18). There is an increased activity of connective tissues, particularly near the kidney tract. The circulatory disorders and inflammatory cells develop necrosis around the border of tissues changed the normal shape of kidney are found to seen in fish exposed to heavy metals. The MRB 14 treated fishes were less abnormalities to compare the control fishes are noticed.

The muscle sample of fish maintained as control shows normal histological features (Plate. 19). Muscle samples of fishes exposed to heavymetals are characterized by disappearance of striations. The tissues involved in necrosis with a homogenous liquid appearance and loss of staining properties. Enlarge lesions in epidermis completely changed the architecture of external cell layer. Wherever no lesions are found in fishes treated with MRB 14.

The fish exposed to heavy metals reflect several histological alterations. In heavymetal exposed fishes show distinct changes in histology with proliferation of epithelial cells, fusion and degenerative changes in the lamellae with the accumulation of edema. Several earlier studies reported that edema with frequent lesions in gill epithelium of fish exposed to heavy metals (Figueiredo et al., 2007) specifically cadmium (Ishikawa et al., 2007) and nickel (Pane et al., 2004).

The characteristic appearance of liver fibrosis in the heavy metal exposed fish has been supported by report of sunfish in Texas reservoir contaminated with selenium enriched power plant (Ishikawa et al., 2007). The higher bioaccumulation of heavy metals in the kidney could be on specific metabolic process. Macrophages are key cells present in kidney dealing with foreign material and cellular debris (Kaoud et al., 2011).
Kaoud et al., (2011), the presence of hemosiderin pigment in the heavy metal intoxicated kidney tissues supported by exogenous environmental factors like contaminants influence macrophage pigment composition of Mercury-Exposure in Nile Tilapia.

The tissues of muscle samples in intoxicated group show damaged structure with the invasion of cytoplasm. The lesions on the exposed group in turn cause muscle destruction. The affected muscle region arise giant granulomas that undergone necrotic changes in the tissues of heavy metal exposed fish. The present investigation was supported by Kabata arthuri infection in muscle fibres of sutchi cat fish (*Pfangasius sutchi*) due to accumulation Hg and Cu (Dutta, et al., 2008).

**6.4.4. GROWTH RATE OF TEST FISH**

Due to the treatment of bacteria the growth rate of fish is also found to be high when comparing to control, as well as fishes exposed to heavymetal. It extremely larger in size of length and weight. The range of growth tabulated in table 15, regarding the result the weight of heavymetal exposed fishes were less (165.5) to compare the bacterial treated fish (212.5). The similar findings were reported by Palaniappan *et al.*, 2009; Csuros *et al.*, 2002 and Nies, (1999) in *Catla catla*. Regarding their result less accumulation of heavymetals in tissues of freshwater fishes having better growth less mortality.
Table 13. 96 hrs of LC$_{50}$ value of *C. carpio* for mixed heavymetals (Cd, Ni, Pb, Zn and Cu)

<table>
<thead>
<tr>
<th>Duration (h)</th>
<th>2.5 ppm</th>
<th>5ppm</th>
<th>10ppm</th>
<th>15 ppm</th>
<th>20ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. carpio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>72</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>96</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Mortality rate

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*C. carpio*

- At 24 hours, the mortality rate is 1% for all metal concentrations.
- At 48 hours, the mortality rate is 1% for all metal concentrations except 20 ppm.
- At 72 hours, the mortality rate is 2% for all metal concentrations.
- At 96 hours, the mortality rate is 1% for all metal concentrations except 20 ppm.
Table 14: Bioaccumulation of heavy metals in various tissues of fish exposed to mixed heavy metals after a period of six weeks (ppm)

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Gills</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>HM</td>
<td>HM+MRB 14</td>
<td>C</td>
</tr>
<tr>
<td>Cd</td>
<td>0.089</td>
<td>0.790</td>
<td>0.552</td>
<td>0.083</td>
</tr>
<tr>
<td>Ni</td>
<td>0.034</td>
<td>1.043</td>
<td>0.343</td>
<td>0.065</td>
</tr>
<tr>
<td>Pb</td>
<td>0.083</td>
<td>1.883</td>
<td>0.230</td>
<td>0.078</td>
</tr>
<tr>
<td>Zn</td>
<td>0.010</td>
<td>0.400</td>
<td>0.120</td>
<td>0.021</td>
</tr>
<tr>
<td>Cu</td>
<td>0.032</td>
<td>0.850</td>
<td>0.385</td>
<td>0.030</td>
</tr>
</tbody>
</table>

C- Control fish with out any stress

HM- Heavymetal induced fish

HM+ MRB 14- MRB 14 bacterial treated feed with heavymetal exposed fish
## Table 15: Growth Rate of test fish in control and various treatments

<table>
<thead>
<tr>
<th>Character</th>
<th>Time</th>
<th>Weight (mg)</th>
<th>Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control fish</td>
<td>Initial</td>
<td>112.5</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>After 6 weeks</td>
<td>185.5</td>
<td>11.6</td>
</tr>
<tr>
<td>Heavymetal treated fish</td>
<td>Initial</td>
<td>110.2</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>After 6 weeks</td>
<td>165.5</td>
<td>11.7</td>
</tr>
<tr>
<td>Heavymetal+MRB treated fish</td>
<td>Initial</td>
<td>108.5</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>After 6 weeks</td>
<td>212.5</td>
<td>12.6</td>
</tr>
</tbody>
</table>
Plate 16. To show the histology of gills maintained as control and with various treatments

Fish maintained as control fish  Fish exposed with mixed heavymetal  Fish treated with MRB 14 bacteria

Arrow point mentioned the epithelial lesions, lamellar vasodilatation of gills due the impact of heavymetals.

Plate 17. To show the histology of liver tissue maintained as control and with various treatments

Fish maintained as control fish  Fish exposed with mixed heavymetal  Fish treated with MRB 14 bacteria

Arrow point mentioned the cytoplasmic vacuolization and internal bleeding in the liver tissues of common carp due the impact of heavymetals.
Plate 18. To show the histology of kidney tissue maintained as control and with various treatments

Fish maintained as control fish  Fish exposed with mixed heavymetal  Fish treated with MRB 14 bacteria

Arrow point mentioned the necrosis around the border of tissues of kidney in common carp due to the impact of heavymetals.

Plate 19. To show the histology of muscle tissue maintained as control and with various treatments

Fish maintained as control fish  Fish exposed with mixed heavymetal  Fish treated with MRB 14 bacteria

Arrow mark mentioned the enlarge lesions in epidermis in muscle tissues of common carp due to the impact of heavymetals.