Chapter V

Conclusions
The present study was designed to find (1) The potential use of selected experimental freshwater bivalve species, *Corbicula striatella*, *Parreysia corrugata* and *Parreysia cylindrica* for presence of As, Cd, Cu, Pb and Zn in water by determining their bioaccumulation and by developing the realistic oxidative stress biomarker responses (2) to develop a bioaccumulation as well as biomarker response database which might be used for future biomonitoring of heavy metal pollution in freshwater ecosystem, and (3) to find out the most appropriate sentinel species among the three experimental species of freshwater bivalves to monitor the heavy metal pollution in freshwater ecosystem.

**Bioaccumulation study**

The patterns of accumulation of heavy metal studied comparatively in three native freshwater bivalve species after exposure to chronic concentrations of As (0.1719 ppm), Cd (0.1411 ppm), Cu (0.033 ppm), Pb (1.50 ppm) and Zn (1.8589 ppm) separately upto 20 days in laboratory revealed a significant increase in the levels of all heavy metal concentrations, body burden and bioconcentration factor (BCF) in the whole soft body tissues of experimental bivalves with increase in exposure period as compared to the bivalve maintained as control. It was observed that different species of bivalves showed different uptake levels for different metals. The freshwater bivalve, *Corbicula striatella* accumulated the highest concentration of copper (60.6 μg/g), lead (263.19 μg/g) and zinc (1579.3 μg/g), while *Parreysia cylindrica* accumulated highest concentration of arsenic (47.63 μg/g) and cadmium (67.51 μg/g) among the studied bivalve species after 20 days of exposure. The highest values of metal body burden for Cu (4.24 μg/individual), Pb (21.06 μg/individual) and Zn (142.14 μg/individual) in *Corbicula striatella*, and highest body burden values
for As (19.05 µg/individual) and Cd (31.73 µg/individual) in *Parryesia cylindrica* after 20 days of exposure were observed. Higher value of bioconcentration factor (BCF m-w) was obtained for Cu (2211.68), Pb (204.94) and Zn (1040.18) in *Corbicula striatella*, while for As (406.40) and Cd (651.01) in *Parryesia cylindrica* after 20 days of exposure.

Based on these results, it shows that the magnitude of heavy metal accumulation in bivalve tissues depend on the type of heavy metal, exposure period and bivalve species. Concentration of metals observed in the body of control animals indicates the presence of these metals in natural ecosystem of these bivalves. A reduced metal level in control bivalves indicates slow and gradual depuration of metals by bivalves.

The observed differences in tissue metal concentration in bivalve species might be due to variation in body size, growth, fitness, reproductive condition, genotype of the animal difference in metabolic rate and weight. Variability in metal body concentrations between closely related species are mainly caused by interspecific differences in the biokinetics of uptake, elimination, species-specific ability/capacity to accumulate trace metals and different physiological rates such as pumping, filtration, respiration and species specific digestive physiology and absorption rate of a metal across gut epithelium. Both physiological/biochemical responses, metal geochemistry, differences in metal efflux rates might be responsible for the differences in metal bioaccumulation as observed in the experimental bivalve species. Interaction of metals in body tissues seems to vary from species to species. At the same time the responses of the organism is specific for different element and substance. The interspecific difference in the metal concentrations evidenced that different organisms display a
range of capacities varying from low accumulation of certain elements to very high accumulation.

In this study, BCF mollusc-water (BCF m-w) refers to the concentration of a particular metal in the tissue of bivalves per concentration of that metal in water. Tolerant species of bivalves tend to restrict water tissue transfer, and thus have less accumulation in tissue. Respectively high bioaccumulated values show that these bivalve species are best bioindicators for monitoring these metals pollutants in freshwater. On the other hand other species exhibited low bioconcentration factor (BCF m-w). Such low value indicated limited ability/potential of bivalves to these metals to be absorbed from the water to bioaccumulate in bivalve tissue. The bivalves with low bioconcentration factor for the accumulation of metal are not good for monitoring of above mentioned metal pollutants.

Heavy metal accumulation was determined from different soft body tissues of three experimental bivalve species to propose the probable bioindicator organ/tissue for biomonitoring of As, Cd, Cu, Pb and Zn metal pollution in freshwater ecosystem. All three species of bivalves accumulated the higher concentration of arsenic, cadmium, copper, lead and zinc in the digestive glands as compared to others studied tissues. Higher concentration of different metals in the digestive glands may be due to high quantity of metallothioneins (MTs), its involvement in the storage and detoxification of metals as well as digestion and absorption of food etc. Higher accumulation of copper, lead and zinc was observed in the digestive glands of the bivalve, *Corbicula striatella*, while highest accumulation of arsenic and cadmium was observed in the digestive glands of *Parreysia cylindirca* as compared to other studied bivalve species. Therefore, digestive glands of the bivalve, *Corbicula striatella* is being proposed
as a potential biomonitor organ for biomonitoring of Cu, Pb and Zn, while in *Parreysia cylindrica* for biomonitoring of As and Cd pollution in freshwater ecosystem.

The results indicate that, the concentrations, body burdens and bioconcentration factors (BCFs) for Cu, Pb and Zn were highest in the *Corbicula striatella* and for As and Cd in *Parreysia cylindrica*. Thus, these results indicated that, *Corbicula striatella* is sentinel organism for the biomonitoring of Cu, Pb and Zn, while *Parreysia cylindrica* for As and Cd in freshwater ecosystem. The obtained results also revealed that, digestive glands accumulated highest concentration of all studied metals as compared to others studied organs and pointed out its potential as a biomonitoring organ for biomonitoring of heavy metal pollution in three experimental species of freshwater bivalve.

**Antioxidants as biomarkers**

In the present study after chronic exposure to As, Cd, Cu, Pb and Zn for 10 and 20 days a significant increase in the levels of oxidative stress indicator parameters like lipid peroxidation (MDA) and glutathione-s-transferase activity and decrease in the levels of antioxidant scavenger molecule (GSH) and activity of antioxidant defense enzymes (superoxide dismutase, catalase and glutathione peroxidase) was observed in the digestive glands of all three experimental freshwater bivalve species. A maximum increase in the levels of MDA and glutathione-s-transferase activity and maximum decrease in the levels of GSH and activity of antioxidant defense enzymes was found in the digestive glands of the *Corbicula striatella* on exposure and bioaccumulation of Cu, Pb and Zn, while in *Parreysia cylindrica* for As and Cd amongst the studied bivalve species. Results also indicate that amongst the tested heavy metals Cd induces more
formation of lipid peroxidation (MDA), and glutathione-s-transferase activity and much decrease in the levels of GSH and activity of antioxidant enzymes (SOD, CAT, GPx and GST). The study indicates that *Corbicula striatella* is the best sentinel organism for Cu, Pb and Zn biomarker studies, while *Parreysia cylindrica* for As and Cd amongst the studied bivalve species.

It was observed that the formation of MDA and changes in the levels of GSH and activity of antioxidant defense enzyme treated with heavy metals were dependent on chemical nature of the heavy metal, dose, period of exposure and bivalves species. Oxidative stress responses are well marked in digestive glands indicating it as an ideal tissue for oxidative stress biomarker studies in freshwater bivalves for heavy metal pollution in freshwater ecosystem.

Present study on freshwater bivalves support the concept that exposure to heavy metals may enhance the production of the reactive oxygen species (ROS) and induce oxidative stress in the digestive glands of the freshwater bivalves after chronic exposure. Present study represents the first comparative and comprehensive report on activities of the investigated oxidative stress indicator parameters like lipid peroxidation, antioxidant scavenger molecule (GSH) and antioxidant defense enzymes (SOD, CAT, GPx and GST) in the digestive glands of three freshwater bivalve species, *Corbicula striatella*, *Parreysia corrugata* and *Parreysia cylindrica*. Formation of lipid peroxidation and alteration in antioxidant scavenger molecule (GSH) and activity of antioxidant defense enzymes seemed to be better biomarkers of oxidative stress caused in freshwater experimental bivalves after chronic exposure to heavy metals (As, Cd, Cu, Pb and Zn).

**Biochemical study:**
a) **Quantitative estimation**

Present investigation clearly showed that, after chronic exposure to heavy metals As, Cd, Cu, Pb and Zn for 10 and 20 days a marked depletion in the protein, ascorbic acid, DNA and RNA content in mantle, gills, digestive glands and whole soft body tissues of three experimental freshwater bivalve species was observed as compared to bivalves maintained as control.

The highest decrease in protein, ascorbic acid, DNA and RNA content was found on exposure and bioaccumulation of Cu, Pb and Zn in different tissues of *Corbicula striatella*, while in *Parreysia cylindrica* for As and Cd amongst the studied bivalves species. The results indicate that amongst the tested heavy metals cadmium induces more depletion in total protein, ascorbic acid, DNA and RNA contents in all three experimental freshwater bivalve species. It was also found that the decrease in total protein, ascorbic acid, DNA and RNA contents treated with heavy metals depend on chemical nature of the heavy metal, dose, period of exposure and bivalve species.

Amongst the studied tissues maximum depletion of biochemical constituents was observed in digestive glands of all three experimental freshwater bivalve species, which point out its role in the accumulation of metals. Therefore, digestive glands of the studied experimental freshwater bivalve species emerged as an important bioindicator organ for biomonitoring of the As, Cd, Cu, Pb and Zn pollution in freshwater ecosystem.

The results indicate that, *Corbiculla striatella* is the best sentinel species for Cu, Pb and Zn biochemical studies, whereas *Parreysia cylindrica* for As and Cd amongst the studied bivalve species.

The changes in the levels of protein, ascorbic acid, DNA and RNA in the different soft body tissues of experimental freshwater
bivalve species after chronic exposure to As, Cd, Cu, Pb and Zn can be considered as the indices of stress. Therefore, alterations in normal biochemical parameters provide the earliest indicators of toxic effect on tissues. This has been referred to as reliable tools for evaluating the degree of exposure of any chemicals much before any gross sign become visible.

A change in major biochemical components of organisms is useful to know toxicity caused by different heavy metals and protective mechanism of the body against toxic effects. The results obtained in this study indicated that, the bivalves exposed to different metals for 10 and 20 days exhibited cell injury through the formation of lipid peroxidation and alterations in the natural antioxidant defense mechanism (Chapter III) which further leads to oxidative stress and causes damage to the different important cellular macromolecules.

b) Protein profile by electrophoresis

The present study illustrates that, chronic exposure to heavy metals influences the protein expression in three experimental freshwater bivalve species. The initiation of protein expression signature depends on the nature of different heavy metals, period of exposure and bivalve species. In *Corbicula striatella*, after 10 days of exposure to As, Cd, Cu, Pb and Zn some medium range of newly generated proteins were observed which are visualized most considerably in case of Pb and Zn after 20 days of exposure. The major PES was in the range of about 60 to 65 kDa and was overexpressed after exposure to heavy metals as compared to control animals. In *Parreysia corrugata* two sharp protein bands are observed with some diffused ones in the range around 43 kDa of molecular marker protein. Comparatively lower expression was observed in Zn treated animals. In *Parreysia cylindrica* the expression
of newly synthesized proteins was observed similar towards all the heavy metals. After 20 days of chronic exposure approximately 34 kDa proteins were found to more discriminally expressed for heavy metals Cd, Cu and Pb. Differentially expressed proteins in studied bivalve species might be used to develop new protein markers for biomonitoring of heavy metal pollution in freshwater ecosystem.

The present study investigated the potential use of three experimental freshwater bivalve species as bio-indicator organisms after exposing to different heavy metals. The obtained results of bioaccumulation, biomarkers, biochemical study indicates that, *Corbicula striatella* is sentinel animal for the biomonitoring of copper, lead and zinc while *Parreysia cylindrica* for arsenic and cadmium in freshwater ecosystem.

In the present study, the digestive glands of the experimental freshwater bivalve species come forward as an ideal tissue for bioaccumulation, biomarker and biochemical studies in freshwater bivalve, *Corbicula striatella* for Cu, Pb and Zn, whereas in *Parreysia cylindrica* for As and Cd pollution in freshwater ecosystem.