PREFACE

The natural water bodies, both lotic and lentic are most important sources of water to sustain the life. These resources need special attention for conservation, development and management for optimal and sustainable utilization. Water quality is important for drinking, agriculture, aquaculture and industrial use. The aquatic resources of the country are its national wealth.

Pollution of surface and ground water system is one of the major environmental problems, faced worldwide. Heavy metal plays a major role in the pollution of rivers, lakes, ponds and reservoirs. The main routes of metal to the freshwater reservoir are through the rivers, which receives anthropogenic inputs of different origin including industrial effluents. The most important metals from the point of view of water pollution are As, Zn, Cu, Pb Cd, Hg, Ni and Cr. Some of these metals (e.g. Cu, Ni, Cr and Zn) are essential trace metals to living organisms, but become toxic at higher concentrations. Heavy metals are not biodegradable and are persistent in nature and transfer of accumulated heavy metals in the lower trophic organisms to the higher trophic organisms through the food chain leads to an adverse effect, not only to the entire ecosystem but also too human that sits at the top of the food chain of pyramid.

The use of some rivers and lakes as water supplies, threatens are thus posed on human health via drinking water and polluted food. Therefore there is growing need to monitor, detect, assess and manage the impact of pollution, particularly low concentration of increasingly complex mixtures of pollutants, such as heavy metals on aquatic ecosystem.

Chemical analysis of the water and sediment is the most direct approach to reveal the heavy metal pollution status in the environment.

This technique does not show the relation between the metal substance levels in the environment with availability of biological metal substance inside the organism. Furthermore, this technique does not give any indication towards determining the harmful effect on the organism and also the effect at the molecular level.

To overcome this problem, the idea has been proposed to use biomonitoring technique, which includes studies and investigations of not only xenobiotic body load but also their responses and is more efficient and sensitive. Most researchers use benthic organisms as monitors of both the levels and long term influence of pollutant within an ecosystem. Metal accumulation in living organisms lead to concentration several orders of magnitude higher than those of the surrounding water. Measuring the accumulated levels of heavy metals in aquatic organisms could be more reliable water quality indicators than chemical analysis of water column and sediment.

During the past few decades, many species have been studied to determine their potential as biomonitoring organisms and molluscs have become a popular choice for metal monitoring for several reasons. They are somewhat sedentary, regionally abundant, long lived, have adequate tissue mass for analysis, filter feeder; accumulate metals from food, water and also from the ingestion of inorganic particulate materials, hence fulfilling the criteria as good bioindicators. In comparison to fish and crustacean, bivalves have a very low level of activity of enzyme systems capable of metabolizing persistent organic pollutants. Therefore, contaminants concentration in the tissues of bivalves more accurately reflects the magnitude of environmental contamination. Consequently, such organisms have been largely used in programmes of biological monitoring in freshwater. Moreover,

mussels and oysters are widely used as biological indicators in national and international monitoring programs.

In case of bivalves, physiological processes of the organisms, as well as abiotic factors such as the physical and chemical properties of the environment and the chemical nature of the metal influence the metal accumulation. Generally these indicators are able to evaluate the level of metal pollutants, but the problem of individual variability within and between sample batches still remains and this causes problems on the interpretation of the results. For this instance, body size or weight of the organism is one of the parameters that influence the uptake and bioaccumulation of the elements in the body. If the metal concentrations are expressed as concentration per unit body weight (μg/g), then one can expect the highest value to be recorded amongst the smallest individuals and could therefore render a misleading interpretation. The body burdens of metals in most bivalves have been used to identify and map areas with exceedingly high levels of trace metals and organic pollutants hence they can be used as biomonitors for aquatic environment.

It is well established that organisms vary widely in their sensitivity to different pollutants, and that no single species or monitoring system is sensitive or suitable for the detection of all possible toxic pollutants. Bivalve's potential to accumulate metals from a medium into tissue can be estimated using bioconcentration factor (BCF). By comparing BCF, one can compare the potential of different bivalves to uptake metals from water.

In order to use the bivalve as bioindicators in pollution monitoring programmes, there is a need to develop a bioaccumulation database using various bivalve species which might be used in finding the most appropriate sentinel bivalve species for metal pollution

monitoring programme for lentic and lotic ecosystems. Lot of studies on bivalve associated with heavy metal accumulation in marine environment has been done by many researchers, but very few studies have been published related to freshwater bivalves.

The knowledge of concentration of metal in native species is very important with respect to nature management, human consumption of these species and to determine the most useful biomonitor species. Therefore, in the present study different native species of freshwater bivalves, *Corbicula striatella*, *Parreysia corrugata* and *Parreysia cylindrica* were selected to establish a local environmental monitoring network using bivalves as bioindicator species to assess the trends of bioaccumulation of As, Cd, Cu, Pb and Zn in the freshwater ecosystem.

Bioaccumulation only detects the levels of heavy metals, in aquatic organisms, but a true evaluation of the damage inflicted by heavy metals should come from comprehensive biomarker studies. Biomarkers are more telling than bioaccumulation because they deal at molecular, cellular, tissue/organ, physiological changes on the organism level and assess contamination based on a direct measure of change in the organism. Biological responses of organisms may be non-specific, but will reflect real biological harm that is, toxic, usually with sub-lethal effects. Biomarkers in sentinel organisms (‘bioindicators’) can be applied as a supplementary approach for demonstrating links between sub-lethal biochemical and adverse effects in natural populations and also could be developed as early warning sensitive tools and specific indicators of environmental stress. Biomarkers are used as early warning signals of the potential biological effects of environmental pollutants and may help to forecast changes at higher levels of biological organization, so that

unacceptable and irreversible effects at higher levels of biological organization can be avoided. Biomarkers can provide a spatial and temporal integrated measure of minute amounts of bioavailable pollutant levels. The use of biomarkers does not replace chemical monitoring, but it integrates them providing a unique contribution in determining the toxicity of pollutants, even when they are present at low, sub-lethal concentration.

Despite these advances, there are also a number of limitations. It is well understood that no one biomarker has been validated as a unique tool of detecting specific pollutant exposure and effects and cannot provide an adequate base for environmental health assessment, management and sustainability planning. Thus, a suite/battery of biomarkers at different levels of biological organization is required to be effectively applicable in a biomonitoring programme.

Benthic macro-invertebrates on exposure to toxicants bioaccumulates in their body. Heavy metals are known inducers of oxidative stress by directly producing reactive oxygen species (ROS) and causes disturbances in metabolic function activate detoxifying enzymes, and antioxidant system damage, proein, lipid polysaccharides and genetic material. The modulation of enzymatic antioxidants includes superoxide dismutase (SOD), catalase (CAT), glutathione, reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST). Measurement of antioxidant enzymes and lipid peroxidation in mussels can be used as sensitive biomarkers for the biomonitoring of polluted water.

The biomarkers that were selected as endpoints of exposure and effect in this study were antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPX), and glutathione S-transferase (GST)) and lipid

peroxidation (MDA) formation. This suite of multiple biomarker assays using molluscs was subjected to multivariate analysis to provide an integrated biomarker response assessment of the system. As such, measures of oxidative stress have been used as sensitive, early-warning indicators of adverse effects in contaminated aquatic habitats.

While extensive work on biomarkers research is being undertaken in several parts of the world, such studies are yet to receive sufficient attention in India. Freshwater bivalve species such as, *Corbicula striatella*, *Parreysia corrugata* and *Parreysia cylindrica* etc which represent the entire benthic habitat of Maharashtra will be the best organisms for such study.

Biochemical changes in the animals exposed to different metals are likely to be amongst the first expressions of excess metal accumulation and perhaps the most sensitive indicators of stress and are useful in shaping the mechanism of toxicity and severity of a variety of xenobiotics. Qualitative and quantitative study of changes in most important biochemical components of organisms is valuable to identify different toxicants and protective mechanism of the body against toxic property of heavy metal pollution. The ease of biochemical approaches in environmental contamination monitoring and classification of exposure to pollutants for the use in environmental risk evaluation. Low concentrations of a toxicant will cause biochemical responses within individual organisms before these effects are observed at higher levels of biological organization.

Accumulated heavy metals are recognized as inducers of oxidative stress by directly generating reactive oxygen species and causes conflict in metabolic functions, trigger detoxifying enzymes, and antioxidant system damage which lead to oxidative stress and causes damages to proein, lipids, carbohydrates and nucleic acids.

Therefore, measurement of important biochemical constituents such as protein, ascorbic acid, DNA and RNA can be used as susceptible biomarkers for the biomonitoring of heavy metal pollution in freshwater ecosystem.

Proteomics deals with the large-scale analysis of proteins expressed by the genome of an organism. In general, the aim is to search for qualitative and quantitative changes in protein expression that occur as a function of development, disease, environmental insults, or treatment. Because of the complexity of the proteome of most organisms, the complete and rapid mapping of protein expression changes and characterization of posttranslational modifications is challenging and will require an analytical effort that exceeds the technology and throughput that exist in most standard biochemistry laboratories.

The clinical value of electrophoretic protein analysis depends on whether a given change represents acclimatization to stressful conditions or a failure of the supportive physiological and biochemical mechanisms. The SDS-PAGE based method for quantification of protein is a method of choice due to its simplicity and reliability. SDS-PAGE is a technique used in biochemistry, genetics and molecular biology to separate proteins according to their electrophoretic mobility.

This study addressed the use of selected freshwater bivalves as possible biomonitors or indicators to aid in the assessment of the impact of pollutants on aquatic systems. These organisms could provide valuable information on the water quality.

The present research work is divided into four chapters, which deals with different aspects as mentioned below:

I) General introduction

II) Bioaccumulation study

III) Antioxidants as biomarkers

IV) Biochemical study- a) Quantitative estimation
b) Protein profile by electrophoresis

V) Conclusions

Experimental design to carry out the present research work is as below,

For the present study three experimental freshwater bivalve species was separately exposed to chronic concentrations ($LC_{50/10}$) of ionic form of As, Cd, Cu, Pb and Zn separately.

(1) 1st group was maintained as control.

(2) 2nd group was exposed to chronic concentration of As (0.1719 ppm) upto 20 days.

(3) 3rd group was exposed to chronic concentration of Cd (0.1284 ppm) upto 20 days.

(4) 4th group was exposed to chronic concentration of Cu (0.033 ppm) upto 20 days.

(5) 5th group was exposed to chronic concentration of Pb (1.50 ppm) upto 20 days.

(6) 6th group was exposed to chronic concentration of Zn (1.8589 ppm) upto 20 days.

The first chapter "General Introduction" deals with the significance, objectives and literature review of the present study.

The second chapter deals with the "Bioaccumulation studies". In the present study the physico-chemical parameters of tap water and metal added water were analyzed and are summarized in table. The heavy metal concentration in mantle, gill, digestive gland and whole soft body tissues were found to be increased along with the exposure period and metal concentration in bivalves exposed to metals as compared to the bivalves maintained as control. The data revealed a

significant increase in levels of all metal conentrations, body burden and bioconcentration factor (BCF) in the whole soft body tissues of experimental bivalves with increase in exposure period as compared to the bivalves maintained as control. In the present study it was found that amongst the studied freshwater bivalve species, *C. striatella* shows highest concentration, body burden and BCF values for Cu, Pb and Zn while *P. cylindrica* shows highest concentration, body burden and BCF values for As and Cd. In the present study it was also observed that, digestive gland accumulates highest concentration of heavy metals as compared to other studied tissues. The results are presented in the form of tables and graphs and discussed with the citation of literature.

The third chapter deals with "Antioxidants as biomarkers". After chronic exposure to different heavy metals a steady increase in formation of lipid peroxidation (MDA) and glutathione-s-transferase activity and decrease in levels of antioxidant scanvenger molecule (GSH) and activity of antioxidant defense enzymes (superoxide dismutase, catalase and glutathione peroxidase) was observed in the digestive glands of the three experimental freshwater bivalve species. Highest increase and decrease in different biochemical parameters were observed after 20 days of chronic exposure.

A maximum increase in lipid peroxidaton (MDA) formation, GST activity and maximum decreased in levels of GSH and activity of antioxidant defense enzymes (SOD, CAT and GPx) was found in the digestive glands of the freshwater bivalve, *Corbicula striatella* on exposure and bioaccumulation of Cu, Pb and Zn, while in *Parreysia* cylindrica for As and Cd as compared to studied bivalve species. Among the tested heavy metals Cd induce highest increase in lipid peroxidaton (MDA) formation, GST activity and much reduced in levels of GSH and activity of antioxidnat defense enzymes (SOD, CAT

and GPx) in the digestive glands of three experimental freshwater bivalve species. The results are discussed with the citation of literature.

The fourth chapter deals with the "Biochemical study" and is divided into two sections.

a) Quantitative estimation:

The obtained results of qualitative estimation revealed that, the protein, ascorbic acid, DNA and RNA content in mantle, gill, digestive glands and whole soft body tissues of three experimental freshwater bivalve species was significantly decreased as compared to those of control bivalves. The obtained data revealed that, as the exposure period increases there was a significant decrease in biochemical contents in different soft body tissues of three experimental freshwater bivalve species.

A maximum decrease in protein, ascorbic acid, DNA and RNA contents was observed in different soft body tissues of freshwater bivalve, *Corbicula striatella* in response to accumulated levels of Cu, Pb and Zn, while in *Parreysia cylindrica* for As and Cd as compared to other studied bivalve species. Higher protein, ascorbic acid, DNA and RNA depletion was observed in the digestive glands as compared to other studied tissues of three experimental freshwater bivalve species after chronic exposure to As, Cd, Cu, Pb and Zn for 10 and 20 days. In the present study it was also observed that, as compared to other studied metals, Cd induce higher depletion of protein, ascorbic acid, DNA and RNA content in different soft body tissues of the three experimental freshwater bivalve species.

b) Protein profile by electrophoresis:

Protein profile expression of whole soft body tissues of control and experimental bivalves was studied by SDS-PAGE electrophoresis.

The results obtained revealed that, in experimental freshwater bivalve, *Corbicula striatella,* the major protein band in the range of about 60 to 65 kDa was expressed denser in animals treated with As, Cd, Cu, Pb and Zn as compared to respective bands in control animals. The analysis showed that after 10 days of exposure to different metals some medium range of newly synthesized proteins were observed as compared to bivalves maintained as control. These results are visualized most significantly in case of Pb and Zn after 20 days of exposure.

In experimental freshwater bivalve, *Parreysia corrugata,* no significant discrimination is observed after first 10 days of exposure to different metals. Though there was an apparent discrimination seen in the animals after 20 days of exposure. 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 experimental freshwater bivalve, *Parreysia cylindrica* the results observed were similar to that of *Parreysia corrugata*, except the expression of 34 kDa newly synthesized proteins was observed similar towards all the heavy metals. In the animals exposed for 20 days approximately 34 kDa proteins were found to be more clearly expressed in heavy metals Cd, Cu and Pb in *Parreysia cylindrica* as compared to other studied bivalve species. The obtained results are presented in the form of images and discussed with the citation of literature.

Overall results of bioaccumulation, antioxidants as biomarkers and biochemical studies indicate that, *Corbicula striatella* is being proposed as sentinel organism for monitoring of Cu, Pb and Zn, while *P. cylindrica* for As and Cd pollution in freshwater ecosystem.

Protein profile study indicate that PES are the good marker for metal exposure and might be used to develop novel protein markers for biomonitoring of heavy metal pollution in freshwater ecosystem.

Fifth chapter deals with the conclusion drawn from bioaccumulation, antioxidants as biomarkers, biochemical study.

The references regarding the related work done by the several authors are cited at the end of the thesis.