GENERAL INTRODUCTION

The various toxicants, which act as artificial stress inducers, pose a considerable amount of interference on the innate homeostatic condition in natural biological systems. Input of these toxic chemicals is abundant in the environment that levies adversities on the survival of living forms. Amongst most inputs, heavy metals are some of the most prevalent stress, that results from the widely accepted modernizations of agriculture and industrialization system of the present day world. Some of the toxic metals can be described as heavy metals that include metals like cadmium, copper, iron, lead, mercury, zinc and many others. The term heavy metal includes most metals with an atomic number of more than 20, but it also exclude alkali metals, alkaline earth’s lanthanides and actinides (Stanly and Bang, 1987).

Heavy metals arise out of diverse industrial activities and combustion of fossil fuels and are almost ubiquitously present in the environment. Inputs from sewage effluents, anthropogenic and industrial wastes and other disposals add such metals to the environment, which accumulate in many water bodies, in a concentration, exceeding the tolerance level and subsequently enter the food chain, often causing serious health hazards (Bidwell and Dowdy, 1987).

The effects of such toxins on the organisms appear to depend upon its concentration in water and aerial environment and their involvement in the metabolic pattern and on the threshold limiting value (TLV) of a particular species and also of the individuals within the species. The TLV is defined as the maximum concentration of a toxin that an organism can tolerate and may be exposed to, without lethal effect. Since the lethal concentration of a toxin varies from species to species and individual to individual, the TLV is used as a monitoring standard. An individual with a high TLV is more tolerant to the pollution concentration. The adaptive limit of the responses being determined by structural, physiological and behavioural characteristics of each individual within a species. Understanding the development of tolerance of animals, enabling them to survive in highly polluted environments, has of late, gained a momentum (Sathyanathan, 1996).

Urban water bodies serves as sinks for heavy metal pollutants, as they receive domestic and industrial effluents (Fernandez, 1997). Mohaupt et al. (2001) pointed out that urban storm water discharged in the German area and drainage flow in the Deutschland area are the most important sources of heavy metals in the Rhine catchments. The River Rhine also serves as an important external micro pollutant source for the Delft inner city canals (Qu and
Mishra and Khandekar (1994) showed that concentrations of heavy metals as lead (Pb), cadmium (Cd), zinc (Zn) and copper (Cu) in air and water, surface soil and food, in greater Bombay, India, are more in the high traffic zones, with the highest concentrations observed in the winter months (October to February) and the lowest in late summer and monsoon, when pollutants get diluted and dispersed by downpours and turbulent climatic conditions (Mishra and Khandekar, 1994). Wastewaters contaminated with heavy metals such as cadmium and copper, frequently discharged to sewage canals, which drain into the major water bodies (Bugenyi and Lutalo-Bosa, 1994).

Heavy metals can even cause permanent damage to human being, and can even result in death. In the 1950 – 60s in Japan, a town called Haginoshima watered their rice with water contaminated by cadmium from local industries. The absorbed cadmium when eaten, it caused the consumers’ bones to be weak and break easily. The whole body became sore and a light touching was even painful. At least 100 people died. It was called the “Itai – Itai” disease (Karmakar et al., 2008). From the example mentioned above, we can say that species of crops, show differential responses to widely used herbicides or other chemicals, though some varieties remain unaffected by their application but most of them show symptoms of damages, ranging from slight reduction of vigour to complete death of a population.

A study in Turkey showed that cadmium altered the carbohydrate metabolism in Cyprinus carpio by affecting the level of glucose in serum and the glycogen reserves in both muscle and liver tissues. Such changes in the glycogen reserves in muscle and liver tissues and serum glucose levels under the effects of cadmium, might result in impairments of energy requiring vital processes and hence gave us an idea about poor health condition of the fish population there (Cicic and Engin, 2005).

Jacson et al., 2005, studied another report of heavy metal toxicity on a burrowing crustacean the Callianassa kraussi, where they have shown that the combined effect of lead (Pb) and zinc (Zn) can increase the rate of mortality of eggs and larvae of the crustaceans upto 50% more than normal.

Professor Nevo and his school, from the University of Haifa, Israel, for a long time have been busy in identifying the genomic changes at the level of DNA and its subsequent protein products in both marine and terrestrial fauna and flora, in response to different toxin stresses. On a study of shrimp Palaemon elegans, in an other study on a marine gastropod Monodonta turbinata in the Mediterranean coast of Israel, they studied not only the pattern of the toxic
substances and their influences not only on the physiology of the organisms, but also on the effects of these pollutants on the pattern of genetic diversity, within and between populations, which was the basis of adaptive evolution (Nevo et al., 1985). In their observations on the allozyme frequencies of organisms for the isozymes Phosphoglucose Isomerase (PGI) and Phosphogluco Mutase (PGM) in *P. elegans* and *M. turbinata*, exposed to Pb, Cu, Cd, Zn and Hg, both in nature and in laboratory, they showed that changes in allozyme frequencies of the organisms are indeed sensitive to pollutants. Such changes due to resistance to toxicity can be explored and be used as promising biological indicators of pollution. They also showed that different allelic isozymes of either PGI or PGM, display a differential tolerance to specific pollutants. It has been claimed that this differential patterns indicate the adaptive nature of the allelic isozymes and provide the qualitative and quantitative basis for detecting and monitoring marine pollution (Lavie et al., 1982).

We must mention here that the major water bodies including ponds, lakes, canals and rivers in and around the city of Kolkata, India, which are quite rich in flora and fauna, also receive effluents from industries, agriculture, households and vehicular traffic, through sewage system around the Metropolis (Bandopadhyay et al., 2006).

To investigate the situation in Kolkata we tried to detect the type and level of toxicity of some of the heavy metals in the inhabitant, flora and fauna, which are being constantly exposed to such pollutants are decided to make an estimate of toxicants in the aquatic resources under study by Atomic Absorption Spectrophotometry.

Analysis of the metal content of the niche and its inhabitant organisms do not provide the complete information about the adaptive response of these living systems, to such toxic environments. With the help of molecular biological tools, we made an attempt to characterize of the genomic changes at the level of protein and DNA, in a range of terrestrial, aerial and aquatic fauna and in the floral forms (Lewontin, 1974).

Enzyme markers are ideal candidates for such quantitative genetic analysis. Allelic isozyme markers and ecological factors provide important predictive methods for identifying the elite genotypes, characterized by single or multiple disease resistance properties (Nevo et al., 1985), high protein content and a variety of some quantitative traits. Adaptive significance of genetic polymorphism has therefore been studied by protein and DNA diversities on genetic ecological correlations.
Isozymes or isoenzymes are enzymes that differ in amino acid sequences, having similar substrate specificity and enzyme action, but have different molecular origin or as protein variants controlled by allelic variants of a single gene. Usually these enzymes display different kinetic parameters, such as $k_m$ or different regulatory properties (Ferguson, 1980). They are coded by different genetic loci, which usually arise through gene duplications and divergences. Isozymes differ from allozymes, which are enzymes that arise from allelic variations at one gene locus. Isozymes can often be distinguished from one another by biochemical properties such as electrophoretic mobility. The existence of isozymes permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stages (Hunter and Merkert, 1957). Some common examples of isozymes are Esterase (Est), Alkaline Phosphatase (A1P), Phosphoglucomutase (PGM), Malate Dehydrogenase (MDH), Lactate Dehydrogenase (LDH), Alcohol Dehydrogenase (ADH) etc.

The changes in the isozyme / allozyme markers of the tolerant organisms can be sensitive to and vary adaptively to the level, type and concentration of the pollutants, and such changes can be explored and potentially be used as promising genetic monitoring system and thus they can be used for detecting the level and tolerant doses of a species to pollutants (Bandopadhyay et al., 2006). Natural selection selects across the entire genome and in different genetic systems, for mechanisms that both generate maintain polymorphism as adaptive strategies to cope with variable, changing and unpredictable environments (Nevo, 1994). The intention of this study was to explore the changes caused by the pollutants at the genetic level, involved in such a defense system that may have led the organisms to adapt and survive in different toxic environments.

The detection of genetic variation at the species, population and within population level, is of great importance for knowing properties for sustainability of a particular species in a particular environment (Das et al., 2005). Genetic variation at species level helps to identify the taxonomic units and to determine the species distinctiveness. Variation at the population level can provide an idea about different genetic classes, the genetic diversity among them and their evolutionary relationship with their wild type relatives. The genetic variability within population is extremely useful to gather the informations on individual identity, breeding pattern, degree of relatedness and distribution of genetic variation among them (Schierwater et al., 1994).

For the determination of genetic variability like isozyme analysis, another important technique that can be used is the analysis of Randomly Amplified Polymorphic DNA
(RAPD). RAPD is based on amplification of discrete regions of the genome by polymerase chain reaction (PCR), in presence of synthetic oligomers (8 – 10 bases in length) of arbitrary sequence (with G+C > 60%), this along with DNA templates, thermo-stable polymerase, dNTP mixtures and appropriate buffers for enzymes, generates RAPD profiles, allowing examination of genomic variation without a prior knowledge of DNA sequence (Welsh and Mc Clelland, 1990; Williams et al., 1990). RAPD technique is relatively simple and inexpensive, compared to the other methods (Ward and Grewe, 1995) as it does not require prior knowledge of DNA sequence, for examination of genomic variations. (Islam and Alam, 2004).

Water is very intimately related to the biological life. It is essential therefore that there should be a tolerable level of pollution for an aquatic organism to receive. Polluted water also interferes with the presence of oxygen therein. The effect of toxins on a species appears to depend upon the concentration of a particular toxin in water, the metabolism and a threshold limiting value (TLV) of a particular species and of course of the individuals within the species. Hutchison (1965) pointed out that each of the overall responses represents the outcome of many integrated internal, external and physiological factors like survival rate, reproduction rate and growth rate etc. for particular responses of the individual organism.

The present study therefore programmed to explore a multilocus ecological test of the genetic heterogeneity of a species or populations of species in response to ecological stresses, especially in response to heavy metal toxicity, in and around the Kolkata metropolis and suburbs. Here it should be mentioned that carp fish culture is the mainstay of Indian fresh water aquaculture. The genus “Labeo” exhibits intensive species diversification and constitutes commercially important carp fishes with nutritional benefits for mankind.

About 31 species of Labeo have so far been reported in Indian water (Talwar and Jhingran, 1991), out of which only a few have been domesticated for carp culture (Das et al., 2005). Among them Labeo bata being a most widely cultivated minor carp species throughout West Bengal and account for a great bulk of its production and especially in and around Kolkata it is a well known edible fish, that’s why this species has been chosen as the model in the present study and as Labeo bata is being easily bred in sewage fed water bodies, it is a good specimen to study the genetic and ecological diversities in response to heavy metal toxicides.

On the other hand Lemna minor is a species of free floating aquatic plant belonging to the duckweed group of plants. This rapidly growing plant is used as a model system for studies in basic plant biology, in eco-toxicology, in production of biopharmaceuticals and as a source of
Figure 1: Map of Kolkata and Suburb showing Selected Areas.
animal feeds for agriculture and aquaculture. As duckweeds float, it is especially susceptible to metal toxicants (Mukherjee *et al.*, 2004) and because of its high reproductive rates, it adapts quickly to changes in the environment. An experimental study was conducted on *Lemna minor* and *Lemna minuta* to see the growth response of these two species of duckweeds against high, medium and low nutrient condition, where it was found that *Lemna minor* had a better stability, as compared to *lemna minuta* against low nutrient medium (Njambuya *et al.*, 2011). So being a good and stable indicator, as well as for its easy availability this plant was taken as a test organism for aquatic pollution studies and also for the wastewater treatment.

For the study and characterization of adaptive polymorphism, in response to heavy metals pollution in aquatic environment, the three sites were chosen on the basis of source of pollutants. The first was NAIHATI (Site 1), which was chosen as the minimally exposed site for these different heavy metals and toxicants. The second site was the BYPASS site (Site 2), which is an extended part of East Calcutta Wetland, which is highly polluted with anthropogenic wastes, along with municipal garbage and wastes of slaughterhouses, tanneries, heavy vehicular traffic etc. Lastly the third site was the KHARDAH site (Site 3) that was chosen as the waterbodies of the site was sewage fed and people do the aquaculture of this area, the sources of sewerages are numerous paper mills and industries of Titagarh area.

Genetic variation at the species level helps us to identify the taxonomic units and the species distinctiveness. Variations at the population level can provide us an idea about different genetic classes, the genetic diversity among them and their evolutionary relationship with wild relatives. Thus the genetic variability within population is extremely useful to gather the information on individual identity, breeding pattern, degree of relatedness and distribution of genetic variations among them (Schierwater *et al.*, 1994 and Das *et al.*, 2005).

In the present thesis therefore we tried to explore the nature, mode and the results of the following queries:

A] What is the extent of heavy metal toxicity in the sewage fed aquatic systems and in respective resident organism in Kolkata and suburbs?

B] What is the nature of adaptive changes in the aquatic floral and faunal populations at the molecular level against environmental stresses?