INTRODUCTION

Water pollution due to ever-increasing global urbanization and industrialization is adversely affecting the water quality and biotic components of aquatic environment (Pathak and Gopal, 2009). Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life (Karbassi et al., 2006; Chang et al., 2009). The contamination of fresh water systems with a wide range of pollutants has become a matter of concern over the last few decades. The pollution of aquatic ecosystems with metals is a serious threat to the environment due to their persistent nature, long distance transport, and toxicity to aquatic organisms (Huang et al., 2005). Contamination of aquatic ecosystems (e.g. lakes, rivers, streams etc.) with metals has been receiving increased worldwide attention and the literature has many publications on this (Canpolat and Çalta, 2003; Tekin-Özan and Kır, 2006).

Heavy metals such as cadmium (Cd), lead (Pb), and chromium (Cr) are of toxicological importance. The U.S. Environmental Protection Agency (EPA) in its National Sediment Quality Survey provided a number of guideline values for many recognised toxic chemicals, including Pb, Zn, Cu, and Cd. Cadmium is one of the major metalloids for which there has been concern due to its toxicity in a wide range of animal models and also in humans (Arai et al., 2004; Liu et al., 2011). The toxic nature of cadmium and their hazardous effects on human health have been clearly documented. Cadmium cause kidney damage and cause symptoms of chronic toxicity, including impaired kidney function, poor reproductive capacity, hypertension, tumours and hepatic dysfunction (Luckey and Venugopal, 1977; Waalkes, 2000). At the level of the organism, cadmium toxicity is associated with liver and kidney injury, osteomalacia, osteoporosis, skeletal deformations, neurological, and other deficits (Garg et al., 2009).

Metals transferred through the aquatic food webs to fish and humans are of environmental and health concern (Chen et al., 2000). Throughout the world, where industrial effluent and hazardous waste are growing problems, a number of biological
assays have been developed and evaluated for aquatic toxicity testing (USEPA, 1989; Sponza, 2002a, b). Laboratory bioassays are a common procedure to evaluate toxicological endpoints at organism level, in a large number of test species from across several taxa, and across the main ecological or trophic positions (i.e. from bacteria to fish, and from decomposers to final consumers) (Chapman, 2007; Piva et al., 2011).

Bioassay is a method of estimating environmental changes from biological responses (Terawaki et al., 2009). Bioassays in which test organisms are exposed to various doses of the pollutant are used for toxicity evaluations. This is achieved by monitoring the biological integrity of these organisms and comparing them with those which have not been exposed to the pollutant. Blaise et al. (1988) reported that since living material responds to the total effect of actual and potential disruptions, biological assays have become important tools in assessing harmful chemical activity. Toxicity bioassays give a rapid estimation of the bioavailability fraction of toxicants, even at low doses, and take account of the synergistic and/or antagonistic effects of all components interacting with the biota (Kiss et al., 2003).

Toxicity tests have been shown to be very useful in environmental and chemical hazard assessments because they can be done relatively quickly and inexpensively compared to multiple chemical analyses of synthetic organics (Burton and Scott, 1992) plus the data are easily interpreted. A number of reviews were published concerning the use of various assays for assessing quality of freshwater (Burton, 1991; Hansen et al., 2007). Many types of bioassays are available and tests can be conducted in the laboratory or the field and monitored manually or automatically (Duncan, 1993). The objective of a toxicity test is to define the concentrations at which a test material is capable of producing some selected response, usually deleterious, in a population under controlled conditions of exposure. The appropriate way to do this is by use of the "quantal response" (i.e. by having only two experimental alternatives dead or alive, all or none) from which the relation between concentration and percentage effect can be defined (Ward and Parrish, 1982).
Toxicity bioassays can be classified according to the test species involved (Farre and Barcelo, 2003). The use of a battery of bioassays involving different bioindicators species at different tropic levels is an efficient and essential tool for predicting environmental hazards to the aquatic ecosystem. Routinely used, fish-lethality assays involve exposure to the toxicant for a maximum of 96 h. The results are reported as the percent volume that is lethal to 50% of the organisms within the prescribed period of time (LC50). Standard lethal tests such as the median lethal concentration (LC50, 96 h) are recommended for preliminary exploration to establish initial estimates of the toxicity of a substance. According to Reish and Oshida (1987) the 96-hour test is the most common. In many cases the effects of test material occur rapidly and are well defined in these time periods.

As such, regulatory guidelines for aquatic pollutants in natural ecosystems have been traditionally based on acute lethality tests such as the 96 h LC50 (USEPA, 2001), although impacts on development, growth, and reproduction have also been considered (Rand and Petrocelli, 1985). Lethality test are particularly useful in the predictive assessment of environmental quality of chemicals discharged so that substantial safety factors and margins can be met (Ezemonye et al., 2008). Recently, toxicology and ecotoxicology testing rely on acute toxicity testing as the norm for identification and classification of environmental hazards (Castano et al., 2003). A range of acute toxicity bioassays have been developed to establish the toxicity levels of compounds for aquatic organisms. These tests are based on the use of microorganisms, plants, invertebrates and fish (Farre and Barcelo, 2003). A range of whole organism bioassays is used for the assessment of water quality and to monitor these materials. Such tests provide useful results in assessing risks to human health and aquatic life from the release of contaminants into surface waters.

Acute toxicity experiments to determine the short-term toxicity of metals on aquatic organisms are frequently used as a measure of aquatic health (Rand, 1995). A relatively short-term lethal or other effect, usually defined as occurring within 4 days for fish and macro invertebrates and shorter time for smaller organisms (APHA-AWAA-WPCF, 1985). Acute toxicity studies help in the detection, evaluation, and abatement of pollution by providing reliable estimates of safe concentration, from
which water quality criteria can be derived (Ahsunullah et al., 1981). Utilization of acute toxicity studies for assessing water quality can be employed by examining different life stages of important species (NAS/NAE, 1973). Thus, an acute toxicity test may be used to assess the potential effects of pollutants at high concentrations in a relatively short duration. The objective of acute toxicity testing is to determine the concentration of a particular chemical that will elicit a specific response or measurable end-point from a test species in a relatively short period of time, usually 2 to 7 days. The response of the organism to the increasing concentration of the chemical is used to determine the endpoint of interest. For acute toxicity tests, mortality is expressed as the median lethal concentration (LC50), which is the estimated concentration of the test material that will kill or immobilize 50% of the test organisms in a predetermined period of time (Traas and Van Leeuwen, 2007).

The aim of chronic toxicity testing is to determine whether prolonged exposure to chemicals will have significant adverse effects on ecosystems. In chronic toxicity tests, effects are studied over prolonged periods of exposure, often over entire life cycles and usually the endpoints are primarily sublethal (such as growth) or measurements of reproductive output. Subchronic studies are of longer duration than acute exposure but generally do not exceed a period equivalent to one-third of the time taken for a species to reach sexual maturity. Apart from survival, chronic toxicity studies are based on end-points like individual growth (body length and body weight), abnormal development (teratogenicity), hatching time, hatchability, reproduction (total number of young, brood frequency, etc.) and behavioural aspects, etc. These data are then subjected to concentration-effect modelling or hypothesis testing to derive the no observed effect concentration (NOEC) (Traas and Van Leeuwen, 2007).

Sublethal effects define the toxicity of the environment and understand the potential danger of pollutant inputs. The toxicological assessment of water contamination through sublethal bioassays becomes relevant because they allow the early detection of adverse effects on particular test organisms (Almeida et al., 2002). Mechanism-based, sublethal bioassays provide a more practical and more sensitive index of bioavailability, and at the same time provide strong correlations between increased contamination of sites and the degree of biomarker response (Jimenez-Tenorio et al., 2007). Modeling sub-lethal effects thus requires quantitative
assumptions on energy budgets, and assumptions on how the metabolic processes are affected by toxicants (Jager et al., 2006). Long-term effects may be related to changes in appetite, growth, metabolism, reproduction, and even death and mutations (Kopperdahl, 1976).

The process of toxicity-direct analysis based on classical bioassays is still too laborious to be widely applicable and the use of rapid biological tests systems, based on cells or sub-organisms, to evaluate toxicity opens a new window (Reemtsma, 2001). These new tests can assess toxicity by their effects on living cells or organelles directly, rapidly, cost effectively and without ethical problems ensuing from the use of higher organisms, such as fish. The choice of organisms (biological indicators) for bioassays must take into account many factors, such as sensitivity and reliability, distribution and environmental relevance, availability over the course of the year; furthermore, a toxicity test also requires a reproducible endpoint that can be accurately, predictably, and reliably measured (Chapman, 2002a).

The utility of fish for assessing environmental conditions in aquatic ecosystems has gained prominence in recent years (Ikem et al., 2003). The knowledge that fish show distinct physiological and behavioural responses to low levels of pollutants has been exploited in the development of fish monitors to act as indicators of water quality. Recently, the technique on fish bioassay has attracted attention as a method for constant monitoring of aquatic contamination (Terawaki et al., 2009). Fish remain as the most used and most ideal species for aquatic toxicity tests. Apart from their obvious ecological relevance, which they share with other aquatic species, they have an added commercial, cultural, and health significance to humans (Hayes et al., 1990). Acute toxicity studies are the very first step in determining the water quality requirements of fish (Pandey et al., 2005). Lammer et al. (2009) reported that fish acute toxicity test is a mandatory component in the base set of data requirements for ecotoxicity testing.

Although a numerous literature is available on cadmium effects on fishes, information on bioassay cum a range of responses in fish particularly in the Indian major carp is slight significant. To do bioassay in the present study, it was intend to understand how cadmium induced alterations in the body of fish and whether these
alterations used for environmental monitoring. To extend the understanding the study of responses of carp to environmental cadmium, an attempt was made in this study to evaluate the activity of an Indian major carp *Cirrhinus mrigala* after exposing them for 24 and 96 h to acute and sublethal concentrations. *Cirrhinus mrigala* could be suitable monitoring organism to study the bioavailability of water bound metals in freshwater habitats (Palaniappan and Karthikeyan, 2009).

Hence in the current investigation the freshwater fingerlings *C. mrigala* was used as an experimental animal on the basis of important criteria. It is one of the most common Indian carp representatives of an ecologically important group and withstands a wide range of experimental conditions. It occurs in the principal rivers of India and is a moderately fast growing freshwater major carp. Moreover, it is widely edible and available, is amenable to laboratory testing, easily maintained and genetically stable so uniform populations can be tested. In addition, it is of great commercial importance, renowned for its taste and other purposes including type of test, sensitivity to the pollutants were taken into consideration. The study further focused on more reliable evaluation of the risk of cadmium to freshwater fish and to strengthen the base line data that could be used to estimate a comparative sensitivity to cadmium.