1. INTRODUCTION

Pesticides and agriculture are closely intertwined and immediate halt in the usage of the former would practically be impossible. Regardless of the increasing public concern about the negative effects of pesticides on animal health, it continues to be used worldwide. As a result contamination of water resources such as ponds, lakes, rivers, lagoons and the oceans along with the organisms dwelling therein have been contaminated with persistent pesticides over the years (Tsuda *et al*., 1999; Ejobi *et al*., 2007; Adeyemi *et al*., 2008).

Pesticides have harmful effects on species biodiversity. These compounds have adverse effects either directly or indirectly on the normal life cycles of animals and their habitats. Different species of fish, amphibians, birds and aquatic mammals have been listed as endangered because of chronic exposure to pesticides and other related agrochemicals. Measures can be taken to lower the level of exposure to animals and their habitat. The flow of energy is disturbed when any level of the food chain/web is affected by compounds such as pesticides and other agrochemicals. This can lead to widespread adverse effects causing an imbalance in the ecosystem (Barlas, 1999).

Tea (*Camellia sinensis*) due to its tremendous export potential is one of the most important cash crops in India. Tea as a plantation crop was introduced in Darjeeling hills in 1852 but with time it has spread over vast stretches of the sub-Himalayan foothills and the adjoining regions of North Bengal covering an area of 1,07,479 hectares in the foothills and plains (Biswa and Mukhopadhyay, 2013). Pest, pathogens and weeds are severe constraints in the productivity and quality of tea. As a result, the tea planters use a broad range of pesticides to overcome these problems for high yield and economic returns. The principal control measures taken to keep the population of major tea pests down is by applying synthetic pesticides, e.g. chlorinated hydrocarbons, organophosphates, carbamates and synthetic pyrethroids. Indiscriminate use of pesticides not only in tea gardens but also in agricultural fields have resulted in serious problems concerning environmental pollution, both terrestrial and aquatic. Because of the known toxicity of pesticides in food products, there is increasing public concern of late regarding the pesticides residue in tea also. Information for pesticide residues in Indian tea ecosystem, specifically in the tea growing soils and adjacent water bodies is very scanty (Bishnu *et al*., 2009). Recently, Pal *et al*. (2011) and Singh *et al*. (2015) have reported the presence of pesticide residues in the water bodies, sediments and tissues of fish in and around the areas of tea garden in North Bengal.

Natural and man-made foreign compounds (xenobiotics) enter the aquatic ecosystems by various routes, including direct discharge, land overflow, atmospheric deposition and food chain transfer (Livingstone *et al.*, 1992). Natural xenobiotics comprise a broad range including plant products, animal toxins and natural hydrocarbons, whereas the fabrication of man-made xenobiotics (contaminants) increases on a daily basis in quantity and variety. Knowledge of the fate and effects of xenobiotics on biota is imperative in relation to evolutionary and ecological processes, whereas the same for contaminants is decisive for pollution monitoring, impact assessment and environmental management (Livingstone, 1993).

The complete chemical analysis for all possible environmental contaminants in sediments, water, air and from every species and sample of interest is not possible. Studies of entire ecosystems are expensive and do not provide specific information about the toxic levels of pollutants to individual species (Landis and Yu, 1995). Therefore, a true cascade of toxicity tests or bioassays must be performed which can be classified into three groups:

1) single species acute tests that give results in a short period of time

2) single species chronic tests that show results in weeks, months or years, and are used to analyse exposure effects on growth and reproduction, and

3) multispecies tests that allow analysis of the properties of ecological structures (Heliovaara and Rauno, 1993). These three kinds of tests are used to analyse effects of pesticides in different organisms.

Risk assessment needs reliable scientific information and one such root of information is understanding the metabolic fate and toxicokinetics of compounds. Toxicokinetic refers to the progression of a xenobiotic through the body which is carried out by processes including absorption, distribution, metabolism and excretion. Metabolism is one of the most significant factors that can influence the overall toxic profile of a pesticide. The biotransforming enzymes react with the xenobiotic leading to its metabolism by a two-phase process called phase I and Phase II reactions. In phase I reactions, adding or unmasking the functional groups by oxidation, reduction or hydrolysis give rise to a polar compound and the greatest importance is credited to oxidation enzymes involved in the metabolism of the majority of xenobiotics. This may result in reducing the efficacy of the metabolised xenobiotic, described as bioelimination or generates an active metabolite. The second-phase reaction involves the conjugation of metabolites produced in the first phase or seldom, the original substances also,

with phase II conjugating enzymes which give rise to more polar compounds and facilitates in easy elimination from the body (Siroka and Drastichova, 2004).

In general, these enzymatic reactions are advantageous as they help in the elimination of foreign compounds but however, sometimes these enzymes may transform a harmless substance into a reactive form, a process known as metabolic activation (Guengerich, 2001). Biotransformation is the enzyme-catalyzed process of chemically modifying drugs and other xenobiotics to increase their water solubility in order to facilitate their elimination from the body. Actually, biotransformation is the process that alters the chemical properties of a xenobiotic away from lipophilicity to hydrophilicity that favours the elimination of xenobiotics in urine and faeces (Parkinson *et al*., 2010).

Among the phase I biotransforming enzymes, the cytochrome P450 (CYP 450) system lines first because of its high catalytic versatility and the sheer number of xenobiotic it detoxifies or activates to reactive intermediates (Guengerich, 2006; Wickramasinghe, 2017) and as such also regarded as one of the most important biomarker. In the fish, CYP 450 has primarily been examined as a biomarker signifying pollution of the aquatic environment by industrial or agricultural sewage. However, fish may differ from other species in response to xenobiotics (Siroka and Drastichova, 2004). The highest concentration of CYP 450 enzymes concerned with xenobiotic biotransformation is found in liver endoplasmic reticulum (microsomes), but CYP 450 enzymes are present in virtually all the tissues (Lewis, 2001).

The CYP 450 comprises a large multigene family of heme-thiolate proteins which play a significant role in the metabolism of xenobiotics and endobiotics and hepatic clearance is a principal way for elimination of xenobiotics (Pelkonen and Turpeinen, 2007). It is now possible to differentiate metabolic reactions and interactions and to assign which CYP 450 is involved in the metabolism of a certain xenobiotic by different *in vitro* approaches (Pelkonen and Raunio, 2005; Pelkonen *et al*., 2005; Hodgson and Rose, 2007).

North Bengal being one of the major tea producing areas in India, the use of pesticides is necessary to combat the pest, but the application of these pesticides may affect the non-target organisms apart from its designated pest. As in India, very few research have been carried out regarding CYP 450 as a detoxifying enzyme or a biomarker to assess the contamination of the aquatic environment. The rationale of the present work was to study the role of hepatic CYP 450 and its dependent enzyme activities in some air breathing fishes as a potential detoxifying enzyme of pesticides.