The dissertation work done by me during the tenure from November to March, 2007 at the National Institute of Oceanography is focused on the study of optimization of the laboratory techniques for measurement of Cytochrome P-450 (CYP-450) enzyme induction of various species of marine organisms exposed to polyaromatic hydrocarbons (PAHs) present in the marine environment in terms of 7-ethoxy resorufin-O-deethylase (EROD) activity in marine organisms

Cytochrome (CYP) P-450, one of the class of Mixed Function Oxygenase (MFO) system belongs to a large family of isozymes divided into several classes, is involved in the biotransformation of the endogenous and organic contaminants. It is an inducible enzyme being induced by the exposure to certain classes of xenobiotics such as PCBs, PAHs, and PCDDs etc. Ethoxyresorufin-O-deethylase (EROD) enzyme belongs to this CYP super family, has a deethylase activity, which makes these accumulated lipophilic compounds into the water-soluble form so that they can get easily eliminated out of the body. Thus EROD activity is a specific assay for the xenobiotically inducible form of cytochrome P-450, thus the measurement of this activity in the marine organism is a good means of evaluating organism response to PAHs contamination.

In order to study the enzyme induction, the samples of various species of marine organisms were collected from different locations along the Goa coast. Initially optimization of the method of estimation of the EROD activity was accomplished using the enzyme extracted from the fish *(Sardinella longiceps)* with many trials and errors. Enzymes were extracted from the livers of the fishes using different kinds of homogenizing buffer of different pHs and chemical composition and also with different kinds of homogenizer (viz. manually operated glass homogenizer and high speed Ultra Turax). After standardization of the method of enzyme extraction from the tissues of the marine organisms (using phosphate buffer of pH-7.4 and Ultra Turax) the optimization of the conditions of the experiment for the enzyme reaction was performed with respect to varying concentration of the substrate and the NADPH. Lastly, in this sequence of the method development, the most important step of the enzyme induction study was carried out by measuring the enzyme activity of the fish as a function of time.

After successfully standardizing the procedure, I have determined the EROD activities in the other marine organisms such as oysters (*Saccostrea cucullata*) and snails (*Morula granulata*). In order to assess the state of pollution in the coastal environment around Goa samples of sediment, water and marine organisms were collected from different sites (Tirakol, Anjuna, Sinquerim and Dona Paula) along the Goa coast. The EROD activities of the marine organisms were measured following the technique optimized in the laboratory.

Besides, the total protein contents of all the enzyme extracts from each of the samples were determined following Lowry's method using BSA standard. Further, in support of biomarker studies, water quality parameters and the concentration of PAHs in the tissues of marine organisms and the sediment samples from the same sites were also measured following the standard techniques of extraction and analysis.

The significant finding of my dissertation work is that the CYP-450 responses increase with the concentration of PAHs in the surrounding coastal water as well as in the tissues of the marine organism. Thus the measurement of CYP-450 induction (EROD activity) provides a valuable clue in respect of the contamination of the environment by persistent organic contaminants.