CHAPTER VI

SUBLETHAL EFFECTS OF THE KMML TITANIUM DIOXIDE INDUSTRIAL EFFLUENTS ON THE BIOCHEMICAL COMPOSITION OF THE FISH *OREOCHROMIS MOSSAMBICUS* AND THE SNAIL *PILA VIRENS*
INTRODUCTION

Studies on the deleterious effects of pollutants on aquatic organisms have gained much importance currently, and much work has been done in this field. In spite of this the information regarding the nature of action of the pollutants and physiological changes in organisms is meagre (Madhupratap et al., 1979). Aquatic organisms are particularly sensitive to environmental contamination, and pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes (Nemcsok et al., 1987). Pollutants are not only harmful to adult fish, but may also cause disturbances of development in embryonic stages (Weis and Weis, 1977). Several cases have been reported in which toxic effects of pollutants may be changed due to changes in pH, temperature, hardness, salinity and dissolved oxygen content of the water (Zitco and Carson, 1976; Pascoe et al., 1986; Prakasam, 1989). The harmful effects especially sublethal, retard the development of surviving individuals and/or are of harmful influence on the normal metabolic processes (Nemcsok et al., 1987).

The acidification of natural softwater by acid deposition together with its impact on aquatic organisms, especially fishes, has been a subject of intense study. (Haines, 1981; Howells 1983; Playle et al., 1989). Hydrogen ions reduce active sodium and chloride uptake at the gills and increase effluxes resulting in net ion loses (McDonald, 1983a). Biochemical composition of the tissues of different fishes under experimental conditions has been studied by various investigators. Effect of starvation on the biochemical composition was studied in the North Sea Cod by Love (1960) and in the herring, Clupea harengus, by Wilkins (1967). The effect of stress on fish has been studied by Leloup (1960).

Intense agricultural activities and industrial development during the last few decades have resulted in the increase in the concentration of metals in rivers and lakes, and this has adversely affected fishes which are high in the food chain.
Some of these effects are recognisable by interference with normal biochemical and physiological mechanisms (Crespo and Balasch 1980; Nemcsok and Boross, 1982; Dalal and Bhattacharya, 1994). Choudhary et al., (1981) determined the effect of malathion, an organophosphorous insecticide, on growth and body composition of an air breathing fish H.fossils. Evgen'eva and Kocherehkina (1994) studied the effect of various toxicants on histogenesis of Russian sturgeon (Acipenser culdenstadtii) fry muscle tissues. Some haematological and biochemical effects of cadmium in fish was studied by Larsson et al., (1976). Oxidation stress in fish exposed to model xenobiotics was studied by Pedrajas et al., (1995) and they identified oxidised forms of certain enzymes as early biomarkers of oxidative stress. Chromic acid exposure, even at sublethal levels is sufficient to induce marked disturbances in the histology, physiology and behaviour of salmonids (Jones et al., 1985, 1987) Rashatwar and Ilyas (1984) conducted biochemical studies on the effect of phosphamidon in a fresh water fish Nemachelias denisonii (Day). Changes in carbohydrate metabolism in Oreochromis was the subject of Dange (1986), while he was studying the effect of short term exposure to different types of pollutants on this fish. Bhakthavathsalam (1984) studied the importance of protein metabolism during acute exposure of Anabas testudineus to lindane. The work of Larsen et al., (1980) is noteworthy, in that he concentrated on biochemical and haematological effects of a titanium dioxide industrial effluent on fish. Raj (1984) studied the mercuric chloride induced changes in the protein, lipid and cholesterol levels of the liver and ovary of the fish Channa punctatus. Studies of Bhaskar and Govindappa (1986) deals with the biological impact of acidity and alkalinity on the physiology and biochemistry of the fresh water fish T. mossambica. Nuvan induced physiological, biochemical and behavioural changes in Barbus stigma were investigated by Ghosh (1986). Jothikumar et al., (1986) conducted experiments to study the toxicity and biochemical responses of carp to dinitrobenene plant effluents.
Total oxygen consumption, glycogen content and succinic dehydrogenase levels in liver, muscle and heart in normal and thiodon exposed *S. mossambicus* were studied by Vasanthi and Ramaswamy (1987). Rao *et al.*, (1987) studied the effect of Benthioacarp on protein metabolism of the same fish. Behavioural and biochemical studies of onset and recovery from acid stress in Arctic char (Salvelinus alpinus) were conducted by Jones *et al.*, (1987). Sublethal effects of organochlorine insecticide (endosulfan) on protein, carbohydrate and lipid contents of liver tissues of Oreochromis mossambicus were observed by Ganesan *et al.*, (1988). Ghazaly (1992a,b) studied haematological and physiological responses to sublethal concentrations of cadmium and sublethal effects of nickel on carbohydrate metabolism of *Tilapia nilotica*. The related works dealing with the analysis of biochemical constituents under stress conditions include those of Folmer *et al.*, (1993, 1993a), Bhatnagar *et al.*, (1992); Van (1996); Coello *et al.*, (1996) and Sinha and Mandal (1996). Biochemical investigations were carried out by Vinuchandran *et al.*, (1998) on the kinetics of isosomal acid phosphatase of *Oreochromis mossambicus*.

As far as the studies on the biochemical composition of molluscs under stress conditions are concerned, majority of the investigations are being carried out on bivalve molluscs. Moorthy *et al.*, (1985) studied the glucose metabolism of *L. marginalis* exposed to pesticide. The related investigations in this field include those of De Zwaan and Zandu (1972); Badman and Chin (1973); Madar and Pora (1981); Garamina (1984); Kulkarni *et al.*, (1984) and Sunila and Lindstorm (1985).

There are also reports on the biochemical composition of snails under stress conditions, especially on exposure to heavy metals and other pollutants. Laskowski and Hopkin (1996) conducted studies on the effect of heavy metals on fitness in snail Helix aspersa. Rajyalakshmi *et al.*, (1996) studied the Butachlor impact on protein, free amino acid and glutamine contents, and on activity levels of aminotransferases, glutamate dehydrogenase and glutamine
synthetase in the fresh water snail Pila globosa (Swanson). Suryanarayanan and Alexander (1973) worked on the biochemical constituents of the red muscles of the gastropod *Pila virens*. Zhu *et al.*, (1994) studied the lipids of slugs and snails and showed the unique sterol and fatty acid composition of slugs and snails, as well as similarities and differences in sterol composition between the two. Pivorarov (1994) worked on certain biochemical aspects related to the neurons on the snail exposed to toxicants. Rao *et al.*, (1983) studied the toxicity of phenthoate and changes in the organic constituents of the snail Pila globosa under sublethal and lethal impacts.

On reviewing the literature, it was found that not much work has been done on the effects of titanium dioxide industrial effluents on the biochemical composition of fishes or snails, except for the works of Larsen *et al.*, (1980) who conducted studies on the biochemical and haematological effects of a titanium dioxide industrial effluent on fish, and of Vijayamohan (1991) who studied on the effect of effluents from Travancore Titanium Products (TVM) on the biochemical composition of certain fresh and brackish water fishes. Since the acid waste water from the Titanium Dioxide Pigment Plant of KMML, Sankaramangalam enters Vatta kayal and the adjoining inlets and canals as well as the paddy fields, and water logged areas around the factory, through various outlets, especially during the monsoon season, a detailed study was conducted to evaluate the toxicity of this industrial effluent on *Oreochromis mossambicus*, which is commonly found in the canals and inlets connected to Vatta kayal, and on *Pila virens*, which is also commonly found in the paddy fields and swamps in the area.
MATERIALS AND METHODS

The animals used in the present study were the fish, *Oreochromis mossambicus* and the gastropod mollusc, *Pila virens*. The fishes and snails were brought from the field in live condition and stored in well aerated aquarium tanks. They were acclimatized in the laboratory for two weeks before being subjected to experimental studies. The fishes and snails were well fed during the acclimation period.

The effluent samples were collected freshly from the four outlets and brought to the laboratory and stored in refrigerator. Three different sublethal concentrations of the effluents, viz., 0.2%, 0.6% and 1.0% were prepared in fresh water for samples I and III; 1%, 2%, and 3% for sample II and 0.02%, 0.06% and 0.1% for sample IV. The effluent samples were kept outside the refrigerator for 3-4 hours to bring to room temperature, before preparing the desired concentrations. Twenty litres effluent mixed water (hereafter referred to as ‘test solution’) was taken in 3' × 2' × 1' glass tanks. Controls were also arranged having twenty litres of water in each case.

Ten fishes and ten snails each were introduced into each tank. Test solution was changed every 24 hours and the experiment was extended for seven days. During the experimental period, the snails were fed with hydriilla and the fish with tubifex worms. Physicochemical characteristics of the test solution and the control water were recorded periodically. At the end of the seven day period, the fishes and snails were taken out, wiped thoroughly and the total weight was determined. Muscle and liver from all the fishes, and muscle and hepatopancreas from all the snails were dissected out. The wet weight of each tissue was noted and dried to constant weight in an oven at 70°C. The dried tissue samples were then used for the determination of glycogen, protein and lipid. Estimations were carried out in duplicate by the following methods.
Glycogen was analysed by Anthrone colorimetric method (Sam Siefert et al., 1950). The total lipids were determined by the methanol chloroform method of Barnos and Blackstock (1973). The protein concentration of the tissue was estimated by deploying Folin phenol reagent using bovine serum albumin as standard (Lowry et al., 1951).