INTRODUCTION
INTRODUCTION

Pollution is defined as 'an undesirable change in the physical, chemical or biological characteristics of our land and water that may or will harmfully affect human life or that of desirable species, our industrial processes, living conditions and cultural assets; or that may or will waste or deteriorate our raw material resources'. Pollution increases not only because as people multiply the space available to each person becomes smaller, but also because the demands per persons are continually increasing year by year. As the earth becomes more crowded there is no longer 'away', one persons 'trash basket' is another's living space.

Pollution is caused by pollutants, that are released in the environment as a result of anthropological activities. It has infact shocked people that uncontrolled man's activities can have a widespread unintended effects on living organisms. Pollution of our rivers, streams, canals and oceans, seas, etc. is more acute in the industrialized countries, where millions of tones of pollutants are discharged in the rivers and streams and also directly into the sea.

In the present era of green revolution the adoption of new technology in crop production has increased the off-take of biocides. This coupled with ever increasing use of pesticides for public health purposes poses menacing problems in our water bodies. Majority of pesticide problems are contamination in food grains, dairy products, vegetables, fruits, fish, meat, eggs in field storage and market. And also due to drift the air, soil, canals, rivers, tanks and rainwater too
are coaminated with pesticides. The subject today constitutes one of the most serious challenges of public health and environmental pollution.

Pesticides reach the aquatic environment through direct application to control pests or through erosion resulting from heavy rains or from irrigation or from direct discharge of industrial effluents into water resources. In aquatic organisms, the process of bio-accumulation concentrate pesticides. The pesticide of proven potentialities in residual form contaminates aquatic ecosystem and having affinities for living systems creates series of problems to the aquatic biota especially to the fishes. Contamination of aquatic ecosystem initiates a chain of interaction in the organisms. The higher invertebrate feeding upon the micro and macro aquatic forms are ultimately vulnerable to the threats of bio-accumulation of pesticides.

The ever-increasing pressure to increase food production to meet current demands requires protection of crops from pests. The use of pesticides is therefore inevitable and constitutes an integral part of modern crop management practices. It has been estimated that crop damage is as high as 20 percent where pesticides are not used.

Pesticides include chemicals to protect crops from insects (insecticide), weeds (herbicides), disease causing micro-organism (fungicides and bactericides), mites (acaricides), nematodes (nematicides) and rodents (rodenticides). Pesticides also include chemicals that control pests directly hazardous to animals and humans, particularly vectors such as, mosquitoes, flies and fleas, ticks and lice that transmit diseases.
INDIA AND THE WORLD: USE OF VARIOUS PESTICIDES
COMPARISON OF THE VARIOUS TYPES OF PESTICIDES USED IN
INDIA AND THE REST OF THE WORLD

FIG. 1
In India 143 pesticides are registered, with an annual consumption of about 85,000 tones (Agnihotri 1998). Organo-chlorine insecticides form the bulk of pesticides used in India, of which hexachloro-hexane (HCH), DDT, malathion, methyl parathion, monocrotophos, hexachloro-benzene and endosulfan are the most extensively used. (Fig.1). In the past the organo-chlorine pesticides like DDT, BHC and aldrin have been popular among Indian farmers because of their simplicity in application, efficiency and good returns. Their continuous use has resulted in widespread contamination of food commodities, which is a serious matter from the viewpoint of both domestic use and food export. It has also been reported that all major rivers of India are contaminated with DDT (21,900 ng/Lt.), BHC (272,000ng/Lt.), endosulfan (2,890ng/Lt.) and aldrin (1,500ng/Lt.).

The first generation pesticides were adequate to keep the then population well fed, when farms were small and diversified and cultural practices favorable for blocking massive build up of pests. DDT and other potent broad-spectrum pesticides not only ushered in an era of industrialized agriculture, but they were supposed to 'solve' all pest problems forever. But their continuous use resulted in almost senseless saturation of the environment to the point that we now must phase out the use of many of them. Twelve pesticides; endrin, chlordane, heptachlor, dibromochloropropane, toxaphene, pentachloro-nitrobenzene, pentachloro-phenol, ethyl parathion, nitrofen, paraquat, dimethyl sulphate and aldrin were banned on April 1, 1997 (Agnihotri 1998) Alternatives are now available like DDT can be replaced by synthetic pyrethroids, which are biodegradable, effective at low doses and with a low toxicity for mammals. However, these
alternatives are more expensive and require proper handling and application. So while DDT, aldrin is phased out, it is still being used in public health applications, primarily for economic reasons.

The synthetic pyrethroids are insecticides of great importance for the protection of agricultural produce. Though natural pyrethrins are effective their usefulness is limited due to their unstability in the environment. This has led to emergence of number of highly active and photostable 4th generation synthetic substitutes based on the structure of the naturally occurring pyrethrins. Synthetic pyrethroid insecticide, the newest major class of insecticides, is neuro-toxicant with increasing worldwide usage. The increased use of these modern pyrethroids is due to (i) excellent insecticidal activity with low toxicity towards mammals and (ii) rapid metabolism. But these pyrethroids in lethal and sub-lethal dosages can cause damage to nervous system such as axonal swelling and/or breaks and myelin degeneration in the sciatic nerves. There have been no reports of delayed neurotoxicity with the pyrethrins (Gupta 198-). Although pyrethrins are cheap in terms of cost per hectare as they are applied at the rate of 50 gm/ha. but are highly toxic to fish (Kumaraguru et al. 1982).

Water pollution poses a continuous dilemma for public. Its effects are worldwide well recorded but physical and chemical analyses are inadequate to reveal all the effects of polluted water. Since the effects of toxic pollutants are largely biological, the use of bioassay with fish as a responsive animal has gained increasing importance in determining the effects of pollutants on aquatic life to complement analytical data. The present study has been an attempt to explore the anamolous
changes in fishes due to pesticides, and is carried out under following heads:

(i) Acute Toxicity

(ii) Chronic Toxicity

(iii) Oxygen Consumption

(iv) Haematological Studies

(v) Biochemical Studies

The fish selected for the bioassay study is *Heteropneustes fossilis*. The position of the fish in the animal kingdom is as follows:

Phylum : Chordata

Sub-Phylum Vertebrata

Group Gnathostomata

Series Pisces

Class Teleostomi

Order Cypriniformes

Family Saccobranchidae

Genus *Heteropneustes*

Species *fossilis*

The fish is locally known as 'Seenghan' in Sagar Division and is one of the most important freshwater catfish having wide range of distribution. This fish is consumed
FIG. 2 (a): *HETEROPNEUSTES FOSSILIS*
in fresh conditions by the local peoples of this region. The fish has been selected for this study because it is available more or less throughout the year, except in the winter and rains, due to rise in water column and rather low water temperature of the lakes and ponds. The fish has been found fit, as an ideal specimen for different types of experimentation. Being a hardy fish can live for a longer period for the experimentation.(Fig.2)

The most commonly used pesticides, Aldrin (organochlorine) and Fenvalerate (synthetic pyrethrin) have been chosen for the study. These pesticides were selected because of the following criteria;

(i) They are used extensively in agricultural and industrial operations in India,

(ii) They are available and handled easily,

(iii) They display wide range of toxicity,

(iv) Their concentration can be analytically determined, and

(v) They belong to different groups of pesticides.

The important information pertaining to the identity and nature of the pesticides are as follows:

1: IDENTIFY OF INSECTICIDES:

(a) Aldrin:

Aldrin is a cyclodiene insecticide (organochlorinated compound) having two rings, one is chlorinated ring having six molecules of chlorine and other one is unchlorinated. Aldrin is
FIG. 3: STRUCTURAL FORMULA OF ALDRIN.

FIG. 4: STRUCTURAL FORMULA OF FENVALERATE.
synthesized by the Diel-Alder's reaction of hexachlorocyclopentadiene with an excess of bicycloheptadiene at 100°C. The yield is more than 80%, calculated on the hexachlorocyclopentadiene (Melnikov, 1971). The technical Aldrin contains not less than 95 % by weight of compound 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo, exo-5,8-dimethanonapthalene (HHDN) and not more than 5% by weight of insecticidal active related compounds. Aldrin is usually abbreviated to HHDN. The molecular formula is C_{12}H_{8}Cl_{6} and structural formula is given in Fig.3

Primary constituent of Aldrin according to Worthing and Walker (1983):

IUPAC Chemical name: (1R,4S,4aS,5S,8R,8RaR)- 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene

or

1,2,3,4,10,10-hexachloro1,4,4a,5,8,8a-hexahydro-exo-1,4-endo-5,8-dimethanonaphthalene.

Relative Molecular Mass: 364.9

Common synonyms and trade names: ENT15949 (compound,118). HHDN, Octalene, OMS 194.

CAS Registry number: 309-00-2

RTECS Registry number: 102100000

Technical trade name: Aldrin (Aldrex). This is the common name of an insecticide containing 95% of HHDN.

Purity: The minimum content of Aldrin (as defined above) is technical Aldrin is 90%).
FIG. 4: CHEMICAL STRUCTURE OF THE FOUR STEREOISOMERS OF FENVALERATE.
Impurities: Octachlorocyclopentene (0.4%).
Hexachlorobutadiene (0.5%), Toluene (0.6%), a complex mixture of compounds formed by polymerization during the Aldrin reaction (3.7%) and carbonyl compounds (2%) (FAO/WHO, 1968 b).

(b) Fenvalerate:

Fenvalerate is a synthetic pyrethroid having no cyclopropane ring in the molecule. It is prepared by the esterification of (2RS)-2-(4-chlorophenyl)-3-methylbutyric acid (also known as (2RS)-2-(4-chlorophenyl) isovaleric acid, CPIA, or CI-Vacid, with ( alfa RS ) - alfa - cyano-3-phenoxybenzyl alcohol (Ohno et al., 1976). It has four stereoisomers as a result of the two chiral centers in the acid and alcohol moieties RR,RS,SR, and SS (Fig.4) The SS isomer is the most bioactive isomer of Fenvalerate (Yoshioka, 1978).

The composition of the product is a racemic mixture of the four isomers in equal proportions (Table-3) Technical grade Fenvalerate contains 90-94% of Fenvalerate (FAO/WHO, 1980 'b').The molecular formula is C_{25}H_{22}ClNO_{3} and structural formula is given in Fig.41,2

2: PHYSICAL AND CHEMICAL PROPERTIES OF PESTICIDES

(a) Aldrin:

Some physical and chemical properties of Aldrin are given in Table-1. It is stable to heat (200°C) and at 4<pH<8 but oxidizing agents and concentrated acids attack the chlorinated ring. Aldrin is non-corrosive or slightly corrosive to
metals because of slow formation of hydrogen chloride on storage. (Shell 1976, 1984; Worthing and Walker 1983).

(b) Fenvalerate:

Some physical and chemical properties of Fenvalerate are given in Table-2&3. It is stable to heat and moisture and is relatively stable (compared with natural pyrethrins) when exposed to light. It is more stable in acidic than in alkaline media, optimum stability being at pH 4 (FAO/WHO, 1980 'b'. Meister et al., 1983; Worthing and Walker 1987).

It is widely known fact that in the development of new chemical or product which comes into contact with or be ingested by human beings, an estimate should be made of its potential harmfulness to different plants, animals and human beings. The chemical or product when enters a water course either on direct application or as a component of effluent discharge alien to water ecosystem creates the risk to the biota of that water ecosystem. Number of studies have been attempted to explore the harmful effects of these pesticides on the aquatic biota in particular fishes, in terms of toxicity assessments.


Bakthavathsalam and Reddy (1981) evaluated effects of lindane on growth and survival of the fish, Anabas testudineus, exposed for a period of 360 days. Long-term toxicity of pesticides to Gambusia affinis and Gymnocorymbus ternetzi has been determined by Joshi et al. (1981). They also studied effects of chronic exposure of pesticides on survival, feeding, growth, oxygen consumption and swimming performance in both the fishes. Choudhary et al. (1981) reported chronic toxic effects of malathion on body composition, growth rate, hepatosomatic index, gonadosomatic index and condition factor of fish, Heteropneustes fossilis.

Hameed and Vadamalai (1986) determined toxicity of dimethioate EC 30 to Macrones keletius and also studied effect of sub-lethal concentrations of the same pesticide on growth, feeding, oxygen consumption and activity of the fish. Thiodon and ekalux induced effects on food utilization, growth and
conversion efficiency in *Lepidocephalichthys thermalis* have been observed by Palanichamy *et al.* (1986).Mohemad *et al.* (1987) determined sub-lethal effects of aldrin on swimming activity in *Oreochromis mossambicus* (Peters).


Food consumption in fishes due to exposure to fertilizers and varying temperatures has been exclusively studied by, Sarkar (1995). Palanichamy *et al.* (1996) and Ponmani *et al.* (1997) observed rate of food intake and growth in *Mystus vittatus* and *Cyprinus carpio* on exposure to pesticides.
Various studies have been conducted to evaluate the effect of pesticides on the metabolic state of the fishes. Changes in oxygen consumption, blood parameters and biochemical constituents of the tissues have been reported.

Thurstan et al. (1981) observed increased toxicity of ammonia to *Salmo gairdneri* due to reduction in dissolved oxygen of the medium. The fish, *Pleuronectes platessa* had a lower oxygen uptake when acclimatized to higher temperature (Jobling1982). Nagartanamma and Ramamurthi (1982) reported metabolic depression in the freshwater teleost, *Cyprinus carpio* exposed to organophosphate pesticide. Effect of fenitrothion was studied on respiration of *Mystus cavasius* and *Labeo rohita*. Panigrahi et al. (1984) observed that oxygen consumption rate of the fish of lower age group was more to higher age group. Effect of endosulfan and ekalux on oxygen consumption on *Barbus ticto* were studied by Bhusari et al. (1985).

Lakshmi et al. (1990) observed changes in tissue metabolism and the adaptability of the fish *Cyprinus carpio* (Linn) on exposure to environmental acidity and alkalinity. Tilak et al. (1991) studied effect of pesticides on a fresh water fish, *Labeo rohita*. Effect of salinity on oxygen consumption in juveniles of *Penaeus merguiensis* (Deman) have been studied by Prasad et al. (1991). Singh et al. (1991) observed effect of temperature on oxygen uptake in relation to body weight of *Danio spinosus* due to pesticide pollution have been studied by Suryawanshi (1992). Cebrian et al. (1992) observed toxic effects of chlorphyrifos on oxygen uptake and Jabde and Ansari (1993) studied oxygen consumption in *Neemachelius aureus* (Day) acutely exposed to cypermethrin. Arasta (1993)
has studied oxygen consumption in *Mystus vittatus* on exposure to alderex and nuvan. Effect on oxygen uptake of rainbow trout in response to 1,2,4,5-tetrachlorobenzene exposure has been observed by Brauner *et al.* (1994). Reddy and Bashamohideen (1995) studied oxygen consumption in *Cyprinus carpio* on exposure to cypermethrin. Yang and Randall (1997) observed effect of tetrachlorobenzene and tetrachloroguaicol on oxygen consumption of rainbow trout. Wagh and Jagtap (1997) have reported significant changes in oxygen consumption in *Cyprinus carpio* on exposure to thiodon.

Mishra and Srivastava (1983) determined median tolerance limits of the Indian catfish, (*Heteropneustes fossilis*) to malathion and evaluated the effects of the insecticide on carbohydrate metabolism, blood chloride levels and haematological variables. Lal *et al.* (1986) observed haematological and biochemical responses pertaining to the bioenergetic of *Heteropneustes fossilis* after exposure to sub-lethal concentrations of malathion. Thakur and Pandey (1990) observed variations in total and differential leucocytes of an air breathing fish, *Clarias batrachus* exposed to various concentrations of BHC (Benzene hexachloride). Reddy *et al.* (1992) recorded effects of sublethal concentrations of herbicide, diuron on RBC counts, haemoglobin percentage and Packed cell volume of *Sarotherodon mossambicus*.

Effects of paper and pulp mill effluents has also been observed on the haematological parameters of *Oreochromis mossambicus* (Peters) (Varadraj *et al.* 1993). Thakur and Sahai (1994) discussed effects of carbaryl on differential leucocyte counts of *Channa punctatus*, *Channa striatus* and *Garra gotyla gotyla*. Gupta *et al.* (1995) evaluated effect of chlordane and
malathion on certain haematological parameters of *Notopterus notopterus*. Yadav and Hadique (1996) observed increase /decrease in RBC number and haemoglobin content of *Symbranchus bengalensis* and *Heteropneustes fossilis* in low and high concentrations of sevin. Effects of water pollution on the haematological parameters of three fresh water teleosts, *Channa punctatus*, *Channa striatus* and *Heteropneustes fossilis* inhabiting the polluted waters of Hussainsagar lake has been studied (Kumari and Kumar 1996).

Changes in protein content of gills, liver, muscle, kidney and total homogenate of fish, due to endosulfan toxicity have been reported (Subbiah et al. 1985). Khalid *et al.* (1986) determined total protein and glycogen content of liver and muscles of freshwater fishes, *Barbus ticto* and *Rasbora daniconius*, exposed to suquin. Toxic effect of malathion on glycogen reserves of *Heteropneustes fossilis* has been studied by Lal *et al.* (1986). Effect of starvation on total protein and free amino acid in some organs of *Channa punctatus* have been recorded by Gupta *et al.* (1987).

Khillare and Davne (1990) reported variations in the glycogen contents in liver and muscles of *Puntius stigma*, exposed to agrofen and thiodon. Effect of heptachlor on organic components of muscles of *Channa punctatus* have been determined by Baigh *et al.* (1991). Protein, lipid and glycogen content in liver, gill, brain and muscles of *Cyprinus carpio* have been recorded in response to cypermethrin toxicity (Piska and Waghray 1992). Ghosh and Shrotri (1992) determined glycogen content in hepato-pancreas and muscles of *Scylla serrata*, exposed to thiodon. Suryawanshi (1992) and Arasta (1993) have studied effect of pesticides on the
physiological aspects of the fresh water fishes and reported changes in the organic reserves of the exposed group of fishes. In the liver of *Channa punctatus*, Reddy *et al.* (1994) determined sub-lethal toxic impact of hexachloro-hexane on the enzyme and metabolic profiles of carbohydrate metabolism.

Yadav and Hadique (1996) observed toxic effect of sevin on liver glycogen in *Symbranchus bengalensis* and *Heteropneustes fossilis*. Borah and Yadav (1996 'a') studied effect of starvation on biochemical parameters of *Heteropneustes fossilis*. Muley *et al.* (1996) have determined endosulfan toxicity to *Tilapia mossambica* in terms of alterations in biochemical components of the exposed fish. Kumar and Chauhan (1996) have studied toxic effect of malathion and thio tox to *Rasbora daniconius*. They recorded depletion in lipid contents in the tissues of the fish. Borah and Yadav (1996 'b') evaluated toxicity of rogor to *Heteropneustes fossilis* and determined liver protein and glycogen content of the exposed fish.

Effect of dichlorovos on protein and RNA contents in liver and kidney in *Rasbora daniconius* have been studied by Kaurav and Jain (1998). Davne and Khillare (1998) also observed variations in protein, glycogen, cholesterol and ascorbic acid in gills, muscles and liver in pesticides exposed fish, *Thynnichthys sandkhol*. Biochemical alterations in *Mystus vittatus* on exposure to aldrin have been studied by Arasta *et al.* (1998).

Srivastava and Srivastava (1990) studied skeletal anomalies in *Heteropneustes fossilis* on exposure to malathion. Suryawanshi (1992) and Arasta (1993) have studied tissue
ascorbic acid content of *Danio spinosis* and *Mystus vittatus* respectively, on exposure to various pesticides. Mishra and Mukhopadhyay (1996) have determined ascorbic acid requirement of catfish fry *Clarias batrachus*. Davne and Khillare (1998) and Arasta *et al.* (1998) have observed variations in the ascorbic acid levels in gills, liver and muscle tissues in *Thynnichthys sandkhol* and *Mystus vittatus* on exposure to thiodon, carbamate, dimethoate and aldrin respectively.

The above cited review of the literature on the effects of chemicals and pesticides on different freshwater fishes, show that still much work is awaited to make new enquiries about the metabolic changes in the tissues of fish via way of understanding biochemical responses. And at the same time ascertain the previous findings, the present investigation has been therefore undertaken to explore, observe and record changes in organic constituents of the different tissues, blood parameters and rate of oxygen consumption capacity of the fish, *Heteropneustes fossilis* during the exposure to the pesticides aldrin and fenvalerate, and to understand the physiological state of the fish.