Dynamics of Some Environmentally Significant Pesticides in a Tropical Waterway — A Toxicological Approach

A THESIS SUBMITTED TO
THE COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Philosophiae Doctor
in
MARINE ENVIRONMENTAL CHEMISTRY
Under the Faculty of Marine Science

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JULY 1992

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CERTIFICATE

This is to certify that this thesis is a bonafide record of research carried out by Smt. Sujatha.C.H. under my guidance, in partial fulfilment of the requirements for the degree of Philosophiae Doctor of the Cochin University of Science and Technology.

Cochin - 682 016
July, 1992

Dr. Jacob Chacko
Supervising Teacher

Corrections suggested by the examiner have been incorporated

Sujatha

Certified that the corrections suggested by the Examiners have been incorporated wherever necessary

4.12.1992

DECLARATION

I hereby declare that this thesis entitled, "Dynamics of some Environmentally Significant Pesticides in a Tropical Waterway - A Toxicological Approach" is an authentic record of the research carried out by me under the supervision of Dr. Jacob Chacko, Head, Chemical Oceanography Division, School of Marine Sciences, Cochin University of Science and Technology, and that no part of it has previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar title or recognition.

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ACKNOWLEDGEMENTS

I express my deep sense of gratitude to my supervising teacher, Dr. Jacob Chacko, Head, Chemical Oceanography Division, School of Marine Sciences, Cochin University of Science and Technology, for initiating me into this topically-relevant research on pesticide chemistry and for his inspiring guidance, help and ceaseless encouragement throughout the progress of this research work, which has enabled me to successfully complete this investigation in time.

With heartfelt gratitude, I remember the encouragement rendered and the keen interest evinced by the late Prof. (Dr) P.N.Krishnan Nambisan, former Director of the School of Marine Sciences.

I am grateful to Prof. (Dr) N.R.Menon, Director, School of Marine Sciences, Cochin University of Science and Technology, for his valuable help and support.

I take this opportunity to thank Dr.N.Chandramohanakumar, Senior Lecturer, in the Chemical Oceanography Division for his co-operation and advice during the progress of this study.

I am greatly indebted to Dr.S.Muraleedharan Nair, Lecturer in Chemical Oceanography, for his invaluable, insistent help and indelible inspiration throughout the duration of my research.

I record my thankful gratitude to Dr.A.Mohandas, Head, School of Environmental Studies for his constant encouragement and for the facilities provided.

I appreciate the encouragement given by my colleagues in the School of Marine Sciences and School of Environmental Studies and thankfully acknowledge their whole-hearted co-operation at every stage of this work.

I am thankful to all the staff members of School of Marine Sciences, who have always been willing to render me assistance for the successful completion of my research.

I gratefully acknowledge the financial support received from the State Committee on Science and Technology (Kerala) and from the Council of Scientific and Industrial Research, (New Delhi) in the form of a Junior Research Fellowship and a Senior Research Fellowship respectively. I also thank the Cochin University of Science and Technology for the facilities provided.

This work would not have been feasible but for the keen interest, love, encouragement and support from my grandfather, parents, husband, son, sisters and others.

PREFACE

The application of scientific methods to agricultural practices had succeeded in ushering in an era of increased foodgrain production but has also brought with it a plethora of environmental problems. The unwary agriculturists caught in the daze of the advertisement blitz of the pesticide manufacturers have unwittingly become party to a wanton abuse of pesticides and of other persistent plant protection synthetic chemicals that are the bane of the hazard which now threatens man's very existence.

Most of the pesticide manufacturing concerns have sought to tread upon the virginity of the uneducated farmers of this sub-continent by propagating and dumping into this country diverse chemicals that have been banned in the developed world. Pesticide use in India has recorded a steep increase over the three decades and India is now the largest producer and consumer of pesticides in South Asia.

Available reports indicate that although the country uses just over two percentage of the world's pesticides, half of the world's pesticide poisoning cases and almost three quarters of the deaths take place here. Poor handling and indiscriminate (ab)use of pesticides by small holders who are virtually ignorant of the toxic nature of the chemicals they handle have led to the present environmental crisis. Not all the agrochemicals applied is taken up by the crop and a shocking large amount leached through the soil/sediment into the water spread of the region (the rivers, lakes and coastal waters). The residual levels of the pesticides, thus, far exceed the prescribed, permissible, maximum limits. These persistent chemicals which reach the aquatic system get partitioned among its constituent phases viz., the aqueous phase, the biota and the sediments. Adequate knowledge of the nature and extent of this distribution is an essential prerequisite for quantifying pesticide pollution. Increasing instances of pesticide pollution are but writings on the wall for policy makers as well as for agricultural and environmental scientists.

Bioaccumulation and biomagnification of agrochemicals along the food chain is precariously dependent upon the nature of distribution of the chemicals among the three segments of the aquatic system. The theme of this investigation, the results of which are presented herein, has been the evaluation of the aquatic levels of some of the common xenobiotics and a quantification of their sediment-sorptional phenomena as well as their toxicological impact on an estuarine bivalve, *Villorita cyprinoides var. cochinensis*.

Part of the studies reported in this thesis has been published/communicated as research papers as indicated below:

- Toxic effects of DDT, BHC and Endosulfan on an estuarine bivalve. Jacob Chacko and Sujatha, C.H. International symposium on the fate and effects of toxic chemicals in large rivers and their estuaries, Quebec, Canada (1988).

- Malathion sorption by sediments from a tropical estuary. Sujatha, C.H. and Jacob Chacko. Chemosphere. 22 (2) 168-180 (1991).

- Pesticide sorption by sediments from a tropical estuary. Sujatha, C.H. and Jacob Chacko. Proceedings of IAPSO symposium, Vienna. 90: (1991).

- Sujatha, C.H. and Jacob Chacko. Effect of sublethal concentrations of Endosulfan and Malathion on an estuarine bivalve, *Villorita cyprinoides var. cochinensis* (Hanley). Second European conference on Ecotoxicology, Amsterdam, (1992).

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CONTENTS

CHAPTER I

CHAPTER I

INTRODUCTION

Mankind has witnessed a large diversity of environmental degradations such as changes in climatic conditions and biogeochemical cycles, eco-accidents, appearance of xenobiotics and a general world-wide deterioration in environmental quality. The effects have far reaching consequences when pertaining to aquatic systems. Since the seas cover over 70% of the earth's surface, deterioration of the aquatic environment could lead to catastrophic effects such as formation of large areas of anoxic sea basins, massive fish-kills etc.

Many an environmentalist whose conscience has been aroused by the prospects of a bleak world looming large over him, has opted to devote his time and effort for assessing the damage already caused and for devising means for a more judicious exploitation of the aquatic resources at his disposal.

Estuaries are unique water systems as they are the interface between river water and saline water. They are often surrounded by highly industrialized and densely populated regions and, consequently, are particularly prone to the hazards of anthropogenic pollution. Being a link between the limnetic and marine environments they are characterized by an array of physical, chemical, biological and geological processes, the quantification of which would require complex experimental strategies.

In the context of the immensely prominent role that estuaries have played in the evolution and the development of civilization, research on estuarine processes becomes increasingly relevant to mankind's future on Mother Earth if this future is to be one of sustainable development.

From the glory of the economic importance that was once the hallmark of estuaries, they have now, sadly, been down-graded to dumping grounds for indiscriminate disposal of industrial, commercial and urban wastes, which can broadly be categorized into the following :

- Atmospheric pollutants (including oxides of sulphur and nitrogen, hydrocarbons, ozone, ammonia etc.,)
- Fresh water pollutants (agrochemicals and their residues, domestic and urban sewage etc.,)
- Industrial effluents (of diverse-origin characteristic of the industries that punctuate the banks of the estuary)
- Marine pollutants (oil and other organics from the open seas).

Of all the pollutants that are discharged into the aquatic system, the xenobiotics, which include trace metals, pesticides, tributyl tin oxide, PCBs, PAHs etc., are the most hazardous as evidenced by their persistence, toxicity and bioaccumulation potential.

Synthetic agrochemicals have been widely used to increase not only the production of food and fibre but also to ensure protection from epidemic diseases as well as from obnoxious plants and animals. Thus, pesticides occupy an unique position among the many chemicals that man encounters everyday. Though these agrochemicals are cheap, easy to use and extremely effective, they leave behind residues in food which pose health hazards not only to livestock and wildlife but also to fishes,birds, mammals and even to human beings. Long term exposure is known to adversely affect a number of biological functions such as nervous system, reproduction, hormonal balance, temperature regulating mechanism, liver function etc.

Tributyltin(TBT) compounds have been extensively used over the last decade as biocides in anti-fouling paints. Although, TBT compounds are extremely effective in preventing the settlement of plants and animals on ship hulls, other marine organisms besides those targeted are significantly affected by the TBT compounds leaching from anti-fouling paints. Recent concern over the environmental impact of TBT compounds derived from pleasure vessels and fish farm nets, in enclosed waters, has prompted a number of studies on their toxicity to marine fauna. Sensitivity to TBT compounds varies among aquatic species, with gastropods and bivalves being the most susceptible followed by crustaceans, algae and fish. Although, yacht anti-fouling paints containing TBT compounds are now banned in countries like the U.K., they continue to be advertised and marketed in other countries, which are free from such restrictions.It is, therefore, important that ecotoxicological information concerning TBT and its compounds is made available to such countries before irrepairable damage is done to their marine environment.

Following the second World War, organochlorine pesticides made a major contribution to the agricultural output and in the control of disease vectors. While the persistence of these compounds after application was of considerable benefit to the user, it also introduced toxic effects to non-target organisms. These contraindications led to the development of organophosphorus pesticides. A number of such compounds have been screened for pesticide activity and have been introduced into the market. OP pesticides are believed to be potent inhibitors of the enzyme, acetylcholinesterase (AchE) (which modulates the neurotransmitter substance, acetylcholine, at the nerve cell junctions) and are thereby held responsible for bringing about physiological and metabolic changes in several non-target organisms, including a variety of edible aquatic organisms such as fish, crab, mussels etc. Like many other OP insecticides, Malathion and Methyl parathion are widely used to control crop pests presumably because it is biodegradable and leaves residues only for a short time. Eventhough, these organophosphorus compounds decompose rapidly in the environment, they may be toxic to target and non-target organisms alike. Since they are relatively non-persistent insecticides, repeated applications may be necessary for the control of pests and this might result in cumulative reduction of AchE.

The bulk of pesticide production in India includes Hexachlorocyclohexane (HCH), Dichloro diphenyl trichloroethane (DDT), Endosulfan, Malathion, Parathion and Carbamates. At present, in India, about 50,000 tonnes of pesticides are used annually for agriculture (Sarkar and Sen Gupta, 1988). Pesticides used on farmlands and also from effluents are washed out into rivers, lakes and ultimately into the seas. Pollution of the environment due to pesticide application has become a scareword among the public and a password among the environmentalists. However, increasing public awareness of the inherent dangers, has resulted in the initiation of measures to combat the menace of pollution.

Scientific investigations have established that residues left by chemical pesticides are highly toxic and can persist in the soil for over twenty years causing irreversible gene mutation with serious implication for life on earth. Research has also shown that DDT in concentrations as low as one millionth part in water significantly reduces photosynthesis on phytoplankton. Since the plankton is the primary producer in the aquatic system, the deleterious effect could get transmitted along the food chain and further lead to catastrophic consequences.

The dynamics of movements of pesticides from agricultural runoff and industrial effluents to the aquatic environment and their impact on the ecosystem are phenomena which are least understood. Information on pesticide residue levels in water, soil, plants and animal samples is scattered and diffuse in the Indian context. This dearth of authentic and systematic scientific information has been the major constraint in the development of any meaningful evaluation or management of the environmental impact.

Scope of the present study

The Cochin Estuarine System (CES), adjoining the Greater Cochin area, receives considerable amounts of domestic sewage, urban wastes, agricultural runoff as well as effluent from the industrial units spread all along its shores. Since preliminary investigations revealed that the most prominent of organic pollutants discharged to these estuarine waters were the pesticides, the present study was designed to analyse the temporal and spatial distribution profile of some of the more toxic, persistent pesticides —— organochlorines such as DDT and their metabolites; HCH-isomers; a cyclodiene compound, Endosulfan and a widely distributed, easily degradable, organophosphorus compound, Malathion, besides investigating their sorptional and toxicological characteristics.

Although, there were indications of widespread contamination of various regions of the CES with DDT, HCH-isomers etc., due to inadequacies of the monitoring programmes and due to a glaring void of baseline data the causative factors could not identified authentically.

Therefore, seasonal and spatial distributions of some of the more commonly used pesticides in the CES were monitored systematically, (employing Gas Chromatographic techniques) and the results are analysed in Chapter III. Sampling locations were fixed so as to represent the divergence of the estuary.

Estuarine sediments are able to accumulate persistent and hydrophobic organic chemicals from the overlying water and the characterization of the accumulated materials can provide useful information about the environmental quality of the estuary. Kinetic investigations of pesticide sorption is regarded as an excellent tool for assessing the fluvial transport mechanism of pesticides in the aquatic environment. Although, much work has been done on the adsorption of pesticides by soils, clays and mineral surfaces (Hermosin *et al.*, 1987; Smith and Willis, 1987 and Sattar, 1990), only scanty information is available on the nature of the sediment-pesticide sorption, occurring in aquatic systems. The studies detailed in Chapter IV relates to the above aspects.Variation in the sorption characteristics of two organophosphorus pesticides, Malathion and Methyl parathion was assessed by performing suitable sorptional experiments on three environmentally divergent sediment samples obtained from the CES.

Prediction of the fate of these pesticides require quantification of the sorption phenomenon which is dependent on the nature of the equilibrium distribution of pollutant between the aqueous phases as well as on the nature of the estuarine sediments. The experimental results from the equilibrium study were subjected to simple regressional analysis and the results were analysed by the three isotherms: Langmuir, Freundlich and Elovich. The relevance of partition coefficients of the solute and of the organic carbon content of the sediment involved in controlling and regulating the sorption process has been examined. The sorption variabilities of the two pesticides, Malathion and Methyl parathion have been quantified in terms of the Sorptive Index, S for varying exposure times.

Sorption of contaminants, for which the dissolved species, is strongly dependent on the "in situ" properties of the aqueous phase, can be affected by various environmental parameters like hydrogen ion concentration, ionic strength etc. Elaborate studies were carried out to evaluate the influence of pH and salinity on the time-dependent sorption of pesticides by natural sediments suspended in water samples collected from the sites within the CES. Besides, the significance of synthetic seawater was also studied in an attempt to identify any differences in sorptional behaviour that might be dependent on the ionic composition.

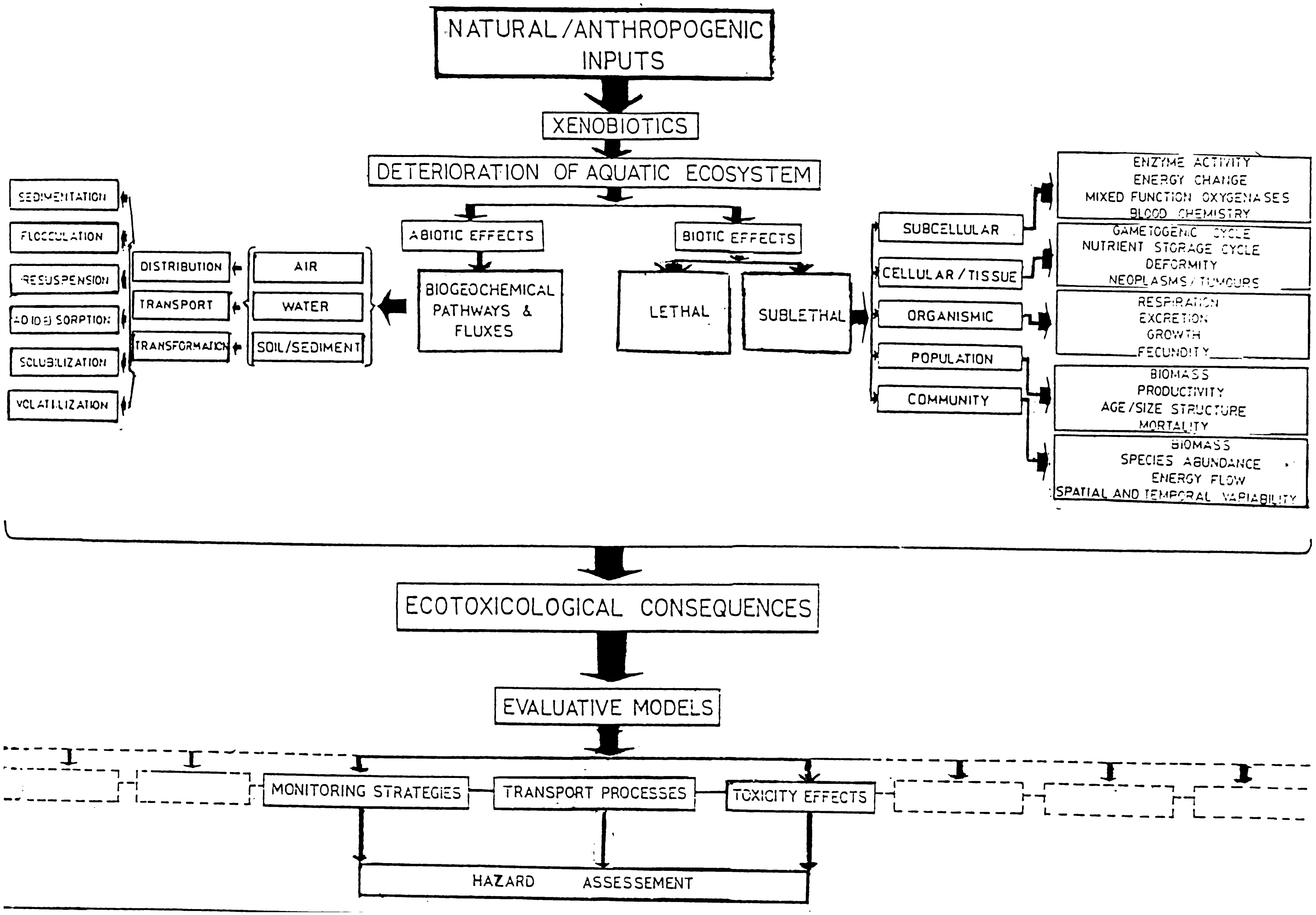
The complexities and variabilities associated with estuarine ecosystems can be overwhelming and confounding. Three fundamental concerns need to be probed into before evolving strategies for effective protection of the aquatic system from anthropogenic inputs:

- Mode of exposure of the biological components of an ecosystem to the stress

- Response of the ecosystem to the disturbance

- Mode of recovery from or adaptation to the stress.

In an effort to understand the complexities involved with the interactions between xenobiotics and the physical and biogeochemical environment (Figure 1), a variety of testing methods and procedures have been developed. Of these, the toxicological approach has been made use of for the


NATURAL/ANTHROPOGENIC INPUTS
XENOBIOTICS
DETERIORATION OF AQUATIC ECOSYSTEM
ABIOTIC EFFECTS
BIOTIC EFFECTS
BIOGEOCHEMICAL PATHWAYS & FLUXES
LETHAL
SUBLETHAL
AIR
WATER
SOIL/SEDIMENT
DISTRIBUTION
TRANSPORT
TRANSFORMATION
SEDIMENTATION
FLOCCULATION
RESUSPENSION
(AD)(DE) SORPTION
SOLUBILIZATION
VOLATILIZATION
SUBCELLULAR
CELLULAR/TISSUE
ORGANISMIC
POPULATION
COMMUNITY
ENZYME ACTIVITY
ENERGY CHANGE
MIXED FUNCTION OXYGENASES
BLOOD CHEMISTRY
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establishment of the pollutant threshold levels and also for the assessment of the nature of the biochemical effects produced in organisms subjected to pollutant stress.

The establishment of simple and rapid protocols for toxicity testing, could be expected to make significant contributions to the design of appropriate pollution-abatement strategies. **Chapter V** reports the results of a toxicological approach designed to assess the sublethal effects manifested in bivalves, exposed to some of the commonly encountered biocides.

Sublethal effects are evaluated on the response of the morphological, histological, physiological or ethological changes in the organisms induced at any stage of their life cycle but expressed in later phases in terms of reduced survival potential. The identification of small changes occurring in the organisms is the first and essential step in the understanding of sublethal effects, and in practice, these effects are generally measured through physiological and biochemical alterations. Sublethal, physiological and subcellular biochemical responses of marine organisms serve both as operational and as research indicators of environmental quality. These responses make suitable tools for the monitoring of biological effects, which have the following advantages:

- Relatively short response time
- Sensitivity to changes in the environment and ability to be measured with precision
- Existence of a quantitative or predictable relationship between physiological/cellular responses and environmental stress or pollution.

Mussels have been widely employed in toxicity evaluation and water quality management programmes in view of their acknowledged role bioindicators of toxicity levels. Bivalve molluscs which feed and respire by drawing a current of water into their bodies (under the influence of the ctenidial cilia) can accumulate pollutants to very high levels and can bring about serious alterations in their various physiological parameters (such as filtration rate, respiration, biochemical constituents etc.,). Bivalves have been reported to be able to tolerate very high levels of pesticides due to tissue-specific, inherent, metabolic, adaptive, capabilities which confer a greater survival chance on the animal.

The effects of some of the biocides (TBTO, Endosulfan, Malathion and Methyl parathion) on the respiratory rates (oxygen consumption) and variations in energy content (glycogen, lipid and lactic acid) of an estuarine bivalve, *Villorita cyprinoides var. cochinensis* have been estimated and discussed in Chapter V. TBTO was also included along with the other three pesticides, in view of the very scanty reports available in the literature on the sublethal effects of TBTO.

In the present attempt, therefore, the metabolic consequences of biocidal exposure have been traced, with a view to understanding the adaptations undergone by bivalves in pesticide-polluted ecosystems. The data generated herein would update and significantly broaden the aquatic toxicity data-base and thus afford a better understanding of the hazards caused by these chemicals in the aquatic environment.

Details of the location of the study area, the sampling procedures and the various methods employed, in order to realize the objectives of the studies reported in Chapters III, IV and V are incorporated in Chapter II. The salient features of the present investigation are summarized in Chapter VI.

CHAPTER II

CHAPTER II

MATERIALS AND METHODS

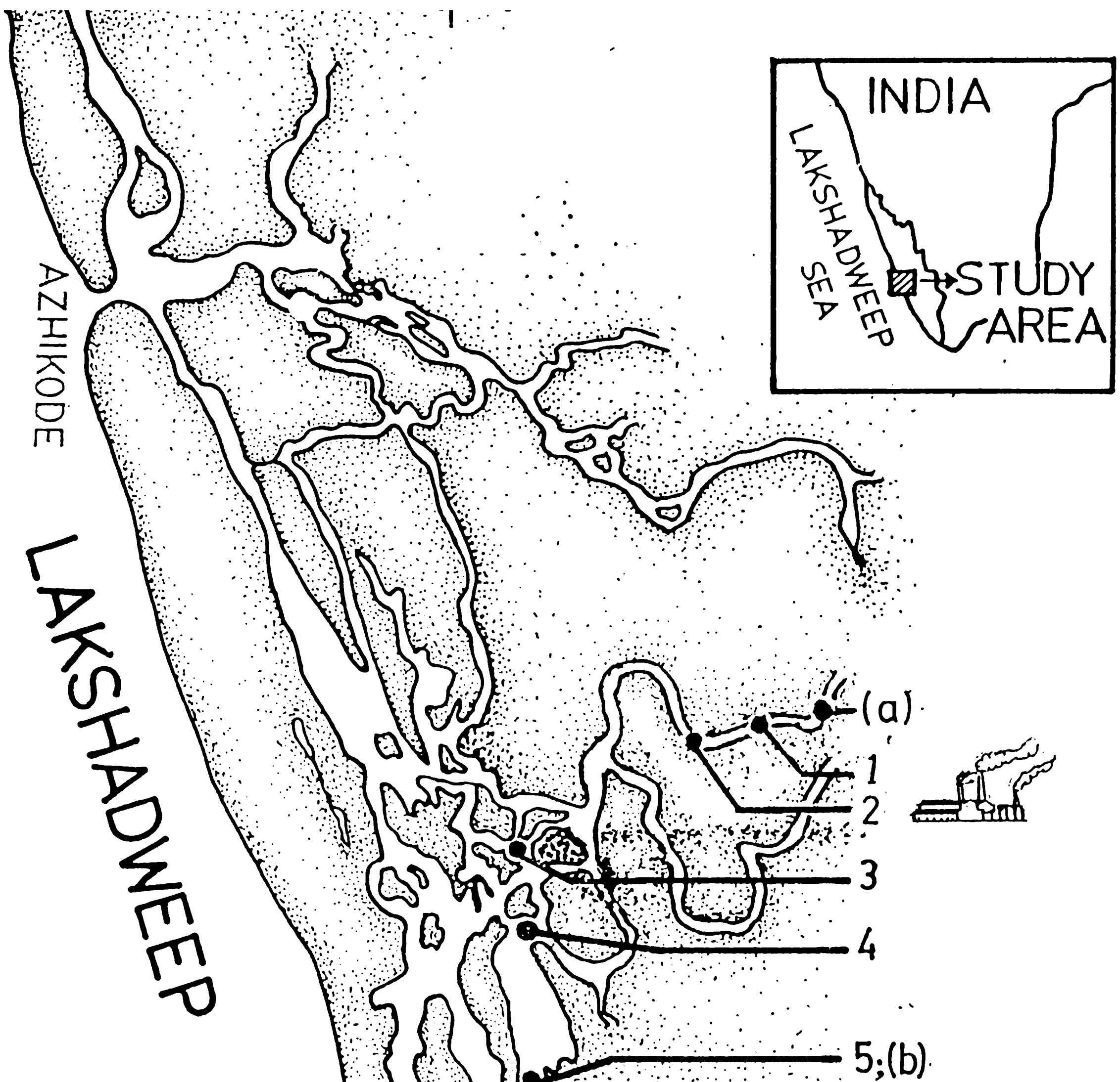
The procedural details of sampling strategies and of the experimental methodologies adopted for studies reported in Chapters III, IV and V are presented hereunder on a chapterwise basis.

2.1. CHAPTER III —— TEMPORAL AND SPATIAL DISTRIBUTION OF THE MAJOR PESTICIDES IN THE COCHIN ESTUARINE SYSTEM

2.1.1. Description of location

The Cochin Estuarine System (also referred to as the Cochin Backwater System-CBS) in the Kerala State on the west coast of India, extends between 09°40'-10°10'N; 76°10'-76°30'E (Figure 2). The fluvial dynamics of this water body are influenced by the discharge from two rivers, Periyar and Muvattupuzha, as well as by the tidal influence from the Arabian sea acting through the Cochin barmouth. The sampling sites (1-6) were spread along the northern arm of the Cochin Backwater and were selected to reflect the different environmental characteristics of the estuary. Station 1, located far upstream was riverine in nature, while Station 6, at the coastal zone barmouth, represented the confluence region of the estuary and the Arabian sea.

Station 2 was about half a kilometre downstream from Station 1 and represented a site that received enormous quantities of industrial wastes from the several fertilizer, insecticide, aluminium, zinc etc. manufacturing units located in its vicinity.



e 2.

of Cochin Backwater System showing location of sampling sites.

Station 3, Cheranellur was at the confluence of two tributaries of the river Periyar and consequently represented a large inflow of agricultural runoff.

Station 4, Vaduthala was characterized by leachings from coconut-husk retting grounds, besides other agricultural discharges.

Station 5, Bolghatty received huge quantities of municipal sewage and was hence typical of urban influence.

2.1.2. Sampling procedure

Sampling was carried out during the premonsoon (January to May), monsoon (June to August) and postmonsoon (September to December) periods. In each period three collections were made.

Water samples were collected from a depth of ≈ 2m below the surface by using a Niskin water sampler, was immediately transferred into prewashed glass bottles and was stored at -5°C till analyses were performed.

2.1.3. Experimental procedure

Each water sample was filtered through GF/C Whatman filter paper under reduced pressure. One litre of the filtrate was collected and extracted with n-hexane (HPLC grade) in a glass separatory funnel and dried with anhydrous sodium sulphate. The extract was then concentrated to 2 ml using a Kuderna-Danish evaporator to about 2 ml. This was then subjected to a clean up procedure involving elution [using 500 ml of a 6 % solution of diethylether in petroleum spirit, (HPLC grade)] through a florisil column (conditioned for 24h at 130°C) in order to remove the fat present in the extracts. The eluate so collected was again concentrated to a volume of 2 ml. Aliquots of 0.2 µl of these concentrates were injected into a gas liquid chromatograph (Shimadzu 15A) equipped with ECD (Ni^{63}) (APHA, 1985). The packed column OV-17 (0.23mm i.d; 3m length) was employed for the determination of organochlorine and organophosphorous compounds. The instrument was programmed to operate at the following temperatures:

- Column temperature : 240°C
- Injection port temperature : 270°C
- Detector temperature : 300°C

The initial calibration of the GC was carried out by injecting suitable amounts of standard solutions of pesticides prepared in HPLC grade acetone. Analysis of the solvent blanks confirmed the absence of any of the pesticides under investigation in the solvent. The analytical reproducibility was checked by duplicate measurements. A laboratory blank (solvent) spiked with the organochlorine surrogate standards purchased from Supelco Inc. (USA) was also analysed with each batch of water samples to determine the analytical contamination and the percentage recoveries of the following analytes:

- o,p'-DDT
- p,p'-DDE
- p,p'-DDD
- α-HCH
- γ-HCH
- δ-HCH
- Endosulfan and
- Malathion

The analytical data have been presented on a weighted-average basis.

2.1.4. Hydrographical parameters

The hydrographical parameters [pH, salinity, dissolved oxygen (DO) and dissolved organic carbon (DOC)] of the water samples were determined by employing standard methods (APHA, 1985 and Grasshoff, 1983).

2.2. CHAPTER IV —— SORPTIONAL BEHAVIOUR OF ORGANOPHOSPHORUS PESTICIDES ON DIVERGENT SEDIMENTS

For the sorption study, three sediment types were collected from three distinctly different locations within the Cochin estuarine system. A location far upstream in the estuary (Station a), away from the influence of industrial and anthropogenic pollution and mainly characterized by riverine sediments, was chosen to obtain a relatively less polluted sediment (LPS) sample. A typically polluted sediment (PS) was collected from Vaduthala (Station b), a location prone to severe anthropogenic pollution (coconut-husk retting, industrial effluents etc.,). A Sample of the sediment obtained from the confluence point of the estuary and the open sea, i.e., the Barmouth (Station c), was denoted as the confluence sediment (CS) and could be expected to have a much greater marine influence(Figure 2).

The sediment samples were collected using a prewashed polyethylene-lined van Veen grab and stored at -5°C till the analyses were performed. They were air-dried and homogenised before being weighed out for the experiments.

2.2.1. Pesticides

The pesticides chosen for the study, Malathion (MT) and Methyl parathion (MPT) are organophosphorus pesticides widely used in agriculture against a large variety of insect pests.

These pesticides were technical formulations (50% emulsifiable concentrates) obtained commercially. Stock solutions of 1000 ppm were prepared using double-distilled, deionized water. The experimental test concentrations (5, 10, 25, 50, 75 and 100 ppm) of the pesticides were prepared by appropriate dilutions with distilled water.

2.2.2. Natural water

Water samples representing varying environmental characteristics of the Cochin Estuarine System were collected (using a Niskin water sampler) from the sampling sites of the three sediments and were denoted as riverine water sample (Station a), estuarine water sample (Station b) and coastal water sample (Station c).

2.2.3. Synthetic seawater

Synthetic seawater was prepared according to the standard APHA (1985) procedure. The composition and concentration of constituents are given below:

Compound in order of addition	Concentration (mg l^{-1})
NaF	3
$SrCl_2.6H_2O$	20
H_3BO_3	30
KBr	100
KCl	700
$CaCl_2.2H_2O$	1,470
Na_2SO_4	4,000
$MgCl_2.6H_2O$	10,780
NaCl	23,500
$Na_2SiO_3.9H_2O$	20
Na_4EDTA	1
$NaHCO_3$	200

2.2.4. Experimental design

Equilibrium study

Adsorption experiments were performed by suspending 1g quantities of each sediment type (LPS, PS and CS) in standard aqueous solutions (20 ml) of the two pesticides, Malathion, and Methyl parathion in acid washed, 100 ml beakers. The suspensions were thoroughly mixed to facilitate attainment of equilibrium, (Prior to the experiments, the sediment samples were analysed to determine their background pesticide levels). Proper controls consisting of similar aliquots of the pesticides, but devoid of the sediment samples, were maintained so as to assess the contributions, if any, from microbiological degradation, photolysis, volatilization or possible adsorption of pesticides by glassware, to the observed decrease in pesticide concentrations. No recognizable losses due to any of the above processes were observed. Each of these solutions, along with duplicates, was kept aside for different time intervals ranging from 0.5 days to 10 days for equilibration. At the expiry of each designated time interval, the respective solutions were filtered, extracted with chloroform and preconcentrated using a Kuderna-Danish evaporator. The residues were treated with 0.2 ml of p-nitrobenzyl pyridine and 0.2 ml of cyclohexylamine (SIGMA; 0.2% solution in redistilled acetone) and the solution was made upto 6ml with ethylacetate. The absorbance of the resulting solution was measured at 520 nm. Commercially available pesticide samples were used as the calibrants. The concentrations of the two pesticides in the chloroform extract were obtained from the intensity of the resulting chromophores (Gelts and Walts, 1964). The amount of pesticide adsorbed by the sediment was obtained by computing the difference between the initial (dosed) concentration (a) and the concentration observed in the solvent after the expiry of the designated time interval (x). As has been reported in

Chapter IV, pesticide sorption by sediments took a period of 8 days for the attainment of equilibrium.

The above experimental design is schematically represented in Figure 3.

Evaluation of the effect of environmental factors

The experimental set up for the evaluation of the effect of environmental factors was the same as that used for the equilibrium study discussed above.

Effect of pH

Each of the three types of sediments was simultaneously exposed to varying concentrations (10, 50 and 100 ppm) of Malathion and Methyl parathion dosed on natural and synthetic waters maintained at three different hydrogen ion concentrations (5, 7 and 8.5), obtained by appropriate dilutions with hydrochloric acid and sodium hydroxide solutions.

Effect of salinity

To study the effect of salinity each of the three sediment types was simultaneously exposed to varying concentrations (10, 50 and 100 ppm) of Malathion and Methyl parathion dosed in natural waters maintained at their characteristic salinities (of $0\text{x}10^{-3}$, riverine — Station a; $12\text{x}10^{-3}$, estuarine — Station b and $29\text{x}10^{-3}$, coastal — Station c) and in synthetic seawater maintained at three comparable ionic strengths (of 10, 20 and 30 $\text{x}10^{-3}$ salinities). Deionized water was used as the control in all these cases.

Each of these solutions, along with duplicates was kept

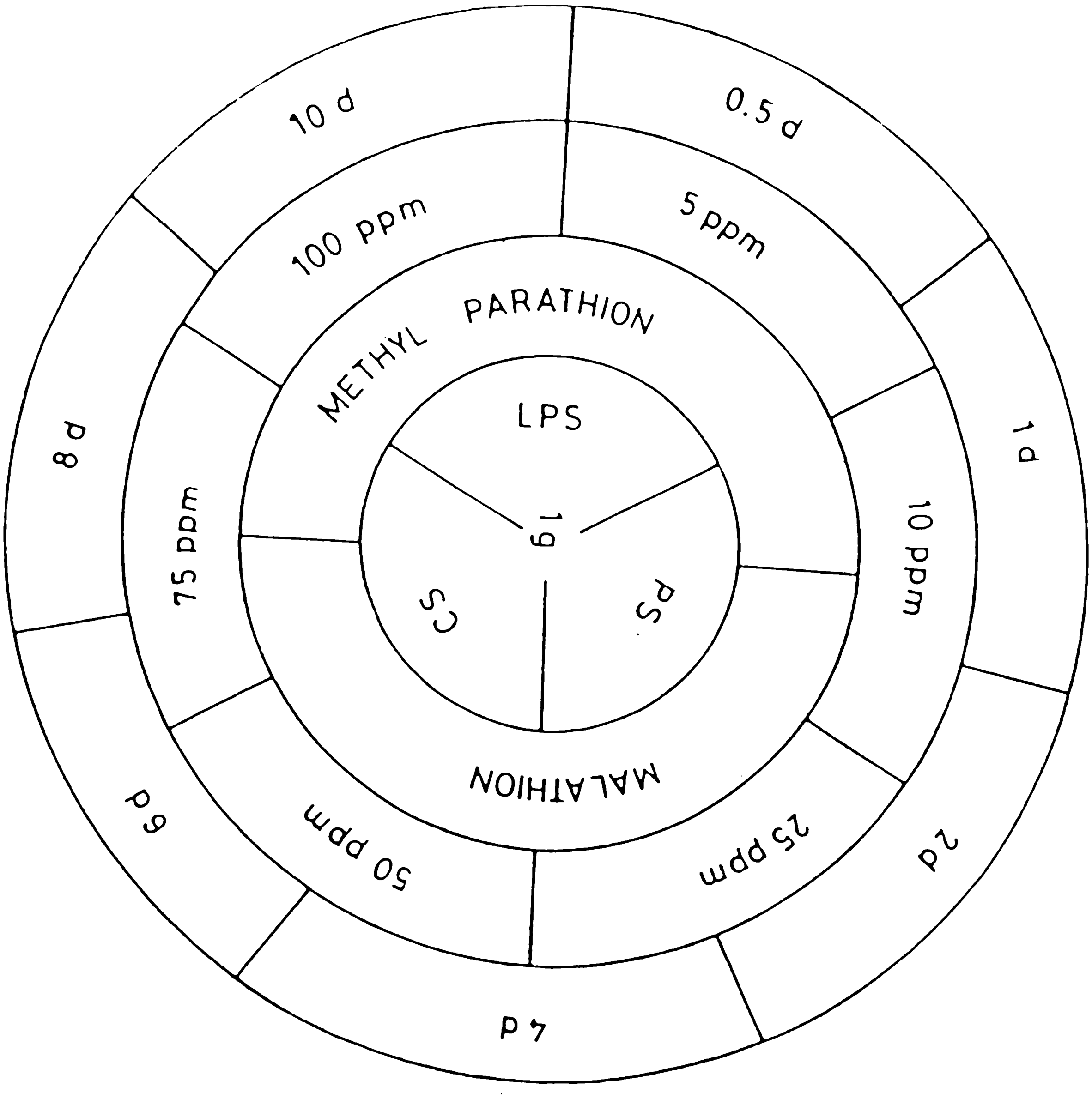


Figure 3.

Schematic representation of experimental design.

aside for 10 days, since the attainment of equilibrium required a period of 8 days. The experimental set up for the studies using varying pH and salinities were therefore, designed to assess only the 8-day equilibrium value. After this equilibration time the respective solutions were analysed for the pesticide residues and the amount of pesticide adsorbed by the sediment was computed (2.2.4). From this value the percentage adsorbed was calculated.

Sediment organic carbon content (SOC) was determined by the El-Wakeel and Riley (1957) method and sediment organic matter (SOM) was calculated by multiplying SOC by 1.72. Particle distribution studies were carried out by the combined sieving and pipette analysis method (Krumbein and Pettijohn, 1938). The sand-silt-clay percentages were plotted in a triangular diagram (Shepard, 1954) to find out the sediment types.

2.3. CHAPTER V —— TOXICOLOGICAL EFFECTS OF SOME BIOCIDES ON THE BIVALVE, *Villorita cyprinoides var. cochinensis*

2.3.1. Experimental animal

An estuarine bivalve, *Villorita cyprinoides var. cochinensis* was collected from Chilavannur, a site in the Cochin Backwater system , about 6 km southeast of the Cochin barmouth. They were brought to the laboratory in plastic containers with utmost care so as to provide minimum stress. These animals were inspected for any external sign of injury and only the healthy ones were retained for acclimation to the habitat salinity. They were then transferred and acclimatized to the test conditions (salinity = 10×10^{-3}; pH = 7.2 ± 0.03; DO = 7.8 ± 0.2 mg l^{-1} and temperature = 29 ± 2^{0}C).

The test animals were fed (with blue green algae, *Synechocystis salina*) during the acclimation period but were not fed during the course of the acute toxicity studies. Clams of the size 20 ± 2mm were chosen for the toxicity experiments.

2.3.2. Toxicants

Four biocides, namely Tributyl tin oxide (TBTO; FLUKA-99%), Endosulfan (35% emulsifiable concentrate), Malathion (50% emulsifiable concentrate) and Methyl parathion (50% emulsifiable concentrate) were used. One ml of TBTO was dissolved in 1000 ml acetone to obtain a 1000 ppm stock solution. Since the other three biocides were obtained as emulsifiable concentrates of varying strengths, and their stock solutions (1000 ppm) were prepared in triple distilled water as follows:

Endosulfan : 2.86ml (of 35% EC) in 1000ml
Malathion : 2ml (of 50% EC) in 1000ml
Methyl parathion : 2ml (of 50% EC) in 1000ml

Test concentrations of all the above biocides were prepared by appropriate dilutions of respective stock solutions.

2.3.3. Experimental design

After the one week acclimation period, ten animals each were transferred to separate 10 liter, prewashed, glass troughs. These were filled with water of experimental salinity and were dosed with the respective test concentrations of each biocide. Triplicate experiments were performed for each concentration of the biocides. Appropriate controls were maintained. For experiments with TBTO, the control was also dosed with the same quantity of acetone that had been used for making up the standard solutions of TBTO. The water was changed daily and the concentrations of the biocide in the experimental troughs were maintained at their respective levels throughout the exposure period (96h) by daily renewal of the test media. Mortality of the organisms was noted every 12h. The animals were considered dead, when the valve gap was at least ≈ 3 to 6 mm and when they showed no response to external stimulus. The 96h LC50 values were calculated from the cumulative mortality percentages in each concentration using the log-probit method (Litchfield and Wilcoxon, 1949). The following sublethal concentrations were chosen from the LC 50 values of each of the biocides:

Toxicants	LC50 value mg l^{-1}	Sublethal concentrations (mg l^{-1}) I (low)	II (medium)	III (high)
TBTO	0.080	0.006	0.008	0.010
Endosulfan	4.000	0.200	0.400	0.600
Malathion	6.000	0.400	0.600	0.800
Methyl-parathion	8.000	0.600	0.800	1.000

During the sublethal tests, no mortality was observed and animals were found to be actively filtering throughout the duration of the experiment. Separate sets of animals (control and experimentally dosed) from each of the troughs were withdrawn for the determination of metabolic rate and biochemical constituents as described in the respective sections (vide § — metabolic rate and biochemical constituents).

Metabolic rate/Oxygen consumption

Oxygen consumed by the whole animal was measured under controlled conditions before and after treatment with the different concentrations of the biocides. The oxygen content of the test medium was initially determined (i.e., before release of the clams). The experimental clams were exposed to a measured volume of the medium containing the different sublethal concentrations of each of the biocides. Control animals were maintained in a measured volume of the experimental medium devoid of the biocides. Gas exchange from the atmosphere was prevented by sealing the containers (conical flasks) with liquid paraffin. The duration of the experiment was 2h. For each of the test concentrations, three replicates were used. After the experiment, the test medium was siphoned out for the determination of the DO content. All DO measurements were made by the Winkler (Grasshoff, 1983) method. The oxygen consumed by the control and the experimental clams was calculated as follows:

Oxygen consumed by control/experimental clams $= O_w - O_c$

Decrease in the oxygen consumed by experimental clams $= OC_c - OC_e$

Where,

O_w = Oxygen in water without clams

O_c = Oxygen in the water in which control/experimental clams were kept

OC_c = Oxygen consumed by control clams

OC_e = Oxygen consumed by experimental clams.

After the experiments, soft tissues of each of the animals were scooped out, dried and weighed. The oxygen consumed was expressed as mg O_2 h^{-1} mg^{-1} dry weight of tissue. Percentage deviation from control values was used as an index for interpreting the experimental data.

Biochemical constituents

The biochemical constituents determined were glycogen, lipid and lactic acid. For each of these constituents six analyses were made by withdrawing six animals each from both the experimental as well as the control troughs. In order to compensate for any individual effects, the tissue of groups of six animals (chosen at random) were pooled together, homogenised and dried between the folds of filter papers. Each of these samples was then divided into six sub-samples, which were analysed for glycogen, lipid and lactic acid.

Estimation of glycogen

Glycogen was estimated by the method of Pflüger as modified by Hassid and Abraham (1957). A known weight of the tissue was warmed in potassium hydroxide solution (3 ml of 30%) for ≈ 30 minutes, and then allowed to react with 0.5 ml of saturated sodium sulfate solution. Glycogen was precipitated out from the solution by the addition of 95% ethanol and was purified by repeated precipitations. Subsequently, the purified glycogen was completely hydrolysed in dilute hydrochloric acid at 100^oC, for 3h and the resultant sugars were estimated spectrophotometrically at 520nm by the method of Heath and Barnes (1970). The concentration of glycogen in the sample was computed from the calibration graph obtained for standard glucose and was expressed as μg glucose. g^{-1} of tissue. The glucose value was converted to glycogen using the conversion factor 0.93.

Estimation of total lipid

The lipid content in the tissue sample was determined by the sulphophosphovanillin method described by Barnes and Blackstock (1973). A known weight of the homogenised tissue (≈ 0.5g) was dissolved in ≈ 3ml of chloroform-methanol (2:1) solution. The sample was mixed well and allowed to react with ≈ 2 ml of a 0.9% sodium chloride solution. It was then capped with non-absorbent cotton and allowed to stand overnight at 4-5°C for the separation of the layers. The mixture was transferred to a separatory funnel and the lower layer was drained out. Concentrated sulfuric acid (0.5ml) was then added and the solution was warmed for ≈10 minutes in a boiling waterbath. When cool, phosphovanillin reagent (5 ml) was added to the solution. It was shaken well, and the intensity of the colour was measured at 520nm. The lipid concentration was calculated from the standard graph prepared using cholesterol standards and was expressed as μg lipid. g^{-1} of tissue (as equivalent of cholesterol).

Estimation of lactic acid

Lactic acid was quantitatively converted to aldehyde by heating with concentrated sulfuric acid and was complexed with p-hydroxy diphenyl reagent (Barker, 1957).

The tissue of the organism was dried by pressing between the folds of filter paper and the wet weight was noted It was then homogenised with trichloro acetic acid (10 ml of 10%) and purified sea-sand (which had been collected from an environmentally clean beach area and washed thoroughly to remove the organic detritus). The mixture was centrifuged for ≈ 30 minutes, and the supernatant liquid was decanted and treated with copper sulphate solution (≈ 1 ml of 20%). This solution was then diluted to 10 ml, shaken well with calcium hydroxide powder (1 g) and kept aside for ≈ 30 minutes before being centrifuged again. Duplicate aliquots (1ml) of the supernatant liquid were pipetted out into clean test tubes and chilled after addition of 1 drop of 4% copper sulphate solution. This was then treated with sulfuric acid (≈ 3ml of 6%) and allowed to hydrolyse by placing in a waterbath. After cooling, the solution was treated with p-hydroxy diphenyl amine reagent (2 drops of 1.5% solution), kept aside for ≈ 30 minutes and the absorbance measured at 560 nm. Lithium lactate was used as the standard and the concentration of lactic acid was expressed as μg lactic acid. $g^{-1}$of the tissue.

All spectrophotometric readings were taken on a Hitachi 150-20 UV-VIS spectrophotometer. Cell to cell and blank corrections were employed for all sets of readings.

2.4. REAGENTS AND CHEMICALS

All reagents and chemicals used were BDH AR grade unless otherwise specified.

CHAPTER III

CHAPTER III

TEMPORAL AND SPATIAL DISTRIBUTION OF THE MAJOR PESTICIDES IN THE COCHIN ESTUARINE SYSTEM

3.1. INTRODUCTION

Synthetic agrochemicals have made an overwhelming contribution to the control of insects and pests in all areas of human involvement——agriculture, veterinary, public health house-hold etc. Nevertheless, the side effects resulting from the use of pesticides, have become increasingly severe. These compounds, being non-selective are poisonous not only to the target species but also to warm-blooded animals including man.

The use of chemicals for the control of insects and pests has a long history. In the past, chemical control of pests was accomplished through a relatively small number of inorganic copper or arsenic compounds or by naturally occurring insecticides such as Pyrethrum and Rotenone. The greatest revolution in the usage of pesticides came with the development of the synthetic organic pesticide, DDT. However, an early realization of its harmful side effects triggered concerted research efforts all across the globe into the synthesis of alternate organic pesticides and before long there was an exponential increase in the number of such compounds.

There are several hundred pesticides in common use at the present time. Of these, ubiquitous xenobiotic compounds, the persistent organochlorines get disseminated through drainage basins and storm-water channels and get accumulated to appreciable levels in estuaries and other coastal aquatic systems.

The use of these persistent organochlorines has serious implications, with regard to the environmental quality and human health in tropical areas than in temperate zones due to the specific climatic conditions and the socio-economic status of the people inhabiting in these areas.

Pesticides are biological, physical or chemical agents used to kill plants or animals that are harmful to man and these include acaricides, rodenticides, insecticides, nematicides, herbicides, molluscicides and fungicides. Of these, the insecticides are the most widely used. Since the publication of the "Silent spring" (Carson, 1962) which aroused widespread concern about the ill-effects of persistent, man made chemicals in the environment, the trend has been towards the development of more specific and more easily degradable compounds. Although the use of these insecticides has been severely restricted by legislation and a greater awareness of their ill-effects, these compounds are still regarded as the single major environmental hazard.

Prominent among the range of insecticides now in common use are the organochlorines (DDT, HCH, Cyclodienes, etc.,), the organophosphorus compounds (Malathion, Methyl parathion, Diazinon etc.,), carbamates (Carbaryl, Sevin, Aldicarb etc.,) and the pyrethroids (Fenvelerate, Resmethrin, Cyfluthrin etc.,).

This study has focussed attention only on the organochlorines and the organophosphates in view of their greater involvement in the aquatic environment. The physicochemical properties and structural formulae are presented in Table 1 and Figure 4 respectively.

Table 1.

Physical properties of some organochlorine and organophosphorus pesticides.

Name	Mol.Formula	Mol. Wt.	M.Pt/B.Pt(°C)	VP (mm Hg $\times 10^{-6}$ - 20 to 25°C)	Solubility	
					In water (ppm)	In organic solvent
α-BHC	$C_6H_6Cl_6$	290.8	159-160°	25.0	1.63	Soluble in benzene, chloroform
β-BHC	$C_6H_6Cl_6$	290.8	312	0.28	0.70	Soluble in benzene chloroform
γ-BHC	$C_6H_6Cl_6$	290.8	112.9	9.4-45.0	7.3-10.0	Soluble in acetone, benzene, chloroform
δ-BHC	$C_6H_6Cl_6$	290.8	139.5	-	0.90	Soluble in benzene, chloroform
p,p'-DDT	$C_{14}H_9Cl_5$	354.5	108.5	0.15	0.001-0.004	Very soluble in benzene,chloroform. Soluble in ethanol and hydrocarbons.
o,p'-DDT	$C_{14}H_9Cl_5$	354.5	73-75	5.5 at 30°C	-	Soluble in most organic solvents.
p,p'-DDE	$C_{14}H_8Cl_4$	318.1	88-90	6.5 at 30°C	-	Soluble in most organic solvents.
p,p'-DDD	$C_{14}H_{10}Cl_4$	320.1	112	1.0 at 30°C	-	Soluble in most organic solvents
α-Endosulfan	$C_9H_6Cl_6O_3S$	406.9	108-110	0<75°C	Insoluble	Soluble in most organic solvents
β-Endosulfan	$C_9H_6Cl_6O_3S$	406.9	208-210	0 < 75°C	Insoluble	Soluble in most organic solvents
*Malathion	$C_{10}H_{19}O_6PS_2$	330.3	156-157	1.6×10^{-4} (mbar)	145	Very soluble in organic solvents
Methyl Parathion	$C_8H_{10}NO_5PS$	263.2	35-36	1.3×10^{-4}	50-60	Very soluble in common organic solvents

(*colourless oily liquid)

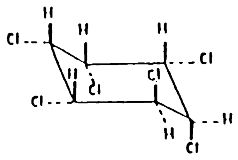
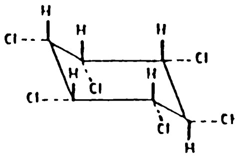
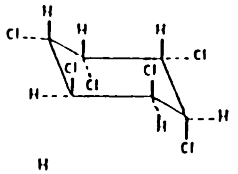
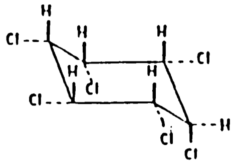
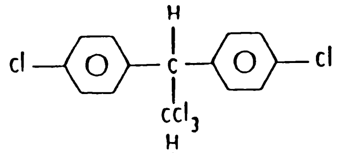
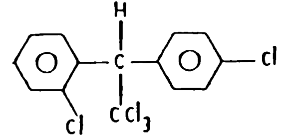
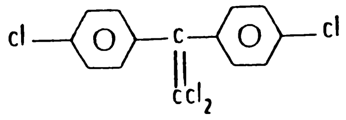
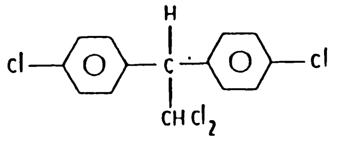
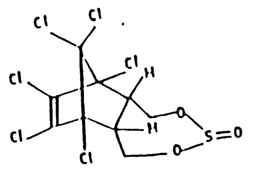
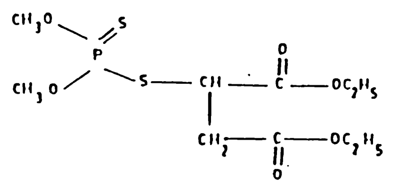
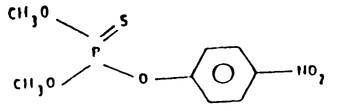
COMMON NAME	STRUCTURE	NOMENCLATURE
α-HCH		α- 1,2,3,4,5,6 - Hexachloro Cyclohexane
β-HCH		β- 1,2,3,4,5,6 - Hexachloro Cyclohexane
γ-HCH		γ- 1,2,3,4,5,6 - Hexachloro Cyclohexane
δ-HCH		δ- 1,2,3,4,5,6 - Hexachloro Cyclohexane
p,p'-DDT		1,1,1 - Trichloro-2,2-bis (P - Chlorophenyl)-ethane
o,p'-DDT		1,1,1 - Trichloro-2-o-Chlorophenyl-2-p-chlorophenyl-ethane
p,p'-DDE		1,1-Dichloro-2,2-bis (P-chlorophenyl)-ethylene
p,p'-DDD		1,1-Dichloro-2,2-bis (P-chlorophenyl)-ethane
Endosulfan		6,7,8,9,10,10-Hexachloro 1,5,5a,6,9,9a - Hexahydro -6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Malathion		S-(1,2-dicarbethyoxy ethyl) 0,0-dimethyl dithiophosphate
Methyl Parathion		0,0-dimethyl 0,P-nitropheyl phosphorothioate

Figure 4.

Structural formulae of pesticides.

3.1.1. Organochlorines

Organochlorine pesticides have been so widely dispersed in the environment that there is now no significant part of the biosphere which remains uncontaminated by them. Residues have been detected even in Arctic seals and Antarctic penguins besides a host of other marine animals (Jernelov, 1980).

The principal concern about these chemicals arises from their toxicity, persistence, and strong propensity to undergo bioaccumulation, all of which are a consequence of their properties such as high chemical stability, resistance to photochemical degradation, inflammability, high lipophilicity and miscibility with a large number of organic solvents and resistance to biological breakdown processes (Safe, 1982).

The DDT family of compounds has been used primarily for the control of mosquitoes and other insects. DDE is both a metabolite and a photochemical degradation product of DDT and has been observed to be environmentally persistent and to have a greater environmental impact than the original compound. DDE has an environmental half life of ten years or more and can therefore be persistent in the environment for several decades. DDD, another metabolite of DDT is itself often used as a pesticide under the name Rhothane and is frequently detected as a residue in human fat along with DDT and DDE.

The most extensive use of DDT now occurs in tropical developing countries like India partly because, it is cheap, persistent, appreciably effective and causes minimum harm to human beings in comparison with other insecticides and partly because possible substituents are much more expensive, less persistent and more toxic to human beings. Most of the harmful effects of DDT on nontarget organisms result from excessive and illegal use of these compounds in agricultural applications.

The excess finds its way into ponds, lakes, rivers and, through the estuaries, ultimately into the sea.

Several pesticides such as hexachlorocyclohexanes, the hexachlorocyclopentadiene-derived hydrocarbons and hexachlorobenzenes etc., have also been used for the control of pests. Of these, the hexachlorocyclohexanes (HCH) are the most widely used insecticide in India and other developing countries. The technical product is a mixture of several stereo- isomers α, β, γ, and δ (See Figure 4) of which the γ-isomer, Lindane, is responsible for the insecticidal action. HCH is persistent in the biosphere chiefly because of its β-isomer.

Endosulfan (Figure 4) is a broad spectrum insecticide and an acaricide widely used in agriculture and forestry throughout the world. This pesticide is applied in the agricultural fields as liquid in India, nearly 2,725 metric tons were applied in 1986-87 (Rajendran and Venugopalan, 1991) and due to its favourable environmental properties, formulated as emulsifiable concentrate and low toxicity to a large number of beneficial organisms, it is one of the few organochlorine pesticides that are still permitted to be used in crops. It is however quite persistent in the environment and consequently accumulates in the tissue of plants and animals.

Various instances of mass mortality of fish have been reported immediately following the massive application of pesticides. Schmitt and Winger (1980) cited several instances where spot and commercial fisheries had to be closed because of the occurrence of high pesticide residues in the fish. The brain mitochondrial OS-Mg^{2+}-ATPase of bluegill sunfish was almost totally inhibited by DDE and DDT (Koch, 1971). Campbell *at al.* (1974) reported that DDT significantly inhibited Na^{+}, K^{+}-activated ATPase in the kidney and gills of rainbow trout.

At sublethal concentrations, DDT induced locomotor hyper activity in the bluegill (Ellgaard *et al.*, 1977). Both β- and γ-isomers of HCH have been shown to produce severe hepatotoxic and nephrotoxic effects in young animals (Srinivasan *et al.*, 1984; 1988; Srinivasan and Radhakrishnamurthy, 1988 and Ravinder *et al.*, 1989). An elevated concentration of HCH, and a variety of consequent harmful effects were reported in birds/seals that fed on fish, exposed to pesticide.

3.1.2. Organophosphates

Organophosphates form the second generation pesticides which were developed as potential substitutes for the organochlorines. These being more easily degradable and consequently less persistent, are often preferred to organochlorines in conventional agricultural applications. The more prominent organophosphate insecticides include Malathion, Methyl parathion, Dichlorovos, Diazinon, etc. Although the organophosphorus based pesticides are highly toxic to humans, they are generally easily biodegradable and may also be susceptible to hydrolysis in aqueous solutions, particularly at higher pH values (Preston, 1989). They would, therefore, not be expected to exist for sufficient times in the marine environment as to cause either acute or sublethal effects unless introduced directly (Preston, 1989 have reported a spill of Methyl parathion in the Mediterranean by the sinking of a ship carrying them). They are readily metabolized (by mammalian enzymes) and rapidly degraded in the environment. Thus, any environmental damage caused by these compounds will tend to be localized in the area of application. The principal action of the organophosphate compounds is to inhibit the production of acetylcholine esterase.

3.2. Sources, transport and fate

Studies on transport and fate of chemicals provide information on the extent of partitioning and on the residence time of these chemicals in air, water and sediment as well as on their potential bioaccumulation in the biota. An integrated evaluation of this information would help to assess the potential impact of these chemicals in the environment. Widespread and unregulated (often unscientific) agricultural applications have resulted in an easy entry of the pesticides into the aquatic ecosystem. The persistence of the pesticides in the sediments and their long range transport in the atmosphere are two important factors that contribute to their accumulation in the aquatic environment.

Soils treated with pesticides act as traps releasing the residues to the atmosphere and also by contributing to the residue load in the surface runoff. As soils receive the major part of the globally used pesticides, and since the residues are transported eventually to the water bodies, persistence in the soils ultimately constitutes a threat to the aquatic environment. Apart from these, the level of pesticide residues in the open waters depends on many factors, such as the nature of the drainage basin, the extent of flow, the load of particulate matter, the level of productivity, the depth of the water body etc.

During application, a considerable quantity of the sprayed pesticide fails to hit the target area and is thus lost to the atmosphere as spray drift. Fox and Matthiessen (1982) have reported that the concentration of Endosulfan in water detected after spraying the delta, Botswana ranged from 0.5 to 4.2 μg l^{-1} which is equal to or more than the 96h LC50 values for many species of fish.

Pesticides that are transported to the aquatic environment are primarily of agricultural origin.But may also arise as effluents from manufacturing and formulating plants. The sources of contamination of the aquatic ecosystem would be categorized into nonpoint and point sources. The former refers to contamination of a widespread and diffuse nature as in the case of agricultural runoff, while the latter refers to a single source of contamination like effluents from a pesticide manufacturing plant. Agricultural practices like tilling also contribute significantly to the erosion of soils and hence to the transportation of pesticides to the aquatic environment. Significant quantities of pesticides are thus transported by sediment which constitutes an important nonpoint source. Urban sewage is yet another nonpoint source of pesticide residues to aquatic environment. Harper *et al.* (1977) have reported on the presence of organochlorine residues in domestic sewage effluents.

An estimate of a point source of pesticide residues in the aquatic environment could be obtained from the fact that fish collected near the point of entry of the effluent of pesticide manufacturing plant in Lake Orsjoen, Norway have been reported to contain 14 mg kg^{-1} ΣDDT (Kveseth, 1981). River sediments from Tombigbee-mobile river system were reported to contain ≈ 400 and 170 ppm of DDT and DDE respectively (Li, 1975).

Transport of pesticides from nonpoint or point sources to the aquatic environment is regulated chiefly by processes such as solubilization, precipitation, volatilization, leaching, surface erosion, ad(de)sorption, complexation etc. The greater the water solubility, the greater the mobility. For instance, Lindane, which has a higher water solubility, is more mobile than many other organochlorine compounds.

In general, pesticide adsorption to the soil or sediment is inversely related to the water solubility of that compound. Pesticide residues are subjected to a variety of dispersive forces/transformations in the environment as depicted in **Figure 5.** They may be transported through the aquatic system by diffusive or sorptive processes. There is also a continuous interchange of pesticides between sediments and water that is influenced by water movement, turbulence, temperature, pH, ionic strength etc.

In short, the aquatic environment acts as the ultimate sink for the diverse kinds of pesticides in use all over the globe. The residues that reach the hydrosphere are either concentrated in certain parts of the aquatic ecosystem or remain in solution for extended periods of time or are adsorbed onto the particulate matter, and thereby get deposited in the sediments.

India is the largest manufacturer of basic pesticide chemicals among the South Asian and African countries excluding Japan. Of the 126 odd pesticides that have been registered for use in agriculture and public health, about 125,000 tonnes of 57 technical grade pesticides are manufactured in India (DDT ≈ 10,000, BHC ≈ 50,000, Endosulfan ≈ 4,000, Malathion ≈ 20,000 and Methyl parathion ≈ 2,000; Indian Pesticide Directory, 1985).

Persistent chemical pesticides might be disseminated through drainage basins and might get accumulated in estuaries. Estuaries are often areas of high biological productivity (Odum and Heald, 1972 and Haedrich, 1983) and are ecologically significant as nursery areas for aquatic fauna, including important commercial seafood species (Williams, 1980). However, they are often exposed to heavy pollution loads due to their proximity to urban and industrial development and they act as sinks for river-borne fertilizers and pesticides originating from agricultural runoff and industrial discharges including

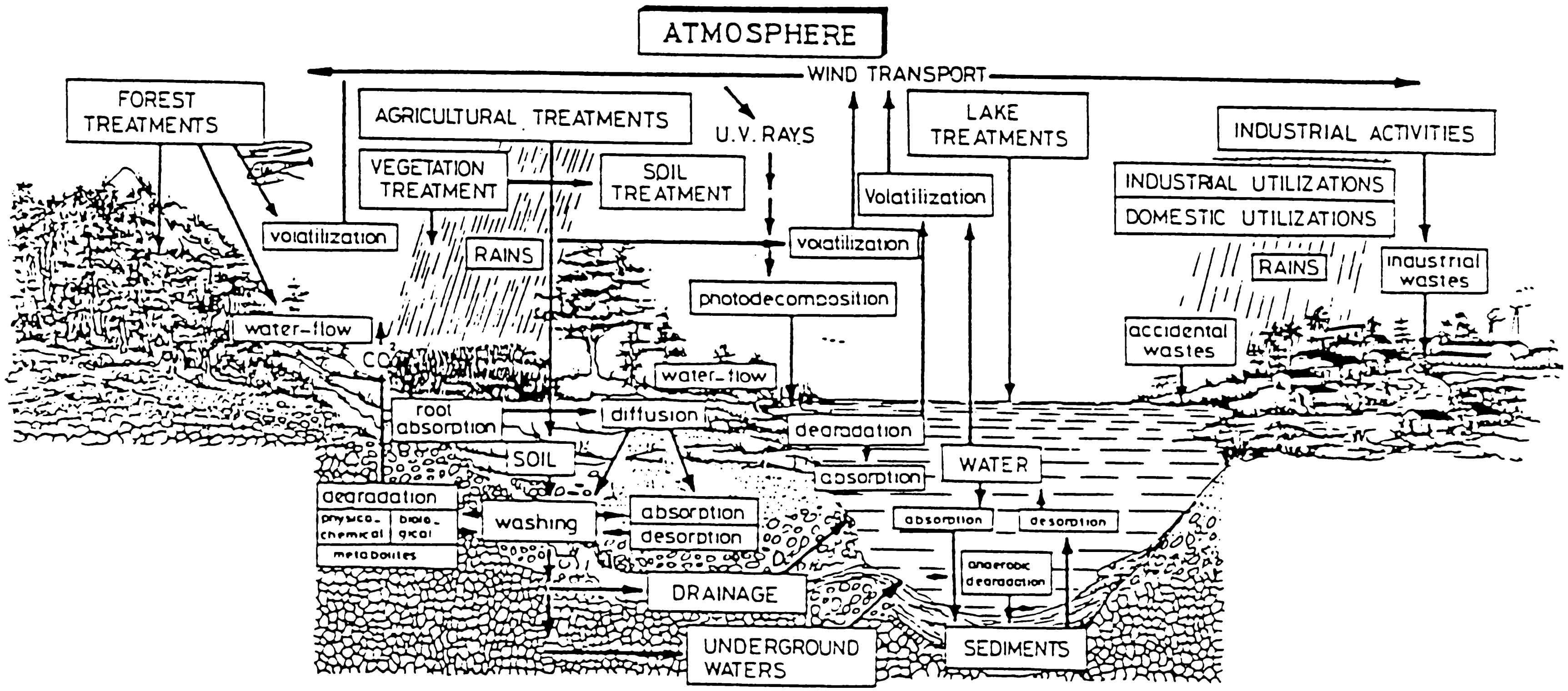


Figure 5.

Pesticide dispersion in the environment [Cited from *Aquatic ecotoxicology: Fundamental Concepts and Methodologies*, (Alain Boudou and Francis Ribeyre eds), CRC press, Florida, 1989) - modified for the present study].

heavy metals (Meade, 1981; Bieri *et al.*, 1982; Church *et al.*, 1984 and Nixon *et al.*, 1986).

The Cochin Estuarine System situated on the southwest coast of India is no exception to the above reality. Like many other major estuarine systems, the Cochin Backwater is also subjected to increasing human interferences and receives considerable amounts of pollutants from industrial units, domestic sewage works, storm-water channels, port area which handles large quantities of crude and refined petroleum products, coconut husk retting yards, fishery industries, etc. The cumulative effects of these pesticides on the coastal environment can be expected to be considerable as is evident from their accumulation in marine organisms (Kannan and Sen Gupta, 1987).

Thus the scale of ecological havoc being brought by pesticides is alarming. Besides, studies about the distribution of environmental persistence of dangerous chemicals are often very important to evaluate both their toxic actions and bioaccumulation. Although several investigations have been carried out on the occurrence, sources, pathways and effects in the environment of such ubiquitous chemicals, most of the reports relate to studies in the northern hemisphere (Duinker *et al.*, 1988; Boerde, 1989: Morris *et al.*, 1989 and Granby and Kinze, 1991). Studies on the residual levels of various chlorinated hydrocarbons in water samples of the southern Indian ocean seem to be confined to a few (Tanabe *et al.*, 1982; Ramesh *et al.*, 1990; Sarkar and Sen Gupta, 1983 and Takeoka *et al.*, 1991).

Among the man-made xenobiotic compounds, the persistent organochlorines like DDT,HCH and Endosulfan have been the most extensively studied from the view point of environmental quality and ecological impact. Only scanty data are available on the residual levels of various organochlorine pesticides in the seas around India (Kannan and Sen Gupta, 1987; Sarkar and Sen Gupta, 1986; 1987; 1988a and b and 1989).

Investigation on the residual effects of pesticides in the environment giving prominence to the water media are imperative when the pesticides tend to have a direct or indirect impact on human beings as well as on animals. Therefore, a systematic effort has been made to analyse the distribution of some of the more common pesticides carried by agricultural runoff released from the manufacturing plants into the Cochin Estuarine System. The complex processes that govern the overall distribution of these persistent organochlorines via large rivers to the estuaries,the addition of such chemicals to the estuaries directly, and the eventual movement of these chemicals into the associated bays are yet only poorly understood. Available previous reports on the status of pollution in the Cochin Estuarine System relate mainly to the trace metals and other organic pollutants (Sankaranarayanan and Rosamma Stephen, 1978; Paul and Pillai, 1983a and b; Nair, *et al.*, 1989; Nair *et al.*, 1990; Nair, *et al.*, 1991 and Ouseph, 1992).

Because of the increasing importance assigned to pesticide compounds like organochlorines and the tendency to deal with them as a generic group in regulatory actions, it is imperative that the nature and profile of their distribution be assessed quantitatively and rigorously.

In the absence of any authentic reports on the status of contamination of this estuary by these toxicants, there exists a lot of uncertainity about even the orders of magnitude in which these ecologically important compounds are present in the estuary. This investigation was therefore, designed to remedy this grave situation and generate baseline data in respect of these pesticides being freely used in this part of the Indian sub-continent.

3.3. RESULTS AND DISCUSSION

During the last decade there has been growing evidence of the considerable extent to which tissues of aquatic organisms of all trophic levels have become infected with potentially hazardous organic compounds, particularly chlorinated pesticides like DDT and its residues. In contrast, comparatively sparse information is available on the concentrations of these compounds in natural waters (including estuaries) and the published values differ by several orders of magnitude (Table 2).

Chlorinated pesticide residues have been reported from many rivers around the world; α-HCH, γ-HCH, Heptachlor, Heptachlor-epoxide, Aldrin, Dieldrin, Endrin and DDT were all encountered in water from the river Rhine (Greve, 1972). Four years after the ban of γ-HCH, its levels in river waters in Japan were reported to range from 46 to 307 $\mu g\ l^{-1}$ (Yamato *et al.*, 1975). The organochlorine residue levels (ng l^{-1}) recorded in the Danube river were γ- HCH: 69; p,p'-DDE: 22; p,p'-DDT: 42 (Sackmauerova *et al.*, 1977). Studies on Endosulfan concentrations at 81 locations in Germany, showed values which ranged between 10 and 6350 $\mu g\ l^{-1}$ (Murty , 1986).

In a survey of 11 watersheds in south western Ontario (Frank and Broun, 1981) DDT group compounds were observed to be present at appreciable concentrations in 93% of the 949 stream water samples analysed, DDE being the predominant component of DDT. Kellogg and Bulkely (1976) studied the seasonal variation in the concentration of Dieldrin in Des-Moines river in Iowa during 1971 to 1973 and found that the concentration in the water was low in early spring, increased rapidly thereafter and decreased in late summer, the average concentration ranging between 10 to 50 ng l^{-1} in 1971, 10 to 40 ng l^{-1} in 1972 and 1 to 31 ng l^{-1} in 1973.

Table 2.

Pesticide content (ng l^{-1}) at various geographical locations.

No.	Geographical location	Pesticide	Content	Reference
1.	California coast	DDT-residues	2-6	Cox (1971)
2.	Marseilles (upper layer)	ΣDDT	80-180	Harvey et al. (1973)
3.	California coast	DDT-residues	0.1	Williams and Robertson (1973)
4.	North Central Pacific	DDT-residues	0.01	Williams and Robertson (1973)
5.	N.W. Mediteranian coastal waters	Σ DDT	0.03-0.07	Elder (1974)
6.	Inshore and offshore water off California	ΣDDT	0.9 & 0.5	Pavlou et al. (1974)
7.	Sargasso sea, 1973	DDT-residues	0.06	Bidleman and Olney (1975)
8.	Inshore water of N.E.Pacific	ΣDDT (DDE + DDT)	0.3-1.1	Scura and Mc Clura (1975)
9.	Baltic Sea (Surface)	ΣDDT	5.2	Dawson and Riley (1977)
10.	N.W. Mediterranean coastal waters	γ-HCH	0.5-2.7	Elder (1976)
11.	Gulf of Mexico	DDT-residues	0.6	Giam et al. (1976)
12.	Sinve lugoons (Danube delta)	ΣDDT	32-426	Bilal and Illiescu (1984)
13.	North Atlantic	α-HCH	0.02-0.07	Wough et al. (1987)
14.	Mediterranean coast of Morocco	γ-HCH p,p'-DDE p,p'-DDD p,p'-DDT	0.072-0.091 0.022-0.087 0.023-57 0.025-0.425	Kessabi et al. (1988)
15.	Indian Ocean	ΣHCH	0.10-9.30	Sarkar and Sen Gupta (1988a)
16.	Arabian Sea	ΣHCH	0.06-9.40	Sarkar and Sen Gupta (1988b)
17.	Canadian polar shelf	α-HCH γ-HCH	7.1 0.8	Patton et al. (1989)
18.	Arabian Sea	ΣDDT	15.81-441	Sarkar and Sen Gupta (1989)
19.	Gulf of Mexico	γ-HCH	0.01-0.15	Theodor et al. (1989)
20.	Atlantic Ocean	ΣHCH (α and γ-isomers)	0.069-0.133	Fisher et al. (1991)
21.	Kerch Straits between the Azov and Black Sea	ΣDDT	8-20	Mee (1992)

Comparatively fewer reports pertaining to the levels of organophosphorus pesticides in the aquatic environment are a clear reflection of the easier biodegradability of these group of pesticides. While, high concentrations of Parathion (upto 1.9 mg kg^{-1}) were reported from pond mud (Nicholson *et al.*, 1962) which was found to increase with the progress of the agricultural season, Coppage *et al.* (1975) had reported on high Malathion values (as high as 500 μg l^{-1}) in the river waters in Texas. Miles and Harris (1978) recorded high levels of organophosphorus residues in the water of Holland Marsh in southern Ontario.

The present study was aimed at elucidating the distribution pattern of the common pesticide species encountered in the aquatic environment around the metropolis of Greater Cochin. The pesticide contaminants analysed in the study area belonged to the following four main structural groups:

- DDT-metabolites (o,p'-DDT; p,p'-DDE; p,p'-DDD)
- HCH-isomers (α-HCH, β-HCH, γ-HCH)
- A cyclodiene compound — Endosulfan and
- An organophosphorus compound — Malathion.

Stations, sampling techniques and analytical procedures have been described in Chapter II — Materials and Methods.

3.3.1. Distribution of total organochlorine pesticides (ΣDDT + ΣHCH + Endosulfan)

A well defined seasonal dependence of the pesticide concentration was observed at the six sampling sites and the concentration factors compared well with those reported for other water systems elsewhere (Castro *et al.*, 1988; Kessabi *et al.*, 1988; Phillips, 1989; and Montanes *et al.*, 1990).

The residual levels of total organochlorine pesticides are presented in Table 3 and in Figure 6. The generally high concentrations of organochlorines observed in the estuarine waters in contrast to the open ocean water (Table 2) could be attributed to the high lipophilicity and particle affinity of these persistent organics. Of these organochlorine pesticides, DDT and its derivatives were the most dominant forms. Total organochlorine concentrations during the premonsoon season were found to vary between 20.47 and 58.58 $\mu g\ l^{-1}$ with the maximum being observed at Station 2 and the minimum being observed at Station 4. During postmonsoon season, low concentrations (14.83 to 16.86 $\mu g\ l^{-1}$) were observed at Stations 3 and 4 while Stations 2 and 5 showed high values (39.03 and 40.21 $\mu g\ l^{-1}$ respectively). During monsoon, appreciable concentrations of pesticide were detected only at Stations 3 and 4. While Stations 1 and 6 remained pesticide-free zones, only traces of the pesticides were observed at Stations 2 and 5. It has been reported that surface wash off-from soil and airborne emissions are the most wide-spread sources of low level contamination of surface waters by pesticides (Risebrough 1969; Hasset and Lee, 1975; and Montanes *et al.*, 1990). The observed concentration trends are reflective of the monsoonal flushing of the aquatic system which is followed by a gradual perceptible increase in the pesticide concentrations through the postmonsoon to the premonsoon season. Stations 3,4 and 5 have been referred to as "sheltered sedimentary environment" (Pillai, 1989) which restricts the free flow of fresh water into the open sea and causes recirculation of the fresh water within this zone. As a consequence the suspended particulate and sedimentary matter are subjected to a much greater influence of re-suspension/ re-solubilisation. This is probably the reason for the higher values observed at the Stations in this zone.

Table 3.

Seasonal distribution of total organochlorine pesticides in Cochin Backwaters ($\mu g\ l^{-1}$).

Stations	Total Pesticides		
	Pre-monsoon	Monsoon	Post-monsoon
1	21.379	ND	19.956
2	58.582	0.034	39.031
3	29.765	16.887	14.830
4	20.471	44.444	16.856
5	33.635	2.468	40.212
6	23.241	ND	27.833

ND : Not detected.

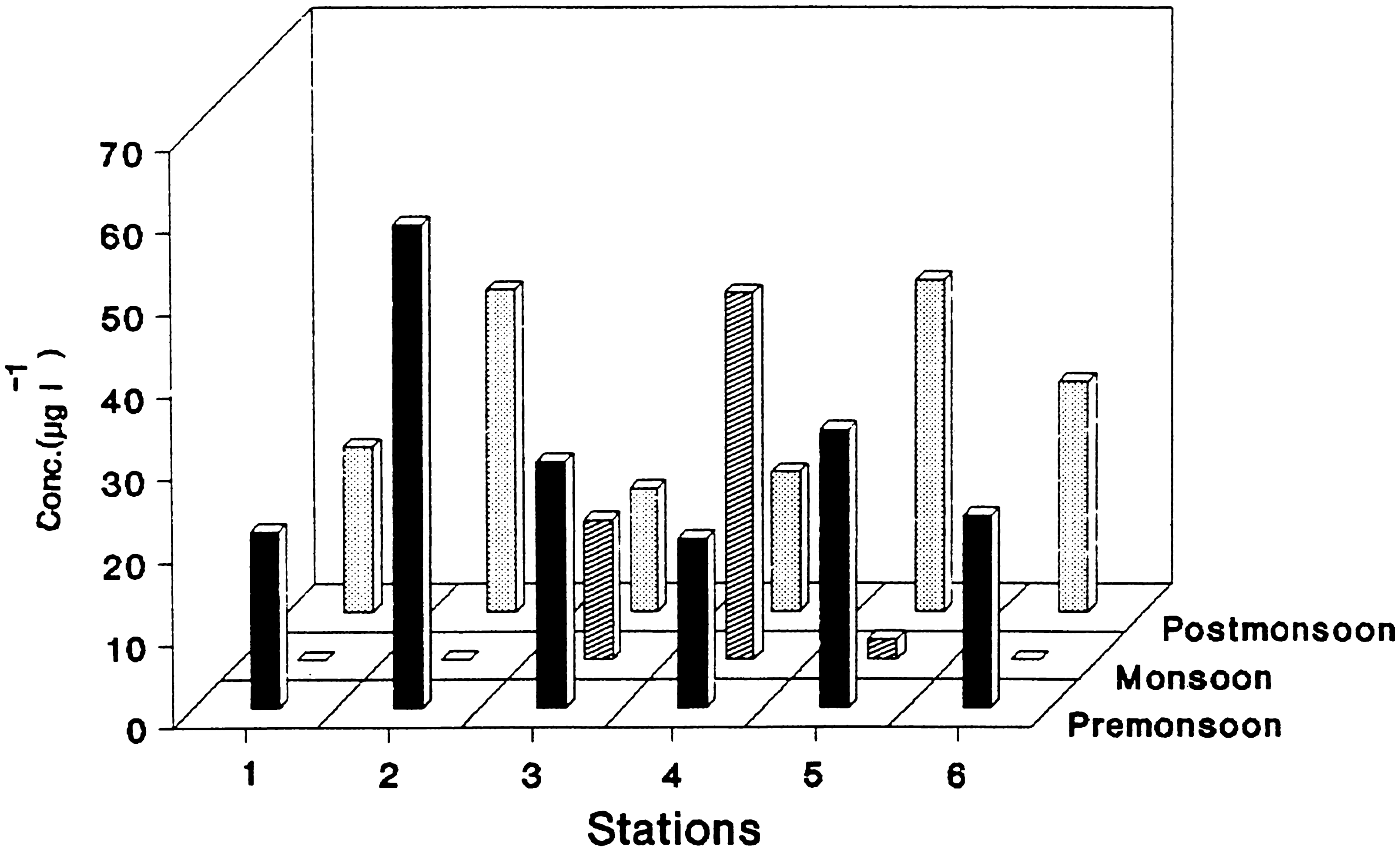


Figure 6.

Distribution profile of total organochlorine pesticides in Cochin Backwaters.

Distribution of ΣDDT

The concentration profile of ΣDDT is presented in Table 4 and in Figure 7. ΣDDT has been reported to be the most prominent among the pesticides commonly observed in coastal waters (Nimmo, 1985; Sloterdijk, 1985 and Sarkar and Sen Gupta, 1987) as has been observed in the present study also. ΣDDT concentrations observed at the different Stations varied between 0.03 and 55.42 $\mu g\ l^{-1}$. Sarkar and Sen Gupta (1989) have reported that DDT concentrations in the eastern Arabian sea ranged between 15.81 to 444 $ng\ l^{-1}$. Granby and Kinze, (1991) have reported that ΣDDT bioaccumulated into the blubber of harbour porpoises collected from Danish North sea and ranged between 0.7 to 61.4 $mg\ kg^{-1}$.

Studies conducted for assessing the level of pollution of pesticides from New Zealand coastal waters revealed that the concentrations of total DDT were fairly uniform (1.2-2.3 $\mu g\ \ell^{-1}$; Solly and Harrison, 1972). This uniformity, coupled, with the very low proportion of parent product (o,p' and p,p'-DDT) to the weathered products (o,p'and p,p'-DDE and DDD) suggested a diffuse source and/or prolonged redistribution throughout the harbour by the action tides, wind and biota. Similar levels of total DDT were observed in Galveston Bay, Texas and San Francisco Bay, California (Murray *et al.*, 1981; and Chapman *et al.*, 1987).

The maximum value during the premonsoon season (55.42 $\mu g\ l^{-1}$ was observed at Station 2, while that during the postmonsoon season (38.68 $\mu g\ l^{-1}$) was recorded at Station 5. The concentration observed at Station 2 during the postmonsoon season was only slightly less than that at Station 5. The ΣDDT loading was thus generally higher at Station 2 during the above two seasons.During monsoon ΣDDT was the only pesticide observed at any appreciable concentrations in the estuary and therefore

Table 4.

Seasonal distribution of organochlorine pesticides in Cochin Backwaters (μg l^{-1}).

Stations	Σ DDT			Σ HCH			Endosulfan		
	Pre-Monsson	Monsoon	Post-Monsoon	Pre-Monsoon	Monsoon	Post-Monsoon	Pre-Monsoon	Monsoon	Post-Monsoon
1	19.453	N.D	19.181	1.067	N.D	o.272	0.858	N.D	0.503
2	55.422	0.034	37.556	1.125	N.D	0.447	2.035	N.D	1.028
3	27.991	16.428	14.349	0.482	0.139	0.275	1.292	0.319	0.206
4	18.992	44.269	16.138	0.341	0.175	0.242	1.138	N.D	0.477
5	31.514	2.455	38.682	0.400	0.013	0.518	1.721	N.D	1.013
6	22.168	N.D	26.663	0.237	N.D	0.321	0.835	N.D	0.849

ND:Not detected

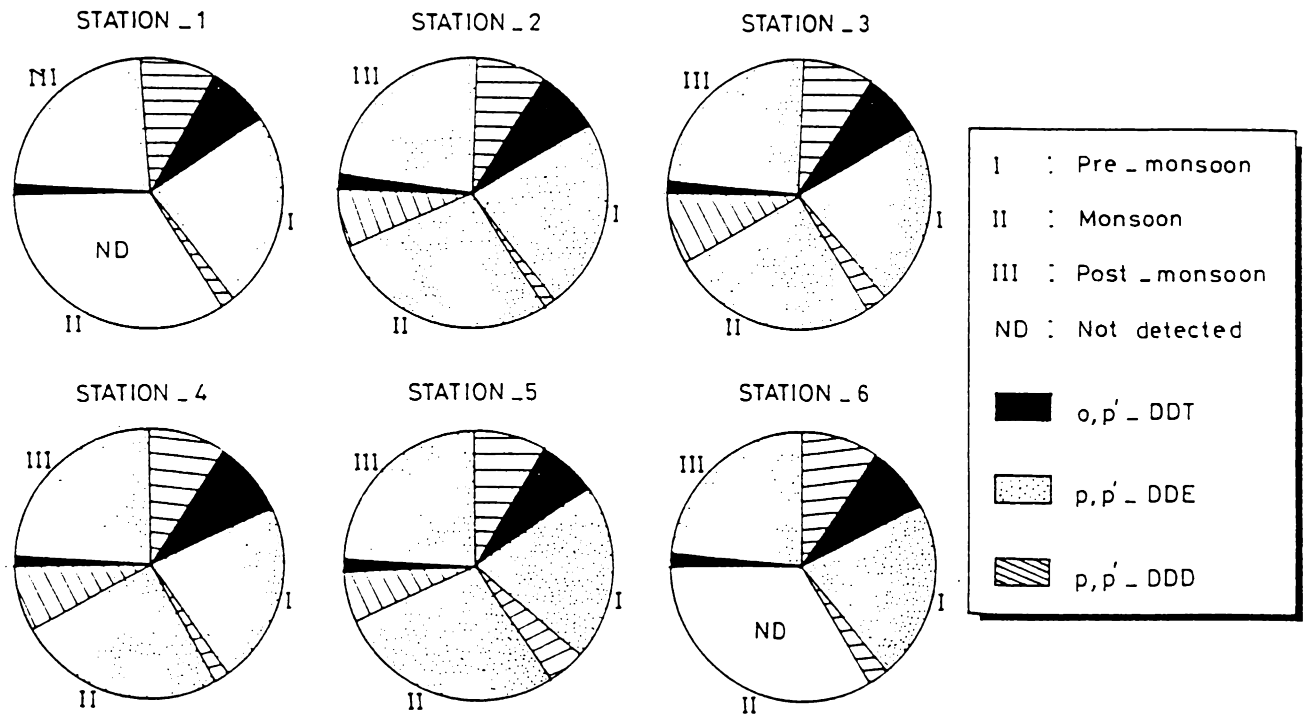


Figure 7.

Distribution profile of Σ DDT in Cochin Backwaters.

the distribution profile of total organochlorine pesticides discussed earlier (3.3.1.) largely reflects the seasonal variations in ΣDDT. The high concentrations of ΣDDT observed during the monsoon at Stations 3 and 4 could be a consequence of the relatively high dissolved organic carbon content reported earlier (Sujatha and Chacko, 1991) for these Stations. Odum *et al.* (1969) and Simmons and Kotz (1982) have reported that the organic matter in the streams made a significant contribution to the solubilization of the organochlorine and the polybrominated biphenyl compounds.

The effect of the industrial effluents, which results in a grossly elevated ΣDDT level at Station 2 during premonsoon period is moderated by increased fresh water influx during the other seasons. The high concentrations observed at Station 5 during both the premonsoon and postmonsoon periods are only a reflection of the agricultural land runoff accumulating at this Station. The higher content of silt and clay characteristics of the Stations 2 to 5 (Nair, *et al.*, 1991) accounts for the generally higher ΣDDT concentrations observed at these Stations compared to Station 1. The high concentrations of ΣDDT and its metabolites observed in these estuarine waters could be attributed to the remnants of the chemical that might have ultimately found their way into the estuary from the large quantities that had been used in this part of India for the National Malaria Eradication Programme. Similar high residual values of ΣDDT metabolites have been reported in other environmental species as well (Falandysz, *et al.*, 1988).

Distribution of o,p'-DDT

The distribution pattern of o,p'-DDT is presented in **Table** 5 and Figure 8. While o,p'-DDT was not detected at any of the Stations during the monsoon period, its values were generally higher during the premonsoon season than during postmonsoon

Table 5.

Seasonal distribution of DDT metabolites (o,p'-DDT, p,p'-DDE and p,p'-DDD) in Cochin Backwaters (µg l^{-1}).

Stations	o,p'-DDT			p,p'-DDE			p,p'-DDD		
	Pre-Monsoon	Monsoon	Post-Monsoon	Pre-Monsoon	Monsoon	Post-Monsoon	Pre-Monsoon	Monsoon	Post-Monsoon
1	0.776	N.D	0.588	13.957	N.D	13.401	4.719	N.D	5.191
2	1.838	N.D	1.633	39.674	0.026	26.658	13.910	0.008	9.265
3	1.859	N.D	0.564	19.505	12.073	10.335	6.627	4.355	3.450
4	0.751	N.D	0.514	12.796	33.330	11.573	5.444	10.939	4.051
5	3.589	N.D	1.375	20.950	1.963	28.249	6.975	0.493	9.058
6	1.541	N.D	1.005	15.166	N.D	18.825	5.462	N.D	6.833

ND: Not detected.

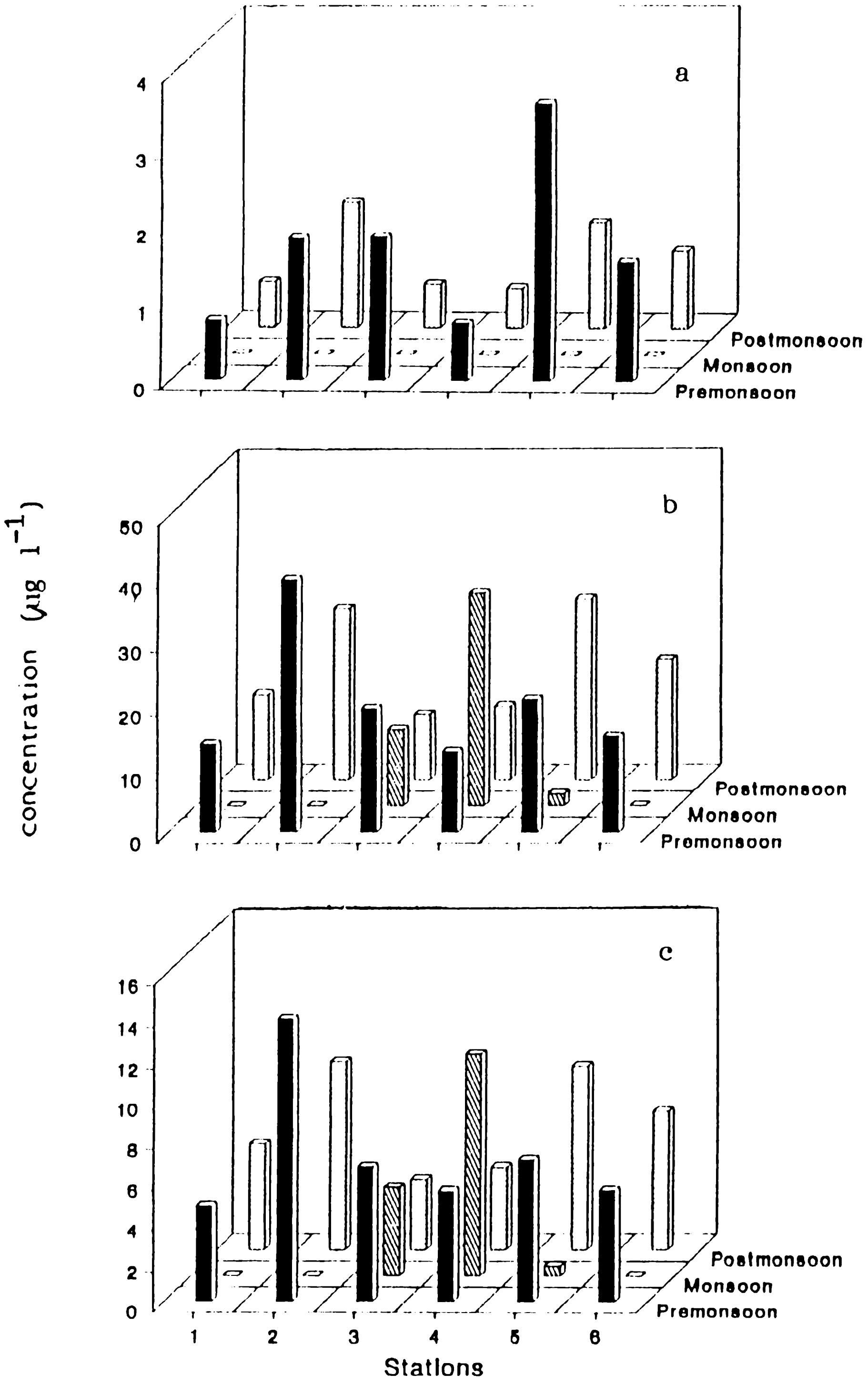


Figure 8.

Distribution profile of o,p'-DDT(a), p,p'-DDE(b) and p,p'-DDD(c) in Cochin Backwaters.

season and fluctuated between 0.51 (Station 4-postmonsoon) and 3.58 (Station 5-premonsoon) μg 1^{-1}. Stations 2 and 3 recorded similar values of o,p'-DDT during premonsoon; however, during postmonsoon the o,p'-DDT concentration was reduced to a third of its premonsoon value at Station 3. The premonsoon concentration of o,p'DDT at Station 5, besides being the highest concentrations of this metabolite in the area of investigation, was also the highest concentration observed for this metabolite during the other seasons over which the investigation was carried out.

o,p'DDT was exceptional among all other DDT metabolites in that while it recorded the lowest concentration at all Stations during pre- and postmonsoon, it remained totally undetectable during the monsoon period.

Distribution of p,p'-DDE

Most of the water samples collected during this study contained detectable levels of p,p' DDE. It was observed to be the prominent DDT derivative and the predominant component of organochlorine pesticide in water, accounting for over 50% of total pesticide in the water of the Cochin estuary (Table 5 and in Figure 8). Frank and Broun (1981) reported that p,p'-DDE was the predominant component of ΣDDT and represented ≈ 70 and 45% for the first and second years respectively of the total DDT lost. Generally high values have been reported for p,p'-DDE residues in other aquatic systems as well (Buhler *et al.*, 1975; O'Shea *et al.*, 1980 and Britt and Howard , 1983).

From Stations 1 to 6, p,p'-DDE values generally alternated between high and low concentrations during pre-and postmonsoon. The postmonsoon period as compared to the premonsoon period generally brought about a reduction in the p,p'-DDE values except at Station 5 and 6, where a reverse trend was observed.

During monsoon, p,p'-DDE was present at appreciable levels at Stations 3 and 4 while they were either nondetectable or present only at very low levels at the other Stations. The maximum p,p'-DDE concentration (39.67 $\mu g\ l^{-1}$) was recorded at Station 2 during the premonsoon period, the preceding high value of 33.33 $\mu g\ l^{-1}$ was observed at Station 4 during the monsoon period. The high concentrations of p,p' DDE content at Station 2, which is located in the vicinity of a pesticide manufacturing concern is probably an indication of the extent of pollution caused by this industry. The observed higher proportion of p,p'-DDE in ΣDDT may be a reflection of the greater persistence of p,p'-DDE (EPA, 1980) and is to be expected in the estuarine waters which provide a longer period for the disintegration of pollutants.

DDT is transformed into DDE through a dehydrochlorination process (Walker, 1975) and it is the most abundant among the DDT metabolites disposed in the aquatic environment and accumulated in the tissue of animals.

Distribution of p,p'-DDD

The overall distribution profile of p,p'-DDD (Table 5 and in Figure 8) residue was more or less identical to that of p,p'-DDE. The p,p'-DDD metabolite was present at a maximum concentration of 13.91 $\mu g\ l^{-1}$ at Station 2, during the premonsoon period. This concentration was nearly twice the p,p'-DDD levels recorded at all other Stations during the same period and also higher than the values recorded at most of the Stations during the postmonsoon period. The influence of seasonal changes are also borne out by the fact that the apex Stations (1 and 6) remained as p,p'-DDD free zones during monsoon period.

Among the various factors which regulate the proportionate concentration of the different metabolites of DDT, microbial degradation has a prominent place.Highlighting one such study (Johnsen, 1976), it has been inferred that anaerobic metabolization of p,p'-DDT in the biota would lead to the formation of p,p'-DDE, whereas p,p-DDE would be formed under aerobic conditions.

Distribution of ΣHCH

The distribution profile of ΣHCH is presented in Table 4 and in Figure 9. The α and δ-HCH were observed to be the important HCH residues found in the study area. The distributional characteristics of ΣHCH resembled that of ΣDDT and the weighted average concentrations of ΣHCH ranged between 0.013 and 1.125 μg l^{-1} with the maximum being recorded at Station 2, during the premonsoon period. Hexachlorocyclohexane detected in different regions of the Indian Ocean was observed to be mainly composed of the three isomers: α,β and γ-HCH, of which α and γ-isomers contributed around 45-48% whereas, the β-isomer only 5-10% of the total HCH concentrations (Sarkar and Sen Gupta , 1987; 1988 a and b). The HCH isomer loads in Danish North Sea and Baltic waters were low, ranging from 0.06 to 3.20 mg kg^{-1} (Granby and Kinze, 1991).

In the monsoonal period, ΣHCH isomers were not detectable at Stations 1, 2 and 6 but were present at comparatively high concentrations at Stations 3 and 4 during the monsoon period. Stations 2, 5 and 6 reflected higher values during the postmonsoon period. Reports on HCH-isomer studies carried out on the west-coast of India (Sarkar and Sen Gupta, 1987; 1988a and b and 1989; Tanabe *et al.*, 1982 and 1983), reported overall concentrations which ranged between 0.44 to 200 ng g^{-1} (coastal sediment), 0.06 to 9.40 ng l^{-1} (Arabian sea) and 0.10 to 9.30 ng $^{-1}$ (Southern Indian Ocean). Gaul (1984) found higher γ-HCH concentrations from the inner Danish seawater than in the rest

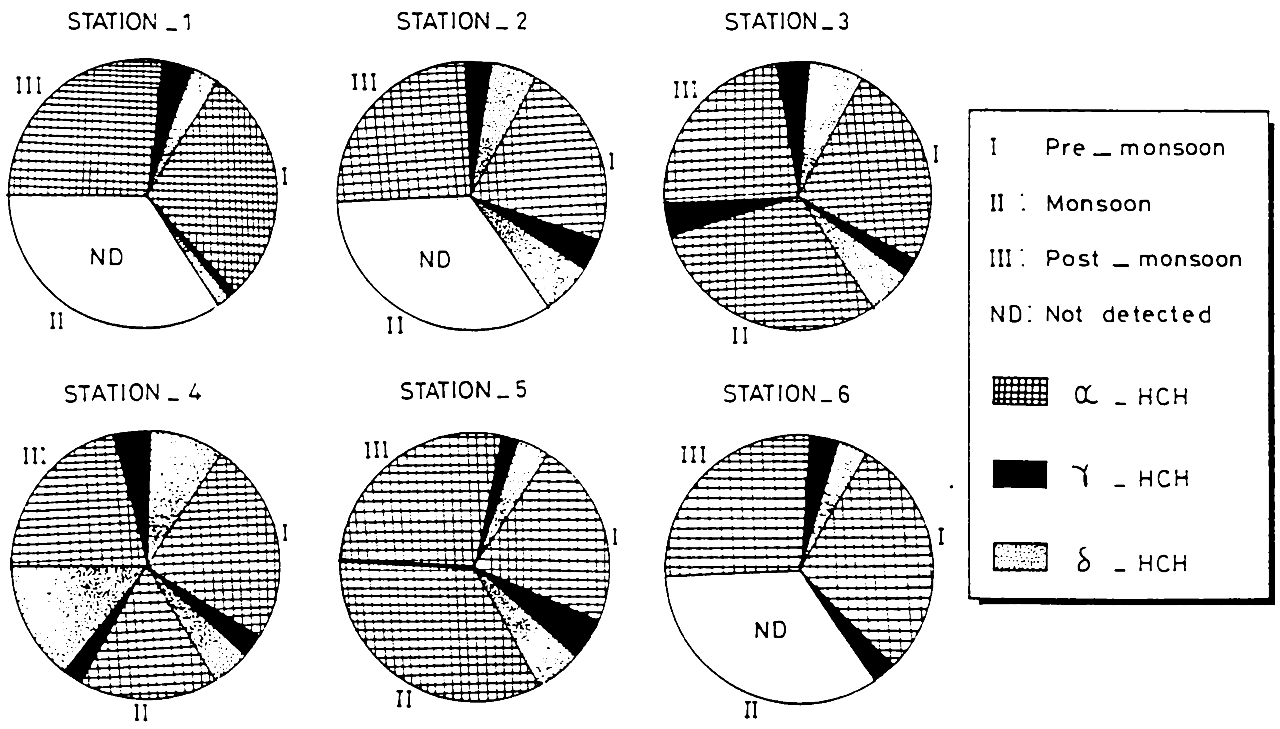


Figure 9.

Distribution profile of ΣHCH in Cochin Backwaters.

of the Baltic sea and suggested that this might be due to the use of Lindane (>99% γ-HCH) as an insecticide in Danish agriculture. Furthermore, coastal rainwater in Denmark showed relatively high concentrations of γ-HCH during spring and summer, probably because of the use of Lindane (Hilbert *et al.*, 1990). In Rhine river samples, α-HCH ranged from 0.06 to 0.22 $\mu g\ l^{-1}$ and γ-HCH from 0.04 to 0.13 $\mu g\ l^{-1}$ (Wegman and Greve, 1978).

α-HCH

α-HCH was found to be present at appreciable concentrations throughout the study area which included the industrial belt of this positive estuary. It also formed the highest percentage (Table 6 and Figure 10) of all isomers of the HCH family and its distributional characteristics were influenced by both regional as well as seasonal variations. A similar trend, (the α-isomer being about ten times higher than γ-isomer) was reported by Granby and Kinze (1991). Predominance of α-HCH may be related to the rapid photo- and bacterial degradation of γ-isomer to the more stable α-isomer (Benezet and Matsumura, 1973 and Malaiyandi and Shah, 1984). Technical BHC formulations characteristically consist of 70% of the α-isomer.

A definite pattern seemed to evolve out of the distribution profile. The α-HCH concentrations were invariably the highest at all Stations during premonsoon except at Stations 5 and 6 where the premonsoonal levels were exceeded by the postmonsoon values. Lowest concentrations were observed at the respective Stations during the monsoon period, while the postmonsoonal concentrations were generally intermediate between the former two.

The maximum concentration (1.002 $\mu g\ l^{-1}$) at Station 1 and the next higher concentration at Station 2 (0.793 $\mu g\ l^{-1}$) were

Table 6.

Seasonal distribution of HCH-isomers (α, β and γ) in Cochin Backwaters ($\mu g\ l^{-1}$).

Stations	α - HCH			γ - HCH			δ - HCH		
	Pre-Monsoon	Monsoon	Post-Monsoon	Pre-Monsoon	Monsoon	Post-Monsoon	Pre-Monsoon	Monsoon	Post-Monsoon
1	1.002	N.D	0.215	0.020	N.D	0.028	0.045	N.D	0.029
2	0.793	N.D	0.341	0.118	N.D	0.036	0.214	N.D	0.071
3	0.375	0.124	0.192	0.032	0.015	0.029	0.075	0.001	0.053
4	0.262	0.083	0.153	0.033	0.012	0.028	0.045	0.080	0.060
5	0.275	0.013	0.428	0.058	N.D	0.033	0.067	N.D	0.057
6	0.217	N.D	0.256	0.019	N.D	0.033	0.001	N.D	0.032

N.D: Not detected

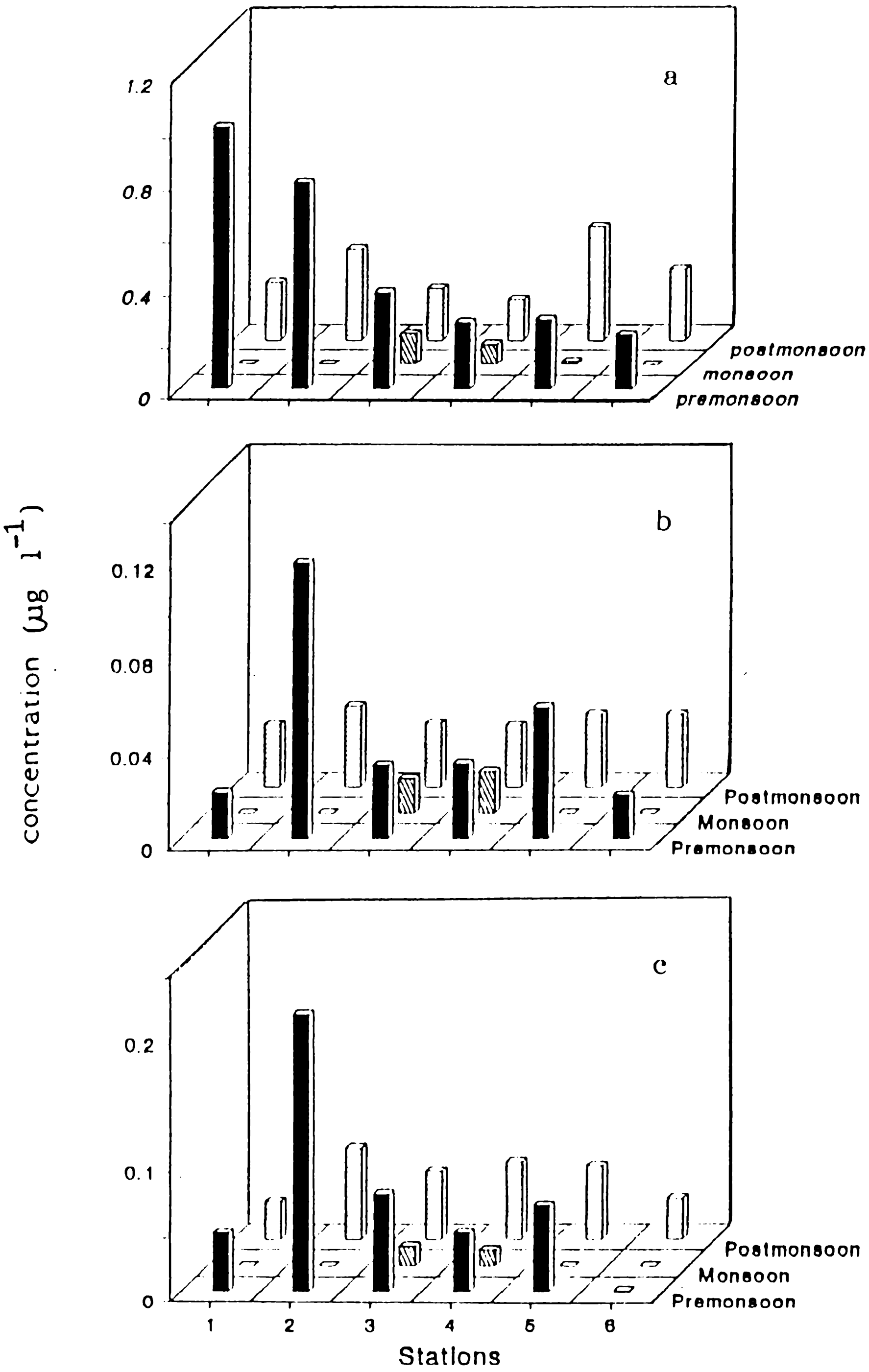


Figure 10.

Distribution profile of α-HCH(a), γ-HCH(b) and δ-HCH(c) in Cochin Backwaters.

observed during premonsoon period. The premonsoon concentrations of α-HCH decreased exponentially from Stations 1 to 6, agricultural and industrial discharges being responsible for the higher inputs. However, the postmonsoonal concentrations increased from Station 1 through Station 2 to Station 5 with Stations 3,4 and 6 exhibiting intermediate concentrations. While the highest postmonsoonal level (0.428 µg l^{-1}) of α-HCH was observed at Station 5, the highest monsoonal concentration (0.124 µg l^{-1}) was recorded at Station 3.

Freshwater is known to contain a many fold increase in the concentrations of humic acids than saline water. Therefore, during periods of increased fresh water flow, the possibility of a greater accumulation of pesticides in particulate matter, by adsorption onto the humic acids, is increased several fold. The fluvial dynamics serve to dislodge the pesticide from its association with organic matter (whose content is incidentally higher at Stations 3, 4 and 5) and is probably responsible for the marginally higher concentrations observed at these Stations. Agricultural and industrial runoff also account for the higher amounts of α-HCH isomers observed at Stations 3,4 and 5. Moreover the "sheltered sedimentary environment" (as mentioned earlier) of these three Stations may also contribute to the elevation of the pesticide content.

γ-HCH

Seasonal and spatial variations of Lindane are presented in Table 6 and in Figure 10. Lindane concentrations were generally low at all the Stations and ranged between 0.012 to 0.118 µg l^{-1}. During monsoon, Lindane levels were invariably the lowest, it being nondetectable at all Stations except at 3 and 4 which was a reflection of localized agricultural inputs.During postmonsoon, Lindane concentrations did not show any appreciable variations (average value: 0.031 µg l^{-1} ± 0.003), thus neglecting any regional influences. During premonsoon, no regular pattern emerged out of the concentrations observed. Very similar values were observed at Station 1 and 6 as well as at 3 and 4. The proximity of Station 2 to the pesticide manufacturing unit and the large amount of urban runoff/municipal sludge and sewage that characterized Station 5 account for the higher concentrations of Lindane recorded at these Stations.

Presence of Lindane ($\mu g\ g^{-1}$ range) in sewage sludge collected from sewage treatment works in the U.K. has been reported (Mc Intyre and Lester, 1982) and attributed to its wide-spread use in wood preservation and insecticidal formulations, in addition to its use in agriculture. Lindane was detected at concentrations in the range of 0.01-0.15 $\mu g\ l^{-1}$ in the seawater filtrate sample analyzed from the open ocean waters off the U.S. east coast and in the Gulf of Mexico (Theodor *et al.*, 1989).

δ-HCH

δ-HCH concentrations are presented in Table 6 and in Figure 10. Premonsoonal concentrations were higher than postmonsoonal, while monsoonal levels were the lowest. The only exception was the high monsoonal concentrations recorded at Station 4. Station 2 recorded a pronounced δ-HCH value during premonsoon which was about 2-3 times higher than that observed at the other Stations. The next higher value was the unusually elevated monsoonal level detected at Station 4. During monsoon, all other Stations were more or less free of δ-HCH. Postmonsoonal concentration of δ-HCH was maximum at Station 2. The δ-HCH distribution pattern thus followed the trends observed for the other HCH-isomers.

Endosulfan

Table 4 and Figure 11 which depict the distribution profile of Endosulfan in the Cochin estuarine waters, prominently portray a seasonal influence. The values ranged between 0.21 to 2.04 $\mu g\ l^{-1}$ with the premonsoonal loading being always higher than the postmonsoonal one. Throughout the monsoonal season, the cyclodiene pesticide remained undetectable at all Stations except at Stations 3. Station 2 recorded maximum concentrations both during pre- and postmonsoon, while Station 5 recorded immediately lower values. The pesticide manufacturing concern and the urban influence were responsible for this feature. Postmonsoonal effects were found to reduce Endosulfan concentrations to more than half of its premonsoonal levels. Station 6, however, remained a conspicuous exception to this behaviour. During the monsoon season, Station 3 alone had recorded the presence of Endosulfan content (0.32 $\mu g\ l^{-1}$) and this could be attributed to the prominent agricultural runoff which characterized the sampling site. A similar cyclodiene compound, Dieldrin, has been reported to have an uniform distribution on ranging from 0.3 to 0.5 ng g^{-1} in the Manukau Harbour, New Zealand (Fox *et al.*, 1988). In Rhine and Mars rivers the Endosulfan concentrations were 0.81 and 0.09 $\mu g\ l^{-1}$ respectively (Wegman and Greve, 1978).

3.3.2. Organophosphorus Pesticide - Malathion

The distribution profile of Malathion in the study area is presented in Table 7 and in Figure 12. Premonsoonal values were invariably higher than postmonsoonal concentrations. During monsoon, Malathion was detected only at Station 3. The maximum concentration was recorded at Station 2 during premonsoon and the minimum value was recorded at Station 1 during postmonsoon. During premonsoon, Stations 3 and 4 as well as Stations 5 and 6 recorded very similar values whereas during postmonsoon,

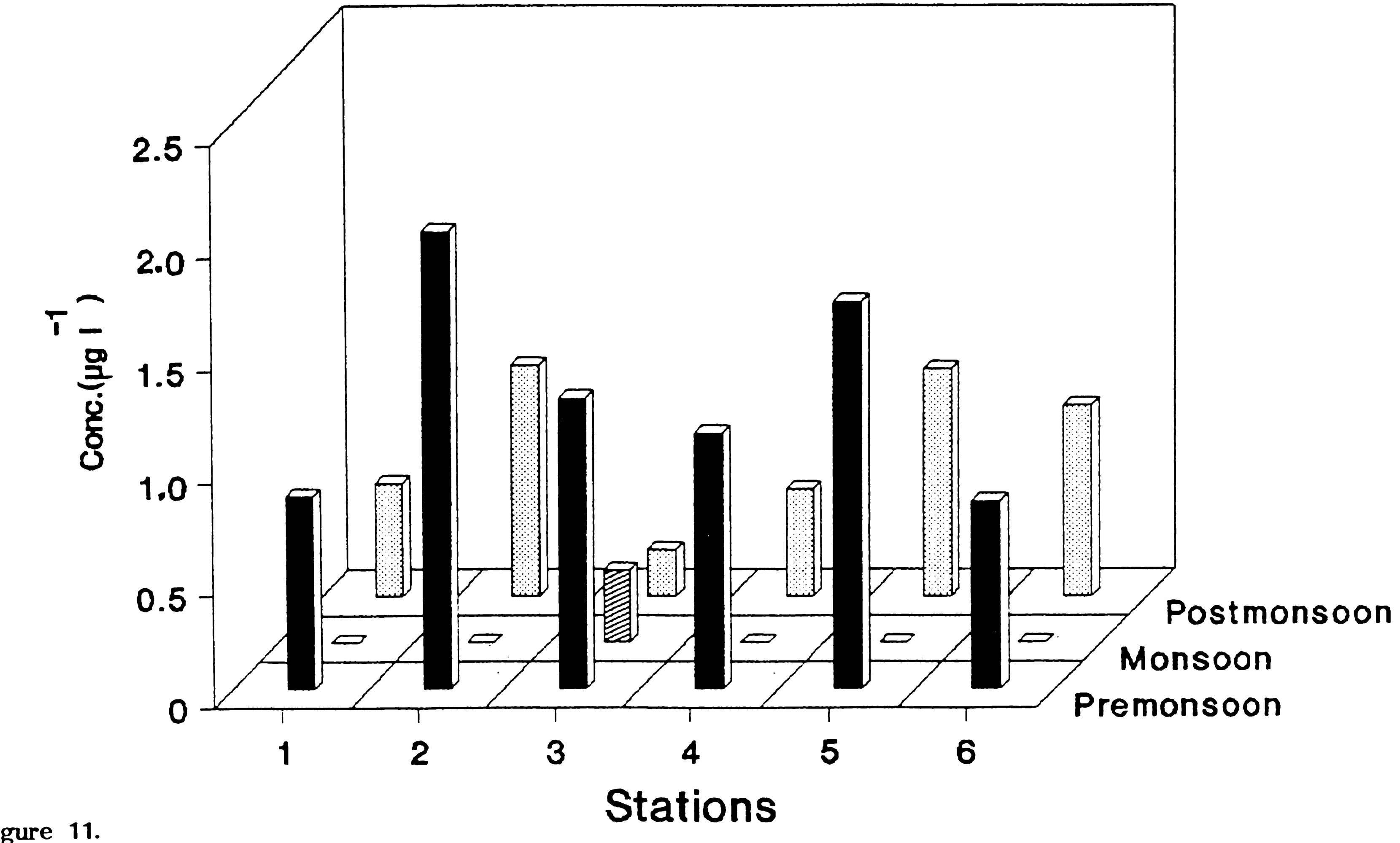


Figure 11.

Distribution profile of Endosulfan in Cochin Backwaters.

Table 7.

Seasonal distribution of Malathion in Cochin Backwaters (µg l^{-1}).

Stations	Malathion		
	Premonsoon	Monsoon	Postmonsoon
1	2.533	ND	1.373
2	13.013	ND	4.269
3	4.689	1.792	1.475
4	4.439	ND	2.649
5	3.367	ND	2.822
6	3.346	ND	2.197

ND = Not Detected

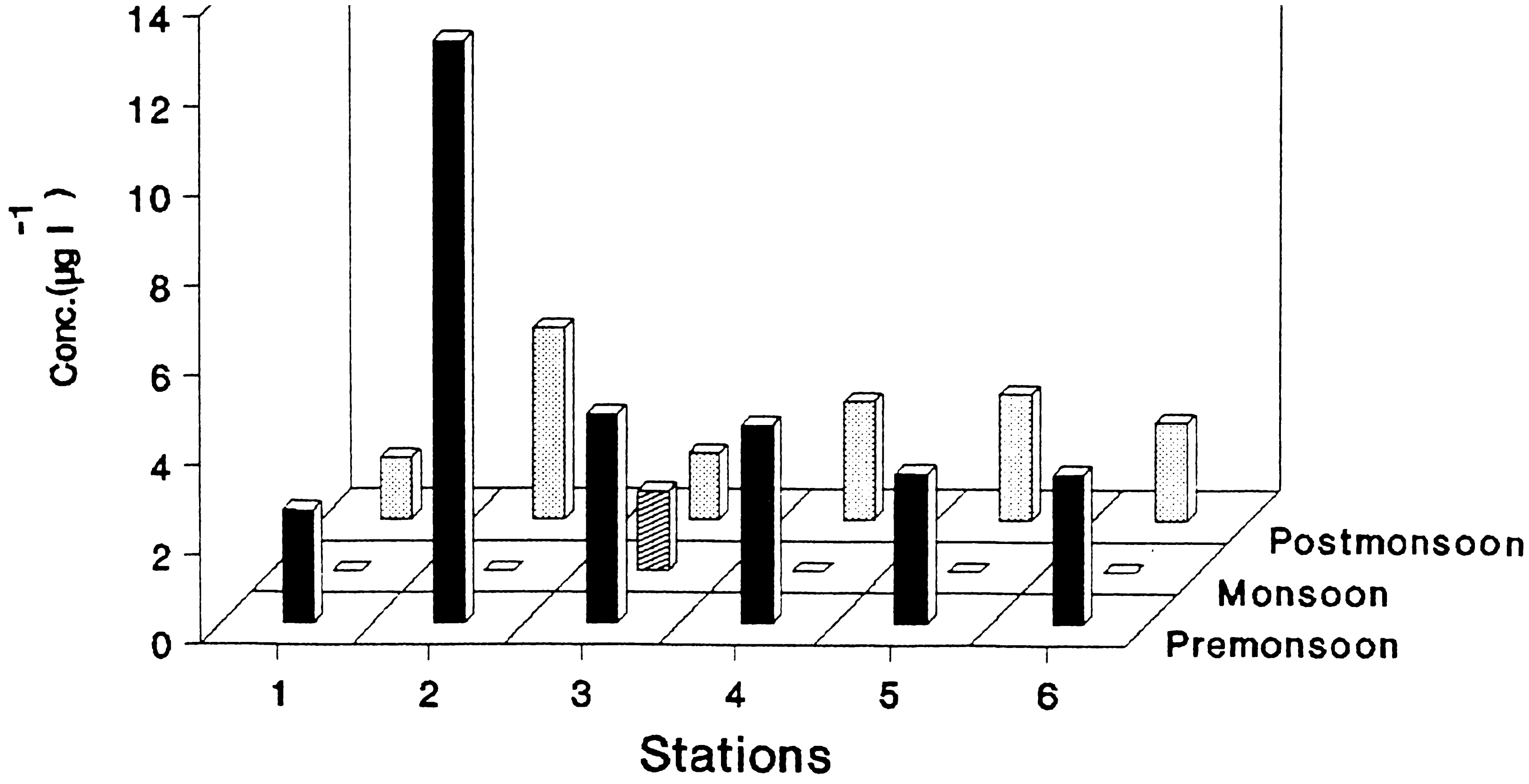


Figure 12.

Distribution profile of Malathion in Cochin Backwaters.

Stations 1 and 3 as well as Stations 4, 5 and 6 recorded comparable values.

Very few reports are available on the aquatic levels of Malathion due to its non-persistent characteristics (cited earlier). Available reports on aquatic levels of other organophosphorus pesticides pertain mainly to Dichlorvos, Fenitrothion, Methyl parathion etc. The concentrations of Fenitrothion in stream and Fenthion in estuary (following aerial spraying) were monitored by earlier workers who recorded a maximum value of ≈ 18 and 16 $\mu g\ l^{-1}$ (Morrison and Wells, 1981 and Wang *et al.*, 1987) respectively. The concentrations recorded from stream waters in southern Ontario, ranged from 0.24 to 1.80 $\mu g\ l^{-1}$ (Broun and Frank, 1980). Trace levels of Dichlorvos were reported in Ireland seawater (Tully and Morrissey, 1991).

3.4. Correlations of pesticides with the hydrographical parameters

Seasonal variations of the hydrographical parameters (pH, salinity, DO. and DOC.) viz-a-viz the total organochlorine pesticide concentrations (ΣDDT, ΣHCH and Endosulfan) and an organophosphorus pesticide (Malathion) at the different Stations are presented in Figure 13. The ΣDDT, ΣHCH and Malathion concentrations were found to be significantly influenced (though negatively) by the hydrogen ion concentration, pH ($P < 0.01$; Table 8), although it did not have any significant effect on the distribution of Endosulfan. It was reported that the HCH contamination in surface water from Seto Inland Sea has a negative correlation with salinity and hence decreasing residue levels are rather common with an increase in distance from coastal inlets (Tanabe and Tarsukawa, 1981). The dissolved oxygen concentration was negatively correlated only with the ΣHCH levels. Dissolved organic carbon

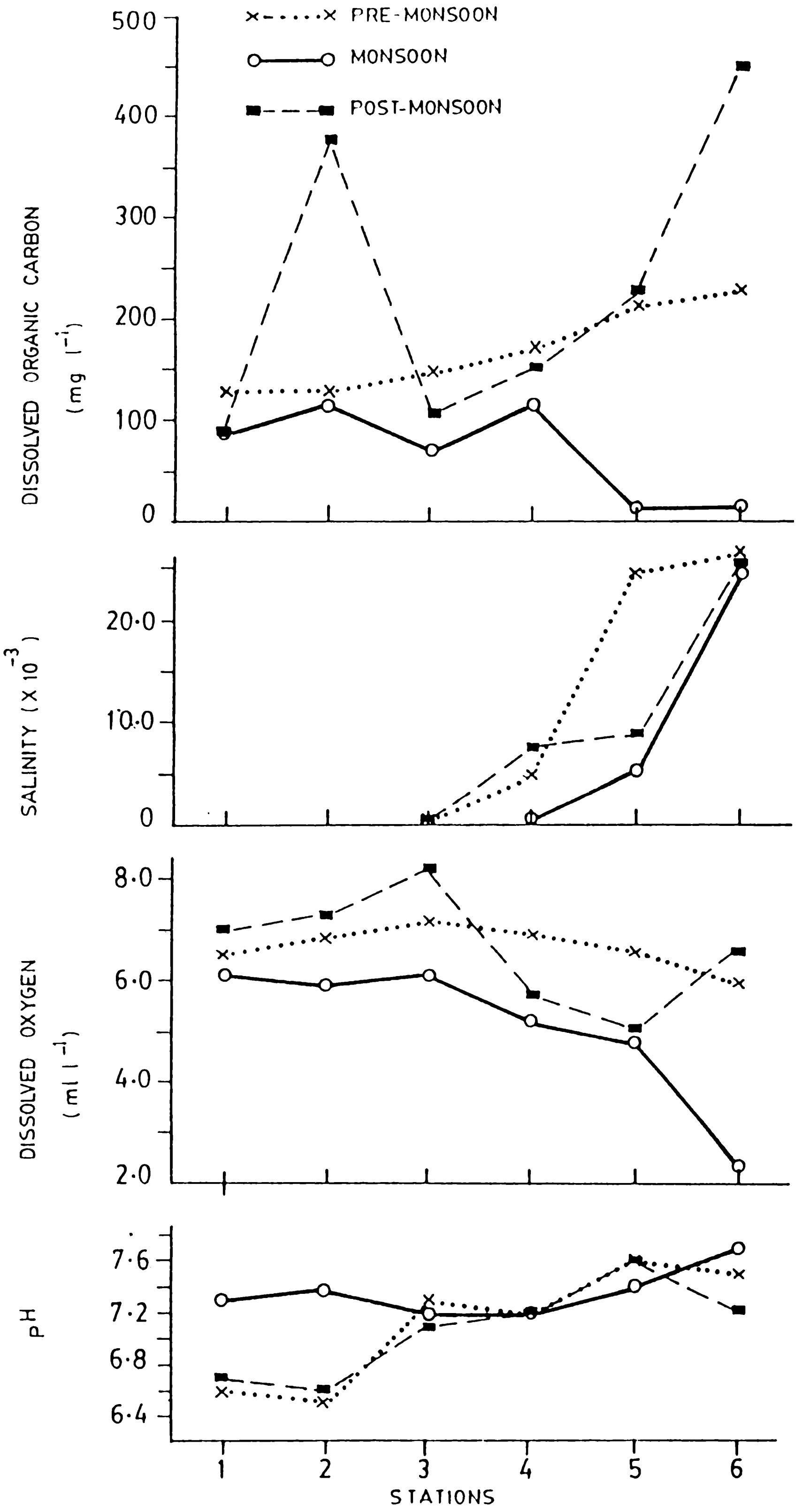


Figure 13.

Seasonal variation of hydrochemical parameters.

Table 8.

Correlation between pesticide levels and hydrographical characteristics.

Hydrographical Characteristics	Pesticide			
	ΣDDT	ΣHCH	Endosulfan	Malathion
pH	- 0.405	- 0.641*	- 0.301	- 0.495*
Salinity	- 0.055	- 0.187	0.152	- 0.089
DO	0.340	- 0.615*	0.430*	0.389
DOC	0.465*	0.238	0.455*	0.242

(*$p < 0.05$, $n = 18$)

was observed to be significant (positive) in relation to ΣDDT and Endosulfan concentrations.

The studies reported herein constitute the first attempt to ascertain the baseline data of ΣDDT, ΣHCH, Endosulfan and Malathion in this tropical water way viz., the Cochin Backwater.

An overall exponential decline in both organochlorine as well as organophosphorus pesticides have been observed from the upstream riverine zone to the confluence region. Studies on the metabolites of DDT have exposed the absence of o,p'-DDT concentration during the monsoon season. When the estuarine area under study largely remained a "free zone" as far as Endosulfan and Malathion were concerned. During the same season, Stations 3,4 and 5, however, recorded a significant loading of ΣDDT and of ΣHCH. One of the reasons for this feature might be the fluvial dynamics that play a prominent role in dislodging the pesticides from its association with the organic matter (which is incidentally higher at Stations 3, 4 and 5). The presence of small concentrations of almost all the pesticides at the mid-estuarine region (Station 3) could be attributed to the prominant agricultural runoff which characterized this sampling site. Profound seasonal changes resulted in a premonsoonal accumulation of pesticides along the waterway. Station 5 (Bolghatty), adjacent to the coastal Station, 6, however, was being excluded from this generalisation on account of the fact that the postmonsoonal values were considerably higher than the premonsoonal values. The Stations designated as 2 and 5 could be characterized in most cases, as "pesticidal loading sites" in view of the proximity of Station 2 to the pesticide manufacturing unit and of the large amount of urban runoff/municipal sludge and sewage that described Station 5.

The two well identified sources of pesticides which controlled/regulated the fluvial loading of pesticides in this waterway were (i) agricultural land runoff and (ii) the effluent discharges from the manufacturing units. It is hoped that these results which have enabled the identification of the "hot spots" would serve as the much wanted baseline data to trigger further studies on the pesticidal levels in the Periyar river and the Cochin Backwater System.

CHAPTER IV

CHAPTER IV

SORPTIONAL BEHAVIOUR OF ORGANOPHOSPHORUS PESTICIDES ON DIVERGENT SEDIMENTS

4.1. INTRODUCTION

Estuaries are often conceptualized as large reactors in which chemicals are mixed and processed along the salinity and suspended-sediment gradients (Church, 1986). Therefore,it is imperative that we understand the transport of pollutants in estuaries as a process that is affected by estuarine hydrodynamics which determine the eventual contamination of biota at all levels of food chain. The fate of chemical wastes in estuarine systems is thus a complex problem and is generally quantified by assessing the source input, the partitioning, the transport pathways, the enrichment at accumulation sites, the history of contaminants etc., because estuaries are heterogeneous, chemically reactive, hydrodynamically variable and sedimentologically dynamic (Nichols, 1990).

Since most pollutants are distributed while adsorbed or complexed to the surface of suspended solids, understanding the transport of sediments is also vital in determining the fate of these pollutants (Bero and Gibbs, 1990). Sediments act as major repository for many persistent chemicals introduced into surface waters with their low concentrations of dissolved oxygen and increased levels of reduced gases, ammonia and other toxicants, they are often inhospitable to aquatic organisms being directly toxic to them or being a source of contaminants for bioaccumulation in the food chain. Although it is now well established that ad(de)sorption phenomena at solid-solution interfaces significantly regulate the fate of contaminants in the hydrosphere, lack of a thorough understanding of relationship between sediment-contaminant concentrations and bioavailability emphasizes the need for laboratory toxicity and bioaccumulation tests designed to estimate biological damage caused when aquatic organisms are exposed to contaminant sediments (**Figure 14**).

The dispersion of contaminants in the aquatic system is defined by processes such as suspension of solids in turbulent aquatic systems, vertical movements of particles in the water column, water movement through solids in aquifers etc. Sediment represents a complex and dynamic matrix and is characterized by its properties such as the organic carbon content, particle size, clay content, cation exchange capacity, pH etc. (Karickhoff, 1984; Hermosin *et al.*, 1987; Podoll *et al.*, 1987;Ribo, 1988 and Marca and Opperhuizen, 1989).

Sediments thus play an important role in determining the potential environmental effects of pollutants and fundamentals to predict the fate of these pollutants are:

- an understanding of the sorption processes which influence the degradation, volatilization, transportation and hence the bioavailability of pollutants in the aquatic system and

- the quantification of the sorption phenomenon which is dependent on the nature of the equilibrium distribution of the pollutant between the aqueous and the solid phases as well as on the rate at which this distribution is approached.

Various types of natural sorbents are used for adsorption studies: biotic or abiotic; organic or inorganic; or chemical composites varying in size from macromolecules to gravel. Natural sorbents can indirectly mediate solution phase process

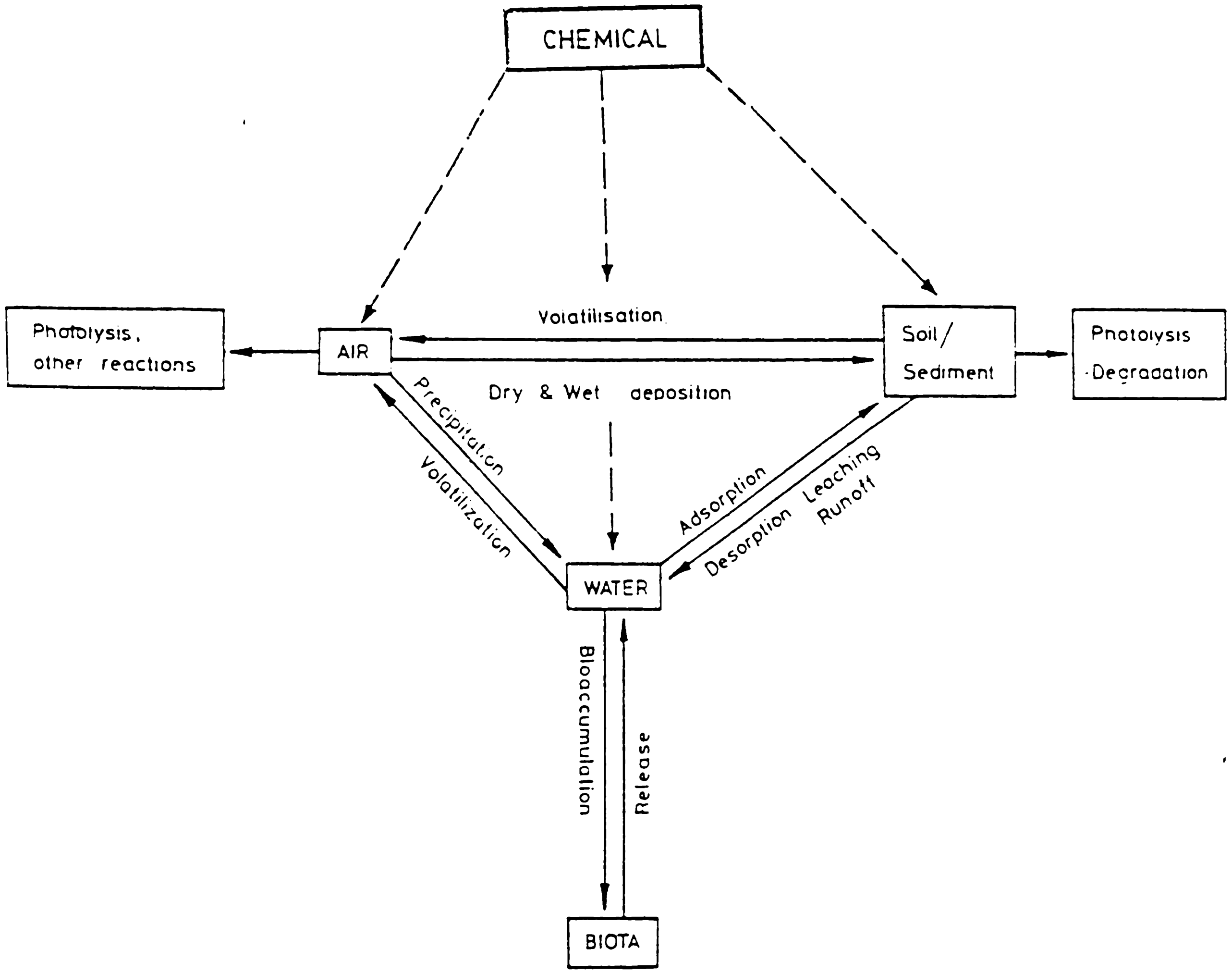


Figure 14.

Pesticide transport and transformation process in the environment.

by altering the solution phase pollutant concentration, and also by introducing into the solution a "buffered" suite of inorganic and organic species that can significantly affect pollutant reactivity in the aquatic phase (Karickhoff, 1984). Bar-Yosef *et al.* (1988) had studied the sorption of fluoride by using montmorillonite and kaolinite. Activated carbon was employed to remove heavy metals and naturally occurring colour in surface waters (Corapcioglu and Huang, 1987; Samuel and Osman, 1987). The application of the carbon activated technique in the removal of soluble aromatic hydrocarbons was demonstrated by Mohammed and Badawy (1979). Champion and Olsen (1971) studied the adsorption of C^{14} labelled DDT from aqueous solution onto the solid particles such as strongly basic anion exchange resin and weakly acidic cation exchange celluloses, as well to sedimented and non-sedimented promise clay. Rizwal and William (1971) studied the adsorption of two uracil herbicides viz. Isocil and Bromocil onto illite, montmorillonite, silica gel, humic acid and kaolinite surfaces. Haque and Schmedding (1976) conducted experiments on the adsorption of three polychlorinated biphenyls from aqueous solutions onto the surfaces of sand, clay, soil and humic acids.

Generally, the extent of sorption of organic pollutants depends on the nature of the sorbate-sorbent and solute-solvent interactions. Many organic chemicals, especially those that are nonpolar and insoluble in water, sorb strongly to sediments which are complex mixtures of alumino silicate minerals (clay), metal oxides, water and humic material. If the fraction of a chemical that gets adsorbed to a sediment is large, the rate of loss of the chemical by other transformation processes will be lessened. In effect, the adsorption process would serve to decrease the concentration of the chemical present in the aqueous phase. Thus, transport and ultimate disposition of organic pollutants, especially pesticides, in the aquatic environment involve complex physicochemical phenomena, which regulate the sorption or the partitioning processes occurring across the water/sediment interface.

Karickhoff (1984) and Schrap and Opperhuizen (1989) investigated sorptional behaviour of non-polar organic compounds (polycyclic aromatics and chlorinated hydrocarbons) with respect to river and pond sediments and attributed the differences in their sorption rate was due to variations in their organic carbon content. On the basis of extensive studies on pesticide sorption in natural systems, Lambert *et al.* (1965) and Lambert (1968) also arrived at a similar conclusion that the role of organic matter in sediment sorption studies as being similar to that of an organic solvent in solvent extraction processes and argued that the partitioning of a neutral organic compound between soil organic matter and water should correlate well with its partitioning between water and an immiscible organic solvent. Organophosphorus compounds have been reported to demonstrate a greater affinity for surfaces of organic matter present in the soils (Harris, 1966; Swoboda and Thomas, 1968; Saltzman *et al.*, 1972 and Sattar, 1990).

Poinke and Chesters (1973) have reviewed the interaction of pesticide with sediment-water systems and have outlined compositional distinctions between soils and sediments, that can affect sorption. The distinction between the two systems has been mainly attributed to particle size composition and its potential impact upon their environmental behaviour. Richardson and Epstein (1971) found that DDT and Methoxychlor preferred to concentrate more in fine particle sizes (clay), whereas, the comparatively more soluble Endosulfan preferred coarser material.

Factors like molecular weight of adsorbate, the particle size of adsorbent and the solution pH were also encountered by Mangialardi and Paoline (1990).

Adsorption rate is determined as a function of time by estimating the change in concentration of the solute which has been in contact with various sediment types. Earlier investigations (Haque and Schmedding, 1976 and Samuel and Osman, 1987) on the behaviour of pollutants in field soils, had clearly established the existence of an equilibrium between the fractions in the liquid and the solid phases and had indicated that the equilibrium would be more precisely determined by a suitably designed adsorption experiment (Leistra and Smelt, 1981; Nichols *et al.*, 1982; Nair, 1990 and Sujatha and Chacko, 1991). Tsicritsis and Newland (1983) had investigated the kinetics of Permethrin adsorption in soil to ascertain the time span required for the attainment of equilibrium between Permethrin adsorbed and Permethrin in solution. Generally, the processes of adsorption of environmental chemicals on soils and their constituents like clays, humins, humic acids, fulvic acids etc., are evaluated by the application of suitably selected isotherm models (Li and Felbeck, 1972; Bowman, 1978 and Crombie-Quilty and Mc Laughlin, 1983), which tend to define a relationship between the amount of an environmental chemical adsorbed per unit weight of the adsorbent and the concentration of the adsorbate in the solution when equilibrium is attained.

Among the pesticide family of compounds, the organophosphorous (OP) insecticides have gained much wider acceptance due to their propensity for relatively shorter persistence time in the environment as well as because of a tendency for these compounds to be strongly adsorbed to the soil (Sharom *et al.*, 1980). However, although these OP compounds persist for only less than 3 to 6 weeks in the environment (Smith and Willis, 1987), they are known to cause acute toxic effects to the aquatic life (Yule and Duffy, 1972; Schoor and Brausch, 1980; Jash and Bhattacharya, 1983 and Agarwal and Nair, 1989). Therefore, detailed studies on the fate of such compounds are essential to comprehend their toxic nature and to assess their suitability for use in the environment.

This chapter describes the results of a laboratory investigation designed to assess the sorption kinetics of two widely used organophosphorus pesticides viz., Malathion and Methyl parathion. Such studies could be expected to throw much light on the nature of partitioning of the pesticide in the aquatic system and thereby to provide a valuable insight into the complexities of bioaccumulation. Further this study was mainly attempted to quantify the exposure concentration of these OP pesticides in the ecosystem as well as to evaluate in which medium (water/sediment) of the environment these chemicals are likely to accumulate and persist for the specified period of time.

Details of the experimental design and analytical procedures and methodologies employed are described in Chapter II — Materials and Methods.

4.2. RESULTS AND DISCUSSION

The distribution profiles of the two pesticides, Malathion and Methyl parathion in three environmentally divergent sediment samples (representing the diversity of the Cochin estuarine sediments which reflect the natural variations in the environmental conditions as well as the localised effects caused by anthropogenic activities) viz., a) relatively less polluted sediment (LPS), b) polluted sediment (PS) and c) confluence sediment (CS) were envisaged by exposing these sediment types to varying pesticide test concentrations for different chosen periods of equilibration time.

Several theoretical and empirical relationships have been tested for mathematically describing the pesticide adsorption capacity and for quantifying the associated equilibria (Kanazawa, 1987 and Hermosin *et al.*, 1991). As adsorption process proceeds, the sorbed solute tends to be desorbed into the solution and the rates of adsorption and desorption will approach an equilibrium state, viz. the adsorption equilibrium. The position of the equilibrium would be defined by the characteristic of the solute, the adsorbent, the solvent, the temperature and the chemical environment (e.g.pH and salinity). An adsorption isotherm expresses the relationship between the amount of the solute adsorbed per unit weight of the adsorbent and its equilibrium concentration in solution at constant temperature. The shape of the adsorption isotherm provides information on the nature of the adsorption process (monolayer/multilayer, first/second order etc.).

A detailed descriptions of the models employed in the study and the results obtained are presented below.

4.2.1. Equilibrium model

Since the processes controlling the concentration of chemicals in the environment are dynamic, the kinetic and equilibrium rate constants are the most relevant quantitative descriptors for evaluating the net change and the rate of change in concentration of the solute at equilibrium.

The kinetic patterns of the pesticide adsorption in the present study could be adequately represented by the first order rate equation.

$$\log (a-x) = \left[-\frac{Kr}{2.303} \right] t + \log a$$

where "a" is the initial dosed pesticide concentration and "x" is the amount of the pesticide adsorbed onto the sediment after an elapse of time "t" (Kanazawa, 1987).

A rate graph (Figure 15) was obtained by plotting the percentage of the pesticide adsorbed (at any given dosed concentration) against exposure time. The values of the rate constant (Kr, calculated from the rate equation) and of the equilibrium constant (Ke, calculated as $Ke = \frac{x}{a-x}$) are presented in Table 9. Irrespective of the nature of the sediment, Malathion recorded higher values for the equilibrium constant as compared to Methyl parathion. However, with values of rate constant a reverse trend was observed.

The observed variations in these values could be attributed to:

- the different geochemical characteristics of the sediments (nature, size, SOC, collection site)
- the structural variation between the two pesticides
- the specific interaction of the pesticide with the negatively charged functional groups in the sediment types
- the difference in the sorptional behaviour of intrinsic and extrinsic sites of the sediment
- the mechanisms of mass transfer occurring across a boundary film surrounding an individual solid particle as well as diffusion processes within the particle as envisaged in the dual resistance diffusion theory (Weber and Miller, 1988) and
- the organic complexation capabilities of the two pesticides.

It has been proposed that the sediment sorption with respect to two pesticides be quantified in terms of a new parameter —— the Sorptive Index, S, where S is defined as the ratio

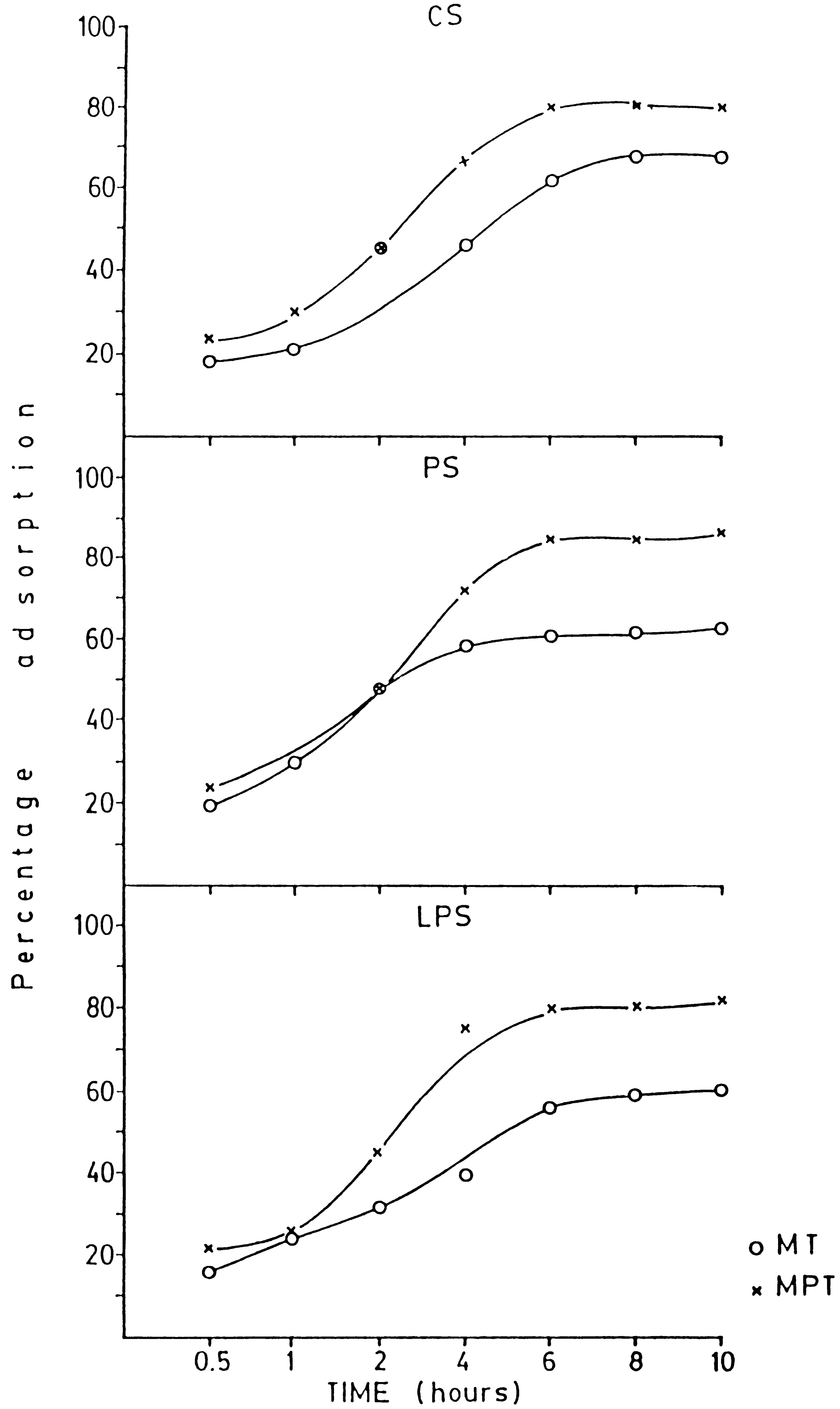


Figure 15.

Rate of adsorption of MPT and MT by LPS, and PS and CS.

Table 9.

Kinetic parameters of first order rate equations.

Sediment	Pesticide	Ke n = 14	Kr n = 14
LPS	MT	0.572±0.06	0.107±0.01
	MPT	0.357±0.16	0.138±0.04
PS	MT	0.716±0.21	0.067±0.02
	MPT	0.293±0.11	0.126±0.03
CS	MT	0.570±0.11	0.082±0.04
	MPT	0.392±0.12	0.132±0.04

between the equilibrium concentrations of the respective pesticides in a particular sediment. Thus,

$$S = \frac{C_{MPT}}{C_{MT}}$$

where C_{MPT} and C_{MT} are the equilibrium concentrations of Methyl parathion and Malathion in the sediment for given dosed concentrations of the pesticide.

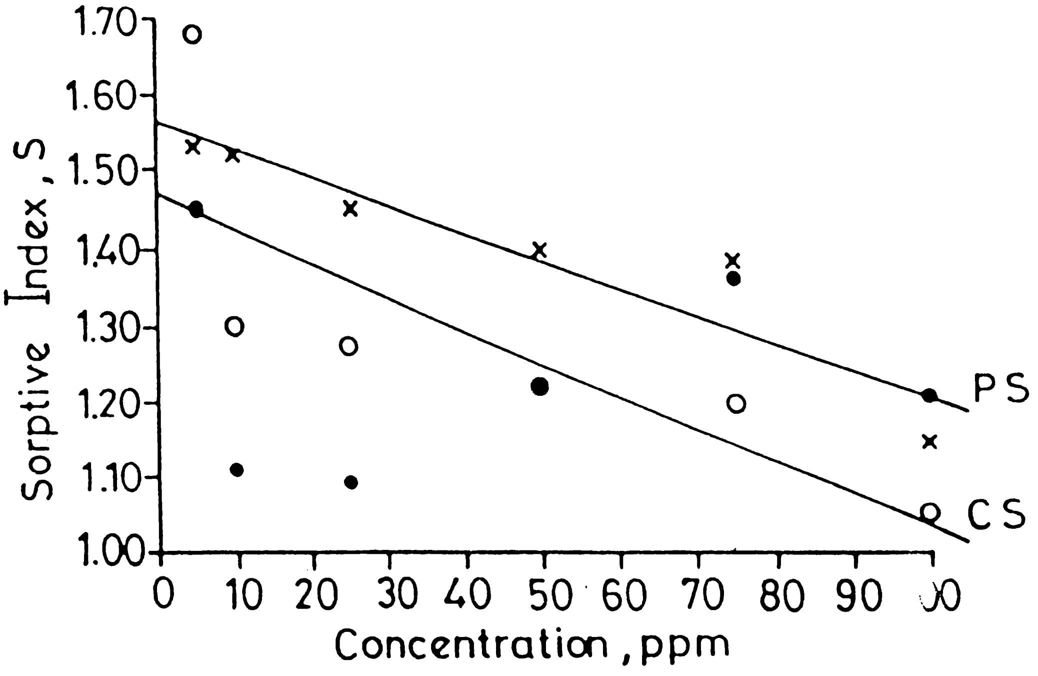
Values of the Sorptive Indices for varying dosed concentrations of two pesticides are presented in Table 10 and Figure 16. The dosed pesticide concentration was found to be significantly correlated, though negatively, to the Sorptive Index for PS and CS. In other words the pesticides were observed to be adsorbed at higher rates at lower initial concentrations. Sorptive Index dropped from 1.53 to 1.15 and from 1.68 to 1.05 respectively in PS and CS when the concentration of the pesticide was increased from 5 to 100 ppm. At lower concentrations of the pesticide, rapid adsorption occurs at the surface while at higher concentrations, (when the surficial adsorption sites would have been almost fully used up, the amount of pesticide adsorbed would tend to plateau off) there would even be a marginal increase in the pesticide adsorption as a result of the multilayer-adsorption process.

Generally the sorption of pesticides and other solutes in the aquatic system is influenced and regulated by a multitude of physicochemical factors, which include characteristics of the sediment (texture, surface condition, chemical nature etc.) as well as properties and composition of the aquatic phase (Morrill *et al.*, 1982; Voice and Weber, 1983 Nesterova *et al.*, 1984 and Sujatha and Chacko, 1992).

Table 10.

Sorptive index of LPS, PS and CS.

Sediment	Sorptive Index (S) = $\frac{C_{MPT}}{C_{MT}}$					
	5 ppm	10 ppm	25 ppm	50 ppm	75 ppm	100 ppm
LPS	1.45	1.14	1.09	1.21	1.36	1.21
PS	1.53	1.52	1.45	1.40	1.38	1.15
CS	1.68	1.30	1.28	1.21	1.20	1.05



	a	1.244		a	1.557		a	1.484
● LPS	b	-1.162	x PS	b	-0.003	o CS	b	-0.004
	r	-0.003		r	-0.939		r	-0.799

Figure 16.

Influence of pesticide concentration on the Sorptive Index.

Sediments are largely eroded soils that have been subjected to continuous re-dispersion and particle size fractionation commencing with runoff and continuing with subsequent water-system processes. These processes are highly dependent upon the dynamics of the specific stream, river, pond or lake and upon the dispersion properties of the parent soil. Sediment within a given water compartment may contain a very narrow range of particle sizes. For example, suspended sediment within a river system may be largely clayey, bottom sediment from the middle of the river could be largely sandy and bottom sediment from the edge of the river largely could be silty. Functional dependence on particle size could decide the degree of sorption of the pesticides in the different aquatic environment.

The organic matter in the sediment also markedly influences its adsorptive capacity. This has been vividly reflected in the increased adsorptive capacities (Sorptive Indices) observed for PS and CS. Table 11 gives the sediment characteristics of LPS, PS and CS. The SOC content of PS and CS were, respectively, about five and three fold higher than that of LPS. LPS, which contains a higher amount of coarser particles and a lower organic carbon content, has the lowest Sorptive Index.

Although the adsorption of pesticides, in general, could be appreciably affected by the organic matter content of the sediment, the adsorption of one pesticide in preference to another might only be a consequence of the structural differences (vide Chapter III) existing between Malathion and Methyl parathion. Between the two pesticides employed in the study, Methyl parathion was clearly more polarizable than Malathion and hence could be expected to have a greater electrostatic interaction with the charged or polar sites of the sediment. Detailed investigations are, however needed to distinctly identify and delineate the various factors

Table 11.

Sedimental characteristics of LPS, PS and CS.

Sedi-ment	SOC	SOM	Particle Size			Nature of Sediment
	mg g^{-1}	mg g^{-1}	$> 63\ \mu$ (Sand)%	$63 - 4\ \mu$ (Silt)%	$< 4\ \mu$ (Clay)%	
LPS	0.69	1.19	90.04	3.50	6.46	Sand
PS	3.22	5.51	19.49	43.71	36.80	Clayey Silt
CS	1.73	2.91	18.51	53.15	28.34	Clayey Silt

responsible for the observed variations in the adsorption capacities of these pesticides.

An attempt has also been made to explain the sediment sorption characteristics on the basis of the partition coefficients. As the knowledge of the partition coefficient is similar to rate constants stated earlier, it is important in estimating the exposure concentrations of chemicals in the environment.

Partition coefficients for the three sediment types at different Malathion concentrations are given in **Table 12**. There was over a two fold increase in the Kp values for PS (values ranging from 25.5 - 56.9) and CS (values ranging from 20.0 - 46.6) when the Malathion concentration was raised to 100 ppm. However, the concentration dependence of Kp was more pronounced in the case of CS (the value was doubled at 75 ppm) as compared to PS (the value was doubled only at 100ppm). The Kp value for LPS was found to be 35.42 ± 3.89, irrespective of the change in Malathion concentration. Upto a limiting concentration of 50 ppm, the sediment collected from a less polluted environment tends to retain a comparatively higher amount of the pesticide (32.6 - 38.8) than that from a polluted (25.5 - 27.6) and a confluence (20.0 - 32.6) region.

4.2.2. Adsorption Isotherms

Although various mathematical models have been proposed for quantifying the sorption of organic compounds on natural solids, most of the investigators have used the isotherm approach. An isotherm expresses the relationship between the amount of an environmental chemical (solute) adsorbed per unit weight of the adsorbent, and the concentration of the solute in solution at equilibrium.

Table 12.

Variation of partition coefficient with Malathion concentration.

Concentration of Malathion in ppm	Kp		
	L P S	P S	C S
5	32.6	25.5	20.0
10	40.6	20.8	28.7
25	35.0	23.5	28.8
50	38.8	27.6	32.6
75	30.0	32.6	40.6
100	35.5	56.9	46.6

Three of the more common mathematical equations employed for studying the adsorption phenomena are:

- Langmuir isotherm
- Freundlich isotherm and
- Elovich isotherm

In this investigation, the validity of these three isotherms on the sorptional characteristics of the sediment types described earlier (Chapter II) has been tested by using the pesticide, Malathion.

Langmuir isotherm

The Langmuir adsorption equation (Langmuir, 1916 and 1918) was initially designed for the adsorption of gases by solids. This model is based on the following assumptions:

- The energy of adsorption is constant and is independent of the extent of the surface coverage

- Adsorption occurs on localized surface sites

- There is no interaction between adsorbed compounds and adsorbate molecules

- The maximum adsorption possible is that of a complete monolayer.

In terms of concentration, the Langmuir adsorption equation has the general form:

$$m = \frac{K_1 K_2 C}{1 + K_2 C}$$

where,

m = the amount of pesticide adsorbed per unit weight of sediment at equilibrium.

c = the equilibrium concentration of pesticide remaining in solution

K_1 = a constant reflecting the ultimate pesticide adsorption capacity and

K_2 = a constant related to the energy of adsorption or net enthalpy of adsorption.

The equation can also be written in the form:

$$\frac{C}{m} = \frac{1}{K_1} C + \frac{1}{K_1 K_2}$$

A plot of $\frac{C}{m}$ versus C would yield a straight line if the experimental data confirmed to the Langmuir equation.

Freundlich isotherm

Even though the Langmuir model has a sound theoretical basis it often adequately fail to describe the adsorption process (Bourg, 1989). Adsorption isotherm of Freundlich (1926) has a parabolic shape and that the adsorption for many dilute solutions could be described by the following equation:

$$\frac{x}{m} = K C_e^{\frac{1}{n}}$$

where,

$\frac{x}{m}$ = the amount of pesticide adsorbed per gram of the sorbent at equilibrium,

Ce = the equilibrium concentration of the pesticide and

K and $\frac{1}{n}$ = (constants) respectively called the sorption-capacity coefficient and the sorption-energy or internsity coefficient.

The Freundlich equation is an empirical expression that encompasses the heterogeneity of the surface and the exponential distribution of sites and their energies (Young and Growell, 1962). Though it is assumed that each class of surface sites adsorbs individually according to the Langmuir equation, the Freundlich isotherm can be shown to:

- correspond to a unique distribution of relative adsorption site affinities which is essentially log-normal and

- that the empirical parameters 'K' and 'n' may be used to mathematically characterize the site distribution, thus providing information about surface heterogeneity of the adsorbent (Sposito,1980).

Thus it is the most widely used mathematical description for the multi-layer adsorption and is expressed in the log form (Freundlich, 1926) as

$$\log \frac{x}{m} = \frac{1}{n} \log Ce + \log K$$

A plot of log $\frac{x}{m}$ versus log Ce would yield a slope of $\frac{1}{n}$ and log K as the intercept. Steep slopes (i.e. $\frac{1}{n} \approx n$) indicate high adsorptive capacity at high equilibrium concentrations, which rapidly diminishes at lower equilibrium concentrations covered by the isotherm. Flat slopes (i.e. $\frac{1}{n} << 1$) indicate that the adsorptive capacity is only slightly reduced at the lower equilibrium concentrations (Samuel and Osman, 1987).

Singhal and Kumar (1978) investigated the adsorption of

Telone (a soil fumigant) from alcoholic solution on hydrogen and sodium saturated bentonites in the presence of 0.5 and 1.0% humic acids and the isotherm followed the Freundlich relation. The Freundlich plots for the adsorption of benzene, toluene, o-xylene and ethyl benzene on the activated carbon showed a good fit (Mohammed and Badawy, 1979). The sorption of two hydrophobic compounds, Lindane and nitro benzene was found to exhibit both the Langmuir and Freundlich isotherm model (Weber and Miller, 1988). The interaction of most of the pesticides with soils and clay minerals has been found to follow the Freundlich's adsorption isotherm.

Elovich isotherm

The simplified form of the Elovich equation can be represented as:

$$q = \frac{1}{b} \ln t + \frac{1}{b} \ln (ab)$$

where, 'q' the amount of pesticide desorbed at time 't' and 'a', and 'b' are constants. A plot of 'q' versus 'ln t' should give a linear relationship with a slope of $(\frac{1}{b})$ and an intercept of $(\frac{1}{b})$ ln (ab) (Chien and Clayton, 1980). The kinetics of phosphate desorption from various soils were found to be well fitted in Elovich isotherm (Elkhatib and Hern, 1988).

The values of intercept, slope as well as the correlation coefficients obtained by applying the Langmuir and Freundlich equations to the sediment systems are given in Table 13. The general shapes of the respective isotherms and the experimental plots of log $\frac{x}{m}$ versus log Ce for Freundlich isotherm are depicted in Figure 17 and 18 respectively. Since the Elovich equation yields a separate plot for each of the six Malathion concentrations used, the corresponding values from the Elovich

Table 13.

Kinetic parameters of Langmuir and Freundlich isotherms.

Equation	Sediment type	Intercept k	Slope n	Correlation coefficient r
	L P S	1.56	0.99	0.997
Freundlich	P S	1.25	1.21	0.975
equation	C S	1.16	1.31	0.998
Langmuir	L P S	0.03	0.05	0.044
equation	P S	6.28	-7.23	-0.774
	C S	0.29	-8.04	-0.897

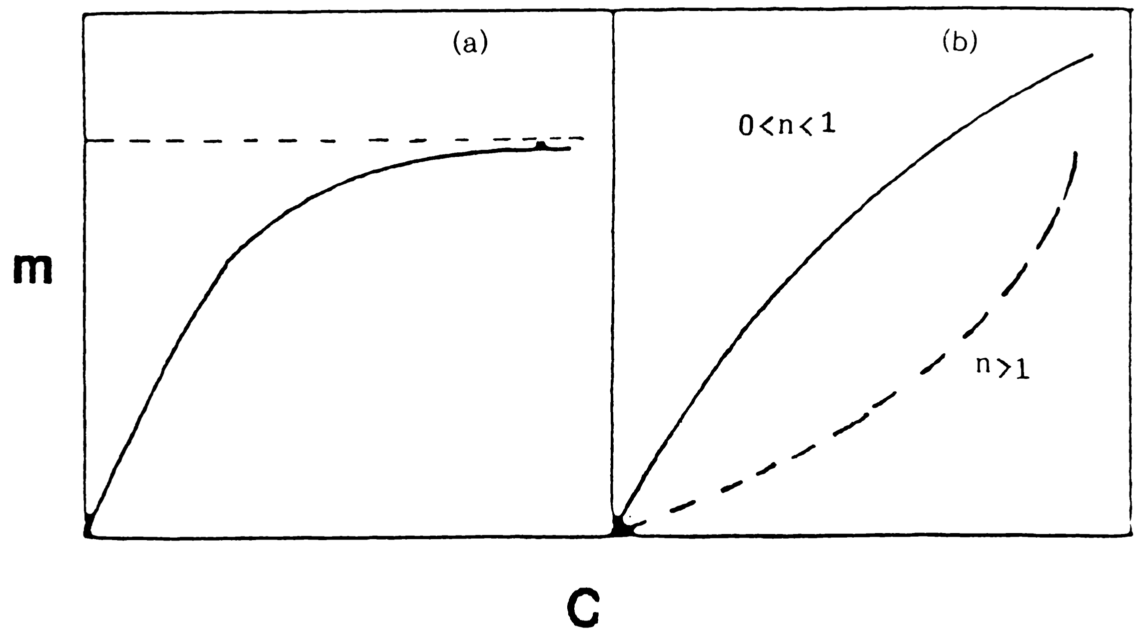


Figure 17.

General shapes of Langmuir (a) and Freundlich (b) isotherms.

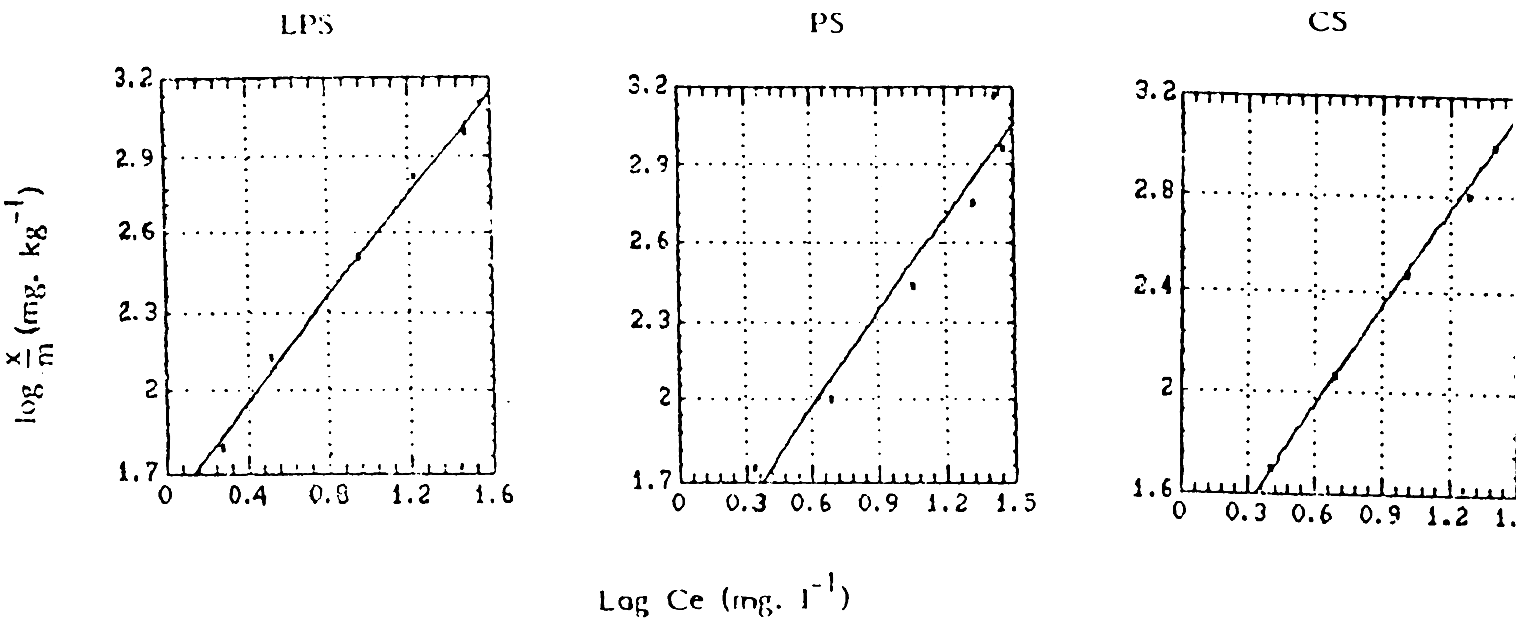


Figure 18.

Plots of log x/m vs log Ce (Freundlich isotherm) equation are given along with the respective plots (Figures 19a and b). From the correlation coefficients, it can be seen that the sorption process in all the three sediments conforms to both the Freundlich as well as the Elovich models but not to the Langmuir equation.

The basic premise of the Langmuir adsorption isotherm conceives that molecules are adsorbed on definite sites on the surface of the adsorbent, each site being capable of accommodating only one molecule (the monolayer), that the area of each site is a fixed quantity determined solely by the geometry of the surface and that the adsorption energy is the same at all the sites (Samuel and Osman, 1987). Besides, the adsorbed molecules cannot migrate across the surface, or interact with neighbouring molecules. Thus the Langmuir concept precludes intrinsic pesticide-sediment interactions as well as pesticide-pesticide interactions and envisages only a weak pesticide-sediment interaction confined to the surface of the sediment. The poor correlation observed for the Langmuir isotherm, clearly indicates that the adsorption process for the sediments under investigation does not proceed via a monolayer type interaction. The fact that the energy of adsorption (denoted by the term $\frac{1}{n}$) in the Freundlich equation varies as a function of the surface coverage, whereas the corresponding term, k_2, in the Langmuir equation is a constant further corroborates the inapplicability of the Langmuir model in adsorption studies involving heterogeneous surfaces.

Adsorption of solute on sediments occurs mainly at the sites of the negatively charged molecules. Besides, the organic carbon moieties in the sediments could also bind solute molecules by weak forces of attraction. CS and PS are seen to have appreciably larger amounts of SOC than LPS. Thus, in LPS, interaction mainly occurs between the pesticide molecules and the negatively charged functional groups within the matrix of

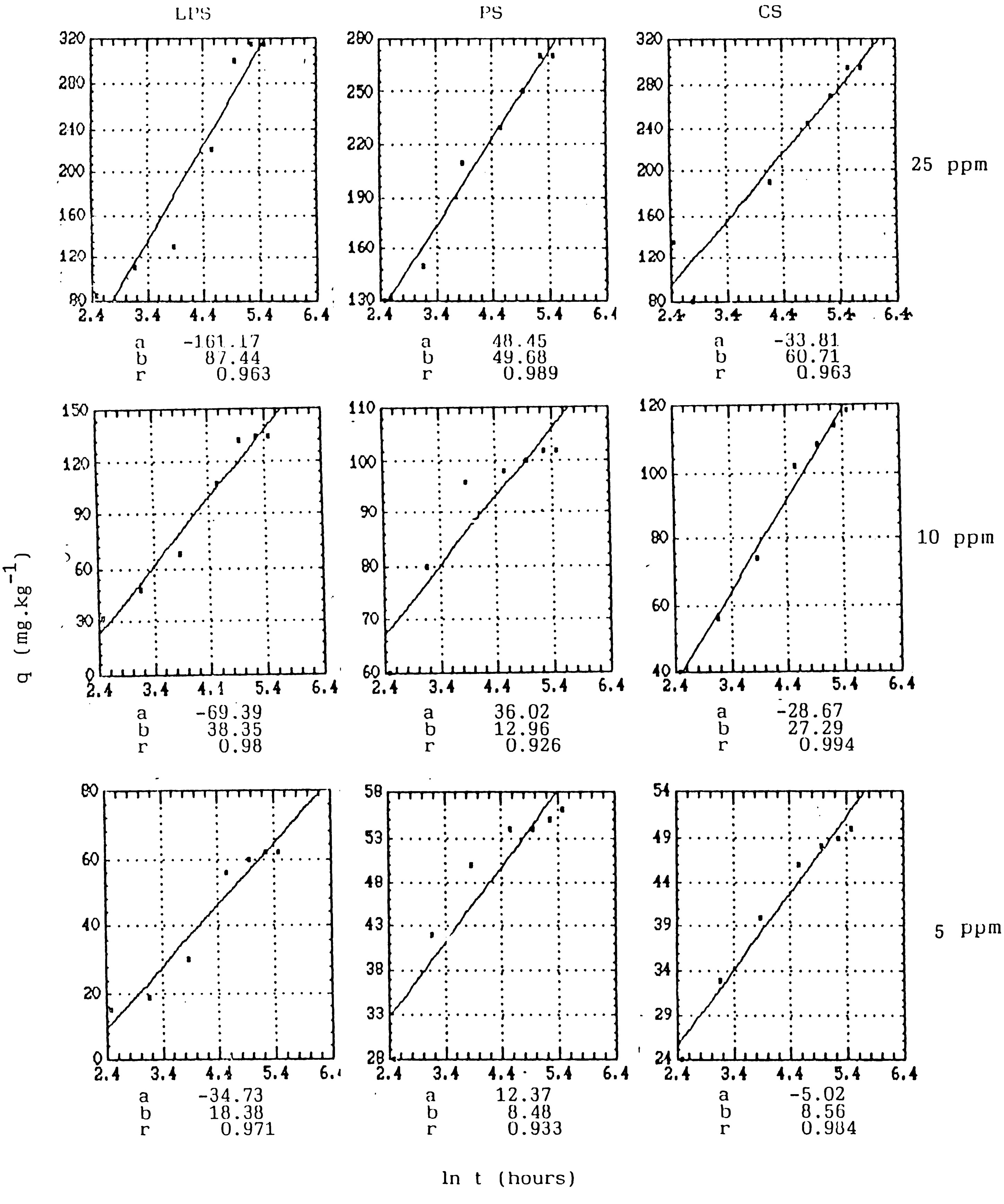


Figure 19a.

Elovich equation plots of LPS, PS and CS at varying Malathion concentrations.

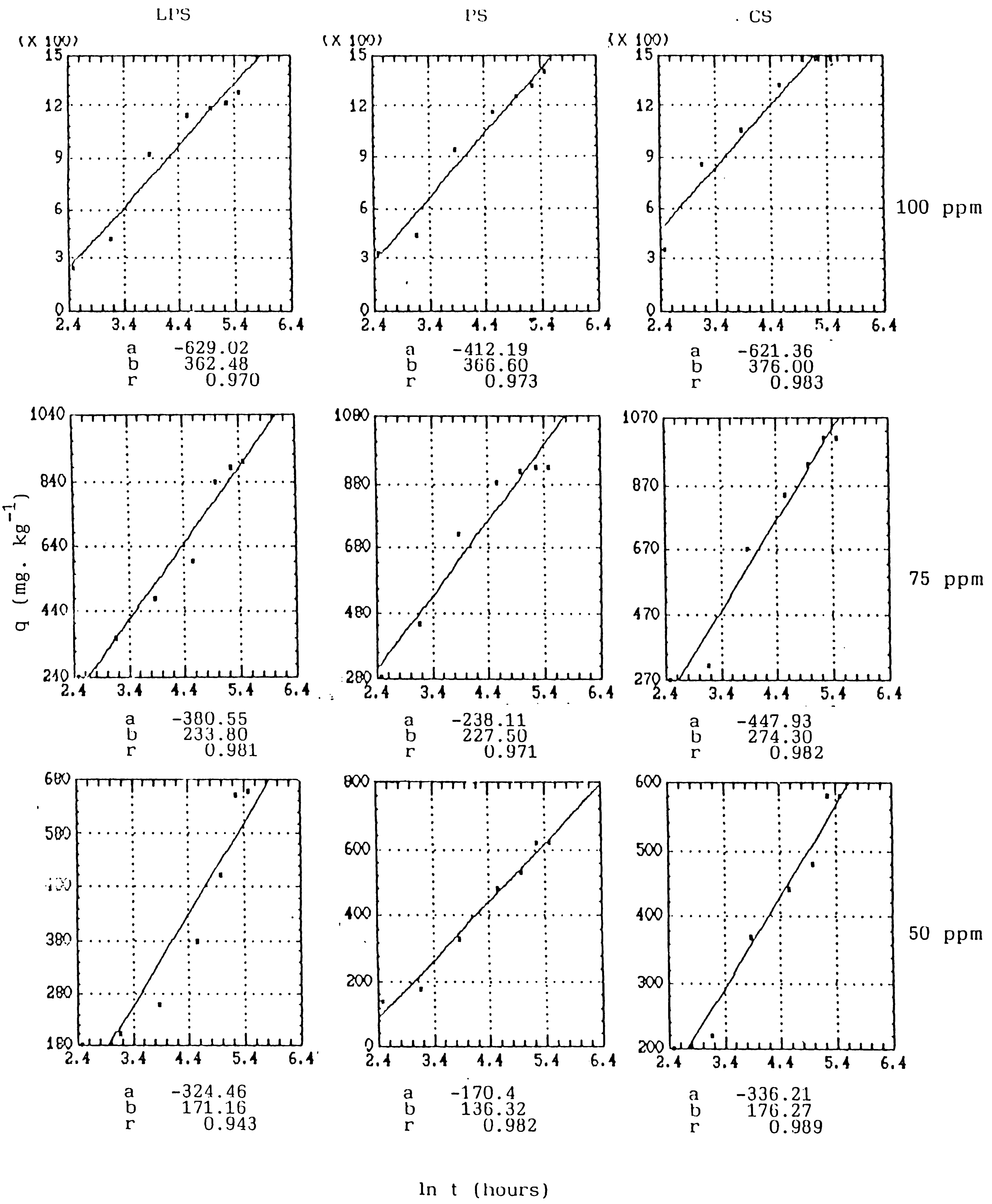


Figure 19b.

Elovich equation plots of LPS, PS and CS at varying Malathion concentrations.

the sediment, while the interactions in PS and CS occur not only at these negatively charged sites but also at the binding sites offered by the SOC. This further explains the poor `r' value for LPS as well as the significant negative correlation for PS and CS obtained by applying the Langmuir model to the system under study. The negative slopes of PS and CS are added evidence to the decrease in adsorption (monolayer) with increase in pesticide concentration (Crombie-Quilty and Mc Laughlin, 1983).

The Freundlich and Elovich models have the multilayer type of heterogeneous adsorption as its focal theme and are therefore governed by such physicochemical parameters as: partition coefficient and solubility of the solute, particle size and the organic carbon content of the sediment.

It is well recognized that partitioning of a chemical between the phases of a suspended sediment is a reversible phenomenon which can be quantified in terms of its partition coefficient K_p, where K_p is the ratio of the concentration of the chemical in the sediment to its concentration in the liquid phase (Karickhoff *et al.*, 1979). For non-ionic chemicals like pesticides, the K_p values for various clay and silt-sized sediments have been found to be proportional to the SOC (Richard, 1980).

LPS has a relatively lower content of organic matter than PS and CS, consequently resulting in the availability of a larger number of free adsorption sites which facilitates a rapid and massive filling up of these sites by the pesticide molecules. This is reflected in the Kp values which quickly reach a maximum at 50 ppm Malathion concentration. The higher values of SOC of PS and CS as compared to LPS (Table 11) are clear evidence of the direct proportionality of SOC to adsorptivity of the sediment. Brusseau and Rao (1989) have reported that organic matter in soils and sediments is commonly pictured as a three-dimensional network of polymer chains, with a relatively open, flexible structure perforated with `voids'. With such a structure attributed to sorbent organic matter, the diffusive mass transfer of sorbate into the interior of organic matter is conceptually viable and thus organic matter can be considered as primary sorbents.

Sorption of solutes increases with a decrease in solubility (i.e. with increase in Kp) and is directly proportional to the SOC (Podoll *et al.*, 1987). The trend is clearly borne out in the SOC values (which increase in the order PS<CS<LPS and Kp values which decrease in the order PS>CS>LPS) obtained in this study (Figures 11 and 12). The higher values of Kp are presumably due to the specific interactions, between the proton donating phosphorus atom and the negatively charged functional groups present at both the external and internal adsorption sites in the sediments.

The plot of log $\frac{x}{m}$ versus log Ce (Figure 18) affords a straight line for the three sediment types convincing conformity to the Freundlich model. The significant differences in slope ($\frac{1}{n}$) and intercept (log K) reflect variations in the level of competitive effects in the adsorptivity of the pesticide. The steep slopes observed for the LPS, PS and CS (0.99, 1.21 and 1.31) indicate high adsorptive capacity at high equilibrium concentrations (Table 13). Since all the three categories of sediment fit into the Freundlich model, ($r = 0.997$, 0.975 and 0.998 at $n=6$), the loading factor on sediments, $\frac{x}{m}$, is a function of the equilibrium concentration of the pesticide.

The results obtained have also been analysed by the Elovich model and are depicted in the plots of 'q' versus 'ln t' (Figure 19a and b). The correlation coefficients, (0.971 ±

0.019, n=18) depicted on the plots themselves, obtained for each sediment for the respective Malathion concentrations establish that the adsorption process is in good agreement with this model as well.

Based on the results reported herein, it is concluded that Malathion sorption by sediments with a pollution gradient (from the Cochin estuary) occurs via a heterogeneous, multilayer adsorptive process that can be described by either the Freundlich or the Elovich isotherm. Significant correlations existed between organic carbon content and partition coefficient as well as between organic carbon content and adsorptivity.

4.2.3. Environmental factors affecting adsorption

Adsorption isotherms have been widely used to describe the sorption kinetics of contaminants in aquatic systems. However, the environmental fate of the pesticide, apart from being influenced by the chemistry of the molecule, would be significantly affected by a number of extraneous environmental factors that can alter the nature and involvement of the pesticide in the sorption process. For example, temperature, pH, moisture and oxidisable organic matter contents of water have long been known to influence the degradation of pesticides (Barge, 1971; Rajaram and Sethunathan, 1975; Greenhalgh *et al.*, 1980 and Shelton and Parkin, 1991). The dependence of the partition coefficient of the herbicide, Chlorsulfuran, on the pH of the aqueous phase is in agreement with the dependence of solubility on pH, and with the influence of pH on the rate of hydrolysis (Ribo, 1988). Murphy (1991) observed that added aluminium had a decisive role in regulating the adsorption of pesticide, Aldicarb, onto a high organic soil.

It was, therefore, of concern to investigate the effects of pH and salinity on the sorption of Malathion and Methyl parathion by the three sediment types referred to earlier.

Hydrogen ion concentration

The hydrogen ion concentration has been shown to exert a decisive influence on the uptake/release mechanisms of adsorbents to and from the sediments (Christensen, 1984; Bourg, 1989;Boers, 1991). Laquer and Manahan (1987) have noticed that the pH of the solution was found to be an important factor that can control the adsorption of phenol onto siltstone. The importance of pH on the fate, stability, bioavailability and toxicity of Carbaryl and Aldicarb in the aquatic environment has been well established (Kuhr and Dorough, 1976 and Murthy and Raghu, 1991). Although in view of their non-ionic character, sorption of organophosphorus and organochlorines are not normally expected to be significantly influenced by variations in pH, reports on the modification of pesticide toxicities (Bender, 1969 and Johnson and Julin, 1980) by changes in pH seemed to suggest that the hydrogen ion concentration could bring about changes in the nature of adsorption of such pesticides in the aquatic environment. The results of the studies designed to test the above hypothesis are presented below. The investigations deal with the assessment of sorption of two important pesticides, (Malathion and Methyl parathion) at three concentrations (10, 50 and 100 ppm) onto three naturally occurring (but environmentally divergent) sediment samples exposed to three pH regimes representing acidic (5.0), neutral (7.0) and alkaline (8.5) conditions. These experiments were carried out with both natural waters (collected from coastal, estuarine and riverine environments within the Cochin backwater system) and with synthetic seawater so as to discern the effects, if any, of the medium on the sorption process. The pH of each of these water samples was adjusted to the experimental pH values by appropriate addition of hydrochloric acid or sodium hydroxide solutions.

Details of the experimental methodology adopted have been described in Chapter II — Materials and Methods and the results are discussed here under.

Malathion

A graphical representation of the influence of pH on Malathion adsorption at the three dosed concentrations (10, 50 and 100 ppm) is presented in Figure 20. The values plotted are the averages of duplicate measurements corresponding at three different concentrations of Malathion. The x-axis is non-scaled and represents the three hydrogen ion concentrations. The results obtained are summarized below.

Low concentration (10 PPM): With synthetic, coastal and estuarine waters, an acidic pH was required to effect significant sorption of Malathion by the sediments whereas with riverine water effective removal of the pesticide occurred only in an alkaline condition.

Intermediate concentration (50 ppm): At an intermediate Malathion concentration of 50 ppm, for the riverine and estuarine waters, the three sets of sediments recorded similar trends in their sorption behaviour and that their adsorption rates increased more or less steadily with the decreasing pH values. A reverse trend was visualized in the adsorption profile for the riverine water when the Malathion concentration was increased from 10 to 50 ppm, irrespective of the diversity of the sediment used; i.e. the adsorption was favourably shifted in the direction of the acidic pH. Synthetic seawater was also found to cause a similar behaviour with all the three sediment types. With coastal waters, the adsorption of

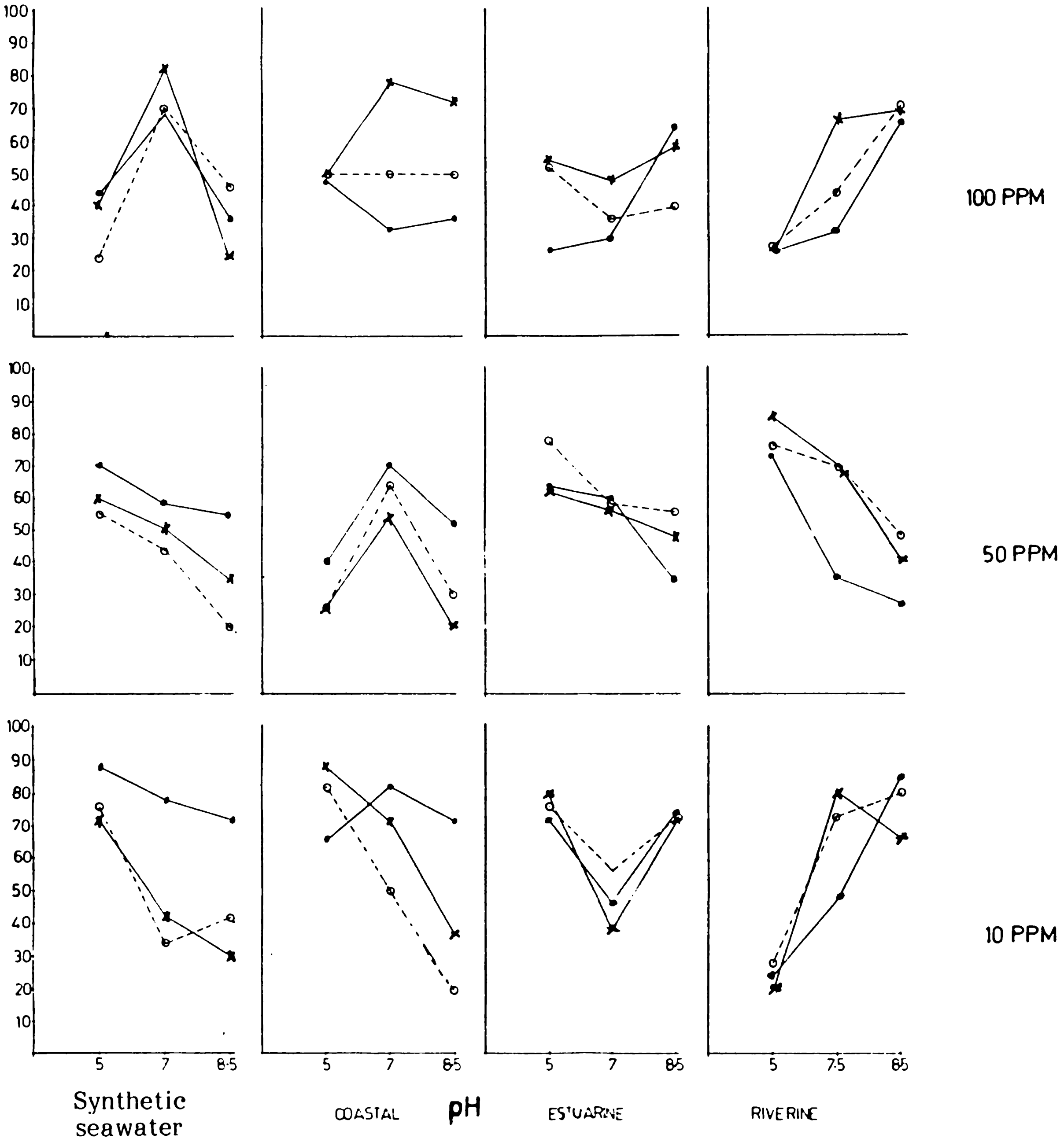


Figure 20.

Effect of pH on the adsorption of Malathion by divergent sediments

(polluted, x——x ; confluence, o- - - -o and less polluted, ●——●).

Malathion was found to be independent of the nature of the sediment and was maximum at neutral pH.

Thus, with riverine, estuarine and synthetic waters, sorption of Malathion was found to be most effective at an acidic pH, whereas, a neutral pH favoured effective sorption in coastal water samples.

High concentration (100 ppm): When the dosed pesticide concentration was maximized, the similarity in the sorptional behaviour underwent a drastic change and it became virtually impossible to project any general trend in the adsorption pattern.

Alkaline pH provided an excellent condition, in riverine water, for the removal of initially higher dosed concentrations, whereas with synthetic water, neutral pH favoured a maximum adsorption.

The above results have been meaningfully generalized in a schematic representation as given in Figure 21.

Methyl parathion

Another important OP pesticide viz. Methyl parathion was also subjected to similar sorption experiments. The graphical representation of the Methyl parathion adsorption at three dosed concentrations is given in Figure 22. The results obtained are summarized here under.

Low concentration (10 ppm): Irrespective of the nature of the water used in the experiments (whether coastal, estuarine or riverine) acidic pH was found to favour the adsorption of Methyl parathion and the percentage adsorption was found to decrease with increasing pH values. With synthetic seawater,

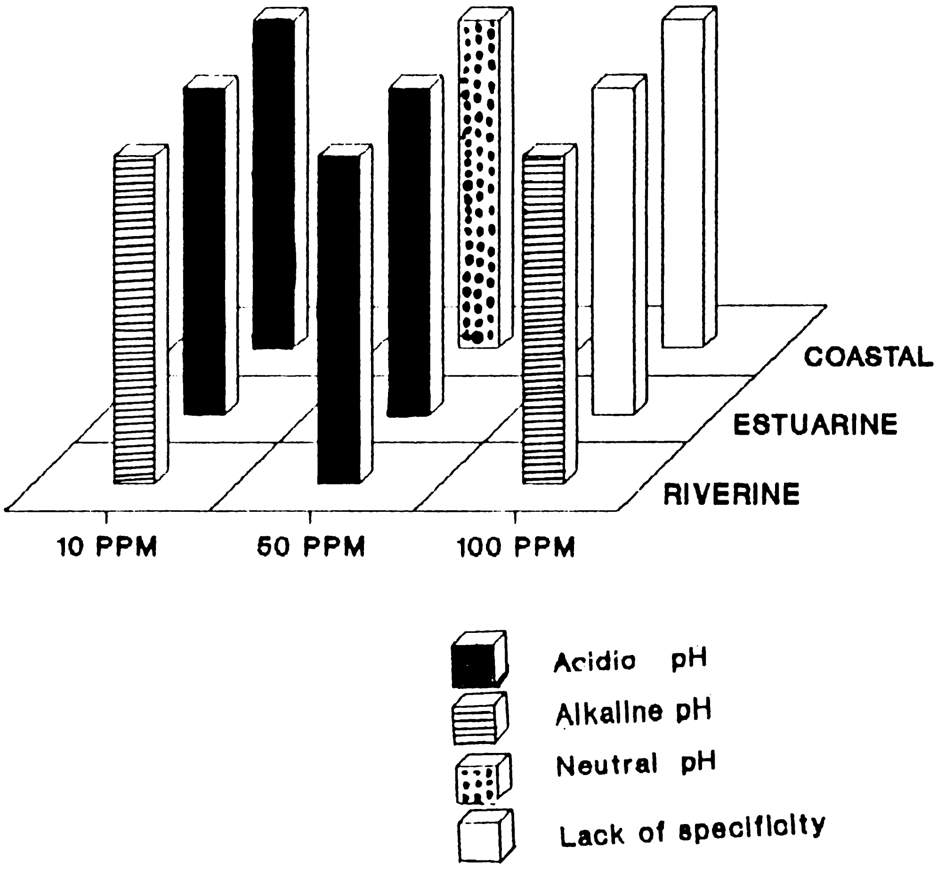


Figure 21.

Diagrammatic representation of the influence of pH on the adsorption of Malathion by aquatic sediments.

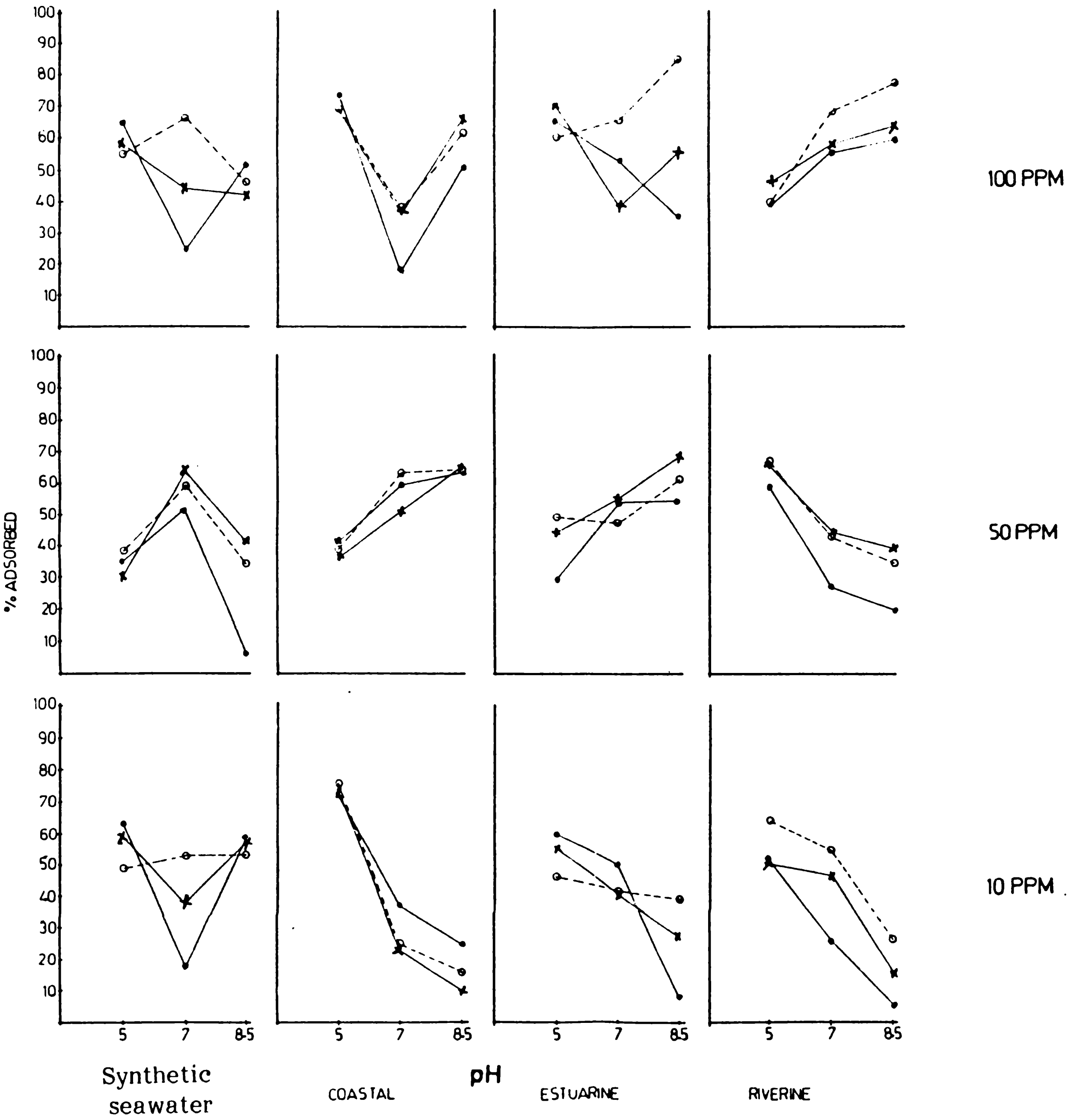


Figure 22.

Effect of pH on the adsorption of Methyl parathion by divergent sediments (polluted, x———x ; confluence, o- - - -o and less polluted, ●———●).

however, adsorption was found to be a minimum especially for LPS and PS at neutral pH.

Intermediate concentration (50 ppm): At the intermediate concentration the adsorption of Methyl parathion in riverine water resembled the lower concentration. In coastal and estuarine waters, the adsorption was found to be a maximum at alkaline pH. With synthetic seawater, the adsorption profile exhibited the maximum adsorption at neutral pH which is reversal to that observed for low concentration.

High concentration (100): At higher concentration of Methyl parathion no definite trend was visible in the nature of adsorption. In riverine water, the maximum adsorption occurred at high hydrogen ion concentrations. A similar behaviour was observed at higher concentrations of Malathion as well.

The adsorption profile is depicted in Figure 23.

A critical evaluation of the sorptional behaviour of the two pesticides would indicate that the influence of the effect of pH was more specific at low concentrations which exhibited an increased adsorption at acidic pH. It followed that adsorption of pesticides onto sediment would not be favoured by the pH conditions prevalent in the estuarine/coastal waters. Significant adsorption of pesticides could be expected to occur only in riverine waters which were inherently acidic in nature. The overall result can be represented as below:

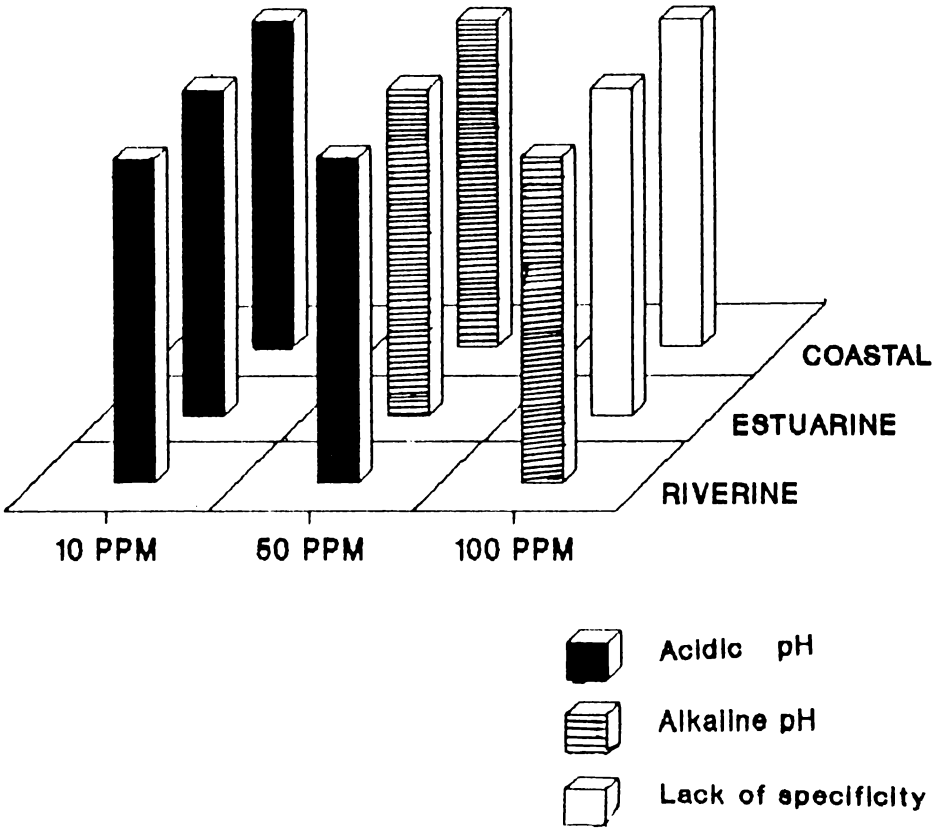


Figure 23.

Diagrammatic representation of the influence of pH on the adsorption of Methyl parathion by aquatic sediments.

Natural water sample	Concentration of pesticide		
	Low	Intermediate	High
R	OH^- H^+	H^+	OH^-
E	H^+	OH^-H^+	LS
C	H^+	OH^- OH	LS

R - Reverine
E - Estuarine
C - Coastal

OH - Neutral pH
H^+ - Acidic pH
OH^- - Alkaline pH
LS - Lack of specificity

Ionic strength (salinity)

Although the effect of ionic strength on the adsorption of non-pesticidal compounds viz., trace metals, nutrients etc., has been reported (Thanabalasingam and Pickering, 1985; Nair, 1990;), only very few attempts have been directed at quantifying pesticide adsorption (Murphy, 1970; Wolfe *et al.*, 1977 and Tulp *et al.*, 1979).

The studies were carried out at the three designated salinities of synthetic seawater (10, 20 and 30 x 10^{-3}) besides the salinities of the natural waters (coastal, estuarine and riverine). Procedural details of the experimental set up are given in Chapter II — Materials and Methods.

Malathion

The graphical representation of the influence of salinity on Malathion adsorption at the three dosed concentration are given in Figure 24 and results are summarized here under.

Low concentration (10 ppm): Salinity was found to exert a profound influence on the sorptional behaviour of Malathion onto the three sets of sediments. At the low concentration of Malathion, the adsorption capacity of polluted as well as confluence sediment was higher at water of maximum salinity. A reverse trend was observed for less polluted sediment in synthetic seawater.These characteristics of the less polluted sediments did not persist when the synthetic seawater salinity gradient was replaced with the salinity gradient of naturally collected seawater. Generally, irrespective of the nature of the sediment used in the experiment, the adsorption in natural waters was more or less directly proportional to the ionic concentration of the medium.

Intermediate concentration (50 ppm): At the intermediate concentration of Malathion, the percentage adsorption in natural waters exhibited a uniform pattern for three sets of sediments and was found to decrease with increasing ionic strength. Synthetic seawater, showed an overall increase in the percentage adsorption when the pesticide concentration was increased to 50 ppm. Riverine water recorded the highest adsorption of the pesticide at the intermediate concentration.

High concentration (100 ppm): At the high Malathion concentration of 100 ppm, adsorption increased from low saline (riverine) to high saline (coastal) waters. With synthetic seawater, confluence and polluted sediments showed a rather steady decrease in the rate of adsorption with decrease in salinity. The foregoing results are depicted below:

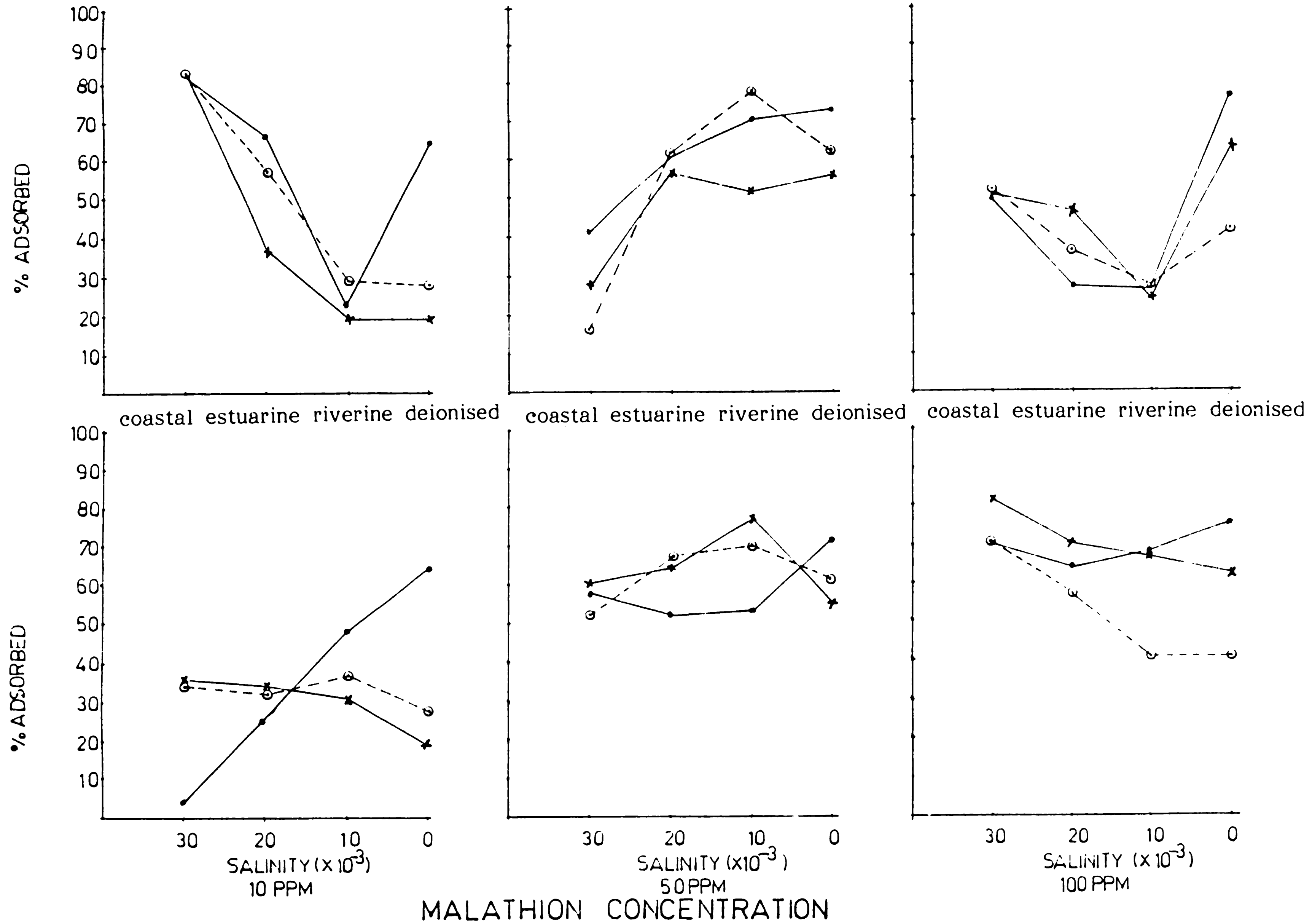


Figure 24.

Effect of ionic concentration on the adsorption of Malathion by divergent sediments (polluted, x———x ; confluence, o----o and less polluted, ●———●).

Adsorption Maximum

Salinity	Riverine	Estuarine	Coastal
Pesticide Concentration	Medium	Medium	Low/ High

Methyl parathion

The graphical representation of the influence of salinity on Methyl parathion adsorption at three dosed concentrations is depicted in Figure 25 and results obtained are summarized as follows.

Low concentration (10 ppm): The adsorption profile of Methyl parathion differed widely between synthetic as well as natural waters. Irrespective of the nature of the sediment, a maximum adsorption of >70% was observed in coastal waters whereas in synthetic saline water, the adsorption was reduced to <60%.

The adsorption capacity of LPS in synthetic seawater was found to be less than those of PS and CS. Though, the adsorption for the three types of sediments in the natural waters was alike, the coastal waters favoured maximum adsorption of Methyl parathion by all the sediments.

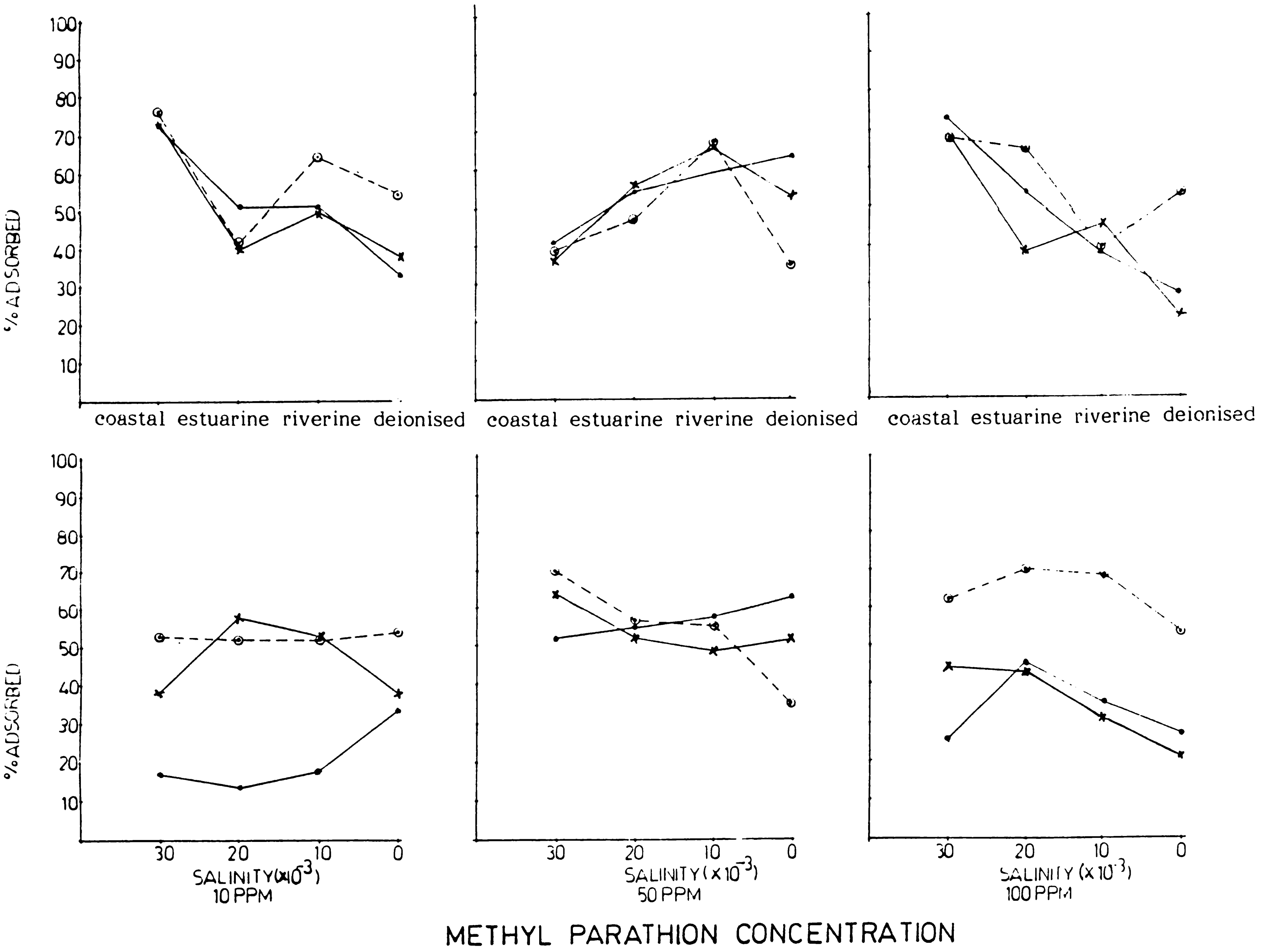


Figure 25.

Effect of ionic concentration on the adsorption of Methyl parathion by divergent sediments (polluted, x———x ; confluence, ⊙- - - - ⊙ and less polluted, ●———●).

Intermediate concentration (50 ppm): In synthetic seawater, the trend observed in adsorption pattern was more or less similar to that of Malathion (40 to 70%) and no specific trend emerged from the adsorption pattern exhibited by the sediment in synthetic seawater (the adsorption percentage varied between 25 to 65%.). Among the natural waters, the riverine water exhibited a maximum pesticide uptake.

High concentration (100 ppm): For synthetic seawater, the influence of salinity on adsorption showed no definite pattern with the three sediments, whereas, among natural waters, maximum adsorption was observed in coastal waters.

In natural waters, the adsorption decreased more or less steadily with decrease in ionic concentration. In general, the foregoing results on natural water samples can be represented as given below:

Adsorption Maximum

Salinity	Riverine	Estuarine	Coastal
Pesticide Concentration	Low/ Medium	Medium/ High	Low/ High

The following general trends emerged from the analysis of ffects of salinity on the adsorption variability of the ides:

- At an intermediate concentration of the pesticide, the riverine water favoured maximum adsorption, whereas no specificity was observed in estuarine water.

- The higher salinity of coastal water favoured the pesticide adsorption at its lower and higher concentrations.

4.3. PESTICIDE BUDGET OF COCHIN ESTUARY —— projections and predictions.

The estuarine environment around the metropolis of Cochin is as much exposed to its inherent variability and dynamism as any other estuary and the factors affecting the distribution of any contaminant would be the overall effect of the numerous forces that characterise estuarine reactivity. Hydrogen ion concentration and ionic strength are one but two of these which are significantly important though not overwhelming in its influence. The studies described above represent an attempt to predict the fate of pesticide in this estuary in terms of projected variations in these two salient features. The Cochin estuary is prone to the seasonal influence whereby appreciable saline water intrusion and a consequent increase in pH occurs during the non-monsoonal months. The southwest and the northeast monsoon lead to a fall in the salinity and concomitant processes of resuspension, resolubilization, coagulation, sedimentation, volatilization etc. lead to drastic variations in the hydrogen ion concentrations. It was in this context that this laboratory-oriented study was undertaken to delve deeper into the mysteries of estuarine sorption processes.

The CES could be generally categorized into three segments as indicated below:

Estuarine area	Lower	Mid	Upper
pH	>7.6 (slightly alkaline)	7 to 7.5 (neutral)	<7 (slightly acidic)
Salinity $\times 10^{-3}$	29	5 to 15	0

The pH and the salinity values employed in this study fell within the three segments identified above, so as to translate and apply the results of the study into the respective segments of the Cochin Backwater environment.

4.3.1. pH dependence

The results of the sorption studies indicate that the low pH prevalent in the upper estuary, which is largely riverine in character would not be conductive to the operation of a significant pesticide removal mechanism in this segment of the CES, provided the pesticide concentrations were kept low. Fluvial transport to the upper estuary would upset its characteristics and would consequently render the above hypothesis invalid. In short, the upper region of the CES could be expected to withstand increased dissolved pesticide concentrations. Similar was observed for the higher Malathion concentrations; but a reversal of the above trend was however observed with intermediate concentrations.

In the middle estuary (characterised by neutral pH) and in the lower reaches of the estuary (which is alkaline in nature) at all the concentrations, Malathion adsorption could be expected to be a minimum, thereby causing an increase in the aquatic levels of this pesticide.

The observed increased concentrations of Malathion in the waters of the Cochin estuary (Chapter III) clearly corroborate the projections of the (laboratory) sorption experiments.

With Methyl parathion (in low concentration), however, lower pH values in the upper estuary served to enhance the adsorption onto the sediments, thereby decreasing its availability in the aqueous phase. This divergence (as compared with that of Malathion) could only be attributed to perceptional differences arising out of structural variations.

4.3.2. Salinity dependence

The influence of ionic concentration on the sorption of the pesticides was seen to be more pronounced at high salinities. Higher ionic strength was found to enhance adsorption of Malathion at both low and high concentrations. The decrease in dissolved Malathion concentration associated with increase in salinity reported earlier (Chapter III) is a reflection of this observation. At the intermediate concentration employed in the experiment a maximum adsorption was observed when the ambient water had low salinities (riverine). The influence of pH on the adsorption of pesticides supported this observation which led to a decrease in the aquatic pesticide concentration. These results amply support the hypothesis that pesticide sorption in the natural aquatic environment which is governed by numerous physicochemical factors can indeed be predicted on the basis of laboratory experiments designed to quantify these forces individually.

CHAPTER V

CHAPTER V

TOXICOLOGICAL EFFECTS OF SOME BIOCIDES ON THE BIVALVE, *Villorita cyprinoides var. cochinensis*

5.1. INTRODUCTION

The increasing awareness of environmental problems during the last two decades has kindled much interest on the environmental fate and behaviour of xenobiotic chemicals. Recently, a vast number of pesticides have been liberally used in agriculture to combat the pest menace and thereby to produce higher yields from crops. These pesticides eventually find their way into the aquatic environment and indiscriminately affect the non-target organisms such as fishes, clams and prawns, which are of great economic importance to the common man. Generally, the potential impact of these pollutants is more on the aquatic organisms, because, in the hydrosphere, pesticides and such other substances are transported to much great distances than on land, and hence many more non-target organisms are likely to be exposed to them than in the terrestrial environment.

Synthetic pesticides, especially organochlorine and organophosphorus insecticides, are becoming increasingly abundant in chemical wastes which pollute natural aquatic communities particularly in inshore coastal areas. Since the early times of their use, these pesticides were recognised as extremely toxic to fish and other aquatic organisms and were considered hazardous because of their ability to kill or immobilize freshwater, estuarine and marine organisms even at extremely low concentrations.

Organotin compounds are widely used as additives in PVC stabilizers, and antifouling paints, and as biocides (fungicides and bactericides). The use of antifouling paints containing organotin compounds in recent years has resulted in the enormously elevated levels of these compounds being reported in areas of high pleasure-craft activity. Tributyl tin (TBT) compounds were found to have an effect on deformities in bivalves (Rexorode,1987) and retardation of limb regeneration and ecdysis in *Uca pugilator* (Weiss et al., 1987). TBT is generally lipophilic in character, and these compounds have a low solubility in seawater (Maguire *et al.*, 1983 and Laughlin et al., 1986). Since many estuaries are shallow with restricted circulation, it is only natural that estuarine animals would be more affected by TBT compounds than marine life from other environment.

Comprehensive investigations on the environmental hazards of pesticides have necessitated the testing of the toxicities of different pesticides to diverse aquatic organisms. The quality of the aquatic environment has long been assessed by chemical analyses, and also by biological surveillance of plant and animal communities. More recently, the biological responses of living organisms have been used to evaluate the environmental impact of aqueous wastes (Mac Kay et al., 1989).These bioassays can be used to forecast the impact of a substance prior to its release, or to monitor the actual effects manifested in nature.

There are innumerable chemicals that are routinely used in large quantities in the environment and it is virtually impossible to test the effects of all of them on entire ecosystems or even on many of the important species. It is estimated that only 5-10% of the known chemicals have been ever tested for toxicity, and less than 1% of the 50,000 or so compounds manufactured in any one country (United States) have been tested for their toxicity to aquatic organisms (Martell et al., 1988). These estimates clearly indicate that with all these efforts it has only been yet possible to merely scratch the tip of the ice-berg. Extensive and systematic toxicity tests are, therefore, necessary to evaluate the effects of potential pollutants on aquatic ecosystems.

The purpose of a toxicity test is to determine how toxic an agent is to the test species. Bioassays differ from toxicity test and the former are regarded as a tool for determining the concentration of a specific reagent or an effluent, either directly or by assessing the known response of an organism to the chemical. In toxicity test, the effect of a toxicant —— be it death, physiological, biochemical or behavioural changes or long-term effects on growth and reproduction —— on a specific organism is tested. Mount (1977) emphasized this distinction, stating that bioassay test measured only the potency or strength of a substance and as long as the response was sensitive enough, the test organism —— bacteria, invertebrates, or fish —— could be substituted one for the other.

Various testing methods are available for different toxicity studies. Short term or acute toxicity tests extending over a period of 96h is one of the most commonly employed tests for the evaluation of environmental impact of chemicals. Acute toxicity testing enables a preliminary evaluation of the possible environmental hazard posed by chemicals over a short period. Acute tests can be used to ascertain both the lethal and the sublethal effects. Studies on acute effects of pollutants are useful in assessing the threshold of response to a particular toxicant. Elucidation of the threshold concentrations at which physiological and biochemical changes occur in the tissue of aquatic organism should provide a more sensitive method for assessing permissible discharge limits. Chronic toxicity tests are those conducted over extended durations (varying from 30 days to several months) or spanning the entire life cycle or partial life cycle tests, embryo/ larval tests, bioconcentration/bioaccumulation tests, avoidance and other behavioural tests, biochemical/physiological tests etc.

Lethal tests are performed to determine the range of tolerance of the test species to the pollutants and evaluate the significance of environmental concentrations. The median lethal concentration or the LC50 value is the most often used measure of short term toxicity. This is the concentration at which 50% mortality of the test population is recorded in a given time. Dose-response relationships express the response of a population of individuals as a function of the toxicants concentration and of the duration of exposure. This type of analyses assumes that there is a relationship between the intensity and duration of exposure which resulted in the existence of a threshold concentration. This is called the incipient lethal concentration or time-independent LC50 (Giesy and Graney, 1989).

It has been frequently pointed out that acute tests, in which the observed response is death, do not take into account sublethal effects which occur at lower concentrations and which may have considerable ecological significance. The sublethal concentrations in the long run may cause more deleterious effects than the lethal concentrations, because subtle and small effects on the animal may alter their behaviour, feeding habits etc. Behavioural or morphological changes make the animal more conspicuous in the environment and more susceptible to predation or parasitization, thereby reducing the ability of the population to survive and reproduce (Murty, 1986). Likewise, subtle effects at the tissue or cellular level may affect the metabolism of the animal, and hence its ability to withstand stress. Some of the most useful sublethal tests are those directly related to the mode of action of the toxicants e.g,the effect of organophosphorus pesticides on the inhibition of acetylcholinesterase. The inhibition of this enzyme is well known as the means by which these pesticides disrupt the nervous systems of target/nontarget organisms at all trophic levels (Weiss,1959).

Toxic materials exist as atoms or molecules and as such elicit their toxic effects by interacting with biological systems at the cellular and subcellular level of organization (Giesy et al., 1988 and Versteeg et al., 1988). Therefore, the biochemical and physiological measures of toxicant-induced stress would be useful, as sensitive, specific predictors of effects at the level of whole animals to the effects of toxicants. Furthermore, since changes must occur at the biochemical, cellular and tissue levels of organization before effects can be observed at the organism level of organization, it has been suggested that such measures may prove to be useful to measure acute or more chronic effects at higher levels of organization (Mc Kee and Knowles, 1986). For this reason, changes in energy content (protein, glycogen, lipid and lactic acid content), RNA/DNA ratio, etc. have been suggested as potential short term, functional measures of effects which can be used to predict the effects of acute/chronic exposures to xenobiotics on the survival and fecundity of organism (Mitz and Giesy, 1985a and b; Mc Kee and Knowles, 1986; Graney and Giesy, 1986; 1987; 1988; Giesy *et al.*, 1988; Versteeg *et al.*, 1988 Reju, 1990 and Suresh, 1990).

The utility of biochemical and physiological parameters, which are often classified as "clinical" measures, is in determining the mode of action of chemicals under laboratory conditions and in determining the probable causes of lethal or sublethal effects under field conditions (Wedemeyer *et al.*, 1984; Versteeg and Giesy, 1985; 1986 and Versteeg *et al.*, 1988). There are several biochemical parameters which can be used in predicting chronic responses from acute exposures. The basis for the use of energetics is that energy is required to resist the effects of toxicants and to maintain homeostasis. Eventually, the organism expends all of its energy reserves, passes into the exhaustion stage, and ultimately meets death.

Concentrations of a variety of energy-related biomolecules in plasma/haemolymph/tissue, change predictably in response to stress. Under acute stress, these changes (such as increased plasma lactate concentrations) result either from increased locomotive activity or from hormonal changes designed to mobilize energy stores in order to respond immediately to stress-induced energy demands. The increased energy demand associated with stress may reduce energy stores such as glycogen and lipids and /or alter their distribution among tissues. Reduction or distribution of energy stores is generally considered to be a non-specific indicator responsive to any kind of stress, natural or man-made, and can be applied as a biochemical indicator in both field monitoring and laboratory hazard assessment experiments.

The class bivalvia is of interest to toxicologists, as they are sedentary filter-feeding invertebrates which accumulate toxicants from the environment. Since the pollutants are accumulated by these organisms to levels far above those found in the surrounding medium, they are widely used for monitoring the levels of pollutants in the water bodies.

The accumulation of chlorinated hydrocarbons by marine organisms has aroused grave concern, thereby triggered the establishment of aquatic monitoring programme all over the globe. The sedentary nature and rapid accumulating ability of the bivalves make them ideally suited for use as indicator/sentinel organisms in pollution monitoring programmes.

Bivalve molluscs form one of the most important commercial fishery resources that are widely distributed in the coastal waters (estuaries and inland systems). They are rich in protein and being so readily available, constitute a cheap source of high protein diet for the coast-dwelling common populace of this estuary.

Viewed against this background pollution of coastal water-bodies could be expected to lead to serious ramifications. Information on the tolerance levels and toxicological effects of pollutants are essential prerequisites for the development of pollution abatement strategies.

Reports on elevated levels of pesticide residues in estuarine/coastal environment are numerous. DDT, Dieldrin and PCBs were reported from tissues of marine animals from the Liverpool Bay (Riley and Wahby, 1977). The contaminant levels reported in the ovary and liver of Baltin flounder in 1981 were (values in $\mu g\ kg^{-1}$ wet weight): DDD-DDE, 3.1 to 92 in ovary; α-HCH, 0.7 to 6 in ovary and 2.9 to 13.9 in liver; and γ-HCH, 0.4 to 5.6 in ovary and 3.6 to 13.4 in liver (Westernhagen *et al.*, 1981). From the Aegean sea, all of the 142 fish and mollusc samples collected from 1975 to 1979 were contaminated with DDT and PCBs; ΣDDT in fish species was 23 to 1,200 $\mu g\ Kg^{-1}$ and PCBs was 310 to 2,600 $\mu g\ Kg^{-1}$ (Killkidis *et al.*, 1981). A survey of fish in Japan revealed the presence of total Chlordane in 1977 to 1979; Chlordane concentrations in Japanese fish were in the range of 3 to 489 $\mu g\ Kg^{-1}$ (Yamagishi *et al.*, 1981). Pesticides like Lindane, p,p'-DDE and p,p'-DDT were detected in fish samples collected from lake Mariut, Egypt (Saad, 1982). Nettleship and Peakall (1987) analysed organochlorine residue in the high Arctic species of colonially-breeding seabirds from Prince Leopold Island. HCH-isomers were detected in water and biota in the shore of Gallego river, Spain by Hernandez *et al.*,(1991).

Hansen (1980) found that in fish exposed for 9 days, Lindane was accumulated at a concentration between 10 and 70 µg l^{-1}. *Perna viridis* according to Phillips (1985) could be utilized as a bioindicator of PCBs and organochlorines. Atlantic salmon has been shown experimentally to accumulate increased levels of tin in various organs from exposure to low concentration (0.1-1.0 µg l^{-1}) of Tributyltin in solution (Davies and Mckie, 1987).The rate of Dieldrin accumulation by a fresh water filter-feeder, *Sphaerium corneum*, was determined in the field and under controlled conditions in the laboratory (Boryslawskyj *et al.*, 1987).

The vast majority of studies on xenobiotics relate to the assessment of sublethal effects on the aquatic species. Such effects due to pollutant-stress are now being recognised by regulatory agencies in formulating pollution control measures.

Sublethality of Permithrin, Cypermethrin and Fenvalerate to salmon, lobster and shrimp was observed by Metcalfe and Zitko (1980). D'Silva (1980) worked out the toxic effect of Malathion on a few marine invertebrates and it was found to be highly toxic to *Perna viridis*. A marked decline in immunological competency in the clam, *Mercenaria mercenaria* exposed to organic pollutants was reported by Anderson *et al.* (1981). The behavioural parameters of the fish, *Channa punctatus*, were enhanced under stress of Endosulfan exposure (Gopal *et al.*, 1985). Acute toxicity of Endosulfan on crab has been evaluated by Vijayakumari *et al.*, (1987). Modification in tissue metabolism was reported in the muscles of penaeid prawn exposed to sublethal concentration of Phosphomidon (Reddy and Rao, 1986). Asok and Anil (1988) assumed the subacute toxicity of

Aldrin (0.14 ppm) and Malathion (5.84 ppm), and the sublethal doses of both the pesticides caused hypochloremia at 15, 25, 30, 35, and 70 days after exposure. Effect of Aldicarb on the haematological and biochemical status of *Puntius conchonius* was studied by Pant and Singh (1983) and Pant *et al.* (1987). Weiss and Ma (1987) reported the toxic effect of pesticide, Diflubenzuron, on larval horseshoe crabs, *Limulus polyphemus*. Sublethal growth effects of Tributyltin to marine bivalves were studied by Valkirs and Davidson (1987).

Rajyalakshmi and Reddy (1988) reported that administration of sublethal doses of Carbaryl and Lindane to *Heterometrus fulvipes* resulted in a depression in the lipid content of hepatopancreas, pedipalpal muscle, etc.

Neurobehavioural changes were observed in the freshwater fish *Channa punctatus* when exposed to Fenitrothion (Ram and Gopal, 1991). Effects of organophosphorus pollution on genetic structure in two species of estuarine crab were observed by Mortimer and Hughes (1991).

Pesticide-induced physiological and behavioural responses were recorded on different estuarine organisms. Eknath and Menon (1978), studied the effect of organochlorine pesticides on the life and activity of non-target marine organisms. The combined toxicity effect of salinity and a pesticide, Quinalphos, was investigated on *Villorita cyprinoides* (Jacob and Menon, 1986). The toxicity of Fenvelerate to the prawns, *Penaeus indicus* and *Metapenaeus monoceros* was reported by Babu *et al.* (1988). The comparative toxic actions of pesticides were investigated on the euryhaline fish *Etroplus maculatus* (Gopalakrishnan 1990) and on the fresh water gastropod, *Pila virens* (Reju 1990).

Although the general awareness of the hazard of environmental pollution had resulted in the upsurge of interest in toxicological studies pertaining to the Cochin estuary, pesticide accumulations/interactions have been the least explored. It was this lacuna, that motivated the design of laboratory oriented chemotoxicity tests to quantify the effects of some indigenous pesticides on the estuarine clam *V.cyprinoides*.

This investigation was, therefore designed to probe into the changes undergone at the molecular level [eg. metabolic rate, energy content (variations in the glycogen, lipid and lactic acid content) etc.,] by clams exposed to the stress of the xenobiotics.

The biocides used in the investigation were:

- An antifouling agent, Tributyl tin oxide, TBTO
- An organochlorine pesticide, Endosulfan
- Two organophosphorus pesticide, Malathion and Methyl parathion.

These pesticides are the most prominent among a group of new generation pesticides being aggressively advertised/ popularised by the manufacturing concerns, and being widely used by the agriculturists.

Experimental details on sampling and conduct of lethal and sublethal toxicity tests were described earlier (Chapter II — Materials and Methods).

5.2. RESULTS AND DISCUSSION

5.2.1. Effect of biocides on respiration/metabolic rate

The respiratory system of the aquatic animals seems to be the prime target of most pollutants and the respiratory potential is an important physiological parameter that can be used to assess the toxic stress. Pesticides, heavy metals and other xenobiotics have been known to affect the oxygen consumption and the metabolic pathways in bivalves (Bayne *et al.*, 1985; Jacob and Menon, 1986; Prabhudeva and Menon, 1986; Mohan *et al.*, 1986a and b; Krishnakumar, 1987 and Sathyanathan *et al.*, 1988). The changes brought by the four biocides on the metabolic rate of the test animals are depicted in Figure 26 and the results are described below.

An overall decreasing trend in oxygen consumption was observed in all the animals, irrespective of their exposure times (24,48,72 or 96h), the maximum decrease being observed at the expiry of 48h duration. This reduction was more pronounced in the higher dosed concentration of the pollutants. At the medium sublethal concentration, the effect was intermediate to that observed for the low and the high concentrations. This was true of all animals no matter what the exposure concentrations or the exposure periods were.

Among the xenobiotics, TBTO, exhibited pronounced decline in the consumption. TBTO treated animals consumed only well below a tenth of oxygen consumed by the control animal. No appreciable differences were observed (in oxygen consumption) between animals exposed to 72 and 96h for the intermediate concentration of TBTO.

In the case of Endosulfan, exposure of the animals to 48h, resulted in decreased oxygen consumption in comparison to its

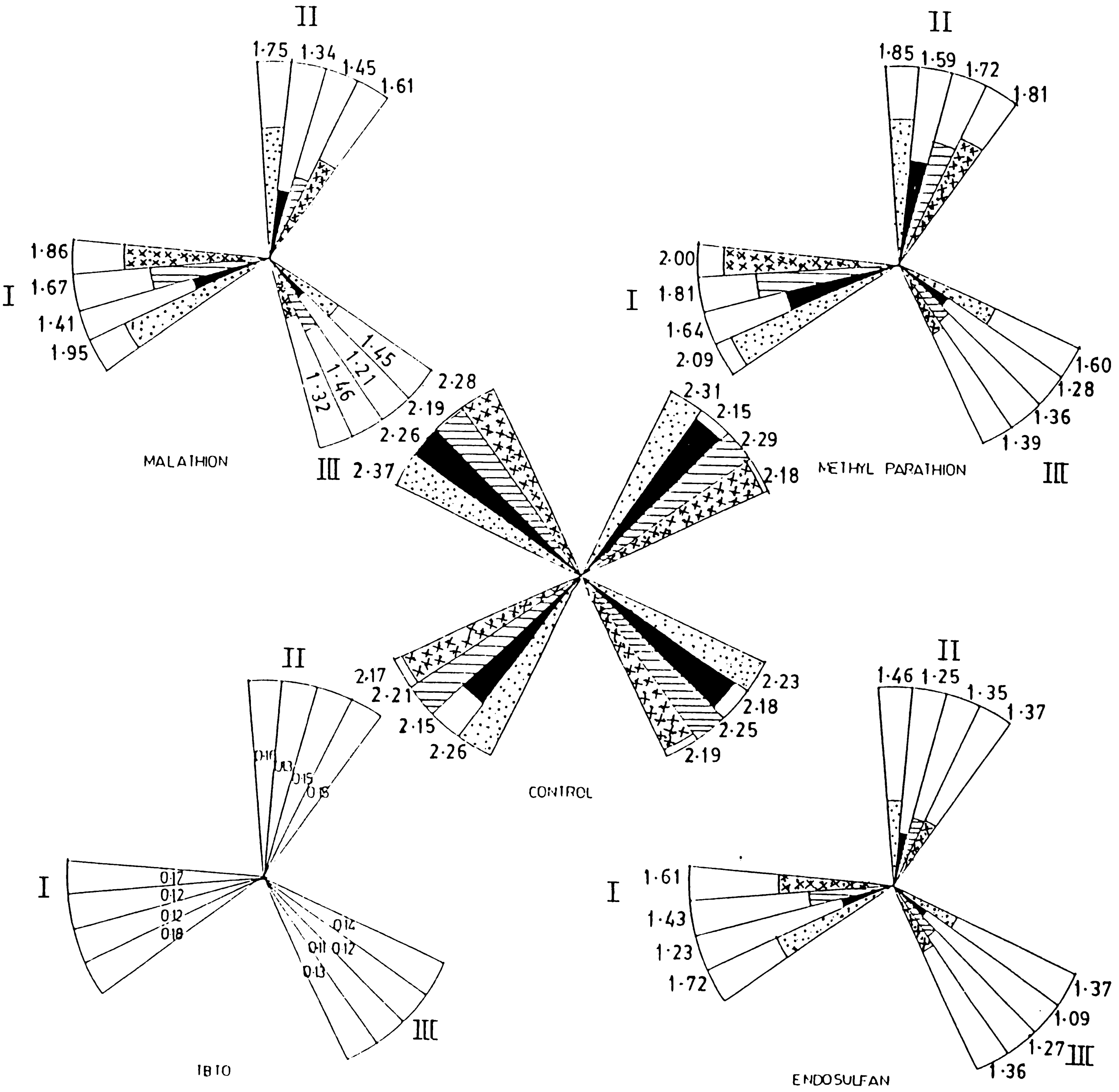


Figure 26.

Influence of exposure period (24 ░ ; 48 ■ ; 72 ▨ and 96 h ⊠) and biocide concentration (I-low; II-intermediate and III-high) on the oxygen consumption (ml O_2 g^{-1} h^{-1}) of V. cyprinoides.

24h-value; thereafter it recorded a slight increase. The oxygen consumption of animals exposed to the intermediate concentration of Endosulfan followed the same trend as observed for TBTO.

The animals exposed to the low sublethal concentrations of Endosulfan, Malathion and Methyl parathion consumed oxygen at the rate of 1.70, 1.95 and 2.09 ml O_2 g^{-1} h^{-1}, respectively. At high concentrations of the pesticides, consumption of oxygen by animal dropped to nearly half the control value(24h).

Percentage deviation from control

The percentage deviation (from control values) of oxygen consumption in animals exposed to the four pollutants is shown in Figure 27. Irrespective of concentrations, the rate of oxygen consumption in TBTO dosed animals declined by more than 90% of the control value. Among the other three pollutants, maximum decline (half that with TBTO) was observed when the animals were exposed to Endosulfan for a period of 48h. For the first two sublethal concentrations, the percentage decline in oxygen consumption (at 48h) was almost of the same magnitude for both Endosulfan and Malathion.

At the high concentration of Malathion, the 96h exposure period resulted in oxygen depression of ≈ 40%; however, for the other concentrations, it was reduced to <30%. Animal exposed to Methyl parathion, followed the same trend as observed with the other two pesticides. At the high concentration of the pesticide, the percentage decrease observed at 72h and 96h was strikingly similar.

Oxygen consumption is a valuable indicator of the energy expended to meet the demands of environmental stress. The overall effect of the four pollutants on the bivalve pointed to

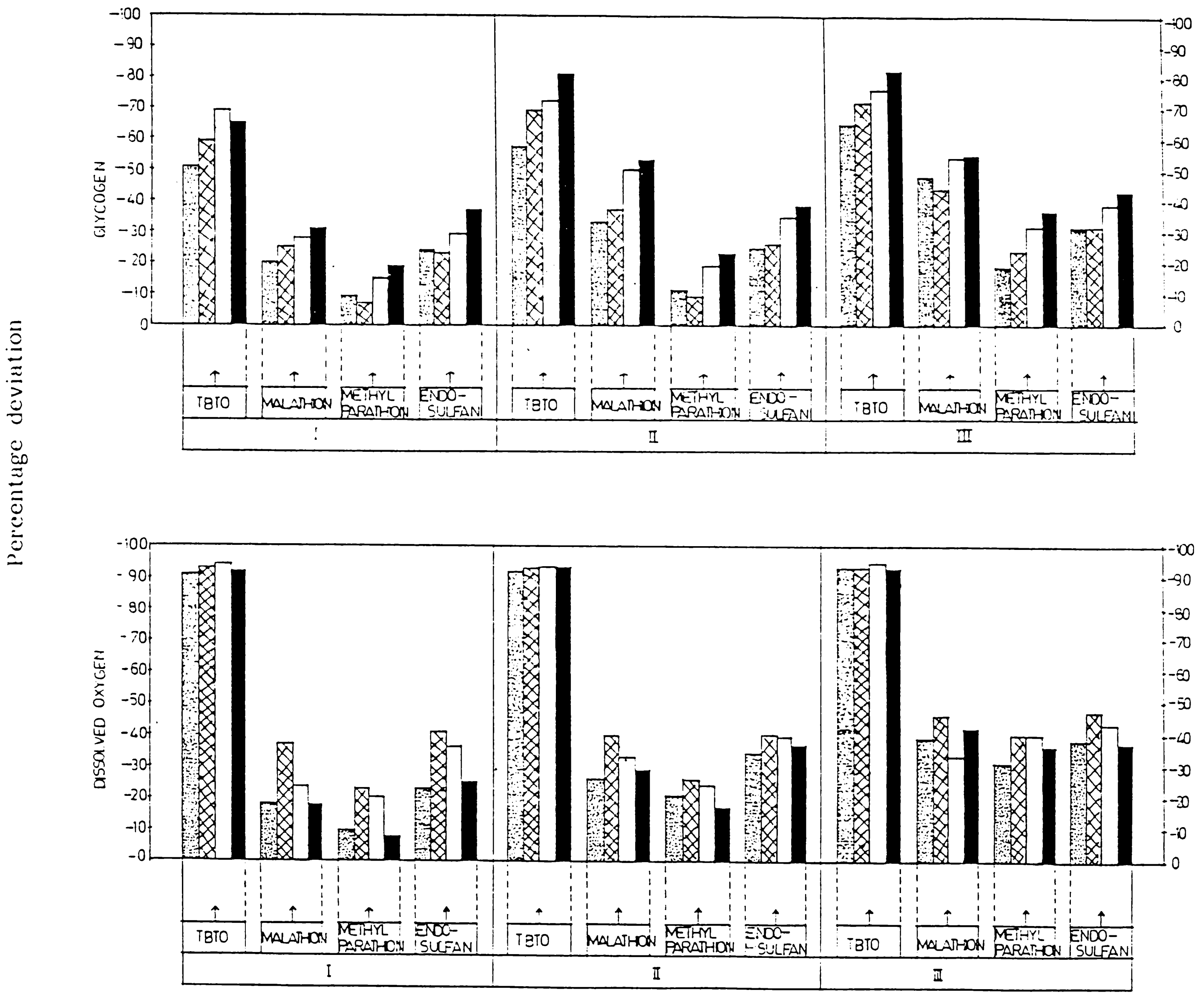


Figure 27.

Percentage deviation (from control) of oxygen consumption and glycogen content in *V. cyprinoides* to varying concentrations (I-low; II-intermediate and III-high) of the biocides for designated period the existence of a distinct relationship between the applied stress and the metabolic rate as observed. Lomte and Jadhav, (1982) reported that when pollutants gained entry through the gills of bivalves, the first physiological function to be affected was oxygen consumption.

Animals exposed to TBTO showed the least uptake of oxygen. The reduction in oxygen consumption was >90% for the three sublethal concentrations of TBTO, whereas, the other three biocides, recorded <50% reduction. When compared to Malathion and Methyl parathion, Endosulfan induced animals recorded greater reduction in oxygen consumption, possibly due to hypoxia induced by the pollutants.

The experimental results reported above agree with the generally observed increased relationship between respiratory rates and pollutant concentration. The drastic suppression in the rates of oxygen consumption may be due to the considerable damage inflicted upon the gill tissue and to the formation of mucus layers over it, causing decreased flow of water onto the gill surface, thereby leading to reduced oxygen intake.

Decreased oxygen consumption produces metabolic stress which could enhance the vulnerability of the organism to the toxicants. The decrease in oxygen uptake has also been attributed to valve closure, decreased ciliary activity and filtration rate, or direct impairment of the metabolic activity (Shapiro, 1964).

Although variations in oxygen consumption of aquatic organism exposed to pollutant-stress have been the subject of several investigations, only a few of them have dealt with the influence of pesticides, the large majority being concerned with the effect of metals (Mathew and Menon, 1983; Prabhudeva and Menon, 1986, and Hartwell *et al.*, 1991).

Scott and Major (1972) found that oxygen consumption was reduced in *Mytilus edulis* by ≈ 12% on exposure to 300 ppb of copper. Several bivalves have well developed and efficient mechanisms to partially regulate their rates of oxygen consumption even when present at low tentions (Bayne and Livingstone, 1977). Prabhudeva and Menon (1986) suggested that oxygen consumption was a product of two important factors, namely ventilation volume and the quantity of gas withdrawn from each litre of water. Therefore, changes in oxygen uptake from the waters by the animal and variations in the amount of water propelled through the gills resulted in fluctuations in oxygen consumption.

Murty *et al.* (1983) reported that oxygen consumption of *Labeo rohita* exposed to Fenitrothion, progressively decreased with increasing concentration of the insecticide. Endosulfan was reported to lower the oxygen uptake in the fresh water bivalve, *Indonaia caeruleus* (Mane *et al.*, 1983).

Uptake of pollutants in bivalves is intimately associated with the gills which transport the toxicants to the respiratory surfaces. Changes in the respiratory surface will alter the oxygen uptake rate and will eventually create a physiological imbalance in the organism.

The observed decline in oxygen consumption, after 24h, indicated the inability of the clams to adapt to the stress-induced by the biocides. This could be due to impairment of the physiological condition of the animal, resulting from poor oxygen transport.

Oxygen consumption has, thus been an useful measure of the sublethal pollutant effects on animals, because energy processes are direct indicators of the overall physiological state. The respiratory response appears to be a better index for use in monitoring pollutants and in predicting their chronic toxicity to aquatic animals.

5.2.2. Biocide-induced changes in biochemical constituents

Studies on the toxicity of insecticide to fresh water organisms have been mainly concerned with effects on fish. The present study undertaken to ascertain the effects of these biocides on the biochemical variations observed in *Villorita cyprinoides* exposed to dosed concentrations of TBTO, Endosulfan, Malathion and Methyl parathion, assumes relevance in the light of the sparse information available on toxicant-induced variation in biochemical constituents of bivalves. The concentration recorded for each sample is the mean values obtained from six replicates.

Variations in tissue glycogen

Glycogen is a branched homopolysaccharide polymer, which upon hydrolysis yields D-glucose, the readily mobilizable storage form of energy.

Variations in tissue glycogen level of the test species exposed to different concentrations of the biocides for varying periods of time are depicted in Figure 28.

An overall decreasing trend was noticed irrespective of the dosed concentration of the pollutants. Although, glycogen content generally responded negatively to increasing stress, it recorded a sudden depression (getting reduced to nearly half of the control value) when the animal was exposed to the high concentrations for 72h. Among the pollutants tested, TBTO was found to produce the maximum decline in glycogen content.

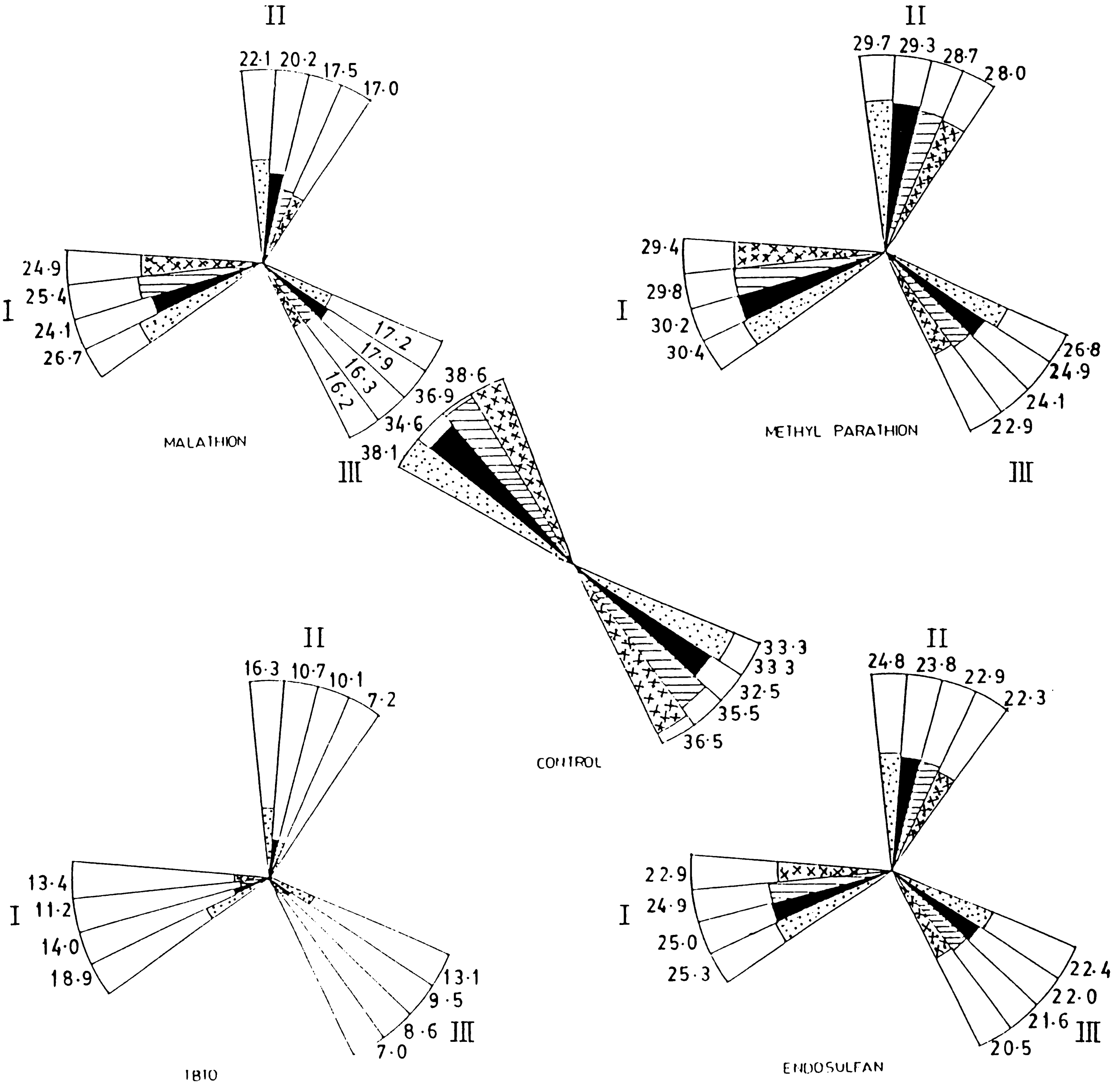


Figure 28.

Influence of exposure period (24 ░ ; 48 ■; 72 ▨ and 96 h ⊠) and biocide concentratio (I-low; II-intermediate and III-high) on the glycogen content (mg g^{-1} wet wt)of V.cyprinoi

Glycogen values in animals exposed to Endosulfan, were not found to be affected by exposure times or by the dosed concentrations. The values were therefore comparable and ranged between 23.213 ± 1.490.

In the case of Malathion, the animals exposed to the intermediate concentration recorded a progressively decreasing glycogen content, whereas, no clearly discernible trend was observed at the other two concentrations.

The effect of Methyl parathion on glycogen content of *Villorita cyprinoides* was similar to that due to Endosulfan. The deviations from control values were uniform at all the three test concentrations.

Percentage deviation from control

Deviations of glycogen content (expressed as percentages) from the control values are depicted in Figure 27.

TBTO, as noted earlier, was the most toxic of the four pollutants tested. All deviations from control values exceeded 50%, and a direct proportionality was observed between exposure concentrations and percentage deviations. The maximum reduction (85%) in glycogen content was detected for the high concentrations.

For the three sublethal concentrations of Endosulfan, <50% deviation was observed for the four exposure periods. At 24 and 48h exposure periods, the percentage of deviation was almost similar. However, at the expiry of the 72 and 96h periods a concurrent elevation in percentage reduction was recorded for all the test concentrations.

Although, Malathion produced greater deviations in glycogen percentages and appeared to be more toxic than Endosulfan, this behaviour was not in consonance with their lethal toxicity doses which were 6 and 4 ppm, respectively.

Malathion induced deviations increased with increasing exposure periods, except at the high concentrations which caused a decrease in the deviation at the expiry of the 48h period.

Methyl parathion proved to be the least toxic to the bivalves and produced the lowest deviations, which were <10% at the low, and the intermediate concentrations used. At the high concentrations, the effect of Methyl parathion, was found to be similar to that produced by Malathion at its low concentration.

In the present investigation, an overall decline in the energy content was noticed for all the biocides, viz, TBTO, Endosulfan, Malathion and Methyl parathion. Earlier workers had also reported similar trends. Glycogen levels were depleted upon exposure of the organism to metal stress, pesticides etc., (Reddy and Gomathy, 1977; Mukhopadhyay and Dehardrai, 1980; Gill and Pant, 1981; Sastry and Sunita, 1983; Chaudhary, 1984; Nath and Kumar, 1988; and Tabche *et al.*, 1991). Studies relating to pollutant-induced variations in the glycogen content of organism sampled from Cochin estuary include those on *Villorita cyprinoides* (Lakshmanan and Nambisan, 1985; Sathyanathan *et al.*, 1988 and Suresh and Mohandas, 1987).

Decrease in glycogen content is a manifestation of the adaptive response of an organism exposed to biocidal stress. The increased energy demand associated with the stress disturbs the carbohydrate metabolism (Shaffi, 1979a and b) and causes glycogen depletion which activates glycogenolysis (Ramamurthi *et al.*, 1968 and Ramalingam, 1988).

The overall decrease observed in the present investigation is a clear reflection of the above effect, and is well supported by the results of several earlier investigations (Reddy, 1980; Sastry and Sunita, 1983; Bhagyalakshmi *et al.*, 1984a and b; Venu Gopal *et al.*, 1990 and Ferrando and Andren-Moliner, 1991).

Pesticides in particular, have been observed to cause predictable alterations in energy biomolecules in blood and other tissues (Murthy and Devi, 1982 and Gluth and Hanke, 1985). Giesy and Graney (1989) have reported that pesticide toxicosis caused increased glycogen concentrations in mussel tissue with in hours of the onset of exposure and then to decrease with continuous exposure.

A marked decrease in liver glycogen of fish on exposure to sublethal levels of Malathion and Methyl parathion was observed by Mukhopadyay and Dehardrai (1980) and Srivastava and Singh (1981).

The overall decrement in glycogen is indicative of the increased rate of glycogenolysis, and the breakdown of glycogen and also suggests that during acute exposures, pesticides can cause predictable alterations in energy biomolecules and this could be attributed to an adaptation to the stressor.

Variations in tissue lipid

The lipids are heterogenous group of compounds (related to the fatty acids) which have, in common, the property of being soluble in organic solvents, but only sparingly soluble in water. Thus, lipids include oils, fats, waxes and related compounds.

Lipids are important dietary constituents because of their high energy value, of the fat-soluble vitamins and essential fatty acids contained in the fat of natural foods. In the body, fat serves as an efficient source of energy —— both directly and potentially when stored in adipose tissue. Lipids play many structural and metabolic roles, as it is the provider of a large proportion of dietary calories that they exert their greatest impact on metabolism and health.

The present investigation has been an attempt to understand the mechanism of biocide induced alterations in the total lipid content. In most of the cases, this energy source was found to be significantly lower in concentration in animals exposed to toxicants than in control animals. This clearly demonstrates the important role of lipid as supplementary energy reserves, which are utilized in the absence of or when there is a shortage of the primary metabolite, the carbohydrate.

The lipid content of the organism was kinetically influenced by all the four pollutants; the extent of deviation being dependant upon the toxic nature of the xenobiotics and of the test organism. The toxicant-induced variations are depicted in Figure 29.

Of the four biocides tested, TBTO was, clearly, the most toxic and exhibited a pronounced concentration/time-dependence. At the low concentration of TBTO, a 24h exposure resulted in lowering the lipid content to one half of the control value, whereas, it dropped to merely a third of the control value after 96h exposure to the high concentration.

An uniformly decreasing trend was observed in the case of Endosulfan-treated animals. The decreasing pattern, though time dependent, was not concentration-specific. The three sublethal test concentrations of this cyclodiene compound caused a

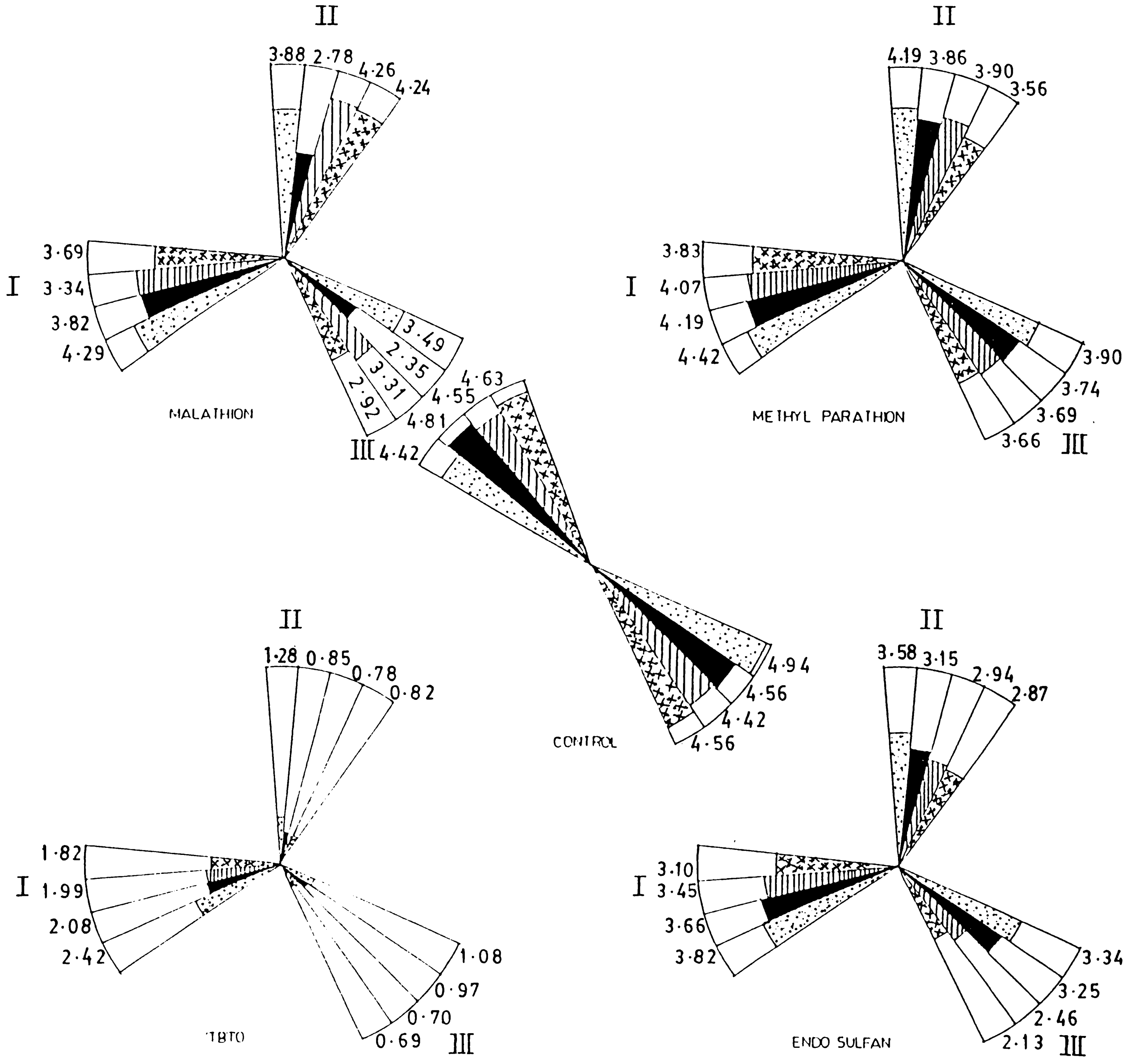


Figure 29.

Influence of exposure period (24 ░ ; 48 ■ ; 72 ▨ and 96 h ▩) and biocide concentrati (I-low; II-intermediate and III-high) on the lipid content (mg g^{-1} wet wt) of V. cyprinoid gradual decrease in the lipid levels after 24 and 48h exposure, whereas, after 72h exposure periods, lipid levels (at highest concentration of biocide) were drastically reduced to that of the control values.

Although, Malathion exposure also resulted in producing a progressive decline in lipid content, two special features made this study exceptional. At the low concentration, both 48 and 72h exposure periods recorded values of comparable magnitudes. But at the other two concentrations, the animal responded by increased lipogenolysis, whereby lipid concentrations fell to their lowest values.

Methyl parathion was similar to Endosulfan in its lipid lowering effect. Higher concentrations did not produce any appreciable variations in the lipid content.

Percentage deviation from control

Deviations of lipid contents (expressed as percentages) from the control values are depicted in Figure 30.

At the low TBTO concentration, the percentage deviation observed at 48h remained unchanged until 72h and recorded a further increase only thereafter. However, exposure of the animal to the intermediate concentration for periods longer than 48h, did not produce any further variations in its lipid content. High concentration of TBTO, produced changes in the lipid content only during the first half of the experiment, and the percentage deviation from control remained unchanged after 72h.

For the sublethal concentrations of Endosulfan, the deviations in lipid content varied between 30 and 60%. With the low and the high concentration of Endosulfan, minimum

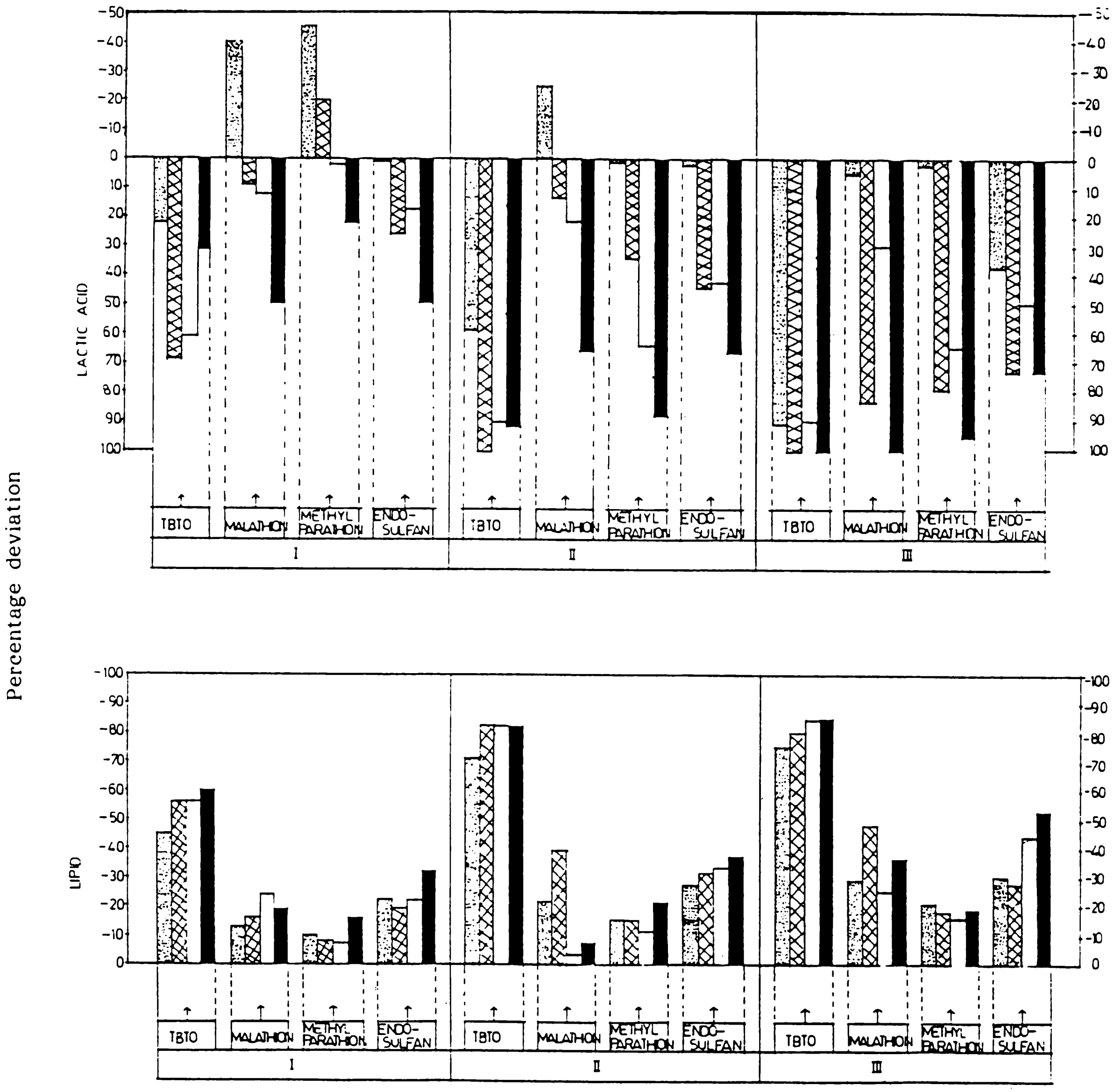


Figure 30.

Percentage deviation (from control) of lipid and lactic acid contents in V. cyprinoides to varying concentrations (I-low; II-intermediate and III-high) of the biocides for designated

(24 ▨ ; 48 ▩ ; 72 □ and 96 ■).

deviations were observed at 48h, whereas at the intermediate concentration, a gradually increasing trend was observed.

Malathion was peculiar in its effects on the percentage deviation of the lipid content. While 48h exposure of the animals to the intermediate and the high concentrations resulted in severe depletion (≈ 40%) of their lipid contents, 72h and above exposures to the intermediate concentration proved to be the least toxic (Producing only <10% deviation). Thus, the results indicate that the deviations were not concentration/time-dependent.

In the case of Methyl parathion significant effects were observed only for the higher concentration. A percentage deviation of ≈ 20% was observed when the test organisms were exposed to the intermediate and the high concentrations. At the low concentration, the deviation in the lipid content was <10% even when the animals were exposed upto 72h.

Next to carbohydrates, fats are the best energy source of the body. Therefore, under conditions of stress, the bivalves utilise lipid reserves to meet the increased energy demands and consequently entail a depletion of their lipid levels.

Tissue lipids of *Villorita cyprinoides* were monitored to assess the response of bivalves to varying exposure durations and pesticide concentrations. The general decrease in lipid content associated with increased xenobiotic stress, which emerged from the results of this study, is akin to most earlier reports (only sparse information was available on the bivalves). A significant concentration dependent-decrease in the lipid content was observed in *Cirrhinus mrigala* (Rao *et al.,* 1984) and *Heterometrus fulvipes* (Rajyalakshmi and Reddy, 1988) when exposed to Carbaryl.

The considerable decrease in total lipids might be a sequel to the efforts of the organism to replenish any glycogen deficiency (caused by any extraneous reason) and/or to mitigate the multi-furious toxic effects of the xenobiotics present in the animal (Rao and Rao, 1979 and 1981). Organic xenobiotics have been found to alter blood and tissue lipid levels of fish during chronic exposures (Chen and Sonstegard, 1984 and Verma *et al.*, 1984). Chronic exposure to the cod *Gadus morhua* to crude oil resulted in an increase in fatty acids and phospholipids and decrease in the triglyceride concentration. These effects indicated an enhanced mobilization and utilization of stored triglycerides to meet the increased energy demands (Dey *et al.*, 1983). The decrease in total phospholipids observed, when *Oncorhynchus kisutch* and *Oreochromis mossambicus* exposed to PCBs and Methyl parathion (Leatherland *et al.*, 1979 and Rao and Rao, 1984) respectively, could be attributed to their use of energy to resist a stress.

Tilapia mossambicus exposed to Methyl parathion recorded lower concentrations of total lipids and phospholipids (Rao and Rao, 1979). Ferrando and Andren-Moliner (1991) reported significant lowering of the lipid level in *Anguilla anguilla* exposed to Lindane.

When the animal body became contaminated with lipophilic material it seems likely that one of the first effects will be a reduction in their fluidity. This may occur either because of unsaturated double bonds of the constituent fatty acids are used up for the formation of `loose' bonds with the alien material, or because of the alteration of saturated/unsaturated ratio of the tissue.

Quantitative comparisons of the effects of pollutants used in the present study disclosed that lipids, the secondary energy reservoir, could serve as an immediate energy fuel in the bivalves, and the rate and extent of tissue lipid variation indicated that pollutant-impact stimulated lipogenolysis. Thus, utilization of lipid reserves may constitute a prime role in augmenting carbohydrate metabolism. These variations could serve as valuable indicators of the environmental pollution.

Variations in tissue lactic acid

Lactic acid is the final product of anaerobic carbohydrate metabolism and is the primary source of energy in aquatic organisms (Pickering *et al.*, 1982).

The present study was undertaken to elucidate the effects of biocides on lactate metabolism of an edible protein-rich bivalve, *Villorita cyprinoides*. The toxicant-induced variations are depicted in Figure 31.

Exposure to the biocides, generally resulted in augmenting lactic acid concentrations. Intermediate and high concentrations of TBTO, resulted in producing maximum elevations in lactic acid content. However, at the low concentration, the increase in lactic acid concentration was exposure dependent upto 72h.

Animals exposed to Endosulfan, recorded lactic acid concentration which increased progressively with higher dosed concentrations and exposure times.

The low and the intermediate sublethal concentration of Malathion showed a time-dose relationship. For both these concentrations, the lactic acid content at the 96h interval was twice the 24-h value. The high concentration of Malathion showed a similar trend as that of the two lower concentrations except at 72h. Although lethal toxicity-wise, Endosulfan was

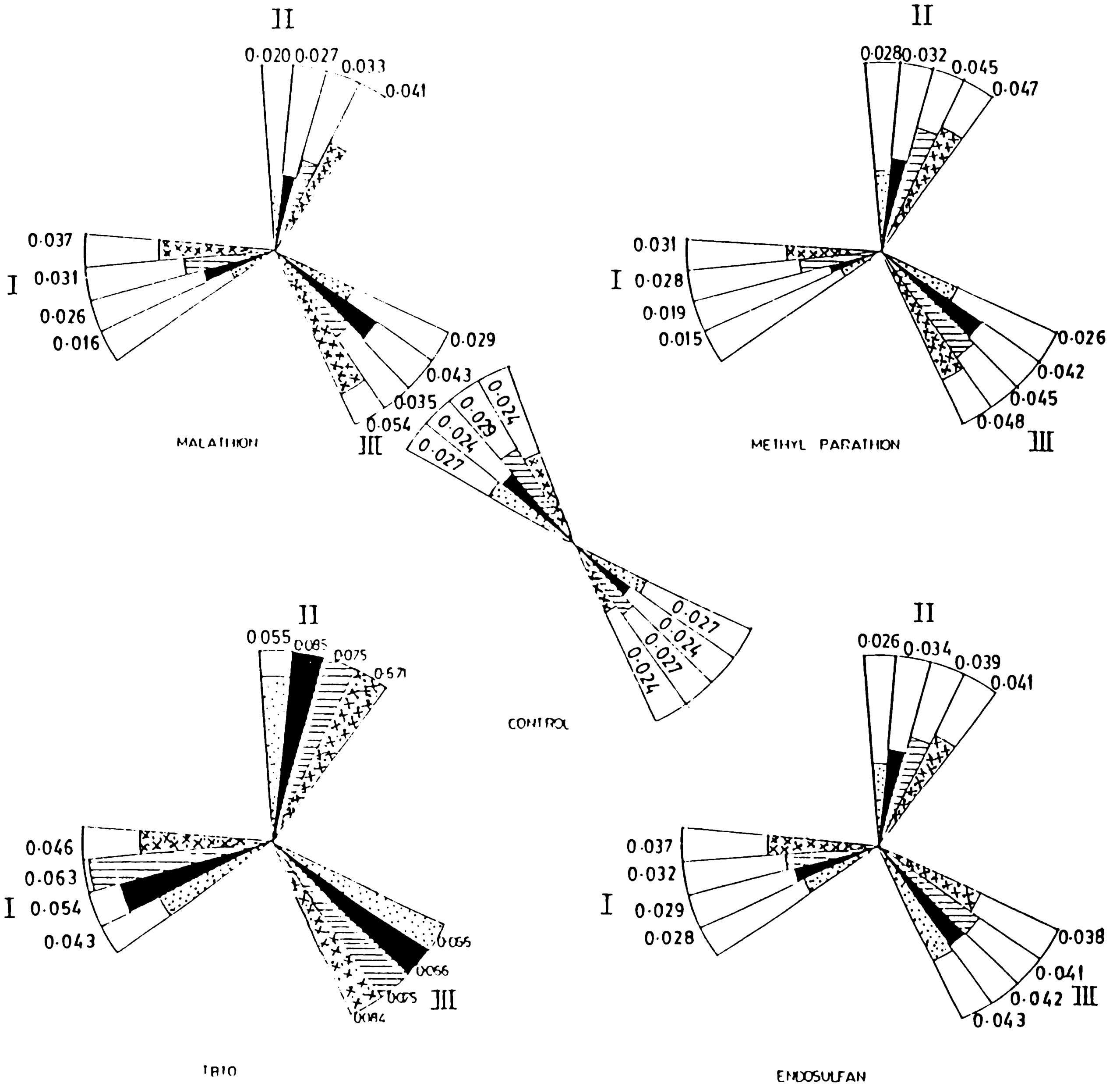


Figure 31.

Influence of exposure period (24 ░ ; 48 ■ ; 72 ▨ and 96 h ⊠) and biocide concentratio (I-low ; II-intermediate and III-high) on the lactic acid content (mg g^{-1} wet wt) of <u>cyprinoides</u>.

more toxic than Malathion, a reversal in the trend was observed as far as lactic acid contents were concerned.

96h-exposure of the bivalve to any of the designated concentration of Methyl parathion resulted in almost doubling the lactic acid concentration observed at 24h. 24h exposure, to the intermediate and high concentrations of Methyl parathion was found to effect a two-fold increase in the lactic acid content.

Percentage deviation from Control

Although the percentage deviations from control (Figure 30) generally increased with the increasing concentrations of TBTO, maximum deviations were observed for the 48h exposure. The effect of 96h-exposure to the low concentration was peculiar in that it resulted in lowering of the lactate content.

Endosulfan was inferior to TBTO in its effects on lactic acid content. 24h exposures to the low and intermediate concentrations produced conspicuously low lactate contents.

Of the four biocides, only Malathion and Methyl parathion produced negative responses in lactic acid contents. With Malathion, this decrease was inversely related to dosed concentration. The low and the intermediate concentrations generated similar trends, with depressions being affected by 24h exposures,and elevations being observed at all other exposures. 96h exposures to the high concentration produced the maximum positive deviation (96%) from control.

Intermediate and high concentrations of Methyl parathion produced positive responses at all exposure times, although the effect was negligibly small at the 24h exposure. The low concentration of Methyl parathion exerted a strikingly different influence on lactic acid content i.e., negative deviations (45 and 20%) were observed for the 24 and 48h durations.

High concentrations of all biocides were similar in their effects, and affected noticeable difference in the deviation; the 72h responses were always less than the 48 and 96h responses.

An overall elevation in lactic acid content in tissue is suggestive of the inadequate oxygen supply in the cells which is characteristic of the anaerobic lactate metabolism, hence, the high persistence of tissue lactate in bivalves exposed to pesticide stress. Thus the rate of lactate production was taken as an index of the physiological stress Mohan *et al.* (1987) also attributed the high lactic acid content in the fresh water mussel *Lamellidens marginalis* to pesticide induced glycolysis.

The experimental data revealed that pollutant-stress caused a decrease in glycogen content but concomitant increase in the lactate production implying that glycogen reduction was one of the main sources of lactate production (Black *et al.*, 1962). Similar results were reported for pesticides, metals, effluents etc. (Brown and Newell, 1972; Hunn, 1972; Mc Leay and Brown, 1975; Ramaligam, 1985 Mohan *et al.*,1987 and Ferrando and Andren-Moliner, 1991). Although elevation in lactic acid content was an evidence of the impairment of the aerobic metabolism (Mc Leay and Brown, 1974), elevation in lactate:pyruvate ratio alone could be taken as conclusive proof of the effect. Wedemeyer *et al.* (1984) reported that short term stresses caused rapid depletion of blood glucose and glycogen and an increase in lactic acid content. Elevated concentrations of lactate were reported in the blood of Indian cat fish exposed to a mixture of Aldrin and Formithion (Srivastava and singh, 1981). The insecticides, Thiotex and

Dichlorvos, were reported to increase the lactate concentration in the blood of the freshwater fishes (Verma *et al.*, 1983).

The biocide-induced lactic acid increase observed in this investigation indicate that the bivalves were under respiratory stress, and that the anaerobic breakdown of sugars was more effective than the aerobic pathway. A similar inhibition of pyruvic dehydrogenase coupled with enhanced lactic dehydrogenase has been reported by Verma *et al.* (1981) and Goel *et al.* (1984) in fish chronically exposed to pesticides.

The present study, thus, suggests that biocides interfered with carbohydrate metabolism by impairing glucose utilization, and rendering the animal under respiratory stress. The evaluation of the physiological and biochemical effects of contaminants on bivalves may, therefore, be a powerful tool in predicting and quantifying the impacts of these material on aquatic ecosystem.

5.3. CORRELATIONS

This section deals with a novel interpretative approach conceived to scoop out apparently submerged interrelationships existing between the different pairs of the physiological indices.

The physiological parameters were paired according to the following scheme:

- Lactic acid Vs Glycogen
- Lactic acid Vs Lipid
- Lactic acid Vs Dissolved oxygen
- Lipid Vs Glycogen
- Lipid Vs Dissolved oxygen
- Glycogen Vs Dissolved oxygen

The deviations from control of the individual parameters of each of these pairs were plotted to obtain the respective correlation coefficients. [The animals were exposed for four time-intervals 24, 48, 72 and 96h, to three different sublethal concentrations (low, intermediate and high) of each of the biocides. Therefore, n = 12 for each pair of the parameters].

Correlation coefficients obtained for the individual biocides revealed the existence of significant (Table 14) correlations between the three pairs of the physiological parameters, lactic acid Vs glycogen (r = 0.84 and 0.66); lactic acid Vs lipid (r = 0.71 and 0.46) and lipid Vs glycogen (r = 0.78 and 0.61) in bivalves exposed to Endosulfan and Methyl parathion, whereas, Malathion exposure significantly regulated the correlations between lactic acid and glycogen (r = 0.42) as well as that between lipid and dissolved oxygen (r = 0.61). In summary it may be mentioned that correlations between lactic acid and glycogen, lactic acid and lipid, and between lipid and glycogen have emerged as dependable indicators of pollutant stress.

Significant correlations were obtained between various pairs of physiological parameters for TBTO except those between lactic acid vs glycogen. Since a 24h exposure to TBTO had itself resulted in pronounced and drastic impact on the physiological parameters, any further proportional change commensurate with longer durations of exposure, was too unlikely to be expected. The low correlation coefficient (r = 0.12) observed for TBTO between lactic acid and glycogen levels merely portrayed the above trend, which was amply illustrative of its higher toxicity as compared to the other three pesticides.

An attempt was also made to obtain a theoretical projection of the cumulative effect of the source of the toxicants that

Table 14.

Interrelationship between biochemical indices of bivalves exposed to the different Xenobiotics

Physiological parameters	Correlation coefficients				
	Overall effect (n = 48)	Individual effect (n = 12)			
		TBTO	Malathion	Methyl parathion	Endosulfan
Lactic acid Vs Glycogen	0.46*	0.12	0.42	0.66*	0.84*
Lactic acid Vs Lipid	0.64*	0.78*	0.31	0.46	0.71*
Lactic acid Vs DO	0.54*	0.49	0.23	0.38	0.49
Lipid Vs Glycogen	0.86*	0.78*	0.16	0.61*	0.78*
Lipid Vs DO	0.90*	0.49	0.61*	0.38	0.20
Glycogen Vs DO	0.86*	0.44	0.41	0.62*	0.15

(*$p < 0.05$)

find their way into the aquatic system. For this, the deviations from control (referred to above) for each of the physiological parameters in the above pairs (obtained for the four biocides, which are likely to be present together in the CES), were pooled together to elicit the correlation coefficients existing between the parameters, when the bivalves are considered to be simultaneously exposed to all the four biocides. These plots (n = 12 X 4 = 48) are presented in **Figure 32**. For better appreciation of the results of this study on the correlations, a summary of the 'r' values for the different pairs of the physiological parameters (individual: n = 12 and overall: n = 48)are presented in **Figure 33**.

The results exposed a demonstrable dependence between the various biochemical parameters and indicated that all the pairs of parameters would be significantly correlated if the effects of the xenobiotics were considered in their totality. Similar variations were reported for *Sarotherodon mossambicus* which recorded a 43% reduction in its oxygen consumption when exposed to sublethal concentrations (0.001 ppm) of Thiodan for 48h and caused significant drop in the glycogen content (Vasanthi and Ramaswamy, 1987). This reduction in stored glycogen content indicated its utilization through anaerobic glycolysis to meet the energy requirement in pesticide-induced hypoxia. Reports on significant enhancement of plasma glucose and reduction of glycogen in various species of fishes exposed to Lindane, Methyl parathion, Malathion etc., are numerous (Rao and Rao, 1979; Mukhopadhyay and Dehardrai, 1980; Dubale and Shah, 1981; Lal *et al*., 1986 and Vasanthi and Ramaswamy, 1987).

The reduction of glycogen and lipid and the elevation of lactic acid in *Villorita cyprinoides* exposed to these biocides, may also be attributed to the physiological stress caused by the xenobiotics. Absorption of insufficient oxygen from the environment leads to impairment of the respiratory metabolism,

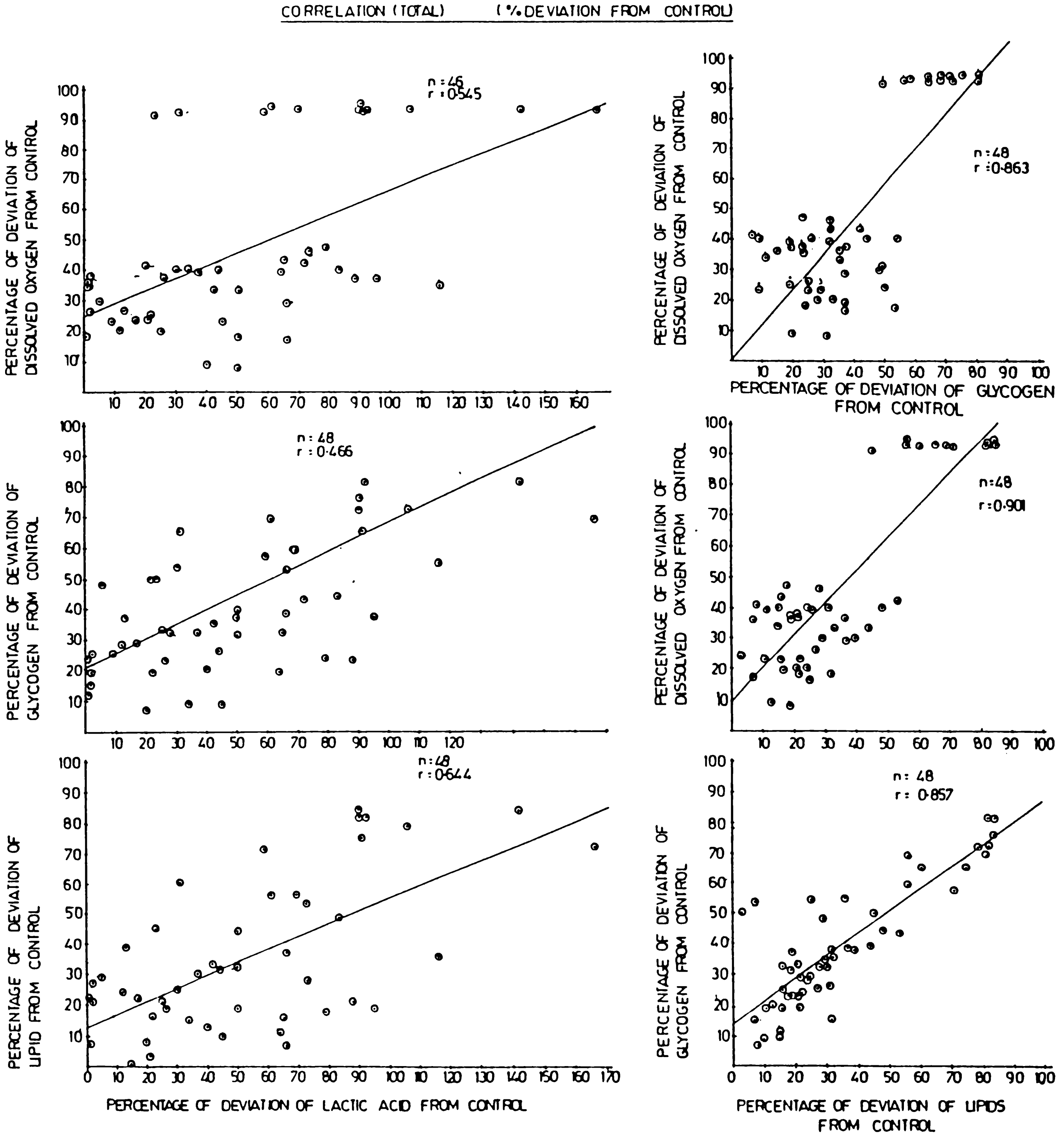


Figure 32.

Correlations between the percentage deviation (from control) of physiological paramete of the biocide - exposed bivalves.

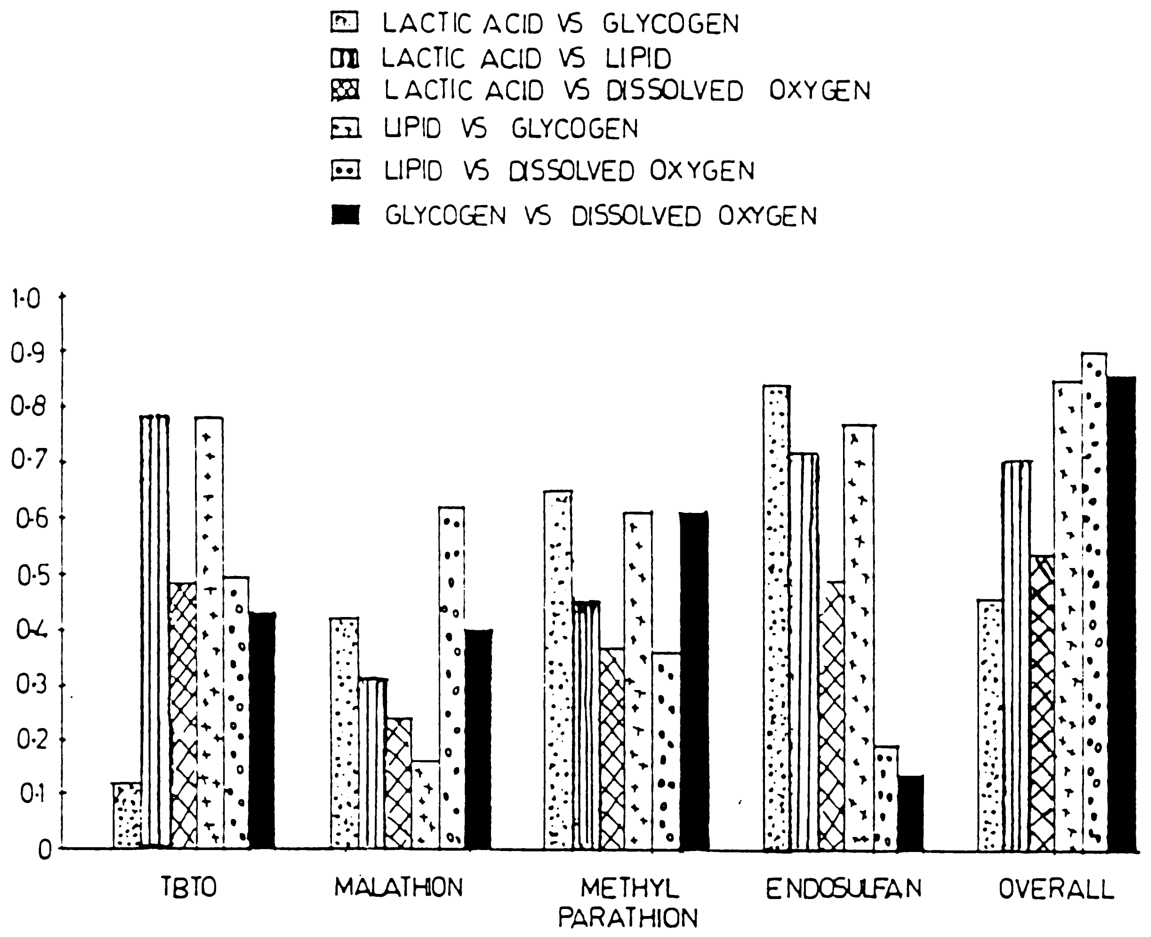


Figure 33.

Relative significance of correlation coefficients between the various physiological parameters of bivalves under biocide stress - individual (n=12) and overall (n=48).

and entails utilization of stored intracellular glycogen. Biocide-induced stress would therefore lead to depleted levels of glycogen and lipid and to elevated levels of lactic acid. The considerable decrease in total lipid which is an immediate source of energy during toxic stress was indicative of induced gluconeogenesis. The increase in free fatty acids with concomitant decrease in total lipid and phospholipid contents in the tissues was suggestive of increased lipolysis occurring during exposure to pesticides. Formation of free fatty acids due to the breakdown of lipid might yield the energy required to mitigate the additional impact of toxicants. The results of the present study lead to the conclusion that TBTO, Endosulfan, Malathion and Methyl parathion induced considerable stress on the respiratory metabolism, and variations in energy expenditure of the bivalve. If water pollution hazards are to be evaluated, tissue degeneracy is a much effective and logical criterion than other toxicity evaluation strategies.

Biochemical changes in bivalves presuppose a knowledge of the chemical constituents of its body as well as the transformations that these chemical constituents undergo within the body. Such study is also essential for the understanding of the balance that exists between the animal and its environment. In such economically important animal groups as the clams, a knowledge of the chemical composition of the body is important not only for the evaluation of their utility for food, but also for a more balanced utilization of the fishery resources of the region.

Besides ascertaining the relevance of physiological parameters in toxicological investigations, the above data, thus, remain a grim reminder of the realities of the aquatic system and emphasis the need for further in-situ/mesocosm studies for rigorous evaluation of toxicant impacts.

CHAPTER VI

CHAPTER VI

SUMMARY

Industrial pollutants, consisting of heavy metals, petroleum residues, petrochemicals, and a wide spectrum of pesticides, enter the marine environment on a massive scale and pose a very serious threat to all forms of aquatic life. Although, earlier, efforts were directed towards the identification of pollutants and their major sources, because of a growing apprehension about the potential harm that pesticides can inflict upon various aquatic fauna and flora, research on fundamental and applied aspects of pesticides in the aquatic environment has mushroomed to a point where it has become difficult to even keep track of the current advances and developments.

Chapter I exposes this grim scenario which urged me to initiate chemotoxicological studies on some of the more common pesticides. Besides defining the aim and scope of the work, an attempt has also been made to focus attention to the urgent need for evolving regulatory mechanisms to severely restrict the use of pesticides.

Chapter II is a compilation of the experimental methodologies adopted in this investigation.

Chapter III begins with a general commentary on the source, transport and fate of pesticides in aquatic ecosystems. Reports on the residual levels of pesticides in the CES, investigated seasonally and temporally using Gas chromatographic methods of analyses ensue. This represents the first attempt to generate data for the establishment of baseline levels of DDT, HCH, Endosulfan and Malathion in this Backwater system. The most prominent organochlorine pesticides were the DDT and HCH compounds. Though their concentration-levels were generally below the permissible limits, their potential for delayed effects on the aquatic organisms remained a distinct possibility.

A progressive reduction in concentrations of both the OC and OP pesticides, were generally observed from the upper estuary to the lower estuary. However, during monsoon, the estuary was characterized by the absence of o,p'-DDT, Endosulfan and Malathion residues. The Stations designated as 2 and 5 were observed to be, in most cases, "pesticidal loading sites" and clearly reflected their proximity to the pesticide manufacturing unit and to the outfall of urban runoff/municipal sludge. The analytical results have, thus, enabled the identification of the pesticide "hot spots" in the estuary. It is hoped that these results which have enabled the identification of the "hot spots" would serve as the much wanted baseline data to trigger further studies in the pesticidal levels in the River Periyar and the adjoining Cochin estuarine system.

The two well identified sources of pesticides which control/regulate their fluvial loading in this water way were observed to be (1) agricultural land runoff and (2) effluent discharges from the manufacturing units.

The Chapter IV has evaluated the sorption characteristics of Malathion and Methyl parathion by three environmentally divergent sediment samples. Malathion sorption by sediments with a pollution gradient occurred via a heterogeneous, multilayer adsorptive process that could be described by either the Freundlich or the Elovich isotherm. Significant correlations existed between organic carbon content and partition coefficient as well as between organic carbon content and adsorptivity.

Sediment sorption with respect to the two pesticides was quantified in terms of a new parameter 'S', the Sorptive Index. At lower concentrations of the pesticide, rapid adsorption occurred at the surface while at higher concentrations, when the surficial adsorption sites were almost fully used up, the amount of pesticide adsorbed tended to plateau off; there was even a marginal increase in the pesticide adsorption as a result of the multilayer-adsorption process. The results of the studies conducted to assess the influence of environmental factors on pesticide sorption were also included in this chapter. The studies reported in Chapter IV have enabled the quantification of the sorption process, which partitions the pesticides among the constituent phases of the aquatic system. From a knowledge of the residual levels of pesticides in coastal waters (such as reported in Chapter III), it should now be possible to comment, though vaguely, on the sorptional features of the pesticide in the Cochin Backwater System. However, detailed investigations are required for evolving a rigorous predictive model.

Pesticide residues pose a greater hazard in the aquatic ecosystems than in the terrestrial ecosystems. Therefore, a concerted effort was undertaken to monitor and evaluate the effects of some xenobiotics on the estuarine bivalve *V. cyprinoides*. The approach was based on the premise that an environmental perturbation would result in responses in measurable biological parameters like metabolic rate, glycogen, lipid, lactic acid etc. The results have been presented in Chapter V. Tributyl tin oxide was observed to inflict a greater deleterious effect upon the organism than any of the other biocides.

The overall effect of the four pollutants on the bivalve pointed to the existence of a distinct relationship between the applied stress and the metabolic rate. Thus the respiratory response appeared to be a better index for use in monitoring pollutants and in predicting their chronic toxicity to aquatic animals. An overall decline in the energy content (glycogen and lipid) and an elevation in the lactic acid content was attributed to be a manifestation of the adaptive response of an organism exposed to the biocidal stress. The overall elevation in lactic acid content was suggestive of the inadequate oxygen supply in the cells which had resorted to anaerobic lactate metabolism. The biocide-induced lactic acid increase observed in this investigation indicated that, in bivalves under respiratory stress, anaerobic breakdown of sugars was more effective than the aerobic pathway.

Determination of physiological responses in an organism exposed to pollutant stress affords a possibility for early detection of potential biological harm. It is obvious that simultaneous observations of a number of physiological parameters would be necessary to distinguish between responses due to temporary environmental or natural stresses and those which are an early reflection of a potentially lethal condition. Such studies conducted on individual organism could signal the onset of the biological damage, which the population and the communities may shortly be exposed to.

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