STUDIES ON THE EFFECT OF THE ORGANOPHOSPHORUS PESTICIDE EKALUX(R) EC 25 ON THE BACTERIAL FLORA OF *VILLORITA CYPRINOIDES* VAR. *COCHINENSIS* (HANLEY)

Thesis submitted to
Cochin University of Science and Technology
in partial fulfilment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY

by

K. R. SREEKUMARI, M.Sc.

DIVISION OF MARINE BIOLOGY, MICROBIOLOGY AND BIOCHEMISTRY
SCHOOL OF MARINE SCIENCES
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 016

SEPTEMBER 1988

ASATO MĀ SAḌ GAMAYA

TAMASO MĀ JYOTIR GAMAYA

MṚTYOR MĀ AMṚTAM GAMAYA ---

DEDICATED TO

MY BELOVED SISTER LATE

MISS. SHEEMA.S. PILLAI

CERTIFICATE

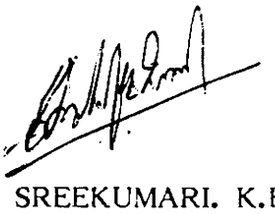
This is to certify that this thesis is an authentic record of research carried out by Ms.. Sreekumari. K.R., under my supervision and guidance in the School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements of the Ph.D. Degree of Cochin University of Science and Technology and no part thereof has been presented before for any other degree in any University.

Dr. P. Lakshmanaperumalsamy,
Reader,
Department of Environmental Sciences,
Bharathiyar University,
COIMBATORE - 641 046.

Coimbatore,
22 ..9..1988.

DECLARATION

I hereby declare that this thesis entitled "Studies on the effect of the organophosphorus pesticide Ekalux(R) EC 25 on the bacterial flora of Villorita cyprinoides var. cochinensis (Hanley)" has not previously formed the basis of the award of any degree, diploma or associateship in any University.



SREEKUMARI. K.R.

Cochin - 16,
22 ..9..1988.

AN EXPRESSION OF GRATITUDE

This humble piece of work . . . . . .

Its successful completion . . . . . .

Definitely is a boon from the Almighty, who has been an unfailing source of strength and comfort to me throughout all these years of earthly strife.

Words are few to express my heartfelt gratitude to my supervising teacher Dr. P. Lakshmanaperumalsamy, Reader, Department of Environmental Sciences, Bharathiyar University, Coimbatore, for all the help, guidance and encouragement given to me. It was an exhilarating experience, to be guided by him.

I am deeply indebted to Dr. M. Chandrasekaran, Lecturer, Department of Applied Chemistry, CUSAT, for the keen interest, valuable advice, and sincere encouragement, he has given during the course of this work.

My sincere thanks to the Director and faculty members of the School of Marine Sciences, especially Dr. N.R. Menon and Dr. R. Damodaran for their kind hospitality in making me feel at home during the tenure of my research in the institution.

I feel obliged to Dr. D. Chandramohan, Scientist-E, NIO, Goa; Dr. R. Natarajan, Professor, CAS in Marine Biology, Porto Novo; Dr. I. Karunasagar, Associate Professor and Head, Department of Fishery Biology, College of Fisheries, Mangalore and Dr. K. Dhevendaran, Reader, University of Kerala for the valuable discussions, I had with them.

I am grateful to the administrative and library staff of CUSAT for their courtesy and help.

I also thank the Director, CIFT, Cochin and the Director, CAS in Marine Biology, Porto Novo for permitting me to utilise the available library facilities.

I wish to place on record my gratitude to the University Grants Commission for the financial support in the form of Research Fellowship in the DRS programme.

I also thank Mr. M.G. Mani and Miss. Preetha, Coastal Impex for the ardous task of typing the manuscript neatly.

I am greatly indebted to my dear friends, Mr. K. Nandakumar, Mr. K.P. Jayakumar, Mr. K. Faisal and Mr. P.M. Mohammed Sagir whose love and care kept me enthusiastic throughout the study period.

I owe my gratitude to all my colleagues and friends: Dr. R. Pradeep, Dr. I.S. Bright Singh, Dr. Rosamma Philip, Mr. Jose P. Jacob, Mr. C. Mohandass, Miss. P. Mercy Mani, Miss. Suneela, S.S, Miss. P.V. Lekha, Mrs. P.G. Meena, Mrs. K.A. Dhoulath, Mrs. Geetha Bhaskar, Miss. Supriya, R, Miss. Rani Jose, Mrs. Jalaja, V, Mrs. Sreekala, K.R, Miss Rosily, N.P., Miss. Nolly, J, and Miss Accamma Joseph, for being the source of encouragement, support and inspiration to me at various stages of this work.

My parents have been great pillars of strength, whose constant support has lead me to draw this work to an end. I also thank my brothers, 'Saramma' and relatives, who have offered their prayers for the grand success of my research work.

.....

CONTENTS

****

INTRODUCTION

1. INTRODUCTION

1.1. Preface

There is growing concern throughout the world at the way man is affecting and often damaging his environment. Kinne (1984) has defined pollution as "the introduction by man, directly or indirectly of substances or energy into the marine environment (including estuaries), resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of seawater and reduction of amenities". The most dangerous group of pollutants comprises pesticides and related chemical compounds (Kinne, 1980). Currently over 500 active pesticidal chemicals, formulated into more than 5000 commercial products primarily as herbicides (36%), insecticides (33%) and fungicide) (24%) are available to farmers (Munnecke, 1978). It has long been recognized that with such a large chemical market of pesticides, there is also the problem of environmental pollution. Unwanted discharge of pesticides into the environment can occur from numerous sources.

Microbes are present in all environmental situations in which pesticides are used and they will therefore encounter such anthropogenic chemicals, however inadvertantly, and probably react with them in some way. In simplest terms, such interactions can be considered as 1. the action of microbes on pesticides and 2. the action of pesticides on microbes. The destruction of beneficial bacterial flora or shifts in the balance of populations of different species may have marked effects on agricultural productivity. So, the study of the effects of pesticides on

bacteria is clearly of great importance. On the other hand, it has been found that some microorganisms, though destroyed by particular pesticides, may flourish in the presence of others and even use them as energy sources. The ecological problems related to the persistence of toxic chemicals in the environment, can obviously be affected by the action of microorganisms which degrade pesticides and so prevent them from having long-acting effects. This again is an important aspect to be studied deeply (Hill and Wright, 1978).

The pesticides are largely or entirely foreign to the marine environment. The most endangered sea areas are those near the primary source of pollutant release such as industrial sites on the coasts, estuaries and bays. Estuaries and mud flats have shown to trap, retain, and accumulate pollutants in their sediments. At the same time they have important ecological functions for recruiting and supporting life in adjacent sea and land areas (Kinne and Bulnheim, 1980). Occurence of a variety of pesticides in estuarine and coastal environment clearly indicate that a certain quantity of the pesticides used in the terrestrial realm is ultimately reaching the aquatic system.

The bacteria associated with clam, play a role in nutrition, growth and disease susceptibility of the host. The quantitative and qualitative changes of flora depend on various factors and the influence of pesticide on number and type of bacterial flora are unexplored. The present investigation is aimed at collecting the basic information and to understand some physiological characteristics of bacteria associated with clam which has been subjected to pesticide treatment.

1.2. Review of Literature

The era of modern synthetic pesticides largely dates from 1939 when the insecticidal properties of DDT were discovered. Pesticides today, include insecticides for insect control, acaricides for mite control, nematicides for the control of eelworms, rodenticides for the control of rats and mice, fungicides and bactericides for the control of plant diseases and storage rots and herbicides for the control of weeds. Pesticides are used in agriculture for three main purposes: to produce a larger yield of crop, to produce a crop of higher quality and to reduce the input of labour and energy into crop production. The extend to which different types of pesticides are used varies with the differing agricultural and sociological conditions which exist in various parts of the world. Apart from normal application to crops in the field, pesticide usage is important in a number of other situations. The use of insecticides and fungicides is essential in both the preservation of stored products and in the disinfection of storage premises and flour mills. Pesticides of all types are used in forestry (Hill and Wright, 1978).

The entry of pesticide into aquatic environment occurs intentionally and unintentionally. The principal direct and intended modes of pesticides entry include, the use of pesticide sprays and granular formulations to control water inhabiting pests; disposal of domestic and industrial wastes; disposal of unused pesticides; on site cleaning of application and mixing equipment and decontamination procedures; disposal of commodities containing excessive residues; and the use of biocides to prevent fouling of industrial cooling waters. Pesticides may unintentionally and indirectly enter aquatic systems by drift from aerial or ground

application of pesticides and movement via wind, water and soil erosion (Nicholson, 1967; Graetz et al. 1970; Hill and Wright, 1978).

1.2.1 Degradation of pesticides by means other than bacterial action

The chemistry, degradation and mode of action of pesticides were thoroughly studied (Getzin and Rosefield, 1968; Wolfe et al., 1976; Hill and Arnold, 1978: Williams and Biddleman, 1978; Plimmer, 1979; Freed et al. 1979; Ku et al. 1979; Zhdamirov and Popov, 1979; Ohkawa et al. 1980; Sharon and Miles, 1981). The state of knowledge of the metabolism of pesticidal chemicals by aquatic vertebrates and invertebrates has been described in detail (Garnas and Crosby, 1977). The literature shows that the degradation of pesticides is taking place by means of photolysis, oxidation, reduction etc in not less an amount than the microbial degradation.

1.2.2 Microbial degradation of pesticides

A model ecosystem for the evaluation of pesticide biodegradability and ecological magnification was proposed in which the plants were treated with radio-labelled pesticide and the progress of the pesticide and its degradation products were followed through the system (Metcalf et al. 1971). The biochemical transformation of pesticides by soil fungi (Bollag, 1972), properties of an immobilized pesticide hydrolyzing enzyme (Munnecke, 1977), microbial interactions with pesticides in estuarine surface slicks (Ahearn et al. 1977) were studied. The role of fungi and bacteria in accomplishing the biodegradation of organic compounds was stressed by considering the variety of reactions which these microorganisms can employ to initiate attack on different classes of organic molecules

(Chapman, 1978). The involvement of microbial degradation in the environmental hazard evaluation process was described (Gledhill and Saeger, 1978). Insecticidal chemicals which are introduced into the environment eventually face various natural "weathering" forces that alter their locations and chemical characteristics. Among the biologically mediated changes in insecticides, microbial processes play the most significant role (Matsumura and Benezet, 1978). The biodegradability of methyl parathion, benzo (b) thiophene, dibenzothiophane, quinoline, and benzo (f) quinoline, can be affected when nutrients that are assimilated more readily are present in the medium. This fact should be considered in the development of ecosystem to be used for assessing whether pollutants are biodegraded (Chou and Bohonos, 1979). Degradation of pesticides by algae and aquatic microorganisms were studied and sevin and malathion were used for studying the metabolic responses of aquatic bacterial population and found out the degradation of the insecticides (Matsumura and Essac, 1979). Monitoring biotransformation and biodegradation of xenobiotics in simulated aquatic microenvironmental systems were undertaken (Portier and Meyers, 1982). The biotransformation of 1', 2' - Dihydrorotinone, a widely used natural pesticide by Streptomyces griseus was studied (Sariaslani and Rosazza, 1985). The transformation products were isolated by solvent extraction method and characterized by spectral methods. Studies on the metabolic product of the dechlorination of 1, 1, 1 - trichloro 2, 2 - bis (P-chlorophenyl) ethane by Aerobacter aerogenes were carried out (Wedemeyer, 1967). Degradation of insecticides by a soil fungus Trichoderma viride (Matsumura and Boush, 1968) and the metabolism of DDT and related insecticides my microorganisms (Focht and Alexander, 1970; Anderson and Lichtenstein, 1971; Pfaender and Alexander, 1972; Patil et al. 1972; Hicks and Corner, 1973) were studied. The herbicide

2- (2,4,5 - trichlorophenoxy) propionic acid (Silvex) was reported to be degraded extensively by a mixed culture of Pseudomonas sp. and an Achromobacter sp. isolated from pond water. Neither of the two organisms separately was able to metabolize the herbicide (Ou and Sikka, 1977). The degradation of Lindane by cell free preparation of Clostridium sphenoides was reported (Heritage and MacRae, 1977). Twenty two strains of soil bacteria including representatives of various genera were shown to degrade aldrin to its epoxide, dieldrin (Fergusan and Korte, 1977). The interactions of atrazine with soil microorganisms were studied (Percich and Lockwood, 1978). The distribution and degradation potential of kepone resistant bacteria in the James River and upper Chesapeake Bay was studied (Orndorff and Colwell, 1978). Lee and Ryan (1979) reported the microbial degradation of organochlorine compounds in the estuarine waters and sediments. Sublethal levels of chlordane were introduced into the growth medium of Aeromonas proteolytica. Chlordane inhibited the synthesis of an extracellular endopeptidase by almost 40%, but exhibited no such inhibition of the extracellular aminopeptidase, also produced during the growth cycle (Nakas and Litchfield, 1979). The biodegradation of 2-4-D incubated in river water at $25^{o}C$ was dependent upon the bacterial activity rather than the initial quantity of species of organisms present (Nesbitt and Watson, 1980a). The rate of 2,4,-D degradation in the Avon river related to the nutrient concentration, sediment load and dissolved organic carbon content of the water (Nesbitt and Watson, 1980b). The anaerobic degradation of the herbicide diuron, 3-(3,4 - dichlorophenyl - 1,1) dimethyl urea) was studied (Attaway et al. 1982). Methoxychlor was found to be sufficiently persistant in soil and its residues were present

even 18 months after the soil treatment (Golovleva et al. 1984).

Transformation of the herbicide 2-(3,4 - dichlorophenyl)-4-methyl-1,2,4 - Oxadiazolidine -3,5 -dione was studied in pure cultures of Nocardia rubra strains 0319 and 0795 and Penicillium fellutanum. The herbicide was reported to transform both, in the presence of an additional energy source and without it (Kuznetsova et al. 1984). The bacterial and fungal cometabolism of DDT and its breakdown products (Subba Rao and Alexander, 1985), microbial degradation of the herbicide mecoprop (2-(2 methyl -4-chlorophenoxy) propionic acid) (Lappin et al. 1985), the removal of lindane, 2,4-D and 2,4,5-T from water by microbial cells immobilized on magnetite (MacRae, 1985) and the microbial transformation of the herbicide metalachlor by a soil actinomycete (Krause et al. 1985) were carried out.

Metabolism of carbaryl and carbofuran by soil enrichment and bacterial cultures (Rajagopal et al. 1978), degradation of carbofuran by Azospirillum lipoferum and Streptomyces spp. isolated from flooded alluvial soil (Venkateswarlu and Sethunathan, 1984) were reported. Mineralization of aldicarb to CO_2 as well as formation of various metabolites and extractable and nonextractable C^{14} were measured in (C^{14}) aldicarb-treated surface and subsurface soils (Ou et al. 1985).

The metabolism of some organophosphorus insecticides by micro-organisms (Ahmed and Casida, 1958) and the malathion degradation (Matsumura and Boush, 1966; Bourquin, 1975; Paris et al. 1975; Bourquin, 1977) were studied. Studies were carried out on the ability to attack organic phosphorus compounds by microorganisms isolated from the bottom sediment of an eutrophic lake. It was found that different types of bottom

sediments of the same lake contain different numbers of microorganisms capable of breaking down these substances. The number of such organisms was different in various sampling periods (Strzelczyk et al. 1972). Permethrin degradation in soil by microbial cultures was studied (Kaufman et al. 1977). A cell suspension of Klebsiella pneumoniae converted the organophosphorus pesticide fensulfothion to a product that was shown by chemical oxidation, GLC, infrared spectrometry and mass spectrometry to be fensulfothion and sulphide (Wood and MacRae, 1977). The first report of parathion utilization by a defined microbial culture and by symbiotic microbial attack and of dissimilation of an organophosphorus pesticide in a chemostat was given (Daughton and Hsich, 1977). The studies on the phosphorus containing pesticide breakdown products and their qualitative utilization as phosphorus sources by bacteria (Cook et al. 1978), metabolism of organophosphorus insecticides in aquatic organisms with special emphasis on fenitrothion (Miyamoto et al. 1978) and phosphate and soil binding factors limiting bacterial degradation of ionic phosphorus containing pesticide metabolites (Daughton et al. 1979) are worth mentioning. Bacteria capable of utilizing aspan, azodrin, dasanit, diazinon, malathion, dylox, methyl parathion and vapona (organophosphorus) as sole phosphorus sources, isolated from water and sewage were utilized for the studies of microbial cleavage of the insecticides (Rosenberg and Alexander, 1979). Studies on the accelerated mineralization of two organophosphorus insecticides in the rhizosphere (Hsu and Bartha, 1979), interactions between the structural aminopolysaccharide, chitin and the organophosphate pesticide azinphosmethyl (Guthion) in a controlled continuous flow-through microcosm (Portier and Meyers, 1980) and the

acute impact of an organophosphorus insecticide on microbes and small invertebrates of a mangrove estuary were carried out. Rates of transformation of methyl parathion and diethyl phthalate by Aufwachs microorganisms was studied (Lewis and Holm, 1981). A strain of Pseudomonas sp. has been isolated which rapidly and efficiently utilizes the herbicide glyphosate (N - phosphonomethyl glycine) as its sole phosphorus source in a synthetic medium (Moore et al. 1983). Pseudomonas alcaligenes C_1, a soil bacterium, metabolized fensulfothion (Sheela and Balakrishnapai, 1983). The bacterial metabolism of three dithioate pesticides, malathion, gusathion and dimethoate was examined in an effort to obtain cell free enzyme system for possible use in pesticide disposal and detoxification processes (Barik et al. 1984). A rapid method was reported to determine microsomal metabolism of organophosphate pesticides (McLean et al. 1984). Multiphasic kinetics for transformation of methyl parathion by Flavobacterium sp. was done by Lewis et al. (1985). Bacterial reduction of fensulfothion and its hydrolysis product 4-methyl sulfinyl phenol was reported (MacRae and Cameron, 1985).

From the literature, it is understood that more work has been done on organochlorides than on organophosphates and carbamates. So, there is the need for further studies in organophosphorus pesticides.

1.2.3 Bivalves as pollution monitoring organisms

Mussels have long been used as indicators of pollution since their sedentary filter feeding mode of life predisposes them to the accumulation of pollutants (Roberts, 1976; Bayne, 1978; Park, 1979). The use of bivalves as pollution indicators for assessment of environmental quality has been reported (Cosson-Mannevy, 1979; Bayne et al. 1979; Krieger et al. 1981).

1.2.4 Effect of pesticides on bivalves

Number of reports on the effect of pesticides on bivalves are available (Stainken and Rollwagen, 1979). Oysters, in particular, were studied by Emmanuelsen et al. (1978). They have suggested that relatively short term exposures of low levels of dieldrin do not cause histopathological damage to Crassostrea virginica. Toxicological studies were carried out in mussels by various authors (Cowan, 1978; Dybern and Jensen, 1978; Frenne, 1978; Krieger et al. 1978; Salanki and Varankal, 1978). The reaction of the marine bivalves Katelysia opima and Donax cuneatus (both commercially important species in India) to various pesticides and narcotants were studied under laboratory conditions by Mane et al. (1979). Physiological studies were done on mussels ie. the oxygen uptake by the hepatopancreas, mitochondria of mussels in the normal condition and when affected by DDT (Golovenko and Petrov, 1979). Lethality of aminocarb and the components of aminocarb formulation to a fresh water clam was reported (McLeese et al. 1980). It was experimentally proved that the mussel sensitivity to DDT depends on the age of molluscs, duration of their contact with the pesticide and concentration of the pesticide (Zaitsev et al. 1981). The changes in the carbohydrate metabolism in selected tissues of freshwater mussel, Lamellidens marginalis were studied during induced toxicity of phosphamidon, an organophosphorus pesticide (Moorthy et al. 1983).

1.2.5 Bacteriology of bivalves

Bacteriological monitoring programme of oyster shell stock harvesting areas (MacLean, 1977; 1978), microbial flora of mussels in the natural beds and farms (Pillai, 1980; Philip, 1987), microbial flora of marine bivalve larvae, post larvae, adults, (Prieur, 1981; 1982) have

been carried out. Preliminary bacteriological studies on some bivalve molluscs showed that Vibrionaceae were more abundant in these invertebrates than in the surrounding seawater (Prieur et al. 1985; Philip, 1987).

1.2.6 Villorita cyprinoides var. cochinensis (Hanley)

Villorita cyprinoides var. cochinensis (Hanley), popularly known as black clam is a hardy bivalve abundantly distributed along the mesohaline and oligohaline stretches of the Cochin backwaters. The biochemical constituents (Suryanarayanan and Alexander, 1972) salinity tolerance (Nair and Shynamma, 1975), the rate of uptake of copper (II) from the surrounding medium (Lakshmanan and Nambisan, 1983) and heavy metal load (Pillai et al. 1986) of the black clam have been investigated. The combined effects of oil and pesticides on the bivalve Villorita cyprinoides var. cochinensis (Hanley) have been carried out (Jacob, 1988).

1.2.7 Effect of pesticides on bacteria

Bacterial populations in nontreated and herbicide treated waters were subjected to three different herbicides, diuron, dichlobenil and diquat to know the effects of the herbicides on them (Yazer et al. 1975). Laboratory salt marsh environments were treated with malathion, an organophosphorus insecticide, and aerobic heterotrophic bacteria were monitored to determine changes in their microbial ecology (Bourquin,1977). Effect of two organophosphate insecticides on the phosphate dissolving soil bacteria were studied (Congregado et al. 1979). The toxic effects of pollutants on microorganisms were described (Colwell, 1979).
The effect of seven thiophosphorus pesticides and their degradation products on estuarine ammonium oxidisers were examined. The data

suggested that the parent compounds of the thiophosphorus pesticides have little effect on ammonium oxidation in estuarine sediments, but that certain metabolites which accumulate from the decomposition of these pesticides, especially in sediments under low O_2 levels may significantly reduce ammonium oxidation (Jones and Hood, 1980). Warburgh manometry (to measure the increased uptake of O_2 as the evidence of pesticide degradation) was shown to be useful rapid method for detecting effects of insecticides on aquatic microbial populations (Murray and Guthrie, 1980a).

The effects of carbaryl, diazinon and malathion on native aquatic populations of microorganisms was also studied (Murray and Guthrie,1980b). Carbaryl caused an increase in aerobic heterotrophic bacterial numbers and a decrease in dissolved O_2 and total algal number, particularly Cyanophyta. Malathion had very little effect on bacterial and algal counts. Diazinon had little effect on bacteria. Although carbaryl, malathion and diazinon appeared initially to inhibit algal growth, the inhibition was short lived and numbers returned to normal or higher. The effect of water pollutants (12 types of fungicides and herbicides as well as three surfactants) on microbial activity was studied (Daubner and Toth, 1980). In situ heterotrophic uptake of mixed C^{14} aminoacids and direct viable cell (DVC) count of Chesapeake Bay water samples were not significantly affected by the insecticide- kepone at concentration of 0.01 mg/l (Orndorff and Colwell, 1980). The inhibitory and bactericidal concentrations of paraquat for bacteria of human origin are above levels likely to be often encountered environmentally (Peterson et al.1981). Escherichia coli, Streptococcus faecalis, Salmonella typhimurium and Staphylococcus

aureus were tested in pure culture and in various combined mixed cultures to determine effects of the presence of carbaryl in water (Guthrie et al. 1981). A contribution to the knowledge of increased effect of compounds-with fungicidal and herbicidal activity when combined - on the bacteria Escherichia coli, Pseudomonas fluorescens and Pseudomonas aeruginosa was given (Toth et al. 1981). In water environment, in the presence of pairs of compounds formed by a fungicide and a herbicide, by two different fungicides or fungicide and petrol, the exert of synergism attained a value of 1.9 to 47.1, which expressed the product of increased inhibition of growth or respiratory activity of bacteria as against the actual values ascertained when applying both compounds individually. Bean and Southall (1983) have carried out studies on the effect of pyridazinone herbicides on growth and aflatoxin release by Aspergillus flavus and Aspergillus parasiticus. Experiments have provided direct biological evidence that the enzyme 5-enol pyrmoyl shikimate-3-phosphoric acid synthase (EPSP synthase) is a major site of glyphosate (a herbicide) action in Escherichia coli and that the amplifications of aro A gene from Escherichia coli results in tolerance to the herbicide glyphosate (Rogers et al. 1983). The effects of pesticides on cyanobacterium Plectonema bonyanum and cynophage LPP-1 were studied (Mallison and Cannon, 1984). The assessment of the effects of simazine and endothall (herbicides) on bacterial population in aquatic ecosystem and the determination of the effects of those herbicides on growth and respiration of common bacterial isolates obtained from aquatic ecosystem were done (Beckmann et al. 1984). Sensitivity and adaptations of selected rhizobia and agrobacteria to paraquat was studied (Roslycky, 1985).

1.2.8 Effect of pesticides on the bacteria associated with plants

Effects of captan on the nonparasitic microflora of apple leaves (Hislop and Cox, 1969) and effects of fungicides on the microflora of potato leaves (Bainbridge and Dickinson, 1972) were studied. A very important aspect of the effects of fungicides on soil fungi concerns the phylloplane microflora. Given the most saprophytic phylloplane fungi have their origin in soil, the inhibition/stimulation effect of a foliar-applied fungicide on an organism may be highly significant in subsequent events when the leaf eventually becomes incorporated into the soil. Ethirinol did not affect populations of saprophytic filamentous fungi or ballistosporic yeasts whilst zinel slightly reduced populations of fungi but had no effect on Sporobolomyces spp. and Cladosporium spp. when flag leaves of winter wheat was studied (Jenkyn and Prew, 1973). The effect of fungicides and other agrochemicals on the microbiology of the aerial surfaces of plants was described (Hislop, 1976). Three nitrogen fixing bacteria isolated from leaf and root surfaces of water hyacinth on Jenson's nitrogen free medium were tested for their tolerance against commonly used pesticides. A mixed stand of aquatic macrophytes, including Elodea sp. and Chara sp., was treated with paraquat. Total numbers of epiphytic bacteria and viable numbers in different physiological groups were studied. The epiphytes of Elodea sp., which had died after 15 days, had all increased in number by ten days. Those on Chara sp., which survived, did not increase except for a short lived initial rise. These results were explained by the presence of paraquat in the water and the release of nutrients by the treated plants (Ramsay and Fry, 1976). The heterotrophic activity of the bacterial epiphytes of Elodea canadensis and Chara vulgaris was

studied by measuring the mineralization of C^{14} - glucose in situ. The experimental data proved that epiphytic bacteria are influenced by the release of nutrients from the host plant. The kinetic studies showed how the activity of the epiphytic bacteria was affected by herbicide treatment of their host plants. Such kinetic data combined with counts of bacteria can also provide information on the activity of individual bacteria (Fry and Ramsay, 1977). The epiphytic microbial community was quantitatively and qualitatively altered when a standard pesticide schedule that comprised applications of an insecticide, a bactericide, and a fungicide was applied to McIntosh apple trees (Andrews and Kenerley, 1978). Similar studies on the effect of pesticide on associated microflora of animals are lacking.

1.3. Research Approach

Marine molluscs, mainly those in shallow waters are very good indicators of pesticide pollution. Since they are filter feeders, they have the capacity of accumulating pesticides in their soft tissues. The accumulation may affect the associated bacterial flora which may be of immense support to the animal.

Ekalux (R) EC 25, a wide spectrum contact and stomach insecticide is of wide use in the agricultural fields adjoining the study area, the Cochin backwater system. This pesticide contains 25% (w/w) of the organophosphate active ingredient 'Quinalphos' (O,O, diethyl-O-quinoxalinyl (2) thiono phosphate) and 75% (w/w) of stabilizers, and adjuvants. There is every possibility of this pesticide reaching the backwater system via land-runoff as there are six major rivers emptying into this water body.

The literature reviewed in section 1.2 shows that the effect of pesticide on estuarine microorganisms and the impact of ecologically significant xenobiotics on the coastal zone are studied and practically no report is available on the effect of pesticide on the microflora associated with animals.

For the present investigation, the black clam Villorita cyprinoides var. cochinensis (Hanley), a most common clam genus present in this estuarine system has been selected as test organaism and Ekalux (R) EC 25 as toxicant. The work has been planned as follows:

1. To estimate the total heterotrophic bacterial population of the clam Villorita cyprinoides var. cochinensis (Hanley) and water samples a) immediately after collection, b) after two days of acclimation and c) after 96 hrs. treatment with the organophosphorus pesticide Ekalux(R) EC 25,

2. To find out the occurence of various bacterial genera and the effect of treatment with pesticide on their composition,

3. To determine the distribution of various hydrolytic enzyme producing bacteria by evaluating their ability to release enzymes such as lipase, amylase, caseinase, gelatinase, urease, DNase, chitinase and cellulase, and the effect of pesticide treatment on these characters,

4. To study the antibiotic resistant character and the effect of pesticide treatment on the resistance pattern,

5. To investigate the heavy metal resistant nature and the effect of pesticide treatment on it,

6. To evaluate the effect of pesticide concentration on the growth of the bacteria,

7. To assess the effect of different environmental parameters such as temperature, pH and sodium chloride concentration on growth of the selected isolates and to find out the generation time of the selected isolates and

8. To assess the effect of different environmental parameters such as temperature, pH and sodium chloride concentration on the phosphate releasing ability of the selected isolates.

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1. Area of Collection

The Vembanad Lake, the largest estuarine system in the south west coast of India extends between latitudes $9^{o}28'$ and $10^{o}10'$N and longitudes $76^{o}13'$ and $76^{o}30'$E. It has a length of about 90 Km between Alleppey in the south and Azhikode in the north, with a total area of about 300 Sq.Km. The samples for the experiment were collected from the Vembanad Lake, near Kumbalam Island (Fig. 1).

2.2. Description of Samples

The Vembanad Lake is rich in clam resources. The edible clam belonging to Villorita spp. is abundant in certain parts of the estuary and the shells accumulated in the lake during the past hundreds of years meet the raw material requirements of a cement factory and also the lime shell demand of central Kerala. The most common species of the genus Villorita present in the estuarine system is Villorita cyprinoides and its variety cochinensis (Fig. 2). They are filter feeders and are mostly distributed in the sandy regions of the estuary.

2.2.1 Systematic Position

Phylum	-	Mollusca
Class	-	Pelecypoda (Bivalvia)
Order	-	Eulamellibranchiata
Suborder	-	Heterodonta
Series	-	Sphaeriacea
Family	-	Corbiculidae

Genus	-	Villorita (Griffith and Pidgeon, 1984)
Species	-	Cyprinoides
Variety	-	Cochinensis (Hanley)

2.2.2 Method of Collection

The black clam Villorita cyprinoides var. cochinensis (Hanley) and the water samples for the experiment were collected from the Vembanad Lake near the Kumbalam Island, during the period of September 1985 to May '86. The clams were collected using a toothed dredge and brought to the laboratory in live condition. The water sample and the clams were also collected in sterile polyethylene bags separately and transported to the laboratory in an insulated ice box.

2.3. Acclimation

The live clams were acclimated to the experimental condition by keeping them in aerated tanks containing seawater (15‰) for 48 hrs.

After 48 hrs. of acclimation, the clams of size 23 to 25mm were selected randomly for the experiment. The seawater used for experiments was collected from Arabian sea, off Cochin in black polyethylene carbouys of 50 l capacity. Before being employed for the experiments, the seawater was filtered through a fibre glass filter (length 32 cm, breadth 16 cm) containing glass wool and activated charcoal using a 0.15 HP. pump and stored in the dark in fibre glass tanks of 200 l capacity upto 15 days. Salinity adjustments were done by diluting with deionised water, whenever necessary. The pH of the experimental water was 8.2 $\pm$ 0.1. All the experiments were carried out at room temperature (28 $\pm$ 2^{o}C).

2.4. Estimation of LC_{50} values for the animals

The pesticide used was Ekalux(R) EC 25 supplied by Sandoz India Ltd. Bombay. Ekalux(R) EC 25 is a wide spectrum insecticide containing 25% (w/w) of the organophosphate active ingredient 'Quinalphos' (0, 1, diethyl-o-quinoxalinyl (2)-thionophosphate) and 75% (w/w) of stabilizers, emulsifiers and adjuvants.

Static bioassays were conducted in glass troughs (15 x 30 cms) with 4 l of test water (15‰). Ekalux(R) EC 25 was mixed with acetone in 1:1 ratio and six concentrations were fixed in a geometric series. Ten animals were used for each test concentration and they were kept in each trough for 96 hrs. The experiments were carried out at room temperature (28 ± 2^{o}C) and the animals were not fed during the period of the experiment. Duplicates and controls were run for all the experiments. The test media was changed every 24 hrs., with fresh medium. Clams were inspected every 12 hrs. and were considered dead, if the valves gaped beyond 5 mm. and showed no movement even under mechanical stimulation. The 96 hr. LC_{50} values were determined by probit analysis (Finney, 1971).

2.5. Pesticide Application

The glasswares used for the experiment were very clean, dried troughs. Each trough contained 4 l of filtered seawater adjusted to 15‰ salinity. The organophosphorus pesticide Ekalux(R) EC 25 (Sandoz India Ltd. Bombay) was added to the experimental medium at sublethal concentrations to the animals. The concentrations used were 0.000125ml/l, 0.00025 ml/l and 0.0005 ml/l. In each trough, ten clams were maintained for 96 hrs. Control trough, without addition of pesticide were also

maintained. During the course of the experiment the test animals were not fed.

2.6. Bacteriological Analysis

2.6.1 Estimation of Bacteria

The water samples and clams collected in sterile polyethylene bags and transported to the laboratory in insulated ice box were used for estimation of total aerobic heterotrophic bacteria (THB). After 48hrs. of acclimation, before the treatment of pesticide and after the treatment (96 hrs.) of pesticide, the bacteria associated with the clam and water were estimated. Appropriate controls were also maintained and bacterial populations were estimated.

The colony forming units (CFU) of THB in water and the clams were estimated following standard plate count method using nutrient agar (NA).

2.6.1.1 Processing of the sample

The clams were taken out of the experimental trough using sterile forceps and kept in sterile petridishes. Water samples were collected using sterile pipettes. The flesh of five clams were separated out using sterile needles and forceps. Each of the solid samples were taken in a sterile homogenizer, where they were fully mascerated. Sterile filtered suspension medium of 50% aged seawater was used as diluent. The water and clam samples were serially diluted to 10^{-4} using the diluent.

2.6.1.2 Plating procedures

Nutrient agar having the following composition was used for the enumeration of THB.

Nutrient Agar Medium

Peptone	-	5.0 g
Beef extract	-	5.0 g
Sodium chloride	-	15.0 g
Agar (BDH)	-	20.0 g
Tap water	-	1000 ml
pH	-	7.5 ± 0.2

Pour plate technique was employed. One ml aliquote of inoculum (10^{-2} to 10^{-4} dilution) was introduced into sterile petriplate. About 20ml of the medium ($\approx 40^{o}C$) was poured into each petridish, and mixed thoroughly by rotating the dishes clockwise and anticloskwise, for 4 to 5 times and allowed to solidify. The plates were incubated at an inverted position at room temperature (28 ± $2^{o}C$) for 7 - 15 days. All the estimations were made aseptically and in duplicate.

2.6.2 Enumeration of Bacteria

The plates showing 30 - 300 colonies were selected. Counts were made and expressed as number of colonies per millilitre of water and per gram (dry weight) of solid sample.

The experiment was repeated three times to get the results confirmed.

2.7. Isolation, maintenance and identification of bacteria

After recording the morphological characters, opacity and pigmentation, twenty colonies per sample were isolated randomly into nutrient agar slants.

The isolates were repeatedly streaked onto nutrient agar plates, checked for their purity and were transferred to soft nutrient agar

(0.5% agar) in small glass vials with rubber stopper and preserved under sterile liquid paraffin at 20°C in a cold room.

The isolates were grouped into various genera based on their morphological and biochemical characters as suggested by Shewan et al (1960), Cowan (1974) and Buchanan and Gibbons (1974).

2.8. Production of hydrolytic enzymes

Bacterial isolates were divided into various physiological groups on the basis of their ability to elaborate different hydrolytic enzymes such as amylase, caseinase, gelatinase cellulase, chitinase, DNase, lipase and urease.

2.8.1 Amylase

Amylase production was tested on the agar medium of Harrigan and McCance (1972) supplemented with starch as the substrate.

Composition of the medium

Peptone	-	10.0 g
Meat Extract	-	10.0 g
Starch (soluble)	-	2.0 g
Sodium chloride	-	15.0 g
Agar	-	20.0 g
Tap water	-	1000 ml
pH	-	7.2 ± 0.2

The medium was autoclaved and poured into sterile petridishes. They were kept for surface drying for 24 hrs. Isolates were streaked onto the agar plates and incubated at room temperature (28 ± 2°C) for 7 days. The production of amylase was detected by flooding the plates with iodine solution (Potassium iodide, 2 g; Iodine, 1 g; distilled water, 300 ml). Unhydrolyzed starch formed a blue colour with iodine. The

amylolytic colonies developed clear zones around them.

2.8.2 Caseinase

Caseinase production by the isolated bacteria were checked by employing casein agar medium of Harrigan and McCance (1972) with slight modification in preparation as described below.

Composition of the medium

Peptone	-	10.0 g
Meat Extract	-	10.0 g
Sodium chloride	-	15.0 g
Agar	-	20.0 g
Tap water	-	750 ml
pH	-	7.2 ± 0.2

The medium was autoclaved at 15 lbs pressure for 15 min. 30.0g of casein in 250ml of distilled water was sterilized separately and mixed with the above medium,before pouring into plates. Cultures were inoculated onto the medium by surface streaking and incubated at room temperature (28 ± 2^{o}C) for 7 days. Caseinase enzyme production was detected by the presence of clear zones around the colonies after flooding with mercuric chloride solution.

2.8.3 Gelatinase

Ability of the bacterial isolates to produce gelatinase was tested employing Frasier's gelatin agar (modified) medium of Harrigan and McCance (1972).

Composition of the medium

Peptone	-	10.0 g
Meat Extract	-	10.0 g
Gelatin	-	4.0 g
Sodium chloride	-	15.0 g
Agar	-	20.0 g
Tap water	-	1000 ml
pH	-	7.2 ± 0.2

The prepared medium was autoclaved and poured into sterilized petridishes. Isolates were inoculated by surface streaking onto the solidified agar medium and incubated at room temperature (28 ± 2°C) for 7 days. The plates were flooded with 8 - 10 ml of the test reagent (mercuric chloride, 15.0 g; concentrated HCl, 200 ml; and distilled water 1000 ml). The hydrolysis was identified by clear halos around the colonies.

2.8.4 Cellulase

Cellulase production by the isolates were determined by using the agar medium of Harrigan and McCance (1972) supplemented with cellulose as the substrate.

Composition of the medium

Peptone	-	5.0 g
Meat Extract	-	3.0 g
Sodium chloride	-	15.0 g
Cellulose	-	20.0 g
Agar	-	20.0 g
Tap water	-	1000 ml
pH	-	7.2 ± 0.2

The medium was prepared and sterilized in an autoclave at 15 lbs pressure for 15 minutes. The medium was poured into sterile petridishes. The dishes were left overnight for surface drying. The cultures were streaked onto the agar and left for incubation for 15 days. The cellulase production is detected by the appearance of a hallow zone around the well grown bacterial colony.

2.8.5 Chitinase

Chitinase production was detected by using the following medium.

Composition of the medium

Peptone	-	5.0 g
Meat Extract	-	5.0 g
Sodium chloride	-	15.0 g
Agar	-	20.0 g
Tap water	-	1000 ml

Chitin precipitate was supplemented to the molten medium till the medium became turbid and pH was adjusted to 7.5 using IN NaOH or IN HCl. The medium was autoclaved and poured into sterilized petridishes. The cultures were streaked onto the surface of the agar medium and incubated at room temperature (28 ± 2°C) for 7 - 14 days. Colonies which developed clear zones around them were counted as positive.

2.8.6 DNase

DNase agar medium was used to detect the production of DNase by the isolates.

Composition of the medium

Tryptose	-	20.0 g
Deoxyribonucleicacid	-	2.0 g
Sodium chloride	-	5.0 g
Agar	-	12.0 g
Water	-	1000 ml
pH	-	7.3 ± 0.2

The ingradients were dissolved by heating and autoclaved at 115°C for 10 minutes. The medium was poured into sterile petridishes. The plates were inoculated with the cultures and incubated at 28 ± 2°C for 72 hrs. The plates were flooded with IN HCl. Clearing zone around the colonies indicated DNase activity.

2.8.7 Lipase

Production of lipase was tested on Tributyrin agar medium.

Composition of the medium

Peptone	-	10.0 g
Sodium chloride	-	15.0 g
Yeast extract	-	3.0 g
Tributyrin	-	10.0 g
Agar	-	12.0 g
Tap water	-	1000 ml
pH	-	7.5 ± 0.2

The medium was autoclaved and poured into sterilized petriplates. Isolates were streaked onto the agar medium and incubated at room temperature (28 ± 2°C) for 3 days. Lipase production was detected by the appearence of a clearzone around the colonies.

2.8.8 Urease

Ability of the bacterial isolates to produce urease was tested with Christensen's urea agar medium (1945).

Composition of the medium (Basal)

Peptone	-	1.0 g
Sodium chloride	-	15.0 g
Potassium dihydrogen phosphate	-	2.0 g
Agar	-	20.0 g
Tap water	-	1000 ml
pH	-	6.8 ± 0.2

The prepared medium was heated to dissolve and autoclaved at 115°C for 10 minutes. The medium was cooled to 50°C and 100 ml of sterile 20% aqueous solution of urea and 10 ml of 0.1 percent phenol red were added and tubed aseptically. Bacteria were inoculated into the tubes and incubated at room temperature (28 ± 2°C) for 3 days. Ureolytic activity was detected by the change in colour of the medium from light yellow to pink.

2.9. Antiblotic sensitivity test

Antibiotic sensitivity tests were carried out by disc diffusion method. Nutrient agar supplemented with 2% sodium chloride was used as the basal medium. Isolates enriched in nutrient broth (6 hrs.) containing 2% sodium chloride were seeded over nutrient agar with sterile cotton swabs. Using flammed forceps, antibiotic discs were placed on the agar surface sufficiently separated from each other to avoid overlapping of inhibition zones. The discs were lightly pressed with the forceps to make

complete contact with the medium. After 30 minutes, (pre-diffusion time) the plates were incubated at 37°C for 18 - 24 hrs. The antibiotics used, their concentration and interpretation of inhibition zones are given in Table 1. Diameter of inhibition zones were measured at the end of incubation period, compared with the interpretive chart of Kirby-Bauer sensitivity test method modified in July 1969 (Schering Corporation, U.S.A, Bloomfield, N.J.) and classified accordingly. For convenience of discussion, intermediate strains were also included under resistant forms. Thus, strains were classified into two types, namely sensitive and resistant.

2.10. Metal sensitivity test

Metal sensitivity was also tested on nutrient agar medium supplemented with 2% NaCl. Filter sterilized salts of five metals were incorporated in the sterile molten basal medium at different concentrations (Table 2) before dispensing into petriplates. Cultures enriched as described in the above section were spot inoculated on the metal incorporated medium. For comparison of growth, basal medium without addition of metals were also inoculated and incubated at 28 ± 2°C for 18 - 24 hrs. If no growth was seen after 24 hrs., plates were reincubated for an additional 24 hrs. If growth was observed within 48 hrs., the isolates were treated as resistant to that concentration. The critical concentration was fixed as 10 ppm for mercury and 100 ppm for other metals for differentiation of the strains into sensitive and resistant forms (Austin et al. 1977). Minimal inhibitory concentration (MIC) of the resistant strains were found out and the maximum tolerance levels were also worked out.

2.11. Effect of various pesticide concentration on the growth of bacteria

Effect of various pesticide concentrations on the growth of bacteria was tested in three media viz. nutrient agar, mineral agar (Molin and Ternström, 1982) and mineral agar with glucose.

Composition of the mineral agar medium

Purified agar (Oxoid)	-	10.0 g
Disodium hydrogen phosphate	-	3.0 g
Potassium dihydrogen phosphate	-	2.3 g
Ammonium chloride	-	1.0 g
Magnesium sulphate	-	0.5 g
Calcium chloride	-	0.005 g
Sodium chloride	-	9.0 g
Tap water	-	1000 ml
pH	-	6.8 ± 0.2

The prepared medium was autoclaved at 15 lbs pressure for 15 minutes. Various concentrations of filter sterilized solutions of the pesticide Ekalux(R) EC 25 (0.005%, 0.01%, 0.02%, 0.05% and 0.1%) were added to the three molten media at about 50°C and mixed well by shaking. About 15 - 20 ml of the medium was poured into the petridishes and left for surface drying.

Isolates were grown in nutrient broth at room temperature (28 ± 2°C) for 18 hrs. This was used as the inoculam. A standard loopful of inoculam was transferred onto the agar plates, incubated at room temperature (28 ± 2°C) for 7 days. Growth was checked and recorded.

2.12. Estimation of phosphate released from the pesticide and inorganic phosphate

2.12.1 Selection of isolates

Isolates (100 numbers) which have shown good growth in agar medium with highest pesticide concentrations were selected and tested for their ability to release phosphate from the pesticide Ekalux(R) EC25 and tricalcium phosphate.

2.12.2 Preparation of inoculum

Inoculum was prepared by suspending 24 hrs. bacterial culture in sterile saline and the optical density was adjusted to 0.2 at 600 nm. From this, 0.5 ml was used as inoculum in the following experiments.

2.12.3 Inoculation and incubation procedures

Composition of the medium

Peptone	-	1.0 g
Yeast extract	-	1.0 g
Glucose	-	5.0 g
Calcium chloride	-	0.1 g
Ferric chloride	-	0.01 g
Manganese chloride	-	0.1 g
Magnesium sulphate	-	0.05 g
Ammonium sulphate	-	0.5 g
Sodium chloride	-	3.0 g
Distilled water	-	1000 ml
pH	-	7 ± 0.2

The medium in 10 ml aliquots were distributed in each test tube and autoclaved. The pesticide solution was filter sterilized and was added at the time of inoculation to give 1% concentration in the sterile medium. Similarly the inorganic phosphate (tricalcium phosphate) solution was also filter sterilized and added at the time of inoculation to give 1% concentration in the sterile medium. The inoculum was added and incubated at room temperature (28 ± 2°C) for 5 days. Control tubes were also run simultaneously without inoculum. After incubation, the medium was centrifuged (10000 rpm) and the culture filtrate was used for the estimation of phosphate, following the method of Murphy and Riley (1962) in a UV-Visible spectrophotometer (Hitachi - 200).

2.13. Effect of environmental parameters on growth of selected bacteria

2.13.1. Selection of bacteria

Based on the amount of phosphate released from the pesticide and tricalcium phosphate, four isolates belonging to two different genera viz. Pseudomonas and Vibrio and also coming under two categories viz. untreated and pesticide trated samples, were selected. Pseudomonas and Vibrio were the two most prominent genera represented in the total heterotrophic bacterial population found associated with the clam. This factor also was taken under consideration for the selection of isolates for further study.

2.13.2 Preparation of inoculum (See section 2.12.2).

2.13.3 Measurement of growth

From the prepared inoculum, 0.5 ml was transferred to culture medium (10 ml) in tubes and incubated at room temperature (28 ± 2°C) for 48 hrs. Turbidity resulted due to the growth of the inoculated

bacterium was measured at 600 nm in a Hitachi model 200 spectrophotometer. The growth was expressed as index of growth. Growth index is the average of turbidity in all tubes.

2.13.4 Effect of temperature on growth.

The effect of temperature on the growth of the bacteria was assessed by growing them at different temperatures ($4^{o}C$, $15^{o}C$, $25^{o}C$, $37^{o}C$, $45^{o}C$, and $60^{o}C$). The prepared medium (nutrient broth) was distributed in 10 ml aliquotes in the test tubes. After autoclaving, they were inoculated with 0.5 ml of prepared inoculum, incubated for 48 hrs. and finally growth was measured.

2.13.5 Effect of pH on growth

The effect of pH on growth of selected bacteria was determined by growing them at various pH levels (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12) in nutrient broth medium, separately adjusted to various pH with IN NaOH or IN HCl. 10 ml aliquots of the media were dispensed into test tubes and autoclaved. The media were inoculated with 0.5 ml of prepared inoculam, incubated for 48 hrs. at room temperature ($28 \pm 2^{o}C$) and the growth was measured.

2.13.6 Effect of Sodium chloride concentration on growth

Effect of Sodium chloride concentration on the growth of selected bacterial isolates were determined in nutrient broth medium containing 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0 and 7.5% NaCl. The medium was dispensed in 10 ml aliquots into test tubes and autoclaved. They were inoculated, incubated at room temperature ($28 \pm 2^{o}C$) for 48 hrs. and finally growth was measured.

2.14. Effect of environmental parameters on phosphate release from the organophosphorus pesticide by the selected bacteria

Preparation of inoculum, composition of the medium and estimation of phosphate are as in section 2.12.2 and 2.12.3.

2.14.1 Effect of temperature on the phosphate release

The effect of temperature on phosphate release by the bacteria from the organophosphorus pesticide was assessed by growing them in the medium (Section 2.12.3) containing pesticide at different temperature ($4^{o}C$, $15^{o}C$, $25^{o}C$, $37^{o}C$, $45^{o}C$ and $60^{o}C$) and estimating the phosphate released after 5 days. The prepared medium was distributed in 10 ml aliquotes into the test tubes and autoclaved. The filter sterilized pesticide solution was added at the time of inoculation to get 1% concentration and mixed thoroughly. The cultures were inoculated and incubated for 5 days. After that, the phosphate released was estimated.

2.14.2 Effect of pH on the phosphate release

The effect of pH on phosphate release was determined by growing them at various pH levels (2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) in the medium (Section 2.12.3) separately adjusted to various pH with IN NaOH or IN HCl. 10 ml aliquots of the media were dispensed into test tubes and autoclaved. The sterile pesticide solution was added at the time of inoculation to get 1% concentration and mixed thoroughly. The isolates were inoculated, incubated for 5 days and the phosphate released was estimated.

2.14.3 Effect of Sodium chloride concentration on the phosphate release

Effect of NaCl concentration on the phosphate release from the pesticide by the bacteria was determined using the medium (Section 2.12.3) containing 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0 and 7.5% NaCl. The medium was dispensed in 10 ml aliquots into test tubes and autoclaved. Pesticide solution was filter sterilized, added to the tubes containing medium to get 1% concentration and mixed throughly at the time of inoculation. Bacteria were inoculated and incubated for 5 days. Phosphate estimation was done after incubation.

2.15. Generation time

The generation time of the selected bacterial isolates was estimated in nutrient broth medium. Medium was prepared and distributed in 100 ml aliquots into 250 ml conical flasks and were autoclaved. A known concentration of cells (10^3/ml) was inoculated into the prepared medium and incubated at room temperature (28 $\pm$ 2^{o}C) in a rotary shaker. At intervals of 15 minutes, upto a period of 90 minutes and at intervals of 30 minutes, upto a period of 6 hrs., aliquotes were removed and the growth was measured. Generation time was determined from the exponential phase of the curve of a semilogarithmic plot.

Fig. 1. Map showing the sampling area

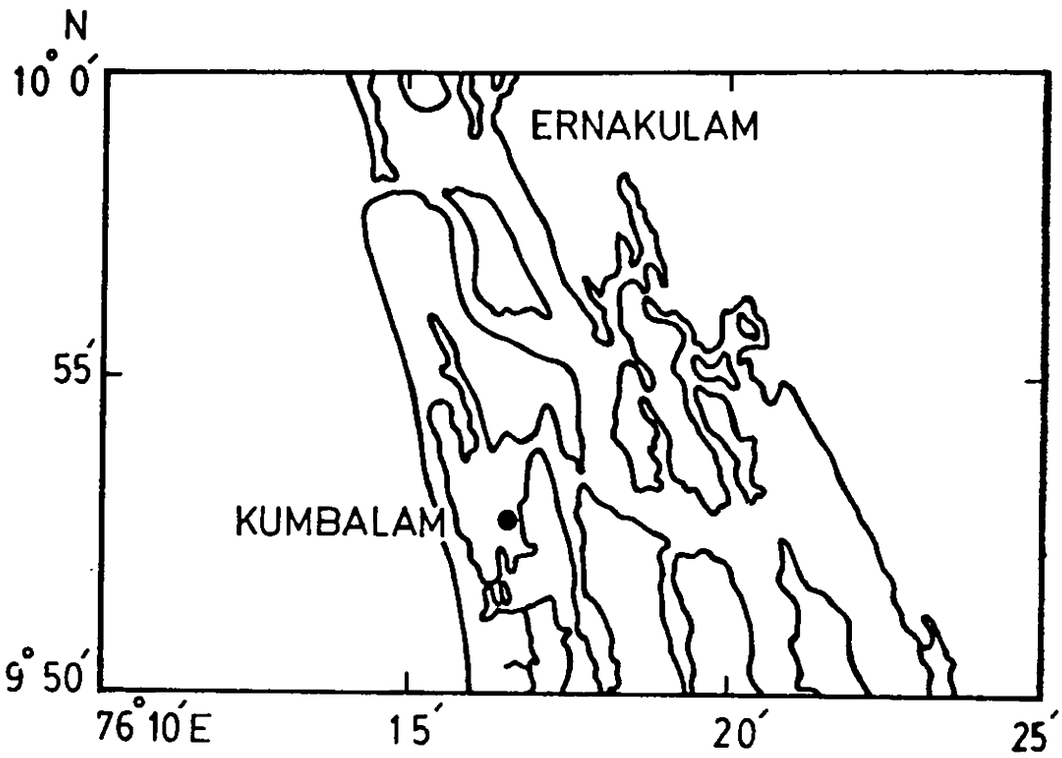


FIG-1

Fig. 2. Villorita cyprinoides var. cochinensis


cms.
camlin

Table 1. Antibiotic resistance - Interpretive chart

Antibiotics (Concentration/disc)	Symbol	Resistant ≤ mm	Intermediate mm	Sensitive ≥ mm
Ampicillin (10 mcg)	A	11	12 - 13	14
Bacitracin (10 µg)	B	8	9 - 12	13
Chloramphenicol (30mcg)	C	12	13 - 17	18
Gentamycin (10 mcg)	G	12	..	13
Neomycin (30 mcg)	N	12	13 - 16	17
Oxytetracycline (30 mcg)	O	14	15 - 18	19
Penicillin-G (10 µg)	P	11	12 - 21	22
Polymyxin-B (300 µg)	PB	8	9 - 11	12
Streptomycin (10 mcg)	S	11	12 - 14	15
Sulphadiazine (300mcg)	Sz	12	13 - 16	17

* Span Diagnostics, Udhna, India.

Table 2. Metalic salts and their different concentrations used in the study

Metals	Salts used	concentration tested (ppm)
Mercury	$HgCl_2$	5, 10, 15, 20, 25, 30, 40
Copper	$CuSo_4.5H_2O$	50,100,150,200,300
Cadmium	$CdCl_2H_2O$	100,200,300,350,400,450,500
Zinc	$ZnCl_2$	100,200,300,350,400,450,500
Lead	$Pb(NO_3)_2$	700,900,1000,1100,1200,1500,2000,2500

RESULTS

3. RESULTS

The black clam Villorita cyprinoides var. cochinensis (Hanley) collected from the Vembanad lake near Kumbalam island were brought alive to the laboratory and kept in aerated tanks containing seawater (15‰) for 48 hrs. for acclimation. Alongwith clams, water samples were also collected. Total heterotrophic bacteria (THB) in water and in clams immediately after collection (Native flora - NF); after 2 days of acclimation and at the beginning of the experiment (Initial - 0 hr) and after 96 hrs. of treatment with the pesticide Ekalux(R) EC 25 were estimated (Treated - T). Control troughs (Untreated - Ut) were also maintained for comparison.

Water used in the experiments was held at 15‰ salinity and 8.2 ± 0.1 pH. All the experiments were carried out at room temperature (28 ± 2°C).

3.1. Total heterotrophic bacterial population (THB)

The results obtained for the different experiments are presented in Table 3. The water and clam (from the site of collection) contained 6.5×10^4/ml and 2.98×10^6/g of THB respectively. After two days of acclimation in the laboratory ie. at the starting of the experiment (0 hr.) bacterial populations were 2.52×10^5/ml in water and 1.64×10^6/g in clam. At the termination of the experiment (after 96 hrs. of experiment), untreated samples showed an increase in the bacterial content. The number doubled in water whereas in clam it increased to more than 10 folds when compared to 0 hr. population.

Addition of organophosphorus pesticide Ekalux(R) EC 25 at sublethal concentrations in water resulted in changes in the THB levels. THB after the experiment (96 hrs.) were high in water and clams treated with pesticides than the 0 hr population. In treated clam, THB was slightly higher than in untreated clam. THB recorded a decrease alongwith increase of pesticide concentration in water and clam samples. In water, a maximum THB of 1.95 x 10^6/ml at 0.000125 ml/l concentration was found reduced to 1.05 x 10^5/ml at 0.0005 ml/l concentration and in clams, a maximum THB of 3.58 x 10^7/g at 0.000125 ml/l concentration decreased to 2.4 x 10^7/g at 0.0005 ml/l concentration.

3.2. Generic composition of the heterotrophic bacteria

Gram negative bacteria were dominant (85.1%) while the rest (14.9%) were gram positive bacteria. In general, species of Vibrio, Aeromonas, Pseudomonas, Acinetobacter, Alcaligenes, Moraxella, Flavobacterium - Cytophaga group, members of Enterobacteriaceae, Bacillus, Coryneform group and Micrococcus were encountered in water and clam during the period of study (Table 4). Among the groups, Pseudomonas (25.7%) was the dominant genus followed by Vibrio (18.6%) and members of Enterobacteriaceae (14.5%).

All the above said genera except Moraxella and Flavobacterium-Cytophaga group constituted the native flora. Members of Enterobacteriaceae dominated the native flora followed by Pseudomonas, Aeromonas and Vibrio. All the other groups showed lesser dominance. Gram negative bacteria were 79.4% and gram positive bacteria were 20.6% in the native flora.

At 0 hr (Initial) of the experiment, 88.6% of the total bacteria were gram negative while the gram positive were 11.4%. Vibrio was the dominant (29.9%) genus followed by Pseudomonas (19.6%), members of Enterobacteriaceae (14.4%) and Aeromonas (10.3%). Acinetobacter and Alcaligenes which were very less in the native flora, was observed at higher level. Further, Moraxella and Flavobacterium - Cytophaga group which were not found as part of native flora appeared at 0 hr.

After treatment with various concentrations of pesticide for 96 hrs, the generic composition of the various flora on the samples showed appreciable changes when compared with 0 hr. The occurrence of gram negative bacteria decreased slightly from that of 0 hr population, after 96 hrs of treatment with pesticide. The percentage decrease was slightly more in treated samples (83.36%) than those of untreated samples (87.2%). A slight increase of gram positive forms were observed and more in treated (16.64%) than untreated (12.8%). Although Pseudomonas exhibited dominance in both untreated and treated samples, it was high in treated samples (31.25%) when compared to that of untreated (20.5%) samples. Aeromonas (19.2%) which was the second dominant group in the untreated samples declined to 10.83% in the treated samples. However, it maintained slight increase in percentage from the inital. Vibrio, though appeared in same percentage in both treated and untreated (16.67%) samples, it demonstrated second dominance in the treated samples and decreased from the initial. All other genera recorded insignificant changes in the percentage composition, except Alcaligenes which got reduced drastically in treated samples (2.5%). The percentage of Bacillus decreased while Coryneform group and Micrococcus increased in treated than the untreated.

The native flora of water was constituted by species of Pseudomonas, Vibrio, Acinetobacter, Bacillus, Micrococcus, and members of Enterobacteriaceae (Table 5). Among them, members of Enterobacteriaceae (47.61%) was found dominant, followed by Pseudomonas (23.81%). Gram negative bacteria (85.72%) were more in the native flora of water.

In water, at 0 hrs, species of Aeromonas, Moraxella and Coryneform group were encountered in addition to the genera except Acinetobacter, observed in the native flora. Among the groups, Pseudomonas (37.93%) was dominant followed by members of Enterobacteriaceae (24.14%) and Vibrio (13.79%).

The untreated water showed varied results when compared with treated water. Except Moraxella and Micrococcus, all other groups which were found at 0 hr, appeared in the untreated water. In Ut water, among the different groups, Bacillus was dominant (27.78%) followed by members of Enterobacteriaceae (16.67%), Coryneform group, Pseudomonas and Alcaligenes. All other groups were present in insignificant percentages.

All the genera could be encountered in water which was treated with pesticide. Pseudomonas recorded dominance followed by Aeromonas and Micrococcus. Other groups formed the lesser dominant genera. Gram negative and gram positive bacteria were 75.36% and 24.64% respectively. Gram negative forms decreased from 85.62% to 66.67% showing an inverse relationship with the increase in pesticide concentration.

In native flora of clams, (Table 6) excluding Acinetobacter, all other genera which were found in the water samples were recorded. In clams, Aeromonas, Pseudomonas and Enterobacteriaceae showed the order of dominance while Enterobacteriaceae and Pseudomonas were

order of dominance in water. No Aeromonas was encountered in water. 83.83% were gram negative among the native flora of clams.

Among the groups, Vibrio (36.23%) was found dominant followed by Aeromonas (13.04%), Pseudomonas (11.59%) and Alcaligenes (11.59%) at 0 hr 88.40% were gram negative in clams.

In the Ut clams, except Bacillus and Flavobacterium - Cytophaga group, all the other genera which were recorded at 0 hr were encountered. Among the, Pseudomonas and Aeromonas showed dominance (23.33% each) followed by Vibrio (20.0%) and all other groups were present in lesser percentage. Gram negative and gram positive components showed a wide difference (96.66% gram negative and 3.34% gram positive) in untreated clam.

All the genera could be encountered in pesticide treated clam. Pseudomonas followed by members of Enterobacteriaceae, Vibrio and Moraxella were dominant. Gram negative bacteria were less (85.84%) in treated than in untreated (96.66%) clam. An increase in gram negative bacteria with increase in the concentration of pesticide was noticed, while a reverse in gram positive bacteria was observed with increase of pesticide concentration.

3.3. Hydrolytic enzyme producing bacteria

Lipolytic bacteria were more (77.82%) among the entire bacterial flora of water and clam followed by urease, chitinase, gelatinase, caseinase, amylase and DNase elaborators (Table 7). None of the isolates extended cellulase enzyme.

Among the total, lipase producers were 77.23% in the native flora representing the most dominant group. Urease was extended by 70.85% followed by chitinase elaborators. All the other hyrolytic enzyme producers occured in lesser percentages.

At 0 hr amylase and lipase producers were 83.85% and 76.4% respectively. The proteolytic enzyme producers (caseinolytic and gelatinolytic) showed almost similar percentages whereas the other groups recorded less.

The bacterial flora after 96 hrs. of the experiment, behaved in a different manner. While lipase producers (78.3%) dominated in untreated samples followed by urease producers (70.85%), the reverse was seen in the treated samples. Urease elaborators constituted 74.59% and lipolytic forms were 72.54%. Amylase producers which were the dominant group at 0 hr samples, reduced in both treated and untreated samples. However, a slight increase (42.84%) from the untreated (38.3%) was recorded.

When the results of the untreated and treated are compared, it was found that in general, percentage of lipase, gelatinase, caseinase, DNase, and chitinase producers got reduced in the treated samples, while amylase and urease producers increased.

The percentage of lipase producers showed an increasing trend representing a linear relationship with the increase of pesticide concentration while all other hydrolytic enzyme producers got reduced in percentage with respect to the increase in concentration of pesticide.

The native flora of water contained higher percentage (80.95%) of lipolytic bacteria (Table 8). Ureolytic forms were 66.7% followed by chitinolytic forms (57.1%). Other enzyme elaborators exhibited lesser dominance and DNase producers were least (28.6%).

At 0 hr, the dominance of amylolytic forms (79.3%) was seen among the hydrolytic enzyme producers in water. Lipolytic and chitinolytic bacteria formed 58.6% each. Other groups were less.

Lipolytic forms (83.3%) retained the dominant position in the untreated water samples, after 96 hrs. Urease and chitinase producers followed it by representing 66.7% each. Other enzyme elaborators were less dominant. In the treated water, the dominance was recorded by the urea utilizers (80.47%). Lipase producers however, represented the second dominant group. While amylolytic and ureolytic forms showed an increase, lipolytic, proteolytic, chitinolytic and DNase producing bacteria were lesser in treated water samples than untreated ones.

The percentage of lipase producers increased linearly with concentration of pesticide. All other groups exhibited reduction with the increase in concentration.

Three fourth of the native flora of clams were ureolytic. Lipase enzyme elaborators were also well represented (73.5%) followed by chitinolytic forms (Table 9).

Lipolytic forms were 94.3% at 0 hr. Amylolytic bacteria also were more (88.4%). Gelatinase, caseinase, urease, and chitinase producers were also more than 60% in clam samples at o hr. Only less represented group was DNase elaborators (33.3%).

A dominance of ureolytic forms (75%) was recorded in untreated clam. The second position was occupied by lipolytic bacteria. In treated clam, the dominance was reverse, ie. lipolytic bacteria were more followed by ureolytic forms. Lipase, gelatinase, caseinase, DNase and chitinase producers were more in treated samples when compared to untreated samples while the rest (amylase and urease elaborators) were more in untreated samples.

In the case of clams also, similar to water, when the concentration of the pesticide increased, lipase producers alone increased and all other enzyme producing groups showed a reduction.

3.4. Antibiotic resistant bacteria

Penicillin-G resistant bacteria (95.4%) were more among the entire flora followed by sulphadiazine (92.29%), while gentamycin resistant forms were less (24.58%) (Table 10).

The native flora and bacteria isolated at 0 hr., in general, exhibited a trend similar to total flora. In untreated samples, in general, there was a slight increase in the percentage of resistant forms. Bacitracin, chloramphenicol and oxytetracycline resistant forms increased slightly while other drug resistant forms decreased in treated than untreated samples.

The percentage of resistant bacteria showed an inverse relationship with the concentration of pesticide used. This response was observed for all antibiotics tested.

The native flora of water contained 100% penicillin-G resistant bacteria (Table 11). More than 70% of bacteria were also found resistant to sulphadiazine and polymyxin-B. The least number showed resistance to gentamycin. The percentage of bacteria resistant to other antibiotics

varied.

A similar pattern of dominance of penicillin resistant forms followed by sulphadiazine resistant ones and least percentage resistance towards gentamycin were observed at 0 hr also.

Untreated water samples, also contained more number (94.12%) of penicillin resistant bacteria. Similar percentages were found resistant to bacitracin followed by sulphadiazine (88.24%). Least percentage was resistant to chloramphenicol.

Maximum percentage of penicillin resistant forms (92.86%), followed by sulphadiazine (88.09%) and bacitracin resistant ones were seen in treated samples. Least percentage of bacteria resistant towards gentamycin was noticed.

A decrease in percentage of ampicillin, bacitracin, gentamycin, penicillin-G,polymyxin-B and sulphadiazine resistant bacteria was noticed in treated than in untreated water. The percentage of antibiotic resistant forms decreased with increase in concentration of pesticide used.

Native flora of clams harboured a maximum of 94.20% each of penicillin and sulphadiazine resistant bacteria. The sulphadiazine resistant forms which were second to penicillin resistant forms in water samples could obtain a higher position in clams. However, the least percentage was resistant to gentamycin (Table 12).

0 hr population of clams contained 97.14% of sulphadiazine resistant forms, followed by 92.86% of penicillin resistant ones. Here also, there was a shift of sulphadiazine resistant forms from second (water - 0 hr.) to first position. Gentamycin resistant forms recorded the least percentage.

All the isolates from untreated clam were penicillin resistant followed by sulphadiazine (98.36%) resistant forms. Here, the trend was the same as that of untreated water. However, bacitracin resistant forms could record only 70.49% in clam, whereas, they were one of the maximum represented groups in water of the same time of the experiment. Similar to water, the clam also harboured least number of chloramphenicol resistant bacteria.

Treated clam showed the same trend as that of water in holding maximum (95.05%) penicillin resistant forms followed by sulphadiazine resistant ones (93.41%) and least number of gentamycin resistant isolates. Treated clam harboured less of ampicillin, gentamycin, neomycin, oxytetracycline, penicillin-G, polymyxin-B, streptomycin and sulphadiazine resistant strains than the treated clam. However, a slight increase in chloramphenicol resistant bacteria could be noticed.

The percentage of resistant forms, against all the antibiotics tested decreased when the concentration of the pesticide increased.

Among the bacterial species encountered as native flora (Table 13), isolates of Pseudomonas showed resistance to all the antibiotics at significant levels except against gentamycin and oxytetracycline. Less number of Vibrio showed resistance to gentamycin and chloramphenicol. All Vibrio isolates showed resistance towards neomycin and bacitracin. On the other hand, Aeromonas recorded least number of resistant strains against gentamycin and exhibited significant levels of resistant forms against all other antibiotics. Bacillus (100%) were resistant against penicillin-G, sulphadiazine, ampicillin, bacitracin and polymyxin-B. Other groups showed varying levels of resistance.

Isolates at 0 hr (Table 14) also showed observations similar to that of native flora. Thus, members of Pseudomonas were sensitive to gentamycin, oxytetracycline and neomycin, while many were resistant to other antibiotics. In general, higher levels of Vibrio and Aeromonas were resistant to all the antibiotics than Pseudomonas. Members of Enterobacteriaceae were sensitive to gentamycin and chloramphenicol, while significantly resistant against other antibiotics. Similar to Vibrio, Alcaligenes which was another major group, also showed resistance to all the antibiotics.

In general, higher percentages of resistant bacteria were found in untreated water than in native flora and 0 hr. population (Table 15). Members of Pseudomonas, Vibrio, Aeromonas and Enterobacteriaceae elaborated resistance against all the antibiotics at significant levels except to chloramphenicol. Other groups which were very less in numbers were sensitive to gentamycin, chloramphenicol and polymyxin-B and resistant to the rest of the antibiotics.

The principal groups, Pseudomonas, Vibrio, Aeromonas, members of Enterobacteriaceae, Coryneform group and Micrococcus of the treated clams were sensitive towards gentamycin and exhibited resistance to all the other antibiotics, at significant level. Comparatively, all other groups showed increased percentages of resistant bacteria to all the antibiotics (Table 16).

Chloramphenicol resistant bacteria of all the genera increased in treated when compared to untreated samples. For other antibiotics there was an increase or decrease in all the genera. However, a uniform pattern could not be seen.

Multiple antibiotic resistance

Multiple antibiotic resistance was recorded by most of the isolates tested (Table 17). 97.78% of strains from native flora, 98.97% of the flora at 0 hr, all the isolates from untreated and 97.92% from treated samples exhibited multiple antibiotic resistance. The pattern which was followed by maximum number of isolates was PSSzAB (P=Penicillin-G; S=Streptomycin; Sz=Sulphadiazine; A=Ampicillin; B=Bacitracin).

Most of the strains of native flora (20%) showed resistance to combination of five antibiotics whereas higher percentages of the flora at 0 hr and untreated, showed resistance to a combination of seven and eight antibiotics respectively. In the case of pesticide treated clam, combinations of resistance to seven antibiotics were more common.

3.5. Heavy metal resistant bacteria

Effect of heavy metals on the growth of bacteria was tested by subjecting them to various heavy metals such as mercury, zinc, cadmium, copper and lead. None of these heavy metals could affect the growth of the bacterial strains significantly (Table 18). Zinc and lead could not exert any influence on the isolates and all showed resistance against these two metals. However, percentage of resistant strains for mercury, copper, and cadmium were 93.7%, 77.4% and 98.0% respectively.

All the native bacterial flora showed resistance to zinc, cadmium and lead while 93.75% and 77.08% elaborated resistance towards mercury and copper respectively. At 0 hr, percentage of mercury resistant forms were lesser (82.35%) than copper resistant bacteria (86.27%). There was no change with respect to the other metal resistant forms. In untreated

sample, mercury resistant isolates were 95.62% and least (66.67%) were copper resistant forms. However, in the untreated samples, an increase of mercury resistant and decrease of cadmium and copper resistant bacteria from 0 hr. could be observed. Although bacteria occured in treated samples exhibited exactly a similar trend (increase) in their resistance pattern as seen in untreated against the five heavy metals, higher percentages of resistance forms were noticed in treated rather than in untreated samples.

Percentages of mercury, cadmium and copper resistant bacteria increased when the concentration of the pesticide increased showing a direct relationship.

The native flora of water contained 100% of mercury, zinc, cadmium and lead resistant forms. 83.3% of the isolates were resistant to copper (Table 19). At 0 hrs, all the isolates from the water samples, were resistant to zinc, cadmium and lead. Mercury and copper resistant forms were equally represented (86.67%).

In the untreated water, 71.43% were copper resistant while all of them were resistant to other metals. In treated water, mercury resistant forms were less (91.67%) and copper resistant forms were more (78.81%) than the untreated samples.

The increase in concentration of pesticide resulted in an increase in percentage of mercury and copper resistant forms while, 100% of the isolates were resistant in all the concentrations, against the other three metals.

Native flora of clams harboured 100% zinc, cadmium and lead resistant bacteria. 91.67% of them were resistant to mercury and 75% to copper (Table 20). All the isolates belonging to the 0 hr. population were resistant to zinc, cadmium and lead. Resistance towards copper was shown by 86.11%. 80.55% were resistant to mercury. 94.44% of the isolates from the untreated samples were resistant to mercury. A considerable number were resistant towards cadmium (88.89%) and only 66.67% were resistant to copper. Mercury resistant bacteria increased while copper and cadmium resistant forms decreased from 0 hr. The treated clam contained more mercury, cadmium and copper resistant forms than untreated clam. Similar to water, here also the resistant forms exhibited an increase in percentage along with an increase in pesticide concentration.

All the isolates belonging to various genera encountered, showed resistance against zinc, cadmium and lead (Table 21). Also, 100% of members other than Pseudomonas and Vibrio were resistant to mercury. 50% and 33.33% of members of Bacillus and Enterobacteriaceae respectively, showed resistance to copper and 100% of other groups were resistant to all other heavy metals.

All the isolates belonging to various genera showed resistance (100%) against zinc, cadmium and lead (Table 21-24). However, slight variations in the resistance against cadmium among Pseudomonas, Vibrio, members of Enterobacteriaceae and Moraxella in untreated and treated samples were encountered. More than 75% of Pseudomonas and Vibrio isolated during various conditions exhibited resistance towards mercury and copper.

More members of bacterial genera, belonging to pesticide treated samples in general, were resistant to copper when compared to untreated and there was a slight variation in cadmium resistant forms among untreated and treated samples.

Multiple heavy metal resistance

All the isolates tested showed multiple heavy metal resistance (Table 25). Irrespective of the source, higher percentages of isolates taken from all the samples recorded resistance against a combination of five heavy metals. However, untreated samples were found to harbour a considerable percentage (29.17%) of bacteria which showed resistance to a combination of four heavy metals. In treated samples, percentage of five metal combination resistant forms were more when compared to untreated. At the sametime, four and three metal combination resistant forms were less in treated than the untreated samples. As the concentration of the pesticide increased, in treated samples, the percentages of bacteria showing resistance to combinations of three, four and five heavy metals also got increased.

Minimal Inhibitory concentrations (MIC) of heavy metals

MIC of heavy metals varied. While 25,150,300,300 and 1000 ppm were MICs of bacterial flora isolated from all the samples for mercury, copper, zinc, cadmium and lead respectively, there was a slight increase of MIC for mercury (30 ppm) for the bacteria isolated from pesticide treated sample (Table 26). However, such an increase was not found for other metals.

3.6. Effect of pesticide concentrations on the growth of bacteria

All the isolates did not grow in mineral agar (MA) medium containing pesticide as the sole source of carbon establishing that the isolates were unable to utilize the pesticide as sole source of carbon. In the presence of a carbohydrate, glucose (Mineral agar supplemented with glucose and pesticide - MAGP), many isolates exhibited growth. Higher percentage of isolates showed growth in the nutrient agar medium supplemented with pesticide (NAP) than in MAGP. As the concentration of pesticide increased, the percentage of tolerant forms decreased in both media (Table 27-29). Half of the native and 0 hr bacteria population could grow even at 0.1% pesticide concentration, when tested in NAP whereas in MAGP one fourth could grow (Table 27).

Number of isolates withstanding the different concentrations of pesticide increased in treated samples when compared to untreated in both the media. However, such difference was well pronounced in MAGP rather than NAP. At the sametime, a reduction in percentage was observed in treated samples when compared to 0 hr population in MAGP while no such clear demarcation could be found in NAP.

In NAP medium, more number of isolates taken from samples treated with higher concentrations of pesticide exhibited growth. The same isolates recorded lesser percentages as the concentrations of pesticide in their sources increased, when grown in MAGP (Table 27-28).

About 33.33% and 4.76% of isolates of native flora of water showed growth at 0.1% pesticide in NAP and MAGP respectively. Among 0 hr population, similar percentages (33.33%) exhibited tolerance to 0.1% pesticide in NAP and only 18.52% showed tolerance in MAGP (Table 28).

The isolates of untreated samples behaved differently in both the media. In NAP higher percentage recorded growth while in MAGP the percentages were lesser than 0 hr. In the treated samples, a decrease in percentage of tolerant isolates from the untreated samples, when tested in NAP and an uplift in MAGP were seen.

Bacteria which were isolated from samples treated with three different concentrations, when tested in NAP medium, an increasing trend of resistant forms was observed. In MAGP medium, it was reverse. Although an increase of tolerant bacteria could be observed in treated sample than untreated in both NAP and MAGP media, in general, the tolerant forms were less than what was observed at 0 hr.

Half of the isolates from the native and 0 hr bacterial flora of clam were withstanding 0.1% pesticide in NAP and around 30% in MAGP (Table 29). In untreated samples it reduced to 9.5% and 11.1 % in NAP and MAGP respectively. However, in treated samples around 50% and 14% of the isolates showed growth at 0.1% pesticide concentration.

The percentage of pesticide tolerant bacteria decreased as the concentration of pesticide in the samples increased when tested in NAP. A reverse observation was made when tested in MAGP.

3.7. Effect of environmental parameters on the growth and phosphate release of bacteria

Effect of environmental parameters on the growth and phosphate release was assayed by subjecting four strains of bacteria, belonging to species of Vibrio and Pseudomonas isolated from untreated (K 22 and K 41 respectively) and treated (K 243 and K 181 respectively) samples to different levels of temperature, pH, and sodium chloride concentration.

3.7.1 Effect of environmental parameters on growth

3.7.1.1 Effect of temperature

Species of Vibrio (K 22) and Pseudomonas (K 41) isolated from untreated samples grew to the maximum at 25°C, while the species of Pseudomonas (K 181) and Vibrio (K 243) isolated from treated samples showed maximum growth at 37°C (Fig. 3). However, all the four isolates exhibited moderate growth 93.43% and 84.78% at temperature between 15 and 37°C. At 4°C, K 243 and at 45°C, K 181 showed about 40% to 50% of the growth which was noticed at 25 and 37°C.

3.7.1.2 Effect of pH

The isolates of the untreated samples grew to their maximum at pH 7 whereas, the isolates of treated samples recorded maximum growth at a higher pH 8 (Fig. 4). All the isolates could grow to a moderate level at hydrogen ion concentrations of 5, 6, 9 and 10.

3.7.1.3 Effect of sodium chloride concentration

Irrespective of the source of samples, whether untreated or pesticide treated, all the four isolates were able to record maximum growth at 1% sodium chloride concentration and could grow at wider range of sodium chloride concentrations (Fig. 5). They could record significant levels of growth at concentrations between 0.5% and 5% of sodium chloride.

3.7.2. Effect of environmental parameters on phosphate release

3.7.2.1 Effect of temperature

Phosphate release by the four strains of bacteria recorded a similar trend observed with respect to growth at various temperatures

(Fig. 6). Maximal phosphate release coincided with maximal growth. Vibrio sp. (K 22) and Pseudomonas sp. (K 41) isolated from untreated samples released maximum phosphate at 25°C. Pseudomonas sp. (K 181) and Vibrio sp. (K 243) isolated from treated samples recorded maximal phosphate release at 37°C. All the isolates released significant levels of phosphate at 4, 15 and 37°C. The phosphate release was highly reduced at higher temperatures.

3.7.2.2 Effect of pH

Maximal release of phosphate was recorded at pH 7 for the isolates taken from untreated samples and at pH 8 for the isolates of treated samples (Fig. 7). However, isolates of both the treated and untreated samples could also release appreciable levels of phosphate at other pH levels where they could record moderate growth.

3.7.2.3 Effect of sodium chloride concentration

It was observed that all the four strains could release maximum phosphate at 1% NaCl concentration, where they recorded maximal growth (Fig. 8). However, at other levels of NaCl concentrations (upto 3%) a moderate amount of phosphate was released by all isolates.

3.8. Generation time

Generation time varied for different isolates. Vibrio (K 22) and Pseudomonas (K 41) strains isolated from untreated samples recorded generation time of 43 minutes and 54 minutes respectively. Interestingly, both the Pseudomonas sp. (K 181) and Vibrio sp. (K 243) isolated from treated samples were doubling their number at 48 minutes.

Fig. 3. Effect of temperature on growth of the selected isolates

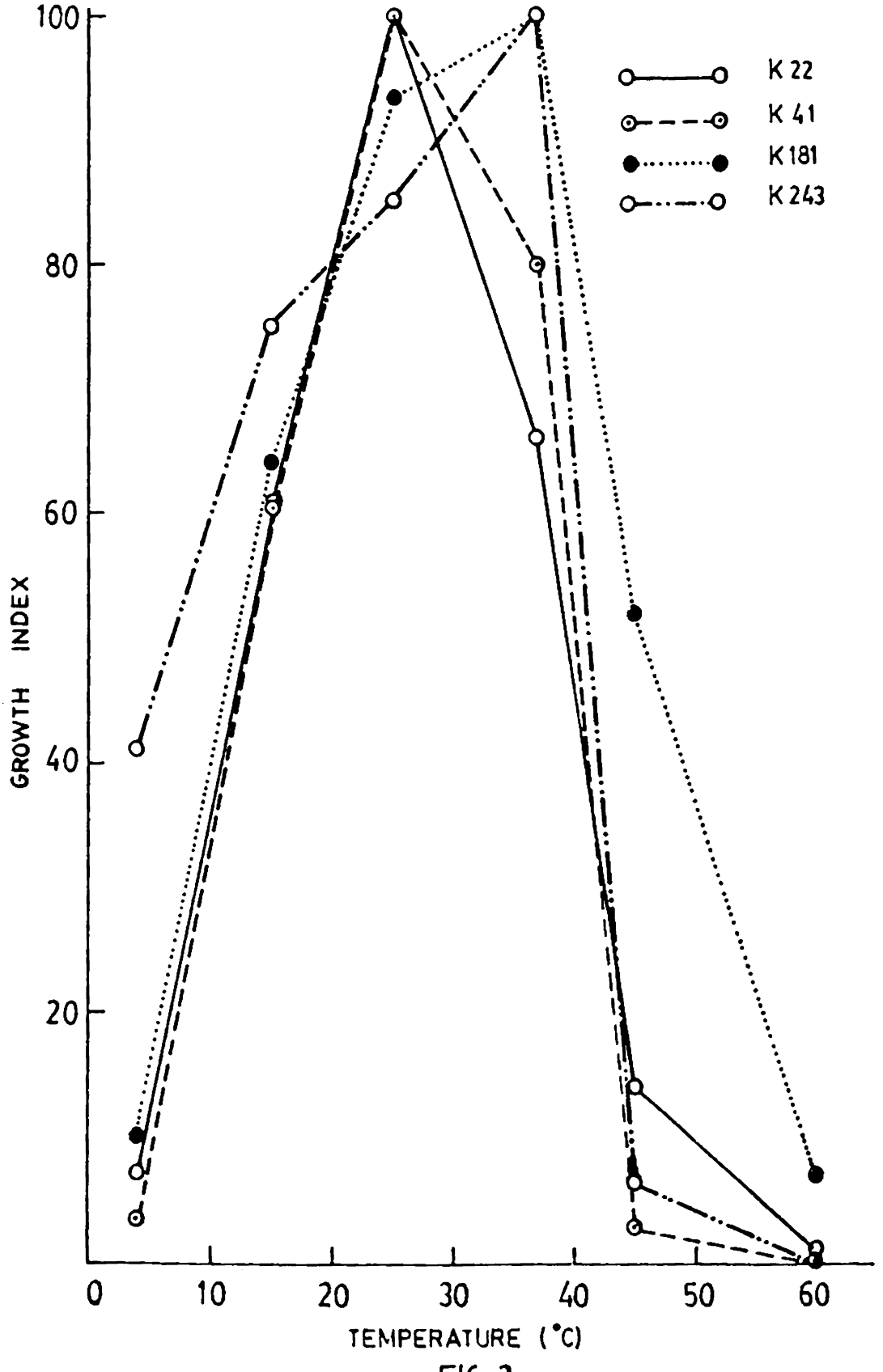

100
80
60
40
20
GROWTH INDEX
0
10
20
30
40
50
60
TEMPERATURE (°C)
K 22
K 41
K 181
K 243

FIG-3

Fig. 4. Effect of pH on growth of the selected isolates

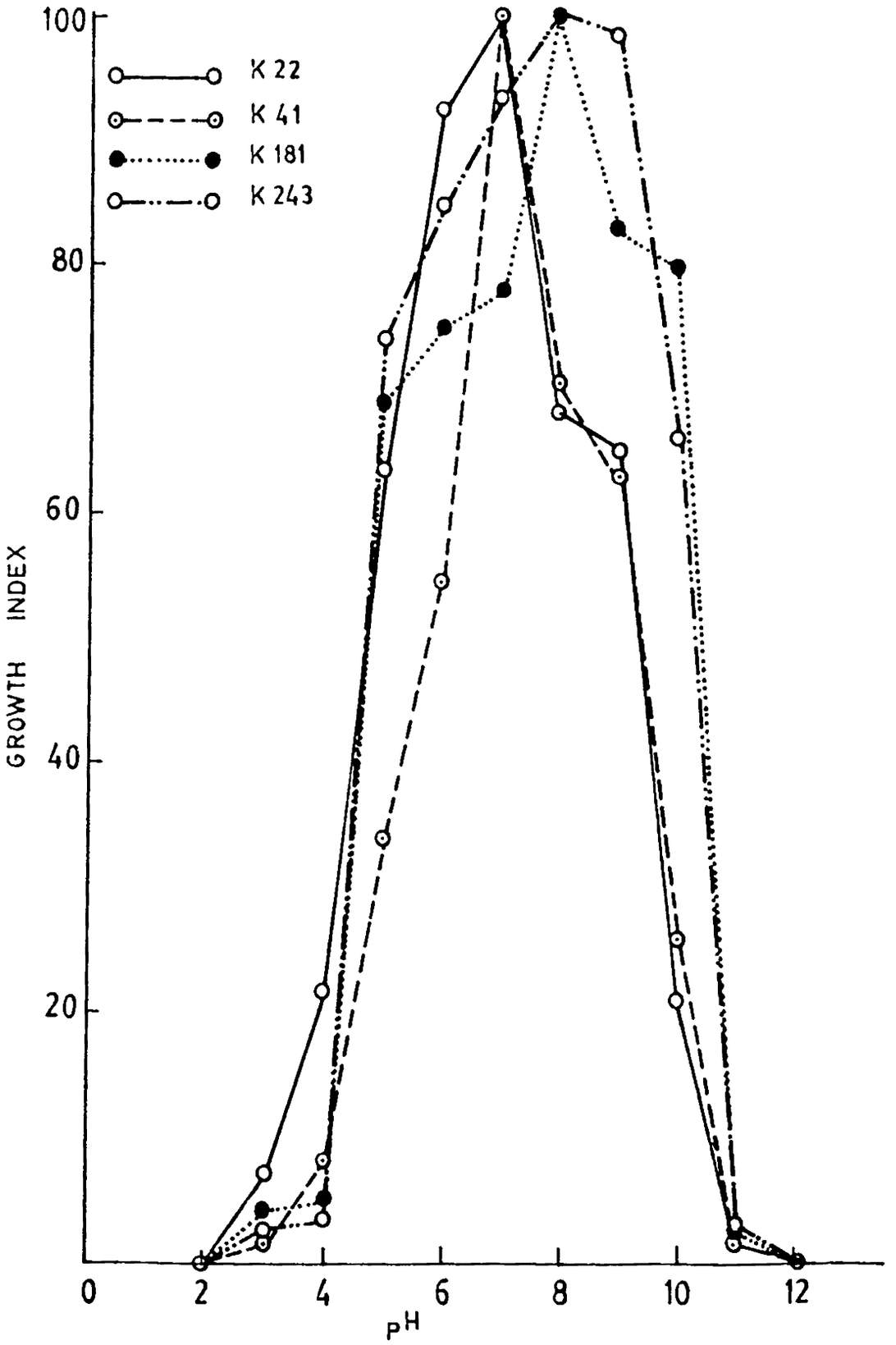


FIG-4

Fig. 5. Effect of sodium chloride concentration on growth of the selected isolates

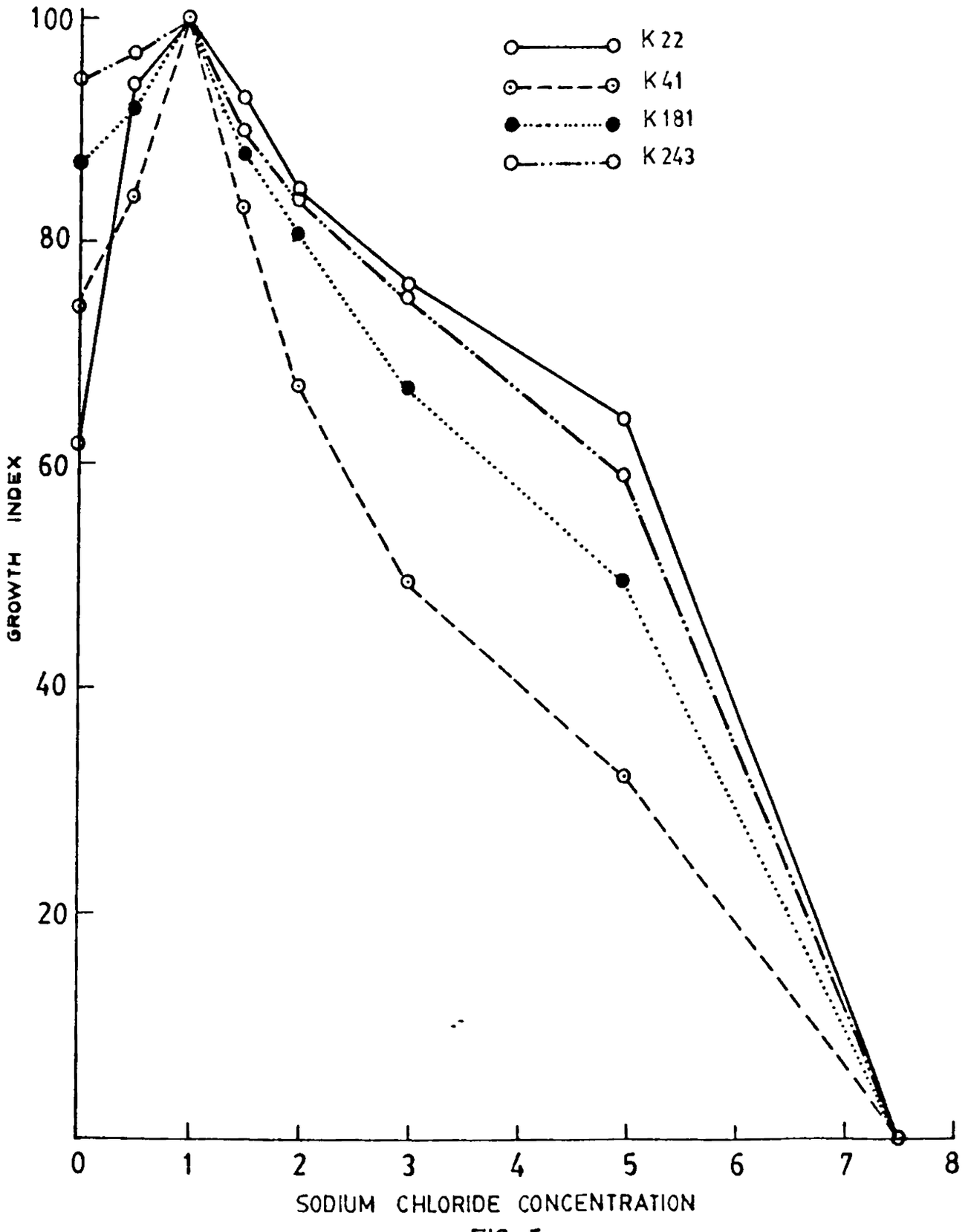

K 22
K 41
K 181
K 243
GROWTH INDEX
100
80
60
40
20
0
1
2
3
4
5
6
7
8
SODIUM CHLORIDE CONCENTRATION

FIG-5

Fig. 6. Effect of temperature on phosphate release by the selected iso

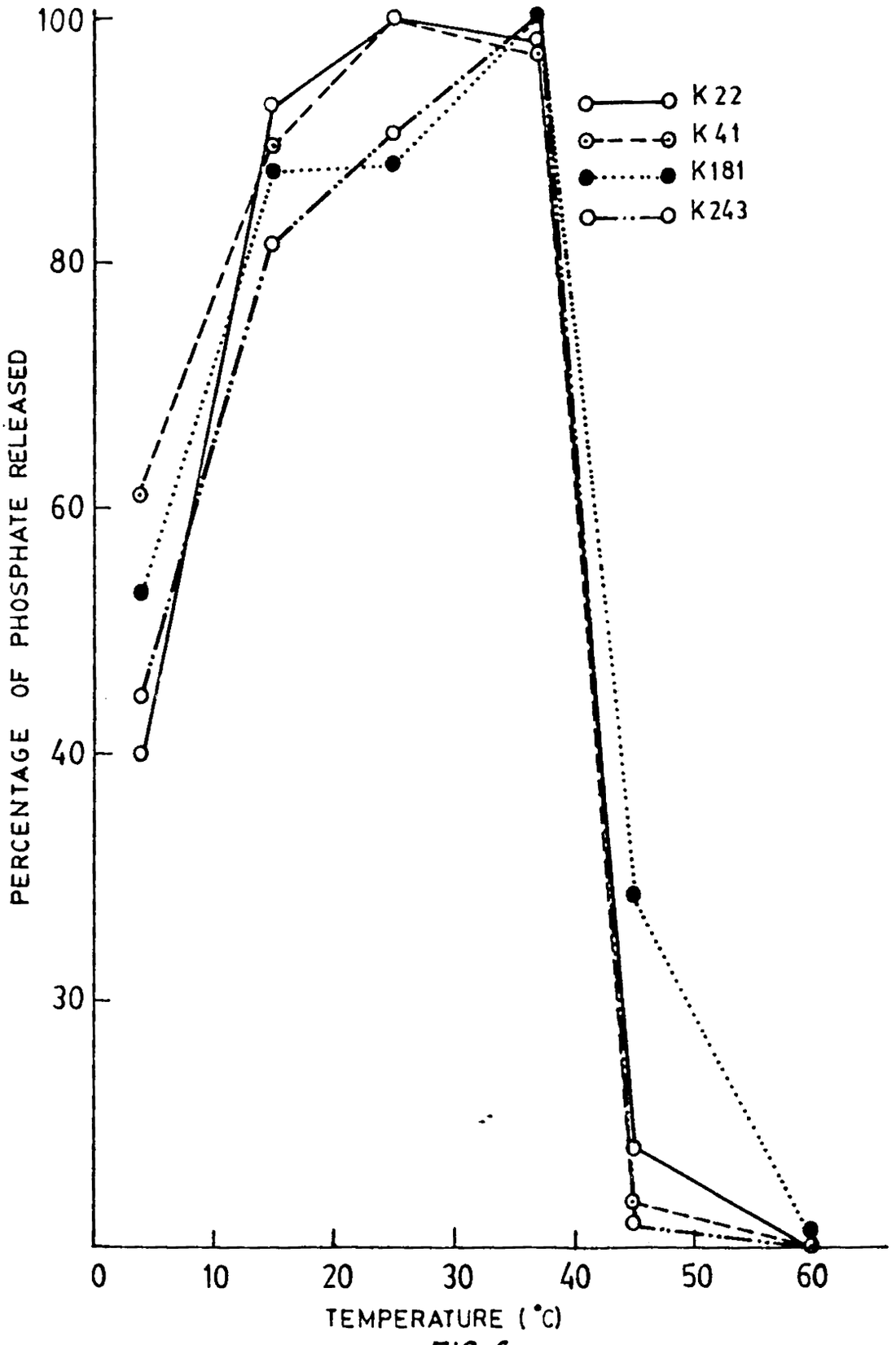

100
80
60
40
30
PERCENTAGE OF PHOSPHATE RELEASED
0
10
20
30
40
50
60
TEMPERATURE (°C)
K 22
K 41
K181
K243

FIG-6

Fig. 7. Effect of pH on phosphate release by the selected iso

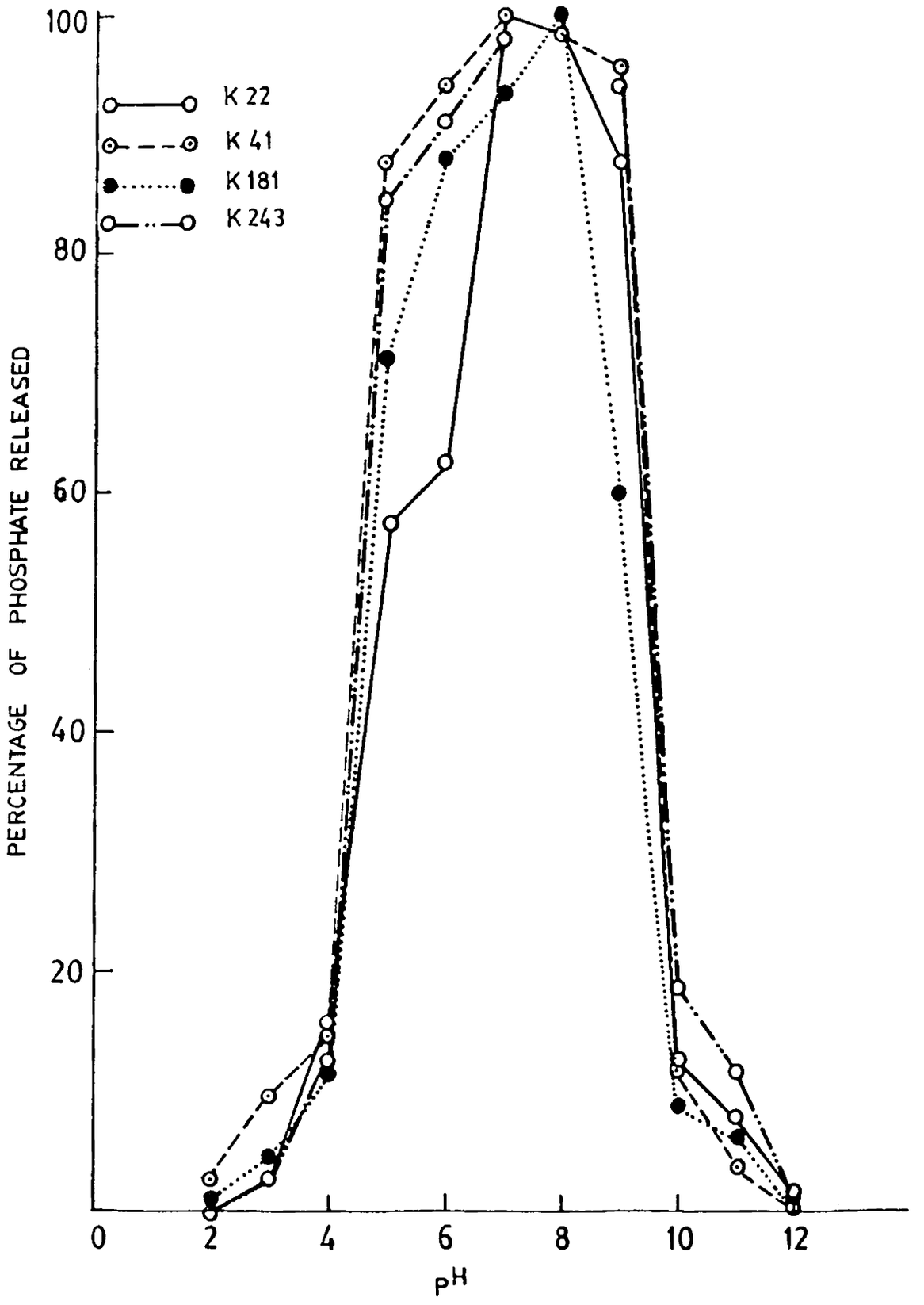

100
80
60
40
20
0
2
4
6
8
10
12
K 22
K 41
K 181
K 243
PERCENTAGE OF PHOSPHATE RELEASED
pH

FIG-7

Fig. 8. Effect of sodium chloride concentration on phospl release by the selected isolates

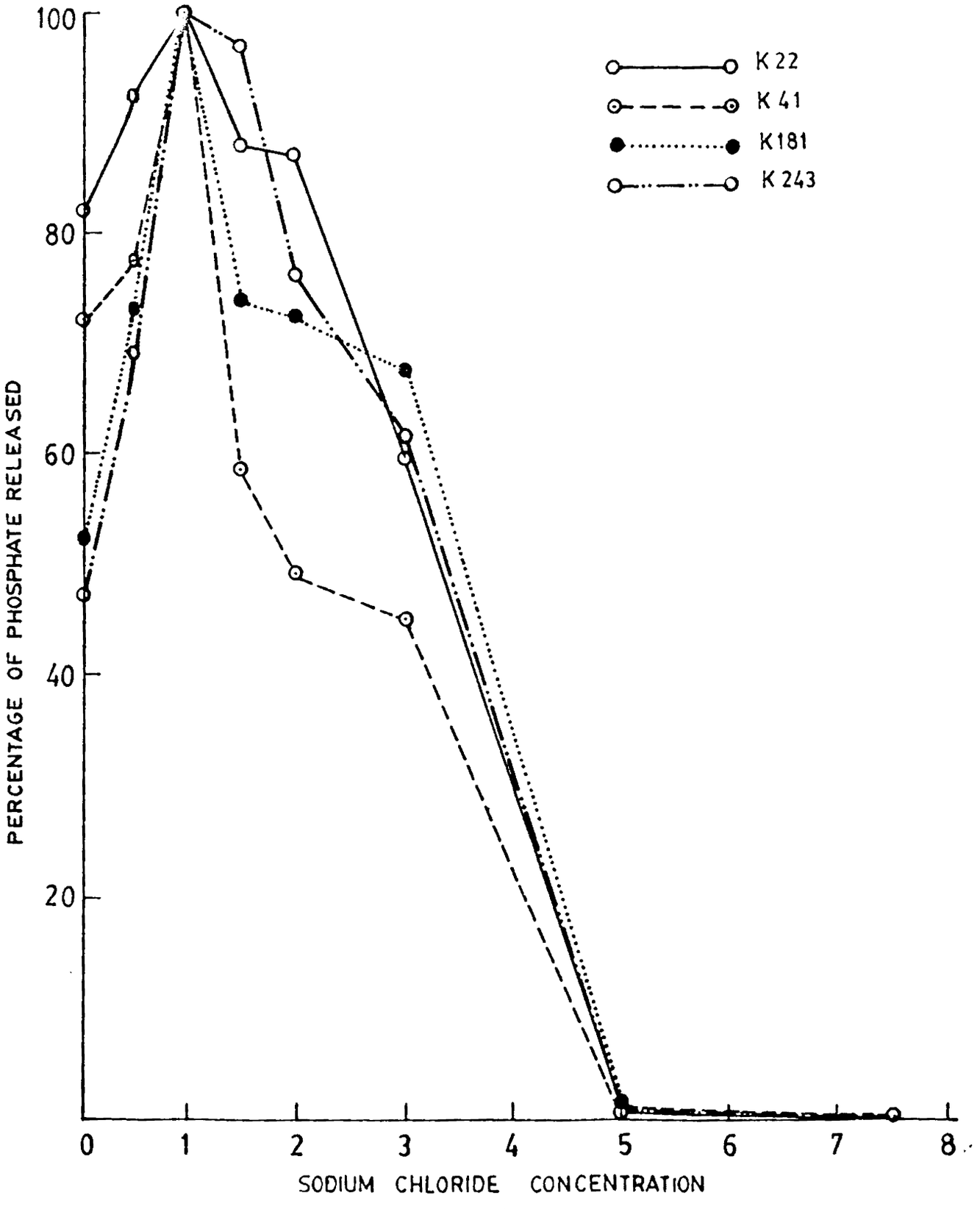

100
80
60
40
20
PERCENTAGE OF PHOSPHATE RELEASED
K 22
K 41
K181
K 243
0
1
2
3
4
5
6
7
8
SODIUM CHLORIDE CONCENTRATION

FIG - 8

Table 3. Distribution of THB in pesticide treated and untreated water and clam

Sample / Concentration (ml / l)	Samples	
	Water (x 10^3/ml)	Clam (x 10^3/g)
NF	65	2975
I	252	1644
UT	595	28017
*T	741	28887
* Distribution of THB in water and clam treated with various concentrations of pesticide		
0.000125	1952	35862
0.00025	167	24183
0.0005	105	24006

NF = Native flora I = Initial (O hr.)

UT = Untreated-Control (96 hrs); T = Pesticide treated (96 hrs)

Table 4. Generic composition of THB isolated from water and clam

Sample	*Pseudomonas*	*Vibrio*	*Aeromonas*	Enterobacteriaceae	*Acinetobacter*	*Alcaligenes*	*Moraxella*	*Flavobacterium -cytophaga*	*Bacillus*	Coryneform group	*Micrococcus*	Gram-negative	Gram positive
NF	22.20	13.30	17.80	26.70	2.20	2.20	0.00	0.00	5.60	5.60	4.40	79.40	20.60
I	19.60	29.90	10.30	14.40	10.30	8.20	3.10	2.10	2.10	3.10	6.20	88.60	11.40
UT	20.50	16.67	19.20	10.30	5.10	9.00	6.40	0.00	6.40	5.10	1.30	87.20	12.80
T	31.25	16.67	10.83	11.25	3.33	2.50	4.58	2.92	3.33	7.08	6.25	83.36	16.64
Total	25.70	18.60	13.30	14.50	2.90	4.50	3.80	1.80	4.00	5.70	5.20	85.10	14.90

NF = Native flora I = Initial (0 hr)

UT = Untreated - Control (96 hrs); T = Pesticide treated (96 hrs)

Table 5. Generic composition of THB isolated from water

Sample/ Concentration (ml/l)	Pseudo-monas	Vibrio	Aero-monas	Entero-bacteriaceae	Acineto-bacter	Alcali-genes	Moraxella	Flavo-bacterium-cytophaga	Bacillus	Coryne-form group	Micro-coccus	Gram negative	Gram positive
NF	23.81	4.76	0.0	47.61	9.50	0.0	0.0	0.0	4.76	0.0	9.52	85.72	14.28
I	37.93	13.79	3.45	24.14	0.0	0.0	3.45	0.0	3.45	3.45	3.45	89.65	10.35
UT	11.11	5,56	5.56	16.67	0.0	11.11	0.0	0.0	27.78	16.67	0.0	55.55	44.45
*T	25.94	8.40	16.57	8.37	5.46	5.36	3.44	1.85	5.46	7.04	12.14	75.36	24.64
	* Generic composition of THB in water treated with various concentrations of pesticide												
0.000125	19.05	14.29	33.33	14.29	0.0	0.0	4.76	0.0	0.0	4.76	9.52	85.62	14.28
0.00025	42.10	5.26	5.26	5.26	5.26	10.53	0.0	0.0	5.26	5.26	15.78	73.70	26.30
0.0005	16.67	5.56	11.11	5.56	11.11	5.56	5.56	5.56	11.11	11.11	11.11	66.67	33.33

NF = Native flora; I = Initial (0 hr)

UT = Untreated - Control (96 hrs); T = Pesticide treated (96 hrs)

Table 6. Generic composition of THB isolated from clam

Sample/ Concentration (ml/1)	Pseudomonas	Vibrio	Aeromonas	Enterobacteriaceae	Acinetobacter	Alcaligenes	Moraxella	Flavobacterium-cytophaga	Bacillus	Coryneform group	Micrococcus	Gram negative	Gram positive
NF	22.06	16.18	23.53	20.59	0.0	2.94	0.0	0.0	5.88	7.35	2.94	83.83	16.17
I	11.59	36.23	13.04	10.14	1.45	11.59	2.90	2.90	1.45	2.90	7.25	88.40	11.60
UT	23.33	20.00	23.33	8.33	6.67	8.33	8.33	0.0	0.0	1.67	1.67	96.66	3.34
*T	32.83	19.25	8.89	19.42	2.74	1.65	12.22	3.28	2.74	6.99	4.43	85.84	14.16
* Generic composition of THB in clam treated with various concentrations of pesticide													
0.000125	45.16	17.74	0.0	1.61	3.23	1.61	0.0	4.84	3.23	20.97	1.61	64.19	35.81
0.00025	28.33	11.67	21.67	33.33	0.0	3.33	33.33	1.67	3.33	0.0	6.67	90.00	10.00
0.0005	25.00	28.33	5.00	23.33	5.00	0.0	3.33	3.33	1.67	0.0	5.0	93.33	6.67

NF = Native flora; I = Initial (0 hr)

UT = Untreated - Control (96 hrs); T = Pesticide treated (96 hrs)

Table 7. Hydrolytic enzyme producing bacteria isolated from water and clam

Sample/ Concentration (ml/l)	Lipase	Amylase	Gelatin-ase	Caseinase	Urease	DNase	Chitinase
NF	77.23	45.70	47.35	51.95	70.85	38.55	58.65
I	76.40	83.85	58.95	59.50	52.85	25.25	62.65
UT	78.30	38.30	52.50	46.65	70.85	46.65	60.00
*T	72.54	42.84	43.31	45.90	74.59	45.34	54.50
Total	77.82	49.90	53.66	50.89	68.91	43.76	59.00
	* Hydrolytic enzyme producing bacteria in water and clam treated with various concentrations of pesticide						
0.000125	62.80	55.95	50.30	60.10	81.90	52.75	55.35
0.00025	75.00	37.40	42.15	46.65	74.35	48.80	55.35
0.0005	80.25	35.15	37.50	31.40	67.50	34.45	52.80

NF = Native flora; I = Initial (0 hr)

UT = Untreated - Control (96 hrs); T = Pesticide treated (96 hrs)

Table 8. Hydrolytic enzyme producing bacteria isolated from water

Sample/ Concentration (ml/1)	Lipase	Amylase	Gelatinase	Caseinase	Urease	DNase	Chitinase
NF	80.95	42.90	47.60	52.40	66.70	28.60	57.10
I	58.60	79.30	48.30	55.20	44.80	17.20	58.60
UT	83.30	33.30	50.00	50.00	66.70	50.00	66.70
*T	65.30	50.87	26.13	41.87	80.47	39.07	47.97
	Hydrolytic enzyme producing bacteria in water treated with various concentrations of pesticide						
0.000125	57.90	61.90	47.40	61.90	90.50	47.60	52.60
0.00025	66.70	52.60	16.70	36.80	84.20	47.40	52.40
0.0005	72.20	38.10	14.30	27.80	66.70	22.20	38.90

NF = Native flora; I = Initial (0 hr)

UT = Untreated - Control (96 hrs); T = Pesticide treated (96 hrs)

Table 9. Hydrolytic enzyme producing bacteria isolated from clam

Sample/ Concentration (ml/1)	Lipase	Amylase	Gelatinase	Caseinase	Urease	DNase	Chitinase
NF	73.50	48.50	47.10	51.50	75.00	48.50	60.30
I	94.30	88.40	69.60	63.80	60.90	33.30	66.70
UT	73.30	43.30	55.00	43.30	75.00	43.30	53.30
*T	79.80	34.80	60.50	49.90	68.70	51.60	61.00
	Hydrolytic enzyme producing bacteria in clam treated with various concentrations of pesticide						
0.000125	67.70	50.00	70.00	58.30	73.30	58.10	66.70
0.00025	83.30	36.70	58.30	56.50	68.30	50.00	58.30
0.0005	88.30	17.70	53.20	35.00	64.50	46.70	58.10

NF = Native flora ; I = Initial (O hr)

UT = Untreated - control (96 hrs); T = Pesticide treated (96 hrs)

Table 10. Distribution of antibiotic resistant bacteria in water and clams

Sample/ Concentration (ml/l)	Ampicillin	Bacitracin	Chloram-phenicol	Gentamycin	Neomycin	Oxytetra-cycline	Penicillin-G	Polymyxin-B	Streptomy-cin	Sulpha-diazine
NF	72.22	65.55	43.30	20.20	68.90	33.70	96.60	75.55	63.30	92.22
I	76.29	73.20	43.30	19.59	56.70	41.21	91.75	55.70	71.13	88.70
UT	83.30	75.60	32.10	42.30	79.50	53.80	98.70	56.40	79.50	96.20
*T	79.03	78.60	52.40	16.23	66.50	56.17	94.53	45.83	77.93	92.03
Total	77.71	73.24	42.78	24.58	67.90	46.22	95.40	58.37	72.97	92.29
* Antibiotic resistant bacteria in water and clam treated with various concentrations of pesticide										
0.000125	88.90	86.40	60.50	19.80	79.00	63.00	96.30	56.80	79.50	95.10
0.00025	79.00	80.20	53.10	14.80	65.40	56.80	96.30	47.40	79.00	95.10
0.0005	69.20	69.20	43.60	14.10	55.10	48.70	91.00	33.30	75.30	85.90

NF = Native flora; I = Initial (O hr)

UT = Untreated - control (96 hrs); T = Pesticide treated (96 hrs)

Table 11. Distribution of antibiotic resistant bacteria in water

Sample/ Concentration (ml/l)	Ampicillin	Bacitracin	Chloram-phenicol	Gentamycin	Neomycin	Oxytetra-cycline	Penicillin-G	Polymyxin-B	Streptomy-cin	Sulphadia-zine
NF	47.62	42.86	42.86	9.52	47.62	23.81	100	71.43	33.33	85.71
I	59.26	48.15	62.96	0.0	40.74	22.22	88.89	59.26	62.96	66.67
UT	88.24	94.12	17.65	41.18	64.71	58.82	94.12	64.71	64.71	88.24
*T	77.51	85.98	62.49	16.74	70.20	72.52	92.86	47.70	74.30	88.09
* Antibiotic resistant bacteria in water treated with various concentrations of pesticide										
0.000125	100	100	68.42	28.57	94.74	84.21	100	57.14	77.78	100
0.00025	71.43	85.71	66.67	11.11	71.43	66.67	95.24	52.63	73.68	83.33
0.0005	61.11	72.22	52.38	10.53	44.44	66.67	83.33	33.33	71.43	80.95

NF = Native flora; I = Initial (O hr)

UT = Untreated - control (96 hrs); T = Pesticide treated (96 hrs)

Table 12. Distribution of antibiotic resistant bacteria in clam

Sample/ Concentration (ml/1)	Ampicillin	Bacitracin	Chloram-phenicol	Gentamycin	Neomycin	Oxytetra-cycline	Penicillin-G	Polymyxin-B	Streptomy-cin	Sulpha-diazine
NF	79.71	72.46	43.48	23.19	75.36	36.23	94.20	76.81	72.46	94.20
I	82.86	82.86	35.71	27.14	62.86	48.57	92.86	54.29	74.23	97.14
UT	81.97	70.49	36.07	42.62	83.61	52.46	100	54.10	83.61	98.36
*T	79.61	76.31	49.35	15.86	65.28	51.09	95.05	45.25	79.11	93.41
* Antibiotic resistant bacteria in clam treated with various concentrations of pesticide										
0.000125	85.48	82.26	58.06	22.58	74.19	58.33	96.67	56.67	80.65	100
0.00025	81.67	78.33	53.33	15.00	63.33	51.61	95.16	51.67	80.00	93.55
0.0005	71.67	68.33	36.67	10.00	58.33	43.33	93.33	27.42	76.67	86.67

NF = Native flora; I = Initial (O hr)

UT = Untreated - control (96 hrs); T = Pesticide treated (96 hrs)

Table 13. Distribution of antibiotic resistant bacteria among different genera

A. Native flora

Genera	Ampicillin	Bacitracin	Chloram-phenicol	Gentamycin	Neomycin	Oxytetra cycline	Penicillin-G	Polymyxin-B	Streptomycin	Sulphadiazine
seudomonas	75.0	80.0	65.00	30.00	85.00	35.00	95.0	85.0	70.0	80.0
ibrio	75.0	100	25.00	16.67	100	50.00	91.67	75.0	75.0	91.67
eromonas	87.5	87.5	62.5	31.25	81.25	62.50	100	62.5	75.0	100
ntero- cteriaceae	45.83	25.00	29.17	0.0	41.67	4.17	91.67	83.33	45.83	91.67
cineto- cter	0.0	0.0	0.0	0.0	0.0	50.0	100	100	0.0	100
lcaligenes	100	0.0	0.0	0.0	10.0	0.0	100	50.0	50.0	100
loraxella	-	-	-	-	-	-	-	-	-	-
lavobacterium- ytophaga	-	-	-	-	-	-	-	-	-	-
acillus	100	100	40.0	20.0	40.0	40.0	100	100	60.00	100
oryneform roup	100	60.0	40.0	60.0	40.0	40.0	100	60.0	80.00	100
icrococcus	100	75.00	50.0	25.0	100	25.0	100	50.0	75.0	100

Table 14. Distribution of antibiotic resistant bacteria among different genera

B. Initial

Genera	Ampicillin	Bacitracin	Chloram-phenicol	Gentamycin	Neomycin	Oxytetra-cycline	Penicillin-G	Polymyxin-B	Streptomycin	Sulphadiazine
Pseudomonas	73.68	63.16	52.63	5.26	26.32	15.79	100	52.63	47.37	68.42
Vibrio	89.66	86.21	48.28	31.03	58.62	44.83	96.55	62.07	68.97	96.55
Aeromonas	80.00	100	40.00	0.0	80.0	50.00	90.00	90.00	90.00	100
Enterobacte-riaceae	42.86	64.29	28.57	21.43	64.29	42.86	64.29	50.00	85.71	85.71
Acinetobacter	0.0	0.0	0.0	0.0	0.0	0.0	100	100	0.0	100
Alcaligenes	100	75.00	62.50	37.50	75.00	62.50	100	50.0	75.00	100
Moraxella	100	66.67	33.33	66.67	100	100	100	100	100	66.67
Flavobacte-rium - cytophaga	100	100	50.00	50.00	50.00	50.00	100	0.0	100	100
Bacillus	100	0.0	0.0	0.0	100	0.0	100	0.0	100	100
Coryneform group	33.33	66.67	66.67	0.0	100	66.67	66.67	66.67	66.67	66.67
Micrococcus	66.67	50.0	16.67	0.0	16.67	33.33	100	0.0	66.67	100

Table 15. Distribution of antibiotic resistant bacteria among different genera

C . Untreated (control - 96 hrs)

Genera	Ampicillin	Bacitracin	Chloramphe-nicol	Gentamycin	Neomycin	Oxytetra-cycline	Penicillin-G	Polymyxin-B	Streptomycin	Sulphadiazine
Pseudomonas	81.25	62.50	37.50	50.00	75.00	43.75	100	50.00	87.5	100
Vibrio	84.62	92.31	30.77	46.15	84.62	69.23	92.31	92.31	92.31	92.31
Aeromonas	93.33	86.67	40.00	66.67	100	53.33	100	73.33	100	93.33
Enteroba-teriaceae	87.50	75.00	12.50	50.00	100	75.00	100	12.50	75.00	100
Acinetobacter	0.0	50.00	0.0	0.0	0.0	0.0	100	25.0	0.0	100
Alcaligenes	100	71.43	14.29	14.29	85.71	42.86	100	42.86	85.71	100
Moraxella	80.00	60.00	60.00	20.00	80.00	100	100	20.00	80.00	100
Flavobacterium cytophaga	-	-	-	-	-	-	-	-	-	-
Bacillus	100	100	20.00	20.00	60.00	0.0	100	100	20.00	100
Coryneform group	100	75.00	25.00	25.00	75.00	100	100	50.00	75.00	75.00
Micrococcus	0.0	0.0	0.0	0.0	0.0	0.0	100	0.0	100	100

Table 16. Distribution of antibiotic resistant bacteria among different genera

D. Pesticide treated (96 hrs)

Genera	Ampicillin	Bacitracin	Chloram-phenicol	Gentamycin	Neomycin	Oxytetra-cycline	Penicillin-G	Polymyxin-B	Streptomycin	Sulphadiazine
Pseudomonas	90.67	86.67	60.00	12.00	74.67	73.33	100	45.33	89.33	98.67
Vibrio	70.00	80.00	32.50	12.50	72.50	52.50	85.00	47.50	77.50	92.50
Aeromonas	76.92	80.77	61.54	23.08	53.85	50.00	92.31	30.77	65.38	96.15
Enterobacter-iaceae	62.96	66.67	33.33	22.22	77.78	29.63	92.59	55.56	81.48	70.37
Acineto-bacter	50.00	37.50	12.50	0.0	12.50	37.50	100	0.0	37.50	75.00
Alcaligenes	66.67	83.33	100	0.0	50.00	50.00	100	66.67	66.67	100
Moraxella	81.82	54.55	90.91	0.0	81.82	90.91	100	81.82	54.55	90.91
Flavobacterium Cytophaga	100	85.71	100	100	100	100	100	57.14	100	100
Bacillus	87.50	62.50	87.50	12.50	50.00	37.50	100	75.00	50.00	100
Coryneform sp	64.71	82.35	47.06	17.65	58.82	35.29	82.35	35.29	70.59	76.47
Micrococcus	100	93.33	26.67	13.33	40.00	40.00	100	33.33	100	100

Table 17. Distribution of multiple antibiotic resistant bacteria in water and clam

Sample/ Concentration (ml/1)	Number of antibiotics									
	1	2	3	4	5	6	7	8	9	10
NF	2.22	0.0	7.78	6.67	20.00	15.56	13.33	18.89	7.78	5.56
I	1.03	1.03	6.19	13.40	18.56	17.53	20.62	12.37	8.25	4.12
UT	0.0	2.56	6.41	5.13	6.41	10.26	25.64	30.77	10.26	5.13
*T	2.08	1.25	7.08	5.83	10.42	17.08	19.17	16.67	15.42	3.75
Total	1.33	1.21	27.46	7.76	13.85	15.11	19.69	19.68	10.43	4.64
	Multiple antibiotic resistant bacteria in water and clam treated with various concentrations of pesticide									
0.000125	1.23	1.23	1.23	4.94	9.88	17.28	22.22	19.75	16.05	2.47
0.00025	1.23	1.23	6.17	8.64	7.41	17.28	18.52	13.58	19.75	6.17
0.0005	3.85	1.28	14.10	3.85	14.10	16.67	16.67	16.67	10.26	2.56

NF = Native flora; I = Initial (O hr)

UT = Untreated - control (96 hrs); T = Pesticide treated (96 hrs)

Table 18. Distribution of heavy metal resistant bacteria in water and clams

Sample/ Concentration (ml/1)	Metals				
	Mercury	Zinc	Cadmium	Copper	Lead
NF	93.75	100	100	77.08	100
I	82.35	100	100	86.27	100
UT	95.62	100	91.67	66.67	100
*T	95.83	100	98.92	70.30	100
Total	93.70	100	98.00	77.40	100
	* Heavy metal resistant bacteria in water and clam treated with various concentrations of pesticide				
0.000125	90.32	100	96.77	51.70	100
0.00025	96.55	100	100	74.19	100
0.0005	100	100	100	85.00	100

NF = Native flora, I = Initial (O hr)

UT = Untreated - control (96 hrs); T = Pesticide treated (96 hrs)

Table 19. Distribution of heavy metal resistant bacteria in water

Sample/ Concentration (ml/1)	Metals				
	Mercury	Zinc	Cadmium	Copper	Lead
NF	100	100	100	83.30	100
I	86.67	100	100	86.67	100
UT	100	100	100	71.43	100
*T	91.67	100	100	78.81	100
	Heavy metal resistant bacteria in water treated with various concentrations of pesticide				
0.000125	75.00	100	100	71.43	100
0.00025	100	100	100	75.00	100
0.0005	100	100	100	90.00	100

NF = Native flora; I = Initial (O hr)

UT = Untreated - control (96 hrs); T = Pesticide treated (96 hrs)

Table 20. Distribution of heavy metal resistant bacteria in clam

Sample/ Concentration (ml/1)	Metals				
	Mercury	Zinc	Cadmium	Copper	Lead
NF	91.67	100	100	75.00	100
I	80.55	100	100	86.11	100
UT	94.44	100	88.89	66.67	100
*T	97.03	100	98.55	68.29	100
	* Heavy metal resistant bacteria in clam treated with various concentrations of pesticide				
0.000125	95.45	100	95.65	47.62	100
0.00025	95.65	100	100	73.91	100
0.0005	100	100	100	83.33	100

NF = Native flora, I = Initial (O hr)

UT = Untreated - control (96 hrs) ; T = Pesticide treated (96 hrs)

Table 21. Distribution of heavy metal resistant bacteria among different genera

A. Native flora

Genera	Metals				
	Mercury	Zinc	Cadmium	Copper	Lead
Pseudomonas	92.31	100	100	92.31	100
Vibrio	87.50	100	100	75.00	100
Aeromonas	100	100	100	100	100
Enterobacteriaceae	100	100	100	50.00	100
Acinetobacter	100	100	100	100	100
Alcaligenes	100	100	100	100	100
Moraxella	-	-	-	-	-
Flavobacterium - cytophaga	-	-	-	-	-
Bacillus	100	100	100	33.33	100
Coryneform group	100	100	100	100	100
Micrococcus	100	100	100	100	100

Table 22. Distribution of heavy metal resistant bacteria among different genera

B . Initial

Genera	Metals				
	Mercury	Zinc	Cadmium	Copper	Lead
Pseudomonas	88.89	100	100	77.78	100
Vibrio	76.92	100	100	76.92	100
Aeromonas	100	100	100	100	100
Enterobacteriaceae	100	100	100	100	100
Acinetobacter	0.0	100	100	100	100
Alcaligenes	75.0	100	100	100	100
Moraxella	100	100	100	100	100
Flavobacterium - cytophaga	100	100	100	100	100
Bacillus	0.0	100	100	50.0	0.0
Coryneform group	100	100	100	50.0	100
Micrococcus	100	100	100	100	100

Table 23. Distribution of heavy metal resistant bacteria among different genera

C . Untreated (control - 96 hrs)

Genera	Metals				
	Mercury	Zinc	Cadmium	Copper	Lead
Pseudomonas	88.89	100	88.89	77.78	100
Vibrio	100	100	87.50	62.50	100
Aeromonas	100	100	100	77.78	100
Enterobacteriaceae	100	100	83.33	50.00	100
Acinetobacter	100	100	100	0.0	100
Alcaligenes	75.00	100	100	50.00	100
Moraxella	100	100	75.00	75.00	100
Flavobacterium cytophaga	-	-	-	-	-
Bacillus	100	100	100	100	100
Coryneform group	100	100	100	33.33	100
Micrococcus	100	100	100	100	100

Table 24. Distribution of heavy metal resistant bacteria among different genera

D . Pesticide treated (96 hrs)

Genera	Metals				
	Mercury	Zinc	Cadmium	Copper	Lead
Pseudomonas	97.08	100	100	73.53	100
Vibrio	100	100	100	72.22	100
Aeromonas	100	100	100	100	100
Enterobacteriaceae	100	100	90.0	90.00	100
Acinetobacter	100	100	100	50.00	100
Alcaligenes	33.33	100	100	0.00	100
Moraxella	100	100	100	75.00	100
Flavobacterium cytophaga	100	100	100	75.00	100
Bacillus	100	100	100	50.00	100
Coryneform group	100	100	100	75.00	100
Micrococcus	83.33	100	100	50.00	100

Table 25. Distribution of multiple heavy metal resistant bacteria in water and clams

Sample/ Concentration (ml/1)	No. of heavy metals				
	1	2	3	4	5
NF	0.0	0.0	2.08	2.08	77.08
I	0.0	0.0	7.84	13.73	78.43
UT	0.0	0.0	8.33	29.17	62.50
*T	0.0	0.0	3.30	27.00	70.00
	* Multiple heavy metal resistant bacteria occured in samples treated with various concentrations of pesticide				
0.000125	0.0	0.0	0.0	15.00	51.72
0.00025	0.0	0.0	3.45	25.81	67.74
0.0005	0.0	0.0	6.45	44.83	85.00

NF = Native flora; I = Initial (O hr)

UT = Untreated - control (96 hrs); T = Pesticide treated (96 hrs)

Table 26. Minimal inhibitory concentration of the heavy metals

Metal	Native flora	Initial	Untreated	Treated
Mercury	25	25	25	30
Zinc	300	300	300	300
Cadmium	300	300	300	300
Copper	150	150	150	150
Lead	1000	1000	1000	1000

Table 27. Effect of pesticide concentration on the growth of bacteria isolated from water and clam in NAP and MAGP media (Percentage of positive isolates)

Sample/ Concentration (ml/l)	NAP[1]						MAGP[2]				
	Co	A	B	C	D	E	A	B	C	D	E
NF	100	93.33	86.67	73.33	63.33	50.00	75.56	58.89	50.00	35.56	23.33
I	100	94.85	85.57	73.20	61.86	48.45	82.47	69.07	52.58	42.67	24.74
UT	100	89.74	83.33	76.92	57.69	37.10	53.85	32.05	28.21	20.51	14.38
*T	100	94.19	86.66	80.39	63.34	46.74	63.77	48.75	33.73	23.35	15.43
	* Effect of pesticide concentration on the growth of bacteria isolated from water and clam treated with various concentrations of pesticide										
0.000125	100	92.59	83.95	78.21	61.73	43.21	72.84	55.56	40.74	24.69	20.99
0.00025	100	93.83	85.90	80.25	64.10	44.44	65.38	48.72	32.05	24.36	16.67
0.0005	100	96.15	90.12	82.72	64.20	52.56	53.09	41.98	28.40	20.99	8.64

NF = Native flora
I = Initial (Ohr)
UT = Untreated (control-96 hrs)
T = Pesticide treated (96 hrs)

Co = Control
A = 0.005%
B = 0.01%
C = 0.02%
D = 0.05%
E = 0.1%

1 : Nutrient agar medium supplemented with pesticide

2 : Mineral agar medium supplemented with glucose and pesticide

Table: 28 Effect of pesticide concentration on the growth of bacteria isolated from water in NAP and MAGP media. (Percentage of positive isolates)

Sample/ Concentration (ml/l)	NAP[1]						MAGP[2]				
	Co	A	B	C	D	E	A	B	C	D	E
NF	100	90.48	85.71	76.19	52.38	33.33	76.19	61.90	52.38	28.57	4.76
I	100	96.30	81.48	62.96	55.56	33.33	76.37	55.56	37.04	25.93	18.52
UT	100	100	100	94.12	82.35	64.71	74.36	41.18	27.91	22.32	17.65
*T	100	94.90	86.11	84.35	67.01	39.70	75.95	63.71	47.58	33.52	20.65
	* Effect of pesticide on the growth of bacteria isolated from water treated with various concentrations of pesticide										
0.000125	100	89.47	78.95	73.68	52.63	38.10	84.21	73.68	73.68	47.62	42.11
0.00025	100	95.24	88.89	88.89	72.22	38.89	72.22	61.90	52.38	47.37	14.29
0.0005	100	100	90.48	90.48	76.19	42.11	71.43	55.56	16.67	5.56	5.56

NF = Native flora
I = Initial (O hr)
UT = Untreated (control-96 hrs)
T = Treated pesticide

Co = Control
A = 0.005%
B = 0.01%
C = 0.02%
D = 0.05%
E = 0.1%

1 : Nutrient agar medium supplemented with pesticide
2 : Mineral agar medium supplemented with glucose and pesticide

Table 29. Effect of pesticide concentration on the growth of bacteria isolated from clam in NAP and MAGP media (Percentage of positive isolates)

Sample/ Concentration (ml/l)	NAP[1]						MAGP[2]				
	Co	A	B	C	D	E	A	B	C	D	E
NF	100	94.20	86.96	72.46	66.67	55.07	75.36	57.97	49.28	37.68	28.99
I	100	94.29	87.14	77.14	64.29	54.29	87.14	74.29	58.57	48.57	27.14
UT	100	86.89	78.69	72.13	33.03	9.49	33.34	29.51	28.51	18.70	11.11
*T	100	93.96	86.83	79.09	62.03	48.96	59.78	43.89	29.11	19.80	13.73
	* Effect of pesticide on the growth of bacteria isolated from clam treated with various concentrations of pesticide.										
0.000125	100	95.00	90.00	82.26	67.74	56.67	46.67	35.00	20.00	11.67	6.67
0.00025	100	93.55	85.48	80.00	61.67	46.67	63.33	46.67	30.65	17.75	14.52
0.0005	100	93.33	85.00	75.00	56.67	43.55	69.35	50.00	36.67	30.00	20.00

NF = Native flora
I = Initial
UT = Untreated (control-96 hrs)
T = Pesticide treated (6 hrs)

Co = Control
A = 0.005%
B = 0.01%
C = 0.02%
D = 0.05%
E = 0.1%

1 : Nutrient agar medium supplemented with pesticide

2 : Mineral agar medium supplemented with glucose and pesticide

DISCUSSION

DISCUSSION

Effect of the organophosphorus pesticide Ekalux(R) EC 25 on the bacterial flora associated with the edible black clam Villorita cyprinoides var. cochinensis (Hanley) of Cochin backwater was studied in detail in the present work. The animals were collected from the Vembanad lake near Kumbalam island, brought alive to the laboratory, kept in aerated tanks for acclimation for two days and then treated with various sublethal concentrations of pesticide. The total heterotrophic bacterial population (THB) associated with the fresh clams and water, at the beginning of the experiment and after treatment with pesticide for 96 hrs were estimated employing standard procedures outlined in the section materials and methods. Appropriate controls of water and clams were maintained in the same condition and THB was estimated.

The clams harboured higher THB than the water samples. The occurrence of more number of bacteria in clams than water in the present study is similar to the reports of Durairaj et al. (1983), who found that the bacterial population of the surrounding seawater was considerably lower than the corresponding counts of oyster at Tuticorin.

Mussels and other filter feeding shellfishes accumulate bacteria from the surrounding water (Al-Jebouri and Trollope, 1981). Owing to their filter feeding habit, autochthonous bacteria are also liable to be accumulated in the gills and intestine. The composition of the commensal bacteria associated with shellfish in their natural environment may be governed by feeding and living habits, the geography of the area, the seasons and the temperature and quality of the water in which they live

(Philip, 1987). THB to the order of 10^5 to 10^8/g were reported in the bivalves Sunetta scripta and Meretrix casta from Cochin area (Philip,1987). The occurrence of high population in Villorita cyprinoides var. cochinensis (Hanley) agrees well with this report. Also, there are reports of occurrence of bacterial population between 10^4 and 10^5/g in Crassostrea gigas (Colwell and Liston, 1960). During acclimation in seawater (2 days), a decrease in THB was observed in clams. Since the animals were maintained in filtered seawater adjusted to 15‰ salinity, with distilled water, possibly due to dilution and filtration this might have resulted. The untreated samples, kept as controls recorded an increase in the population from that of 0 hr. Under controlled and favourable conditions, the bivalves are known to be very active. The clams in the present study, might have adjusted to new environmental conditions during acclimation and then become active. This might have resulted in the higher accumulation of bacterial forms. The heterotrophic population in pesticide treated clams was more than the control clams. Similarly, the bacterial population in water treated with pesticide also found at higher level, when compared to control. The increase in the population of bacteria in treated clam may be due to the stress. Bacteria reproduce at a faster rate under stress conditions (Portier and Meyers, 1982). THB recorded an inverse relationship with increase of concentration of pesticide in water and clam. An increase in the THB population after treatment with pesticide observed in the present study agrees well with the reports made on soil (Kozlova et al. 1964; Milkowska and Gorzelak, 1966; Sosnovskaya and Pashchenko, 1967; Percich and Lockwood, 1978). Also the report on saltmarsh environment treated with organophosphorus pesticide malathion (Bourquin, 1977), where malathion degrading bacteria in treated water were greater than those in control water during 30 days treatment agrees

with the present result. Congregado et al. (1979) found that number of total bacterial colonies increased during weeks 1 and 2 after treatment with malathion and dimethoate. The increase in the total number of bacteria after the application of the pesticide can be explained by assuming that these microorganisms can synergistically metabolise this insecticide as Gunner and Zuckerman (1968) demonstrated for diazinon. If certain groups of bacteria were inhibited by the pesticide or its metabolites, or groups selected due to competition by other microorganisms, these groups were not detected. No reason can be given for the major fluctuations in percentages of the total population exhibiting the functions tested. These fluctuations were so great that no trends could be seen statistically. However, the method of selection has a degree of bias for the predominant organisms at the time of sampling (Bourquin, 1977). Although inhibiting effects of pesticide sequences have been reported (Jones et al. 1974; Verstraete and Voets, 1974) it is difficult to determine the causative factors (Greaves and Malkomes, 1980). This difficulty serves to emphasize the need for more intensive investigation.

More than 80% of the bacterial flora in water and clam (native flora) were gram negative. Members of Enterobacteriaceae followed by Pseudomonas were dominant in water, while Aeromonas followed by Pseudomonas, members of Enterobacteriaceae and Vibrio constituted more than 80% of the total population in clam. However, this is in contrast to the report of Philip (1987) who observed more of Vibrios in water and clams (Sunetta scripta and Meretrix casta) collected from Fort Cochin which is near the sea.

The medium, ie. water used for the experiment, contained 89.65% of gram negative forms. Pseudomonas followed by members of Enterobacteriaceae and Vibrio were dominant. When the pesticide was added to the medium, the above groups reduced considerably, while Aeromonas increased from the initial.

Vibrio followed by Aeromonas were dominant at 0 hr in clam. The treatment with pesticide resulted in the change of bacterial flora. The dominance of Vibrio and Aeromonas was replaced by Pseudomonas and members of Enterobacteriaceae. When gram negative forms reduced slightly in treated clam, gram positive forms increased slightly.

The dominance of Pseudomonas over other flora in pesticide treated samples could be attributed to their wide tolerance and rare quality of this genus to utilize and degrade wide range of organic compounds. Further, it is a known fact that Pseudomonas have degradative, antibiotic resistant and heavy metal tolerant plasmids in them (Powar and Daginawala 1982) which could have caused their rapid increase in the flora. Although no reports are available to assign plasmid mediated resistance or degradative properties to Vibrio, Aeromonas and other major groups observed in the samples, the results suggest that all other groups which recorded maximum percentages after 96 hrs of treatment of pesticide might have some rare ability, probably plasmid mediated pesticide resistance which warrants further investigation. These observations made in the investigation is in contrast to the reports of Andrews and Kenerley (1978) that fluorescent pseudomonads which formed a sizeable component of phylloplane bacteria were severely depressed on treated leaves. Lactic acid type bacteria and other bacteria capable of growth on APT medium

(Evans and Niven, 1951) were also significantly reduced. In the present investigation, the increase of Pseudomonas seen in clam samples suggests that the microenvironment existing in the animal may be favourable for Pseudomonas.

Bacteria isolated from samples were identified and characterized for their ability to elaborate various hydrolytic enzymes, by growing them on different substrates. The results indicated that bacteria from pesticide treated as well as untreated samples could elaborate enzymes such as lipase, amylase, gelatinase, caseinase, chitinase, urease and DNase, apart from the enzymes required for utilization of pesticides. In the present study, it was observed that lipase producers were in dominant numbers followed by chitinase, proteases, amylase and DNase producers among native flora of water. At 0 hr, amylase producers were found dominant followed by lipase and chitinase elaborators. However, after the experiment, in the pesticide treated water, a change in the hydrolytic enzyme producers was noticed. Urease producers followed by lipolytic bacteria were found dominant and amylolytic forms reduced. In clam, urease producers followed by lipase and chitinase producers were at higher level. At 0 hr, lipase producers were dominant followed by amylase producers. After treatment also, the dominance of lipase producers followed by urease producers and gelatinase producers was noticed, although a slight reduction from 0 hr was noticed for lipase producers. Amylase elaborators were reduced to very low level. Other hydrolytic enzyme producing groups such as DNase and urease elaborators increased slightly from 0 hr. In the case of both clams and water, when the concentration of the pesticide increased, all enzyme producing groups except lipase

elaborators, showed reduction. Most biological activities associated with the degradation of malathion (Bourquin, 1977) were reported to be associated with an effective carboxyesterase system that causes early breakdown of malathion to the acids. The microbial system, all had an effective carboxyesterase system that caused rapid breakdown of malathion to the acids. In the present study, the increase in percentages of lipase producers along with the concentration of pesticide in the samples may be due to such esterase systems.

Ramsay and Fry (1976) observed an increase in numbers of both the exoenzyme producers namely amylase and xylanase in the microflora associated with paraquat treated aquatic macrophytes Elodea sp. and Chara sp. In the present study, a decrease in the amylolytic forms was observed and this may be due to the availability of the substrate for these microorganisms. However, the increase or decrease of any hydrolytic enzyme producers in relation to their association has to be worked out in detail. Also, a detailed investigation is warranted to evaluate the exact relationship of various enzymatic groups of bacteria and pesticide concentration in the microenvironment prevailing in the animal system.

Bacteria isolated from both untreated and treated water and clams were checked for their ability to resist various antibiotics. Thus, their resistance/sensitivity against ampicillin, bacitracin, chloramphenicol, gentamycin, neomycin, oxytetracycline, penicillin-G, polymyxin-B, streptomycin and sulphadiazine were studied.

Penicillin-G resistant bacteria were found at higher level in the native flora, at 0 hr of the experiment, in untreated and pesticide treated water samples. Least percentage was resistant to gentamycin in all the cases except untreated, where the least was represented by chloramphenicol resistant forms.

A decrease in percentage of ampicillin, bacitracin, gentamycin, penicillin-G, polymyxin-B and sulphadiazine resistant bacteria was noticed in treated than in untreated water. The percentage of antibiotic resistant forms decreased with increase in concentration of pesticide used.

Native flora and 0 hr population of clams contained maximum sulphadiazine resistant forms. The sulphadiazine resistant forms which were second to penicillin-G resistant bacteria in water was found to occupy a higher position in clams. However, the least percentage were gentamycin resistant. In untreated and treated clam samples, penicillin-G resistant forms were more common. Similar to untreated water, the clams also harboured least number of chloramphenicol resistant bacteria. But, in treated clams, the least percentage was resistant to gentamycin. The percentage of resistant forms, against all the antibiotics tested decreased when the concentration of the pesticide increased.

Among the various groups tested, Pseudomonas isolated from native flora recorded sensitivity against gentamycin and oxytetracycline while at 0 hr, the same genus showed sensitivity to neomycin in addition to gentamycin and oxytetracyline.

Pseudomonas from untreated samples also recorded sensitivity against chloramphenicol, whereas Pseudomonas of treated samples showed sensitivity against gentamycin alone. In the case of Vibrio, sensitivity was observed with isolates of native flora against gentamycin and chloramphenicol, at 0 hr. against gentamycin alone, of untreated samples against chloramphenicol and of treated samples against gentamycin. Other principal groups such as Aeromonas and members of Enterobacteriaceae also recorded similar observations with respect to sensitivity against the tested antibiotics. In the case of antibiotics other than those mentioned above, all the genera could record significant levels of resistance. Hence, it is clear that among all the ten antibiotics, only gentamycin and chloramphenicol could control the growth of these pesticide utilizing bacteria when compared with other antibiotics. The results also suggest that clams could harbour higher percentage of resistant bacterial strains than water and among the various groups, Pseudomonas could record higher percentage of resistant strains against majority of the antibiotics tested.

Goyal and Adams (1984) has stated that drug resistant bacteria may be concentrated by filter-feeding shellfish which inhabit waters overlying the dumpsites. Incidence of higher percentage of antibiotic resistant bacteria in clams than water in the present study might be due to the filter-feeding habit of the clams as suggested by Goyal and Adams (1984). Further, they also observed that most of the strains were resistant to several antimicrobial agents, similar to the observation made in the study.

More than 97% of the isolates tested, could demonstrate resistance to more than one antibiotic at a time and thus multiple antibiotic resistance

was incurred with the majority of the isolates obtained from the various samples. Interestingly, maximum percentage of isolates obtained at 0 hr. and from treated samples could record multiple resistance against seven antibiotics when compared to that of the native flora which could resist only a combination of five antibiotics, at one time. Further, it was noticed that the isolates of untreated samples showed resistance against a combination of eight antibiotics for which the reason could not be attributed. However, under favourable conditions, resistant character might have been transmitted from one bacteria to other at a higher level.

Harnett and Gyles (1984) could observe multiple resistance to antimicrobial agents as a common feature among the Escherichia coli strains. They could observe multiple resistance against five drugs and a predominant pattern of drug resistance which demonstrated resistance to streptomycin, sulphonamide and tetracycline and resistance to these drugs plus ampicillin and kanamycin. In the present study, predominant pattern of drug resistance was PSSzAB (P = penicillin-G, S = streptomycin, Sz = sulphadiazine, A = ampicillin and B = bacitracin). Although Devanas et al. (1980) reported that multiple drug resistance increased during a survey over an year in sediments, in the present study, in a controlled experiment, it was observed that there was no significant change in the percentage of multiple resistant bacteria between the samples. However, this comparison could not be appreciated as both investigations are not similar in nature.

Resistance to heavymetals, such as mercury (Hg), zinc (Zn), cadmium (Cd), copper (Cu) and lead (Pb) was observed with most of the strains tested during the period of study. None of these metals could affect the growth significantly. Especially, zinc and lead were ignored by the isolates. However,

for other metals, percentage of resistant strains varied significantly well above in the range of 70-100%. Among the five heavy metals, copper could affect the growth of bacteria significantly followed by mercury. However, treated samples could record higher percentage of resistant stains than untreated samples. Also, water contained higher level of resistant strains than of clam samples. All the isolates of bacteria belonging to Pseudomonas. Vibrio, Aeromonas, members of Enterobacteriaceae, Acinetobacter, Alcaligenes and Micrococcus present in the native flora exhibited resistance against zinc, cadmium and lead, while all the members of Aeromonas, Acinetobacter, Alcaligenes, Coryneform group, and Micrococcus showed resistance to all the five heavy metals tested. Bacillus and members of Enterobacteriaceae were affected much by copper than any of the other heavy metals tested. Significant numbers of Pseudomonas and Vibrio isolates could record resistance against mercury and copper when compared to other groups, all the isolates of which showed resistance. However, of all the groups, Vibrio was affected to some extent by mercury and copper than other metals. There was not much change in the trend exhibited by the samples in recording heavy metal resistant isolates at 0 hr and in untreated samples. Treated samples, however, recorded a similar picture in holding heavy metal resistant strains as that of native flora and comparatively they held more resistant isolates than untreated samples.

All the isolates tested showed multiple heavymetal reistance. Irrespective of the source, higher percentages of isolates taken from all the samples recorded resistance against a combination of five heavymetals. As the concentration of the pesticide increased, in treated samples, the percentage of bacteria showing resistance to combinations of three, four and five heavy metals also got increased.

Interactions of bacteria with heavymetal ions have aroused interest in recent years. Timoney et al. (1978) have observed mercury resistant Bacillus population while studying on heavy metals and antibiotic resistance, in the bacterial flora of sediments. They observed mercury resistance frequently linked with resistance to other heavy metals when tested against cadmium, copper and zinc. Devanas et al. (1980) observed cadmium resistant isolates which included yeast and Pseudomonas from benthic flora. Harnett and Gyles (1984) observed heavymetal resistance by Escherichia coli when tested against sodium arsenate, sodium arsenite, lead acetate, lead nitrate, cadmium chloride, cobalt chloride, copper sulphate, zinc sulphate, silver nitrate, mercuric chloride and potassium tellurite. In all the above incidences, heavy metal resistance by bacterial strains were studied in relation with antibiotic resistance whereas hardly any report is available on heavymetal, antibiotic and pesticide resistance.

According to Puleo et al. (1978) minimum inhibitory concentration of mercury for Bacillus from sewage sledge sediments varied from 2 to 15 µg/ml with a tendency towards bimodal distribution and cadmium and zinc MICs were less variable in these organisms. In the case of many of those organisms with resistance to two or more heavymetals, MICs of each heavy metal varied together. In the present study also, MIC of heavy metals varied. There was a slight increase of MIC for mercury (from 25 ppm to 30 ppm) for the bacteria isolated from pesticide treated samples. However, such an increase was not found for other metals.

In the present study, when the bacteria isolated from the samples were subjected to various concentrations of pesticide (as the sole source of carbon) in mineral agar medium, no growth was recorded establishing that the isolates were unable to utilize the pesticide as sole source of carbon. In the presence of a carbohydrate, glucose many isolates exhibited growth. Also more forms

exhibited growth in NAP medium which contains peptone and beef extract when compared to MAGP. Numerous bacteria from a salt marsh environment were reported to be capable of degrading malathion, an organophosphate insecticide, when supplied with additional nutrients as energy and carbon sources (Bourquin, 1977). Also, the report reveals that only eleven of the fifteen bacterial cultures isolated from salt marsh environments after malathion enrichment, degraded malathion as a sole carbon source, whereas all the fifteen isolates degraded the compound within five days when an additional carbon source was added. It was observed that growth of bacteria declined alongwith increase of concentration of pesticide. There was no variation in exhibiting the above trend among the isolates of the different samples.

Inhibitory effects of pesticide sequences have - been reported by Jones et al. (1974) and Verstraete and Voets (1974). Greaves and Malkomes (1980) argue that the successive applications of a herbicide need not necessarily have a greater effect than a single application, especially if the compound has short persistence. Although inhibitory effects of pesticide sequences have been reported, the causative factors are not determined (Greaves and Malkomes, 1980). In the present study, the increase of concetration of pesticide might have directly interfered in the metabolism of the organisms as an inhibitory substance since bacteria cannot utilize any substance beyond an optimal level as suggested by Roše (1968).

Number of isolates withstanding the different concentrations of pesticide were more in treated samples, when compared to untreated samples in both the media. The percentage of pesticide tolerant bacteria decreased as the concentration of pesticide in the samples increased when tested in nutrient agar medium. A reverse observation was made when tested in mineral agar

medium supplemented with glucose.

Isolates from the treated samples, exhibiting multiple resistance in general, increased in percentage as the number of antibiotics in the combination increased. The same trend was seen for multiple heavymetal resistance also, whereas, the percentage of isolates withstanding higher concentrations of pesticide were less.

The rare quality of resistance to more than one type of compound such as antibiotics, heavymetals and pesticides is the most significant observation, that was made in the present study. Further investigation on the isolation and charecterization of plasmids from these strains may perhaps answer this intriguing question, whether it is possible that all the three resistant genes are present in the same plasmid or chromosomal DNA.

The effect of temperature on the growth of bacteria was studied by growing Pseudomonas and Vibrio species at various temperatures. It was observed that species of Vibrio (K 22) and Pseudomonas (K 41) which were isolated from untreated samples could grow to a maximum at 25^{o}C while strains of the same two genera Pseudomonas (K 181) and Vibrio (K 243) isolated from treated samples could grow well to a maximum at 37^{o}C. The results indicated that treatment with pesticide had enhanced the tolerance to a higher temperature and shifted the optimum temperature for the strains from a lower temperature to a higher temperature.

Studies on the effect of pH on the growth of bacteria revealed certain interesting observations with respect to the four isolates tested. While the strains isolated from untreated samples preferred a pH of 7 for their maximum growth, strains isolated from treated samples preferred a higher pH 8. In this case also, the results suggest that pesticide treatment has shifted the optimum pH requirement for maximum growth of the organisms.

When subjected to varied concentrations of sodium chloride in the growth medium, the four isolates tested did not show any significant variation among them in recording maximal growth, as all could record growth to their maximum at 1% sodium chloride concentration. Besides, all the strains irrespective of their source, whether treated or untreated showed tolerance to wide range of sodium chloride concentrations.

These experiments prove that the pesticide affect the physiological charecteristics of bacteria. Since these organisms are estuarine forms, the optimum for sodium chloride has not changed. However, the optimum of temperature and pH were changed.

Any attempt to predict or evaluate potential side effects of herbicides on the microflora and its activities must take account of environmental factors and variability within these factors (Greaves and Malkomes, 1980). Although their reports are based on studies on soil microflora, this also demands the importance of environmental factors on studies on aquatic microflora. Bakalivanov and Hlebarova (1977) reported that herbicide effects on micro-organisms may be smaller after addition of pesticide or larger in the presence of organic material (Vladutu and Soreanu, 1976). Humidity, pH, temperature and other soil factors can also modify the response of organisms to herbicides (Wingfield et al. 1977; Gaur and Misra, 1978, Marsh and Greaves, 1979).

The organophosphorus pesticides exist as phosphorothioates and phosphates. Conversion of the former to the latter appears to be chemically and not microbially mediated (Meikle, 1972). Many microorganisms possess phosphatases capable of hydrolytically releasing the inorganic phosphate from natural organophosphates viz. nucleic acids, nucleotides and phospho-proteins (Mounter and Tuck, 1956; Ahmed and Casida, 1958; Matsumura and Boush, 1968).

Many of the phosphate and phosphorothioate pesticides have been reported to be microbially hydrolyzed in soil (Lichtenstein and Schulz, 1964; Sethunathan and Yoshida, 1969; 1973a,b; Beynon and Wright, 1969). The enzyme phosphatase act upon phosphate readily than phosphothionates as preferred substrates (Motoyama and Dauterman, 1974) and rarely hydrolyze phosphotriases (Huerer et al. 1970). The phosphonate moiety, present in a few pesticides and not particularly common in natural products (Kittredge and Roberts, 1969) is enzymatically cleaved (Alam and Bishop, 1969) but not by phosphatases. But, Bourquin (1977) reported that degradation products of malathion are formed as a result of phosphatase activity. In the present study, the four selected isolates were tested for their ability to release phosphate from the pesticide with reference to varying environmental parameters such as temperature, pH and sodium chloride concentration.

In general, maximum phosphate release from the substrate coincided with maximum growth of the four isolates at their optimal pH, temperature and sodium chloride concentration irrespective of their source of isolation. Further, it was noted that phosphate release showed a linear relationship with growth of bacteria irrespective of the environmental parameters to which it was subjected. The results also evidence that when growth is maximum, enzyme production would also be maximum which has resulted in maximum phosphate release.

The biological degradation of organophosphorus pesticide probably starts from the secretion of microbial enzymes more specifically of phosphatases, in soil (Congregado et al. 1979). Also, the concentration of the organophosphorus compounds may be a significant factor affecting its susceptibility to microbial attack and the degradation may be enhanced by various physico-

chemical and biological factors. The phosphatase producing bacteria may detoxify the pesticide and exhibit synergistic association. At the sametime the change or elimination of certain microbial flora associated with animal by the pesticide, may alter the metabolism and physiology of the organism and may affect the health of the animal.

SUMMARY

SUMMARY

The effect of the organophosphorus pesticide Ekalux(R) EC 25 at sublethal concentrations on the bacterial flora associated with Villorita cyprinoides var. cochinensis (Hanley) is investigated in the present study. The aspects dealt with are 1. Total heterotrophic bacterial population, 2. Generic composition, 3. Hydrolytic enzyme producing bacteria, 4. Antibiotic resistance, 5. Heavy metal resistance, 6. The effect of pesticide concentration on the growth of the bacteria and 7. Effect of temperature, pH and sodium chloride on the growth and phosphate release of selected isolates.

The black clam Villorita cyprinoides var. cochinensis (Hanley) and the water samples for the experiment were collected from the Vembanad lake near the Kumbalam island, brought alive to the laboratory and acclimated in aerated tanks for two days. Later, animals were subjected to various concentrations of the pesticide, Ekalux(R) EC 25 (0.000125 ml/l, 0.00025 ml/l and 0.0005 ml/l) for a period of 96 hrs. Appropriate controls were maintained for both water and clams. Salinity of the water used for maintaining the animals in the troughs was kept constant at 15‰. Temperature in the troughs prevailed between 28 $\pm$ 2^{o}C.

Total heterotrophic bacterial population (THB) was estimated in the water and fresh clams as soon as the collection was made, in the samples at the beginning of the experiment (0 hr.), in the control samples after 96 hrs (untreated) and in the pesticide treated samples after 96 hrs (treated). The THB of the estuarine water and clams contained 6.5 x 10^4/ml and 2.975 x 10^6/g respectively, immediately after collection. Untreated water

and clam samples showed enormous increase in THB from 0 hr population. The treated samples (water and clams) contained higher THB than 0 hr. In general, THB was observed to increase tremendously in the samples treated with pesticide when compared to their native flora. With reference to various concentrations of pesticides, THB recorded an increase with increase of concentration in water and clam samples.

In general, species of Vibrio, Aeromonas, Moraxella, Flavobacterium-Cytophaga group, members of Enterobacteriaceae, Bacillus, Coryneform group and Micrococcus were encountered. Pseudomonas was the dominant one, followed by Vibrio and members of Enterobacteriaceae. The native flora of both water and clams were found to be dominated by members of Enterobacteriaceae, followed by Pseudomonas and Vibrio. At 0 hr., Pseudomonas was found at higher level in water and Vibrio in clams. Pseudomonas and Aeromonas showed dominance in untreated clams followed by Vibrio, while Pseudomonas dominated in treated clams followed by Aeromonas. Bacillus exhibited dominance in untreated water, and in treated water, Pseudomonas were more. Gram negative forms showed an inverse relationship with pesticide concentration in water, and in clams it was reverse.

All the isolates were characterized for the production of various hydrolytic enzymes on different substrates. Lipase, amylase, gelatinase, caseinase, chitinase, DNase, and urease producers were encountered. Among the different hydrolytic enzyme producers, lipase elaborators were at higher proportion followed by urease, chitinase, gelatinase, caseinase, amylase and DNase producers. In general, clams contained higher percentages of hydrolytic enzyme secretors in all stages (native flora, 0 hr., untreated and treated) than water. Treated samples of water and clam harboured more number of ureolytic and lipolytic bacteria than untreated samples. In the case of

both clams and water when the concentration of the pesticide increased all enzyme producing groups except lipase producers showed reduction.

All the isolates were tested for their resistance/sensitivity to various antibiotics. Ampicillin, bacitracin, gentamycin, chloramphenicol, oxytetracycline penicillin-G, polymyxin-B, streptomycin and sulphadiazine were used in the study. In general, penicillin-G resistant bacteria were more among the entire flora followed by sulphadiazine while gentamycin resistant forms were less. Isolates of native flora and those at 0 hr., showed sensitivity towards oxytetracycline and gentamycin, and maximum number exhibited resistance to penicillin-G, sulphadiazine and others. Gentamycin and chloramphenicol were more effective against the isolates of untreated samples while gentamycin alone was highly effective against isolates of treated samples. In other stages, significant percentage of resistant isolates were also recorded. Among the various groups tested, Pseudomonas isolated from native flora, recorded sensitivity against gentamycin, neomycin and chloramphenicol. Other dominant groups such as Aeromonas, Vibrio and members of Enterobacteriaceae also showed sensitivity to gentamycin and chloramphenicol. It was observed that clams harboured higher percentages of antibiotic resistant bacterial isolates than water and among the various groups, higher percentage of Pseudomonas isolates exhibited resistance against majority of the antibiotics tested. More than 97% of the isolates tested demonstrated multiple antibiotic resistance irrespective of their source of isolation. Of all, the untreated samples contained isolates with multiple antibiotic resistance against a combination of eight antibiotics when compared to the native flora, flora at 0 hr., flora of treated samples which showed resistance in combination of five, seven and seven respectively. The antibiotic resistance pattern exhibited by the

maximum number of isolates was PSSzAB (P = penicillin-G, S = streptomycin, Sz = sulphadiazine, A = ampicillin and B = bacitracin).

Heavy metal resistance by bacterial isolates was tested against mercury, zinc, cadmium, copper and lead. None of these metals could affect the growth of bacteria significantly. All the isolates of all the samples showed resistance against zinc and lead and the percentage of resistant strains varied from 70-100% for the other metals. Among the five heavy metals, copper could affect the growth of bacteria to a certain extent followed by mercury. Among the samples, pesticide treated water and clam possessed higher percentage of resistant isolates than untreated samples. All bacterial isolates belonging to the genera Pseudomonas, Vibrio, Aeromonas, members of Enterobacteriaceae, Acinetobacter, Alcaligenes, Moraxella, Flavobacterium-Cytophaga group, Bacillus, Coryneform group and Micrococcus showed resistance against all the heavy metals tested.

All the isolates tested exhibited multiple heavy metal resistance. Irrespective of the source, higher percentage of isolates taken from all the samples revealed resistance against a combination of five heavy metals. As the concentration of the pesticide increased in treated samples, the percentage of bacteria showing resistance to combination of three, four and five heavy metals also got increased.

Minimal inhibitory concentrations (MIC) of mercury varied between 25 ppm and 30 ppm for the maximum number of isolates. MIC of cadmium (300 ppm), zinc (300 ppm), copper (150 ppm) and lead (1000 ppm) however, did not show any variation for the isolates obtained from different samples.

When the bacteria isolated from different samples were subjected to various concentrations of pesticide as the sole source of carbon in the growth medium (mineral medium), no growth was recorded establishing that the isolates were unable to utilize the pesticide as sole source of carbon.

In the presence of a carbohydrate, glucose, many isolates exhibited growth. Higher percentage of isolates recorded growth in the nutrient agar medium supplemented with pesticide. It was observed that growth of bacteria declined along with increase of concentration of pesticide.

Effect of temperature on the growth of bacteria was studied by growing four isolates; two each from untreated (Pseudomonas - K 41 and Vibrio - K 22) and treated (Pseudomonas - K 181 and Vibrio - K 243) samples. Isolates of untreated samples, recorded maximum growth at $25^{o}C$ while isolates of treated samples could grow to their maximum at $37^{o}C$. Isolates from untreated samples showed maximum growth at pH 7, while isolates of treated samples exhibited maximum growth at pH 8. All the four isolates irrespective of their source of isolation grew to maximum at 1% sodium chloride concentration.

Phosphate release from the pesticide component was tested for the four isolates with respect to varing temperature, pH and sodium chloride concentration. With respect to all the three parameters, it was observed that maximum phosphate release coincided with maximum growth at optimum growth temperature ($25^{o}C$ and $37^{o}C$), pH (7 and 8) and sodium chloride concentration (1%).

Organic chemicals that persist in natural waters, even in trace quantities could present environmental problems in several ways. Such trace substances could lead to serious problems if they are susceptible to biomagnification

and subsequently toxic to species at higher trophic levels in food chain. In addition, organic chemicals can impact objectionable taste and odours to waters at levels of nannogram per millilitre.

The approaches and experimental data given here should allow the development of a quantitative baseline of major microbial groups and related enzyme activities for interconnected ecosystems within an ecologically important backwater system. Development of information on enzyme/micro-organisms interactions in relevance to understanding determinants of productivity in aquatic regions. Furthermore, total microbial biomass and species diversity may serve as valid indices of biodegradable substrate turnover and productivity, and should provide information on microhabitat features as they are affected by the addition of a variety of toxic substances. With careful consideration given to the relevant physico-chemical and microbial processes needed to stimulate environmental conditions, use of microcosm approach can generate pertinent information on the impact of toxic substances in aquatic ecosystem.

REFERENCES

REFERENCES

Ahearn, D.G., S.A. Crow and W.L. Cook, 1977. Microbial interactions with pesticides in estuarine surface slicks. Ecol. Res. Ser. U.S. Environ. Prot. Agency pp. 30.

Ahmed, M.K. and J.E. Casida, 1958. Metabolism of some organophosphorus insecticides by microorganisms. J.Econ.Ent., 51 : 59-63.

Alam, A.V. and S.H. Bishop, 1969. Growth of Escherichia coli on some organophosphoric acids. Can.J.Microbiol., 15 : 1043-1046.

Albone, E.S., G. Eglinton, N.C. Evans, J.M. Hunter and M.M. Rhead, 1972. Fate of DDT in seven estuary sediments. Environ.Sci. Technol., 6 : 914-919.

Al-Jebouri, M.M. and D.R. Trollope, 1981. The Escherichia coli content of Mytilus edulis from analysis of whole tissue or digestive tract. J. Appl. Bacteriol., 51 : 135-142.

Ambrosi, D., A.R. Isensee and J.A. Macchia, 1978. Distribution of oxadiazon and phosalone in an aquatic model ecosystem. J.Agric.Food. Chem., 26 : 50-53.

Anderson, J.P.E. and E.P. Lichtenstein, 1971. Effect of nutritional factors on DDT - degradation by Mucor alternans. Can. J. Microbiol., 17 : 1291-1298.

Andrews, J.H. and C.M. Kenerley, 1978. The effects of a pesticide program on non-target epiphytic microbial populations of apple leaves. Can. J.Microbiol., 24 : 1058-1072.

Attaway, H.H., N.D. Camper and M.J.B. Paynter, 1982. Anaerobic microbial degradation of diuron by pond sediment. Pestic.Biochem. Physiol., 17 : 96-101.

Audus, L.J., 1969.(Ed.). The physiology and biochemistry of herbicides. Academic Press Inc, Newyork and London.

Austin, B., D.A. Allen, A.L. Mills and R.R. Colwell, 1977. Numerical taxonomy of heavy metal tolerant bacteria isolated from an estuary. Can. J. Microbiol., 23 : 1433-1447.

Bainbridge, A. and C.H. Dickinson, 1972. Effects of fungicides on the microflora of potato leaves. Trans.Br.Mycol.Soc., 59 : 31-41.

Bakalivanov, D. and S. Hlebarova, 1977. Relationship between soil microflora and afalon and patoran herbicides under fertilizer application. Acta phytopathol. Acad.Sci. Hung., 12 : 101-108.

Barik, S., D.M. Munnecke and J.S. Fletcher, 1984. Bacterial degradation of three dithioate pesticides. Agric.Wastes, 10 : 81-94.

Bayne, B.L., 1978. The potential of bivalve molluscs for monitoring the effects of pollution. Council Meeting 1978, International Council for the exploration of the sea/collected reprints. 20 pp.

Bayne, B.L., M.N. Moore, J. Widdows, D.R. Livingstone and P. Salkeld, 1979. Measurement of the responses of individuals to environmental stress and pollution studies with bivalve molluscs. Philos.Trans. R. Soc. Lond., 286 B : 563-581.

Bean, G.A. and A. Southall, 1983. Effect of pyridazinone herbicides on growth and aflatoxin release by Aspergillus flavus and Aspergillus parasiticus. Appl. Environ.Microbiol., 46 : 503-505.

Beckman, J., P. Tazik and R. Gorden, 1984. Effects of two pesticides on selected aquatic bacteria. Bull.Environ.Contam.Toxicol., 32 : 243-250.

Beynon, K.I. and .A.N. Wright, 1969. Breakdown of the insecticide gardona, on plants and in soils. J.Sci.Fd.Agric. 20 : 250-256.

Bollag, J.M., 1972. Biochemical transformation of pesticides by soil fungi. Crit. Rev. Microbiol., 2 : 35-58.

Bourquin, A.W., 1975. Microbial-malathion interaction in artificial salt marsh ecosystems, effects and degradation. Natl. Envtl. Res. Centre., U.S. Envtl. Protect. Agency. pp 41.

Bourquin, A.W., 1977a. Degradation of malathion by salt-marsh microorganisms Appl. Environ. Microbiol., 33 : 356-362.

Bourquin, A.W., 1977b. Effect of malathion on microorganisms of an artifical salt-marsh environment. J.Environ.Qual., 6 : 373-378.

Brooks, G.T., 1980. Perspectives of the chemical fate and toxicity of pesticides. J.Environ.Sci.Health, Part B, 15 : 755-793.

Buchanan, R.E. and N.E. Gibbons (Eds.), 1974. Bergy's manual of determinative bacteriology. 8th ed. The Williams and Wilkins Co., Baltimore. 1268 pp.

Caceres, O., J. Galizia Tundisi, and O.A.M. Castellen, 1980. Chlorinated pesticide residues in waters of Broa reservoir and its tributaries, Cienc. Cult. (Sao Paulo), 32 : 1659-1662.

Chapman, P.J., 1978. Degradation mechanisms. Misc. Rep. Ser. U.S. Environ. Protect. Agency, 28-66.

Chou, T.W. and Bohonos, N., 1979. Diauxic and cometabolic phenomena in biodegradation evaluations. Misc. Rep. Ser. U.S. Environ. Protect. Agency, 76-88.

Christensen, 1946. Urea decomposition as a means of differentiating Proteus and Paracolon cultures from each other and from Salmonella and Shigella types. J. Bacteriol., 52 : 461-466.

Cole, L.K. and R.L. Metcalf, 1980. Environmental destinies of insecticides, herbicides and fungicides in the plants, animals, soil, air and water of homologous microcosms. Doe Symp. Ser., 52 : 971-1008.

Colwell, R.R., 1979. Toxic effects of pollutants on microorganisms. In : Ecotoxicology. Chapter 13. SCOPE Project 4. 275-294.

Colwell, R.R. and J. Liston, 1960. Microbiology of shellfish, Bacteriological study of the natural flora of Pacific oysters (Crassostrea gigas). Appl. Microbiol. 8 : 104-109.

Congregado, F., D.S. Pujol and A. Juarez, 1979. Effect of two organophosphorus insecticides on the phosphate dissolving soil bacteria. Appl. Environ. Microbiol., 37 : 169-171.

Cook, A.M., C.G. Daughton and M. Alexander, 1978. Phosphorus containing pesticide breakdown products : Quantitative utilization as phosphorus sources by bacteria. Appl. Environ. Microbiol., 36 : 668-673.

Cosson-Mannevy, M.A., 1979. Use of a filter-feeder mollusc Mytilus edulis as a pollution indicator of organochlorine compounds in the marine environment. Paris-6 Univ., 75 (France) 22 March, 130 pp.

Cowan, A.A., 1978. Organochlorine compounds in mussels from Scottish coastal waters. Council meeting, International Council for the exploration of the sea/collected papers. 14 pp.

Cowan, S.T., 1974 (Ed.). Cowan and Steels manual for the identification of medical bacteria. Cambridge Univ. Press, Cambridge.

Daubner, I. and D. Toth, 1980. The effect of some water pollutants on microbial activity. Schweizer-Bart's Chever Lags Buchhandlung, Stuttgart (FRG) 1406-1409.

Daughton, C.G. and D.P.G. Hsich, 1977. Parathion utilization by bacterial symbionts in a chemostat. Appl. Environ. Microbiol., 34: 175-184.

Daughton, C.G., A.M. Cook and M. Alexander, 1979. Phosphate and soil binding factors limiting bacterial degradation of ionic phosphorus containing pesticide metabolites. Appl. Environ. Microbiol., 37 : 605-610.

Devanas, M.A., C.D. Litchfield, C. Mclean and J. Gianni, 1980. Coincidence of cadmium and antibiotic resistance in New York Bight apex benthic microorganisms. Mar. Pollut. Bull., 11 : 264-269.

Dow, R.L., 1981. Pesticides and commercial fisheries Mar. Technol. Soc. J., 15 : 32-34.

Durairaj, S., G. Chinnasamy and M. Syed Mohammed, 1983. A bacteriological study of the natural flora of edible oyster, Crassostrea madrasensis. Fish. Technol., 20 : 111-114.

Duursma, E.K. and A. Hanafi., 1976. Use of pesticides in brackishwater ponds. Distribution of chlorinated pesticides in environmental samples. Bull. Shrimp. Cult. Res. Cent., 2 : 114-142.

Dybern, B.I. and S. Jensen, 1978. DDT and PCB in fish and mussels in the Kattegat-Skagerrak area. Med. Havfiskelab, Lysekil., No. 232: 1- 32.

Eisler, R., 1970. Latent effects of isecticide intoxication to marine molluscs. Hydrobiologia., 36 : 345-352.

Emmanuelsen, M., J.L. Lincer and E. Rifklin, 1978. The residue uptake and histology of American oysters (Crassostrea virginica Gmelin) exposed to dieldrin. Bull. Environ. Contam. Toxicol., 19 : 121-129.

Evans, J.B. and C.F. Niven, Jr. 1951. Nutrition of the hetero fermentative lactobacilli that cause greening of cured meat products. J. Bacteriol., 2 : 599-603.

Fergusan, J.A. and F. Korte, 1977. Epoxidation of aldrin to exo-dieldrin by soil bacteria. Appl. Environ. Microbiol., 34 : 7-13.

Finney, D.J. 1971. Probit Analysis. Cambridge Univ. Press, London : 333pp.

Focht, D.D. and M. Alexander, 1970. Bacterial degradation of diphenyl methane, a DDT model substrate. Appl. Microbiol., 20 : 608-611.

Fossato, V.U. and L. Craboledda, 1979. Chlorinated hydrocarbons (BHC, DDT, PCB) in marine organisms obtained from the central northern Adriatic from June 1976 to June 1978. Proc. of Natl. Scientific meeting, Rome, 5-7 March, Publ. by CNR, Rome (Italy), 2: 831-844.

Fowler, S.W. and D.L. Elder, 1978. PCB and DDT residues in a Mediterranean pelagic food chain. Bull. Environ. Contam. Toxicol., 19 : 244-249.

Freed, V.H., C.T. Chiou and D.W. Schmedding, 1979. Degradation of selected organophosphate pesticide in water and soil. J. Agric. Food Chem., 27 : 706-708.

*Frenne, D. De, 1978. Bioaccumulation by the mussel in Weser-Ems District. In : Fresh marine mussels as food - second mussel symposium 1978. Kleinsteuber, H., K. R. Will (Eds.) Verband Deutsher Sportfischer, E.V., Offenbach (FRG), 91-102.

Fry, J.C. and .A.J. Ramsay, 1977. Changes in the activity of epiphytic bacteria of Elodea canadensis and Chara vulgaris following treatment with the herbicide paraquat. Limnol. Oceanogr., 22 : 556-562.

Garnas, R.L., and D.G. Crosby, 1977. Comparative metabolism of parathion by intertidal invertebrates. Proc. Symp. Pollut. Physiol. Mar. Organisms, 291-305.

Gaur, A.C. and K.C. Misra, 1978. Dynamics of microbial populations in soil as influenced by simazine and ecological factors. Zol. Bakteriol. Abt., 133 : 357-361.

Getzin, L.W. and I. Rosefield, 1968. Organophosphorus insecticide degradation by heat-liable substances in soil. J. Agric. Food. Chem., 16 : 598-601.

Gledhill, W.E. and V.W. Saeger, 1978. Microbial degradation in the environmental hazard evaluation process. Proc. workshop: Microbial degradation of pollutants in marine environments. 434-442.

Golovenko, V.K. and S.A. Petrov, 1979. The oxygen uptake by the hepato-pancreas mitochondria of mussels in the normal and when affected by DDT, Biol. Morya. (Kiev), 50 : 65-69.

Golovleva, L.A., A.B. Polyakova, R.N. Pertsova and Z.I. Finkelshtein, 1984. The fate of methoxychlor in soils and transformation by soil microorganisms. J. Environ. Sci. Health, 19B ; 523-538.

Goyal, S.M. and W.N. Adams, 1984. Drug resistant bacteria in continental shelf sediments. Appl. Environ. Microbiol., 48 : 861-862.

Graetz, D.A., G. Chesters, T.C. Daniel, L.W. Newland and G.B. Lee, 1970. Parathion degradation in lake sediments. J. Wat. Pollut. Control. Fed., 42 : R76-R94.

Greaves, M.P. and H.P. Malkomes, 1980. Effects on soil microflora. In : Interactions between herbicides and the soil. (Ed.) Hance, R.J. Academic Press, London, New York, Toronto, Sydney, San Francisco, 223-253.

Gunner, H.B. and B.M. Zuckerman, 1968. Degradation of "Diazinon" by synergistic microbial action. Nature (London), 216: 1183-1184.

Guthrie, R.K., U.A. Anugwelem and E.M. Davis, 1981. Responses of bacteria to the presence of Carbaryl in water. II Pure culture vs. mixed culture response. Water Resour. Bull., 17 : 1005-1007.

Hanafi, A., and E.K. Duursma, 1976. 1. Use of pesticides in brackishwater ponds 2. Effect of phosvel on fish and shrimp. Bull. Shrimp Cult. Res. Cent., 2 : 143-157.

Harnett, N.M. and C.L. Gyles, 1984. Resistance to drugs and heavymetals, colicin production and biochemical charecteristics of selected bovine and porcine Escherichia coli strains. Appl. Environ, Microbiol., 48 : 930-935.

Harrigan, W.F. and M.E. McCance (Eds.), 1972. Laboratory methods in microbiology, Academic Press, London, New York. 362 pp.

Hassal, K.A., 1985. Pesticides : A wider perspective. Environmentalist, 5 : 105-109.

Heritage, A.D. and I.C. MacRae, 1977. Degradation of lindane by cell free preparations of Clostridium spheroides. Appl. Environ. Microbiol., 34 : 222-224.

Hicks, G.F. Jr. and T.R. Corner, 1973. Location and consequences of 1,1,1 - trichloro -2, 2 - bis (p-chlorophenyl) ethane uptake by Bacillus megaterium. Appl. Microbiol., 25 : 381-387.

Hill, I.R. and D.J. Arnold, 1978. Transformations of pesticides in the environment - the experimental approach. In: Pesticide Microbiology, I.R. Hill and S.J.L. Wright (Eds.), Academic Press, London, New York, San Francisco, 799 pp.

Hill, I.R. and S.J.L. Wright, 1978 (Eds.). Pesticide Microbiology, Academic Press, London, Newyork, San Francisco, 799 pp.

Hislop, E.C., 1976. Some effects of fungicides and other agrochemicals on the microbiology of the aerial surfaces of plants. In: Microbiology of aerial plant surfaces. Dickinson, C.H. and T.F. Preece, (Eds.) Academic Press, Inc., New York. 41-74.

Hislop, E.C. and Cox, T.W., 1969. Effects of captan on the nonparasitic microflora of apple leaves. Trans. Br. Mycol. Soc., 52 : 223-235.

Hsu, T.S. and R. Bartha, 1979. Accelerated mineralization of two organophosphate insecticides in the rhizosphere. Appl. Environ. Microbiol., 37 : 36-41.

Huerer, B., V. Birk and B. Yaron, 1976. Effects of phosphatases on the persistence of organophosphorus insecticides in soil and water. J. Agric. Food Chem., 24 : 611-614.

Hughes, D.N., M.G. Boyer, M.H. Papsi and C.D. Fowle , 1980. Persistence of three organophosphorus insecticides in artificial ponds and some biological implications. Arch. Environ. Contam. Toxicol., 9 : 269-279.

Iview, J.W., D.L. Bull and J.A. Veech, 1980. Fate of diflubenzuron in water. J. Agr. Food Chem., 28 : 330-337.

Jacob, J.P., 1988. Combined toxic effects of oil and pesticides on selected marine invertebrates. Ph.D. thesis. Cochin Univ. Sci. Technol., 172 pp.

Jenkyn, J.F. and R.D. Prew, 1973. The effect of fungicides on incidence of Sporobolomyces spp. and Cladosporium spp. on flag leaves of winter wheat. Ann. Appl. Biol., 75 : 253-256.

Jones, L.S., O.E. Anderson and C. Dowler, 1974. Effects of herbicides in a crop-herbicide rotation on sulfur oxidation in Tifton soil. Agron. J., 66 : 744-747.

Jones, R.D. and M.A. Hood, 1980. The effects of organophosphorus pesticides on estuarine ammonium oxidisers. Can. J. Microbiol., 26 : 1296-1299.

Kaufman, D.D., S.C. Haynes, E.G. Jordan and A.J. Kayser, 1977. Permethrin degradation in soil and microbial cultures. In; Synthetic pyrethroids. Gould, R.F. (Ed.). ACS Synposia Series. No. 42 : 147-161.

Khalil, M.F., J. Labbe, S. Lantague, A.C. Horth and M. Arnac, 1984. Organochlorinated hydrocarbons in the St. Lawrence estuary. Sci. Tech. Eau., 17 : 73-79.

Khan, M.A.Q., F. Korte and J.F. Payne, 1977. Metabolism of pesticides by aquatic animals. In: Pesticides in aquatic environments (Khan, M.A.Q. (Ed.), Plenum., New York, NY (USA)., 191 pp.

Kinne, O., 1980. "Protection of Life in the Sea" - 14th European Marine Biology Symposium. Helgolander Meeresunters., 33 : 732-761.

Kinne, O. (Ed.), 1984. Marine Ecology Vol. 5. Ocean Management. John Wiley & Sons, Chichester. New York. Brisbane. Toronto. Singapore. 1618 pp.

Kinne, O. and H. - P. Bulnheim (Eds.), 1980. "Protection of life in the sea"- 14th European Marine Biology Symposium. Helgolander Meeresunters., 33 : 1-772.

Kittredge, J.S. and E. Roberts, 1969. A carbon-phosphorus bond in nature. Science, 164 : 37-42.

Kozlova, E.I., A.A. Belousova and V.S. Vandar'eva, 1964. Effect of simazine and atrazine on the development of soil microorganisms. Agrobiologiya, 2 : 271-277.

Krause, A., W.G. Hancock, R.D. Minard, A.J. Freyer, R.C. Honcycutt, H.M. LeBaron, D.L. Paulson, S.Y. Liu and J.M. Bollag, 1985. Microbial transformation of the herbicide metolachlor by a soil actinomycete. J. Agric. Food Chem., 33 : 584-589.

Krieger, R.I., S.J. Gee and L.O. Lim, 1981. Marine bivalves, particularly mussels Mytilus sp. for assessment of environmental quality. Ecotoxicol. Eviron. Saf., 5 : 72-86.

Krieger, R.I., S.J. Gee, L.O. Lim, J.H. Ross and A. Wilson, 1978. Disposition of toxic substances in mussels (Mytilus californianus) preliminary metabolic and histologic studies. In: Pesticide and xenobiotic metabolism in aquatic organisms (Khan. M.A.Q., J.J. Lech, and J.J. Menn, (Eds.). American Chemical Society, Washington DC (USA) 259-277.

Ku, C.C., Kapoor, I.P., Stout, S.J., and J.D. Rosen, 1979. Photodegradation of cytrolane (Mephosfolan) systemic insecticide in the aquatic environment using carbon-13 as a mass tracer. J. Agric. Food Chem., 27 : 1046-1050.

Kuznetsova, I.A., Yu. V. Kruglov and Yu.A. Baskakov, 1984. Transforamtion of the herbicide by pure cultures of soil microorganisms. Mikrobiologiya, 53 : 1003-1006.

Lakshmanan, P.T. and P.N.K. Nambisan, 1983. Toxicity of copper on the bivalve Villorita cyprinoides var. cochinensis. Ind. J. Mar.Sci., 6 : 83-85.

Lappin, H.M., M.P. Greaves and J.H. Slater, 1985. Degradation of the herbicide mecoprop (2- (2-methyl-4-chlorophenoxy) propionic acid) by a synergistic microbial community. Appl. Environ. Microbiol., 49 : 429-433.

Lee, R.F. and C. Ryan, 1979. Microbial degradation of organo chlorine compounds in estuarine waters and sediments. In: Microbial degradation of pollutants in marine environments. EPA-600/9-79-012. Environmental Protection Agency. Gulf Breeze, (USA) 443-450.

Lewis, D.L. and H.W. Holm, 1981. Rates of transformation of methyl parathion and diethyl phthalate by Aufwuchs microorganisms. Appl. Environ. Microbiol., 42 : 698-703.

Lewis, D.L., R.E. Hodson, and L.F. Freeman III, 1985. Multiphasic kinetics for transformation of methyl parathion by Flavobacterium sp. Appl. Environ. Microbiol., 50 : 553-557.

Lichtenstein, E.P. and S.R. Schulz, 1964. The effects of moisture and micro-organisms on the persistence and metabolism of some organo-phosphorus insecticides in soils, with special emphasis on parathion. J. Econ. Ent., 57 : 618-627.

Likums, E., 1979. Pesticides in runoff water. Agric. Res., 27 : 11.

MacLean, J.R., 1977. Bacteriological results of monitoring program of oyster shellstock harvesting areas, Maritimes region 1977. Data Rep. Fish. Mar. Serv. (Can.),86 : 12 pp.

MacLean, J.R. 1978. Bacteriological results of monitoring program of oyster shellstock harvesting areas Maritimes region 1978. Data Rep. Fish. Mar. Ser. (Can.).,123 : 16 pp.

Mac Rae, I.C., 1985. Removal of pesticides in water by microbial cells adsorbed to magnetite. Water Res., 19 : 825-830.

Mac Rae, I.C. and A.J. Cameron, 1985. Bacterial reduction of fensulfothion and its hydrolysis product, 4-methyl sulfinyl phenol. Appl. Environ. Microbiol., 49 : 236-237.

Mallison, S.M. and R.E. Cannon, 1984. Effects of pesticides on Cyanobacterium Plectonema boryanum and Cyanophage LPP-1. Appl. Environ. Microbiol., 47 : 910-914.

Mane, U.H., M.S. Kachole and S.S. Pawar, 1979. Effect of pesticides and narcotants on bivalve molluscs. Malacologia, 18 : 347-360.

Marsh, J.A.P. and M.P. Greaves, 1979. The influence of temperature and moisture on the effects of the herbicide dalapon on nitrogen transformation in soil. Soil Biol. Biochem., 11 : 279-285.

Matsumura, F., 1979. Use of a microcosm approach to assess pesticide biodegradability. In: Biotransformations and fate of chemicals in the aquatic environment. (Maki, A.W., K.L. Kickson, and J. Cairns Jr. (Eds.), American Society for Microbiology, Washington DC (USA). 126-135.

Matsumura, F., and G.M. Boush, 1966. Malathion degradation by Trichoderma viride and a Pseudomonas sp. Science, 153 : 1278-1280.

Matsumura, F. and G.M. Boush, 1968. Degradation of insecticides by a soil fungus Trichoderma viride. J. Econ. Entomol., 61 : 610-612.

Matsumura, F. and H.J. Benezet, 1978. Microbial degradation of insecticides. In: Pesticide Microbiology. I.R. Hill and S.J.L. Wright (Eds.) Academic Press, London, New York, San Francisco. 799 pp.

Matsumura, F. and E.G. Essac, 1979. Degradation of pesticides by algae and aquatic microorganisms. In: Pesticides and xenobiotic metabolism in aquatic organisms. Khan. M.A.Q., J.J. Lech and J.J. Menn, (Eds.). Am. Chem. Soc. Symp. Ser. 99. American Chemical Society, Washington, DC 371-387.

McLean, S., M. Sameshima, T. Katayama, J. Iwata, C.E. Olney and K.L.Simpson 1984. A rapid method to determine microsomal metabolism of organophosphate pesticides. Bull. Jap. Soc. Sci.Fish/Nissuishi, 50 : 1419-1423.

Mc Leese, D.W., V. Zitko, C.D. Metcalf and D.B. Sergeant, 1980. Lethality of aminocarb and the components of the aminocarb formulation to juvenile Atlantic salmon, marine invertebrates and a fresh-water clam. Chemosphere, 9 : 79-82.

Meikle, R.W., 1972. Decomposition: Quantitative relationships. In: "Organic Chemicals in the soil environment". Goring, C.A.I. and J.W. Hamaker, (Eds.), Marcel Dekker Inc., New York. 145-251.

Metcalf, R.L., G.K. Sangha and I.P. Kapoor, 1971. Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. Environ. Sci. Technol., 5 : 709-713.

Milkowsa, A. and A. Gorzelak, 1966. Effect of atrazine and simazine on the soil microflora in weed control in the nurseries. Sylwan, 110 : 13-22.

Miyamoto, J., Y. Takimoto and K. Mihara, 1978. Metabolism of organophosphorus insecticides in aquatic organisms with special emphasis on fenitrothion. In: Pesticide and xenobiotic metabolism in aquatic organisms. Khan. M.A.Q. J.J. Lech and J.J. Menn, (Eds.), American Chemical Society, Washington DC (USA),

Molin. G., and A. Ternström, 1982. Numerical taxonomy of Psychrotrophic pseudomonads. J. Gen. Microbiol., 128 : 1249-1264.

Moore, J.K., H.D. Braymer and A.D. Larron, 1983. Isolation of a Pseudomonas sp. which utilizes the phosphate herbicide glyphosate. Appl. Environ. Microbiol., 46 : 316-320.

Moorthy, K.S., M.D. Naidu, C.S. Chetty and K.S. Swami, 1983. Changes in carbohydrate metabolism in tissues of freshwater mussel Lamellidens marginalis exposed to phosphamidon. Bull. Environ. Contam. Toxicol., 30 : 219-222.

Motoyama, N. and W.C. Dauterman, 1974. The role of nonoxidative metabolism in organophosphorus resistance. J. Agric. Food Chem., 22: 350-356.

Mounter, L.A. and K. D. Tuck, 1956. Dialkyfluorophosphatases of microorganisms, II. Substrate Specificity Studies. J. Biol. Chem., 221: 537-541.

Munnecke, D.E., 1978. The persistence of non-volatile diffusible fungicides in soil. Phytopathol., 48 : 581-585.

Munnecke, D.M., 1977. Properties of an immobilized pesticide hydrolyzing enzyme. Appl. Environ. Microbiol., 33 : 503-508.

Murphy, J. and J.P. Riley, 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chim. Acta., 27 : 31-36.

Murray, H.E. and R.K. Guthrie, 1980a. Metabolic responses of aquatic bacterial populations to selected insecticides. Water. Resour. Bull., 16 : 749-751.

Murray, H.E. and R.K. Guthrie, 1980b. Effects of carbaryl diazinon and malathion on native aquatic populations of microorganisms. Bull. Environ. Contam. Toxicol., 24 : 535-542.

Nair, G.S. and C.S. Shynamma, 1975. Studies on the salinity tolerance of Villorita cyprinoides var. cochinensis (Hanley). Bull. Dept. Mar. Sci., Univ. Cochin. 7 : 537-542.

Nakas, J.P. and C.D. Litchfield, 1979. Evidence for the subcellular localization and specificity of chlordane inhibition in the marine bacterium Aeromonas proteolytica. Appl. Environ. Microbiol., 37: 471-479.

Nambu, K., H. Ohkawa and J. Miyamoto, 1980. Metabolic fate of phenothrin in plants and soils. J. Pestic. Sci., 5 : 177-197.

Nesbitt, H.J. and J.R. Watson, 1980a. Degradation of the herbicides 2,4-D in river water 1. Description of study area and survey of rate determining factors. Water Res., 14 : 1683-1688.

Nesbitt, H.J. and J.R. Watson, 1980b. Degradation of the herbicide 2,4 -D in river water 2. The role of suspended sediment, nutrients and water temperature. Water Res., 14 : 1689-1694.

Nicholson, H.P., 1967. Pesticide pollution control. Science. N.Y., 158 : 871-876.

Nimmo, D.R., 1977. Pesticides: their impact on the estuarine environment. In: Proc. Symp. Pollut. Physiol. Mar. Organisms. Vernberg,W., A. Calabrese, A. Thurberg and F.P. Vernberg (Eds.). Academic Press, New York, NY (USA). pp. 259-270.

Ohkawa, H., R. Kikuchi and J. Miyamoto, 1980. Bioaccumulation and biodegradation of the (S) - acid isomer of fenvalerate (Sumicidin) in an aquatic model ecosystem. J. Pestic. Sci. Jap., 5 : 11-22.

Ohkawa, H., K. Nambu, H. Inui and J. Miyamoto, 1978. Metabolic fate of fenvalerate (Sumicidin) in soil and by soil microorganisms. J. Pestic. Sci., 3 : 129-141.

Orndorff, S.A. and R.R. Colwell, 1978. Distribution and degradative potential of kepone-resistant bacteria in the James River and upper Chesapeake Bay. In: Proceedings of the workshop: Microbial degradation of pollutants in marine environments. pp. 396-407.

Orndoff, S.A. and R.R. Colwell, 1980. Effect of kepone on estuarine microbial activity. Microb. Ecol., 6 : 357-368.

Ou, L.T. and H.C. Sikka, 1977. Extensive degradation of silver by synergistic action of aquatic microorganisms. J. Agric. Food Chem., 25 : 1336-1339.

Ou, L.T., K.S.V. Edvardsson, P. Suresh and C. Rao, 1985. Aerobic and anaerobic degradation of aldicarb in soils. J. Agric. Food Chem., 33 : 72-78.

Paris, D.F., D.L. Lewis and N.L. Wolfe, 1975. Rates of degradation of malathion by bacteria isolated from aquatic systems. Environ. Sci. Technol., 9 : 135-138.

Park, J.S., 1979. Field bioassays on shellfish to assess environmental pollution levels of the Masan Bay. J. Oceanol. Soc. Korea., 14: 15-25.

Parrish, P.R., J.M. Patrick, Jr. and J. Forester, 1977. Effects of three toxicants on oysters (Crassostrea virginica) exposed continuously for two years. Proc. Natl. Shellfish Assoc. Md., 67: 121-122.

Patil, K.C., F. Matsumura and G.M. Boush, 1972. Metabolic transformation of DDT, dieldrin, aldrin and endrin by marine microorganisms. Environ. Sci. Technol., 6 : 629-632.

Percich, J.A. and J.L. Lockwood, 1978. Interaction of atrazine with soil microorganisms: Population changes and accumulation. Can. J. Microbiol., 24 : 1145-1152.

Peterson, E., R. Fairshter, J. Morrison and T. Cesario, 1981. Effects of the herbicide paraquat dichloride on bacteria of human origin. Appl. Environ. Microbiol., 41 : 327-328.

Pfaender, F.K. and M. Alexander, 1972. Extensive microbial degradation of DDT in vitro and DDT metabolism by natural communities. J. Agric. Food Chem., 20 : 842-846.

Philip, R., 1987. Studies on proteolytic bacteria in fish spoilage. Ph.D. Thesis. Cochin Univ. Sci. Technol., 299 pp.

Pillai, C.T., 1980. Microbial flora of mussels in the natural beds and farms. In: Coastal Aquaculture, Mussel farming: Progress and prospects. CMFRI, Cochin. pp. 33-38.

Pillai, V.K., A.G. Ponniah, K.K. Valsala and P.V.R. Nair, 1986. Heavy metal load in a few bivalves from the coastal waters of South India. National Seminar on Mussel Watch, Univ. Cochin, 1 : 93-104.

Plimmer, J.R., 1979. Degradation methodology: Chemical-physical effects. In: Proceedings of the workshop: Microbial degradation of pollutants in marine environments. Bourquin, A.W., and P.H. Pritchard (Eds.). EPA, Gulf Breeze, FL(USA) p.423-433.

Portier, R.J. and S.P. Meyers, 1980. Chitin transformation and pesticide interactions in a simulated aquatic microenvironmental system. Dev. Ind. Microbiol., 22 : 543-555.

Portier, R.J. and S.P. Meyers, 1982. Monitoring biotransformation and biodegradation of xenobiotics in simulated aquatic microenvironmental systems. Dev. Ind. Microbiol., 23 : 459-475.

Powar, C.B. and H.F. Daginawala (Eds.) 1982. General Microbiology Himalaya Publishing House, Bombay, 677 pp.

*Prieur, D., 1981. Nouvelles donnees sur les relations entre bacteries et bivalves marins. Haliotis, 11 : 251-260.

Prieur, D., 1982a. Quantitative and qualitative study of the bacterial communities associated with some marine bivalves: Comparisons with the microflora of seawater and sediments. Bacteriologie Marine - Marseille 17-18-19, Mai 1982.

Prieur, D., 1982b. General features of the microflora associated with bivalve molluscs in the marine environment. Bull. Cent. Ebud. rech. Sci., Biarritz. 14 : 181-190.

Prieur, D., T. Barbosa and A. Marhic, 1985. Bacterial communities in bivalve molluscs and sediment in the Bay of Brest. Oceanis, 11 : 287-294.

Puleo, J.R., S.L. Bergstrom, J.T. Peeler and G.S. Oxborrow, 1978. Thermal resistance of naturally occuring airborne bacterial spores. Appl. Environ. Microbiol. 36 : 473-479.

Purnomo, A. and A. Hanafi, 1977. Agricultural pesticides in brackishwater environment and suggestions for protecting aquaculture resources Proc. Technical Sessions, ASEAN Meeting of experts of Aquaculture, 31 Jan-6, Feb., 1977.

Rajagopal, B.S., V.R. Rao, G. Nagendrappa and N. Sethunathan,1978. Metabolism of carbaryl and carbofuran by soil enrichment and bacterial cultures. Can. J. Microbiol., 30 : 1458-1466.

Ramsay, A.J. and J.C. Fry. 1976. Response of epiphytic bacteria to the treatment of two aquatic macrophytes with the herbicide paraquat. Water Res., 10 : 453-459.

Rao, P.S.C. and J.M. Davidson, 1982. Retention and transformation of selected pesticide and phosphorus in soil-water systems: A critical review. NTIS order No. PB 82-256884, 341 pp.

Riskallah, M.R., M.M.El-Sayed and S.A. Hindi, 1979. Study on the stability of leptophos in water under laboratory conditions. Bull.Environ. Contam. Toxicol., 23 : 607-614.

Roberts, D., 1976. Mussels and pollution. In: Marine mussels: their ecology and physiology. B.L. Bayne (Ed.), Cambridge Univ. Press, London (U.K.) pp. 67-80.

Rogers, S.G., L.A. Brand, S.B. Holder, E.S. Sharps and M.J. Bracklin, 1983. Amplification of the aro A Gene from E. coli results in tolerance to the herbicide glyphosate. Appl. Environ. Microbiol. 46 : 37-43.

Rosales-Hoz, M.T.L., 1979. Concerning the dispersion of chlorinated hydrocarbons in the marine environment: scientific note. An. Cent. Cienc. Mar. Limnol. Univ. Nac. Auton. Max., 6 : 33-36.

Rose, A.H., 1968. (Ed.). Chemical Microbiology, Butterworks, London, 279pp.

Rosenberg, A. and M. Alexander, 1979. Microbial cleavage of various organophosphorus insecticides. Appl. Environ. Microbiol., 37 : 886-891.

Roslycky, E.B., 1985. Sensitivity and adaptation of selected rhizobia and agrobacteria to paraquat. Can. J. Soil. Sci., 65 : 555-562.

Salanki, J. and I. Varankal, 1978. Effect of some insecticides on the periodic activity of the freshwater mussel (Anodonta cypnea L.). Acta. Biol. Acad. Sci. Hung., 29 : 173-180.

Sariaslani, F.S. and J.P. Rosazza, 1985. Biotransformations of 1', 2' - Dihydrorotenone by Streptomyces griseus. Appl. Environ. Microbiol., 49 : 451-452.

Sethunathan, N. and T. Yoshida, 1969. Rate of diazinon in submerged soil, accumulation of hydrolysis product. J. Agric. Fd. Chem., 17 : 1192-1195.

Sethunathan, N. and T. Yoshida, 1973a. A Flavobacterium sp. that degrades diazinon and parathion. Can. J. Microbiol., 19 : 873-875.

Sethunathan, N. and T. Yoshida, 1973b. Parathion degradation in submerged rice soils in the Philippines. J. Agric. Fd. Chem., 21 : 504- 506.

Sharon, M.S. and J.R. Miles, 1981. The degradation of parathion and DDT in aqueous systems containing organic additives. J. Environ. Sci. Health., 16 : 703-711.

Sheela, S. and S. Balakrishna Pai, 1983. Metabolism of fensulfothion by a soil bacterium, Pseudomonas alcaligenes C_1. Appl. Environ. Microbiol., 46 : 475-479.

-G4028-

Shewan, J.M., G. Hobbs and W. Hodgkiss, 1960. A determinative scheme for the identification of certain genera of gram negative bacteria with special reference to the Pseudomonadaceae. J. Appl. Bacteriol., 23 : 379-390.

Skaar, D.R., B.T. Johnson, J.R. Jones and J.N. Huckins, 1981. Fate of kepone and mirex in a model aquatic environment: sediment, fish and diet. Can. J. Fish. Aquat. Sci., 38 : 931-938.

Sosnovskaya, E.A. and P.D. Pashchenko, 1967. The effect of herbicides on microflora of the soil under maize. Proc. Conf. Chemicalization Agric., 179-182.

Stainken, D. and J. Rollwagen, 1979. PCB residues in bivalves and sediments of Raritan Bay. Bull. Environ. Contam. Toxicol., 23: 690-697.

Strzelczyk, E., W. Donderski and W. Lewosz, 1972. Occurrence of micro-organisms capable of decomposing organic phosphorus compounds in two types of bottom sediments of the eutrophic lake Jeziorak. Acta. Microbiol. Pol., B : 101-110.

Subba Rao, R.V. and M. Alexander, 1985. Bacterial and fungal cometabolism of 1,1,1 - Trichloro - 2,2 - bis (4-chlorophenyl) ethane (DDT) and its breakdown products. Appl. Environ. Microbiol., 49: 509-516.

Suryanarayanan, H. and K.M. Alexander, 1972. Biochemical investigations on edible molluscs of Kerala. Fish. Technol., 9 : 42-47.

Timoney, J.F., J. Port, J. Giles and J. Spanier, 1978. Heavymetal and antibiotic resistance in the bacterial flora of sediments of New York Bight. Appl. Environ. Microbiol., 36 : 465-472.

Tobby, T.E., 1975. The risk to freshwater fisheries from the use of aquatic herbicides. In: Lectures presented at the fourth FAO/SIDA training course on aquatic pollution in relation to protection of living resources. pp. 155-167.

Toth, D.D., E. Tomasovicova and E. Karelova, 1981. The influence of polluting substances on microorganisms in water environment. Biologia (Bratisl.), 36 : 775-783.

Venkateswarlu and N. Sethunathan, 1984. Degradation of Carbofuran by Azospirillum lipoferum and Streptomyces spp. isolated from flooded alluvial soil. Bull. Environ. Contam. Toxicol., 33 : 556-560.

Verstraete, W. and J.P. Voets, 1974. Impact in sugarbeet crops of some important pesticide treatment systems on the microbial and enzymatic constitution of the soil. Meded. Fac. Landbouw, Rijksuniv. Gent., 39 : 1263-1277.

*Vladutu, I and I. Soreanu, 1976. Efectul Unor ingrasaminte organice asupra recoltei de porumb si activatii enzymatice a solului in functi de aplicarea erbicidului Gesaprium 50. Analele Inst. Cercetari Cereale Plante Technice Fundulea, 41 : 193-202.

Wedemeyer, G., 1967. Dechlorination of 1,1,1-trichloro-2,2- bis (p-chlorophenyl) ethane by Aerobacter aerogenes 1. Metabolic products. Appl. Microbiol., 15 : 569-574.

Wharfe, J.R. and W.L.F. Van Den Brock, 1978. Chlorinated hydrocarbons in macroinvertebrates and fish from the lower Medway estuary, Kent. Mar. Pollut. Bull. 9 : 76-79.

Williams, R.R. and T.F. Biddleman, 1978. Toxaphene degradation in estuarine sediments. J. Agric. Food. Chem., 26 : 280-282.

Wingfield, G.I., H.A. Davies, and M.P. Greaves, 1977. The effect of soil treatment on the response of the soil microflora to the herbicide dalapon. J. Appl. Bacteriol., 43 : 39-46.

Winteringham, F.P.W., 1971. Some global aspects of pesticide residue problems. Israel J. Entomol., 6 : 171-181.

Wolfe, N.L., R.G. Zepp, G.L. Baughman, R.C. Fincher and J.A. Bo-Garden, 1976. Chemical and photochemical transformations of selected pesticides in aquatic systems. Ecol. Res. Series. U.S. Environ. Protect. Agency. No. 600/3-76-067.

Wood, P.A. and I.C. Mac Rae, 1977. Reduction of fensulfothion to fensulfothion sulfide by Klebsiella pneumoniae. Appl. Environ. Microbiol., 34 : 247-250.

Yazer, O., J.M. Shively and N.D. Camper, 1975. Effects of selected herbicides on bacterial populations in nontreated and treated water. Water Resour. Bull., Am. Water Resour. Assoc., 11 : 294-299.

*Zaitsev, Yu.P. and V.K. Golovenko, 1981. Effect of DDT on the black sea mussel. Dopov. Akad. Nauk. Ukr. Rsr. Ser., B (10) : 69-72.

*Zhdamirov, G.G. and V.E. Popov (Eds.) 1979. Pesticide volatilization from aquatic systems. In: Methods and problems of ecotoxicological modelling and prediction. Sci. Cent. Biol. Res., Acad. Sci. USSR., p. 38-42.

* Not referred in original.

LIST OF PUBLICATIONS

LIST OF PUBLICATIONS

1. Sreekumari, K.R., M. Chandrasekaran and P. Lakshmanaperumalsamy, 1984. Lipolytic bacteria in Cochin backwater. National Seminar on Development and Ecosystem organised by the Society for Ecological Conservation and Development, June 5, Cochin.

2. Sreekumari, K.R. and P. Lakshmanaperumalsamy, 1987. Heavy metal resistant bacteria associated with black clam Villorita cyprinoides var. cochinensis (Hanley) and water collected from Cochin backwater. National Seminar on shellfish resources and farming. 19-21 Jan. CMFRI, Tuticorin.

3. Geethamony, S., K.R. Sreekumari and P. Lakshmanaperumalsamy, 1987. Metal resistance of Escherichia coli strains isolated from Vembanad lake. National Symposium on Environmental Biology, 2-4 April, Palayankottai, Abst. No. 67 pp. 25.

4. Sreekumari, K.R. and P. Lakshmanaperumalsamy, 1987. Metal resistant bacteria associated with pesticide treated black clam Villorita cyprinoides var. cochinensis (Hanley). National Seminar on Estuarine Management, Trivandrum, 4-5 June, Trivandrum, Abst. No. EB. 40.

5. Rosily, N.P., K.R. Sreekumari and P. Lakshmanaperumalsamy, 1987. Drug resistant Vibrio sp. associated with eggs and larvae of prawn Penaeus indicus (Milne Edwards). National Seminar on Estuarine Management, State Committee on Science, Technology and Environment, Trivandrum, 4-5 June, Trivandrum, Abst. No. EB 59.

6. Sreekumari, K.R., M. Chandrasekaran and P. Lakshmanaperumalsamy, 1988. Studies on lipolytic bacteria in stored fish Etroplus suratensis (Bloch). Fishery Technology, 25 (1) : 64-68.

7. Sreekumari, K.R. and P. Lakshmanaperumalsamy, 1988. Effect of an organophosphorus pesticide on the bacteria associated with Villorita cyprinoides var. cochinensis (Hanley). The Indian Zoologist, 12 (3 & 4) : 249-253.

8. Sreekumari, K.R. and P. Lakshmanaperumalsamy, 1988. Antibiotic resistance of heterotrophic bacteria isolated from water and black clam in Cochin backwater. (National Symposium on Estuarine Biology, 26-28 Oct. 1986. Abst. No. 76 pp. 59). J. Mar. Biol. Assoc. India, 29 (1 & 2): (in press).