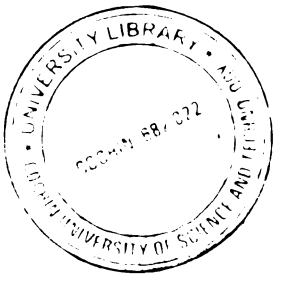
THE INFLUENCE OF ORGANOPHOSPHORUS PESTICIDE-METHYLPARATHION ON PROTEIN, LIPID METABOLISM AND DETOXIFYING ENZYMES IN ROHU (*Labeo rohita*)

Thesis submitted to the

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

for the award of the degree of



DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

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COCHIN-682 016, INDIA

APRIL-2008

DECLARATION

I hereby declare that the thesis entitled **"The influence of organophosphorus pesticide-methylparathion on protein, lipid metabolism and detoxifying enzymes in rohu *(Labeo rohita)*"** is based on the original research work carried out by me under the guidance and supervision of Dr TV Sankar, Senior Scientist, Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin-682 029 and no part of this work has previously formed the basis for award of any degree, associate ship and fellowship or any other similar titles of this or in any other university or institution.

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CERTIFICATE

This is to certify that the thesis entitled "**The influence of organophosphorus pesticide-methylparathion on protein, lipid metabolism and detoxifying enzymes in rohu *(Labeo rohita)***" embodies the original work carried out by Sivaperumal, P under my guidance and supervision in the Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin-682 029, in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Chemistry) and no part of this thesis has previously been formed the basis of award of any degree, diploma, associate ship, fellowship or any other similar titles of this or in any other university or institution.

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LIST OF ABBREVIATIONS

2,4,5-T	:	2,4,5-trichlorophenoxyacetic acid
2,4-D	:	2,4-dichlorophenoxyacetic acid,
AChE	:	acetylcholinesterase
ALT	:	Alanine aminotransferase
ANSA	:	Aminonaphthylsulfonic acid
APS	:	Ammonium per sulphate
AST	:	Aspartate aminotransferase
ATPase	:	Adenosine Triphosphatase synthase
BHC	:	Benzene hexachloride
BSA	:	Bovine serum albumin
BuChE	:	Butyrylcholnesterase
^0C	:	Degree Celsius
Ca^{2+}	:	Calcium ion
$CaCO_3$	:	Calcium carbonate
Cal	:	Calories
CAT	:	Catalase
CB	:	Carbamate
CDNB	:	1-Choloro-2, 4-dinitrobenzene
ChEs	:	Cholinesterases
Cl^-	:	Chloride ion
Conc	:	Concentration
d	:	Density
DDT	:	Dichloro Diphenyl Trichloroethane
DNA	:	Deoxy ribonucleic acid
DNPH	:	2,4 Dinitrophenyl hydrazine
DTNB	:	5,5;-Dithiobis(2-nitrobenzoic acid)
EDTA	:	Ethylene diamine tetraacetic acid
EPSPS	:	5-enolpyruvoylshikimate 3-phosphate synthase
$FeCl_3$	:	Ferric Chloride
Fig	:	Figure
g	:	Gram

GABA	:	γ-aminobutyric acid
GP_x	:	Gluthathione peroxidase
GSH	:	Reduced gluthathinoe
GST	:	Gluthathione-S-tranferase
h	:	Hour
hrs	:	Hours
H_2O_2	:	Hydrogen peroxide
H_2SO_4	:	Sulphuric acid
H_3BO_3	:	Boric acid
HCl	:	Hydrochloric acid
ICAR	:	Indian Council of Agricultural Research Institute
kDa	:	Kilo dalton
Kg	:	Kilogram
KOH	:	Potassium hydroxide
K_{ow}	:	Octanol/water partition coefficient
L	:	Litre
LC_{50}	:	Lethal concentration
LDH	:	Lactate dehydrogenase
LPO	:	Lipid peroxides
M	:	Molar
MCPA	:	4-chloro-*o*-toloxyacetic acid
MDA	:	Malonaldehyde
mg	:	milligram
mg/L	:	milligram per litre
Mg^{2+}	:	Magnesium ion
MIC	:	Methyl isocyanate
min	:	minutes
ml	:	milliliter
mM	:	millimolar
mPa	:	milli pascal
N	:	Normality
Na^+	:	Sodium ion
Na_2CO_3	:	Sodium carbonate

Na_2SO_4	:	Sodium sulphate
NaCl	:	Sodium chloride
NAD	:	Nicotinamide adenine dinucleotide
NADH	:	Reduced nicotinamide adenine dinucleotide
$NaHCO_3$	:	Sodium bicarbonate
NaN_3	:	Sodium azide
NaOH	:	Sodium hydroxide
ng	:	Nanogram
NIOH	:	National institute of Occupational Health
nm	:	Nanometer
nmol	:	Nanomol
NRC	:	National Research Council
O_2^-	:	Superoxide
OH^-	:	Hydroxyl radicals
OP	:	Organophosphorus pesticide
OPs	:	Organophosphorus pesticides
PA	:	Phosphatidic acid
PC	:	Phosphatidylcholine
PE	:	Petroleum ether
pH	:	Hydrogen ion concentration
PI	:	Phospaditylinositol
PL	:	Phospholipids
PS	:	Phsophatidylserine
PUFA	:	Poly unsaturated fatty acids
ROS	:	Reactive oxygen species
rpm	:	Revolutions per minute
SD	:	Standard deviation
SDH	:	Succinic dehydrogenase
SDS-PAGE	:	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SOD	:	Superoxide dismutase
TBA	:	Thiobarbituric acid
TBARS	:	Thiobarbituric acid reactive substances

TCA	:	Trichloro aceticacid
TCA cycle	:	Tri carboxylic acid cycle
TEMED	:	N,N,N',N' -Tetra ethyl methylene diamine
TEP	:	Tetraethoxy propane
TLC-FID	:	Thin Layer chromatography-Flame ionization detector
UV	:	Ultra violet
v/v	:	Volume/Volume
w/v	:	Weight/Volume
WHO	:	World Health Organization
µm	:	micromoles
µg	:	microgram
µl	:	microlitre

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ABSTRACT

Methylparathion (MP) is an organophosphorus insecticide used world wide in agriculture due to its high activity against a broad spectrum of insect pests. The aim of the study is to understand the effect of methylparathion on the lipid peroxidation, detoxifying and antioxidant enzymes namely catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione S-transferase (GST), total reduced glutathione (GSH), lipid peroxidation (LPO), acetylcholinesterase (AChE) and disease diagnostic marker enzymes in liver, sarcoplasmic (SP) and myofirbirllar (MF) proteins in muscles, lipids and histopathlogical changes in various organs of Labeo rohita of size 75 ± 6g at lethal and sublethal level of exposure. The probit analysis showed that the lethal concentration (LC 50%) for 24, 48, 72 and 96h were 15.5mg/L, 12.3mg/L, 11.4mg/L and 10.2mg/L respectively which is much higher compared to the LC50 for juvenile fish. The LPO level and GST activity increased five folds and two folds respectively on exposure to methylparathion at 10.2 mg/L and the level of the enzymes increased, on sub lethal exposure beyond 0.25mg/L. AChE activity was inhibited by 74% at a concentration of 1.8mg/L and 90% at 5.4mg/L. The disease diagnostic marker enzymes AST, ALT, ALP and LDH increased by about 2, 3 ,3 and 2 folds respectively at pesticide concentration of 10.2mg/L when compared to control. On sub lethal exposure, however the enzymes did not show any significant changes up to 0.5mg/L. At a concentration of 10.2 mg/L, there was a three fold increase in myofibrillar proteins while the increase in sarcoplasmic protein was above 1.5 fold. On sub lethal exposure, significant

alteration was noticed up to 30 days up to 1mg/L of methylparathion concentration. Further exposure up to 45 days increased sarcoplasmic proteins (upto 0.5mg/L). In the case of myofibrillar proteins, noticeable changes were observed at 1mg/L concentration right from 15th day. The cholesterol content in brain tissues increased by about 27% at methylparathion concentration of 5.4 mg/L. However at 0.25mg/L sub lethal concentration, no significant alteration was observed in enzyme activity, muscle proteins, lipids and histopathology of the tissues. The results suggest that methylparathion has the potential to induce oxidative stress in fish, and that liver, muscle and brains are more sensitive organs of Labeo rohita, with poor antioxidant potentials at higher concentrations of the pesticide. The various parameters studied in this investigation can also be used as biomarkers of methylparathion exposure.

Key words: Labeo rohita, methylparathion, oxidative stress, biomarkers, detoxifying enzyme, disease diagnostic marker enzymes, acetylcholinesterase, muscle proteins, Lipids, histopathology.

Introduction

1. INTRODUCTION

Ever since the dawn of civilization, man has been struggling to improve his living conditions. Securing relief from hunger, one of the basic human needs, is a major concern for humans. Today India is engaged in the gigantic task of feeding over 1000 million people and a huge cattle population on which the poor farmer is dependent for his livelihood. The Green Revolution of 1960, which has given reasonable hope for the country being not only self sufficient in the production of adequate food and fodder for feeding its teeming human and animal population but has become the largest producer of some important commodities. The role played by the magic chemicals, called "pesticides" are quite significant but have given rise to serious other health problems (Gupta, 1989). With a shuddering chill in the spine we recall the horror of Bhopal. The catastrophe, resulting from the leakage of methyl isocyanate (MIC) gas from the pesticide factory of Union Carbide Limited at Bhopal in the morning hours of 3 December, 1984, in which thousands of human beings and animals died, will never be forgotten. Most of the chlorinated non-degradable pesticides leave residues in various living systems for prolonged periods of their life span and are presumably responsible for a variety of known and unknown toxic symptoms (Gupta, 1985). Even when present in minute quantities, they show adverse effect on ecological systems to which human welfare is closely associated (Gupta, 1986).

Pesticide is a substance, or mixture of substance, that are used to control pests such as plant parasitic viruses, bacteria, nematodes, fungi, insects,

weeds, rodents, and birds. Pesticides include all materials that are used to prevent, destroy, repel, attract, or reduce pest organism. Therefore, pesticides find application in different area viz., forestry, landscaping, agriculture and domestic use.

Pesticides have been in use for over thousands of year. Some of the early demonstrations include burning of sulphur to control insects by Romans and the use of arsenic and pyrethrum by the Chinese. By the early 20^{th} century, two classes of pesticides were primarily used, viz., botanicals, natural chemicals derived from plant material, and preparation of inorganic salts, which were widely used as fungicides, herbicides and insecticides. The era of synthetic pesticides started with the discovery of DDT (Dichloro diphenyl trichloroethane) in 1934, which possessed insecticidal properties. Since then, innumerable preparations have been in use in the name of insecticides, fungicides, herbicides, etc. Development of resistant pests led to the banning of DDT in the 1960s. Globally, about 2.5 million tons of pesticides are targeted on agricultural crops. Even though the use of pesticides had a very positive impact in the overall increase in food production, the risks associated with this include deterioration of human health, water contamination, livestock animal poisoning and death of beneficial insects, wildlife endangerment and pesticide tolerance (Gupta, 2004).

1.1. Role of pesticides in public health

In the year 1948, DDT was imported for the control of diseases such as malaria, filariasis, dengue, Japanese encephalitis, cholera, and louse-borne typhus. Pesticides played a significant role in the control of vector-borne

diseases. In 1947, when India became independent, there were 75 million cases of malaria with about 800,000 deaths. During this period the National Malaria Eradication Program was launched, where in DDT played a crucial role and in the first 7 years of its operation, the malaria cases were drastically reduced. Therefore DDT is still among the magic chemicals as far as public hygiene is concerned. However, recent reports indicate that a large number of cases of dengue fever in different part of India are emerging (Anonymous, 2003), this is a matter of serious concern.

1.2. Trends in pesticide use

The three commonly used pesticides, BHC (only gamma-BHC is allowed), DDT and malathion account for 70% of the total pesticide consumption (Gupta, 2004). These pesticides are still preferred by the small farmers because they are cost effective, easily available, and display a wide spectrum of bioactivity. Out of the total consumption of pesticides in India, 80% are in the form of insecticides, 15% are herbicides, 1.46% is fungicide and less than 3% are others. In comparison, the worldwide consumption of herbicides is 47.5%; insecticides contribute 29.5%, fungicides, 17.5% and others account for 5.5% only (Gupta, 2004). The consumption of herbicides in India is probably low, because weed control is mainly done by hand weeding. In addition to public health and agricultural use, pesticides also find their use in other sectors (Gupta, 2004).

Pesticides can be classified according to their chemical structure. Inorganic pesticides are poisons made from common natural highly toxic and indestructible chemicals like arsenic, copper, lead and mercury; hence can

accumulate in the environment. Natural or organic pesticides are generally compounds extracted from plants. Many plants like tobacco, chrysanthemum, and conifers, have evolved the ability to produce substances that are used for this purpose. Another class of chemicals are fumigants, used to fumigate the insects but due to the danger associated it, finds only limited application.

1.3. Ecological effects of pesticides

All the pollutants released on land will eventually find their way to the rivers and sea as the final repository. Since estuaries are the links to the freshwater and marine systems, they contain a variety of anthropogenic chemicals referred to as xenobiotics, viz. oil derivatives, heavy metals, pesticides, fertilizers, polychlorinated biphenyls, etc. All the organic xenobiotics have the potential to affect normal physiology of aquatic animals.

Pesticides are included in a broad range of organic micro-pollutants that have tremendous ecological impacts. Different categories of pesticides have different effects on living organisms and hence generalization is difficult. Although terrestrial impacts by pesticides do occur, the principal pathway that causes ecological impacts is that of water contaminated by pesticides runoff. Fish and aquatic animals are exposed to pesticides in three ways namely (i) direct absorption through the skin by swimming in pesticide contaminated waters, (ii) direct uptake of pesticides through the gills during respiration, and (iii) drinking of pesticides contaminated water or feeding on pesticide contaminated prey. Exposure of fish and other aquatic animals to a pesticide depends on its biological availability, bio concentration, biomagnifications and its persistence in the environment.

The organophosphorus pesticide (OP) Folidol 600, which contains methyl parathion as the active substance, is extensively applied as a pesticide in agriculture, food storage shelters, pest control programs, and fish culture tanks to kill the aquatic larval stages of predator insects that threaten fish larvae. The kinds and amounts of pesticides applied to environments are almost out of control, and about one-third of organophosphorus pesticides (OPs) are selectively toxic to fishes (Aguiar *et al.*, 1994). In tropical countries several OPs are applied to soil and are washed into nearby water bodies where they affect, in particular, the aquatic organisms.

1.4. Mechanisms of pesticide action

Pesticides can be classified according to their mechanisms of action. For example, insecticides like organochlorines, organophosphates, and carbamates act primarily by disrupting the functioning of nervous system, while herbicides often target photosynthetic pathways (Ecobichon, 1991; DeLorenzo *et al.*, 2001). Mechanism of action of some of the pesticides is given below:

Pesticides	Chemical mode of action
Organophosphates and carbamates	Acetylcholinesterase inhibitors
	Receptor or Agonists
Chlorinate hydrocarbon/pyrethroid	Na^+ Channel,
Cyclodienes, Avermectines	Cl^- Channel
Nitro and Chlorophenols, Bipyridyls	Cytotoxins
Pyreyhrins/ pyrethroids	Allergines
Thiocarbamates	Mitosis blockers
Several groups of fungicides	Sterol inhibitors
Organophosphate fungicides	Chitin inhibitors
Sulfonylureas	Amino acid synthesis blockers
Rodenticides (varies chemistries)	Anticoagulants

1.4.1. Insecticides

Organochlorines are insecticides, affecting the nervous system. Chemically they are relatively unreactive stable compounds and are characterized by their long-lasting effects. Recently, Younis *et al.,* (2002) identified that the DDT target protein in insects. The target protein was shown to be a subunit of the adenosine triphosphatase synthase (ATPase), which is involved in the neuronal repolarization and thus important for normal nerve function. At the level of neuronal membrane, DDT affects the permeability to potassium ions, reducing potassium transport across the membrane and alters the channels, through which sodium ions pass, thereby interfering with the active transport of sodium out of the nerve axon during repolarization. Finally, DDT inhibits the ability of calmodulin, a calcium mediator in the nerve, to transport calcium ions essential for the release of neurotransmitters. Two other organochlorine insecticides, endrin and lindane, in addition to the DDT-resembling effects are also inhibitor of the γ-aminobutyric acid (GABA) receptors in both insect and vertebrate central nervous system (Wafford *et al.*, 1989). The GABA receptor is an ion channel glycoprotein that traverses the cell membrane and functions as a receptor for the neurotransmitter GABA.

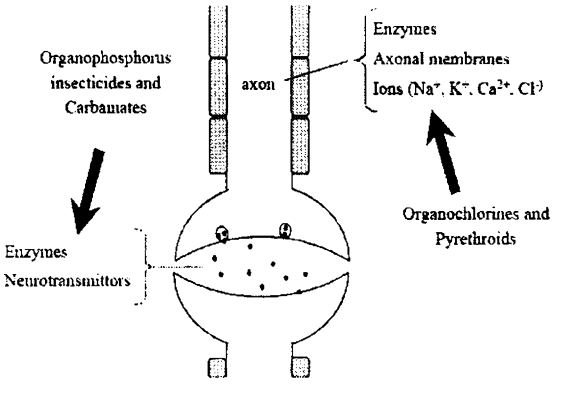


Fig-1. Potential sites of action of classes of insecticides on the axon and the terminal portions of the nerve (Ecobichon 1991).

The organophosphorus group of insecticides is also neurotoxic and this group acts on the nervous system by inhibiting the enzyme acetylcholinesterase (AChE). The reaction between an organophosphorus insecticide and the active site in the AChE protein results in the formation of a transient, intermediate complex that partially hydrolyzes, leaving a stable, phosphorylated, and largely unreactive, inhibited enzyme that, under normal circumstances, can be reactivated only at very slow rate (Fig 2). With many organophosphorus insecticides, an irreversibly inhibited enzyme is formed and the signs and symptoms of intoxication are prolonged and persistent. However, some organophosphorus insecticides are thought to be toxic only after metabolism by the cytochrome P-450 monooxygenase enzyme systems. This bioactivation step creates a metabolite that is a much stronger inhibitor of the AChE than the parent compound (Belden and Lydy, 2000).

Organophosphorus insecticides, like malathion and parathion, replaced the organochlorines in malaria eradication and other global programs for control of disease vectors when some the organochlorines, including DDT, were banned and withdrawn from the market in many countries (Mulla and Mian, 1981).

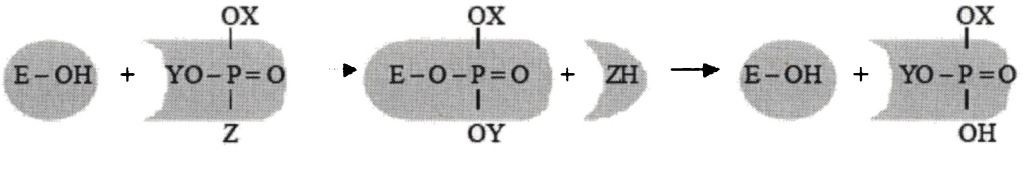


Fig-2. The interaction between an organophosphorus insecticide with the hydroxyl group in the active site of the enzyme acetylcholinesterase (E-OH). The intermediate, unstable complex formed before the release of the "leaving" group (ZH) is not shown. The dephosphorylation of the inhibited enzyme is the rate-limiting step to forming free enzyme. (Ecobichon, 1991)

Carbamates are also AChE inhibitors, attaching to the reactive site of this enzyme (Ecobichon, 1991). However, in contrast to organophosphorus insecicides, carbamates are poor substrates for the cholinesterase-type enzymes, resulting in a short and reversible inhibition of AChE.

Synthetic pyrethroids belong to the newest of the major classes of insecticides. The pyrethroids show two different characteristic acidic portions, chrysanthemic or pyrethric acids resulting in type I and type II syndrome. Type II syndrome involves primarily an action in the central nervous system, whereas with the type I syndrome, peripheral nerves are also involved (Ecobichon, 1991). Both type I and II pyrethroid insecticides affect the sodium channels in the nerve membranes, causing repetitive neuronal discharge, with effects being quite similar to those produced by DDT. There appears to be a prolongation of sodium influx with a delay in the closing of the sodium activation gate, resulting in an increased and prolonged sodium tail current (Narahashi, 1986; Bradbury and Coats, 1989; Ecobichon, 1991). Type II pyrethroids prolongs the sodium channel open-time much more drastically than type I pyrethroids (Narahashi, 1986). Also other sites of action have been noted for the pyrethroid insecticides.

Pyrethroid insecticides inhibit Ca^{2+}, Mg^{2+}-ATPase, thereby interfering with calcium removal from the nerve endings, resulting in increased neurotransmitter release in the postsynaptic gap. In addition, the protein calmodulin, responsible for the intracellular binding of calcium ions to reduce spontaneous neurotransmitter release, also get inhibited. Type II pyrethroids have also been shown to bind to the GABA-receptor chloride channel

complex, blocking chloride ion transport into the nerve cell. To summarize, there are many similarities between the mechanism of action of pyrethriod insecticides and organochlorines, consequently there is a risk for additive or even synergistic effects.

1.4.2. Herbicides

Herbicides are produced to kill or injure plants and therefore affect various mechanisms associated e.g. photosynthesis, respiration, growth, cell and nucleus division, or synthesis of proteins, carotenoids or lipids (Ecobichon, 1991).

The herbicide glyphosate, which is the active substance in the commonly used preparation Roundup™, controls weeds by inhibiting a single plant enzyme, 5-enolpyruvoylshikimate 3-phosphate synthase (EPSPS) (Sikorski and Gruys, 1997; Baylis, 2000). EPSPS is a key enzyme in the aromatic amino acid biosynthetic pathway and a blockage of this enzyme, adversely affects the protein synthesis. Although EPSPS is the only known enzyme target of glyphosate, the herbicide affects many physicochemical and physiological processes (Cole, 1985). Among these are the reduction in photosynthesis and the degradation of chlorophyll, as well as inhibited transport of the plant growth hormone auxin and enhancement of auxin oxidation.

The commonly used chlorophenoxy herbicides, including 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and MCPA (4-chloro-*o*-toloxyacetic acid) mimic the action of the growth hormone auxin in plants (Ecobichon, 1991; Grossmann, 2000). When the herbicides

are present in low concentrations at the cellular site of action, growth by cell division and elongation is usually stimulated. However, with increasing concentrations, a variety of growth abnormalities are induced within 24 h of treatment (Grossmann, 2000).

1.4.3. Fungicides

The breakdown of organic molecules provides energy for the survival of living systems. In fungi, as well as in other eukaryotes, a part of this catabolic process takes place in the mitochondria and lead to the synthesis of the high energy intermediate ATP. Several groups of fungicides disturb the energy supply in fungi and all such compounds are powerful inhibitors of spore germination (Leroux, 1992). Among them are the dithiocarbamates (e.g. maneb and thiram) and the R-S-CCl_3 compounds (e.g. captan and dichlofluanid). These fungicides have a multisite action by inhibiting several enzymes involved in the respiratory processes.

Another group of fungicides, the phenylpyrroles, including fenpiclonil and iprodionel are known to inhibit spore germentation and induce morphological alterations of germ tubes, i.e. inhibit germ-tube elongation (Leroux *et al.*, 1992). In addition these fungicides are able to uncouple oxidative phosphorylation and inhibit electron transport in respiration processes. However, more recent studies indicate that the main effect induced by fenpiclonil is due to the inhibition of wall glycan biosynthesis and to the accumulation of natural sugars. These effects indicate that the mechanism of action may be related to glucose metabolism (Jespers and de Waard, 1995). The effects of phenylpyrroles in fungi are thereby very close to the effect of

the herbicide dichlobenil in plants. According to Delmer *et al.,* (1987), dichlobenil may interfere with a membrane-bound protein involved with the regulation of the ß-glucan synthesis. Consequently there is a risk for additive or even synergistic effects when the phenylpyrroles fungicides and the herbicide dichlobenil are present at the same time in the environment.

1.5. Indian Scenario

Use of pesticides in India began in 1948 when DDT was imported for malaria control and BHC for locust control. India started pesticide production with manufacturing plant for DDT and benzene hexachloride (BHC) (HCH) in the year 1952. In 1958, India was producing over 5000 metric tonnes of pesticides. Currently, there are approximately 145 pesticides registered for use, and production has increased to approximately 85,000 metric tonnes. Rampant use of these chemicals has given rise to several short-term and long-term adverse effects of these chemicals. Despite the fact that the consumption of pesticides in India is still very low, about 0.5 kg/ha of pesticides against 6.60 and 12.0 kg/ha in Korea and Japan respectively, there has been a widespread contamination of food commodities with pesticide residues, basically due to non-judicious usage. In India, 51% of food commodities are contaminated with pesticide residues and out of these, 20% have pesticides residues above the maximum residue level values on a worldwide basis (Gupta, 2004; Agnihotri, 1999). It has been observed that their long-term, low-dose exposure are increasingly linked to human health effects such as immune-suppression, hormone disruption, diminished intelligence, reproductive abnormalities, and cancer. In this light, problems of

pesticide safety, regulation of pesticide use, use of biotechnology, and biopesticides are some of the possible future strategies for minimizing human exposure to pesticides. The worldwide consumption of pesticides is about two million tonnes per year, of which 24% is consumed in the USA alone, 45% in Europe and 25% in the rest of the world (Gupta, 2004)

1.6. Poisoning from pesticides

The rampant uses of pesticides have played havoc with human and other life forms. There is a serious hurdle in documentation because of lack of systematic and authentic data on poisonings. Pesticides account for a small but significant fraction of acute human poisonings. There has been a number of outbreaks of accidental poisoning by pesticides that deserve special mention. In India, the first report of poisoning due to pesticides was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion Folidol E 605 (Karunakaran, 1958).

Subsequently, several cases of human and animal poisonings, besides deaths of birds and fishes, have been reported (Sethuraman, 1977; Banerjee, 1979). In Indore, out of the 35 cases of malathion (diazole) poisoning reported during 1967-1968. In another report from Madhya Pradesh, 12 humans who consumed wheat for 6-12 months contaminated with aldrin dust and γ BHC developed symptoms of poisoning which consisted of myoclonic jerks, generalized clonic convulsions, and weakness in the extremities (Gupta, 1975). There are a number of similar cases of pesticides poising elsewhere in the country (Nag *et al.*, 1977, Anonymous, 1981, Gupta, 1986).

In general, it has been observed that organophosphorus pesticides are responsible for death in more than 70% cases of intentional poisonings (mainly attempted or successful suicides) make up a large proportion of the poisonings by pesticides of high toxicity in certain developing countries (Anonymous, 1990). In Indonesia, Malaysia, and Thailand, for example, the proportion of acute pesticide poisonings that are due to suicide attempts has been reported to be 62.6, 67.9, and 61.4%, respectively (Jeyaratnam, 1987). In India such suicide poisoning may even go up to 70% because such compounds are easily available in many households. However, no systematic population-based data on the role of pesticides on homicides are available.

The Bhopal gas tragedy is a catastrophe that has no parallel in industry history. In the early morning of 3 December, 1984 a rolling wind carried a poisonous gray cloud past the wall of the Union Carbide plant in Bhopal, Madhya Pradesh, India. An estimated 8000 or more people died. More than 4000 animals died within minutes of exposure to the gas and almost 15,000 animals suffered the toxic gas effect while surviving (over three times the officially announced total. Adverse effects included pulmonary edema which was the cause of death in most cases, with many deaths resulting from secondary respiratory infections such as bronchitis and bronchial pneumonia (Pandey, 1986; Jain and Dave, 1986).

1.7. Health hazards of occupational exposure

In addition to intentional exposure (suicides and homicides), workers are exposed to occupational hazards in industrial settings and operational hazards during distribution and use in the field. Pesticides are toxic chemicals

and as such they represent risks to users. In developing countries where users are often illiterate, ill-trained, and lacking appropriate protective devices, the risks are magnified. The Poison Information Centre in National Institute of Occupational Health in Ahmedabad reported that organophosphorus pesticides were responsible for the maximum number of poisonings (73%) among all agricultural chemicals (Dewan and Sayed, 1998). In a study on 190 patients of acute organophosphorus pesticide poisoning muscrinic manifestations were recorded (Bhatnagar, 2001). Studies on 356 workers in four units manufacturing HCH revealed neurological symptoms (21%) with significant increase in liver related enzymes which were related to the intensity of exposure (Nigam *et al.*, 1993). Observations confined to health surveillance in male formulators engaged in production of dust and liquid formulations of various pesticides (malathion, methyl parathion, DDT, and lindane) revealed several types of adverse effects and reproductive problems (Gupta *et al.*, 1984; Gupta, 2004). The thyroid function of formulators exposed to a combination of pesticides in the organized sector demonstrated a significant suppression of T3 while a marginal decrease (7%) was noticed in T4 level. TSH levels were also elevated by 28% but the rise was statistically insignificant (Zaidi *et al.*, 2000).

A cytogenic study revealed a significant increase in chromatid breaks and gaps in chromosomes in the peripheral blood in grape garden workers exposed to pesticides (Rita *et al.*, 1987). Separate studies conducted in malaria spray men spraying HCH, DDT, malathion, and cyfluthrin, showed increased levels of serum IgG (malathion exposure) and serum IgA (cyfluthrin exposure) (Karnik *et al.*, 1993). Observations on malaria spray men exposed

to HCH indicated changes in serum A/G ratio, glucose levels, and HCH residues (Kashyap *et al.*, 1980).

1.8. Assessment of human exposure

Pesticides have been primarily criticized for the presence of their residues in various samples of human blood, human fat, human milk, and fat samples, and food commodities. There is no denial of the fact that there is some element of risk involved in the use of pesticides like in any other product or service. Therefore, the pesticide-residue level is an indicator of the accidental exposure and/or average measure of body burden to persistent pesticides. This could either be due to direct exposure or indirectly through the food chain. In India, National Occupational Health Centre (NIOH) and Indian Council of Agricultural Research Institute (ICAR) are carrying out monitoring of health status and pesticide-residues in various samples. Analysis of samples of human blood, human fat, human milk, and fat samples was carried out by the National Institute of Occupational Health, Ahmedabad. Residues of organochlorine insecticides, especially DDT and HCH have been detected in man and his environment the world over. However, by comparison very high levels of these have been reported in human blood, fat, and milk samples in India (Bhatnagar, 2001). Higher level of these chemicals in human samples and in mother's milk is a reflection of their increased burden and their translactation passage. The toxicological implications of these findings could not be assessed precisely, however, preventive measures are warranted to reduce their body burden to avoid any potential health effect. Residues of pesticides from food commodities were monitored by All India Co-coordinated

Research Project on Pesticide Residues under the Indian Council of Agricultural Research, New Delhi, through their centers located in different parts of the country. Recently, analysis of bottled water, colas and other soft drinks carried out by the Centre for Science and Environment, New Delhi revealed very high content of pesticide residues (Anonymous, 2003a, b, c).

1.9. Consumption of pesticides in India

India is presently the second largest manufacturer of basic pesticides in Asia and ranks 12th globally. Currently, the consumption of pesticide is showing a slight declining trend, probably due to shift of farmers toward biopesticides, natural plant sources and other alternative methods (Das *et al.*, 2002; Gupta, 2003). Despite such a large consumption of pesticides, it is estimated that crop losses vary between 10-30% due to pests alone. In monetary terms, these losses amount to Rs.290,000 million per year (Agnihotri, 1999).

1.10. Objective of the study

Fish have been widely used as models to evaluate the health of aquatic ecosystems and in toxicological pathology studies (Law, 2003), Research in fish has demonstrated that mammalian and piscine systems exhibit similar toxicological and adaptive responses to oxidative stress. This suggests that piscine models, in addition to traditional mammalian models, may be useful for further understanding the mechanisms underlying the oxidative stress response.

The fresh water aquaculture system constitutes one third of the total fish production of India and the major carps (*Catla catla*, *Labeo rohita* and

Cirrhinius mrigala) being the dominant species. Aquaculture areas are not exclusive areas and usually get exposed to influx from surrounding water bodies particularly during rainy seasons. Therefore the contamination likely to be present in the water bodies may affect the aquaculture produce irrespective of its size. Most of the toxicological studies in fish are confined to the effects of pesticides on fingerlings and therefore a study on the growing/ grown out fish is envisaged in this study. The effect of methylparathion on the biochemical enzymatic changes in rohu (*Labeo rohita*) an important candidate species in a polycultural system is the theme of this work. Therefore the objectives of the study include

1. Determination of lethal concentration (LC_{50}) of methylparathion in grown up *L.rohita* (75 ± 6g size).

2. Effect of lethal/sub-lethal concentration of methylparathion on tissue metabolism by evaluating

    a) Acetylcholinesterase activity in brain tissues.

    b) Detoxifying enzyme systems in liver

    c) Alteration in liver diseases diagnostic marker enzymes

    d) Alteration in lipid metabolism in major organs

    e) Changes in tissue muscle protein metabolism

    f) Histopathological alteration in fish organ during exposure to methylparathion

Review of Literature

2. REVIEW OF LITERATURE

Chemical pesticides are well recognized as an economic approach to controlling pests, at the same time such chemicals are highly toxic to other species sharing the environment. Now there is growing concern worldwide over the indiscriminate use of such chemicals that results in environmental pollution and toxicity risk to nontarget organisms. (Venkateswara Rao, 2006). Chemical pollution by pesticides has been increasing in a large scale due to their vast usage for eradiation of various pests and insects and to protect agricultural crops (Matsumura *et al.*, 1972). Pesticides, even in very low concentrations have been reported to interfere with basal metabolism (Lal and Singh, 1986; Reddy and Rao, 1987). Continuous or pulse exposure to pesticides may cause serious problems for nontarget organisums, leading to a number of pathological and disturbed biochemical processes, including changes in energy budgets. The organisms can have direct energy costs to resist the toxicant by avoidance, exclusion, or removal; moreover, they may need energy to repair mechanisms and eventually show pathological effects. All these energy expenses reduce the amount of energy left to invest in normal life and, therefore, increase the probability of dying from additional stress (Calow, 1989). Responses to organophosphorus insecticides by aquatic organisms are broad ranged depending on the compound, exposure time, water quality and the species (Fisher, 1991; Richmonds and Duta, 1992).

Changes in the chemical composition of natural aquatic environments can affect the non-target organisms, particularly fish. Fish have been largely used

to evaluate the quality of aquatic systems as bioindicators for environmental pollutants (Adams and Greeley, 2000). Many environmental pollutants induce oxidative stress inducing effect in fish. Pesticides may induce oxidative stress through generation of ROS (Banerjee *et al.*, 1999). The increase in intercellular levels of ROS to such a level that cellular antioxidant defenses are insufficient to maintain these harmful molecules below a toxic threshold levels is generally referred to as oxidative stress. ROS are highly reactive molecules, which indiscriminately interact with essential macromolecules such as DNA, protein and lipids, leading to the disturbance of physiological processes (Cnubben *et al.*, 2001). Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity (Khrer, 1993).

The oxidative stress resulting from the production of reactive oxygen species (ROS) has gained considerable interest in the field of ecotoxicology (Lemaire *et al.*, 1994). The brain of fish contains low levels of antioxidants and higher levels of oxidizable catecholamines and peroxidizable unsaturated lipids (Lasner *et al.*, 1995). It may be more vulnerable to peroxidative damage compared with other tissues, therefore estimation of LPO has high predictive importance in toxicological research.

The highly reactive hydroxyl radical ($OH^{\cdot}$), which is one of the reactive oxygen species generated in the process leading to oxidative stress, is considered to be responsible for the formation of carbonyl groups in proteins (Farber and Levine, 1986; Oliver, 1987). Protein oxidation can lead to loss of critical sulfhydrly groups in addition to modification of amino acids leading to the

formation of carbonyl and other oxidized moieties (Stern, 1985; Bainy *et al.*, 1996). High oxygen tension in many areas of the circulation favors reactive oxygen species formation and membrane proteins are cross linked. Oxidative modification leads to proteolytic degradation, which may affect the structure, function and integrity of proteins (Davies, 1987; Carney *et al.*, 1991).

Organophosphorus insecticides include derivates of phosphoric acid, phosphorothioic acid, and phosphonic acid, while other organophosphorus pesticides that is widely used and has been shown to have toxic effects in human (De-Bleecker *et al.*, 1993, Thruman, 1989). Organophosphorus compounds (OPC) are widely used for agriculture and domestic purpose for controlling insect pests (Videira *et al.*, 2001). Due to their rapid breakdown in water and their low environmental persistence, organophosphorus pesticides have largely replaced the use of organochlorines in resent years (Li and Zhang, 2001).

2.1. Effect on acetylcholinesterase

Organophosphorus (OPs) insecticides are known to inhibit acetylcholinesterase, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate (Soreq and Zakut, 1993). These inhibit the action of the cholinesterase by phosphorylating or carbamylating the active centre of the enzymes. Cholinesterases (ChEs) are a ubiquitous class of serine hydrolases which physiologically remove acetylcholine from the synaptic cleft. ChEs are widely distributed among vertebrate and invertebrate animals (Bocquene *et al.*, 1997). In invertebrates two isomers occur, acetylcholinesterase (AChE)

which preferentially hydrolyses acetylesters such as acetylcholine, and butyrylcholnesterase (BChE) which preferentially acts on butyrylcholine. The main function of AChE is the rapid hydrolysis of the neurotransmitter, where as BChE has no known specific natural substrate, although it is able to hydrolyse acetylcholine. Acetylcholinesterase hydrolyses the neurotransmitter, acetylcholine, thereby ending transmission of nerve impulses at the synapses of cholinergic neurons in the central and peripheral nervous system. It has been suggested that BChE acts as a scavenging enzyme in the detoxification of natural compounds (Massoulie *et al.*, 1993).

Two principle human cholinesterase are acetylcholinesterase (AChE) found primarily in nervous tissue and erythrocytes and butyrylcholinesterase (BChE) found in liver and plasma (Arufe *et al.*, 2000). Through carbamates and a variety of phosphate esters are direct cholinesterase inhibitors, highly purified phosphorothionate and phosphorodithioate esters are poor anticholinesterase agents (Murphy, 1986). These compounds have substantially less electrophilic and reactive phosphorous groups than the corresponding phosphates, and are generally so stable as to be unable to combine with cholinesterase. Therefore, they own their biological activity to *in vivo* oxidation by microsomal mixed-function oxidase which takes place in the insect gut and fat body tissues and in the mammalian liver. A typical example of metabolic activation is that of parathion, Phosphorothioic acid O,O-diethyl O-(4-nitrophenyl) ester, a poor inhibitor of the cholinesterase enzymes that is converted to paraoxon, Phosphoric acid diethyl-4-nitophenyl ester, which is potential inhibitor of the enzyme.

Blockage of AChE by organophosphorus insecticides or carbamates results in accumulation of endogenous acetylcholine and this is in turn related to the symptoms associated with pesticide poisoning (Arufe *et al.*, 2000; Glynn, 2006). There are reports indicating that several pesticides exert their biological effects through electrophilic attack on the cellular constituents of hepatic and brain tissues (Samanta *et al.*, 1995) with simultaneous generation of reactive oxygen species (ROS) (Lemaire *et al.*, 1994). It has been reported that organophosphrous insecticides may induce oxidative stress on acute exposure in human (Banerjee *et al.*, 2001)

The inhibitory effects of organophosphorus insecticides are dependent on their binding capacity to the enzyme active site and by their rate of phosphroylation in relation to behavior and age (Dutta *et al.*, 1955). Organophosphorus pesticides are converted *in vivo* to the corresponding active phosphate ester or oxon (P=O), which is a potent acetylcholinesterase inhibitor. Studies using animal livers have shown that cytochrome P450 (CYP) enzymes mediate the oxidative desulphuration of the OP parathion to the active metabolite paraoxon (Neal, 1967). Organophosphorus compounds generally are lipophilic and therefore cross the blood-brain barrier in most cases (Vale, 1998). Tomokuni *et al.*, (1985) observed the accumulation of diazinon in the brain of rats and mice after single interperitoneal injection. They also found that brain AChE activity inhibited markedly after injection.

The phosphorus group of organophosphorus compounds attacks the hydroxyl group of a serine amino acid at the active site of the enzyme during AChE inhibition. Organophosphorus compounds may undergo isomerisations

(Corbett, 1974), leading to the conversion of phosphorothionate esters (P=S) to P=O group. Organophosphorus esters contaning the P=S moiety are less reactive and more stable to hydrolytic degradation than the corresponding P=O ester (Fukuto, 1990).

Neurological and behavioral activities of animals can be extremely sensitive to environmental contamination (Doving, 1991; Scherer, 1992; Silbergeld, 1993; Costa, 1996). Measurements of acetylcholinesterase (AChE, EC 3.1.1.7) activity are routinely used as a biomarker of exposure to certain groups of contaminants, such as organophosphate and carbamate insecticides (Williams and Sova, 1996; Grue *et al.*, 1997). Low concentrations of these can inhibit AChE, which leads to accumulation of acetylcholine at central cholinergic synapses and at vertebrate neuromuscular junctions (Hoy *et al.*, 1991; Gupta, 1994; Sancho *et al.*, 1997). As a consequence, these disturbances can affect locomotion and equilibrium in exposed organisms (Little *et al.*, 1990; Richmonds and Dutta, 1992; Hart, 1993; Saglio *et al.*, 1996).

Inhibition of AChE that is responsible for the degradation of acetylcholine will result in excessive stimulation of cholinergic nerves. This will result in tremors, convulsion and finally the death of the aquatic organism (Baxtr *et al.*, 1998). Several factors seem to be involved in affecting the AChE activity caused by OPs such as length to time and exposure concentrations (Uncer *et al.*, 2006). Inhibition of AChE, impairs cholinergic nerve impulses and may result in death of organisms (Salles *et al.*, 2006). As OPs exert their action by inhibiting AChE activity, measurement of AChE activity in the brain of fish has

been described as a method for diagnosing anti cholinesterase pesticides in aquatic solutions (Zinkl *et al.*, 1987; Sams *et al.*, 2000).

Inhibition of AChE was accompanied by an increase in acetylcholine levels (Brzezinski and Ludwicki, 1973). This condition can lead to increase of catecholamines which can affect the activity of enzymes involved in glycogenolysis and glycogen synthesis. Continuous stress may affect the synthesis site of AChE or decrease the levels of excess AChE. Mortality of fish may be due to inhibition of other enzymes, especially those taking part in carbohydrate and protein metabolisms. The inhibitory effect on AChE activity indicates that insecticides might interfere in vital processes like energy metabolism of nerve cells (Ansari *et al.*, 1987). Consequently, inhibition of AChE leads to paralysis and death.

AChE inhibition in brain, was observed earlier, when fish was exposed to other organophosphorous insecticides like chlorpyrifos and profenfos (Kumar and Chapman, 2001; Venkateswara Rao *et al.*, 2003a; Venkateswara Rao *et al.*, 2003b). The effect of malathion, diazinon, endosulfan on brain acetylcholinerase activity in blue gill sunfish and the largemouth bass showed a remarkable reduction in the activity which influenced the optomotor behavior of the fish that could be detrimental to their existence in the environment. (Dutta *et al.*,1992; Richmonds and Dutta, 1992; Dutta *et al.*, 1995; Guozhong and Dutta, 1998; Dutta and Arends, 2003).

Butyrylcholinesterases (BChE, EC 3.1.1.8) are enzymes belonging to a group of hydrolyse classified as B-type esterases (Aldridge, 1953). They are inhibited by organophosphorus (OP) and carbamate (CB) pesticides. As a

result, along with acetylcholinesterase (AChE), BChE inhibition has been used as an indicator of exposure in biomonitoring programs of pesticide contamination (Sanchez-Hernandez *et al.*, 2004). BChE contributes to the inactivating deoctanoylation of ghrelin, a peptide that stimulates growth hormone release from the pituitary (De Vriese, 2004). BChE molecules protect from organophosphate toxicity (Ashani, 1991) by stoichiometrically binding the organophosphates before they reach their biochemical targets. Therefore, some organisms with high amounts of serum or hepatic BChE may be resistant to poisoning by organophosphorus pesticides.

2.2. Effect on enzyme metabolism

The depletion with dissolved oxygen concentration of water polluted with phenolic waters (Phipps *et al.*, 1981) leads to formation of free radicals, especially superoxide (O_2^-), which acts by oxidizing various cellular substrates, especially unsaturated fatty acids, which are very susceptible to free radical damage (Rady, 1993). Generation of oxygen metabolites such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) is believed to be important in the pathogenesis of liver injury (Thurman and Handler, 1989). To counteract these oxidants, cells have several antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GP_x), and catalase (CAT). The resulting oxidative stress is a reason of enhanced lipid peroxidation and changes in structure and function of other important cellular components, such as protein and DNA (Wang *et al.*, 1990).

On long-term exposure to organophosphrous insecticides, ROS simply consume and exhaust antioxidant agents present in the body which is the

reason for the lower antioxidant capacity in chronic exposure (Rajibar, 2002). In contrast, subchronic exposures (40-45 days) of rats to organophosphorus insecticides result in an increased antioxidant capacity and higher lipid peroxidation. This means that in sub chronic exposure, the body is capable of defending itself by overproduction of antioxidants: an ability which fails to cope with the persistent organophosphorus insecticides induced reactive oxygen species in a longer period (Akhgari, 2003; Comporti, 1985).

Glutathione S-transferases (GST) is a multigene family of dimeric, multifunctional, mainly cytosolic enzymes, which play an important role in the biotransformation and detoxification of a number of electrophilic compounds, by conjunction to glutathione. In toxicological studies of subacute exposure, the alteration in the enzymatic activities directly reflects the metabolic disturbances and cell damage in specific organs (Casillas *et al.*, 1983). Elevated GST activity may reflect the possibility of better protection against pesticides toxicity and used as biomarker for pollution monitoring (Oruc *et al.*, 2004). Induced GST activity indicates the role of this enzyme in protection against the toxicity of xenobiotic-induced lipid peroxidation (Leaver and Geroge, 1998).

Glutathione is presumed to be an important endogenous defense against the peroxidative destruction of cellular membranes. Glutathione plays a protective role against free radical mediated or peroxidative damage (DeLucia *et al.*, 1972). Glutathione can act either to detoxify activated oxygen species such as H_2O_2 or to reduce lipid peroxides themselves. Antioxidants such as reduced glutathione (GSH) are central to cellular defense against oxidative stress

(Meister and Anderson, 1983). Glutathione plays a central role in antioxidant system and its depletion is considered as an important biomarker of oxidative stress in fish caused by pollutants (Almar *et al.*, 1998; Pena-Llopsis *et al.*, 2001).

Depletion of glutathione may reduce the cellular ability to destroy free radicals and reactive oxygen species, so that it raises the general oxidative potential in the cells. A reduction in GSH levels led to a significant induction of GST activity during most of the exposure period. GST-mediated conjunction may be an important mechanism for detoxifying peroxidised lipid break down products, which have a number of adverse biological effects when present in high amounts. Induced GST activity indicates the role of this enzyme in protection against the toxicity of xenobiotic-induced lipid peroxidaion (Leaver and George, 1998).

Increase in the levels of thiobarbituric acid reactive substances (TBARS) indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals (Comporti, 1985). Lipid peroxidation (LPO) mediated by free radicals is considered to be a primary mechanism of cell membrane destruction and cell damage (Plaa, 1976). Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced erythrocyte survival, and membrane fluidity (Thampi, 1991).

The decrease of LPO in brain of organophosphorus compound-Dichlorvos intoxicated *C. carpio* was attributed to the pesticide induced GSH increase, an

antioxidant compound. (Hai *et al.*, 1997). Since the organophosphorus insecticides are lipophilic substances, they penetrate the blood-brain barrier (Walker and Coleman, 1995) and enhance LPO by direct interaction with cellular plasma membrane (Hazarika *et al.*, 2003). However, Yang *et al.*, (1996) and Yang and Dettbarn, (1996) in their study with diispropylphosphorofluoridate (DFP), suggested that AChE inhibitor-induced cholinergic hyperactivity initiates the accumulation of free radicals leading to LPO. This may be an initiator of AChE inhibitor-induced cell injury. Hazarika *et al.*, (2003) discussed that LPO induced by malathion and anilofos appears to be mediated through activation of cholinergic receptors.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are liver specific enzymes which are more sensitive measure of hepatotoxicity and histophathalogical changes and can be assessed within a short time (Balint *et al.*, 1997). Increase in ALT and AST indicate tissue damaged in liver, kidney and gill (Rajyasree and Neeraja, 1989; Oluah, 1999). Alterations in alkaline phosphatase (ALP) and acid phosphatase (ACP) activities in tissues and serum have been reported in fish (Jyothi and Narayan, 2000). Elevated lactate dehydrogenase (LDH) activities are a marker for tissue damage in fish (Ramesh *et al.*, 1993; Balint *et al.*, 1997) and serve as a good diagnostic tool in toxicology. Sastry and Siddiqui (1982) reported increases in LDH of liver and brain and decreases in succinic dehydrogenase (SDH) activity of liver and brain of *Channa punctatus* exposed to sublethal concnentrations of sevin. Anjum and Siddiqui (1990) studied the inhibition of brain Ca^{2+} ATPase by monochrotophos, dimethoate and diazinon on *Tilapia mossambica.*

Changes in acid phosphatase (ACP) and alkaline phosphatase (ALP) in brain tissues induced by pesticides have been recorded by Sastry and Sharma (1981). Ghosh (1987) reported that there was an increased activity of lactic dehydrogenase (LDH) in brain, liver and muscle and decreased activity in kidney and intestine of *Clarias batrachus* when exposed to sublethal concentrations of Tara 909, Suquin and Croton 36.

Fish accumulate γ-BHC preferentially in their fatty tissues, such as liver and gonads, but the effects may become apparent only when concentration in such tissues passes a certain threshold. These effects can include damaged immune response, tumors, or respiratory problems, all of which can shorten the life span and decrease the population through both premature mortality and a decreased number of fish surviving to enter the spawning season (Macek, 1968; Anderson, 1996).

2.3. Effect of protein metabolism

A fall in muscle protein is indicative of reduced protein synthesis and low assimilation of food and low amino acid uptake for protein synthesis. Organophosphates are known to methylate and phosphorylate cellular proteins directly (Wild, 1975). Murty and Devi (1982), recorded a decreases in the protein levels in the tissues of *C. punctatus* following acute exposure to technical grade malathion. Similarly, a reduction in the protein content of brain, liver and ovary of *C. punctatus* was recorded due to cython (Narayan Ram and Satyanesan, 1986). Shanmugan, (1977) suggested that tissue proteins breakdown occurs in order to maintain plasma proteins in conditions of protein deficiency.

The decrease in protein content of parathion-intoxicated isopods also indicates a physiological adaptability to compensate for pesticides stress. To overcome the stress situation, animals require high energy which could be derived from protein catabolism as well. Furthermore, this decrease in protein content might also be due to the repair of damaged cell and tissue organelles (Sancho *et al.*, 1998; Rambabu and Rao, 1994).

2.4. Effect of lipid metabolism

The strong lipophilicity of most insecticides promotes incorporation in to biomembranes. Owing to the crucial functions of membranes, the insecticide effects, either acute or chronic are certainly membrane related. Accordingly, studies indicate that insecticide compounds induce perturbations of membrane permeability and enzyme dynamics (Antunes-Madeira and Madeira, 1979 and 1982; Antunes-Madeira *et al.*, 1981). Since basic membrane mechanisms are greatly influenced by the membrane physical state and organization (Sikkema *et al.*, 1995), the primary effects of insecticides may be related to physical changes at the level of lipid-lipid and lipid-protein interactions. Additionally, partition studies (Antunes-Madeira and Madeira, 1989) indicate that membrane organization modulates to great extent, the incorporation of insecticide. Since lipids undergo rapid breakdown, re-synthesis and interconversion in response to different stimuli, it is essential that various lipid fractions in different tissues be considered simultaneously to provide a clear picture of lipid metabolism in response to pesticides (Srinivasulu Reddy and Ramana Rao, 1989)

Decrease in tissue lipid and proteins under pesticide stress could be due to several mechanisms viz., formation of lipoproteins which are utilized for repair of damaged cell and tissue organelles, direct utilization by cells for energy requirements, increased lypolyses, and damage to cellular organization, as noticed in *B. dissimilis* exposed to pesticides (Ghosh and Chatterjee, 1989).

There is a general decrease in the total lipids and glycerol levels in prawns exposed to phosphamidon, methylparathion and lindane, while the free fatty acids and total cholesterol showed a significant increase in prawn tissues. This suggests the mobilization of rich lipids for production of energy during toxic stress caused by pesticides (Srinivasulu Reddy and Ramana Rao, 1989). Increased levels of acetoacetate and β-hydroxybutyrate have been shown in the insecticide administered animals (Domschke *et al.*, 1971). Since TCA cycle enzymes are inhibited during insecticides stress (Reddy and Rao, 1987), the accumulation of acetyl-CoA is likely to cause that diversion. The acetyl-CoA, produced through augmented β oxidation and glycolysis (Reddy and Rao, 1988) will produce more ketone bodies, particularly acetoacetate, which then act as precursors for the synthesis of cholesterol.

The changes suggested that cells adapted their membrane lipids to compensate for presence of pesticides in the environment (Rosas *et al.*, 1980). The effect of different concentrations (5, 10 and 20 mg/L) of phenol on the carp following exposures of 48 and 96h indicated that exposure of carp to high concentrations of phenol led to an increase in phosphatidylcholine (PC) in erythrocyte plasma membrane and eliminated phospaditylinositol (PI), phsophatidylserine (PS), and phosphatidic acid (PA)

The data showed increased synthesis of arachidonic acid (20:4 n-6), the n-3 fatty acids (eicosapentaenoic acid (20:5 n-3), and docosahexaenoic acids (22:6 n-3) displayed a fairly varied picture after exposure to phenol pollutant. Long-term exposure to high phenol concentrations, however, led to elimination of these acids and significantly decreased n-3/n-6 ratios. The data support lipogenesis indicating a clear of metabolic shift from carbohydrate to lipid through acetyl-CoA barriers. The increase in the total lipids in tissues of the frog exposed to sublethal concentration may be to constitute glycolipids, lipoproteins and phospholipids to from the structural entity to meet the prolonged toxic stress as has been shown in fresh water bivalve (Bhalchandra and Lomte, 2004) and fish (Sivaramakrishan *et al.*, 1992) Similarly there are studies indicating decrease in the muscle protein and RNA levels and evalvation in DNA levels and acid phosphatase activity (Das and Mukherjee, 2000).

2.5. Physical and chemical properties of methylparathion

CAS chemical name	*O,O*-dimethyl *O*-(4-nitro-phenyl) phosphorothioate
IUPAC systematic name	*O,O*-Dimethyl *O*-4-nitrophenylphos-phorothioate
Physical state	Pure white crystalline solid or powder (National Fire Protection Association, 1986)
Melting point	37-38 °C
Freezing point	about 29 °C (technical product)
Density/specific gravity	1.358 at 20 °C/40 °C (d^{20}_4 1.358)

Vapour pressure	1.3 mPa at 20 °C
Octanol/water partition coefficient	log K_{ow} = 2.68 (measured) log K_{ow} = 1.81-3.43 (reported range)
Water solubility	55-60 mg/L at 25°C (pure) (Midwest Research Institute, 1975; National Research Council, 1977)
	37.7 mg/L at 19 °C (pure) (Bowman and Sans, 1979)
	57 mg/L at 22°C (anal. grade) (Sanders and Seiber, 1983)
Nonaqueous solubility	soluble in ethanol, chloroform, aliphatic solvents, and slightly soluble in light petroleum
Volatility (pure)	0.14 mg/m^3 at 20 °C.
Odour	Like rotten eggs or garlic (technical grade) (Midwest Research Institute, 1975; Anon,1984)
Other properties	hydrolyses and isomerizes easily (White-Stevens, 1971)
	Half-life in aqueous solution at 20°C, pH 1-5: 175 days (Melnikov, 1971)

2.5.1. Source of human and environmental exposure

Methyl parathion is a representative of the highly active insecticides, the thiophosphorus esters, developed in the 1940s by Schrader, a German chemist. Methyl parathion was introduced as a commercial chemical in 1949. It is synthesized by the reaction of *O,O*-dimethyl phosphoro-chloridothioate with the sodium salt of 4-nitrophenol (Schrader, 1963).

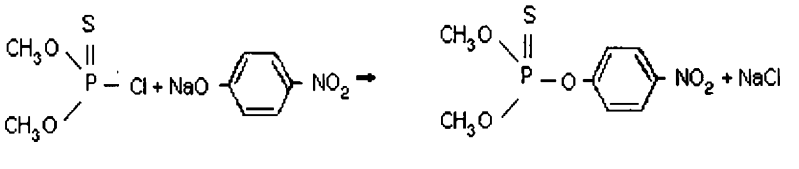


Fig -3 Structure and synthesis of methylparathion

Natural occurrence of methyl parathion is unlikely. Emissions of methyl parathion during the production process can be disregarded when compared with those from its use as an insecticide. The air emission from a factory in the USA was reported to be around 0.1% of the production level (Archer *et al.*, 1978). The major effects of this insecticide are directly caused by spraying and evaporation from water surfaces, leaves, and from the soil (Wooder *et al.*, 1977).

Methyl parathion is used in following formulations:

1. Emulsifiable concentrates (EC) with 19.5%, 40%, 50%, 60% active ingredient (2) Wettable powders containing 40%
2. Dusts 1.5%, 2%, and 3% methyl parathion
3. Microencapsulated methyl parathion
4. Ready-to-use liquid.

The usual carriers are: petroleum solvents and clay carriers (such as propargite). Besides, neat preparations, combinations are available containing parathion, omethoate, tetradifon, prothoate, and petroleum oil.

Methyl parathion is a broad-spectrum insecticide with non-systemic contact and stomach action. The normal method of application is foliar spraying by aircraft or ground equipment (HSDB, 1990). Only foliar application of methyl parathion is known. It is used as a contact insecticide and acaricide. There are different routes of application depending on the type of plant to be protected and the organisms to be killed. The recommended application rate

is 0.5-1 kg active ingredient/ha for vegetables, 1-2 kg/ha for cereals, 1.5-6 kg/ha for fruit trees, 2-5 kg/ha for citrus fruits, and 0.12-1.0 kg/ha for cotton.

2.6. Methyl parathion Entry into the food-chain

Methyl parathion hydrolyses faster than parathion. Because of the physical and chemical properties of methyl parathion, its pollution potential seems to be very small. Therefore, the most probable entry into the food-chain seems to be directly via residues on vegetables or crops. Since animals can degrade methyl parathion and excrete the degradation products within a very short time, a risk from eating meat seems to be unlikely. However, there may be an additional hazard from methyl parathion bound to glucosides (Dorough, 1978).

2.7. Kinetics and metabolism

2.7.1. Absorption

Methyl parathion can be absorbed through the digestive tract, the skin, and the respiratory tract (White-Stevens, 1971). The primary routes of exposure are via skin contact with contaminated plants or material, and via inhalation. Severe accidental intoxications of humans have occurred. The absorption of methyl parathion from the digestive tract is rapid, and it appears in the bloodstream immediately after oral intake. The maximum level was found 1-2 h after treatment. The liver showed a remarkably high concentration (Gar *et al.*, 1958). Labeled studies indicated that maximum concentrations in the blood and brain were reached 1-3h after treatment. An oral dose of 50mg methyl parathion/kg resulted in no detectable levels of methyl parathion in either the brain or blood after 3min, but, after 6-8min, at which point lethal

effects occurred, levels of methyl parathion increased to 182ng/ml in plasma and to 137ng/g in brain (Miyamoto *et al.*, 1963; Yamamoto *et al.*, 1983).

2.7.2. Distribution and metabolic transformation

Accumulation of methyl parathion was observed in tissues. The highest concentrations were found in the lung and the liver (NRC, 1977). Organic nitro compounds, orally administered to ruminants, will undergo reduction of the nitro groups to amino groups. This reaction takes place in the rumen (Karlog *et al.*, 1978).

2.7.3. The metabolism of methyl parathion in rodents

Because of the toxicological made, the methyl parathion first pass through the liver for the metabolism and there is a distinct difference between the oral and intravenous toxicity (Morgan *et al.*, 1977; Braeckman *et al.*, 1983). Conversion of methyl parathion to its toxic metabolite, methyl paraoxon, may occur within minutes following oral administration (Yamamoto *et al.*, 1983). Mouse liver, perfused with methyl parathion, released the toxic metabolite methyl paraoxon into the effluate. Mouse whole blood rapidly detoxified the methyl paraoxon formed (Sultatos, 1987).

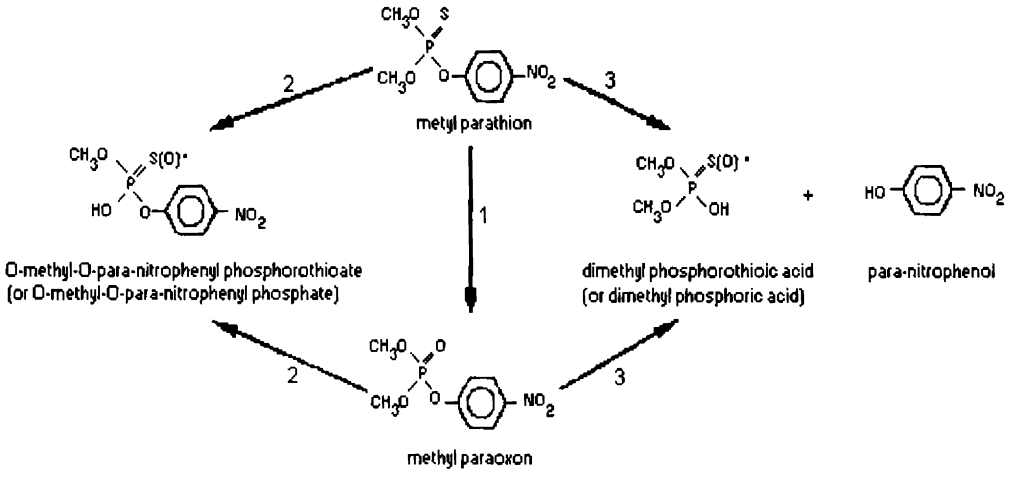


Fig. 4 Metabolism of methyl parathion in rodents. Methyl paraoxon may be metabolized via the same pathways as methyl parathion, resulting in the oxygen analogue, indicated by the presence of (O)* in the figure. From: IARC (1983).

1. Toxification: metabolic formation of oxon.
2. Detoxification glutathione dependent alkyl transferase.
3. Detoxification glutathione dependent aryl transferase.

Only after the metabolic conversion of methyl parathion to methyl paraoxon by liver microsomal oxidases the substances become toxic. Therefore, this is an activation reaction. Methyl parathion and methyl paraoxon are mainly detoxified by conjugation with glutathione (Hennighausen, 1984).

Glutathione (GSH) influences mitosis, mobility, and other GSH-dependent cell functions. Glutathione *S*-transferases are mainly located in the cytosol and display overlapping substrate specificity. They also show peroxidase activity and prevent the peroxidation of membrane lipids. The interaction of methyl parathion with GSH or with the glutathione *S*-transferases therefore is important not only for the non-oxidative detoxification of the insecticide, but also for species-selective toxicity, and for the development of resistance. Placental and fetal human glutathione *S*-transferase catalysed the

dealkylation of methyl parathion exclusively to demethyl parathion via *O*-dealkylation (Radulovic *et al.*, 1986; 1987).

Detoxification is achieved by degradation reactions that involve either demethylation or dearylation. The resulting desmethyl compounds and dimethyl phosphoric acids are essentially nontoxic (NRC, 1977). The reaction products are *O*-methyl-*O*-*p*-nitrophenyl phosphorothioate (or *O*-methyl-*O*-*p*-nitrophenyl phosphate) or dimethyl phosphorothioic acid (or dimethyl phosphoric acid) and *p*-nitrophenol. In addition, hydrolysis of methylparaoxon by tissue arylesterases may occur. Thus, it is possible to follow an exposure to methyl parathion by measuring the urinary excretion of *p*-nitrophenol (Benke and Murphy, 1975). The amount of the active toxic compound (methyl paraoxon) that will be produced after exposure to methyl parathion depends on the kinetics of the oxidation of methyl parathion and on the kinetics of the detoxification reactions. This enzyme system was found in the supernatant of the liver homogenate. The main metabolites were dimethyl parathion (80%), demethyl paraoxon (Fukami and Shihido, 1963; Shihido and Fukami, 1963), diethylparxone and *p*-nitro phenol (Fig-5).

Malaysian prawns (*Macrobrachium rosenbergii*) as well as ridgeback prawns *(Sicyonia ingentis)* decomposed methyl parathion readily to *p*-nitrophenol and *p*-nitrophenyl conjugates. The dominant way of detoxification was the formation of ß-glycosides and sulfate esters (Foster and Crosby, 1987).

The metabolism of methyl parathion in humans is similar to that reported in experimental animals (Fig-5) (Benke and Murphy, 1975; Morgan *et al.*, 1977). The liver is the primary organ for detoxification and metabolism (Nakatsugawa

et al., 1968; 1969). The main metabolites recovered from urine following administration of methyl parathion to human subjects were also *p*-nitrophenol and dimethyl phosphate. Eight hours after application, *p*-nitrophenol excretion was nearly complete. Methyl paraoxon was hydrolysed to dimethyl phosphate and an amount representing 12% of the administered dose was excreted. Its excretion was more protracted than that of *p*-nitrophenol (Morgan *et al.*, 1977). After an oral dose of ^{32}*p*-methyl parathion to mice (17 mg/kg), 75% of the radioactivity was found after 72h as metabolites in the urine and up to 10% was eliminated in the faeces (Hollingworth *et al.*, 1967).

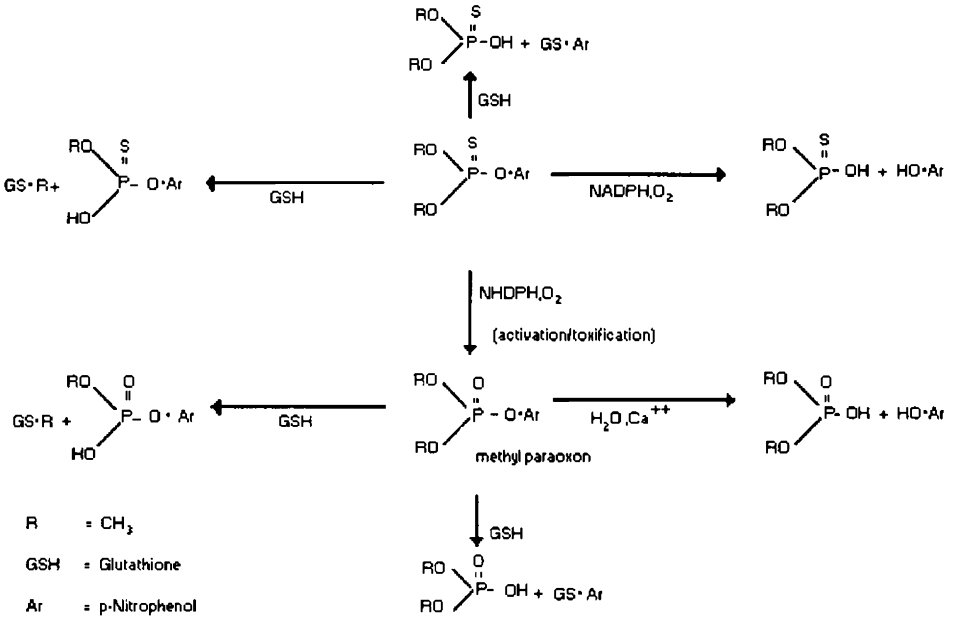


Fig. 5 Metabolism of methyl parathion in mammals. From: Flucke (1984).

2.8. Effects on organisms in the environment

2.8.1. Aquatic animals

LC_{50} values of more than 1 mg/L have been found for some freshwater biota (molluscs, fish, and amphibians). Insect sensitivity to methyl parathion

depends not only on the species but on the life stage. In general, instar I larvae are more affected than instar IV larvae. Many laboratory studies have been performed on the acute toxicity of methyl parathion in fish. The symptoms of methyl parathion poisoning which can be expected to occur in fish include darkening of the skin, hyperactivity, body tremors, lethargy, jerky swimming, scalosis, loss of equilibrium, opercular or gaping paralysis, and death (Rao *et al.*, 1967; Anees, 1975; Midwest Research Institute, 1975). One response that may be considered to be somewhat characteristic of acute methyl parathion poisoning in fish is the extreme forward position of the pectoral and pelvic fins (Midwest Research Institute, 1975; Srivastava and Singh, 1981).

2.8.2. Toxicity in aquatic invertebrates

Exposure of the freshwater mussel *(Lamellidens marginalis)* to sublethal (8mg/L) concentrations resulted in a transient increase (at 12h) followed by a decrease (at 24-72h) in the rate of respiration (Moorthy *et al.*, 1984). Exposure of this species to concentrations ranging from 10 to 50mg/L resulted in a concentration-dependent decrease in heart rate (Rao *et al.*, 1983a).

For crustaceans, long-term toxicity levels appear to be of the same magnitude as acute: a no-effect level on the reproduction of *Daphnia magna* was 0.0012mg methylparathion/L after 21 days (artificial water, 18°C; Dortland, 1980). Exposure of the freshwater crab *(Oziotelphusa senex senex)* to sublethal levels of methyl parathion (0.1-1 mg/L) resulted in complete inhibition of molt, a delay in the onset of molt, or a decrease in the percentage of molting animals (Reddy *et al.*, 1985). A decrease in the carbohydrate

content and increase in acid phosphatase activity in both the hepatopancreas and muscle also occurred (Reddy *et al.*, 1986a; 1986b). Eisler (1970a,b) found a 20% increase in mortality in *Nassa docoleta* after 10 days' exposure to 25 mg/L (well water with a salinity of 24°/$_{\infty}$, 20°C, pH 8).

Exposure of prawns *(Penaeus indicus* and *Metapenaeus monoceros)* to sublethal concentrations of methyl parathion resulted in a concentration-dependent inhibition of acetylcholinesterase activity, which recovered in 7 days (Reddy and Rao, 1988). An increase in tissue levels of ammonia, urea, and glutamine, apparently resulted from the catabolism of purines and glutamate (Reddy *et al.*, 1988; Reddy and Rao, 1990a). There was also an increase in tissue levels of fatty acids and cholesterol (Reddy and Rao, 1989), while the activity of alkaline phosphatase in the hepatopancreas was inhibited, and the acid phosphatase activity enhanced (Reddy and Rao, 1990b). Changes in hepatic glycogen content and haemolymph glucose levels were observed after 5 days of sublethal methyl parathion exposure (Reddy and Rao, 1990b). Cripe *et al.*, (1981) tested the stamina of mysid shrimp *(Mysidopsis bahia)* in swimming against a water current in the presence of methyl parathion. Concentrations of 0.10 and 0.31 µg/L did not affect maximum sustained speeds of the shrimp, but they were significantly reduced on exposure to 0.58 µg/L.

Methyl parathion concentrations of 0.59-0.77 mg/L induced increased mortality after 32 days. No effects on mortality were found at 0.38 mg/L for the technical grade product and 0.59 mg/L for the controlled release formulation (Jarvinen and Tanner, 1982). Mortality in rainbow trout *(Salmo gairdneri)*

increased to 98% after exposure to 2.8 mg methyl parathion/L (technical grade) (well water, 12°C, pH 7.5, 272 mg $CaCO_3$/L) for 96h, followed by 7 days of observation (Palawski *et al.*, 1983).

Exposure of tilapia *(Tilapia mossambica)* to methyl parathion at a concentration of 0.09 mg/L for 48 h resulted in a decrease in various anions and cations in tissues (Rao *et al.*, 1983b), and in inhibition of acetylcholinesterase (20-60%) and ATPase (10-14%) activities. The activities of aspartate and alanine amino-transferase in muscle, gill, liver, and brain increased by 12-31% and 9-31%, respectively (Rao and Rao, 1984a; 1984b). Concentrations of carbohydrate and glycogen decreased in the tissues (Rao and Rao, 1983a). Levels of soluble protein and the activity of glucose-6-phosphate dehydrogenase, a key enzyme of the hexose monophosphate shunt, in muscle, gill, and liver, were increased, and changes in carbohydrate metabolism were also observed in the freshwater fish *Clarias batrachus* (Rao and Rao, 1987).

Materials and Methods

3. MATERIALS AND METHODS

3.1. Animals

Rohu (*Labeo rohita*) weighing about 75 ± 6g and mean body length 23 ± 5 cm were collected from a Thiruvankulam fish farm near Ernakulam Kerala, India. The fish were brought to the laboratory and acclimatized for more than 15 days in plastic tank before starting the experiment. The water had a pH of 6.74 ± 0.4 and temperature 32 ± 2^0C. The fish tanks were well aerated, and the physical and chemical parameters were kept nearly constant.

3.2. Chemicals

Methylparathion-50% (O,O-dimethyl-O-4-nitrophenyl-Phosphorothioate-Bayer, Germany) a synthetic organophosphrous insecticide was obtained from market in Cochin. All other experimental chemicals were purchased from Sigma (USA), Merck (Germany) and SRL (India).

3.3. Range finding test

The range finding bioassay was conducted following APHA-AWWA-WPCF, (1975) and Reish and Oshida, (1987) with fish exposed to a range of sequential concentrations (0.002, 0.02, 0.2, 2, 20 mg/L) of methyl parathion. Eight fish were released into fish tank containing 50L of water and sequential concentrations of methylparathion. 20% water level was replaced on daily basis during the experimental period. The pesticide loss during this procedure was compensated by adding it in to the water. Mortalities were recorded (24, 48, 72 and 96h) and dead fish were removed immediately.

3.4. Static Bioassays

To determine the lethal concentration (LC_{50}) of the pesticide, eight fish of approximately equal size (75 ± 6g) were released into different fish tank, containing different concentrations (1.8, 3.6, 5.4, 7.2, 9.0, 10.8, 12.6, 14.4, and 16.2 mg/L) of methylparathion. Lethal concentration was determined as per the methods of Reish and Oshida, (1987). Control fishes were maintained separately and mortality was recorded at 24, 48, 72 and 96h. The dead fishes were removed immediately and kept frozen (-20°C) pending analysis.

3.5. Lethal and sub lethal exposures

Six concentrations (1.8, 3.6, 5.4, 7.2, 9.0 and 10.2 mg/L) were selected for lethal (96h) exposures. One group was maintained as control in a tank containing methylparathion free water. Fishes were fed with commercial fish feed, and the tanks were kept well aerated. 20 liters of the medium was replaced every 24 h. At end of exposure, fishes were collected, kept frozen pending analysis. For sublethal studies, 8 numbers of fishes were exposed to the sublethal concentrations. The sublethal concentrations calculated based on the LC_{50} for range finding periods of 15, 30 and 45 days.

3.6. Biochemical assay

At the end of the experiments both lethal and sub lethal, fish were killed by decapitation and organs were analysed for detoxifying and disease diagnostic and/or marker enzymes, proteins and lipids. Liver tissues were dissected, washed in physiological saline (0.9% Nacl), and kept at –20°C until analysis. The tissues were homogenized for 5 min in ice-cold 0.1M Tris-HCl buffer

solution pH 7.2 (1:5 w/v) using Polytron homogenizer (Polytron Model PT3000, Kinematica-Switzerland) and centrifuged (Remi-India) at 8000rpm for 30min. Supernatant were used to determination enzymes.

3.7. Lipid peroxidation and detoxifying enzymes

3.7.1. Lipid peroxides

Lipid peroxide (LPO) content in liver was determined by thiobarbituric acid reaction (Ohkawa *et al.*, 1979). To 0.2 ml of homogenized sample, 1.5 ml of 20% acetic acid, 0.2 ml of 8% SDS and 1.5 ml of 20% TBA were added. The mixture was made up to 5.0ml with distilled water and heated in a boiling water bath for one hour. After cooling, the mixture was centrifuged at 3000 rpm for 10 min. Standards (Tetraethoxy propane-TEP) and blank were treated similarly. The pink colour developed was measured at 532nm in a spectrophotometer (Spectronic 20 Genesys-USA). The lipidperoxide content was expressed as nmol of malonaldehyde formed per mg of protein.

3.7.2. Superoxide dismutase (EC 1.15.1.1)

The superoxide dismutase (SOD) was assayed in liver according to the method of Misra and Fridovich (1972) based on the oxidation of epinephrine-adrenochrome transition by the enzyme. To 0.1ml of the sample in a cuvette, 1.4ml of Carbonate-bicarbonate buffer, (0.1M-pH 10.2) and 0.5ml of 3mM epinephrine was added, mixed well and immediately read the change in optical density at 480nm for 2min in a UV spectrophotometer (Shimadzu-Japan). One unit of SOD was defined as the amount of protein needed to decrease the absorbance to 50% inhibition of epinephrine auto oxidation.

3.7.3. Assay of catalase (EC 1.11.1.6)

Catalase (CAT) in liver was assayed according to the method of Takahara *et al.*, (1960). To 2.4 ml of 50mM phosphate buffer, 0.1 ml of the enzyme solution was added and the reaction was started by the addition of 1.0 ml of 30mM H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30sec intervals for 2min in UV spectrophotometer (Shimadzu-Japan). The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as nmol of H_2O_2 decomposed min^{-1}. mg $protein^{-1}$

3.7.4. Glutathione peroxidase (EC 1.11.1.9)

Glutathione peroxidase (GP_x) activity in liver was determined by the non-enzymatic method (Pagila *et al.*, 1967). Homogenized sample (0.2 ml) was added to a mixture of 0.2 ml 0.4 M phosphate buffer, (pH 7.0), 0.2 ml of 0.4mM EDTA, 0.1 ml of 10mM sodium azide, mixed well and 0.2 ml glutathione (61.4mg Glutathione in 100 ml). The reaction mixture was thoroughly mixed and 0.2 ml of GSH, 0.1ml of 30% hydrogen peroxide and 0.1ml NADPH were added. The content of the test tube incubated in a water bath at 37°C for 10min. At the end of incubation period, 0.5ml of 10% TCA was added and centrifuged at 10,000rpm for 5 min. To 1.0 ml of the supernatant, 2ml Tris buffer (0.4M, pH 8.9) and 0.1 ml DTNB (99mg in 25ml methanol) were added. The absorbance was read at 412nm in a spectrophotometer (Spectronic 20 Genesys-USA). The enzyme activity was expressed as nmole of glutathione oxidized min^{-1} mg $protein^{-1}$.

3.7.5. Total reduced glutathione (E.C 1.6.4.2)

The total reduced glutathione (GSH) was determined in liver by the method of Ellman (1959). The method is based on the reaction of reduced glutathione with 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) to give a compound that has absorbance at 412 nm. To 0.5 ml of homogenized liver sample, 0.1ml of 5 % TCA was added and made the volume up to 1.0 ml using distilled water. The contents were mixed well for complete precipitation of proteins and centrifuged at 4000 rpm for 15min. To 0.5ml of clear supernatant, 2.5ml of 0.2M-phosphate buffer and 50µl of 0.6mM DTNB were added and the absorbance was read at 412nm in spectrophometer (Spectronic 20 Genesys-USA), against a blank containing TCA instead of sample. Series of standards were treated in a similar way to determine the reduced glutathione content. The amount of glutathione was expressed as µmol. g wet tissue $^{-1}$

3.7.6. Glutathione S-transferase (EC 2.5.1.18)

Glutathione S-tranferase (GST) activity was determined in liver by the method of Habig *et al.*, (1974). To a reaction mixture containing 1.0 ml of Phosphate buffer (0.3 M - pH 6.5), 0.1 ml of 30mM CDNB, 0.1 ml of tissue homogenate and 1.3 ml of distilled water were added and pre-incubated at 37°C for 5min. 0.1 ml of 30mM GSH was added and the change in the absorbance was measured at 340nm for 3min at 30sec intervals in UV spectrophotometer (Shimadzu-Japan). The enzyme activity was expressed as µmol of chloro-2,4-dinitrobenzyne conjugate formed min^{-1}. mg of protein^{-1}.

3.8. Acetylcholinesterase activity (EC 3.1.1.7)

Acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and propionylcholine (PChE) were assayed by the method of Ellman (1961), taking actylthiocholine iodide, S-butyrylthiocholine, propionylcholine as substrates. To 3ml of sodium phosphate buffer (0.1mM, pH-8), 50 µl of the enzyme was added and incubated at room temperature for 5min. To this mixture 10µl of 10mM DTNB followed by 20 µl acetylthiocholine iodide (75mM) as substrate was added. The increase in absorbance was recorded at 412 nm on a UV spectrophotometer (Shimadzu-Japan) against blank. Butyrylcholinesterase (150mM) and propionylcholinesterase (150mM) were analysed by the same procedure, but using the appropriate substrate. The enzyme activity was expressed as nmol mg protein^{-1} min^{-1}.

3.9. Marker and/or disease diagnostic enzymes.

3.9.1. Alanine aminotransferase (EC 2.6.1.2)

The activity of alanine aminotransferase (ALT) was determined by the method of Mohun and Cook (1957). To 1.0 ml of the buffered substrate (0.1M phosphate buffer, pH 7.4, 0.2M DL-alanine, 2.0mM 2-oxoglutarate, 1.5g dipotassium hydrogen phosphate, 0.2g potassium dihydrogen phosphate, 30mg 2-oxoglutaric acid and 1.78g DL-alanine were dissolved in 100 ml distilled water), in a test tube and 0.1 ml sample was added and incubated at 37°C for 30 min. The reaction was arrested by adding 1.0 ml of 200 mM DNPH and left aside for 20 min at room temperature. 10 ml of 0.4N NaOH was added and read at 540nm in a spectrophotometer (Spectronic 20

Genesys-USA) against the reagent blank. The enzyme activity was expressed as µmol pyruate librated h^{-1} L^{-1}.

3.9.2. Aspartate aminotransferase (EC 2.6.1.1)

The activity of aspartate aminotransferase (AST) was assayed by the method of Mohun and Cook (1957). To 1.0 ml of the buffered substrate (300mg of L-aspartic acid and 50mg of α-ketoglutaric acid dissolved in 20-30 ml of the phosphate buffer and 10% sodium hydroxide added to bring the pH to 7.5 and was made up to 100 ml with phosphate buffer) in a test tube, 0.1 ml of the sample was added and incubated for one hour at 37°C. Then 1.0 ml of 200 mM DNPH was added and left for 20 min. 10 ml of 0.4N NaOH was added and the absorbance was read at 540nm in a spectrophometer (Spectronic 20 Genesys-USA) after 10 min. The blank and standards were also treated similarly. The enzyme activity was expressed as µmol pyruate librated h^{-1} L^{-1}.

3.9.3. Lactate dehydrogenase (EC 1.1.1.27)

The lactate dehydrogenase (LDH) activity was assayed according to the method of King (1965a). The amount of pyruvate formed in the forward reaction was measured colorimetrically. To 1.0 ml of the buffered substrate (2.76g of lithium lactate was dissolved in 125 ml of glycine buffer containing 75 ml of 0.1 N sodium hydroxide to adjust the pH 10), 0.1 ml of the sample was added and the tubes were incubated at 37°C for 15 min. After adding 0.2 ml of 5.0mg NAD solution, the incubation was continued for 30 min and then 1.0 ml of 200mM DNPH reagent was added, and the tubes were incubated further 15 min. Then 7.0 ml of 0.4N NaOH was added and the colour

developed was read at 540nm in a spectrophometer (Spectronic 20 Genesys-USA) against the reagent blank. Suitable aliquots of the standards were also treated in the same procedure. The enzyme activity was expressed as µmol pyruate librated h^{-1} L^{-1}

3.9.4. Alkaline phosphatase (EC 3.1.3.1)

Alkaline phosphatase (ALP) was assayed by the method of King (1965b) using disodium phenyl phosphate as the substrate. The incubation mixture contained the following components in a final volume of 2.8-ml. 1.5 ml of carbonate- bicarbonate buffer (pH 10.0), 1.0 ml of substrate (0.01 M disodium phenyl phosphate) and 0.1 ml of 0.1M magnesium chloride and 0.2ml of enzyme. The reaction mixture was incubated at 37^0C for 15 minutes. The reaction was terminated by the addition of 1.0 ml of 1:2 Folin's phenol reagent and tubes were centrifuged. Controls without enzyme were also incubated and the enzyme source was added after the addition of Folin's phenol reagent. 1.0 ml of 15% sodium carbonate solution was added and incubated for a further 10 minutes at 37^0C and read at 640 nm in a spectrophotometer (Spectronic 20 Genesys-USA) against a blank. The standards were also treated as for samples. The activity of the enzyme is expressed as µmol phenol librated h^{-1} L^{-1}

3.9.5. Assay of acid phosphatase (EC 3.1.3.2)

Acid phosphatase (ACP) was assayed by the method of King (1965b) using disodium phenyl phosphate as the substrate. The incubation mixture contained the following components in a final volume of 3.0 ml - 1.5 ml of 0.1 M citrate buffer (pH 4.9), 1.0 ml of substrate (0.01 M Disodium phenyl

phosphate), 0.3 ml of distilled water 0.2 ml of enzyme solution. The reaction mixture was incubated at 37^0 C for 15 minutes. The reaction was terminated by the addition of 1.0 ml of Folin's phenol reagent. If turbidity appeared, the tubes were centrifuged. Controls without enzyme sources were also incubated and the enzyme source was added after the addition of Folin's phenol reagent. 1.0 ml of 15% sodium carbonate solution was added and incubated for a further 10 minutes at 37^0 C. The blue colour developed was read at 640 nm in a spectrophotometer (Spectronic 20 Genesys-USA) against a blank. Blank and standards were done in the same way. The activity of the enzyme is expressed as µmol phenol librated $h^{-1} L^{-1}$.

3.10. Protein

3.10.1. Extraction of muscle protein (Sarcoplasmic and myofibrilar) fractions

Sarcoplasmic (SP) and myofibrilar (MP) proteins were analysed by the methods of Sankar *et al.*, (2001) and King and Poulter, (1985) respectively. 2g of mince muscle was homogenized with 0.02M sodium bicarbonate buffer, (pH 7.25 buffer), maintaining a meat buffer ratio of 1:5 in a homegeniser (Polytron Model PT3000, Kinematica, Switzerland) at 10000 rpm for 1 minute. The homogenate was centrifuged using a refrigerated centrifuge (REMI R24, India) at 10000 rpm for 15 min maintaining a temperature of 0°C. Supernatant was transferred into test tube and residue was re-extracted and centrifuged as above and the pooled supernatant was taken as sarcoplasmic protein. The residue as extracted with cold 5% NaCl containing 0.02M $NaHCO_3$ (as above) twice and the supernatant was taken as myofibrilar protein.

3. 10.2. Estimation of protein

The protein content in the sample was estimated by the method of Lowry *et al.*, (1951). Sample 0.1ml was made up to 1.0 ml with distilled water. Exactly 4.5 ml of alkaline copper reagent was added to all the tubes and left at room temperature for 10 minutes after which 0.5 ml of Folin's phenol reagent (1:3) was added. The blue colour developed was read after 20 minutes at 640 nm in a spectrophotometer (Spectronic 20 Genesys-USA) against the reagent blank. Bovine serum albumin (BSA) was used as standared 20-100µg, protein concentration calculated by regression and the protein concentrations were expressed as mg g^{-1}.

3.10.3. Electrophoresis separation of proteins - Sodium Dodecyl sulphate polyacrylamide gel electrophoresis-SDS PAGE

Sarcoplasmic and myofibrilar proteins were separated by SDS-PAGE as described by Laemmli (1970) using Mini-PROTEAN II electrophoresis system (Biorad, USA). The proteins were separated using 7.5% gel concentration with staking gel (4%). In the presence of 10% SDS and 2-mercaptoethanol, proteins dissociate into sub units and bind large quantities of the detergent which mask the charge of the proteins giving a constant charge to mass ratio, so that the proteins move according to their molecular weight in an electric field.

After the run (200V, current) in 0.38M Tris-Glycine-SDS buffers, the gel were stained with 0.1% coomassie brilliant blue R250 in methanol, distilled water and acetic acid in ratio 4:5:1 respectively for 30 min. They were distained with 7% acetic acid. The developed gels were documented using Gel

documentation system (Biorad-USA) and the densitometry analysis was made.

3.11. Lipids

3. 11.1. Extraction of total lipids

The lipid content was extracted by Folch *et al.*, (1957). The weighed organs (meat, liver and brain) were subjected to lipid extraction (1:15 w/v) using chloroform-methanol mixture (2:1). The extraction was repeated twice with fresh solvent. The lipid extracts were transferred to a separating funnel and added 20% water. The separated lower layer was filtered, through sodium sulphate and concentrated by flash evaporator and stored at -23°C until analysis.

3.11.2. Lipid class separation

Cholesterol, cholesterol ester, triglycerides and phospholipids were determined by the method of Ogasawara *et al.*, (2002), by TLC-FID method (Iatroscan MK-6s, Japan). The extracted lipid sample was made up with 1ml of the chloroform and 1μl of made up solution was spotted on the chromarods with Drummond Micro dispenser. The spotted chromarods were developed in chloroform- methanol-water-25% ammonia (47:20:2.5:0.28) at 20°C up to 7cm. Then after drying at 110°C for 3min, the chromarods were developed in hexane-diethylether (60:10) up to 10cm in the second stage of development and dried at 110°C for 3min and finally the rods were scanned by Iatroscan-MK-6s (scan speed 40s/rod, 160ml/min hydrogen flow-rate and 1500ml/min airflow rate).

3.12. Histopathological studies

The slices of the gills, brain and liver were fixed in 10% neutral buffered formaldehyde. Fixing prevent autolysis and putrification of tissues. Then they were dipped in different concentration of alcohol in ascending order and finally in absolute alcohol (10 min each) for removing water. They were then kept in methyl benzoate until it sank and dipped in benzene for removing alcohol. The tissues were then infiltered with molten paraffin (60-70 °C) for 1 h and 15 min. A boat was made filled with molten paraffin and the tissues were placed in it. The paraffin was then cooled until it hardened, enclosing the tissue.

Using a rotary microtome, section of 4 to 5μ paraffin infiltered tissues were made. The tissues were de-paraffinised with xylene and treated with 100%, 90% and 70% alcohol (10 min each) for removing undesirable pigment and other materials. The sections were then stained with haematoxylin and counter stained with eosin and dehydrated with 70%, 90% and 100% alcohol for 10 min each. The sections were mounted using dibutylpthalate in xylene and examined under microscope.

3.13. Statistical analysis

Lethal concentration (LC50) was calculated on the basis of initial treatment mortality data. The percentage mortality data were converted to probit values and toxic concentrations were log transformed (Reish and Oshida, 1987). Weighed linear regression was performed on the transformed data to calculate LC50 values. Mean cumulative mortalities were calculated across the treatment duration for each of the three trails and one-way ANOVA was employed followed by Duncan's new multi-range test to calculate the significant difference between control and experimental mean (Daniel, 1987).

Results and Discussion

4. RESULTS AND DISCUSSION

Pesticides are chemicals that are used to destroy pests to protect the desired products. But most often they enter the life of other organisms sharing the same ecosystem, thus becoming hazardous chemicals. Some of the pesticides are used directly in aquaculture to control ectoparasites and insects in nursery and grow-out systems. Although non-persistent pesticides are most often used for the purpose, sometimes more persistent pesticides are also used for the purpose. The toxicity effect is often related to the size of the fish and therefore forms an important part of the study.

4.1. Bioassay – Study of lethal concentration

4.1.1. Range finding tests

The range finding test carried out for concentration of methyl parathion between 0.002 mg/L to 20 mg/L for a period of 96h showed no mortality up to concentration of 2 mg/L while at 20 mg/L methyl parathion concentration, 100% mortality was observed (Table 1). Therefore it was concluded that the lethal concentration 50 % (LC_{50}) of *Labeo rohita* is between 2 and 20 mg/L of methyl parathion.

4.1.2. Lethal concentration (LC_{50})

The lethal toxicity study was performed for the concentration of methyl parathion ranging from 1.8 to 16.2 mg/L. The study shows that practically there was no mortality noticed up to a concentration of 5.4 mg/L. The exposure of fish to 96h, at a concentration of 7.2 mg/L of methyl parathion showed 10% mortality, while at concentration of 16.2 mg/L 100% mortality

Table 1 : Test for range finding of *L. rohita* exposed to methylparathion

S.No	Concentration in mg/L	Percentage of mortality
1	0.002	0%
2	0.02	0%
3	0.2	0%
4	2	0%
5	20	100%

Table 2 : Test for lethal toxicity of *L. rohita* exposed to methylparathion

S.No	Conc. mg/L	log Con	No. of fish taken	Percentage of mortality			
				24h	48h	72h	96h
1	07.2	1.9741	8	0	0	0	10
2	09.0	2.1972	8	10	20	20	30
3	10.8	2.3795	8	20	30	40	40
4	12.6	2.5337	8	30	30	50	60
5	14.4	2.6672	8	30	60	100	100
6	16.2	2.7850	8	40	100	100	100

24 h	48h	72h	96h
R=0.990	R=0.947	R=0.964	R=0.987
R Square=0.979	R Square=0.896	R Square=0.930	R Square=0.974
S.E of Est. 2.111E-02	S.E of Est. 4.730E-02	S.E of Est. 3.886E-02	S.E of Est. 2.390E-02
Y=0.861 + 7.064E-03X	Y=0.894 + 7.064E-03X	Y=0.881 + 3.163E-03X	Y=0.823 + 3.666E-03X

Table 3 : Lethal toxicity value of methylparathion in *L.rohita*

S.No	Exposure Time(h)	LC_{50} mg/L	95% confidential limit	
			Upper limit	Lower limit
1	24	15.5	26.74	13.30
2	48	12.3	13.91	11.03
3	72	11.4	12.65	10.57
4	96	10.2	11.48	08.83

was noticed in 48h (Table 2). The probit analysis shows that the lethal concentration for 50% mortality of the fishes at (LC_{50}) 24, 48, 72, and 96h were 15.5, 12.3, 11.4 and 10.2 mg/L respectively, for *Labeo rohita* of size 75 ± 6g (Table 3).

The toxicity of xenobiotic in living organisms depends up on the size of fish and temperature of exposure. The LC_{50} of the organophosphorus pesticide RPR-II was found to be 0.17 mg/L for *Oreochormis mossabicus* of size 5 ± 1g (Venkateswara Rao, 2006). The 96h LC_{50} of azinphosmethyl, parathion and carbaryl were 7.18, 6.46 and 13.86 mg/L respectively for goldfish (*Carrassius auratus*) of size 2 - 5g weight range (Ferrari *et al.*, 2004). For pyrethroid pesticide cypermethrin the 96h LC_{50} was reported to be 0.139 mg/L for rohu (*Labeo rohita*) of size 8.52 ± 2.54g. These results suggest the changes in LC50 of pesticide as a function of size of the fish.

4.1.3. Behavior of fish

The behavioral responses of tested fishes were observed daily. Normal behavior was observed for fish of control group through out the entire experimental period. The first observed abnormal behavioral changes were noticed immediately in fish exposed to 15.0 and 20.0 mg/L concentrations of methylparathion. Some of the observed behavioral changes include opercular movement, dullness, loss of equilibrium, stop of food intake, erratic and hysteric swimming, swimming at the water surface, circling movement, and gasping. Prior to death, the fish became less active or generally inactive, remained hanging vertically in the water or lay down on their sides at higher concentrations. The clinically observed toxic signs on animals exposed to

methylparathion especially at higher concentrations were darkening on the body surface, erosion or rotting of fins and tails, slimness, loss of fish scales and hemorrhagic patches on the body surface (Rao *et al.*, 1967; Anees, 1975).

4.2. Effect of lethal toxicity on lipid peroxidation and detoxifying enzyme systems in liver

4.2.1. Lipid peroxidation (LPO)

The lipid peroxidation subjects were assessed in terms of thiobarbituric acid reactive substances (TBARS) produced in response to the effect of pesticides. The specific level of lipid peroxidation (LPO) on methylparathion exposure during lethal toxicity exposure for 96h was higher than that of a control fish (Table 4). A direct consequence of failure of antioxidant system was the accumulation of ROS (reactive oxygen species) in the system, which led to higher rate of formation of lipid peroxides. This consequently resulted in tissues damage. Exposure to methylparathion gradually increased LPO and at a concentration of 10.2 mg/L (LC_{50}), the hepatic LPO level increased almost 5 times ($P<0.05$). There are reports indicating the generation of ROS in the presence of organic contaminants like organophosphorus pesticides in aquatic animals (Dorval *et al.*, 2005; Monserrat *et al.*, 2003).

The generation of $O_2^{\cdot-}$, $^{\cdot}OH$ radicals and H_2O_2 in methyl parathion toxicity resulted in increased lipid peroxidase. LPO has been reported as a major contributor to the loss of cell function under oxidative stress conditions (Storey, 1996; Hermes-Lima *et al.*, 1995). Lipid peroxidase disintegrates the biomembrane rich in poly unsaturated fatty acids (PUFA) which are susceptible to oxidation (Rady, 1993). In experimental animals it was

reported that the organophosphorus pesticides, carbmate and endrine increased lipid peroxidation in both liver and kidney by direct interaction with the cell membrane (Hazaarika *et al.*, 2003). Unsaturated fatty acids are more susceptible to the attack by hydroxyl free radicals, generating lipid peroxides. Peroxides of unsaturated fatty acids are more hydrophilic and consequently alter the structure of the membrane, thereby disturbing normal membrane function.

The increased lipid peroxidation in the present study suggested that the ROS induced oxidative damage can be one of the main toxic effects of methylparathion. It has been reported that LPO may be induced by a variety of environmental pollutants (Ploch *et al.*, 1999; Ahmad *et al.*, 2000; Wilhelm-Filho *et al.*, 2001; Oakes and Van der Kraak, 2003; Oakes *et al.*, 2004). LPO is considered a valuable indicator of oxidative damage of cellular components. The results suggested that the exposure to methylparathion enhanced ROS synthesis in the liver of *L. rohita* and that antioxidant defenses were not totally able to effectively scavenge them, thus leading to lipid peroxidation.

4.2.2. Superoxide Dismutase (SOD)

The superoxide dismutase (SOD) activity in the liver of *L. rohita* after methylparathion exposure was higher than that in the control (Table 4). There was a significant ($P<0.05$) increase in SOD activity with increase in the concentration of methylparathion, and a 7 fold increase was noticed at a methylparathion concentration of 10.2 mg/L compared to control. Increase in SOD compares well with increase in LPO and induction of SOD could occur during the increased production of superoxide anion radical. Superoxide,

peroxide, hydroxyl radical and other free radicals derived from oxygen are highly reactive and therefore threatening to the integrity of essential bimolecules such as DNA and RNA, enzymes and other protein and phospholipids responsible for membrane integrity. The activities of the enzymes usually increase as an adaptive response to free radical overload. An increase in the SOD activity indicates an increase of $O_2^{\cdot -}$ production and similar increase in SOD isomers in the liver of *Leuciscus cephalus* were reported as a result of pollution (Lenartova *et al.*, 1997). Significantly ($P<0.05$) increased SOD activity was reported in pesticide poisoning in human subjects compared to control (Banerjee *et al.*, 1999). In contrast, the superoxide radicals by themselves or after their transformation to H_2O_2 cause an oxidation of the cysteine in the enzyme and decrease SOD activity (Dimitrova *et al.*, 1994). Decreases in SOD activity level were also reported in erythrocytes of *Cyprinus carpio* exposed to MS 222 (Bartowiak *et al.*, 1981).

4.2.3. Catalase (CAT)

The catalase (CAT) activity in the liver of *L. rohita* increased (61%) significantly ($P<0.05$) with the increasing concentration of methylparathion up to 5.4 mg/L followed by a decrease at higher concentrations (Table 4). The increase in catalase activity in liver after 96h appears to be due to the increased generation of ROS.

Catalase is an enzyme, which promotes the conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and diatomic oxygen. The formation of highly reactive oxygen species is a normal consequence of essential biochemical reactions including mitochondrial and

microsomal electron transport systems. At higher concentrations, however the cells are unable to increase the production of catalase probably due to cell damage and are limited by their inability to counter effect of methylparathion toxicity. There are also reports indicating the increase in SOD and catalase activities at the combined effect of zinc and lead in *C. carpio* (Dimitrova *et al.*, 1994).

The increased SOD and CAT levels induced by methylparathion in *L. rohita* indicate an elevated antioxidant status attempting to neutralize the impact of the ROS. This result supported the statement (Alves *et al.*, 2002), that the exposure to pesticides elicit pro-oxidant conditions that trigger adaptive responses such as increase in the activity of the antioxidant enzymes.

SOD is responsible for the dismutation of the superoxide anion radical to H_2O_2, which is detoxified by both CAT and GPx activity. Due to the inhibitory effects on oxyradical formation, the SOD-CAT system provides the first defense line against oxygen toxicity (Pandey *et al.*, 2003) and usually used as a biomarker indicating ROS production (Oost *et al.*, 2003; Regoli *et al.*, 2003). Increase in these enzyme activities are probably a response towards increased ROS generation in pesticide toxicity (John *et al.*, 2001). However, there are studies indicating the changes in enzymes in fish after exposure to xenobiotics (Livingstone, 2001) or no change after exposure to pesticides (Oruc and Unce, 2000)

4.2.4. Glutathione peroxidase (GPx)

The Glutathione peroxidase (GPx) activity in the liver of *L. rohita* increased ($P<0.05$) after methylparathion exposure up to a concentration of 5.4 mg/L

followed by a decrease at higher concentration (Table 4). The GPx inhibition observed in the present study reflect a possible antioxidant defense failure which is also reflected in the increased LPO levels and decreased catalase activity leading to cell damage. The activity response is similar to that of catalase as both leads to the detoxification of product formed due to LPO action.

Glutathione peroxidase (GPx) is important in the prevention of cell damage by oxidants. GPx being an antioxidant enzyme removes precursors of free oxygen radicals and is necessary for the conversion of hydrogen peroxide to molecular oxygen and water. GPx reduces reactive oxygen species (ROS) and intervene in hydrogen peroxide detoxification, leading to GPx formation of their corresponding alcohols or water. GPx plays an important role against the LPO, since it is mainly involved in the removal of organic xenobiotics and, to a small extent, hydrogen peroxides. Thus, GPx is considered to play an important role in protecting membranes from damage due to LPO (Oost *et al.*, 2003). There are reports indicating increase in GPx activity on exposure to chemical contaminants (Almedia *et al.*, 2002; Sayeed *et al.*, 2003; Zhang *et al.*, 2004).

There are studies indicating a low GPx activity after exposure of the fish to the pesticides like folisuper (Monteriro, 2006), malathion (Yarsan *et al.*, 1999), lindane (Bainy *et al.*, 1993) and exposure to paper mill effluent (Fartima *et al.*, 2000). The decrease in GPx could be attributed to the negative feedback from excess of substrate or damage by oxidative modification (Tabatavaie and Floyd, 1994). A reduction in GPx activity may indicate that its antioxidant

capacity was surpassed by the amount of hydrogen peroxide, the products of lipidoxidation (Remacle *et al.*, 1992).

4.2.5. Total reduced glutathione (GSH)

The total reduced glutathione (GSH) activity increased ($P<0.05$) gradually with increase in the concentration of methylparthion compared to control (Table 4), and a 3.5 fold increase in glutathione activity was noticed at 10.2 mg/L. During a moderate oxidative stress, the glutathione levels increase as an adaptive mechanism by means of an increased synthesis; however, a severe oxidative stress suppresses glutathione levels due to the impairment of the adaptive mechanism (Zhang *et al.*, 2004). GSH depletion may reduce the cellular ability to scavenge free radicals which affects the general oxidative potential of the cell.

The GSH plays an important role in the detoxification of electrophiles and prevention of cellular oxidative stress (Hasspieler *et al.*, 1994; Sies, 1999; Reed and Beatty, 1980), and hence has a protective role against free radical mediated or peroxidative damage (DeLucia *et al.*, 1972).

Considerable decline in GSH was noted in the tissue during exposure to methylparathion which could be due to an increased utilization of GSH, which get converted into oxidized glutathione and inefficient replenishment of GSH (Monteriio, 2006). In this study there was no decrease in GSH up to the final concentration of 10.2mg/L indicating the effectiveness of the cell to replenish GSH at this high level of pesticide concentration. According to Elia *et al.*, (2003). GSH depletion may reduce the ability to scavenge free radicals raising the general oxidative potential in the cell; this seems to enhance the risk of

oxidative cells in reducing its protective ability. Possible increased peroxide over load could be induced by the high SOD activity (Monterrio, 2006)

A reduction of GSH levels led to a significant induction of GST activity during most of the exposure period. GST-mediated conjunction may be an important mechanism for detoxifying peroxidised lipid break down products, which have a number of adverse biological effects when present in high amounts. Induced GST activity indicates the role of this enzyme in protection against the toxicity of xenobiotic-induced lipid peroxidation (Leaver and George, 1998). Glutathione depletion is considered a biomarker of environmental stress as it was observed in fish stressed either by chemical or natural pollutants (Vaglio and Landriscina, 1999; Pena-Llopis *et al.*, 2002).

4.2.6. Glutathione S-Transferase (GST)

The glutathione S-transferase (GST) activity in the liver of *L. rohita* after methylparathion exposure increased ($P<0.05$) with increase in concentration of methylparathion (Table 4). The activity increased by two fold level on exposure to methylparathion at 10.2 mg/L concentration. Increased GST activity may indicate the development of a defensive mechanism to counteract the effects of methylparathion and may reflect the possibility of a more efficient protection against pesticide toxicity.

Glutathione S-transferase (GST) is a group of multifunctional enzyme involved in biotransformation and detoxification of xenobiotics (Smith and Litwack, 1980; Jokanovic, 2001). Highly reactive electrophilic components can be removed before they covalently bind to tissue nucleophilic compounds which would lead to toxic effects. It plays an important role in protecting tissue from

Table 4 : Effect of different methylparathion concentrations on the liver specific activity (SOD, CAT, GSH, GPx and GST) and level of LPO on L. *rohita* after 96h exposure.

S.No	Conc (mg/L)	LPO	SOD	CAT	GPx	GSH	GST
1	Control	0.69 ± 0.1^{a}	1.7 ± 0.4^{a}	7.9 ± 1.1^{a}	1.6 ± 0.2^{a}	1.5 ± 0.3^{a}	1909.5 ± 583^{a}
2	1.8	0.87 ± 0.2^{a}	2.6 ± 0.2^{a}	7.1 ± 0.8^{ab}	4.3 ± 1.2^{b}	2.9 ± 0.1^{b}	2310.3 ± 309^{a}
3	3.6	1.09 ± 0.3^{a}	4.2 ± 0.8^{b}	10.4 ± 1.1^{c}	7.2 ± 0.6^{c}	3.2 ± 0.6^{b}	2973.3 ± 118^{b}
4	5.4	1.54 ± 0.3^{ab}	3.8 ± 0.6^{b}	12.9 ± 0.3^{d}	11.3 ± 2.2^{d}	3.9 ± 0.6^{bc}	3129.2 ± 180^{bc}
5	7.2	1.98 ± 0.6^{b}	11.3 ± 0.6^{c}	7.2 ± 0.5^{ab}	4.1 ± 1.8^{b}	4.5 ± 0.9^{cd}	3648.0 ± 198^{c}
6	9.0	2.34 ± 0.3^{b}	11.8 ± 1.1^{c}	6.2 ± 0.3^{b}	3.8 ± 0.3^{b}	5.1 ± 0.8^{d}	4660.7 ± 249^{d}
7	10.2	3.53 ± 0.9^{c}	12.4 ± 0.7^{c}	7.9 ± 0.2^{a}	3.6 ± 0.4^{b}	5.2 ± 0.3^{d}	4733.7 ± 292^{d}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significantly (P<0.05Duncan's multiple range test).

LPO - nmol of malonaldehyde formed (mg of protein) $^{-1}$

SOD - one unit of the activity is the amount of protein required to give 50% inhibition of epinephrine auto oxidation.

CAT - nmol of H_2O_2 decomposed min^{-1}(mg protein)$^{-1}$

GPx - nmol of GSH oxidized min^{-1} (mg protein)$^{-1}$

GSH - µmol (g wet tissue) $^{-1}$

GST - µmol of chloro-2,4-dinitrobenzyne conjucated formed min^{-1} (mg of protein)$^{-1}$

oxidative stress (Fournier *et al.*, 1992; Banerjee *et al.*, 1999). The increased GST activity in liver observed in the present study after exposure to methylparathion suggests the increase in detoxification processes in *Labeo rohita.* GST has been reported as a biomarker for assessing the environmental impact of organic xenobiotics generating oxidative stress (Livingstone, 1998; Rodríguez-Ariza *et al.*, 1991). The GST was more active in hepatic tissue than in white muscle and gill, which indicates the effective role of liver in xenobiotic detoxification (Basha and Rani, 2003).

Cytosoloic - free radicals are either removed nonenzymatically or by antioxidant enzymes such as SOD and GPx, which oxidise GSH to reduced glutathione. Reduced glutathione is then oxidised back to GSH by glutathione reductase through oxidation of NADPH to $NADP^+$, which is recycled by the pentose phosphate pathway. Furthermore, glutathione S-transferase (GST) and catalase in conjunction with GSH acts in detoxification and elimination of the organophosphorus insecticide. The protective action of antioxidants would be probably due to an inhibition of free-radical-induced chain reaction, which results in the prevention of peroxidative deterioration of structural lipids in cell membranes. These results clearly indicate that an increase of LPO in all treatment groups indicate oxidative stress.

4.3. Effect of cholinesterase (AChE, BChE and PChE) activity brain

Acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and propionyl-cholinesterase (PChE) activities in brain tissues were gradually inhibited with increase in the concentration of methylparathion, after 96h exposure (Table 5). The orgnophosphorus pesticides act, in general, through inhibiting CNS,

by blocking the transmission of nerve impulse. AChE is the major neurotransmitter in the nervous system and was inhibited by 74% at a concentration 1.8 mg/L itself and 90% of the activity was inhibited at 5.4mg/L methylparathion concentration. Further increase in methylparathion concentration did not inhibited AChE activity significantly. The other neurotransmitters namely PChE and BChE were inhibited by 76 and 65% respectively by methylparathion at 1.8 mg/L concentration. The role of BChE is still unclear, but it is suggested that it scavenges ACh from tissue (Silver, 1974; Brestkin *et al.*, 1977).

Propionylcholine is a choline derivative, which can be hydrolysed to propionic acid and choline, a precursor of acetylcholine. Organophosphorus insecticides (OPs) produce toxicity by inhibiting the cholinesterase enzyme (ChE) in the nervous system of both vertebrates and invertebrates. Propionylcholine had moderately low activity in the brain tissue of rohu *(Labeo rohita)* when compared to AChE, i.e., about 50% of the activity decreased at a concentration of 5.4 mg/L of methylparathion and further increase in the pesticide concentration decreased the activity by only 4%.

The BChE, which is found to have lowest activity in the brain tissue of the fish (34 nmol mg protein^{-1} min^{-1}) decreased by 70% at 5.4mg/L of methylparathion. Further increase in the pesticide concentration decreased the activity by only 7%. The methylparathion and their active metabolites are electrophilic compounds, with moderate to may be high potency, for phosphorylating the serine hydroxyl group located in the active site of AChE. This phosphorylation occurs by the loss of the "leaving group" of the

Table 5 : Effect of different methylparathion concentrations on the brain specific activity (AChE, PChE and BChE- nmol mg protein^{-1} min^{-1}) of *L. rohita* after 96h exposure

S.No	Conc. (mg/L)	AChE	PChE	BChE
1	Control	147 ± 15^{a}	89.8 ± 11^{a}	34.4 ± 7.0^{a}
2	1.8	39.0 ± 6.0^{b}	21.5 ± 6.2^{b}	12.2 ± 3.0^{b}
3	3.6	21.9 ± 3.0^{bc}	19.5 ± 2.3^{b}	11.2 ± 0.1^{b}
4	5.4	14.7 ± 4.0^{c}	17.3 ± 3.6^{b}	10.1 ± 0.9^{b}
5	7.2	13.9 ± 4.0^{c}	15.5 ± 3.9^{b}	09.8 ± 1.3^{b}
6	9.0	12.9 ± 3.0^{c}	11.2 ± 1.4^{b}	07.2 ± 1.3^{b}
7	10.2	11.2 ± 0.7^{c}	10.3 ± 0.7^{b}	07.6 ± 1.5^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,) differ significantly (P<0.05Duncan's multiple range test).

organophosphorus compounds and the establishment of a covalent bond with AChE through the serine hydroxyl group. The resultant phosphorylated AChE is typically very stable and is slowly reactivated only by spontaneous hydrolysis of the phosphate ester. Weiss (1961) and Zinkl *et al.*, (1991) proposed that depression of 70-90% brain ChE activity occurs at the LC_{50}.

AChE activity measurement in fish has been used for monitoring the neurotoxicity of pesticides (Bretaud *et al.*, 2000). The inhibitory effect on AChE activity indicates that insecticides might interfere in vital processes like energy metabolism of nerve cells (Nath and Kumar, 1999).

Organophosphorus insecticides exert their action by inhibiting AChE which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate (Glynn, 2006; Sams *et al.*, 2000; Soreq and Zakut 1993). The inhibitory effects of OP insecticides are dependent on their binding capacity to the enzyme active site and by their rate of phosphorylation in relation to the behavior and age (Dutta *et al.*, 1955). OPs are converted *in vivo* to the corresponding active phosphate ester or oxon (P=O), which is a potent acetylcholinesterase inhibitor. Studies using animal livers have shown that cytochrome P-450 (CYP) enzymes mediate the oxidative desulphuration of the OP parathion to the active metabolite paraoxon (Neal, 1967).

4.4. Effect of disease diagnostic or marker enzymes activity in liver

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) are liver specific enzymes and they are more sensitive measure of

hepato toxicity and histopathologic changes (Balint *et al.*, 1997). These enzymes are used as biochemical markers of hepatic cell necrosis (Henderson *et al.*, 1983).

The alanine aminotransferase (ALT) activity in the liver of *L. rohita* after methylparathion exposure was observed to be higher than that of control (Table - 6). There was a significant ($P<0.05$) increase in ALT activity with an increasing concentration of methylparathion, and more than 2 fold increase was noticed at a pesticide concentration of 10.2 mg/L compared to control. The results indicate the damage to hepatic cells and histopathgical studies support the extent of damage by the result of methylparathion exposure (Fig 16-19).

The aspartate aminotransferase (AST) activity in the liver of *L. rohita* after methylparathion exposure was found to be higher when compared to control (Table 6). There was a significant ($P<0.05$) increase in AST activity with an increase in concentration and a three fold increase was noticed at concentration of 10.2 mg/L when compared to control. When an organ or body tissue is injured, increased levels of AST is released into the blood and greater the degree of tissue damage, the greater is the quantity of AST that is released.

The lactate dehydrogenase (LDH) activity in the liver of *L. rohita* after methylparathion exposure was higher when compared to control (Table 6). There was a significant ($P<0.05$) increase in LDH activity with an increase in the concentration of methylparathion, and a two-fold increase was noticed at 10.2 mg/L. Lactate dehydrogenase mediates the inter-conversion of lactate

and pyruvate, depending up on the availability of NAD. LDH is present in numerous tissues, cytoplasm, enzymes and marker of tissue damage and its increased level is reported in liver necrosis (Ramesh *et al.*, 1993; Lemaire *et al.*, 1991). Injured cells, organs and tissues often release LDH into the haemolymph, which raises the level of this enzyme, and greater the degree of tissue damage greater is the release of LDH. LDH is one of the metabolic requirements of tissue and involved in energy production. LDH activity indicates the switching over of anaerobic glycolysis to aerobic respiration. Also, it can be used as an indicator for cellular damage, clinical practice and cytotoxicity of toxic agents (Bagchi *et al.*, 1995).

Alkaline phosphatase (ALP) is a broad term associated with non-specific phosphomonoesterases with activity optima at alkaline pH. Alkaline phosphatases are enzymes involved in the process of mineralization of calcium carbonate in invertebrates. ALP is mainly localized at the cell membrane; any damage to hepatic cells may result in the alternation of ALP activity. The ALP activity in the liver of *L. rohita,* after the methylparathion exposure was higher when compared to control (Table 6). There was a significant ($P<0.05$) increase in ALP activity with an increase in the concentration of methylparathion, and a three fold increase was noticed indicating the action of methylparathion on cell membrane leading to cell lysis.

Similarly the acid phosphatase (ACP) activity in the liver of *L. rohita* after methylparathion exposure was found to be higher when compared to control (Table 6). There was a significant ($P<0.05$) increase in ACP activity with increase in the concentration of methylparathion. The result shows that

Table 6 : Effect of different methylparathion concentrations on the liver specific activity (ALT, AST, LDH, ALP and ACP) of *L. rohita* after 96h exposure.

S.No	Conc. (mg/L)	ALT	AST	LDH	ALP	ACP
1	Control	099.5 ± 4.7^{a}	240.6 ± 09^{a}	593.9 ± 10^{a}	110.9 ±10^{a}	209.3 ± 05^{a}
2	1.8	102.6 ± 11^{a}	267.6 ± 17^{b}	839.7 ± 14^{b}	227.3 ± 09^{b}	235.6 ± 19^{b}
3	3.6	124.8 ± 5.9^{b}	399.3 ± 17^{c}	862.8 ± 18^{b}	250.3 ± 15^{b}	242.7 ± 13^{bc}
4	5.4	107.5 ± 5.6^{a}	576.1 ± 26^{d}	927.7 ± 15^{c}	185.2 ± 09^{c}	251.5± 09^{bc}
5	7.2	127.5 ± 7.5^{b}	645.5 ± 03^{e}	1003 ± 14^{d}	240.1 ± 06^{b}	262.7 ± 16^{cd}
6	9.0	149.8 ± 6.9^{c}	657.5 ± 14^{e}	1105 ± 12^{e}	296.7 ± 18^{d}	272.7 ± 12de
7	10.2	227.4 ± 8.8^{d}	707.5 ± 07^{f}	1191 ± 27^{f}	357.7 ± 17^{e}	287.5 ± 03^{e}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d,e,f) differ significantly (P<0.05Duncan's multiple range test).

Units: ALT, AST, LDH – µmol pyruate librated $h^{-1}L^{-1}$; ALP, ACP – µmol phenol librated $h^{-1}L^{-1}$

methylparathion leads to the release of this enzyme into cytoplasm consequently leading to the autolytic breakdown of cellular organs. Acid phosphatase (ACP) is known to be localized in lysosomes, and surrounded by a lipoprotein membrane. Increase in ALT and AST indicate the tissue damage in liver, kidney and gill (Rajayasree and Neeraja, 1989, Oluah, 1999). The increase in the activities of these enzymes in the liver consequently affects the circulatory system and results in subsequent damage to the tissue. Ram and Singh (1988) found an elevation in ALP and ACP activity in the liver of carbofuran-treated *Channa punctatus*.

The results indicated that, in response to methylparathion intoxication the activities of ALT, AST, ACP and ALP increased in the liver, which led to damage and disruption of normal liver function (Shakoori *et al.*, 1994). There are reports indicating that increase in ALP could be a result of damage of liver cells and duct obstruction due to proliferation of its cells and/or related to the progressive liver necrosis (Tietz, 1976). Acute intoxication of deltamethrine also reported to increase ALT and AST in Nile tilapia. Similarly increase in these enzymes has been reported in common carp (Velisck *et al.*, 2006 and 2007), and an increase in AST level and decrease in ALT level in rainbow trout (Velisck *et al.*, 2006 and 2007).

4.5. Alteration of muscle proteins

Fish exposed to pesticide or other xenobiotics compensate any possible loss of protein by increasing protein synthesis. The sarcoplasmic proteins (SPP) comprising mainly tissue enzymes demonstrated a marginal dip, at a pesticide concentration of 1.8 mg/L, which gradually increased as the concentration of

pesticides increased ($P<0.05$). At a concentration of 9.0mg/L there was a 44% increase in the SPP (Table 7). It is well known that living organisms respond at cellular levels to unfavorable conditions such as heat or stress condition including exposure to xenobiotics by expressing specific set of stress proteins which are induced as a result of damage to the cells by the toxicant. The disappearance of electrophoretic band (Fig 6) at 3.6 mg/L level could be due to the shock experienced by the organelles at higher concentration and related damages. This clearly indicates the initial induction during the initial exposure followed by the increase in the synthesis of tissue enzymes to counteract the deleterious effect of pesticides as a part of the repair mechanism.

At lower concentrations of methylparathion there were some differences in protein fractions. At higher concentrations of above 5.4 mg/L of methylparathion, the densitometry analysis (Fig 6a) of sarcoplasmic protein (SPP) extracted from meat showed 10 distinct bands between molecular weight of the range 19 - 68 kDa. At methylparathion concentration of 3.6 mg/L, two high molecular weight fractions with approximate molecular weights of 58 and 68 kDa disappeared but again noticed at higher concentrations of methylparathion. Another significant point is the appearance of a protein band at Rf 0.808 (approximate molecular weight 26 kDa) at the same concentration (3.6 mg/L). The low molecular weight proteins, however, decreased with increase in concentrations of methylparathion. To overcome the effect of pesticide toxicity the cellular repair mechanism increased the production of detoxifying enzymes or marker enzymes, which might have contributed to the increase in the sarcoplasmic proteins at high methylparathion concentrations.

Table 7: Changes in sarcoplasmic and myofirbrilar (mg g^{-1}) protein profile in muscle of *L.rohita* after 96h exposure

S.No	Conc (mg/L)	Sarcoplasmic protein	Myofirbrilar protein
1	Control	12.9 ± 1.8^{ab}	24.2 ± 2.2^{a}
2	1.8	12.2 ± 3.3^{a}	37.5 ± 8.2^{ab}
3	3.6	14.5 ± 2.9^{ab}	30.4 ± 5.3^{ab}
4	5.4	16.7 ± 1.4^{ab}	43.5 ± 4.9^{bc}
5	7.2	11.9 ± 2.1^{a}	53.3 ± 13^{c}
6	9.0	18.9 ± 3.1^{b}	54.0 ± 8.9^{c}
7	10.2	18.6 ± 5.7^{b}	69.9 ± 7.7^{d}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significantly ($P<0.05$ Duncan's multiple range test).

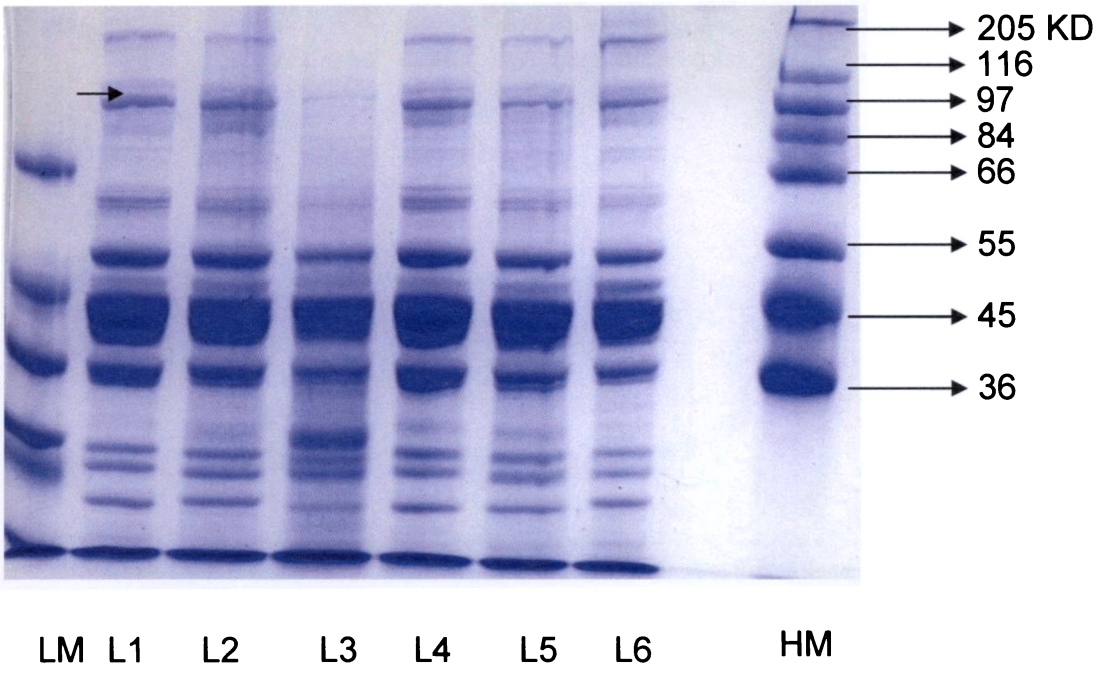
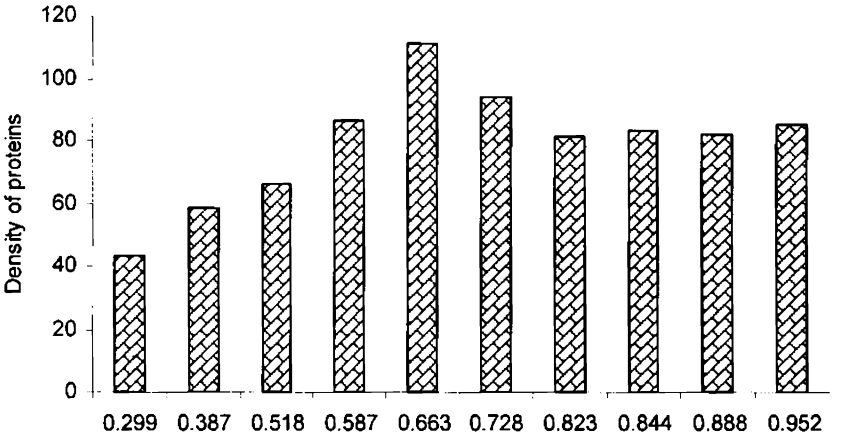
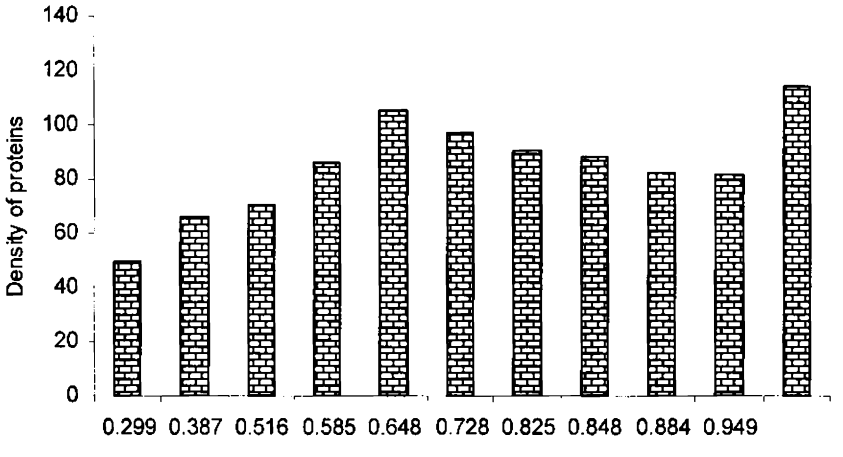


Fig 6: SDS-PAGE of sarco plasmic protein profile in muscle of *L. rohita* after 96hrs exposure (LM- Low molecular weight: L1 -Control, L2 -1.8 mg/L; L3 -3.6 mg/L; L4-5.4 mg/L; L5-7.4mg/L; L6-9.0 mg/ L. HM; high molecular weight).


120
100
80
60
40
20
0
Density of proteins
0.299 0.387 0.518 0.587 0.663 0.728 0.823 0.844 0.888 0.952

L1


140
120
100
80
60
40
20
0
Density of proteins
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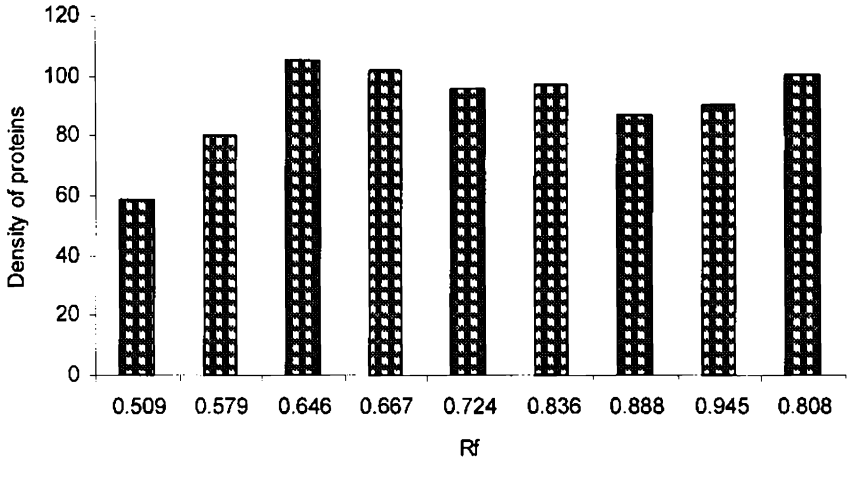
L2


120
100
80
60
40
20
0
Density of proteins
0.509 0.579 0.646 0.667 0.724 0.836 0.888 0.945 0.808
Rf

L3

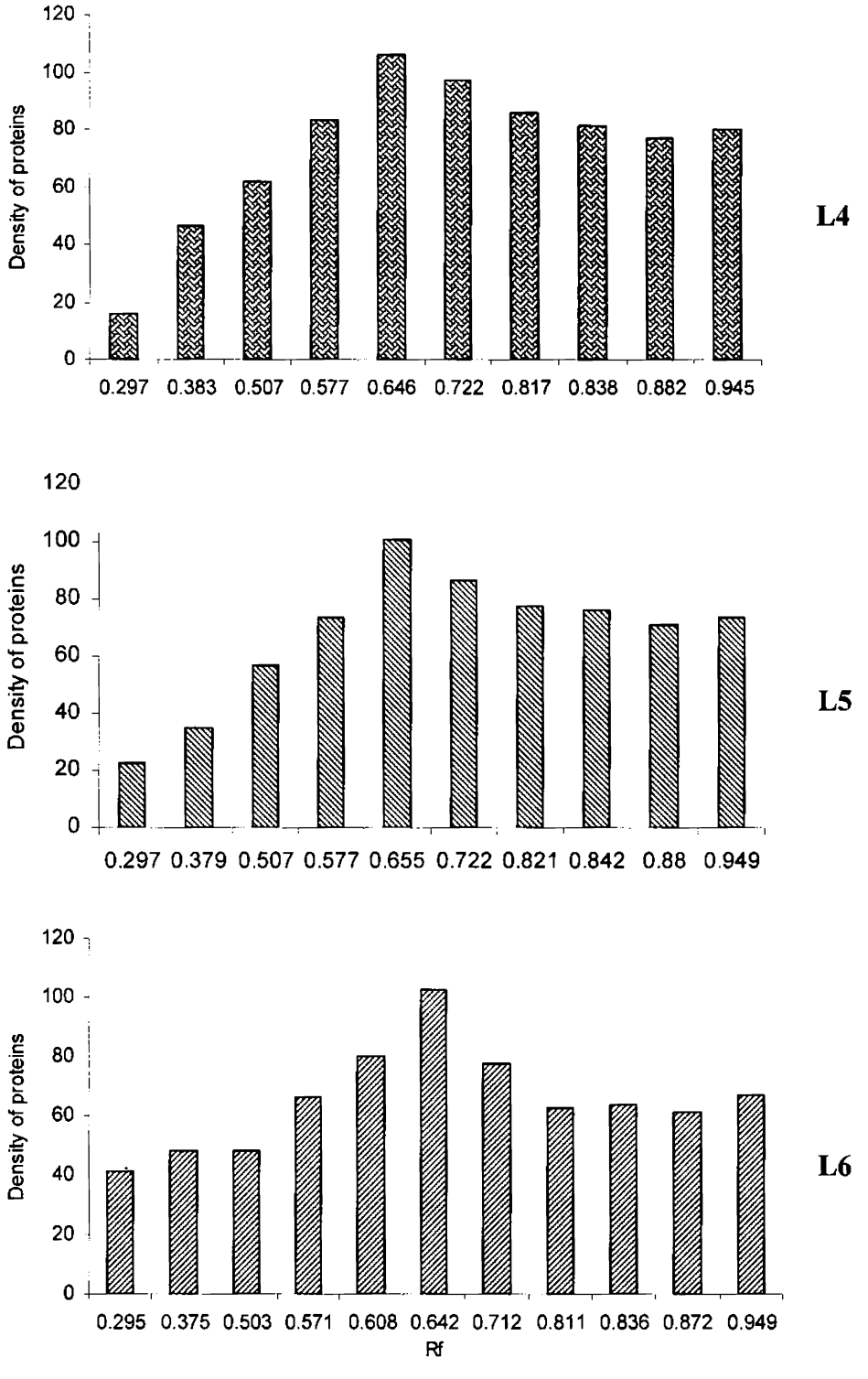


Fig -6a: Densitometry analysis of sarcoplasmic protein profile in muscle of *L. rohita* after 96hrs exposure of methyl parathion. (L 1 -Control, L2 -1.8 mg/L; L3 -3.6 mg/L; L4-5.4 mg/L; L5-7.4mg/L; L6-9.0 mg/ L.

The myofibrillar protein (MFP) also behaved in the same way as that of SPP to the exposure to methylparathion. There was a gradual and steady increase in the MFP as the methylparathion concentration was increased. About three fold increase in MFP was noticed at methylparathion concentration of 10.2 mg/L (Table 7). There was a possible increase in protein synthesis at ribosomal level leading to increased synthesis of contractile proteins. The results suggested that there was an enhanced synthesis of protein, possibly to repair damaged cell organelles, to serve as a compensatory pool to restore enzymes lost due to tissue necrosis and to meet the increased demand to detoxify the pesticides (Gill *et al.*, 1990; Orue and uner, 1999).

SDS-PAGE of myofibrillar proteins, showed two distinct bands probably of myosin (205 kDa) and actin (45 kDa) and eight faint bands (Fig 7). As the concentration of methylparathion increased the intensity of almost all the bands increased particularly the intensity of bands of approximate molecular weight 95kDa, 45 kDa, 42 kDa, 38 kDa and 32 kDa. Similar findings have been noted in the fresh water prawn *Macrobrachium kistensis* on exposure to naphthalene and pesticides (Jaiswal *et al.*, 1991; Nagabhushanam *et al.*, 1987); in the fresh water field crab *Barytelphusa guerini* and the fish Anabas *Scandens notopterus* exposed to endosulfan (Reddy *et al.*, 1991; Yasmeen *et al.*, 1991), and in the fish *Pundulus heteroclitus* and *Notopterus notopterus* exposed to various forms of stress (Umminger, 1970; Narasimhan *et al.*, 1971).

The electrophoretic pattern of the MFP of fishes exposed to methylparathion is given in Figure 7. The MFP profile showed 9 distinct bands between the

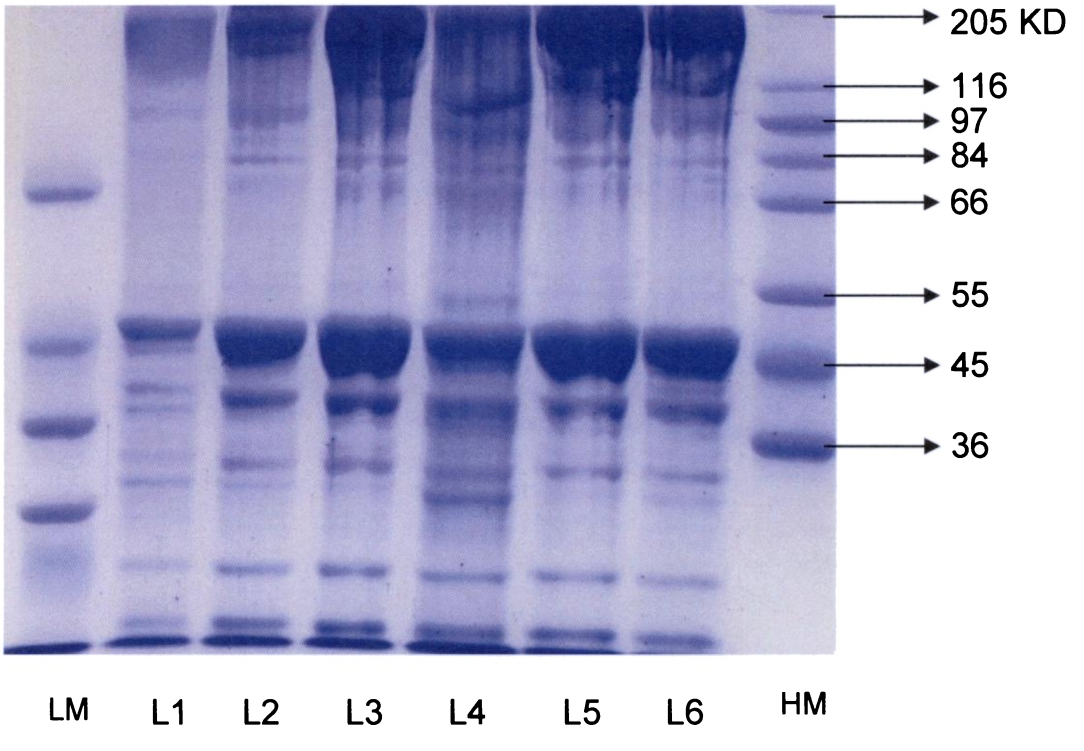
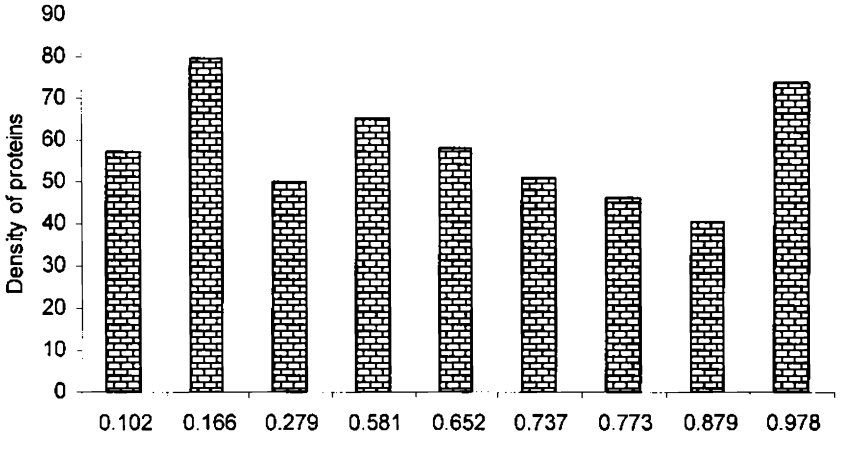


Fig 7: SDS-PAGE of myofibrillar protein profile in muscle of *L. rohita* after 96hrs exposure (LM- Low molecular weight; HM-High molecular weight; L1-Control, L2 -1.8 mg/L;L3 -3.6 mg/L; L4-5.4 mg/L; L5-7.4mg/L; L6-9.0 mg/ L).


Density of proteins
90
80
70
60
50
40
30
20
10
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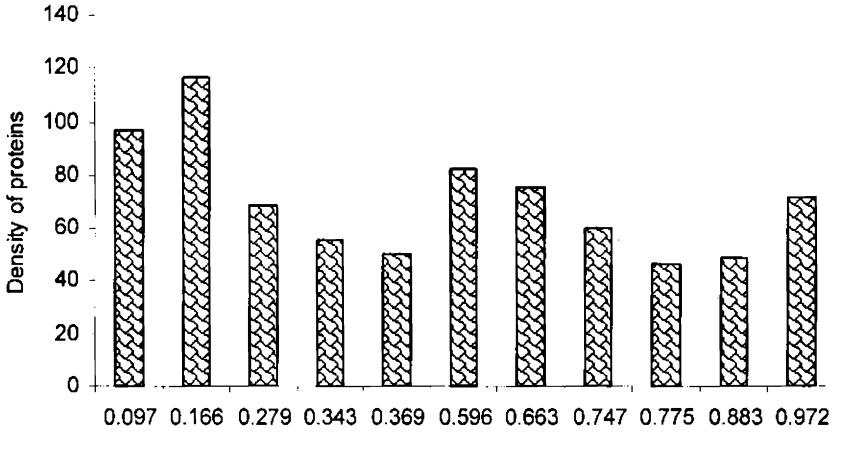
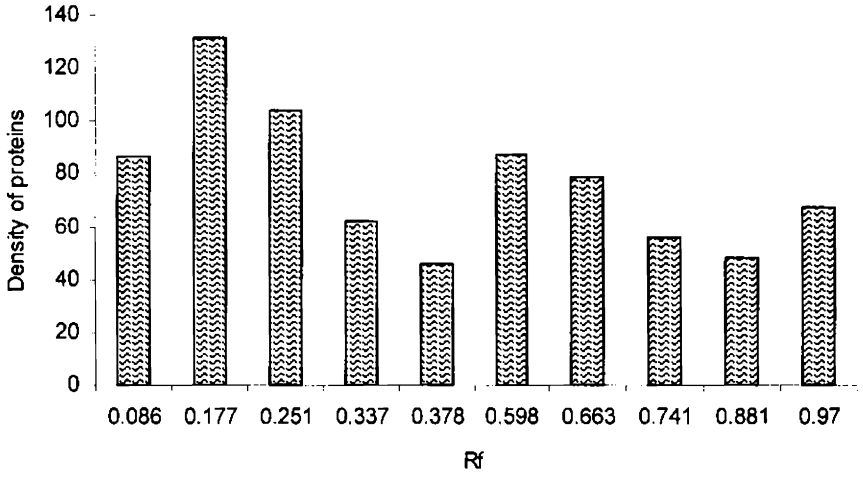
0.166
0.279
0.581
0.652
0.737
0.773
0.879
0.978

L1


Density of proteins
140
120
100
80
60
40
20
0
0.097
0.166
0.279
0.343
0.369
0.596
0.663
0.747
0.775
0.883
0.972

L2


Density of proteins
140
120
100
80
60
40
20
0
0.086
0.177
0.251
0.337
0.378
0.598
0.663
0.741
0.881
0.97
Rf

L3

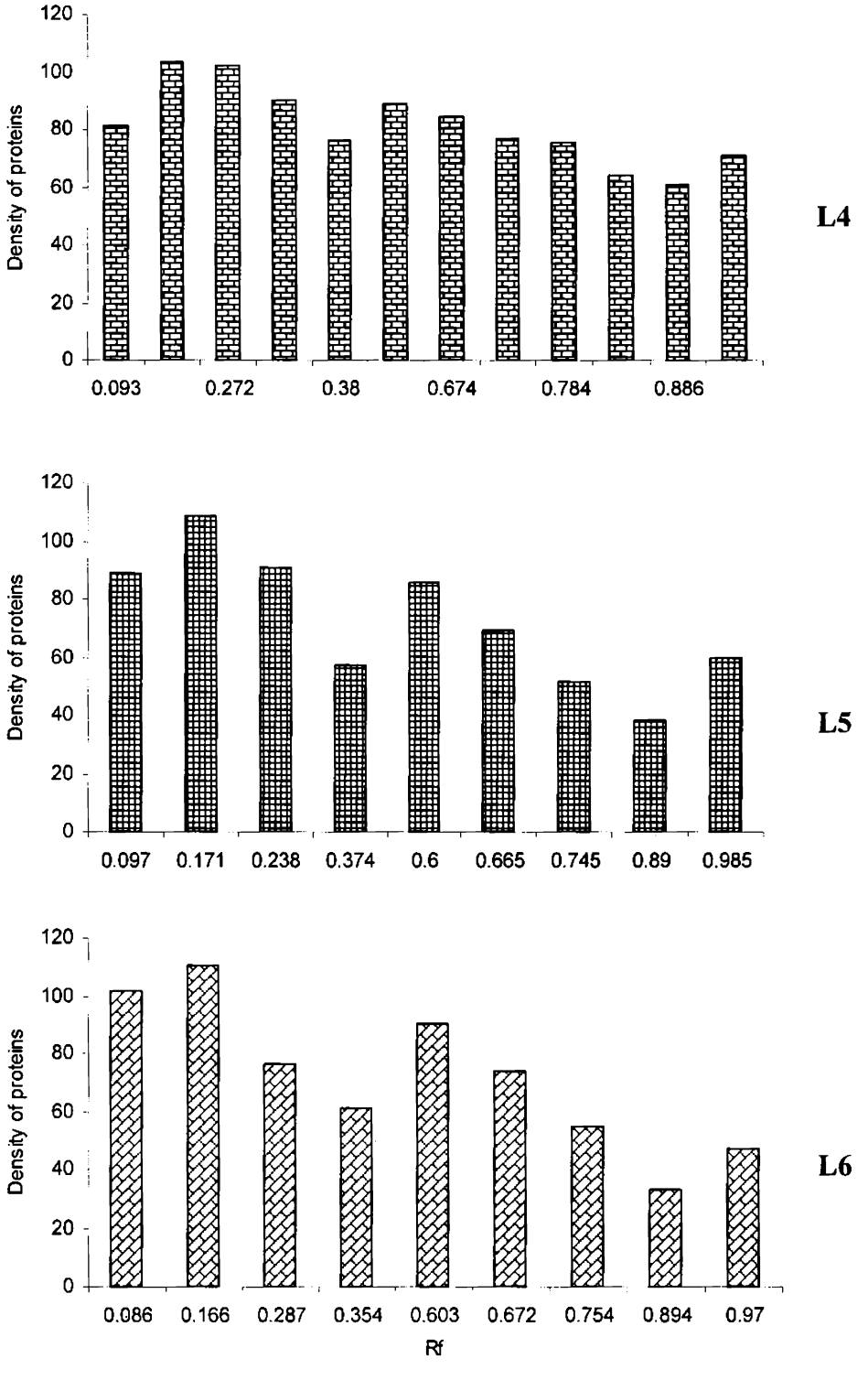


Fig -7a: Densitometry analysis of myofibrillar protein profile in muscle of *L. rohita* after 96hrs exposure of methyl parathion (L1 -Control, L2 -1.8 mg/L; L3 - 3.6 mg/L; L4-5.4 mg/L; L5-7.4mg/L; L6-9.0 mg/ L)

molecular weights 231 and 20 kDa (Fig 7a). At 1.8 mg/L methylparathion concentration, two unique protein fractions appeared at Rf 0.343 (~ 81 kDa) and 0.369 (~ 73 kDa) while a protein fraction of molecular weight 31 kDa (Rf: 0.784) appeared at a concentration of 5.4 mg/L of methylparathion. The quantitative calculation of the protein fractions revealed a decreasing trend in the protein fraction between molecular weights of the range 20 - 31 kDa with increase in the concentration of methylparathion. The protein fractions almost remained constant except at high pesticide concentration, where certain new bands appeared indicating the transcription of certain specific protein. This may be related to the synthesis of some stress proteins in response to the pesticide.

4.6. Alteration of lipids in different organs

Tables 8 and 9 show the changes in cholesterol, cholesterol ester, triglycerides, and phospholipids observed from liver samples, after 96h of exposure to methylparathion. The average cholesterol content in control fish was 1.32 mg/g, which gradually increased with increase in concentration of pesticide (methylparathion). At a concentration of 5.4 mg/L the cholesterol content doubled compared to control. Further increase in pesticide concentration decreased the cholesterol content. In the same way the triglycerides content increased in liver tissue up to a methylparathion concentration of 7.2 mg/L and then showed a decreasing trend.

The cholesterol ester content in the liver exposed to methylparathion showed gradual increase through out the study period of 96h. Among the phospholipids, phosphatidyl choline and phosphatidyl inositol showed a

gradual increase but was not significant ($P<0.05$), and on the other hand phosphatidyl choline (up to 7.2 mg/L), sphingomylein (up to 9 mg/L) and lyso phosphatidyl choline (up to 3.6 mg/L) showed an increase by further increasing the concentration.

The cholesterol and triglycerides content in brain tissues was marginally higher than in liver. The cholesterol content in brain tissues (Table 10) also increased ($P<0.05$) by about 27% with increase in the methylparathion concentration up to 5.4 mg/L and then decreased. At the same concentration, the triglycerides content in the brain also increased (12%). The cholesterol ester content increased by 29% in 7.2mg/L and then decreased. The phospholipids content of the brain tissues (Table 11) were several times higher compared to liver tissues. The phosphatidyl ethylamine in brain tissue increased gradually up to 7.2mg/L and then decreased. The sphingomylein an important phospholipid associated with brain tissues also showed concentration dependent increase up to 7.2 mg/L. Phosphatidyl choline turn over is several fold higher in brain tissue when compared to hepatic tissue. The lyso phosphatidylcholine also increased up to 5.4mg/L.

The changes in cholesterol, cholesterol ester and triglycerides content in the fish muscle, in response to methylparathion exposure is given in Table 12. The cholesterol and triglycerides content in muscle was about 3 fold higher than that of liver, and showed concentration dependent increase.

Among the phospholipids, phosphatidyl serine showed increase in muscle tissue compared to the liver (Table 13), and the value showed an increase up to a certain level of methylparathion and then decreased, but the alteration in

Table 8 : Changes in non-saponifiable lipids and Phosphatidyl ethylamine (mg g^{-1}) in liver of *L.rohita* after 96h exposure

S.No	Conc (mg/L)	Cholesterol	Cholesterol ester	Triglycerides	Phosphatidyl ethylamine
1	Control	1.32 ± 0.02^{a}	0.21 ± 0.02^{a}	1.82 ± 0.03^{a}	3.2 ± 0.02^{a}
2	1.8	1.72 ± 0.01^{a}	0.28 ± 0.01^{b}	1.92 ± 0.01^{b}	3.6 ± 0.02^{b}
3	3.6	2.82 ± 0.03^{c}	0.35 ± 0.03^{c}	2.01 ± 0.03^{c}	3.9 ± 0.03^{c}
4	5.4	3.24 ± 0.03^{d}	0.39 ± 0.03^{cd}	3.62 ± 0.02^{d}	4.6 ± 0.01^{d}
5	7.2	2.81 ± 0.04^{c}	0.38 ± 0.02^{cd}	4.20 ± 0.02^{e}	5.0 ± 0.02^{e}
6	9.0	2.36 ± 0.04^{e}	0.42 ± 0.01^{d}	3.80 ± 0.03^{f}	5.1 ± 0.01^{f}
7	10.2	2.32 ± 0.05^{e}	0.51 ± 0.04^{e}	2.90 ± 0.03^{g}	5.0 ± 0.03^{e}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d,f,g) differ significantly (P<0.05Duncan's multiple range test).

Table 9 : Changes in phospholipids (mg g^{-1}) in liver of *L.rohita* after 96h exposure

S.No	Conc (mg/L)	Phosphatidyl choline	Phosphatidyl inositol	Sphingomylein	Lyso Phospha tidyl choline
1	Control	0.35 ± 0.03^{a}	0.42 ± 0.01^{a}	0.32 ± 0.05^{a}	0.47 ± 0.01^{ab}
2	1.8	0.36 ± 0.03^{ac}	0.47 ± 0.06^{ab}	0.36 ± 0.03^{ab}	0.52 ± 0.02^{b}
3	3.6	0.42 ± 0.06^{b}	0.49 ± 0.06^{b}	0.33 ± 0.02^{a}	0.62 ± 0.01^{c}
4	5.4	0.43 ± 0.01^{b}	0.56 ± 0.01^{c}	0.31 ± 0.01^{a}	0.46 ± 0.05^{a}
5	7.2	0.41 ± 0.01^{c}	0.52 ± 0.01^{bc}	0.38 ± 0.02^{b}	0.49 ± 0.02^{a}
6	9.0	0.42 ± 0.02^{b}	0.58 ± 0.02^{c}	0.42 ± 0.01^{c}	0.46 ± 0.02^{a}
7	10.2	0.43 ± 0.03^{b}	0.53 ± 0.03^{bc}	0.36 ± 0.02^{ab}	0.45 ± 0.03^{a}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c) differ significantly (P<0.05Duncan's multiple range test).

Table 10 : Changes in non-saponifiable lipids and phatidylethylamine (mg g^{-1}) in brain of *L.rohita* after 96h exposure

S.No	Conc. (mg/L)	Cholesterol	Cholesterol ester	Triglycerides	Phosphatidyl ethylamine
1	Control	3.62 ± 0.01^{a}	0.21 ± 0.01^{a}	8.2 ± 0.02^{a}	2.57 ± 0.02^{a}
2	1.8	3.82 ± 0.05^{b}	0.18 ± 0.02^{a}	8.8 ± 0.01^{b}	2.62 ± 0.01^{a}
3	3.6	4.26 ± 0.01^{c}	0.23 ± 0.01^{bc}	8.5 ± 0.01^{c}	2.96 ± 0.02^{a}
4	5.4	4.62 ± 0.01^{d}	0.25 ± 0.02^{cd}	9.2 ± 0.05^{d}	3.13 ± 0.01^{d}
5	7.2	3.82 ± 0.02^{b}	0.27 ± 0.02^{d}	8.2 ± 0.10^{a}	3.36 ± 0.06^{e}
6	9.0	3.92 ± 0.04^{e}	0.21 ± 0.03^{ab}	8.4 ± 0.20^{c}	3.27 ± 0.01^{f}
7	10.2	3.61 ± 0.01^{a}	0.23 ± 0.01^{bc}	8.5 ± 0.01^{c}	3.12 ± 0.02^{d}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d,e,f) differ significantly (P<0.05Duncan's multiple range test).

Table 11 : Changes in phospholipids (mg g^{-1}) in brain of *L.rohita* after 96h exposure.

S.No	Conc (mg/L)	Phosphatidyl choline	Phosphatidyl inositol	Sphingomylein	Lyso Phosphatidyl choline
1	Control	18.2 ± 0.02^{a}	12.0 ± 0.02^{a}	0.62 ± 0.20^{a}	1.0 ± 0.02^{af}
2	1.8	19.1 ± 0.01^{b}	12.8 ± 0.06^{b}	0.67 ± 0.06^{ab}	2.7 ± 0.23^{b}
3	3.6	21.6 ± 0.02^{c}	13.2 ± 0.02^{c}	0.72 ± 0.01^{ab}	3.2 ± 0.27^{c}
4	5.4	20.7 ± 0.01^{d}	14.2 ± 0.02^{d}	0.81 ± 0.02^{bc}	2.1 ± 0.07^{d}
5	7.2	19.7 ± 0.02^{e}	13.2 ± 0.01^{c}	0.92 ± 0.02^{c}	1.8 ± 0.02^{e}
6	9.0	22.3 ± 0.03^{f}	13.6 ± 0.10^{e}	0.61 ± 0.03^{a}	1.2 ± 0.02^{f}
7	10.2	21.0 ± 0.05^{g}	12.9 ± 0.10^{b}	0.62 ± 0.06^{a}	0.8 ± 0.02^{a}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d,e,f,g) differ significantly (P<0.05Duncan's multiple range test).

Table 12 : Changes in non-saponifiable and phosphatidylethylamine (mg g^{-1}) in muscle of *L.rohita* after 96h exposure.

S.No	Conc (mg/L)	Cholesterol	Cholesterol ester	Triglycerides	Phosphatidyl ethylamine
1	Control	1.06 ± 0.01^{a}	0.26 ± 0.05^{a}	1.05 ± 0.02^{a}	0.90 ± 0.01^{a}
2	1.8	1.87 ± 0.03^{b}	0.27 ± 0.02^{a}	1.52 ± 0.01^{b}	0.92 ± 0.03^{a}
3	3.6	1.72 ± 0.04^{c}	0.29 ± 0.02^{a}	1.56 ± 0.02^{bc}	1.24 ± 0.02^{b}
4	5.4	1.97 ± 0.02^{d}	0.32 ± 0.03^{a}	1.72 ± 0.03^{d}	1.12 ± 0.01^{c}
5	7.2	1.24 ± 0.02^{e}	0.31 ± 0.01^{a}	1.52 ± 0.07^{b}	0.91 ± 0.02^{a}
6	9.0	1.32 ± 0.03^{f}	0.42 ± 0.05^{b}	2.01 ± 0.02^{e}	0.82 ± 0.01^{d}
7	10.2	1.21 ± 0.01^{e}	0.28 ± 0.05^{a}	1.61 ± 0.02^{c}	0.72 ± 0.01^{e}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d,e,f) differ significantly (P<0.05Duncan's multiple range test).

Table 13 : Changes in phospholipids (mg g^{-1}) in muscle of *L.rohita* after 96h exposure.

S.No	Conc (mg/L)	Phosphatidyl choline	Phosphatidyl inositol	Sphingomylein	Lyso Phospha tidyl choline
1	Control	0.85 ± 0.01^{a}	0.20 ± 0.01^{a}	0.07 ± 0.01^{a}	0.05 ± 0.01^{a}
2	1.8	1.25 ± 0.02^{b}	0.31 ± 0.02^{b}	0.12 ± 0.01^{b}	0.12 ± 0.02^{b}
3	3.6	1.52 ± 0.01^{c}	0.42 ± 0.02^{c}	0.17 ± 0.02^{c}	0.22 ± 0.02^{c}
4	5.4	1.26 ± 0.02^{b}	0.51 ± 0.01^{d}	0.21 ± 0.01^{d}	0.23 ± 0.02^{c}
5	7.2	1.32 ± 0.01^{d}	0.72 ± 0.01^{e}	0.18 ± 0.02^{c}	0.18 ± 0.01^{d}
6	9.0	1.41 ± 0.02^{e}	0.21 ± 0.02^{a}	0.12 ± 0.01^{b}	0.16 ± 0.02^{d}
7	10.2	0.91 ± 0.02^{f}	0.32 ± 0.05^{b}	0.10 ± 0.02^{b}	0.10 ± 0.02^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d,e,f) differ significantly (P<0.05Duncan's multiple range test).

phospholipids showed significance only in the case of phosphatidyl inosital, sphingomylein and lyso phosphatidylcholine.

The study demonstrated that methylparathion definitely causes cell damage in all tissues and the tissues try to repair this damage by synthesizing the phospholipids and cholesterol, the essential compounds of cell membrane. But when the concentration of methylparathion exceeds 7.2 mg/L there is a failure of repair mechanism indicated by the decrease in the synthesis of these essential requirements. Cholesterol an important non-saponifiable fat plays a crucial role to strengthen the biological membrane and in the membrane synthesis. The increase in quantity of cholesterol might be due to the increased diversion of acetylcholine to aceto acetate formation for cholesterol biosynthesis (Srinivasulu Reddy and Ramana Rao, 1989). Further it is also shown that TCA cycle enzymes are inhibited during insecticidal stress (Reddy and Rao, 1987). On the other hand fluid membrane system consists of phospholipids, which binds the hydrophobic pesticides to the membrane structure thus altering its fluidity as well as signal transmission across the cellular membrane. The activity of integral membrane proteins depends up on the physical and chemical properties of boundary domains, which in turn affects the acetylcholine ester as well as other metabolic activates.

4.7. Histopathological observations in acute toxicity studies

The gill is made up of filaments or primary lamellae arranged in two rows (Fig 14). Secondary lamellae arise from filaments. The secondary lamellae are lined by squamous epithelium. Inside this epithelium is lamellar blood sinuses

separated by pillar cells. At the tip of secondary lamellae is a marginal blood sinus lined by an endothelium. In between the secondary lamellae, the primary filaments are lined by a thick stratified epithelium. This region contains the mucous cells and chloride cells.

Histopathological changes were observed in the gills of *L. rohita* on exposure to methylparathion for 96h compared to control. The hyperplasia of the epithelial lining of the secondary lamellae, necrosis, edema and shorting of the secondary lamella, abnormal raising or swelling of the epithelium are the most common changes noticed at all concentration. At 10.2 mg/L disintegration of primary and secondary lamellae and cell necrosis were observed in methylparathion treated fish gills (Fig 15). The gill epithelium is the site of gaseous exchange, ionic regulation, acid-base balance and nitrogenous waste excretion in fish.

Lamellar fusion and shorting of secondary lamellae could be protective in that it diminishes the amount of vulnerable gill surface area (Mallatt, 1985). Branchial responses that serve to slow the entry of the toxicant have the undesirable effect of threatening to suffocate the fish (Skidmore, 1964). Various cellular, histological and histopathological changes in the gills of the fish by the toxicant signify problems with respiration and acid-base balances. The severe damages in terms of necrosis and ruptures of gill epithelium result in hypoxia and respiratory failure (Richmonds and Dutta, 1989).

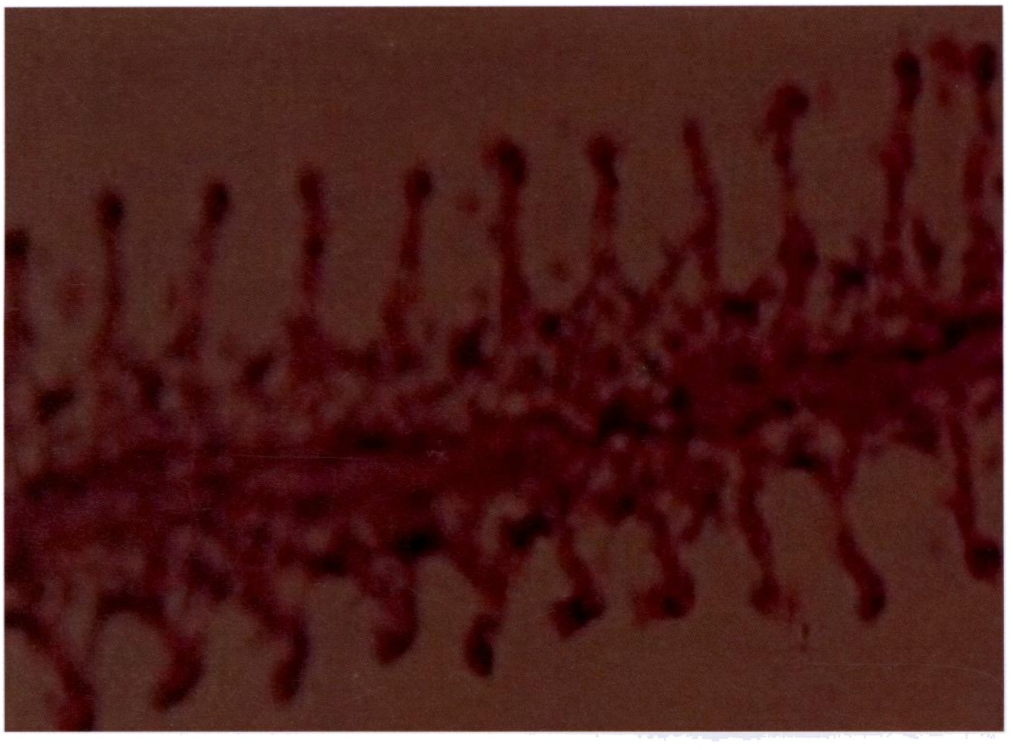
Gill et al., (1988), reported the separation of the lamellar epithelium, necrosis, edema and lifting of epithelium during the exposure of *Puntius conchonius* to 0.19 and 0.31 mg/L of carbaryl and 0.4 and 0.7mg/L of demethoate

(Richmonds and Dutta, 1989) to blue gill sunfish (*Lepomis macrochirus*) to a concentration of 0.05mg/L malathion for 24, 48, 72, and 96h and observed lifting of the epithelial layer and necrosis, edema, lamellar fusion and clavate lamellae. Similar observations were made in experiments on induced diazinon in the gills of bluegill sunfish *L. macrochirus* (Dutta *et al.*, 1993).

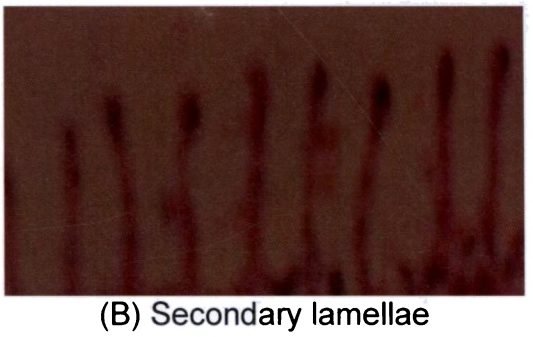
On exposure to 34.8, 46.5 and 53.5 ng/ml and 60.0 ng cyphenothrin/ml water for 96h the gills of *L. reticulatus* showed lifting of epithelial layer from gill lamellae and necrosis, the degeneration of secondary lamellae due to edema, the shortening of secondary lamellae and club-shaped lamellae (Erkmen *et al.*, 2000). Alterations in gills such as those observed in this study and findings from previous studies may result in severe physiological problems, ultimately leading to the death of fish. The result of these alterations in gills could be understood as a defense mechanism against exposure to methylparathion rather than as an irreversible toxic effect.

In the liver cells of *L. rohita* exposed for 96h exposure to methylparathion, the heptocytes have lost their normal architecture and large number of these cells appeared with pyknotic nuclei and sinusoids which in most cases were distended. Severe damage was noted due to marked swelling and degeneration of the endothelial lining cells (Fig 16-19).

In *Clarias gariepinus* exposed to 1/10 LC of fenvalerate for 5 days, similar changes in the liver (Teh *et al.*, 2005) were noticed. The intrahepatic blood vessels were dilated and congested with blood, and inflammatory leucocytic infiltrations were observed. It appears to be a general feature of the liver of intoxicated fish that the degree of structural heterogeneity is enhanced with



(A) Primary lamella and secondary lamellae



(B) Secondary lamellae

Fig 14: The architecture of normal gill tissues in control fish (A and B). Shows intact and well organized Primary lamella and secondary lamellae (Hemotoxyline and Eosin 100x)

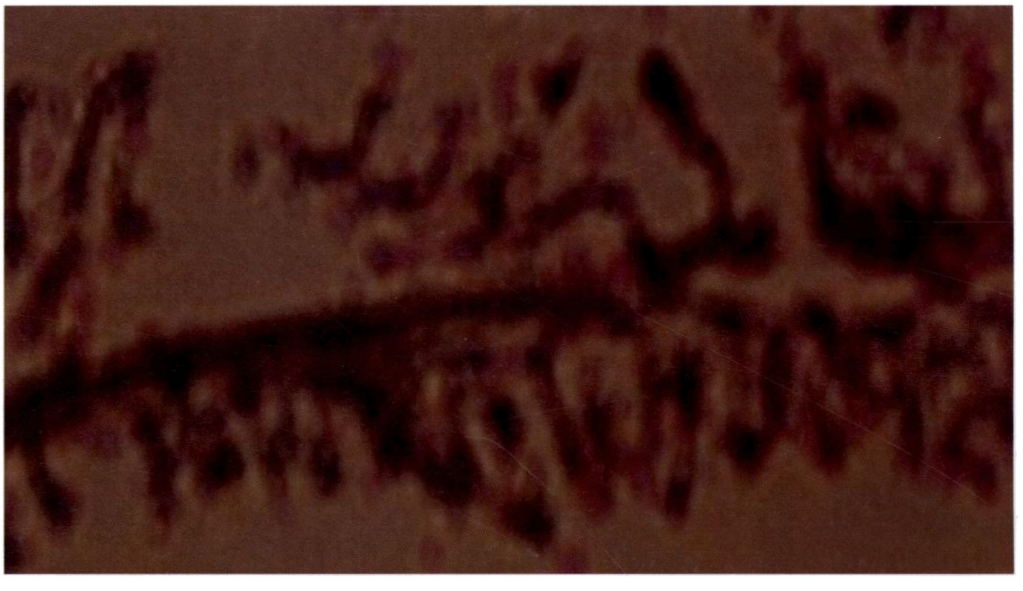


Fig 15: The architecture of fish gills of *Labeo rohita* after 96h exposure to methylparathion (10.2mg/L), shows disintegrated of primary lamellae and secondary lamellae in fish gills (Hemotoxyline and Eosin 100x).

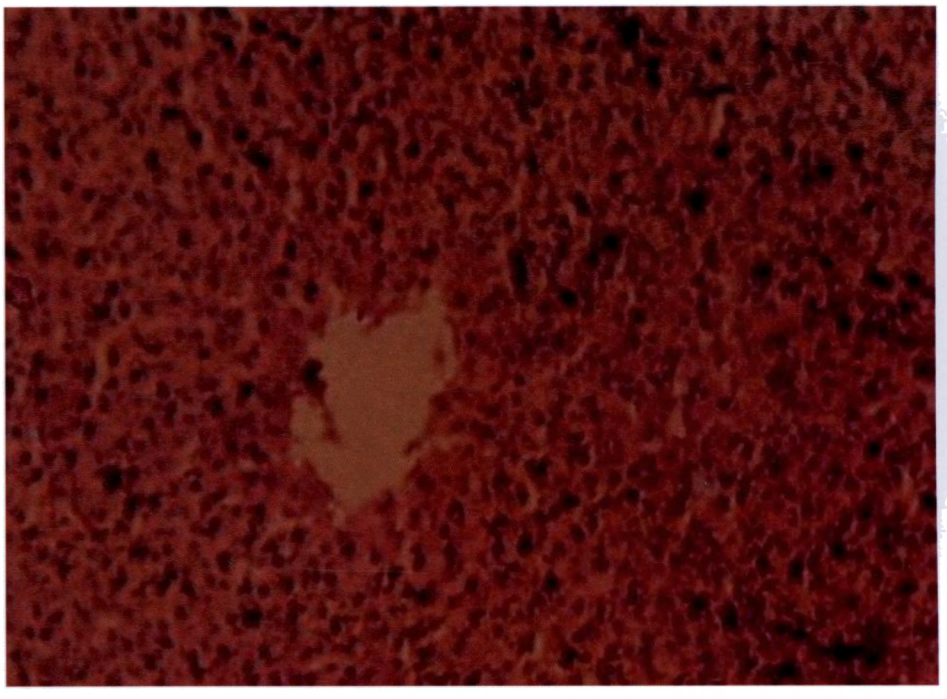


Fig 16: The architecture normal liver tissues of control fish. Shows normal fibrillar structure with striations and continuity with adjacent cells (Hemotoxyline and Eosin 100x)

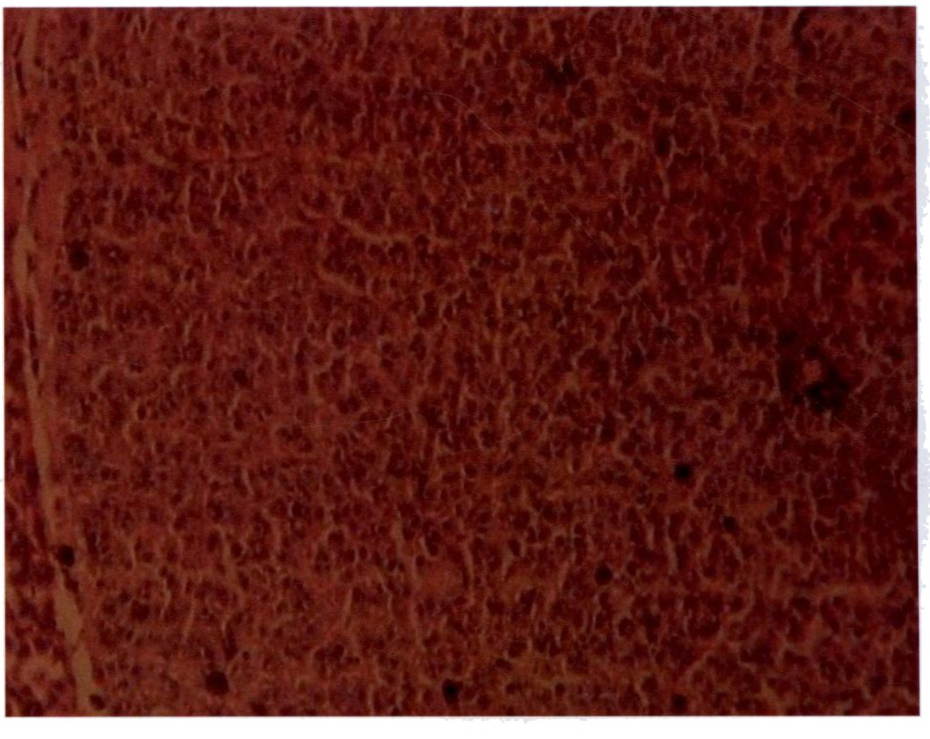


Fig 17: The architecture of fish liver of *Labeo rohita* after 96h exposure to methyl parathion (7.2 mg/L). Shows vascular congestion in liver cells (Hemotoxyline and Eosin 100x).

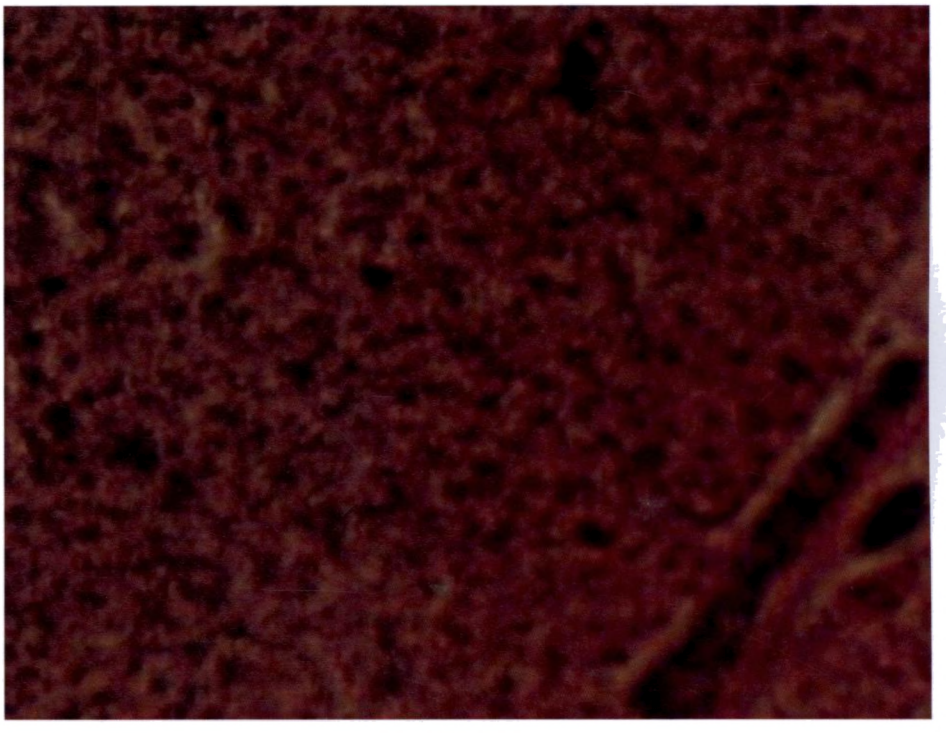


Fig 18: The architecture of fish liver of *Labeo rohita* after 96h exposure to methylparathion (9.0mg/L). Shows focal necrosis in fish liver cells (Hemotoxyline and Eosin 100x).

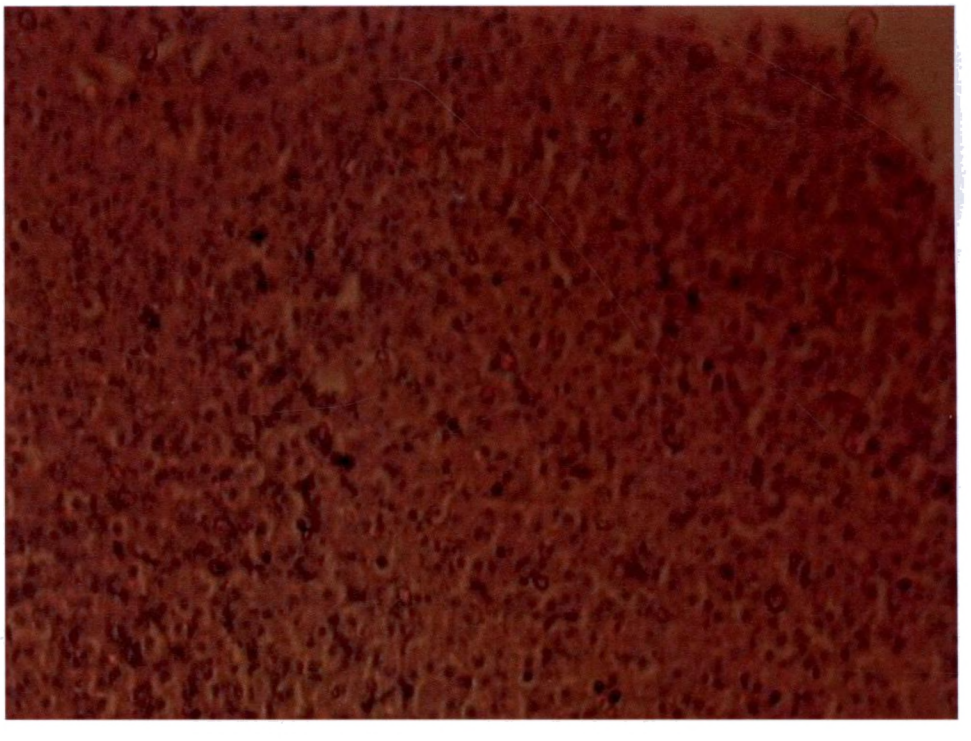


Fig 19: The architecture of fish liver of *Labeo rohita* after 96h exposure to methylparathion (10.2 mg/L). Shows increasing hyperplasia and congestion of liver cells (Hemotoxyline and Eosin 100x).

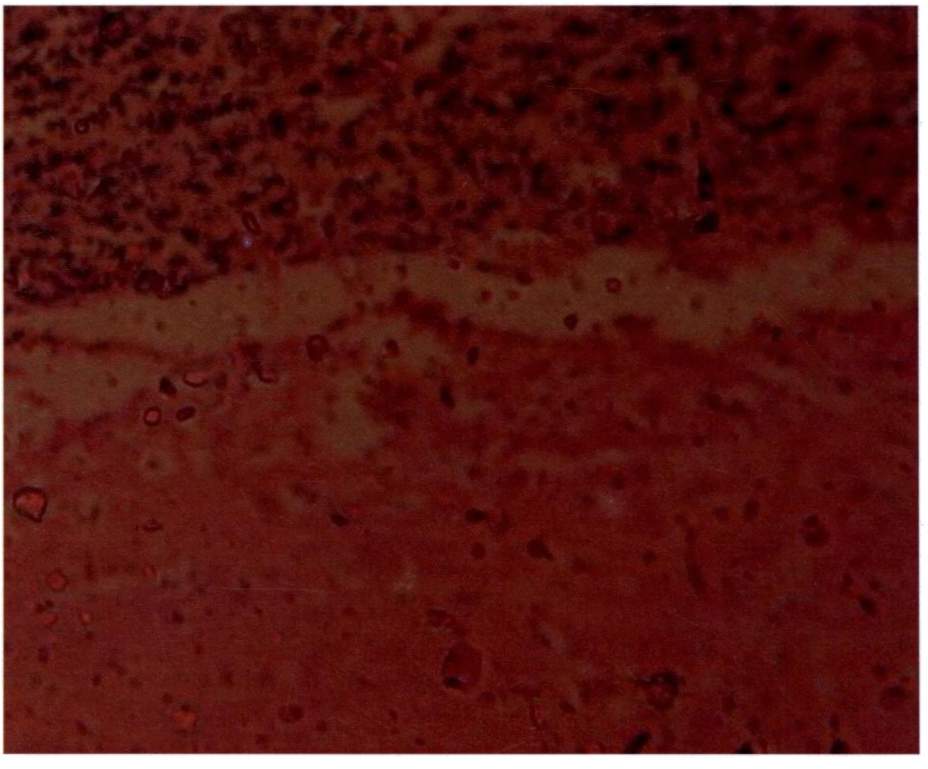


Fig 20: The architecture of normal brain tissue in control fish. Shows intact and well organized brain cells (Hemotoxyline and Eosin100x)

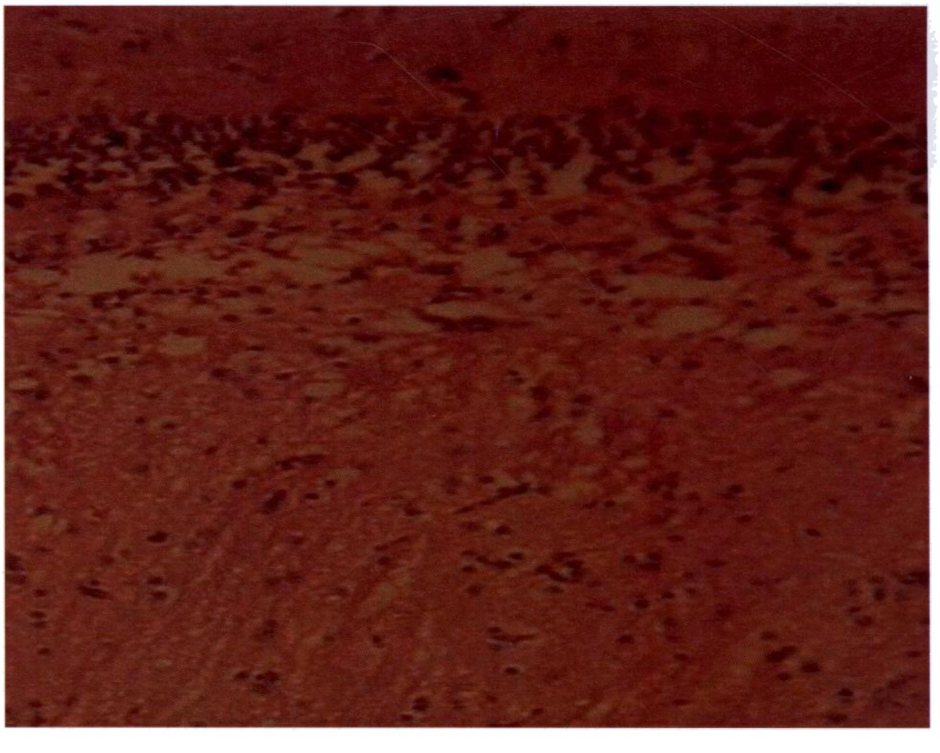


Fig 21: The architecture of fish brain of *Labeo rohita* after 96h exposure to 7.2 mg/L methylparathion, which shows brain cells necrosis (Hemotoxyline and Eosin 100x).

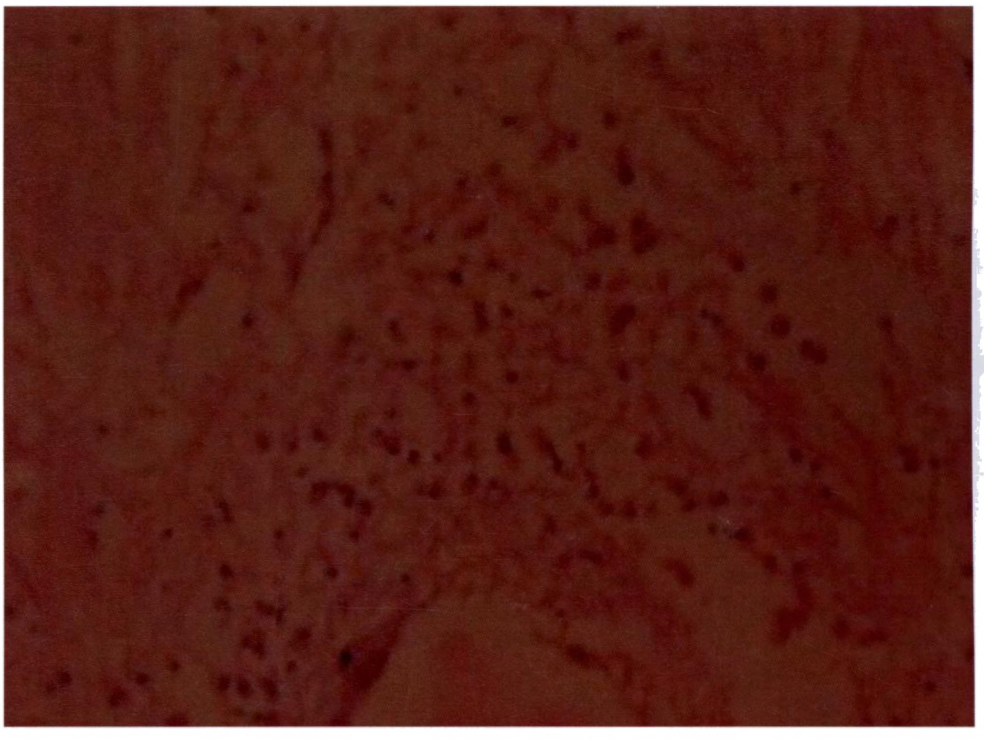


Fig 22: The architecture of fish brain of *Labeo rohita* after 96h exposure to 9.0 mg/L methylparathion, which is shows edema (Hemotoxyline and Eosin 100x).

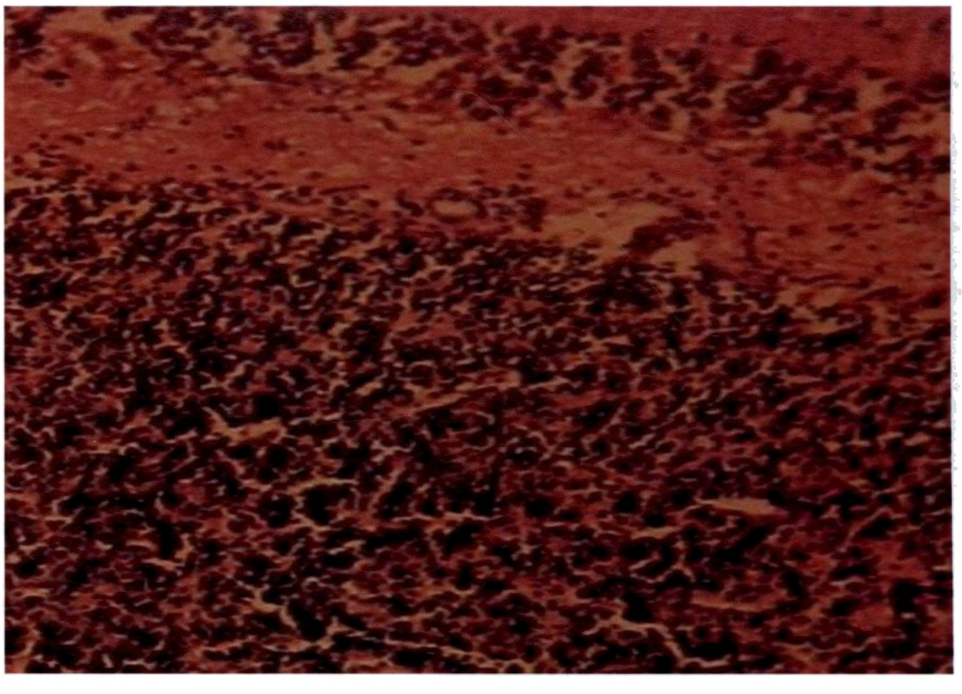


Fig 23: The architecture of fish brain of *Labeo rohita* after 96h exposure to 10.2 mg/L methylparathion, which shows increasing brain cells, edema separation nerve fiber was observed (Hemotoxyline and Eosin 100x).

increasing concentrations of the toxicant (Hawkes, 1980). Narayan and Singh (1991) observed extensive degeneration of cytoplasm with pyknosis of nuclei and loss of glycogen in liver tissue of *Heteropneustes fossilis* on exposure to acute thiodan toxicity.

Tissue changes in liver are linked with histological abnormalities of kidney and gill. Once absorbed, toxicant is transported by blood circulation to liver for transformation and/or storage, and once transformed in the liver it may be excreted through the bile or pass back into blood for possible excretion by kidney or gill (Lindstoma-Seppa *et al.*, 1981).

Histopathological changes in brain tissue of the fish exposed to methyl parathion for 96h showed severe deformations due to ischemia, pyknosis of the cytoplasm, necrosis and separation of neural fibrils and accumulation of disintegrated brain cells (Fig 20-23). Histopathological changes in various areas of the brain as well as increased density of the cytoplasm in neurocytes were observed in the rats on exposure to chlorpyrifos and cypermethrin. The changes were observed most strikingly in the cells of cornu ammonis CA1 and CA3 hippocampus layers, the hypothalamus and the stratum granulosum in area dentate (Latuszynska *et al.*, 2001).

4.8. Effect of sub lethal toxicity on lipid peroxidation and detoxifying enzyme systems in liver

4.8.1. Lipid peroxidation (LPO)

The LPO level in liver of *L. rohita* after exposure to methylparathion at sub lethal level increased compared to the control (Table 14). There was a significant ($P<0.05$), increase in LPO level with an increase in the

concentration of methylparathion. At 0.25 mg/L methylparathion concentration, there was only marginal increase (8%) but at 1.0 mg/L concentration, the lipid peroxidation doubled. At higher concentration (0.5mg/L) 17% increase was noticed compared to control on 15th day, which gradually increased with days of exposure. Exposure to methylparathion at 1.0 mg/L increased LPO over two fold and at the end of 45 days the increase was almost five fold compared to control. It has been reported that increased LPO is one of the major contributors to the loss of cell function during oxidative stress situation (Hermes-Lima *et al.*, 1995).

LPO is considered a valuable indicator of oxidative damage of cellular components. The results suggests that exposure to methylparthion enhanced ROS synthesis in the liver of *L. rohita* and the antioxidant defenses were not totally able to effectively scavenge them, thus leading to lipid peroxidation. LPO has been reported as a major contributor to the loss of cellular function under oxidative stress (Storey, 1996). Considering that the typical reaction during ROS-induced damage involves the peroxidation of unsaturated fatty acids, the results clearly shows that exposure to methylparathion at sub lethal level lead to oxidative stress, with increase of LPO values in liver, compared to the control group. The oxidative stress condition was reported to cause damage in hepatopancreatic tubules in *C. granulatus* exposed to methyl parathion (Hodgson and Levy, 1994).

The increased hydroperoxide lipid production in the present study suggested that ROS-induced oxidative damage can be one of the main toxic effects of methylparathion. It has been reported that LPO may be induced by a variety

of environmental pollutants (Ploch *et al.*, 1999; Ahmad *et al.*, 2000; Wilhelm-Filho *et al.*, 2001; Oakes and Van der Kraak, 2003; Oakes *et al.*, 2004).

4.8.2. Superoxide Dismutase (SOD)

The SOD activity in liver of *L. rohita* after exposure to methylparathion at sub lethal level was compared to control (Table 15). There was a significant ($P<0.05$), increase in SOD activity with increase in the concentration of methylparathion. SOD activity was noticed in rohu during exposure to three different concentrations of methylparathion for 15, 30 and 45 days. On 15 days exposure to 0.25 mg/L concentration there was a two fold increase in SOD activity, which increased by three fold and six fold respectively for 0.5 mg/L and 1 mg/L. Exposure to increased concentration for longer duration showed decreasing activity which was more pronounced at higher concentration. The generation of free radicals due to exposure to methylparathion, induced the LPO reaction, which might have exceeded the ability of the superoxide dismutase to dismute the superoxide radicals, resulting in membrane damage and inhibition of the free radical scavenging enzymes. Similar decrease in SOD activities were reported in tilapia, with average weight of 87gms (Peixoto *et al.*, 2006).

Free radicals ($O_2^{\cdot-}$) are dismutated by SOD to H_2O_2. Induction of SOD could occur during high production of superoxide anion radical. Therefore, an increase in the SOD activity indicates an increase in $O_2^{\cdot-}$ production. Increased SOD levels were reported in similar studies in *Leuciscus cephalus* (Lenartova *et al.*, 1997). In contrast, the superoxide radicals by themselves or after their transformation to H_2O_2 cause oxidation of the cysteine in the

enzyme and decreases SOD activity (Dimitrova *et al.*, 1994). Decrease in SOD activity levels were found in erythrocytes of *Cyprinus carpio* exposed to MS 222 (Bartowiak *et al.*, 1981). SODs are a group of metaloenzymes that plays a crucial antioxidant role and constitute the primary defense against the toxic effect of oxygen (Stegeman *et al.*, 1992).

4.8.3. Catalase (CAT)

The catalases are enzymes that remove hydrogen peroxide, which is metabolized to hydrogen and water. The catalase activity increased by 70% upon exposure to methylparathion (0.25mg/L) for 15 days compared to control. Further increase in the methylparathion concentration however decreased the activity. For particular concentration, CAT activity gradually decreased with increasing duration of exposure (Table 16).

The reduced activity of CAT and SOD in the presence of methylparathion may result in the accumulation of $O_2^{\cdot-}$, H_2O_2 or their products of decomposition. Loss of CAT and SOD activity results in oxygen intolerance and triggers a number of deleterious reactions. It has been proposed that the contribution of CAT might be enhanced if significant amounts of H_2O_2 become available through β-oxidation of fatty acids in peroxisomes (Decremer *et al.*, 1991).

4.8.4. Glutathione peroxidase (GPx)

The GPx activity in liver of *L. rohita* after methylparathion exposure decreased compared to the control (Table 17). There was a significant ($P<0.05$), decrease in GPx activity with increase in the concentration of

Table 14 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of LPO (nmol of malonaldehyde formed (mg of protein) $^{-1}$) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	0.53 ± 0.04^{a}	0.52 ± 0.03^{a}	0.54 ± 0.01^{a}
2	0.25	0.57 ± 0.02^{a}	0.64 ± 0.01^{b}	0.60 ± 0.03^{b}
3	0.50	0.62 ± 0.01^{a}	0.94 ± 0.02^{c}	1.08 ± 0.01^{c}
4	1.00	1.00 ± 0.08^{b}	1.15 ± 0.01^{d}	2.61 ± 0.01^{d}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significantly (P<0.05Duncan's multiple range test).

Table 15 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of SOD* in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	1.02 ± 0.2^{a}	1.28 ± 0.3^{a}	1.35 ± 0.2^{a}
2	0.25	2.45 ± 0.1^{b}	1.81 ± 0.1^{a}	2.25 ± 0.4^{b}
3	0.50	3.13 ± 0.6^{b}	3.39 ± 0.8^{b}	2.36 ± 0.2^{b}
4	1.00	6.41 ± 1.2^{c}	6.39 ± 0.1^{c}	3.35 ± 0.2^{c}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c) differ significantly (P<0.05Duncan's multiple range test).

*Unit: SOD - one unit of the activity is the amount of protein required to give 50% inhibition of epinephrine auto oxidation

Table 16 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of CAT (nmol of H_2O_2 decomposed min^{-1} (mg protein^{-1})) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	8.53 ± 0.8^{a}	7.21 ± 0.8^{a}	8.61 ± 0.7^{a}
2	0.25	14.48 ± 2.5^{a}	10.4 ± 1.9^{b}	9.63 ± 0.7^{a}
3	0.50	9.36 ± 1.7^{a}	7.62 ± 1.2^{a}	6.73 ± 0.8^{b}
4	1.00	7.61 ± 0.4^{b}	7.54 ± 0.7^{a}	5.54 ± 0.9^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b) differ significantly (P<0.05Duncan's multiple range test).

methylparathion. At 0.25 mg/L concentration a 36% decrease in the activity was noticed on 15 days of exposure.

At 1.0 mg/L methylparathion the GPx activity reduced by 76% compared to the control. Almost similar trend was noticed in the case of 30 and 45 days exposed fish (Table 17). The decreased activity of GPx in liver observed in the present study could be related to the excess $O_2^{\bullet -}$ production (Bagnasco *et al.*, 1991) or to the direct action of pesticides on the enzyme synthesis (Bainy *et al.*, 1993). Similar decrease in, GPx activity was noticed in mice erythrocytes by the organophosphorus insecticide malathion (Yarsan *et al.*, 1999). GPx inhibition was reported after combined treatment with the pesticides 2,4-D and azinphosmethyl in the brain of carp, *C. carpio* (Oruc *et al.*, 2004), and in the liver of Nile tilapia, *Oreochromis niloticus* (Oruc and Uner, 2000).

Enzyme activity can be decreased by negative feedback from excess of substrate or damage by oxidative modification (Tabatabaie and Floyd, 1994). A reduced GPx activity could indicate that its antioxidant capacity was supressed by the amount of hydroperoxide products of lipid peroxidation (Remacle *et al.*, 1992).

GPx plays an important role against the LPO, since it is mainly involved in the removal of organic compound and hydrogen peroxides. Thus, GPx is considered to play an important role in protecting membranes from damage due to LPO (Oost *et al.*, 2003). This observation suggested that the major detoxification function of GPx is the termination of the radical chain propagation (Oost *et al.*, 2003). In this context, the GPx inhibition observed in

the present study might reflect a possible antioxidant defense failure responsible for the observed increased in LPO levels.

4.8.5. Total reduced glutathione (GSH)

The GSH activity in liver of *L. rohita* after methylparathion exposure increased compared to the control (Table 18). There was a significant ($P<0.05$) increase in the GSH activity with increase in the concentration of methylparathion. With increase in duration of exposure the GSH level decreased after 30 days irrespective of the concentration of methylparathion.

The GSH plays an important role in the detoxification of electrophiles and prevention of cellular oxidative stress (Hasspieler *et al.*, 1994; Sies, 1999). The considerable decline in the GSH tissue content during exposure to methylparathion may be due to an increased utilization of GSH, leading to the formation of oxidized glutathione and an inefficient GSH regeneration. During a moderate oxidative stress, the GSH levels can increase as an adaptive mechanism by means of an increased synthesis. However, a severe oxidative stress may suppress GSH levels due to the impairment of the adaptive mechanisms (Zhang *et al.*, 2004). GSH depletion as seen in the case of fishes exposed to 1.0mg/L methylparathion may reduce the cellular ability to scavenge free radicals raising the general oxidative potential in the cell. This is well supported by the increased LPO levels shown for the same concentration (Table-14). Cells try to remove the xenobiotics by direct conjugation with GSH or by means of GST, which decrease GSH levels. The observed GSH levels are probable indication of the exhaustion in phase II biotransformation as confirmed by the increased GST activity.

Table 17 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of GPx (nmol of GSH oxidized min^{-1} (mg protein)$^{-1}$) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	4.65 ± 0.37^a	4.39 ± 0.41^a	4.89 ± 0.16^a
2	0.25	3.08 ± 0.10^b	2.43 ± 0.07^b	2.67 ± 0.38^b
3	0.50	2.82 ± 0.20^b	2.40 ± 0.12^b	1.25 ± 0.18^c
4	1.00	1.13 ± 0.15^c	1.61 ± 0.11^c	1.08 ± 0.11^c

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c) differ significantly (P<0.05Duncan's multiple range test).

Table 18 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of GSH (µmol (g wet tissue) $^{-1}$) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	1.19 ± 0.2^a	1.97 ± 0.3^a	1.35 ± 0.19^a
2	0.25	2.11 ± 0.3^b	3.01 ± 0.3^b	2.23 ± 0.44^b
3	0.50	2.58 ± 0.4^b	3.78 ± 0.5^c	2.36 ± 0.21^b
4	1.00	3.91 ± 0.4^c	3.87 ± 0.2^c	3.35 ± 0.19^c

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c) differ significatly (P<0.05Duncan's multiple range test).

Table 19 Effect of sub-lethal concentrations of methylparathion on the liver specific activity of GST (µmol of chloro-2,4-dinitrobenzyne conjugated formed min^{-1} (mg protein)$^{-1}$) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	1761.19 ± 72.4^a	1741.41 ± 116^a	1415.01 ± 376^a
2	0.25	1854.19 ± 82.5^a	3588.39 ± 261^b	2216.81 ± 238^b
3	0.50	3149.27 ± 35.9^b	3034.26 ± 84^c	4575.51 ± 402^c
4	1.00	5249.63 ± 85.8^c	5275.38 ± 162^d	5562.09 ± 257^d

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significantly (P<0.05Duncan's multiple range test).

4.8.6. Glutathion S-Transferase (GST)

The GST activity is involved in xenobiotic detoxification and excretion of xenobiotics and their metabolites (Jokanovic, 2001). It plays an important role in protecting tissue from oxidative stress (Fournier *et al.*, 1992; Banerjee *et al.*, 1999). Highly reactive electrophilic components can be removed before they covalently bind to tissue neucleophilic compounds by the action of this enzyme. Toxic effects of pesticides however inhibit the action of this enzyme. The GST activity in the liver of *L. rohita* after methylparathion exposure in sublethal level increased compared to control (Table 19). There was a significant ($P<0.05$), increase in GST activity with increase in the concentration of methylparathion. Increased GST activity in tissues may indicate the development of a defensive mechanism to counteract the effects of methylparathion and may reflect the possibility of a more efficient protection against pesticide toxicity. The GST activity increases in response to different concentrations of methylparathion for a particular period of exposure signifying the detoxifying mechanism in liver. With increase in the duration of exposure, GST level however, decreased except at higher concentration. This compares well with earlier reports of fresh water characid fish (Monterio *et al.*, 2006).

4.9. Effect on cholinesterase (AChE, BChE and PChE) activity in brain

The lethal studies of methylparathion on the fish tissue metabolism demonstrated significant inhibitory effect on AChE activity in brain tissue of *Labeo rohita*. The phosphate group on the organophosphorus compound

attacks the hydroxyl group of serine amino acids at the active site of the enzyme during AChE inhibition.

The activity of the enzyme decreased with increase in the concentration of methylparathion and also with increase in duration of exposure. During 15 days of exposure at 0.25 mg/L about 18% inhibition of AChE activity was noticed, which increased to 40 and 44% at higher concentrations of 0.5 and 1.0 mg/L respectively. For lowest concentration the activity increased to the level of control and dropped again by 45 days. At higher concentration (0.5 and 1.0 mg/L) long duration of exposure marginally increased the activities. Longer duration of exposure, however, decreased the activity compared to control (Table 20). This indicates that at lower concentration the tissues try to make up for the loss of activity on longer exposure, but at higher concentration the effect was obviously decreased. AChE activity showed a continuous decrease in brain, gill and muscle of tilapia on exposure to RPR2 (Venkateswara Rao, 2006; Venkateswara Rao *et al.*, 2003a, b; Kumar and Chapman, 2001). It is obvious from the results that *L. rohita* of the experimental size (73 ± 5g) can survive even after 44% inhibition of AChE in brain on exposure to methylparathion. In *O. mossbicus* juvenile there is a reduction of brain AChE by 67% on exposure to RPR2 (Venkateswara Rao, 2006), 93% in *O. niloticus* of 50g size on exposure to diazinon (Uncer et al., 2006), and 90-92% in *Cyprinus carpio,* on exposure to diazinon (Balint *et al.*, 1997) were noticed. It is generally accepted that of the total reduction in AChE activity by 20% is caused due to the exposure of organophosphrous pesticides (Feulton and Key, 2001). Some animals are able to survive more

than 50% of ChEs inhibition but it is a life- threatening situation (Ludke *et al.*, 1975).

However fish appear to be capable of surviving much higher levels (>90%) of brain AChE inhibition and doesn't appear to be uniform for all species (Sevgiler *et al.*, 2004). Inhibition of AChE is combated by an increase in acetylcholine levels (Brzezinski and Ludwicki, 1973), which can lead to increase in catecholamines. These changes affect the activity of enzymes involved in glycogenolysis and glycogen synthesis. Mortality of fish could be due to the inhibition of enzymes responsible for carbohydrate and protein metabolism. It is reported that accumulation of acetylcholine inhibits acetylcholinesterase, but activates butyryrlcholinesteras (Salles *et al.*, 2006).

Even though physiological role of BChE in neurotransmission is not fully established it plays the role of muscle relaxant succnylcholine (Kalow and Genest, 1957; Hersh *et al.*, 1974) and is reported to protect organophpsphorus pesticide toxicity in target cells by binding to phosphate before reaching biochemical target (Raveh *et al.*, 1997; Asani, 1991). In the present study control fishes showed a butyrylcholinesterase activity of 129 to 180 nmol mg^{-1} min^{-1} during the exposure period (Table 21). The activity decreased (20%) in 15 days, which marginally increased in 30 days, but decreased on further exposure to 45 days. Fishes exposed 0.5 mg/L also behaved in the same manner, but at higher concentration (1.0 mg/L) the activity increased with duration of exposure.

Table 20 : Effect of sub-lethal concentrations of methylparathion on the brain specific activity of AChE (nmol mg protein^{-1} min^{-1}) in *L. rohita*.

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	211.5 ± 28^{a}	257.1 ± 55^{a}	229.8 ± 24^{a}
2	0.25	174.3 ± 59^{ab}	229.1 ± 11^{a}	228.2 ± 10^{a}
3	0.50	127.8 ± 04^{b}	214.6 ± 08^{a}	158.4 ± 21^{b}
4	1.00	118.7 ± 13^{b}	145.9 ± 12^{b}	125.7 ± 06^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b) differ significantly (P<0.05Duncan's multiple range test).

Table 21 : Effect of sub-lethal concentrations of methylparathion on the brain specific activity of PChE (nmol mg protein-1 min-1) in L. rohita.

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	129.4 ± 07^{a}	183.5 ± 15^{a}	140.5 ± 18^{a}
2	0.25	103.6 ± 19^{b}	177.1 ± 10^{a}	111.5 ± 13^{b}
3	0.50	079.5 ± 13^{c}	118.9 ± 11^{b}	98.15 ± 06^{b}
4	1.00	067.9 ± 01^{c}	059.2 ± 0.7^{c}	95.55 ± 11^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c) differ significantly (P<0.05Duncan's multiple range test).

Table 22 : Effect of sub-lethal concentrations of methylparathion on the brain specific activity of BuChE (nmol mg protein^{-1} min^{-1}) in *L. rohita*.

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	76.72 ± 04^{a}	57.49 ± 15^{a}	76.18 ± 8.1^{a}
2	0.25	57.36 ± 15^{b}	51.26 ± 11^{a}	69.77 ± 8.3^{ab}
3	0.50	48.91 ± 10^{b}	56.49 ± 05^{a}	63.43 ± 8.8^{ab}
4	1.00	46.92 ± 04^{b}	52.55 ± 04^{a}	58.13 ± 8.2^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b) differ significantly (P<0.05Duncan's multiple range test).

4.10. Effect of disease diagnostic or marker enzymes activity in liver

The aspartate aminotransferase (AST) activity in the liver of *L. rohita* after methylparathion exposure in sublethal level was higher compared to control (Table - 23). There was a significant ($P<0.05$) increase in AST activity with increase in the concentration of methylparathion for varying duration. A 6% increase was noticed at the lowest concentration of 0.25 mg/L, which shot up by 24% and 48% respectively, for 0.5 and 1.0 mg/L of methylparathion for the same duration of exposure. During exposure to 30 and 40 days, at lowest concentration (0.25 mg/L) a marginal decrease in AST level was noticed but at higher concentration a significant ($P<0.05$) increase was noticed. This clearly indicates the extent of damage to hepatic cells as a result of methylparathion intoxication. At 1.0 mg/L concentration for 45 days about 82 % increase was noticed. At 0.25 mg/L concentration, the AST activity marginally decreased as a result of the cells getting adjusted to mild concentration of methylparathion.

The alanine aminotransferase (ALT) activity in the liver of *L. rohita* after methylparathion exposure in sub lethal level was higher when compared to control (Table 24). There was a significant ($P<0.05$) increase in ALT activity with increase in the concentration of methylparathion. The result showed a clear alteration in relation to methylparathion concentration and exposure periods for 0.25 and 0.5mg/L concentration. On 15^{th} day of exposure the ALT activity increased with increase in the concentration of methylparathion. On further exposure to 30 and 45 days the activity showed gradual decline. At 30^{th} day, the ALT activity showed a decline indicating the repair mechanism in

the initial periods of exposure to the xenobiotics. However at higher concentration of methylparathion (1.0mg/L) after initial decrease of AST activity up to 30 days the activity increased by 45th day indicating extensive damage to hepatic tissues at higher concentration.

ALT and AST are the most commonly used biochemical markers of hepatocellular necrosis (Jyothi and Narayan, 2000; Friedman *et al.*, 1996; Henderson *et al.*, 1983).The increase in ALT and AST activities noticed in the study supports tissue damage (Oluah, 1998; Oluah, 1999; Zikic *et al.*, 2001).

The Lactate Dehydrogenase (LDH) activity in the liver of *L. rohita* after methylparathion exposure to sub lethal level, was higher when compared to control (Table 25). There was a significant ($P<0.05$) increase in LDH activity with increase in the concentration of methylparathion. The LDH activity on 15th day showed a decreasing trend at a concentration of 0.25mg/L, which increased with the increase in pesticide concentration. After 30 days of exposure, the LDH activity increased ($P<0.05$) several folds at all concentrations of methylparathion. However, the LDH activity showed decrease on 45 days of exposure. The decrease in LDH activities of liver and muscles reflects a possible decrease in the biosynthetic activities and anaerobic capacity, which indicate that the glycolysis of tissues was decreased (Sastri and siddiqui, 1993; Tripathi and Verma, 2004).

Hepatic specific activity of alkaline phosphatase (ALP) and acid phosphatase (ACP) for control and experimental fish increased with increase in the ($P<0.05$) concentration of methylparathion exposure (Table 26-27). For particular concentration, the activity increased with increase in the duration of

Table 23 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of AST (µmol pyruate librated $h^{-1} L^{-1}$) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	498.4 ± 67^{a}	499.4 ± 38^{a}	488.9 ± 40^{a}
2	0.25	529.8 ± 12^{a}	504.1 ± 17^{a}	492.9 ± 85^{a}
3	0.50	617.3 ± 33^{b}	675.4 ± 30^{b}	766.1 ± 32^{b}
4	1.00	712.8 ± 11^{c}	810.5 ± 60^{c}	892.1 ± 62^{c}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c) differ significantly (P<0.05Duncan's multiple range test).

Table 24 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of ALT (µmol pyruate librated $h^{-1} L^{-1}$) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	271.3 ± 16^{a}	250.6 ± 25^{a}	221.1 ± 45^{a}
2	0.25	277.8 ± 16^{ab}	241.3 ± 14^{a}	235.9 ± 17^{ab}
3	0.50	288.1 ± 17^{ab}	264.6 ± 15^{ab}	254.6 ± 11^{b}
4	1.00	332.1 ± 19^{b}	289.6 ± 18^{b}	304.9 ± 06^{c}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c) differ significantly (P<0.05Duncan's multiple range test).

Table 25 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of LDH (µmol pyruate librated $h^{-1} L^{-1}$) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	212.3 ± 07^{a}	238.7 ± 11^{a}	261.8 ± 10^{a}
2	0.25	203.7 ± 10^{a}	465.5 ± 16^{b}	344.1 ± 30^{b}
3	0.50	307.2 ± 04^{b}	961.0 ± 37^{c}	509.2 ± 40^{c}
4	1.00	470.1 ± 14^{c}	962.9 ± 18^{c}	952.3 ± 19^{d}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significantly (P<0.05Duncan's multiple range test).

Table 26 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of ALP (µmol phenol librated $h^{-1}L^{-1}$) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	136.6 ± 14^{a}	131.1 ± 38^{a}	143.7 ± 14^{a}
2	0.25	147.2 ± 10^{a}	148.5 ± 07^{a}	159.7 ± 05^{a}
3	0.50	221.8 ± 42^{b}	234.0 ± 21^{b}	231.0 ± 28^{b}
4	1.00	237.8 ± 14^{b}	241.9 ± 10^{b}	250.4 ± 10^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b) differ significantly (P<0.05Duncan's multiple range test).

Table 27 : Effect of sub-lethal concentrations of methylparathion on the liver specific activity of ACP (µmol phenol librated $h^{-1}L^{-1}$) in *L. rohita.*

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	194.9 ± 33^{a}	216.1 ± 10^{a}	211.7 ± 22^{a}
2	0.25	204.7 ± 02^{a}	224.5 ± 56^{a}	235.1 ± 31^{a}
3	0.50	241.4 ± 41^{a}	248.6 ± 31^{a}	244.7 ± 14^{ab}
4	1.00	250.1 ± 16^{a}	270.7 ± 17^{a}	286.7 ± 19^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b) differ significantly (P<0.05Duncan's multiple range test).

the exposure. The elevation in alkaline phosphatase suggests an increase in the lysosomal mobilization and cell necrosis due to pesticide toxicity. Elevation of ACP activity in brain was reported earlier in stress-exposed *Channa punctatus* (Sastry and Sharma, 1980) and in *Labeo rohita* (Das and mukherjee, 2003). There are also reports indicating increase in the activities of these enzymes in serum as a result of impairment of hepatic tissue and liberation of these enzymes into circulation from the damaged tissues (Oruc and Uner, 1999).

4.11. Alteration of muscle proteins

The effect of sub lethal concentration of methylparathion on muscle proteins, both sarcoplasmic and myofibrillar were evaluated with increasing concentration of methylparathion as well as duration of exposure. The muscle proteins as such did not show any significant difference on exposure to methylparathion at concentrations of 0.25 and 0.5 mg/L (Table 28). The sarcoplasmic protein content more or less remained unchanged even after 15 days of exposure, but higher concentration (1.0 mg/L) showed moderate increase (23%). Considering individual concentration up to 30 days, there was no change in sarcoplasmic protein level at any concentration compared to 15 days sample. But at 45^{th} day there was an increase at all concentrations. During lethal exposure, however there was significant increase in sarcoplasmic protein (Table 7)

The SDS-PAGE of sarcoplasmic protein fraction of muscle extract from control and treated fishes exposed to sub lethal concentration of 0.25, 0.5 and 1.0 mg/L are shown in figure 8 -10. On exposure to 0.25 mg/L, there was no

change in the protein subunits compared to the control. At 0.5 mg/L there was a decrease in the intensity of 3 subunits viz., 16kDa, 54kDa and 20kDa, possibly signifying the effect of methylparathion at that concentration. At 1.0 mg/L concentration the intensity of 116 kDa increased but 84, 34 and 22 kDa fractions almost disappeared. The densitomeric analysis (Fig 8a) shows the disappearance of some of the prominent bands viz 86 kDa, 62 kDa and 56 kDa. On 30^{th} day at 0.5mg/L concentration the intensity of most of the low molecular weight proteins increased, particularly 30, 35 and 38 kDa fractions. The intensities of two protein subunits with molecular weight 48 kDa and 40 kDa increased at 0.5mg/L and 1.0mg/L methylparathion concentration (Fig 9a). In fishes exposed up to 45 days, the intensity of low molecular weight bands showed decreasing trend and is supported by tissues protein levels. The intensity of 34, 22 and 20 kDa fractions showed a decreasing trend substantiating the effect of methylparathion (Fig 10a).

The myofibrillar protein concentration continuously increased ($p<0.05$) with increasing concentration of methylparathion (Table 29). Beyond 30 days at lower concentrations of 0.25 and 0.5 mg/L a decreasing trend was noticed, but at higher concentration it continuously increased. Chronic studies in *L. rohita* fingerlings (Das and mukherjee, 2000) reported a reduction in muscle proteins on 30^{th} and 45^{th} days. The present study, with fishes of bigger size, does not support the finding of a fall in muscle proteins indicating, the possible influence of methylparathion at ribosomal level leading to the synthesis of structural proteins.

Table 28 : Changes in sarcoplasmic (mg g^{-1}) protein profile in muscle of *L.rohita* on sub-lethal concentrations of methylparathion on during the exposure period.

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	11.8 ± 1.8^{a}	11.8 ± 2.2^{a}	12.4 ± 3.4^{a}
2	0.25	11.9 ± 4.8^{a}	11.9 ± 3.2^{a}	12.8 ± 2.7^{a}
3	0.50	11.9 ± 4.4^{a}	11.9 ± 1.3^{a}	19.2 ± 3.2^{ab}
4	1.00	14.5 ± 1.9^{a}	14.5 ± 4.5^{a}	20.7 ± 4.7^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b) differ significantly (P<0.05Duncan's multiple range test).

Table 29 : Changes in myofirbrilar (mg g^{-1}) protein profile in muscle of *L.rohita* on sub-lethal concentrations of methylparathion on during the exposure period.

S.No	Conc (mg/L)	Duration		
		15 days	30 days	45 days
1	Control	27.8 ± 5.2^{a}	30.3 ± 6.7^{a}	28.6 ± 6.7^{a}
2	0.25	25.1 ± 3.0^{a}	39.6 ± 7.6^{a}	30.9 ± 7.9^{a}
3	0.50	29.6 ± 8.2^{a}	64.1 ± 16.2^{b}	69.8 ± 8.9^{b}
4	1.00	31.7 ± 2.8^{a}	71.5 ± 7.2^{b}	81.3 ± 21.2^{b}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b) differ significantly (P<0.05Duncan's multiple range test).

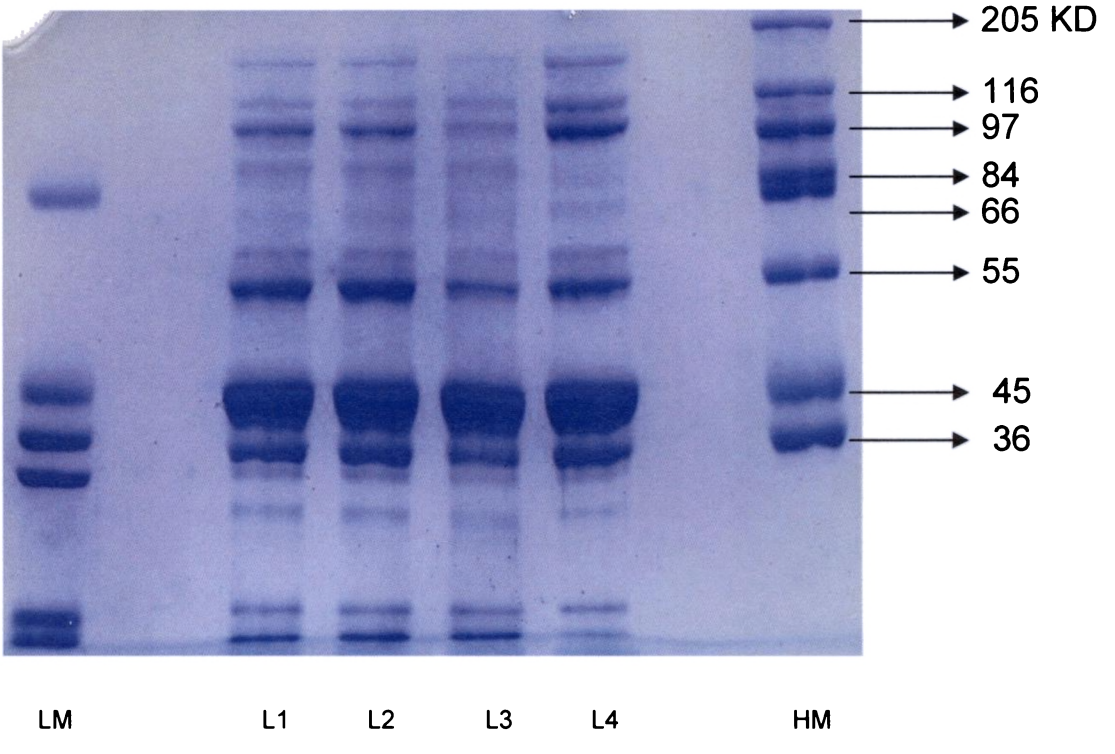


Fig - 8: SDS-PAGE of sarco plasmic protein profile in muscle of *L. rohita* on sublethal concentration of methyl parathion exposure after 15 days (LM- Low molecular weight, HM-High molecular weight, L1: Control, L2: 0.25mg/L; L3: 0.5mg/L; L4: 1.0 mg/L)

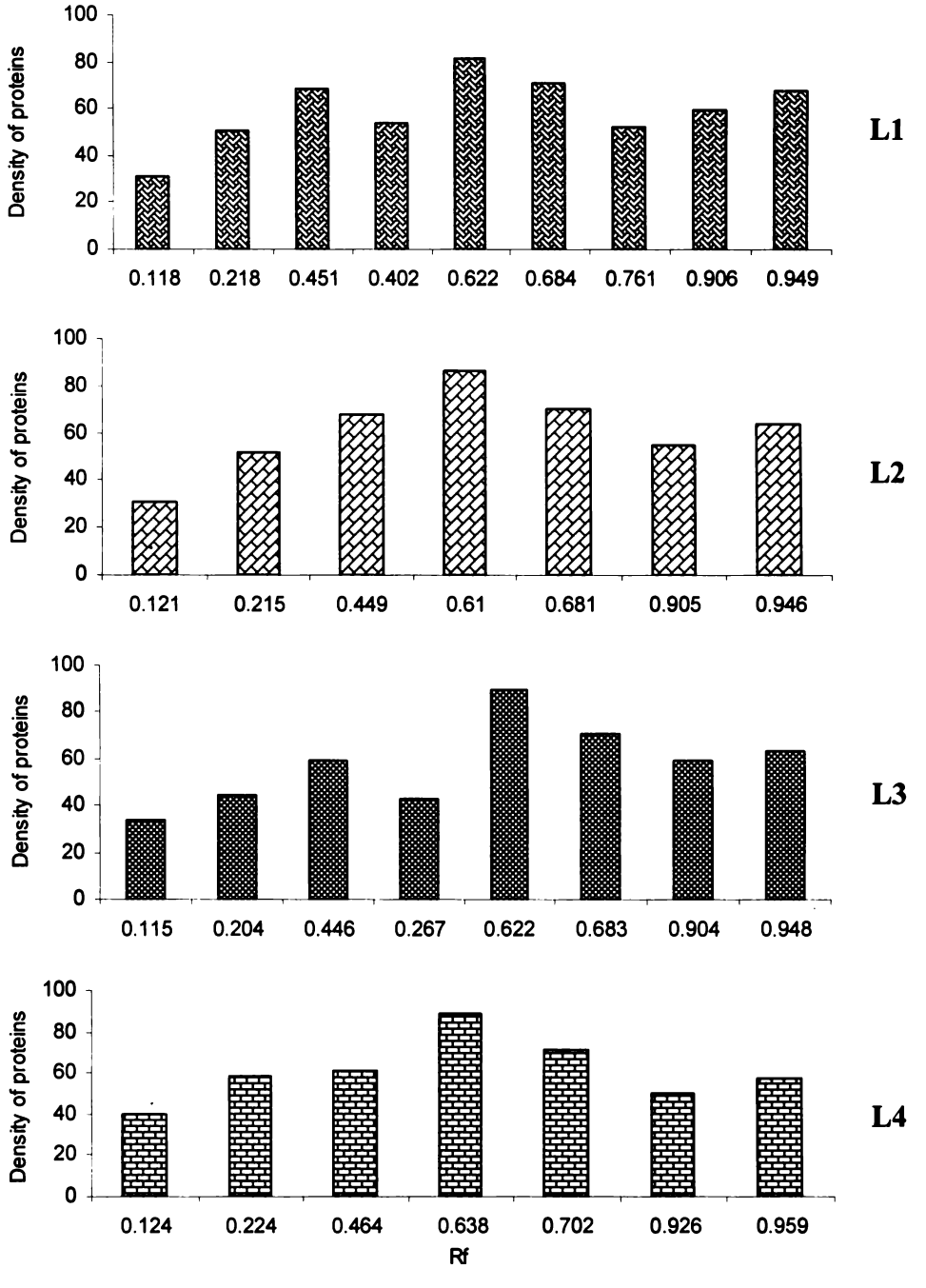


Fig – 8a: Densitometry analysis of sarco plasmic protein profile in muscle of *L. rohita* on sub-lethal concentrations of methyl parathion exposure after 15 days (L1: Control, L2: 0.25mg/L; L3: 0.5mg/L; L4: 1.0 mg/L)

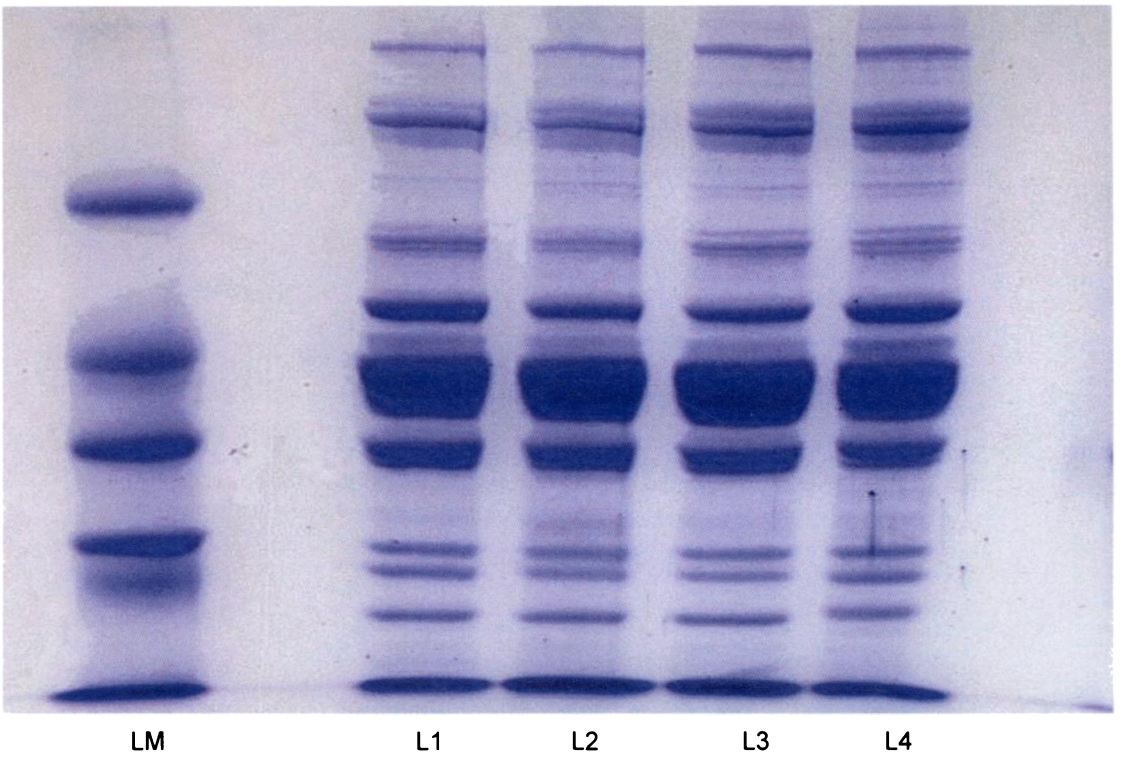


Fig 9: SDS-PAGE of sarco plasmic protein profile in muscle of *L. rohita* on sub-lethal concentration of methyl parathion exposure after 30 days (LM- Low molecular weight, HM-High molecular weight, L1: Control, L2: 0.25mg/L; L3: 0.5mg/L; L4: 1.0 mg/L)

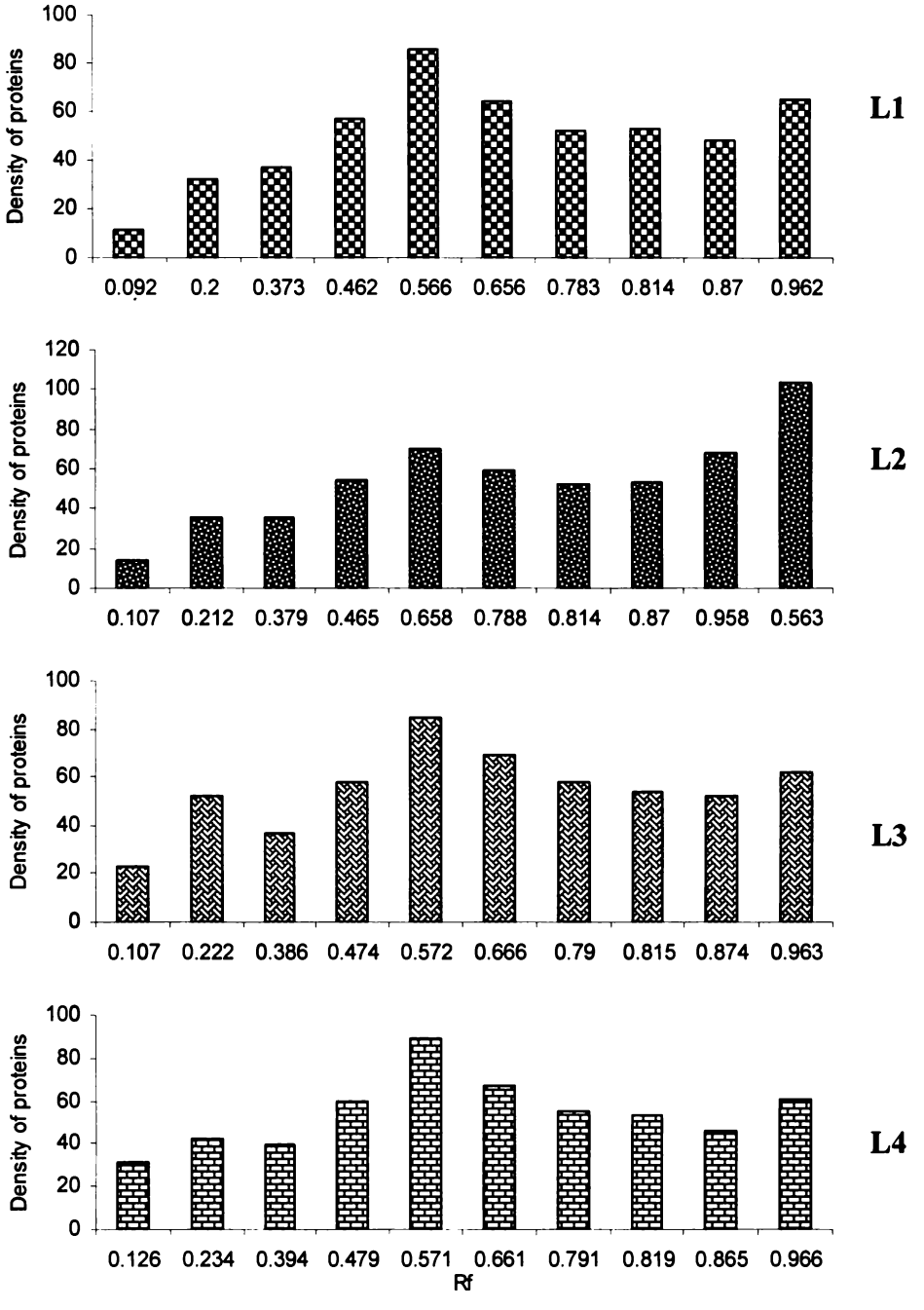


Fig -9a: Densitometry analysis of Sarco plasmic protein profile in muscle of *L. rohita* on sub-lethal concentrations of methyl parathion exposure after 30 days (L1- Control; L2- 0.25mg/L; L3-0.5mg/L; L 4- 1.0 mg/L)

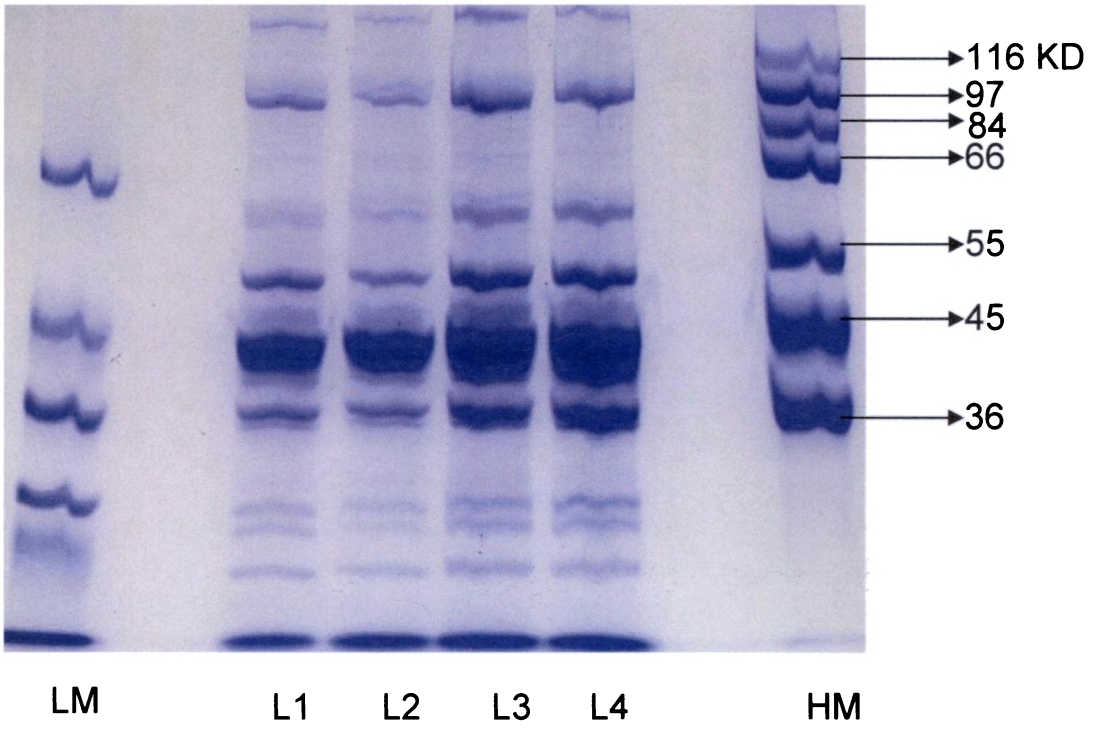


Fig 10: SDS-PAGE of sarco plasmic protein profile in muscle of *L. rohita* on sub-lethal concentration of methyl parathion exposure after 45 days (LM- Low molecular weight, HM-High molecular weight, L1: Control, L2: 0.25mg/L; L3: 0.5mg/L; L4: 1.0 mg/L)

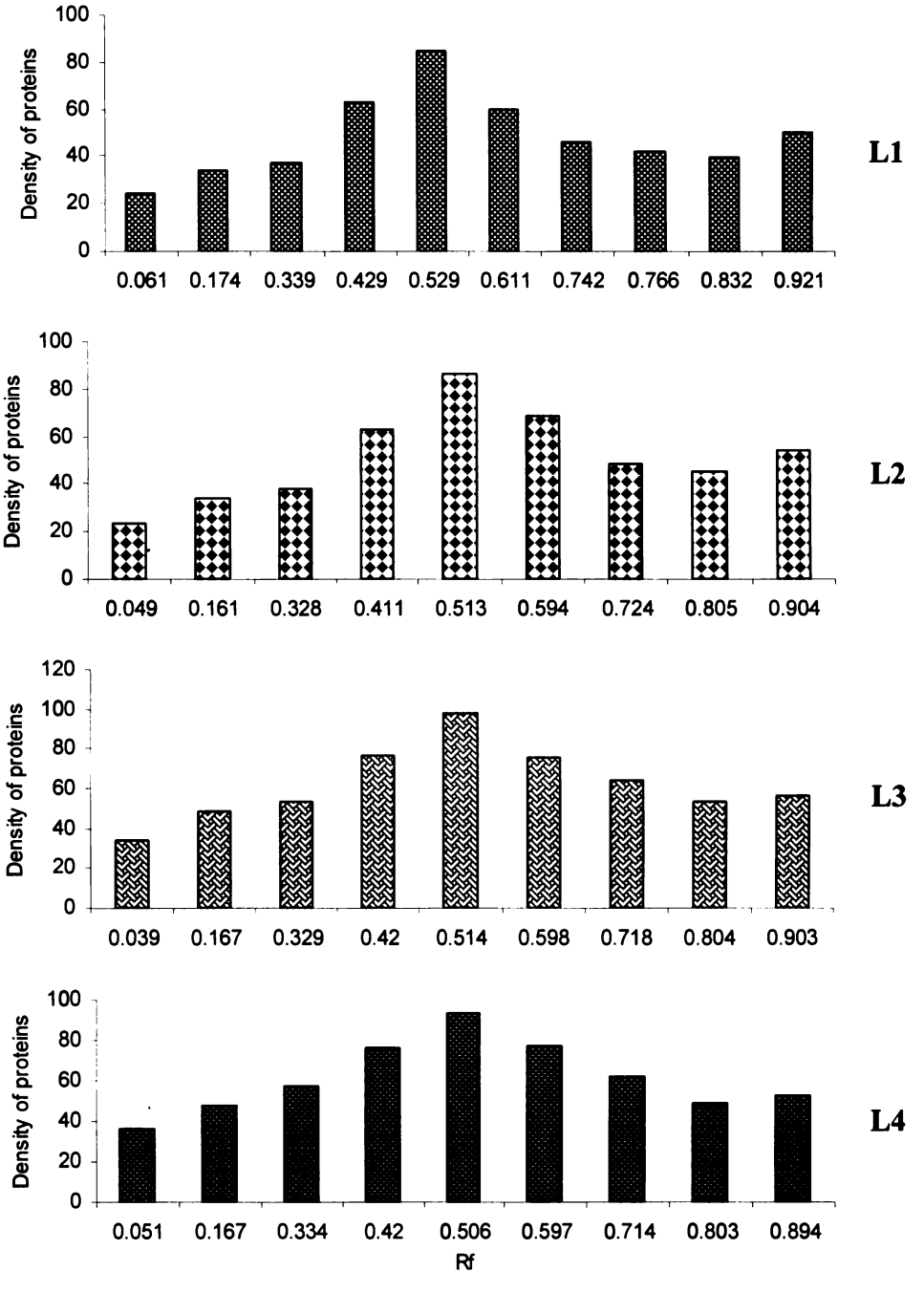


Fig -10a: Densitometry analysis of sarco plasmic protein profile in muscle of *L. rohita* on sub-lethal concentrations of methyl parathion exposure after 45 days (L 1- Control; L2- 0.25mg/L; L3-0.5mg/L; L4- 1.0 mg/L)

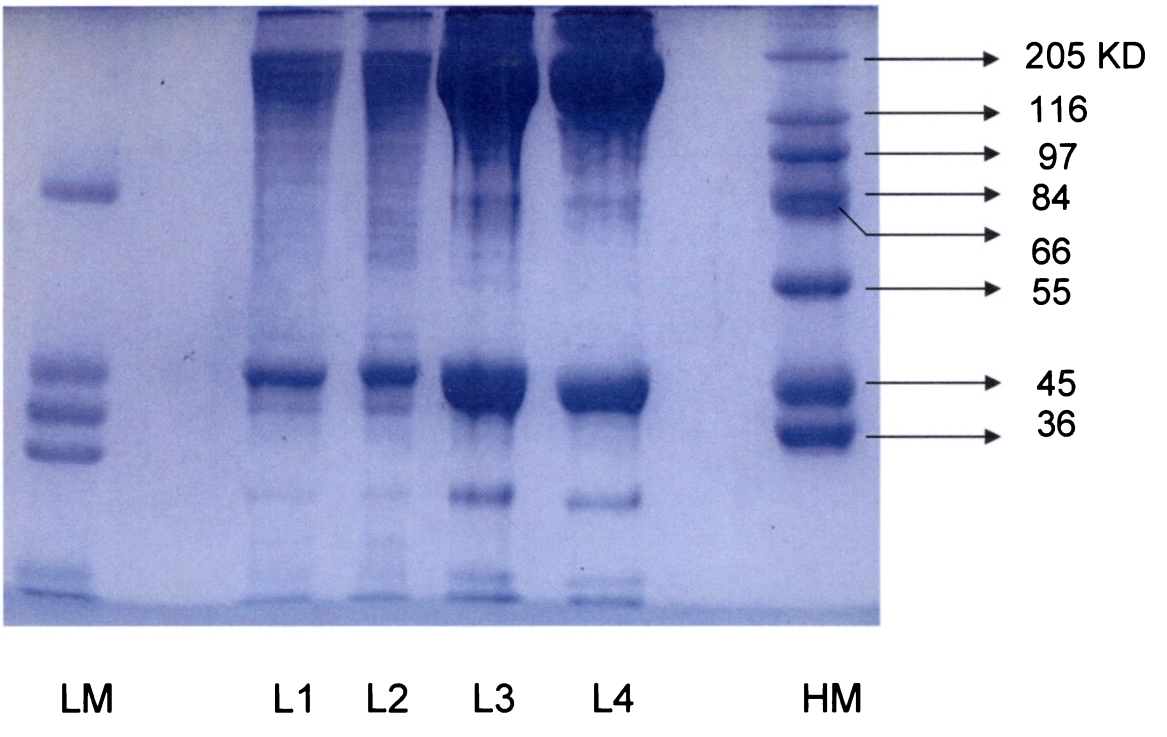


Fig 11: SDS-PAGE of myofibrillar protein profile in muscle of *L. rohita* on sub-lethal concentration of methyl parathion exposure after 15 days (LM- Low molecular weight, HM-High molecular weight, L1: Control, L2: 0.25mg/L; L3: 0.5mg/L; L4: 1.0 mg/L)

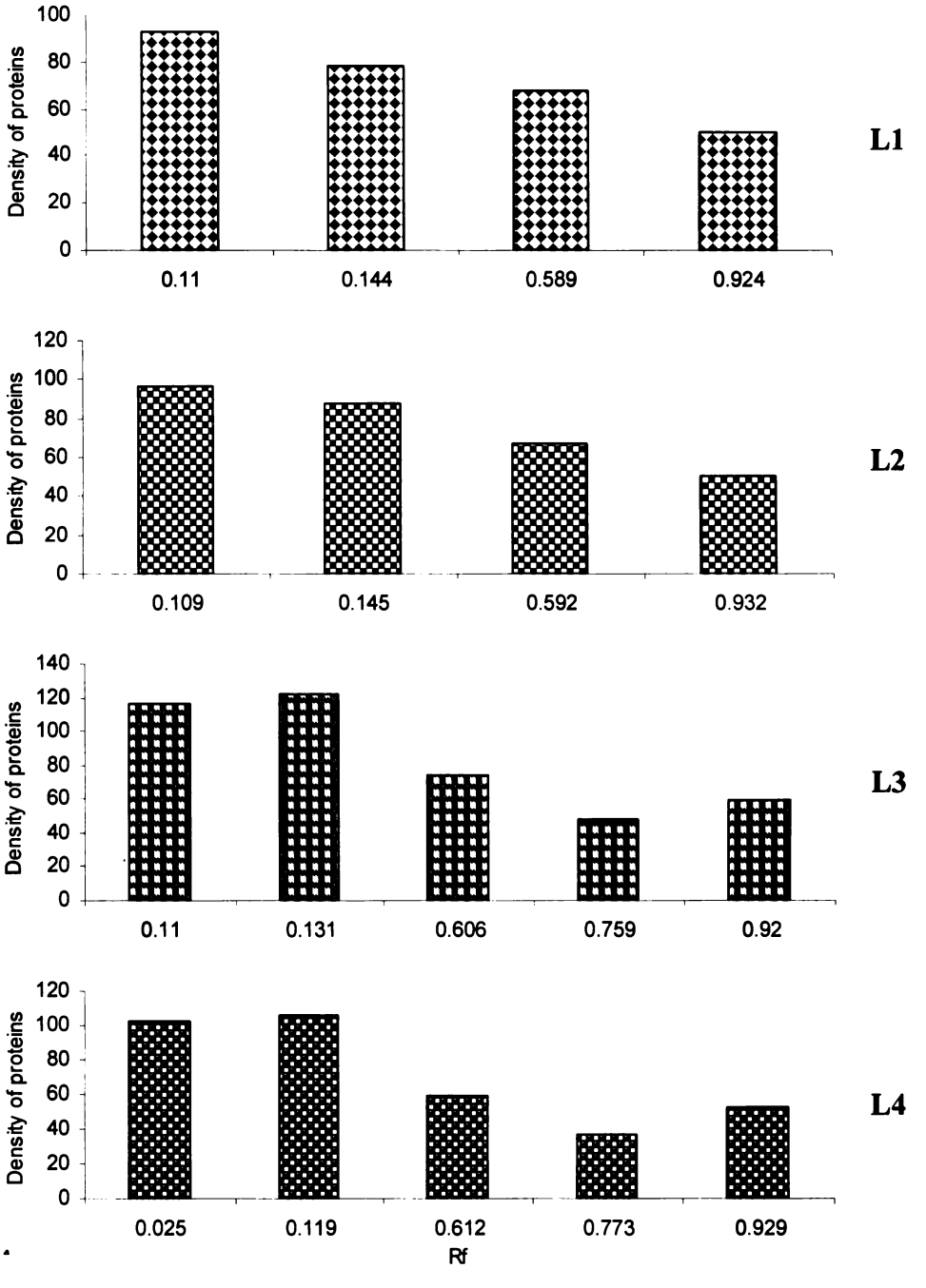


Fig -11a: Densitometry analysis of myofibrillar protein profile in muscle of *L. rohita* on sub-lethal concentrations of methyl parathion exposure after 15 days (L 1- Control; L2- 0.25mg/L; L3-0.5mg/L; L4- 1.0 mg/L)

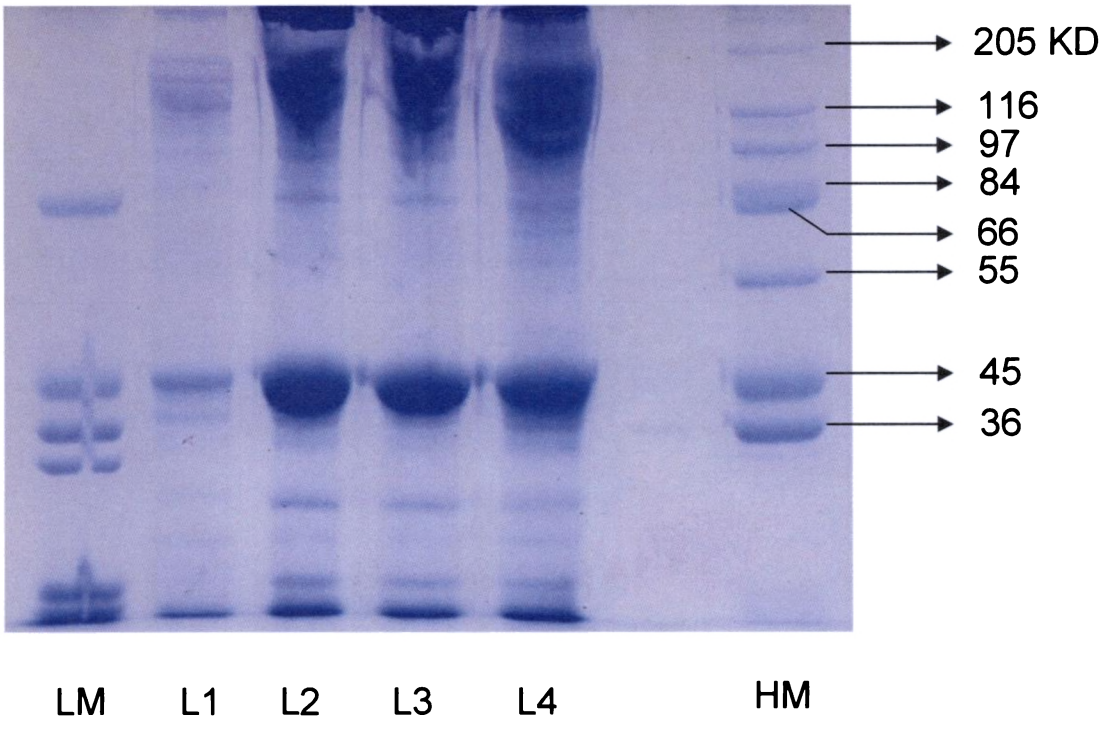


Fig 12: SDS-PAGE of myofibrillar protein profile in muscle of *L. rohita* on sub-lethal concentration of methyl parathion exposure after 30 days (LM- Low molecular weight, HM-High molecular weight, L1: Control, L2: 0.25mg/L; L3: 0.5mg/L; L4: 1.0 mg/L)

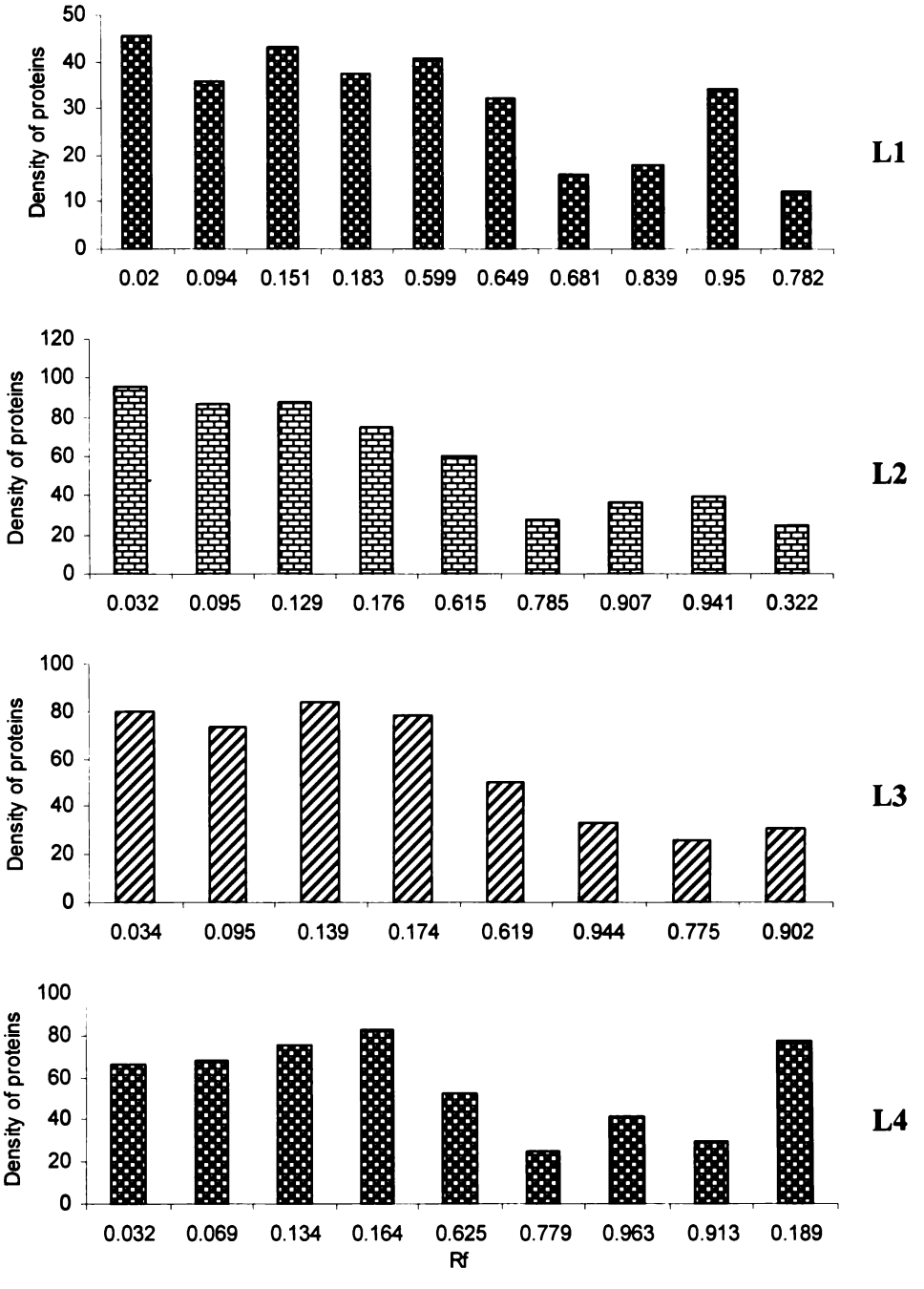


Fig -12a: Densitometry analysis of Myofibrillar protein profile in muscle of *L. rohita* on sub-lethal concentrations of methyl parathion exposure after 30 days (L1- Control; L2- 0.25mg/L; L3-0.5mg/L; L 4- 1.0 mg/L)

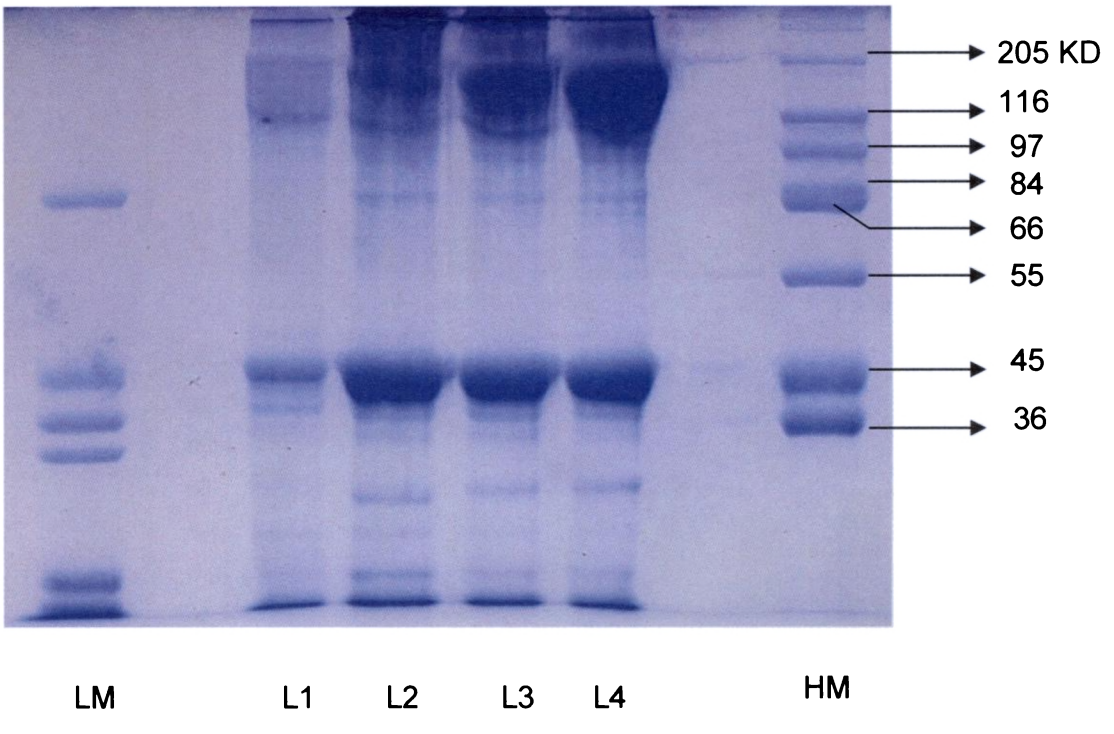


Fig 13: SDS-PAGE of myofibrillar protein profile in muscle of *L. rohita* on sub-lethal concentrations of methyl parathion exposure after 45 days (LM- Low molecular weight, HM-High molecular weight, L1: Control, L2: 0.25mg/L; L3: 0.5mg/L; L4: 1.0 mg/L)

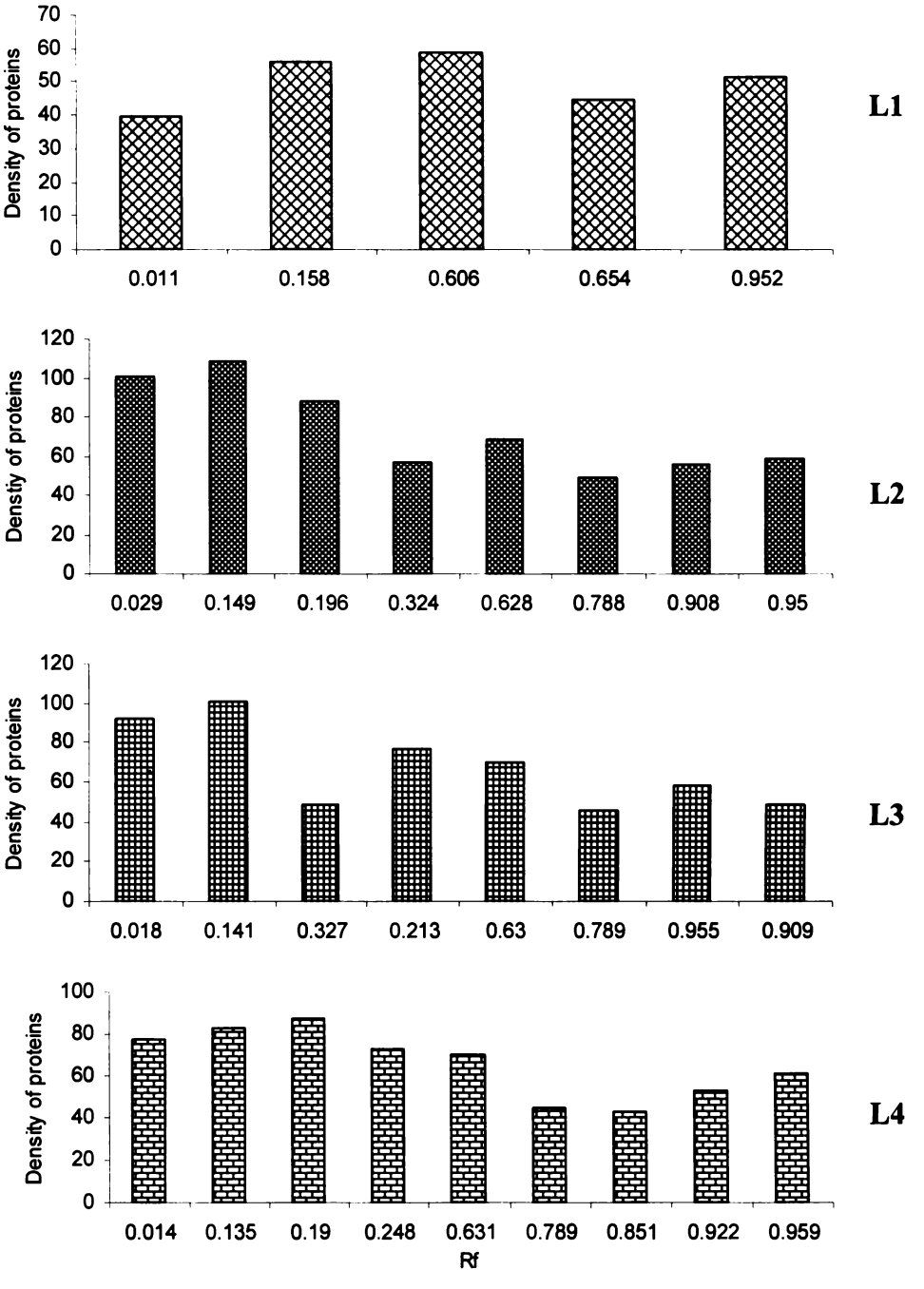


Fig -13a: Densitometry analysis of myofibrillar protein profile in muscle of *L. rohita* on sub-lethal concentrations of methyl parathion exposure after 45 days (L 1- Control; L2- 0.25mg/L; L3-0.5mg/L; L4- 1.0 mg/L)

The major myofibrillar protein bands (Fig 11a - 13a) noticed in the case of control is mainly myosin (205 kDa) and actin (45 kDa). Appearances of two low molecular weight fractions (30 kDa) were seen at methylparthion concentration of 0.5 and 1.0mg/L. The intensity of major bands increased up to 30 days, but decreased by 45 days. By the end of 45 days certain new protein subunits of molecular weights 15, 18 and 26 kDa could be seen appearing. This supports the fact that synthesis of certain stress induced proteins take place in response to methylparation intoxication.

The organophosphates are known to methylate and phosphorylate cellular proteins directly (Wild, 1975) and there are reports indicating increased protein levels in brain, liver and ovary on exposure to pesticides (Narayan Ram and Satyanesan, 1986). Sahib *et al.*, (1984) reported an increase in the protein content in all tissues of malathion exposed *Tilapia mossambicas*, suggesting that pesticides may compensate any possible protein loss by increasing tissue protein synthesis. Similarly increase in liver proteins in *Brabs concnonius* was reported and was attributed to increased protein turnover (Gill *et al.*, 1991). Protein synthesis increases to compensate the protein loss, as a result of tissue necrosis and to meet the increased demand to detoxify the pesticides. The protein study indicated possible damage to tissues on long-term exposure leading to increased production of enzymes. This is reflected in the increased sarcoplasmic protein levels.

4.12. Alteration of lipids in different organs

At sub lethal concentration of methylparathion there were changes in the lipid components. On 15^{th} day, the cholesterol content in liver tissues did not show

any significant change at a pesticide concentration of 0.25 mg/L compared to control. Long term exposure, however, decreased the content up to 0.5 mg/L, but showed an increasing trend on exposure to 1.0 mg/L for 45 days (Table 30).

In brain tissues the cholesterol content showed a marginal increase with increase in the methylparathion concentration for 15 days. Long term exposure at concentration of 0.5 mg/L, showed an increase up to 30 days and decreased by 45th day. At 1.0 mg/L concentration the cholesterol content increased through out the exposure period. In muscle tissue the cholesterol content more or less remained constant up to 0.5 mg/L, but increased by about 62% at 1.0 mg/L. The cholesterol ester marginally increased at all concentrations exposed for 15 days in all the tissue studied. The concentration of cholesterol ester showed a decreasing trend at the end of 45 days of exposure, in liver and muscle tissue, but in brain tissue a reverse trend was noticed. The triglycerides content in brain tissues were found to be higher than in the other two tissues (10mg/g against 1-2 mg/L). The triglycerides content also showed a decreasing trend in liver and muscle tissues on 15 days exposure, but on increasing the duration of exposure it showed a decrease in liver, but in brain tissues the level increased up to 30 days of exposure and then decreased (Table 31).

Among the phospholipids studied, the phosphatidyl ethylamine in brain and liver showed an increasing trend on 15 days exposure, where as in muscle tissues a decrease was noticed at all concentrations exposed. However, with increased duration of exposure increase in the phosphatidyl ethylamine was

noticed at higher concentrations viz., 0.5 mg/L and 1.0 mg/L except in muscle where not much of a change was noticed (Table 31).

Phosphatidyl choline showed an increasing trend in liver and muscle tissues, but in brain a decrease was noticed on exposure to different concentrations for 15 days. On exposure to sub lethal concentration up to 45 days the phosphatidyl choline content decreased up to a methylparathion concentration of 0.5 mg/L but at 1.0 mg/L, the concentration of phospholipids showed an increasing trend. Spingomyeline, which is an important component of brain tissue, showed a significant increase in the tissue on exposure to 1.0 mg/L of methylparathion. Lyso phosphatidyl choline showed a concentration dependent decrease in the liver cells, but increasing trend in brain and muscle tissues were noticed on long-term exposure.

These finding strongly suggest alteration taking place in lipid metabolism and in major organs namely liver, brain and muscle. The strong lipophilicity of methyparathion promotes its incorporation into membrane systems and therefore the crucial functions of the membranes are affected (Lopes *et al.*, 1997). There are reports indicating lipid turnover in vital organs to combat the negative influence of chance contaminant on tissue systems. Therefore, the physical perturbations at the boundary lipids in membrane may also affect the AChE activity in brain cells and thus affect the lipid metabolism (Gurushankara *et al.*, 2006; Vijayavel and Balasubramanian, 2006; Bhalchandra and Lomte, 2004; Richards and Kendall, 2002; Lopes *et al.*, 1997; Antunes-madeira *et al.*, 1994; Antunes-madeira and Maderia, 1993; Hall and Henry, 1992; Sivaramakrishna *et al.*, 1992; Antunes-madeira *et al.*,

Table 30 : Alteration in cholesterol, cholesterol ester and triglycerides (mg g^{-1}) content of tissue in *L.rohita.*

Tissue	Conc. (mg/L)	Cholesterol			Cholesterol ester			Triglycerides		
		Duration			Duration			Duration		
		15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
liver	Control	1.23 ± 0.2^{a}	0.82 ± 0.1^{a}	0.85 ± 0.20^{a}	0.07 ± 0.01^{a}	0.12 ± 0.02^{ab}	0.08 ± 0.01^{a}	2.86 ± 0.3^{a}	2.23 ± 0.75^{ab}	1.65 ± 0.26^{a}
	0.25	1.51 ± 0.3^{ab}	0.86 ± 0.1^{a}	0.83 ± 0.02^{a}	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}	0.08 ± 0.01^{a}	3.46 ± 0.1^{b}	2.93 ± 0.70^{b}	1.72 ± 0.24^{a}
	0.50	1.35 ± 0.2^{ab}	3.06 ± 0.6^{b}	1.54 ± 0.23^{a}	0.46 ± 0.09^{b}	0.12 ± 0.02^{ab}	0.13 ± 0.02^{b}	3.39 ± 0.5^{ab}	1.69 ± 0.30^{a}	1.63 ± 0.43^{a}
	1.00	1.81 ± 0.4^{b}	3.43 ± 0.9^{b}	4.18 ± 0.70^{b}	0.55 ± 0.04^{b}	0.16 ± 0.03^{a}	0.17 ± 0.01^{c}	1.88 ± 0.1^{c}	2.33 ± 0.40^{ab}	1.87 ± 0.61^{a}
Brain	Control	1.22 ± 0.3^{a}	1.12 ± 0.3^{a}	1.02 ± 0.1^{a}	0.18 ± 0.03^{a}	0.25 ± 0.01^{a}	0.23 ± 0.02^{a}	11.20 ± 0.3^{a}	10.0 ± 1.3^{a}	09.4 ± 1.8^{a}
	0.25	1.66 ± 0.6^{a}	1.77 ± 0.4^{b}	1.22 ± 0.1^{a}	0.16 ± 0.03^{a}	0.37 ± 0.01^{b}	0.27 ± 0.01^{a}	11.28 ± 0.2^{a}	11.4 ± 1.9^{ab}	09.5 ± 1.6^{a}
	0.50	1.94 ± 0.3^{a}	2.02 ± 0.4^{b}	1.77 ± 0.6^{a}	0.21 ± 0.01^{a}	0.44 ± 0.02^{c}	0.47 ± 0.01^{b}	11.68 ± 0.8^{a}	14.7 ± 3.6^{bc}	02.5 ± 0.9^{b}
	1.00	5.70 ± 1.5^{b}	7.70 ± 0.3^{c}	10.1 ± 1.9^{b}	0.33 ± 0.05^{b}	0.41 ± 0.01^{d}	0.74 ± 0.04^{c}	13.80 ± 0.9^{b}	16.9 ± 1.3^{c}	02.7 ± 0.3^{b}
Muscle	Control	0.99 ± 0.03^{a}	1.20 ± 0.20^{a}	0.86 ± 0.03^{a}	0.25 ± 0.03^{a}	0.20 ± 0.01^{ab}	0.25 ± 0.03^{a}	1.02 ± 0.1^{a}	1.20 ± 0.3^{a}	1.10 ± 0.3^{a}
	0.25	0.90 ± 0.04^{a}	1.93 ± 0.09^{b}	0.79 ± 0.02^{a}	0.26 ± 0.02^{ab}	0.21 ± 0.02^{b}	0.28 ± 0.01^{a}	1.07 ± 0.3^{a}	1.23 ± 0.2^{a}	1.31 ± 0.1^{ab}
	0.50	0.90 ± 0.03^{a}	0.78 ± 0.20^{c}	1.40 ± 0.05^{b}	0.33 ± 0.04^{c}	0.19 ± 0.03^{ab}	0.18 ± 0.01^{b}	0.81 ± 0.2^{a}	0.78 ± 0.2^{b}	1.70 ± 0.3^{b}
	1.00	1.60 ± 0.09^{b}	0.16 ± 0.02^{d}	1.40 ± 0.11^{b}	0.31 ± 0.01^{bc}	0.16 ± 0.02^{a}	0.29 ± 0.03^{a}	0.72 ± 0.1^{a}	0.16 ± 0.1^{b}	1.30 ± 0.2^{ba}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significatly (P<0.05Duncan's multiple range test).

Table 31 : Alteration in phosphatidylethylamine, phosphatidyl choline and phosphatidyl inosital (mg g^{-1}) content of tissue in *L.rohita.*

Tissue	Conc. (mg/L)	Phosphatidylethylamine			Phosphatidyl choline			Phosphatidyl inosital		
		Duration			Duration			Duration		
		15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
Liver	Control	2.01 ± 0.2^{a}	2.20 ± 0.3^{a}	1.99 ± 0.3^{a}	0.55 ± 0.3^{a}	0.49 ± 0.06^{a}	0.27 ± 0.05^{a}	0.45 ± 0.02^{a}	0.43 ± 0.03^{a}	0.36 ± 0.06^{a}
	0.25	2.25 ± 0.3^{a}	2.56 ± 0.6^{a}	2.01 ± 0.6^{a}	0.69 ± 0.2^{a}	0.50 ± 0.17^{a}	0.27 ± 0.01^{a}	0.46 ± 0.04^{a}	0.49 ± 0.03^{a}	0.17 ± 0.03^{b}
	0.50	2.04 ± 0.4^{a}	7.41 ± 1.9^{b}	3.68 ± 0.7^{ab}	0.51 ± 0.1^{a}	2.47 ± 0.28^{b}	2.81 ± 0.90^{b}	0.76 ± 0.01^{b}	0.73 ± 0.15^{b}	0.49 ± 0.09^{c}
	1.00	3.63 ± 0.9^{b}	7.00 ± 0.9^{b}	5.04 ± 1.6^{b}	3.04 ± 0.2^{b}	2.59 ± 0.20^{b}	2.22 ± 0.50^{b}	0.89 ± 0.10^{c}	0.58 ± 0.13^{ab}	0.96 ± 0.04^{d}
Brain	Control	3.2 ± 0.7^{a}	2.6 ± 0.5^{a}	3.6 ± 0.6^{a}	25.3 ± 2.6^{a}	22.6 ± 1.6^{a}	25.6 ± 2.3^{a}	09.3 ± 0.3^{a}	11.2 ± 2.3^{a}	13.9 ± 1.2^{a}
	0.25	3.9 ± 0.6^{a}	2.2 ± 0.6^{a}	4.4 ± 0.5^{ab}	27.1 ± 2.2^{a}	23.8 ± 6.6^{a}	24.8 ± 6.6^{a}	08.8 ± 0.5^{a}	12.1 ± 2.8^{a}	18.6 ± 2.6^{a}
	0.50	2.7 ± 0.8^{a}	1.6 ± 0.7^{a}	5.2 ± 0.7^{b}	22.1 ± 4.8^{a}	25.2 ± 1.1^{a}	26.3 ± 1.1^{a}	10.6 ± 3.2^{a}	18.9 ± 1.8^{b}	19.1 ± 3.8^{a}
	1.00	7.7 ± 0.6^{b}	4.1 ± 0.3^{b}	5.5 ± 0.4^{b}	23.5 ± 5.1^{a}	28.1 ± 8.3^{a}	28.1 ± 6.3^{a}	09.3 ± 2.1^{a}	20.4 ± 4.7^{b}	15.2 ± 3.7^{a}
Muscle	Control	0.96 ± 0.03^{a}	0.86 ± 0.02^{a}	0.79 ± 0.01^{a}	0.65 ± 0.01^{a}	0.69 ± 0.02^{ab}	0.71 ± 0.09^{a}	0.10 ± 0.01^{a}	0.09 ± 0.02^{a}	0.1 ± 0.01^{a}
	0.25	1.01 ± 0.01^{b}	0.79 ± 0.17^{a}	0.79 ± 0.08^{a}	0.71 ± 0.06^{a}	0.61 ± 0.09^{a}	0.79 ± 0.04^{a}	0.25 ± 0.02^{b}	0.11 ± 0.01^{a}	0.4 ± 0.01^{b}
	0.50	0.72 ± 0.02^{c}	1.66 ± 0.23^{b}	0.89 ± 0.04^{a}	1.27 ± 0.04^{b}	0.72 ± 0.04^{bc}	1.21 ± 0.01^{b}	1.34 ± 0.06^{c}	0.45 ± 0.02^{b}	1.4 ± 0.02^{c}
	1.00	0.16 ± 0.01^{d}	1.86 ± 0.32^{b}	1.80 ± 0.62^{b}	0.31 ± 0.01^{c}	0.16 ± 0.02^{c}	0.29 ± 0.03^{a}	0.72 ± 0.10^{d}	0.16 ± 0.10^{c}	1.3 ± 0.2^{d}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significantly (P<0.05Duncan's multiple range test).

Table 32 : Alteration in sphingomylein and lyso phosphatidyl choline (mg g^{-1}) content of tissue in *L.rohita.*

Tissue	Conc. (mg/L)	Sphingomylein			Lyso phosphatidyl choline		
		Duration			Duration		
		15 days	30 days	45 days	15 days	30 days	45 days
Liver	Control	0.30 ± 0.04^{a}	0.27 ± 0.03^{a}	0.20 ± 0.01^{a}	0.43 ± 0.01^{a}	0.53 ± 0.02^{a}	0.40 ± 0.06^{a}
	0.25	0.38 ± 0.05^{a}	0.31 ± 0.03^{a}	0.22 ± 0.03^{ab}	0.53 ± 0.03^{b}	0.58 ± 0.06^{a}	0.39 ± 0.03^{a}
	0.50	0.39 ± 0.07^{a}	0.62 ± 0.02^{b}	0.30 ± 0.08^{b}	0.21 ± 0.08^{c}	0.71 ± 0.05^{b}	0.52 ± 0.04^{b}
	1.00	0.38 ± 0.01^{a}	0.62 ± 0.04^{b}	0.41 ± 0.02^{c}	0.11 ± 0.05^{d}	0.43 ± 0.02^{c}	1.85 ± 0.02^{c}
Brain	Control	0.52 ± 0.01^{a}	0.56 ± 0.2^{a}	0.5 ± 0.03^{a}	1.1 ± 0.1^{a}	1.4 ± 0.04^{a}	1.2 ± 0.2^{a}
	0.25	0.56 ± 0.05^{ab}	0.51 ± 0.1^{a}	0.5 ± 0.01^{a}	1.6 ± 0.3^{ab}	1.5 ± 0.09^{a}	1.1 ± 0.1^{a}
	0.50	0.74 ± 0.18^{bc}	0.79 ± 0.1^{a}	0.7 ± 0.09^{a}	2.0 ± 0.4^{b}	3.3 ± 0.10^{b}	2.9 ± 0.1^{b}
	1.00	0.76 ± 0.25^{c}	2.24 ± 0.2^{b}	3.3 ± 0.60^{b}	2.2 ± 0.6^{b}	3.6 ± 0.20^{c}	3.9 ± 0.3^{c}
Muscle	Control	0.075 ± 0.01^{a}	0.071 ± 0.01^{a}	0.065 ± 0.03^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}
	0.25	0.085 ± 0.01^{a}	0.082 ± 0.03^{a}	0.085 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.10 ± 0.01^{b}
	0.50	0.100 ± 0.04^{a}	0.120 ± 0.03^{a}	0.230 ± 0.02^{b}	0.04 ± 0.01^{a}	0.08 ± 0.01^{b}	0.10 ± 0.02^{b}
	1.00	0.590 ± 0.03^{b}	0.491 ± 0.04^{b}	0.320 ± 0.02^{c}	0.13 ± 0.03^{b}	0.06 ± 0.01^{c}	0.06 ± 0.02^{c}

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significantly (P<0.05Duncan's multiple range test).

1991; Antunes-madeira *et al.*, 1990; Srinivasulu Reddy and Ramana Rao, 1989; Reddy and Rao, 1987; Rosas *et al.*, 1980).

4.13. Histopathological observations in sub lethal level

Histopathological studies in gill tissues at sub lethal levels of methylparathion revealed that at a concentration of 0.25mg/L no significant changes could be observed at 15, 30 and 45 days. But at concentrations of 0.5 and 1.0 mg/L, congestion of primary lamella, damaged secondary lamella, edema and cell necrosis were observed after 45 days exposure (Fig 24 & 25).

The changes were indicative of diminished oxygen supply to the methylparathion exposed fish, resulting in hypoxic respiratory responses. Gill damage caused by environmental pollutants is important from the aspect of morbidity, as it retards growth and affects reproduction. Richmonds and Dutta (1989) divided the commonly reported gill lesions into two groups viz., the direct deleterious effects of the irritants and the defense responses of the fish. The observed epithelial necrosis and exudation of the gill epithelium are direct responses induced by the action of methylparathion. The defense responses noticed are lifting up of the gill epithelium, hyperplasia, lamellar fusion and clavate lamellae. The lifting up of the epithelium and hyperplasia increases the distance through which the toxicant travel to reach the blood stream. However, a change in the distance from the outer epithelium of the lamellae to the nearest blood lacuna affects the gas-exchange capabilities of a fish (Lease *et al.*, 2003).

Several authors have reported histopathological changes in the gill tissue of fish exposed to miscellaneous pesticides (Lowe, 1964; Eller, 1971; Jauch,

1979; Nowak, 1992; Rijijohn and Jayabalan, 1993). In most cases the changes were characterized by damage in the epithelial cells, hyperplasia and lamellar swelling. The diagnostic values of these observations are limited by the fact that similar changes have been recorded in fish from natural environments, and have been reported to be caused by bacterial and gill diseases, ammonia toxicity and malnutrition (Ribelin and Walsh, 1975). The changes in gill tissues found in the present experiments were mild to moderate congestion of the primary lamellae and hyperplasia of branchial plates (0.35 mg/L exposure).

Histopathological changes were observed in liver of *L. rohita* exposed to methylparathion for 15, 30 and 45 days, In fishes exposed to a concentration of 1.0 mg/L for 45 days, the hepatocytes have lost their normal architecture and hyperplasic cells were crowded. At 0.5 mg/L exposed for 30 days, focal necrosis of hepatocytes was observed (Fig 26,27). Generally in liver tissue the heptocytes have lost their normal architecture and a large number of these cells appeared with pyknotic nuclei. Hypertrophy of hepatocytes is a closely related condition that occurs under chronic toxicity (Myers *et al.*, 1987; Kent *et al.*, 1988). Cellular hypertrophy is associated with exposure to pesticides such as lindane and arochlor 1,2,5,4 (Hinton *et al.*, 1988; Klaunig *et al.*, 1979). The histopathological alterations resulting from exposure to methylparathion lead to a reduction in the functional efficiency of the liver, leading to malfunctioning of several organ systems in the fish.

Fenvalerate induced many histopathological changes in the liver of catfish (*Clarias gariepinus*) such as cytoplasmic vacuolization of the hepatic cells,

inflammatory leucocytic infiltrations, and congestion of blood vessels, necrosis and fatty infiltrations. Similarly it was also found that exposing 7 day old larvae of the fish *Sarcamento splittail* to sub lethal concentrations of esfenvalerate for one week induced vacuolar degeneration and cell necrosis in the liver (Teh *et al.*, 2005).

The effect of insecticides on the liver of different fish species were studied by many investigators. Mandal and Kulshrestha, (1980) studied the effects of sub lethal concentration of sumithion on liver, kidneys and intestine of *Clarias batachus* and observed liver necrosis, vacuolization and breakdown of the cell boundaries. Further vacuolization of epithelial cell of uriniferous tubules and degeneration of the glomeruli in the kidney, lesion formation in the villi and enlargement of mucous cells in the intestine were noticed.

At the initial stage of intoxication, necrosis and vacuolization of hepatocytes were noticed in the liver of *Tilapia mossambica* exposed to the organophosphate monocrotophos (Desai *et al.*, 1984), while fatty acid degeneration was observed on prolonged exposure. Treatment with endrin produced acute pathological changes in the liver of *Channa punctatus* (Sastry and Sharma, 1979). The effect of malathion on the fish *Oreochromis niloticus* and their results showed that this insecticide induced many histopathological changes and lipidosis in the liver and gills of the fishes, like hemorrhage, necrosis and destruction of lamellae of the lungs, and necrosis in the liver (Elezaby *et al.*, 2001). The organophosphorous insecticide (Hostathion) also produced almost similar effect on the liver of the catfish (*Clarias gariepinus*) (Sakr *et al.*, 2001).

Hyperplasia, edema, necrosis, focal necrosis and increase in brain cells were some of the changes noticed in brain tissue of *L. rohita* at sub lethal concentrations of methylparathion. Brain tissue necrosis was observed in 0.5 and 1.0 mg/L pesticide exposed fishes at 45 days (Fig 28, 29). Focal pyknosis of the cytoplasm was observed in the cortex cerebri and the cerebellum. Ludke *et al.*, (1972) reported changes occurring in the purkinje cells in the cerebellum, concentration of the cytoplasm of signal pyramidal cells of CA3 hippocampus layer, and focal pyknosis of the heurocytes of neuclei in lateralis hypotahalami and the cerebral cortex, in rats exposed to alpha cypermethrin. This study, also demonstrated the development of ischemia and pyknosis of the cytoplasm of the neurons in the brain tissues of *L. rohita* exposed to methylparathion. Based on the results and literature data, there is no doubt that methylparathion exerts a neurotoxic effect which is manifested by an inhibition of AChE activity and impairment of neural conductivity in the central and peripheral nervous system.

According to Moore (1985), cellular structures or organelles become damaged after exposure to various pollutants, through various mechanisms. The activation of organic xenobiotics to a more toxic form is the usual mechanism. In fish and molluscs, organophosphorus pesticides toxicity is acquired after their enzymatic oxidation (Hamm *et al.*, 2001; Ludke *et al.*, 1972; Pena-Llopis *et al.*, 2002, 2003). It is reported that MFO system oxidizes the organophosphorus P = S bond replacing the sulphur atom with oxygen (Ecobichon, 1991). It has also been demonstrated that this oxidative desulfuration of organophosphorus pesticides is mediated by MFO and generates ROS (Laval, 1996; Varanka *et al.*, 2002). In turn, ROS can induce

damage in fish liver and molluscs digestive gland (Pena-Llopis *et al.*, 2002 and 2003).

The present study suggests that the hepatopancreatic damages induced by methyl parathion in *L. rohita* is due to LPO of hepatopancreatocytic membranes, as a consequence of the oxidative stress generated after the pesticide oxidative biotransformation mediated by the MFO system. The findings of the present histological investigations demonstrate a direct correlation between pesticide exposure and histopathlogical disorders observed in gills, liver and brain tissues.

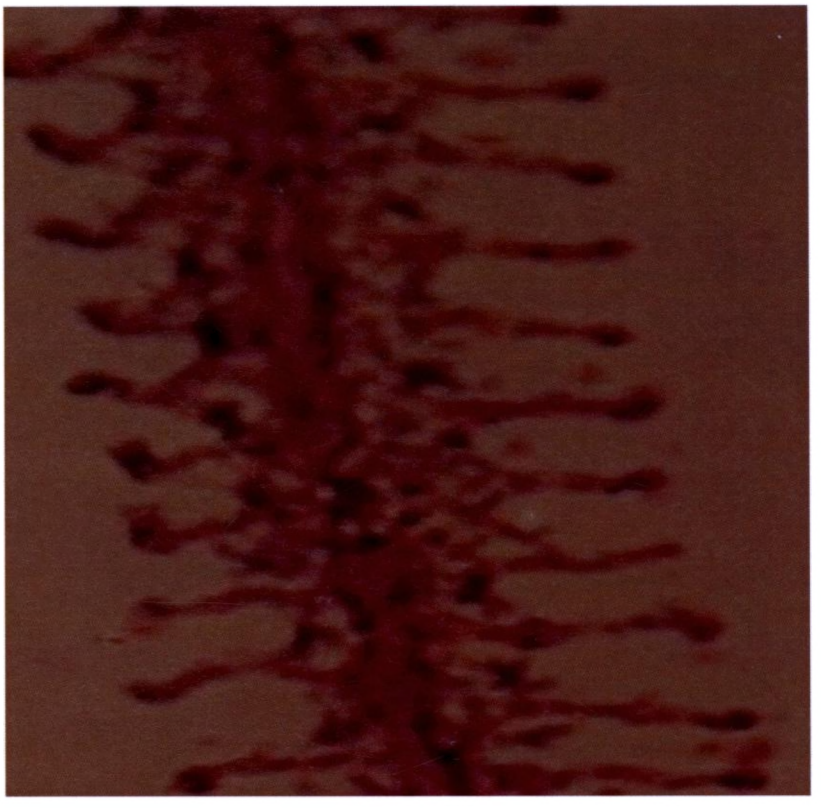


Fig 24: The architecture of control fish gills (Hemotoxyline and Eosin 100x)

Fig 25: The architecture of fish gills of *Labeo rohita* after sublethal exposure to methylparathion, which shows shorting of gills at 1.0 mg/L, 45 days exposure (Hemotoxyline and Eosin 100x).

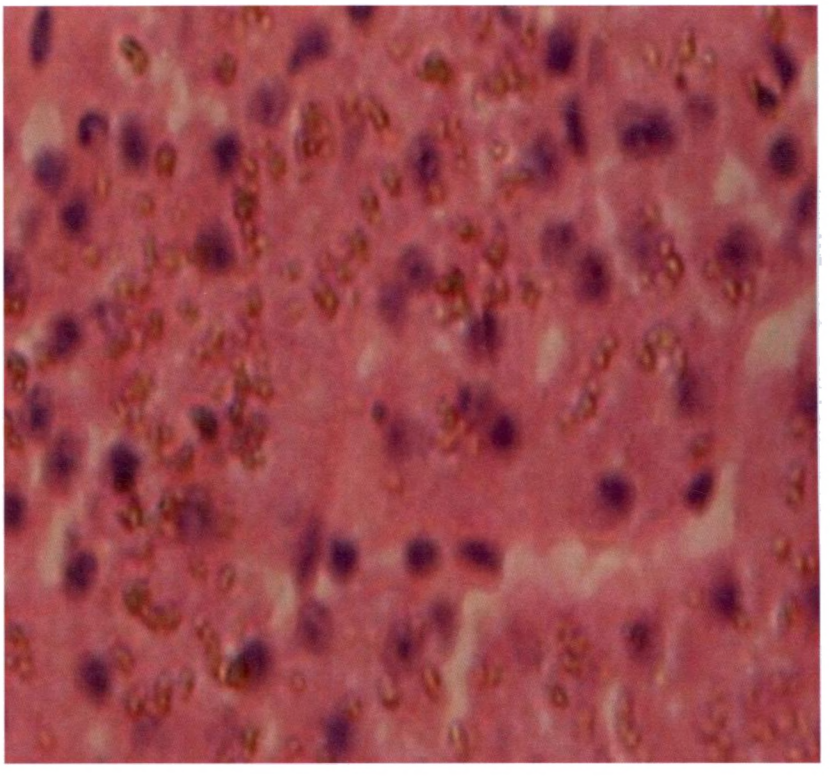


Fig 26: The architecture of control fish liver (Hemotoxyline and Eosin 100x)

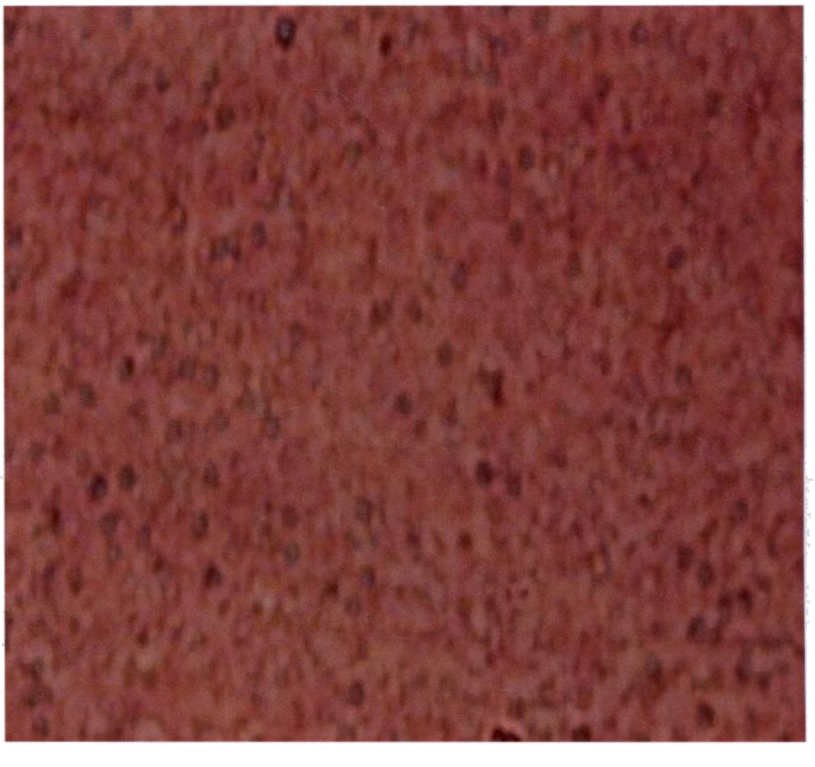


Fig 27: The architecture of fish liver of *Labeo rohita* after sublethal exposure to methylparathion, which shows liver cells are crowded at 0.5mg/l, 30 days (Hemotoxyline and Eosin 100x).

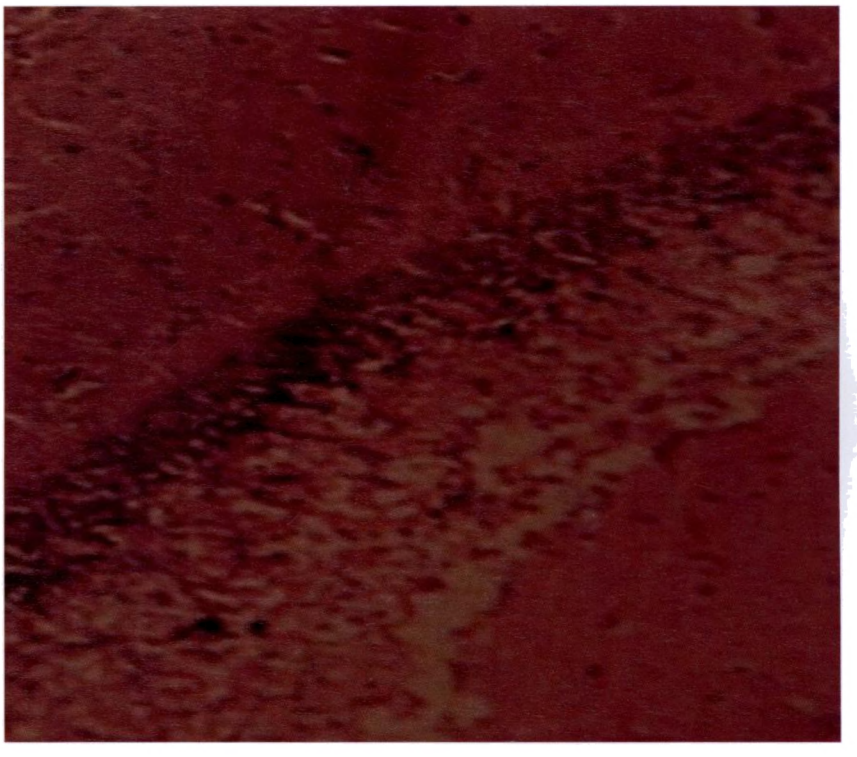


Fig 28:The architecture of control fish brain (Hemotoxyline and Eosin 100x)

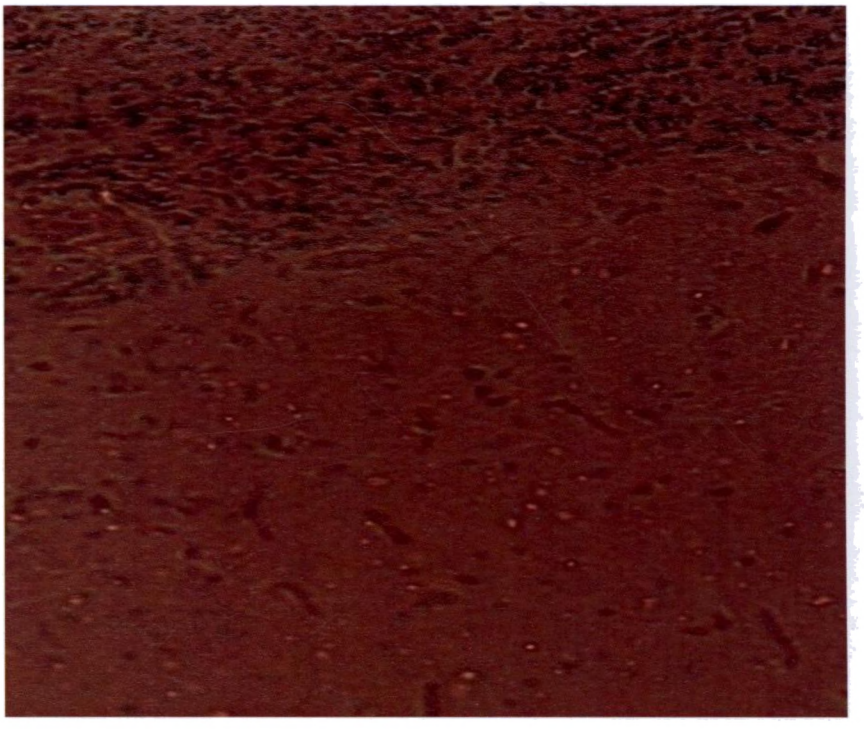


Fig 29: The architecture of fish liver of *Labeo rohita* after sublethal exposure to methylparathion, which shows focal necrosis and cells are increased at 1.0mg/l, 45 days exposure (Hemotoxyline and Eosin 100x).

Summary and Conclusion

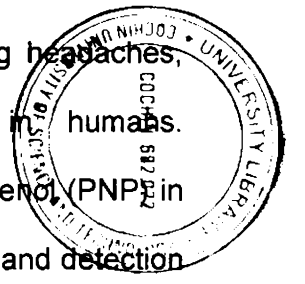
5.0 SUMMARY AND CONCLUSION

Pesticides are used extensively in agriculture but their residues often reach aquatic ecosystems through various means. They can be transferred through food chain, to fish, shellfish and ultimately reach humans. Pesticides are also used directly in aquaculture to control the ectoparasites and insects in nursery and grow out systems. The synthetic organophosphorus pesticides have largely displaced organochlorine pesticides in the last two decades in India.

The development of industrial and agricultural activities contributes enormously to the increase of contaminants in aquatic ecosystems. Its toxicological effects can lead to the deterioration of water quality and causes deleterious effects on health of fish (Handersen and wartten, 1998), thus causing concern tohuman health as well.

In aquaculture, pesticides are used against infestations (Das and Mukherjee, 2003). Food is one of the most important routes by which human beings are exposed to toxic contaminants. Therefore, investigations on the effects of the pesticides in fish have a diagnostic significance in the evaluation of adverse effects of pesticides to human health. Some of the pesticides are persistent in the environment and their presence cause acute toxicity to fish. (Coasts and O'Donnel-Jeffery 1979).

Methylparathion is an organphosphorus pesticide intended only for out door use and is classified as category I (i.e. most toxic) in U.S. EPA. Methylparathion is rapidly metabolized to biologically active methylparaxon. Methylparaxon can chemically bind acetylcholine esterase, which can



ultimately result in a variety of clinical manifestations including headaches, seizures, respiratory depression, paralysis and death in humans. Methylparaxon is detoxified to dimethylphosphate and p-nitrophenol (PNP) in the biological systems. The PNP metabolite is excreted in urine and detection of PNP in urine is considered evidence of recent human exposure.

Most of the work on pesticide toxicity is generally carried out in juvenile fish and a lot of literature is available on LC_{50} alteration in tissue metabolism as indicated by marker enzymes. Pesticide contamination or exposure in fish can happen at any time irrespective of the size of the fish. The information available on the effects of pesticides on grown up fish is relatively scanty. Therefore, the aim of the study is to evaluate the changes in tissue metabolism, protein and lipid profile in grown up rohu (75±6g) in response to lethal and sub lethal concentration of methylparathion.

The results of the lethal toxicity studies showed that there was practically no mortality up to a concentration of 5.4 mg/L. At a concentration of 7.2 mg/L on exposure to 96h 10% mortality was noticed, while at concentration of 16.2 mg/L 100% mortality was noticed in 48h. Probit analysis showed that the lethal concentration 50% for 24, 48, 72, and 96 h were15.5 mg/L, 12.3 mg/L, 11.4 mg/L and 10.2 mg/L respectively, for *Labeo rohita* of size 75 ± 6g.

LPO is considered a valuable indicator of oxidative damage to cellular components. The results suggest that the exposure to methylparathion enhanced ROS production in the liver of *Labeo rohita* and that antioxidant defenses were not totally able to effectively scavenge them, thus leading to lipid peroxidation. The increased hydroxyl radical ($OH^{\cdot}$) production in the

present study suggests that ROS induced oxidative damage can be one of the main toxic effects of methylparathion.

The increased SOD and CAT levels induced by methylparathion in *Labeo rohita* indicate an elevated antioxidant status attempting to neutralize the impact of the ROS. These results support the finding of Alves *et al*, 2002, that the exposure to pesticides can elicit pro-oxidant conditions that trigger adaptive responses by increasing the activity of the antioxidant enzymes.

GP_X is considered to play a very important role in protecting membranes form damage due to LPO (Oost *et al*, 2003). This observation suggests that the major detoxification function of GP_X is the termination of the radicals propagation. (Oost *et al*, 2003). In this context, the GP_X change observed in the present study reflect a possible failure in the antioxidant defense leading to the increased LPO levels. GST activity is involved in xenobiotic detoxification and excretion of xenobiotics and their metabolites, including methylparathion (Jokanovic, 2001). GST plays an important role in protecting tissue from oxidative stress; the increased GST activity in liver indicates the development of a defensive mechanism to counter the effect of methylparathion and may reflect the possibility of a more efficient protection against pesticide toxicity.

The considerable decline in the tissue level of GSH during exposure to methylparathion may be due to an increased utilization of GSH, which get converted into oxidized glutathione and an inefficient GSH regeneration. During moderate oxidative stress, the GSH levels increase as an adaptive mechanism through increased synthesis. However, a severe oxidative stress

suppresses GSH levels due to the impairment of the adaptive mechanism (Zang *et al*, 2004). Methylparathion exposed fish cells usually try to remove the toxicants by direct conjunction with GSH or by means of GST, with observed decrease in GSH, probably an indication of its exhaustion in phase II biotransformation as confirmed by the increased GST activity. In this circumstance, the GSH depletion seems to enhance the risk of oxidative stress due to reduced cell protection ability since a possible increased per oxidative overload could be induced by a high SOD activity, as shown in this study.

Responses to organophosphorus insecticides by aquatic organisms are broad ranged depending on the compound, exposure time, water quality and species (Fischer, 1991). Organophosphorus insecticides exhibit there toxic effect through inhibition of acetylcholine esterase, an enzyme which plays an important role in neurotransmission at cholinergic synapses, by hydrolysing neurotransmitter acetylcholine to choline and acetate (Soreg and Zakut, 1993). AChE activity was inhibited by 74% at a concentration of 1.8 mg/L and by 90% at 5.4mg/L concentration indicating clearly that the methylparathion is highly toxic at lethal and sub lethal level (1.0mg/L).

The biochemical and histological studies showed that even at sub-lethal levels (1.0 mg/L), exposure to methylparathion exhibited deleterious effects. The increase of biomarker enzymes indicate a possible liver necrosis and the treated fishes are faced with a series of metabolic crisis. At lethal and sub lethal concentrations of methylparathion ($P<0.05$) the level of liver specific marker enzymes (AST, ALT, ALP, ACP and LDH) increased indicating liver

injury. The toxic effects of methylparathion is seen at lethal and sub lethal levels.

The protein concentration in *L. rohita* exposed to methylparathion increased at different exposure periods. The increase in the protein content could be the result of an elevation in tissues metabolic activity induced by methylparathion. The increases of the protein content in the fishes exposed to pesticides suggest that any possible protein loss is compensated by increasing the tissue protein synthesis. The loss of enzymes as a result of tissue necrosis or to meet increased demand to detoxify the pesticide necessitates enhanced synthesis of enzyme proteins (Gill *et al.*, 1990). Exposure to methylparathion had significant effects on its protein metabolism, although the fish eventually developed tolerance during long-term exposure; but at lower sub lethal (0.25 and 0.5mg/L) concentration no significant changes were observed.

Increase in the pesticide concentration to lethal and sub lethal levels brought about considerable changes in the lipid conmposition as well ($P<0.05$), suggesting the mobilization of energy rich lipids for production of energy during toxic stress condition caused by methylparathion. The cholesterol and triglyceride content in brain tissue was marginally higher than in liver. The cholesterol content in brain tissue increased to about 27% with increase in methylparathion concentration up to 5.4mg/L, and then decreased. The phospho lipid content in brain tissue was several times higher compared to the liver tissue. The phosphotidyl ethylamine in brain tissue increased gradually up to 7.2 mg/L and then decreased. In sub lethal level, posphotidyl ethylamine in brain and liver showed an increasing trend on 15 days exposure, where as in muscle tissues a

decrease was noticed in all concentration exposed. Generally under any type of stress condition, the animals are bound to spend extra energy to overcome the stress through the oxidation of either carbohydrate or proteins or lipid constituents. The present study demonstrates that fishes exposed to methylparathion develop tissue specific adaptive responses to neutralize the oxidative stress. Further more, the tissue antioxidants status serves as a surrogate marker of oxidant exposure and may be helpful in assessing the risk of oxidative damage associated with consumption of such fish by humans.

The findings clearly indicate that the methylparathion is highly toxic to fish at lethal and sub lethal levels. At 0.25 mg/L sub lethal level, enzymes, proteins and lipid metabolism are not significantly affected but changes were noticed at higher concentrations. A concentration up to 0.5 mg/L of methylparathion can be tolerated by *L. rohita* of 75 ± 6g size group. Also the study points to the fact that the LC_{50} value for methylparathion in fishes vary with size. The accidental contamination of pond water due to various reasons or non judicious use of methylparathion or any pesticides for that matter affect the quantity of the produce which in turn poses a threat to human consumption. The results suggest that the parameters analyzed for methylparathion toxicity could be considered as good biomarkers of the oxidative stress caused by the exposure of methylparathion. Further the studies demonstrate that methylparathion induces oxidative stress in *L. rohita* from the ecophysiological point of view, thus the use of methyl parathion in agriculture and aquaculture must be carefully evaluated.

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Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-a-vis international standards

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Abstract

Heavy metals are an important group of chemical contaminants and food is the major vehicle for entry into the system. Fish constitute a major source of heavy metals in food. Concentration of heavy metals in commercially important species of fish, shellfish and fish products from fish markets in and around the Cochin area was evaluated using an atomic absorption spectrometer. The concentration ranges of Cd, Pb, Hg, Cr, As, Zn, Cu, Co, Mn, Ni, and Se in the samples were <0.07–1, <0.07–1.32, <0.05–2.31, <0.05 to 3.65, <0.1–4.14, 0.6 to 165, 0.15 to 24, <0.02 to 0.85, <0.08 to 9.2, <0.032–1.38 and; <0.03–1.35 mg/kg, respectively. The present study showed that different metals were present in the samples at different levels but within the maximum residual levels prescribed by the EU and USFDA and the fish and shellfish from these areas, in general, are safe for human consumption.

Keywords: Heavy Metals; Mollusks; Cephalopods; Crustaceans; Fish; Fish products; Cochin; Kerala; EU; USFDA; FAO

1. Introduction

Marine organisms, in general, accumulate contaminants from the environment and therefore have been extensively used in marine pollution monitoring programmes (UNEP, 1993; Uthe et al., 1991). In many countries, significant alterations in industrial development lead to an increased discharge of chemical effluents into the ecosystem, leading to damage of marine habitats. Heavy metal discharged into the marine environment can damage both marine species diversity and ecosystems, due to their toxicity and accumulative behaviour (Matta, Milad, Manger, & Tosteson, 1999). As the spawning and nursery grounds of many marine species, including the commercially valuable shrimps and fish, are located in estuarine and coastal areas, they are directly affected by such influx of chemical contaminants into the marine ecosystem (Gibson, 1994).

The accumulation patterns of contaminants in fish and other aquatic organisms depend both on their uptake and elimination rates (Guven, Ozbay, Unlu, & Satar, 1999). Heavy metals are taken up through different organs of the fish and many are concentrated at different levels in different organs of the body (Bervoets, Blust, & Verheyen, 2001; Rao & Padmaja, 2000; Schiarenberg, Gramann, & Pfeiffer, 1994). Fish form an important part of human food and it is therefore not surprising that numerous studies have been carried out on metal pollution in different species of edible fish (Kucuksezgin, Altay, Uluturhan, & Kontas, 2001; Kureishi, Sujatha, & Analia, 1981; Lakshman & Nambisan, 1983; Lewis et al., 2002; Prudente, Kim, Tanabe, & Tatsukawa, 1997; Radhakrishnan, 1994; Ramamurthy, 1979; Sankaranarayanan, Purushan, & Rao, 1978; Senthilnathan & Balasubramanian, 1998; Sultana & Rao, 1998).

Industrial wastes and mining can create a potential source of heavy metal pollution in the aquatic environment (Gumgum, Unlu, Tez, & Gulsun, 1994; Lee & Stuebing, 1990). Under certain environmental conditions, heavy metals might accumulate up to toxic concentrations and cause ecological damage (Guven et al., 1999). Thus, heavy metals, acquired through the food chain as a result of

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pollution, are potential chemical hazards, threatening consumers.

Metals, such as iron, copper, zinc and manganese, are essential metals since they play important roles in biological systems, whereas mercury, lead and cadmium are toxic, even in trace amounts. The essential metals can also produce toxic effects at high concentrations. Only a few metals with proven hazardous nature are to be completely excluded in food for human consumption. Thus, only three metals, lead, cadmium and mercury, have been included in the regulations of the European Union for hazardous metals (EC, 2001), while the USFDA has included a further three metals chromium, arsenic and nickel in the list (USFDA, 1993).

The consequence of heavy metal pollution can be hazardous to man and it often becomes mandatory to check chemical contaminants in foods from the aquatic environment to understand their hazard levels. Therefore, this study aims to take stock of heavy metal concentrations (zinc, copper, cadmium, lead, cobalt, nickel, manganese, chromium, arsenic, selenium and mercury) in the edible portion of mollusks, crustaceans, fish and fish products collected from fish markets in Cochin, Kerala, India, during the period from July 2003 to Jan 2005, in order to evaluate their hazard level in relation to the maximum residual limit for human consumption.

2. Material and methods

2.1. Sample collection

Fresh samples, numbering 67, comprising mollusks, crustaceans, fish and fish products, were collected from fish markets in and around Cochin, India. The samples were placed in ice, brought to the laboratory, washed, separated by species and then stored frozen at −23 °C prior to analysis.

2.2. Reagents

All reagents used were of analytical grade. Working standards of zinc, copper, cadmium, lead, cobalt, nickel, manganese, chromium, arsenic, selenium and mercury were prepared by diluting concentrated stock solutions (Merck, Germany) of 1000 mg/l in ultra-pure water (MilliQ, Millipore-USA).

2.3. Sample preparation

The edible portions of the meat from the samples were removed, homogenized and about 2.5 ± 0.5 g was taken for analysis. Ten milliliters of nitric acid–perchloric acid (10:4) mixture were added to the sample, covered and left overnight at room temperature. Then the samples were digested, using a microwave digester (Milestone ETHOS PLUS, Italy). The completely digested samples were allowed to cool to room temperature, filtered (glass wool), and made up to 50 ml.

Table 1
Working conditions for the analysis of trace elements by atomic absorption spectrophotometer

Metals	Wavelength (nm)	Silt width (nm)	Lamp current (mA)	Gas	Support
Zn	213.9	1.0	5	Acetylene	Air
Cu	324.8	0.5	4	Acetylene	Air
Pb	217.0	1.0	10	Acetylene	Air
Cd	228.8	0.5	5	Acetylene	Air
Ni	232.0	0.2	4	Acetylene	Air
Mn	279.5	0.2	5	Acetylene	Air
Cr	357.9	0.2	7	Acetylene	Air
Co	240.7	0.2	7	Acetylene	Air
As	193.7	0.5	10	Acetylene	Air
Se	196.0	1	10	Acetylene	Air
Hg	253.7	0.5	4	Nitrogen	–

Table 2
Recovery (%) of heavy metals from mollusks, cephalopods, crustaceans, fish and fish products

S. No	Heavy metals	Sample wt (g)	Spiked concentration (mg/kg)	Recovery concentration (mg/kg)	Percentage of recovery (%)
1	Zn	2.5 ± 0.5	0.250	0.240	96
		2.5 ± 0.4	0.500	0.480	96
		2.5 ± 0.5	1.00	1.01	101
2	Cu	2.5 ± 0.3	0.300	0.310	103
		2.5 ± 0.5	0.600	0.589	98
		2.5 ± 0.2	1.20	1.220	101
3	Pb	2.5 ± 0.1	0.025	0.026	104
		2.5 ± 0.4	0.050	0.0485	103
		2.5 ± 0.6	0.100	0.0925	92
4	Cd	2.5 ± 0.6	0.030	0.0280	93
		2.5 ± 0.3	0.060	0.0601	100
		2.5 ± 0.2	0.120	0.119	99
5	Cr	2.5 ± 0.1	0.200	0.199	99
		2.5 ± 0.6	0.400	0.400	99
		2.5 ± 0.2	0.800	0.801	100
6	Ni	2.5 ± 0.3	0.060	0.0601	100
		2.5 ± 0.5	0.120	0.112	99
		2.5 ± 0.2	0.240	0.220	91
7	Co	2.5 ± 0.5	0.250	0.250	99
		2.5 ± 0.4	0.500	0.489	97
		2.5 ± 0.6	0.100	0.999	99
8	Mn	2.5 ± 03	0.050	0.0485	97
		2.5 ± 0.3	0.100	0.0975	97
		2.5 ± 0.4	0.200	0.999	93
9	As	2.5 ± 0.2	0.025	0.0230	92
		2.5 ± 0.5	0.050	0.0488	97
		2.5 ± 0.2	0.100	0.0987	98
10	Hg	2.5 ± 0.6	0.050	0.0489	97
		2.5 ± 0.4	0.100	0.979	97
		2.5 ± 0.3	0.200	0.198	99
11	Se	2.5 ± 0.3	0.050	0.0485	97
		2.5 ± 0.2	0.100	0.0976	97
		2.5 ± 0.7	0.200	0.197	98

2.4. Chemical analysis

All digested samples were analyzed, in triplicate, using an atomic absorption spectrophotometer (Varian 220, USA) as per standard conditions (Table 1). Zinc, copper, cadmium, lead, cobalt, nickel, manganese and chromium were estimated using an air–acetylene flame. Hydride generation and cold vapour techniques (VGA 77) were used for analysis of arsenic, selenium and mercury. The blanks and calibration standard solution were also analyzed in the same way as for the samples.

2.5. Determination of recovery

Homogenized samples (2.5 ± 0.5 g) were spiked with three different concentrations (Table 2) of heavy metals for determination recovery, each run in triplicate and blanks were carried through the whole procedure described above.

3. Results and discussion

The results of analysis showed good recovery when spiked with standards. On average, above 97% recovery was obtained for all the metals studied (Table 2). The concentrations of different metals detected in the edible portion of the mollusks, crustaceans, fish and fish products are given in Tables 3–6, respectively. The mollusk samples analyzed included mussels and clams. Sixteen percent of the samples did not contain cadmium and, in the remaining, the cadmium content was much below the legal limit of 1 mg/kg meat (EC, 2001). Among crustaceans, cadmium was not detected in about 90% of the samples while, in the others, very low concentrations (up to 0.47 mg/kg) were detected. Cephalopods (50% of the samples) and fish (53% of the samples) contained cadmium (0.02–1.32 mg/kg) and only one of the six samples of the brackish water fish *E. suratensis* was found to contain Cd above the EC limit of 0.1 mg/kg but none above the FAO limit (FAO, 1983). Humans are exposed to cadmium through food and the average daily intake for adults has been estimated to be approximately 50 mg (Calabrese, Canada, & Sacco, 1985). The threshold for acute cadmium toxicity would appear to be a total ingestion of 3–15 mg. Severe toxic symptoms are reported to occur with ingestions of 10–326 mg. Fatal ingestions of cadmium, producing shock and acute renal failure, occur from ingestions exceeding 350 mg (NAS-NRC, 1982).

Lead was present in about 66% of the total samples, which included 15% of mollusks (0.07–0.98 mg/kg), 9% of cephalopods (0.07–0.76 mg/kg), 15% of the crustaceans (0.11–75 mg/kg) and 25% of the fish (01–1.32 mg/kg) in their edible portion. The highest concentration was detected in one of the six samples of *Scomberomorus guttatus* (1.32 mg/kg). However, about 70% of the samples contained lead below 0.4 mg/kg (EC, 2001) and 82% below 0.5 mg/kg (FAO, 1983). Lead causes renal failure and liver damage in humans (Emmerson, 1973; Luckey & Venugopal, 1977).

Mercury was not detected in 88% of the total samples and only 3% of the total samples contained mercury above the permitted limit of 1.0 mg/kg (EC, 2001) in the edible portion. Two fish samples, namely *E. affinis* (2.31 mg/kg) and *E. suratensis* (1.76 mg/kg) had the highest concentrations of mercury. For humans, the most significant source of mercury in the diet is fish. In Minamata Bay, Japan, an area heavily polluted with mercury, fish were found to contain from 1 to 20 mg/kg of the edible flesh (NAS-NRC, 1977).

Chromium, arsenic and nickel are group of hazardous metals notified by the USFDA (1993a), even though not covered by EC regulations for fish and other aquatic products. Chromium was detected in almost all the samples and the highest concentration (3.7 mg/kg) was detected in *V. cyprinoides* but the values were within the limits of 12–13 mg/kg (USFDA, 1993a). Chromium is an essential trace element (Mertz, 1969) and the biologically usable form of chromium plays an essential role in glucose metabolism. It has been estimated that the average human requires nearly 1 μg/day. Deficiency of chromium results in impaired growth and disturbances in glucose, lipid, and protein metabolism (Calabrese et al., 1985). Water contributes a major share of chromium in humans (Underwood, 1977).

The arsenic content in the samples varied widely among the different samples; 8, 22, 33 and 80%, respectively, of mollusks, fish, crustaceans and fish products analyzed contained arsenic. Fish and crustaceans contained arsenic above 1 mg/kg with the highest concentration (4.14 mg/kg) in the fresh water fish, *Oreochromis mossambica*, but this is well below the accepted limit of 76 mg/kg (USFDA, 1993 b). The estimated US daily intake of arsenic is approximately 70 μg (USEPA, 1985). Arsenic concentrations as high as 170 mg/kg have been reported in crustaceans and other shellfish (Calabrese et al., 1985). Chronic arsenic poisoning symptoms include pigmented skin lesions, gangrene of the lower extremities (blackfoot disease), along with neuritis and paralysis, anemia and disturbances of the liver and circulatory system (Tseng, 1977; Tseng et al., 1968).

The MRL for nickel is 70–80 mg/kg (USFDA, 1993c), and the samples analyzed showed concentrations only up to 1.38 mg/kg meat. The major source of nickel for humans is food and uptake from natural sources, as well as food processing (NAS-NRC, 1975). The normal range of oral intake of nickel for humans is 300–600 μg/day. An increased incidence of cancer of the lung and nasal cavity has been reported in workers in nickel smelters (NAS-NRC, 1975).

The other metals screened in the samples include zinc, copper, cobalt, manganese and selenium. The zinc content in the samples ranged from 0.6 to 164 mg/kg meat, with a high level in fish (0.66–39.15 mg/kg) and mollusk (3.8–164 mg/kg) samples. The highest concentration was

Table 3
Concentration (mg/kg) of heavy metals in mollusks and cephalopods

		Month of sample collection	Zn	Cu	Cd	Pb	Cr	Ni	Co	Mn	As	Hg	Se
Mollusks													
1	*Perna viridis*	2003 July	37.7 ± 0.14	11.7 ± 0.28	nd	0.37 ± 0.01	0.36 ± 0.04	0.89 ± 0.02	0.17 ± 0.02	0.43 ± 0.02	nd	nd	0.66 ± 0.02
2	*Villorita cyprinoides*	2003 Oct	18.5 ± 0.07	3.9 ± 0.07	0.05 ± 0.28	0.32 ± 0.02	1.36 ± 0.06	nd	0.06 ± 0.03	0.46 ± 0.14	nd	nd	nd
3	*Villorita cyprinoides*	2003 Nov	25.1 ± 0.01	4.69 ± 0.08	0.03 ± 0.21	0.65 ± 0.04	1.66 ± 0.04	nd	nd	0.83 ± 0.4	nd	nd	0.46 ± 0.14
4	*Villorita cyprinoides* (Bo)	2003 Dec	44.0 ± 0.07	7.34 ± 0.05	0.03 ± 0.07	0.39 ± 0.02	1.28 ± 0.04	0.76 ± 0.06	0.4 + 0.28	1.45 ± 0.07	nd	nd	0.13 ± 0.01
5	*Villorita cyprinoides* (Bo)	2004 Jan	20.5 ± 0.21	5.25 ± 0.07	0.14 ± 0.01	0.57 ± 0.04	1.56 ± 0.03	0.05 ± 0.03	0.05 ± 0.02	2.83 ± 0.07	nd	0.33 ± 0.04	0.18 ± 0.04
6	*Perna viridis* (Bo)	2004 Feb	3.8 ± 0.03	1.17 ± 0.09	0.03 ± 0.02	0.98 ± 0.03	0.18 ± 0.02	0.05 ± 0.06	nd	3.75 ± 0.05	0.69 ± 0.22	0.06 ± 0.02	0.09 ± 0.02
7	*Villorita cyprinoides*	2004 mar	12.7 ± 0.21	4.55 ± 0.07	0.15 ± 0.07	0.07 ± 0.29	3.65 ± 0.36	nd	0.5 ± 0.14	2.5 ± 0.14	nd	nd	0.89 ± 0.02
8	*Villorita cyprinoides*	2004 may	165 ± 0.64	24.1 ± 0.07	0.64 ± 0.01	0.09 ± 0.02	2.24 ± 0.08	0.08 ± 0.02	0.76 ± 0.01	0.08 ± 0.02	nd	nd	0.51 ± 0.13
9	*Villorita cyprinoides*	2004 july	33.5 ± 0.45	5.68 ± 0.141	0.18 ± 0.02	0.18 ± 0.04	2.38 ± 0.04	0.07 ± 0.01	0.85 ± 0.04	3.1 ± 0.02	nd	nd	0.25 ± 0.05
10	*Villorita cyprinoides*	2004 Sep	114 ± 0.71	16.0 ± 0.07	0.35 ± 0.02	0.46 ± 0.08	2.20 ± 0.01	0.37 ± 0.03	0.76 ± 0.02	3.7 ± 0.14	nd	nd	0.13 ± 0.03
11	*Perna viridis* (fz)	2004 oct	8.46 ± 0.43	1.44 ± 0.014	nd	nd	2.25 ± 0.04	nd	nd	2.7 ± 0.03	nd	nd	1.16 ± 0.06
12	*Villorita cyprinoides*	2004 Dec	19.9 ± 0.22	5.44 ± 0.028	0.98 ± 0.03	nd	1.28 ± 0.04	0.09 ± 0.02	0.19 ± 0.02	1.07 ± 0.07	nd	nd	0.91 ± 0.01
Cephalopods													
1	*Loligo* sp. whole	2003 Nov	14.4 ± 0.57	10.7 ± 0.14	0.03 ± 0.01	0.55 ± 0.01	0.62 ± 0.14	0.08 ± 0.01	0.04 ± 0.01	nd	nd	nd	nd
2	*Sepia* sp. cleaned	2003 Dec	16.3 ± 0.45	2.5 ± 0.56	0.03 ± 0.01	0.35 ± 0.01	0.52 ± 0.01	0.94 ± 0.03	0.36 ± 0.21	nd	nd	nd	1.35 ± 0.04
3	*Loligo* sp.	2004 Feb	12.3 ± 0.08	9.51 ± 0.08	nd	0.35 ± 0.09	0.64 ± 0.02	0.06 ± 0.02	0.10 ± 0.01	nd	nd	nd	0.44 ± 0.02
4	*Loligo* sp. whole	2004 Oct	7.62 ± 0.08	2.07 ± 0.65	nd	0.76 ± 0.02	1.33 ± 0.07	0.22 ± 0.01	nd	nd	nd	nd	0.15 ± 0.02
5	*Loligo* sp.	2004 Dec	3.99 ± 0.01	0.65 ± 0.071	0.47 ± 0.01	0.76 ± 0.02	nd	0.62 ± 0.02	0.07 ± 0.01	0.43 ± 0.02	nd	nd	nd
6	*Loligo* sp.	2005 Jan	8.13 ± 0.07	2.64 ± 0.07	nd	0.07 ± 0.04	0.53 ± 0.04	nd	0.43 ± 0.02	0.46 ± 0.14	nd	0.60 ± 0.02	nd

Data as mean ± SD in wet weight; sp-species; fz-frozen; Bo-Boiled; nd- not detected.

Table 4
Concentration (mg/kg) of heavy metals in crustaceans

		Month of sample collection	Zn	Cu	Cd	Pb	Cr	Ni	Co	Mn	As	Hg	Sc
1	*Penaeus indicus*	2003 Mar	27.4 ± 0.14	18.9 ± 0.58	nd	0.41 ± 0.01	0.89 ± 0.06	0.06 ± 0.02	0.05 ± 0.01	0.08 ± 0.02	nd	0.28 ± 0.04	0.19 ± 0.04
2	*Penaeus monodon*	2003 Oct	18.9 ± 0.07	14.0 ± 0.06	nd	0.3 ± 0.03	0.25 ± 0.04	0.71 ± 0.02	0.07 ± 0.02	3.1 ± 0.02	nd	nd	0.21 ± 0.01
3	*Penaeus indicus*	2003 Oct	15.6 ± 0.07	12.5 ± 0.07	nd	0.61 ± 0.01	0.34 ± 0.03	0.41 ± 0.02	0.04 ± 0.01	3.7 ± 0.14	nd	nd	0.58 ± 0.04
4	*Penaeus monodon*	2003 Nov	16.5 ± 0.07	13.8 ± 0.07	nd	0.45 ± 0.01	0.76 ± 0.05	nd	nd	2.7 ± 0.03	nd	0.11 ± 0.14	nd
5	*Scylla serrata*	2003 Nov	43.5 ± 0.28	3.94 ± 0.03	nd	nd	0.1 ± 0.01	0.03 ± 0.02	0.05 ± 0.01	1.07 ± 0.07	nd	0.05 ± 0.03	0.35 ± 0.04
6	*Penaeus monodon*	2003 Dec	14.5 ± 0.36	5.74 ± 0.07	nd	0.16 ± 0.01	1.06 ± 0.08	nd	nd	2.87 ± 0.7	nd	nd	0.76 ± 0.05
7	*Penaeus indicus*	2003 Dec	15.8 ± 0.16	11.9 ± 0.05	nd	0.42 ± 0.03	0.33 ± 0.05	nd	nd	2.8 ± 0.14	nd	nd	0.44 ± 0.03
8	*Penaeus monodon*	2004 Jan	15.0 ± 0.15	6.51 ± 0.02	nd	nd	0.31 ± 0.01	0.51 ± 0.02	0.12 ± 0.05	9.2 ± 0.14	nd	nd	0.33 ± 0.4
9	IFDP shrimp	2004 Feb	10.9 ± 0.04	3.48 ± 0.04	nd	nd	0.46 ± 0.02	0.13 ± 0.02	nd	2.45 ± 0.35	2.98 ± 0.03	0.42 ± 0.02	0.11 ± 0.02
10	Block PUD shrimp frozen	2004 Feb	15.8 ± 0.08	4.61 ± 0.04	nd	nd	0.54 ± 0.03	0.12 ± 02	nd	2.77 ± 0.07	1.37 ± 0.42	nd	0.13 ± 0.03
11	Shrimp (fz)	2004 Feb	30.5 ± 0.36	8.63 ± 0.07	nd	nd	0.51 ± 0.01	0.18 ± 0.02	nd	1.32 ± 0.01	1.64 ± 0.03	nd	0.53 ± 0.04
12	PUD shrimp (fz)	2004 Mar	17.6 ± 0.57	4.21 ± 0.02	nd	nd	0.44 ± 0.21	0.2 ± 0.2	0.09 ± 0.01	3.20 ± 0.14	1.25 ± 0.02	0.47 ± 0.02	0.76 ± 0.07
13	BlockPUD (fz)	2004 Mar	5.14 ± 0.71	1.88 ± 0.07	nd	0.11 ± 0.03	0.39 ± 0.02	0.12 ± 0.02	nd	0.52 ± 0.04	0.31 ± 0.14	0.41 ± 0.01	0.16 ± 0.04
14	Block PUD (fz)	2004 Mar	15.4 ± 0.21	4.51 ± 0.08	nd	0.75 ± 0.04	0.22 ± 0.03	0.23 ± 0.03	nd	0.54 ± .02	0.10 ± 0.01	nd	0.16 ± 0.05
15	Shrimp whole Choodan	2004 Apr	16.2 ± 0.21	10.1 ± 0.01	nd	nd	0.42 ± 0.03	0.22 ± 0.04	nd	1.43 ± 0.03	1.53 ± 0.08	nd	nd
16	Shrimp (fz)	2004 Apr	8.17 ± 0.08	2.31 ± 0.04	nd	nd	0.15 ± 0.04	nd	nd	0.81 ± 0.02	nd	nd	nd
17	*Parapenaeopsis stilifera*	2004 July	49.6 ± 0.71	6.91 ± 0.07	nd	0.94 ± 0.06	1.65 ± 0.06	nd	nd	4.75 ± 0.14	nd	nd	0.54 ± 0.32
18	PUD shrimp (fz)	2004 Oct	10.3 ± 0.71	9.41 ± 0.07	0.12 ± 0.01		1.87 ± 0.01	0.28 ± 0.01	0.16 ± 0.03	0.80 ± 0.07	nd	nd	0.19 ± 0.14
19	PUD shrimp (fz)	2004 Nov	12.9 ± 0.07	10.4 ± 0.07	0.07 ± 0.02	0.43 ± 0.04	1.11 ± 0.03	0.09 ± 0.01	0.03 ± 0.04	0.38 ± 0.02	nd	nd	0.22 ± 0.02
20	*Penaeus indicus*	2004 Dec	8.38 ± 0.14	6.22 ± 0.07	nd	nd	0.54 ± 0.02	0.37 ± 0.08	0.1 ± 0.02	1.69 ± 0.07	nd	nd	nd
21	*Penaeus monodon*	2005 Jan	4.91 ± 0.07	12.1 ± 0.07	nd	nd	0.64 ± 0.01	1.38 ± 0.01	nd	1.42 ± 0.07	nd	nd	nd

Data as mean ± SD in wet weight; PUD – peeled undeveined shrimp; IFDP – individually frozen deveined prawn; Fz – frozen; nd – not detected.

Table 5
Concentration (mg/kg) of heavy metals in fish

		Month of sample collection	Zn	Cu	Cd	Pb	Cr	Ni	Co	Mn	As	Hg	Se
1	*Etroplus suratensis*	2003 Dec	12.3 ± 0.14	2.19 ± 0.07	1.32 ± 0.02	0.23 ± 0.01	0.3 ± 0.28	nd	nd	1.01 ± 0.02	nd	nd	0.46 ± 0.22
2	*Labeo rohita*	2003 Dec	12.3 ± 0.04	14.7 ± 0.08	0.02 ± 0.01	0.32 ± 0.01	0.33 ± 0.07	nd	0.02 ± 0.01	1.17 ± 0.02	nd	nd	0.35 ± 0.1
3	*Chanos chanos*	2003 Dec	9.25 ± 0.01	12.5 ± 0.05	0.02 ± 0.01	0.32 ± 0.01	0.2 ± 0.01	nd	0.02 ± 0.02	3.36 ± 0.07	nd	nd	0.03 ± 0.01
4	*Etroplus suratensis*	2004 Jan	11.7 ± 0.14	6.25 ± 0.03	0.03 ± 0.01	0.25 ± 0.02	0.47 ± 0.02	0.15 ± 0.07	0.06 ± 0.01	1.71 ± 0.04	1.515 ± 0.03	nd	0.27 ± 0.1
5	*Scomberomorus guttatus*	2004 Feb	14.5 ± 0.14	3.03 ± 0.04	0.03 ± 0.01	nd	0.34 ± 0.02	0.59 ± 0.02	0.03 ± 0.06	0.59 ± 0.02	1.66 ± 0.03	nd	0.12 ± 0.14
6	*Etroplus suratensis*	2004 Feb	11.5 ± 0.36	5.33 ± 0.02	0.06 ± 0.02	0.76 ± 0.03	0.53 ± 0.02	0.53 ± 0.07	0.06 ± 0.01	1.94 ± 0.03	2.53 ± 0.01	nd	0.53 ± 0.06
7	*Scomberomorus guttatus* (fz)	2004 Mar	39.2 ± 0.08	3.16 ± 0.06	0.04 ± .01	0.24 ± 0.28	0.24 ± 0.05	0.07 ± 0.02	0.02 ± 0.02	0.72 ± 0.02	0.16 ± 0.02	0.28 ± 0.21	0.76 ± 0.04
8	*Catla catla* (fz)	2004 Apr	0.66 ± 0.03	0.13 ± 0.03	nd	nd	0.24 ± 0.06	0.07 ± 0.04	nd	0.85 ± 0.04	nd	nd	nd
9	*Saurida* sp.	2004 July	5.82 ± 0.03	2.27 ± 0.07	0.11 ± 0.02	nd	0.9 ± 0.07	0.03 ± 0.02	0.03 ± 0.01	0.23 ± 0.03	nd	nd	0.95 ± 0.07
10	*Otolithus* sp.	2004 July	12.3 ± 0.43	9.42 ± 0.07	0.11 ± 0.03	0.48 ± 0.01	0.95 ± 0.04	0.09 ± 0.03	0.05 ± 0.07	0.36 ± 0.05	nd	nd	0.04 ± 0.03
11	*Euthynnus affinis* (fz)	2004 Oct	8.61 ± 0.03	0.69 ± 0.02	0.13 ± 0.04	nd	1.3 ± 0.14	0.2 ± 0.14	0.2 ± 0.02	0.43 ± 0.01	nd	nd	0.68 ± 0.01
12	*Scomberomorus guttatus* (fz)	2004 Oct	6.44 ± 0.05	0.83 ± 0.03	0.08 ± 0.01	nd	1.87 ± 0.01	0.4 ± 0.07	0.08 ± 0.03	0.6 ± 0.14	nd	nd	0.26 ± 0.06
13	*Pompus argenteus* (fz)	2004 Oct	6.46 ± 0.05	2.21 ± 0.01	0.09 ± 0.01	0.19 ± 0.01	1.47 ± 0.01	nd	0.05 ± 0.04	0.51 ± 0.01	nd	nd	nd
14	*Scomberomorus guttatus* (fz)	2004 Oct	6.92 ± 0.02	1.19 ± 0.07	nd	nd	1.46 ± 0.07	0.06 ± 0.04	nd	0.14 ± 0.11	nd	nd	0.19 ± 0.02
15	*Scomberomorus guttatus* (fz)	2004 Nov	7.54 ± 0.05	2.00 ± .01	nd	0.10 ± 0.01	1.37 ± 0.01	0.22 ± 0.01	nd	0.28 ± 0.01	nd	nd	0.15 ± 0.02
16	*Etroplus suratensis*	2004 Dec	7.11 ± 0.02	2.32 ± 0.02	nd	0.6 ± 0.14	0.46 ± 0.03	0.42 ± 0.01	0.67 ± 0.02	0.73 ± 0.03	nd	nd	nd
17	*Etroplus suratensis*	2005 Jan	6.33 ± 0.03	1.62 ± 0.03	nd	0.27 ± 0.05	0.31 ± 0.02	0.69 ± 0.02	0.3 ± 0.01	0.58 ± 0.01	nd	nd	nd
18	*Oreochromis mossambicus*	2005 Jan	4.56 ± 0.02	1.12 ± 0.04	nd	0.2 ± 0.01	nd	0.62 ± 0.02	0.12 ± 0.01	nd	4.14 ± 0.01	nd	nd
19	*Etroplus suratensis*	2005 Jan	6.06 ± 0.06	1.92 ± 0.02	nd	0.52 ± 0.02	0.32 ± 0.02	nd	0.57 ± 0.02	0.57 ± 0.01	nd	1.76 ± 0.12	nd
20	Euthynnus affinis	2005 Jan	4.52 ± 0.08	1.64 ± 0.04	nd	0.35 ± 0.04	0.51 ± 0.05	nd	0.43 ± 0.03	0.19 ± 0.4	nd	2.31 ± 0.12	nd
21	*Catla catla*	2005 Jan	1.47 ± 0.07	0.14 ± .02	nd	0.13 ± 0.04	0.52 ± 0.01	nd	0.03 ± 0.05	0.17 ± 0.01	nd	nd	nd
22	*Labeo rohita*	2005 Jan	3.54 ± 0.07	0.17 ± 0.01	nd	0.1 ± 0.01	0.51 ± 0.07	nd	0.17 ± 0.01	1.02 ± 0.02	nd	nd	nd
23	*Scomberomorus guttatus*	2005 Jan	2.08 ± 0.05	0.48 ± 0.04	nd	1.32 ± 0.01	0.52 ± 0.01	nd	0.27 ± 0.01	0.32 ± 0.02	nd	nd	nd

Data as mean ± SD in wet weight; fz: frozen; sp: species; nd: not detected.

Table 6
Concentration (mg/kg) of heavy metals in fish product

		Month of sample collection	Zn	Cu	Cd	Pb	Cr	Ni	Co	Mn	As	Hg	Se
1	Fish kheema	2004 Mar	4.31 ± 0.08	0.66 ± 0.014	0.04 ± 0.03	nd	nd	nd	nd	0.83 ± 0.4	0.30 ± 0.01	0.30 ± 0.01	nd
2	Fish finger	2004 Mar	5.33 ± 0.07	0.85 ± 0.42	nd	nd	0.05 ± 0.01	nd	nd	1.45 ± 0.07	1.52 ± 0.03	0.03 ± 0.01	nd
3	Fish steaks	2004 Mar	6.5 ± 0.14	0.85 ± 0.07	nd	nd	0.24 ± 0.02	0.11 ± 0.02	nd	2.83 ± 0.07	0.15 ± 0.03	0.11 ± 0.01	nd
4	Fish cutlet	2004 Nov	7.9 ± 0.15	14.6 ± 0.14	nd	0.22 ± 0.14	1.81 ± 0.03	0.62 ± 0.02	0.12 ± 0.14	3.75 ± 0.05	nd	nd	nd
5	Fish kheema (fz)	2004 Nov	6.55 ± 0.36	1.1 ± 0.14	nd	nd	0.95 ± 0.14	nd	0.66 ± 0.02	2.5 ± 0.14	0.85 ± .01	nd	nd

Data as mean ± SD in wet weight; fz: frozen; nd: not detected.

detected in black clam *(V. cyprinoides)*. The zinc concentration in the samples compares well with the earlier report in grey mullet from the Tigris river (Unlu, Akba, Sevim, & Gumgum, 1996). Zinc is an essential trace element for both animals and humans. The recommended daily allowance is 10 mg/day in growing children and 15 mg/day for adults (NAS-NRC, 1974). A deficiency of zinc is marked by retarded growth, loss of taste and hypogonadism, leading to decreased fertility. Zinc toxicity is rare but, at concentrations in water up to 40 mg/kg, may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite, and nausea (NAS-NRC, 1974). Zinc appears to have a protective effect against the toxicities of both cadmium (Calabrese et al., 1985) and lead (Sanstead, 1976).

The concentrations of copper in the samples analyzed ranged from 0.13 to 24.1 mg/kg meat, with the highest concentrations in *V. cyprinoides* (24.1 mg/kg) and *Penaeus indicus* (18.9 mg/kg). But the concentrations in the samples were much below the toxic limit of 30 mg/kg (FAO, 1983). Copper is an essential part of several enzymes and it is necessary for the synthesis of hemoglobin. The richest sources of copper are shellfish, especially oysters and crustaceans (Underwood, 1977). No deficiencies of copper in adults have been reported but, in infants, anemia and hypoproteinemia are reported (Underwood, 1977).

Cobalt was detected in 83% of fish, 43% of crustaceans, 83% cephalopods and 75% of mollusks analysed in a range of 0.02–0.85 mg/kg meat. The highest concentration (>0.7 mg/kg) was detected in *V. cyprinoides*. Cobalt is an essential nutrient for man, and is an integral part of vitamin B_{12}. The average daily intake of cobalt, in all forms, ranges from 0.30 to 1.77 mg/day (Underwood, 1977). Cobalt has also been implicated in blood pressure regulation (Perry, Schroeder, Goldstein, & Menhard, 1954), and has been found to be necessary for proper thyroid function (Blakhima, 1970). Excessive ingestion of cobalt is reported to cause congestive heart failure and polycythemia and anemia (Alexander, 1972).

Manganese was detected in almost all the samples and the concentration ranged from 0.08 to 9.2 mg/kg, with the highest concentration bring detected in *V. cyprinoides* and *P. monodon*. Manganese is an essential element for both animals and plants and deficiencies result in severe skeletal and reproductive abnormalities in mammals. It is widely distributed throughout the body with little variation and does not accumulate with age. Total daily intake varies from 2.5 to 7 mg (NAS-NRC, 1977).

Selenium was present in trace levels in almost all the samples. Selenium was detected in 92% of the mollusks, 50% of the cephalopods, 81% of crustaceans and 43% of fish and the concentration ranged from 0.03 to 1.35-mg/kg meat. High levels of selenium were reported in cereal products, meat and seafood (Morris & Levander, 1970). Selenium is an essential element in some oxidation–reduction processes and is a component of glutathione peroxidase, the cellular enzyme responsible for converting

hydrogen peroxide to water and CO_2. However, excessive ingestion or inhalation of selenium can be toxic to man and animals and the symptoms include depression, nervousness, and dermatitis, garlic odour of the breath, gastrointestinal disturbances, and excessive tooth decay (Calabrese et al., 1985). Selenium appears to be protective against a variety of toxic agents, including methyl mercury (Ganther et al., 1972) and cadmium (Mason & Young, 1967).

4. Conclusion

The marginally higher concentrations of Cd, Pb and Hg in fish in the markets samples could be related to industrialization and related activities in these areas. The results of this study supply valuable information about metal contents in fish and shellfish from internal markets around Cochin and indirectly indicate the environmental contamination along the Cochin coastal area. Moreover, these results can also be used to understand the chemical quality of fish and to evaluate the possible risk associated with their consumption. The heavy metal concentrations in the majority of the samples analyzed were well within the prescribed limits set by various authorities, except in a few cases. Therefore, the fish and shellfish from this region, in general, are safe for human consumption.

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