TOXIC EFFECTS OF SELECTED PESTICIDES ON *CHANNA PUNCTATUS* (BLOCH) MAINTAINED IN AQUARIA: A STUDY BASED ON BIOCHEMICAL PROFILES

[redacted]

Thesis submitted for the

Degree of Doctor of Philosophy (Science)

BY

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Certificate

*This is to certify that the thesis entitled "**Toxic effects of selected pesticides on Channa punctatus (Bloch) maintained in aquaria: A study based on biochemical profiles**" submitted by **Gopal Dhar** who got his name registered on 22.1.2001 for the award of Ph.D (Science) degree of North Bengal University, is absolutely based upon his own work under the supervision of Dr. Sudip Barat, Reader, Department of Zoology, North Bengal University, and that neither this thesis nor any part of it has been submitted for any degree or any other academic award anywhere before.*

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DEDICATED TO MY BELOVED PARENTS

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LIST OF ABBREVIATIONS

AChE	:Acetylcholinesterase
ACP	: Acid phosphatase
AKP	: Alkaline phosphatase
ANOVA	:Analysis of Variance
APHA	: American Public Health Association
AT	: Acephate
ATPase	:Adenosine triphosphatase
BCF	: Bioconcentration factor
$CaCO_3$	: Calcium Carbonate
CE	: Chlorpyrifos-ethyl
CO_2	: Free Carbondioxide
CYP2E1	: Cytochrome P450 2E1
CYP45	: Cytochrome P450
Cyt P450	: Cytochrome P450
DDTs	: Dichlorodiphenyl trichloroethanes
DDVP	: Dichlorovos
DNA	: Deoxyribonucleic acid
DO	: Dissolved Oxygen
DPA	: Diphenylamine
EPA	: Environmental protection Agency

ETL	: Economic Threshold Level
FAO	: Food and Agriculture organization
GSH	: Reduced glutathione
H_2SO_4	: Sulfuric acid
Hb%	: Haemoglobin %
HCl	: Hydrochloric acid
HSI	: Hepatosomatic Index
HSP70	: Heat Shock Protein70
IPM	: Integrated Pest Management
KCl	: Potassium chloride
LC_{50}	: Lethal concentration 50
LDH	: Lactic dehydrogenase
LPO	: Lipid Peroxidation
MDA	: Malondialdehyde
MNP	: Methyl nitrophos
mRNA	: Messenger RNA
MS-EXCEL	: Microsoft- Excel
NAD	: Nicotinamide adenine dinucleotide
NAS	: National Academy of Sciences
OFR	: Oxygen Free Radical
OP	: Organophosphate
OSI	: Organosomatic index
PCBs	: Polychlorinated biphenyls
PCA	: Perchloric acid
PHA	: Phytohemagglutinin

PP	: Phytoplankton
PUFA	: Poly Unsaturated Fatty Acid
QP	: Quinalphos
R.B.C	: Red blood corpuscles
REF	: Relatively Environment Friendly
Rf	: Relative mobility
RNA	: Ribonucleic acid
ROI	: Reactive Oxygen Intermediate
ROS	: Reactive Oxygen Species
rRNA	: Ribosomal RNA
RSI	: Renosomatic index
SAM	: S- adenosyl methionine
SCE	: Sister Chromatid Exchanges
SDH	: Succinic dehydrogenase
SDS	: Sodium dodecyl sulfate
SDS-PAGE	: Sodium dodecyl sulfate- polyacrylamide gel electrophoresis
-SH group	: Sulfydryl group
SOD	: Superoxide dismutase
TBA	: Thiobarbituric acid
TCA	: Trichloro acetic acid
TEC	: Total Erythrocyte count
TEMED	: N, N, N', N'- tetramethyl ethylene diamine
tRNA	: Transfer RNA
WHO	: World Health Organization
ZP	: Zooplankton

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LIST OF PUBLICATIONS

- **Dhar, G.**, Mitra Dhar, K., and Barat, S. (2002). Influence of the Organophosphorus Insecticide Phosphamidon on lentic water. **Journal of Environmental Biology (Accepted).**

- **Dhar, G.,** Mitra Dhar, K., and Barat, S. (2002). Cytotoxic effects produced by organophosphate pesticide in *Channa punctatus* (Bloch): The enzymatic changes. **Aquatic Toxicology (Communicated).**

- **Dhar, G.**, Mitra Dhar, K., and Barat, S. (2002). Toxic effects of Dimecron on some biochemical parameters and protein profiles of *Channa punctatus* (Bloch). **Fish Physiology and Biochemistry. (Communicated).**

- **Dhar, G.**, Mitra Dhar, K., and Barat, S. (2002). Alterations in body weight, relative organ weights and behavioral changes induced by Dimecron in *Channa punctatus* (Bloch). **Asian fisheries science (Communicated).**

ABSTRACTS

Dhar, G., Mitra Dhar, K., and Barat, S. (2002). Studies on enzymatic changes produced by Quinalphos in *Channa punctatus* (Bloch). **Parasitology Congress**, held at Department of Animal Science, MJP Rohilkhand University, Bareilly (U.P), India, from October 31-November 2. PP-80.

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Mitra Dhar, K., **Dhar, G.,** and Kundu, S.N. (2002). Magnification of cadmium toxicity in the testis of alcohol-fed mice. **National conference on recent advances in reproductive health**, will be held at Department of Zoology, University of Rajasthan, Jaipur, India, from February 6-8 (Accepted).

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Preface

One of the critical issues faced by the humanity is environmental pollution, which is primarily man-made. Pesticide use in developing countries is indispensable in the struggle against hunger and disease. However, the indiscriminate use of these pesticides especially organophosphorous compounds due to their lower persistence and cost-benefit-effect in agriculture and public health operations has increased the scope of ecological imbalance and thus, many non-target organisms especially the fishes have become victims.

Biomarkers are important tools in the detection of various stresses such as pesticides and other environmental pollutants in aquatic species. Recent emphasis is on studies regarding how natural systems respond to stresses, pollution stress being an important aspect.

An attempt has been made in this thesis to provide wealth of information concerning the various tissue damages, induction of oxidative stress following exposure of sub lethal concentration of organophosphate pesticides. Mechanisms for biotransformations (metabolism) of foreign chemicals such as organophosphate pesticides and biochemical changes that occur in response to those pesticide exposures are the other outcomes of this thesis. Various biochemical end points (e.g., changes in enzymes and other proteins) may also be used as biomarkers of exposure of organophosphorous compounds to understand the intricacies of the pathophysiological alterations that take place in the fish during exposure. Further, cellular combating mechanism against organophosphate toxicity through possible expression of certain genes encoding stress proteins inferred.

Objectives

The objectives of the present investigation

*To understand the behavioral response of pesticide intoxicated subject.

*To explore the extent of damage caused by two organophosphate compounds, dimecron and quinalphos (organophosphate compounds) at sub lethal dose in liver, kidney and muscle of fish.

*To give an idea to the farmers to understand whether the water is polluted due to pesticide or not.

*To find out if there is any induction of oxidative stress following exposure of sub lethal concentration of dimecron and quinalphos.

*To monitor the biochemical and gel electrophoretic changes caused by the toxicants and monitoring of biochemical changes, which will be a tool to evaluate the clinical hazards of this noxious chemical.

*To provide information concerning the toxic limits of pesticide for particular species.

*To understand the mode and route of action of these organophosphate pesticides in non-target aquatic organisms.

*To understand the cellular protective mechanism against chemically induced chronic tissue intoxication.

Review of Literature

Global environmental perturbations affecting the biodiversity and production functions of all kind of ecosystems. In recent years the alarm of environmental degradation has attracted the attention of experts and planners alike and they have been surrounded by a volley of questions: What resource has been affected? How large area has been affected? What caused it? What can be done to cope with the situation? The effective answers rest with viewing the problems in its totality as the process of environmental degradation is mainly a function of various anthropogenic activities and is transboundary in nature. To be precise it is a period of rapid change in population, economy and environment, increasing gap between rich and poor, and increasing disequilibria between the growth and the capacity of the environment to sustain it. It was however agreed in Agenda 21 of the Rio Conference in 1992 about a new concept of resource management, the so-called "sustainable development". It has, of late, been realized that development that meets the needs of the present without compromising the ability of future generations to meet their own needs should be the key issue of development in order to protect the environment from a total collapse.

Aquatic Pollution: Overview

The aquatic ecosystems acting as the ultimate sinks of almost all environmental aberrations, can be the mirrors of environmental degradation following various anthropogenic activities. The drainage basins as a whole and the rivers in particular are being devastated by intensive human activities. River Ganga, the life- line of cultural ethos for instance, has brutally been assaulted to the extent that, barring the region around head water, it has almost lost its originality in terms of ecological intricacies.

The inland aquatic resources are highly threatened due to indiscriminate dumping of industrial wastes, domestic sewage, agricultural run-off and soil erosion (Jhingran, 1991). Thus not only rivers but also other water bodies such as ponds, lakes and reservoirs have been converted into a receiving pot for all kinds of waste, which in turn have vitiated the water quality and biota to the extent of irrepairable state.

The gradual accumulation of toxic substances, heavy metals, pesticides and non-biodegradable chemicals in particular, besides unabated organic loading in the system have assumed serious proportion as the danger of their entry into human body is lurking large.

Environmental pollution by heavy metals is instantly recognized with the Minamata disaster in Japan, when several thousands of people suffered mercury pollution by consuming the oysters caught in the Minamata bay which was the recipient of mercury released from a vinyl chloride plant between 1953 and 1960 (Smith and Sandifer, 1975). Similarly, the level of cadmium in local food stuff in parts of Japan, attributed to irrigation water from the soil heaps of an abandoned mine, caused Itai- Itai Bye disease in 1955, mainly in women over forty (Moore, 1990). Thus the heavy metals may be speedily transferred the ambient environment into the food chain (Adema *et al.*, 1972).

Eutrophication is also a type of pollution affected due to excessive loading of organic matter only which has all the potentialities to upset the ecological balance of an ecosystem, as observed in case of floodplain lakes in the Ganga and the Brahamaputra basins (Sinha and Jha, 1997 a, b).

Although sewage is rich in nutrients, nevertheless, it may pose problems to the sewage-fed biotic produces by being toxic to them directly or to their natural foods due to its physico-chemical characteristics (high BOD, low O_2 tension, suspended solids etc.). Further bioaccumulation of pesticides, heavy metals, detergents may occur in the biotic produces of sewage-fed system (Buras, 1988) and the wide use of pesticides in agriculture has become a pervasive threat to many natural aquatic ecosystems.

Pesticides: One of the prime aquatic pollutants

Environmental transport

There are several routes for pesticides to enter aquatic environments, including spillage, inappropriate disposal of dilute pesticides and washing of equipment used for application, run-off and leaching from the agricultural field and through drainage tiles (Torstensson, 1989). Other possibilities are transportation by wind drift when applying the pesticides and through volatilization of pesticides from soil and vegetation after application (Kreuger, 1999). Pesticides in the atmosphere can then be transported to and deposited on surface waters

(Figure 1). In summary, the main transportation routes of pesticides from the field to surrounding waters are volatilization/deposition, run-off and leaching (Kreuger, 1999). Rainfall and irrigation affect the intensity of the two latter.

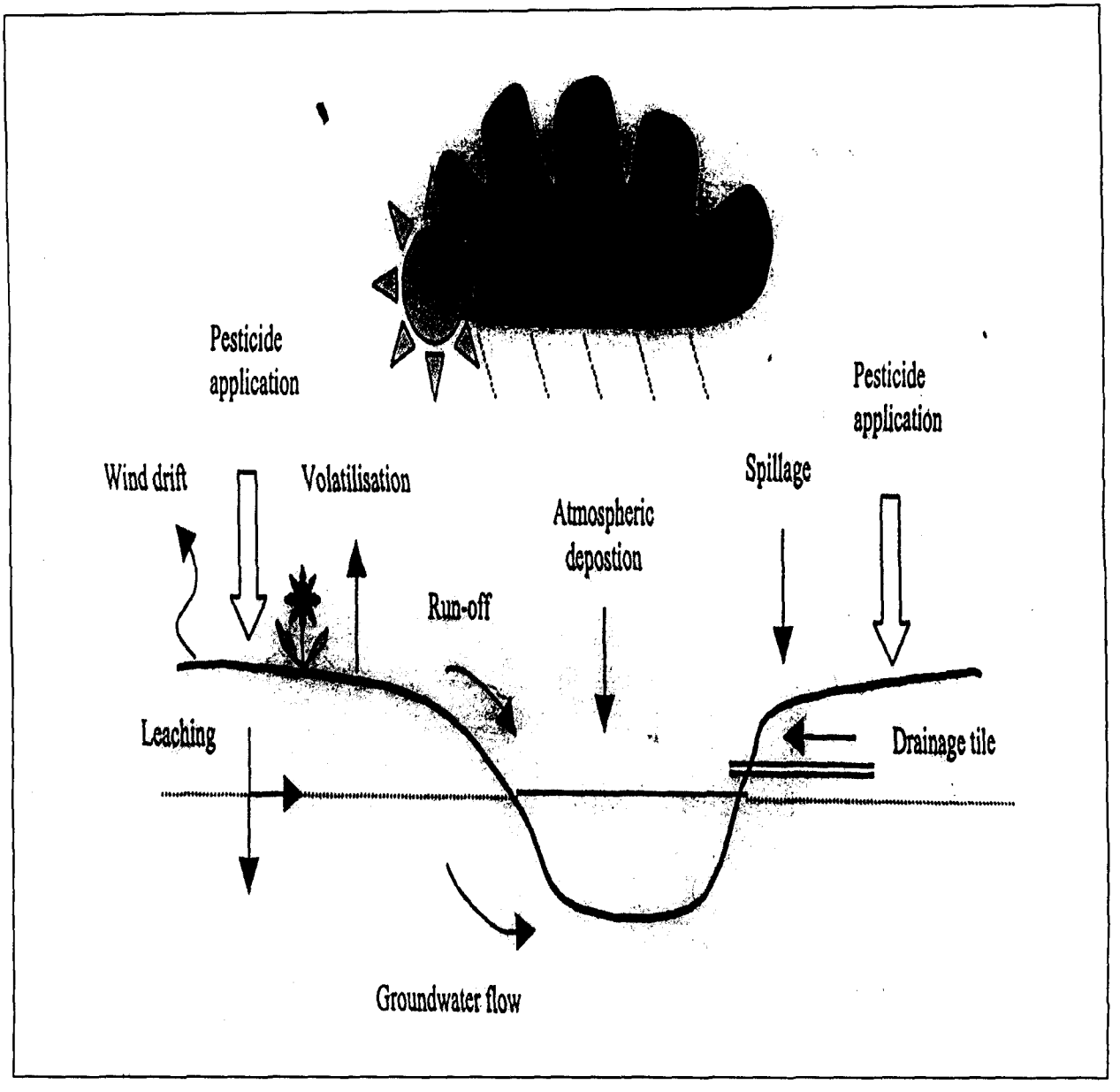


Figure: 1 Possible routes for pesticides used in agriculture to enter surface water and groundwater. Modified after Kreuger (1999)

Bioaccumulation

Bioaccumulation is the general term describing the net uptake of chemicals (usually nonessential ones) from the environment by any or all of the possible routes (i.e., respiration, diet, dermal) from any source in the aquatic environment where chemicals are present (i.e., water, dissolved, colloidal or particulate organic carbon, sediment, other organisms) (Spacie *et al.*, 1995). Bioaccumulation is of concern both for its possible effect on the organism and for the contamination of higher trophic levels, including humans that may occur. The widespread use of pesticides for more than four decades has resulted in problems caused by their interaction with natural biological systems. The complex inter-relationship of these systems has been illustrated in figure 2 (David *et al.*, 1993).

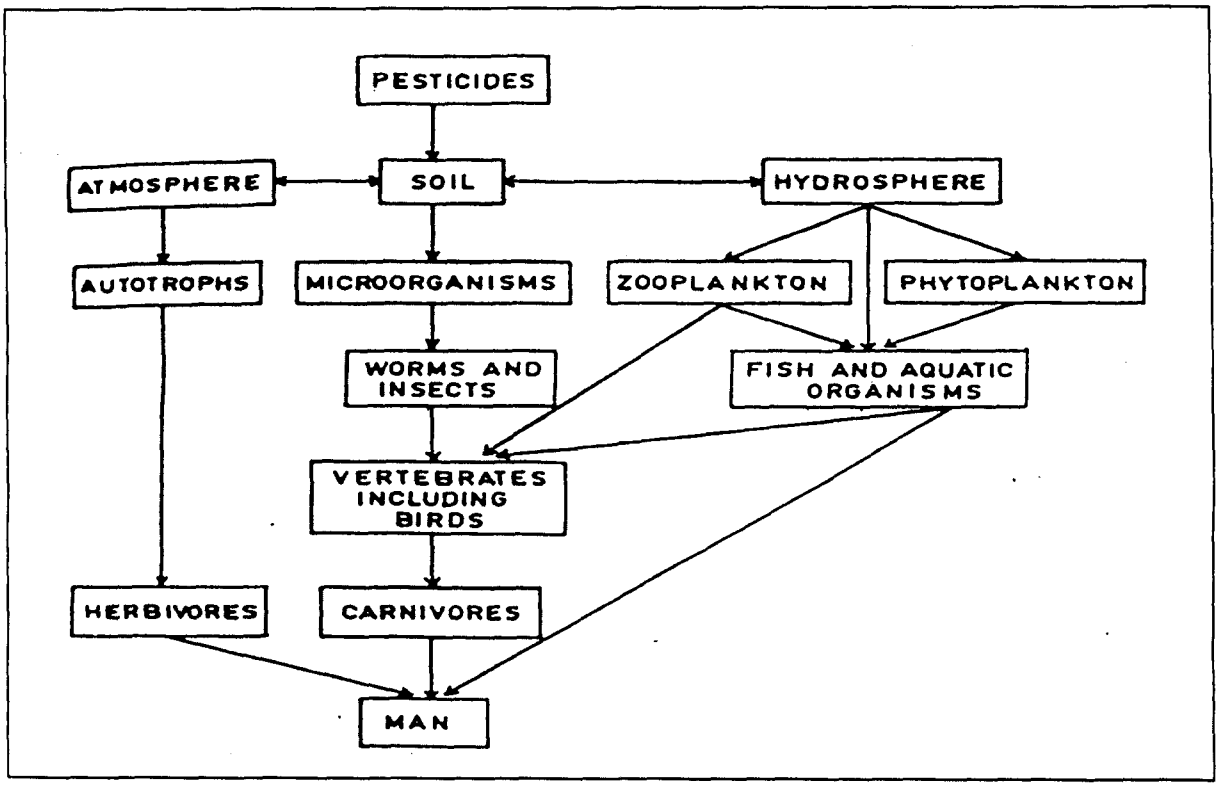


Figure: 2 Schematic diagram of biological transfer of pesticides to man
(David *et. al.*, 1993)

Non-polar compounds with high hydrophobicity, e.g. PCBs and DDTs, tend to accumulate in organisms. Unlike organochlorine pesticides, which are long-lived in the environment and cause biological damage when they accumulate in an organism's system over time, OP and carbamate pesticides are short-lived in the environment and fast acting on their "target pest." Direct mortality of wildlife from organochlorine pesticides was uncommon (Hayes and Wayland, 1975); however, mortality is the primary documented effect on wildlife from OP and carbamate pesticides (Grue *et al.,* 1983). Organophosphorus and carbamate pesticide toxicity is not specific to a target "pest," and lethal effects are seen in nontarget organisms. Generally speaking, newer pesticides are much less persistent and less likely to bioaccumulate than earlier organochlorine pesticides, such as DDT, and organophosphate pesticides, such as parathion. Generally, these characteristics coincide with long biological half-life and they tend to bioaccumulate and possibly biomagnify in a food web. Bioaccumulation can be expressed by the bioconcentration factor (BCF), which is the ratio between the concentration in the organism and its food or the ambient medium. Bioaccumulation is affected by the rate of transformation/degradation of the compound in the organism.

Biomagnification of a compound means, that it is present at increased levels in organisms representing higher trophic levels in the food web. Organisms in aquatic ecosystems may take up pesticides from the food and ingested water, but a diffusion of pollutants between the organisms and the ambient water is also possible (Walker *et al.*, 1996). Differences in feeding, living habits and trophic level of fish can affect their exposure to pollutants like pesticides. Predatory fish may accumulate hydrophobic compounds by eating other organisms, while bottom feeders are in constant contact with pesticides sorbed to the sediment. Herbivorous fishes grazing phytoplankton and periphyton also have a close contact with the sediment. Cullen and Connell (1992) found, that the concentration of dieldrin and DDT with metabolites increased with the age of the fish in an Australian river as a result of bioaccumulation and bioconcentration. A number of workers have investigated the toxicity, uptake and tissue distribution of pesticides in a number of fishes (Anderson and Defoe, 1980; Guiney and Peterson, 1980; Tilak *et al.*, 1980). Because they are generally designed to be persistent pesticides, their residues and breakdown products can remain in the environment

for long periods. Because they are also designed to affect living organisms, they may accumulate in flesh and their impacts may be magnified as they are transferred up the food chain. The air breathing fish, *Channa punctatus* inhabiting the accumulated water in the paddy field besides ponds, are the worst sufferer of residual effect of organophosphate pesticides (though lower persistent) as revealed from some earlier records of investigation (Akhtar, 1984; Sun *et al.*, 1999; Carr *et al.*, 1997).

The poisoning by pesticides from agricultural fields is a serious water pollution problem and its environmental long-term effect may result in the incidence of poisoning of fish and other aquatic life forms. From adjoining crop fields pesticides are drained into ponds (Konar *et al.*, 1997), resulting in depletion in fish yields. Pesticides have been recognized as serious pollutants of the aquatic ecosystem with drastic effects on aquatic fauna. (Sharma *et al.*, 1982; Pal and Konar, 1985; Mani and Konar, 1986; Holden, 1973). Several pesticides are known to reduce growth and fecundity of fish and many invertebrates (Johnson, 1968; McKim *et al.*, 1974.). Poisons are known to affect physico-chemical parameters of water, which ultimately affect the fish (Singh, 1998). Although pesticides produce good many results in the control of pests, their harmful effects on the non-target animals can not be ruled out. Pesticides leave residues in water and mud even several days after their spray in the adjacent crop field. This poses a constant threat to the non-target organism especially to the fishes, because pesticides are known to alter their behaviour pattern (Anderson, 1971), growth and nutritional value (Arunachalam *et al.*, 1980; Yaganobano and Tariq, 1981) and total erythrocyte count (TEC) (Mukhopadhyay and Dehadrai, 1980). Pesticides may affect estuarine microorganisms via spills, runoff, and drift. detrimental effects of pesticides on microbial species may have subsequent impacts on higher trophic levels. Both the structure and the function of microbial communities may be impaired by pesticide toxicity (DeLorenzo *et al.*, 2001). The ecological effects on aquatic organisms caused by pesticides in surface water are dependent both on the peak concentrations the pesticides may reach and the duration of exposure of organisms to pesticides. High concentrations of pesticides can cause acute effects on biota even at a short duration, while low concentrations at long duration may cause chronic effects. If treatment of fields with pesticides coincides with rainfall and subsequent run-off events, high

concentrations of pesticides may be found in surface water recipients. This may give adverse affects on sensitive populations of vertebrates such as fish.

India is considered one of the richest countries in the world with regard to its vast and varied freshwater resources. These aquatic resources are facing the problem of over- exploitation, as a result of which the required niche for the proper development of fish and fisheries has been hampered. So, not only the appropriate water management (Boyd, 1982) but also the agricultural management is very important for sustainable aquaculture development. Keeping in mind the detrimental effects on non-target aquatic resources, rational use of pesticides should be the prime focus of agricultural management

Toxicity:

Toxicity is a relative property reflecting a chemical's potential to have a harmful effect on a living organism. It is a function of the concentration and composition/properties of the chemical to which the organism is exposed (i.e., externally and internally) and the duration of the exposure (Rand *et al.*, 1995). Toxicity can be divided into the broad categories direct and indirect. Direct toxicity results from the toxic agent acting more or less directly at sites of action in and/or on organisms; indirect toxicity occurs as a result of the influence of changes in the chemical, physical, and/or biological environment (e.g., changes in the quality and/or quantity of food organisms or habitat changes and/or losses).

Aquatic toxicity test methods may be categorized according to length of exposure, test situation, criteria of effects to be evaluated, and organisms to be tested. Chronic toxicity tests permit evaluation of the possible adverse effects of the chemical under conditions of long-term exposure at sublethal concentrations. (Rand *et al.*, 1995). Chronic exposure typically induces a biological response of relatively slow progress and long continuance. The chronic aquatic toxicity test is used to study the effects of continuous, long-term exposure to a chemical or other potentially toxic material on aquatic organisms.

In the aquatic environment organisms are not usually exposed to high, acutely toxic concentrations of chemicals unless they are restricted to the vicinity of a chemical release site or spill area. Beyond the initial impact site, dilution and dispersion occur, decreasing these acute concentrations to lower, potentially sublethal levels. In general, a grater biomass is exposed to sublethal concentrations of chemicals than to acutely toxic lethal concentrations. The lower concentrations may not produce death, but they may have a profound effect on the future survival of the organisms.

Sublethal effects may be studied in the laboratory by a variety of procedures. Such effects generally are divided into three classes: biochemical and physiological, behavioral, and histological (Sheehan *et al.*, 1984). Biochemical and physiological effects tests include studies of proteins (e.g., enzyme inhibition, stress proteins), clinical chemistry, hematology, and respiration. Behavior represents an integrated response corresponding to complex biochemical and physiological functions, so chemically induced behavioral changes may reflect effects on internal homeostasis. Behavioral end points may thus be sensitive indicators of sublethal effects. Behavioral effects that have received considerable attention in aquatic organisms are locomotion and swimming, attraction-avoidance, prey-predator relationships, aggression and territoriality, and learning. These are all ecologically significant behaviors (Rand, 1985).

Organophosphate Toxicity:

The air breathing fish, *C. punctatus* inhabiting the accumulated water in the paddy field besides ponds, are the worst sufferer of residual effect of organophosphate pesticides as revealed from some earlier records of investigation (Ghosh and Konar, 1973; Lal and Vohra, 1974; Thomas and Murthy, 1975; Anees, 1978 a b; Dubale and Shaw, 1978; Shafi and Akhtar, 1979, 1982; Akhtar, 1984). These insecticides have also been found to be toxic to algae. (Orus *et al.*, 1990; Marco *et al.*, 1990). Studies pertaining to the effects of pesticides on protein content and nucleic acid levels have been recorded. Dichlorovos (DDVP) reduces the nucleic acid levels and protein content in fresh water fish, *Tilapia mossambicus* (Peters), especially in brain, liver and muscles (Rath and Mishra, 1981) and thus in comparison to

control fish a great loss in DNA, RNA and protein contents is observed in treated fish. In a study with piscine organs the protein quality reduced in both liver and kidney due to exposure of organophosphate pesticides (Awasthi *et al.*, 1984).

The quality of protein is dependent on the synthesis of RNA. Ansari *et al.*, (1988) studied diazinon toxicity in zebra fish exposed for 24 hours to 168 hours and showed a significant time dependent effect on the nucleic acid and protein content. Anees (1974) studied serum protein change of a fresh water teleost, *Channa punctatus* (Bloch), exposed to organophosphate insecticides. Phosphatase activities have been reported extensively in the liver of *Channa gachua* (Dalela *et al.*, 1978). Generally alkaline phosphatase activities in the liver and the muscle increase at lethal concentration and decrease at sublethal concentration in the fish, *Channa sp.* exposed to thiodon and rogor (Verma *et al.*, 1982). Meteleve (1972) studied the effect of dimethoate, methyl nitrophos (MNP) on the carp and found that MNP intoxication increases the blood sugar level and causes the decrease in the glycogen content of the liver. Sublethal concentrations of malathion, an organophosphate insecticide, caused haematological and histopathological damage to channel catfish *(Ictalurus punctatus)* (Areechon and Plumb, 1990).

Deformities were found in the vertebrae and blood samples showed increases in numbers of erythrocytes and decreases in numbers of leucocytes. Jeney and Jeney (1986) also reported a rise in hematocrit levels after high-dose (2500 mg/l) treatment with trichlorphon, an organophosphate used in fisheries for control of planktonic invertebrates and certain parasite infections. The organophosphorus pesticide, sumithion, produced different physiological changes in the fresh water teleost, *S. mossambicus* (Peters) when exposed to a lethal concentration (LC_{50}, 6mg/l) for 48 hr. It was also found that exposure to sumithion depressed tissue respiration and inhibited citric acid cycle enzyme succinic dehydrogenase (SDH) activity. The glycolytic enzyme lactic dehydrogenase (LDH) was increased in sumithion exposed fish suggesting operation of anaerobic glycolytic pathway. Similarly, changes in blood glucose, muscle, liver glycogen content, phosphorylase activity were found. Alkaline phosphatase activity of *S. moassambicus* showed a rise in all tissues (brain, gill, muscle, liver, intestine, kidney) following exposure to sumithion (Koundinya and Ramamurthi, 1982). Sub lethal concentration of dimecron caused conspicuous pathological changes in the liver like

vacuolation of the cytoplasm of hepatocytes, enlargement of nuclei rupture of the cell membrane, liver cord disarray, damage of connective tissue, infiltration of phagocytes and necrosis (Sastry and Malik 1979). Catalase activity in liver and kidney are reduced remarkably by the action of phosphamidon. So the phosphamidon is a partial inhibitor of the enzyme catalase (Thomas and Murthy, 1976).

The rate of oxygen uptake of the bivalve depressed by the sub lethal levels of dimecron (Kulkarni and Keshavan, 1989). Organophosphate pesticides inhibit the acetylcholinesterase activity and also induces changes characteristic of " oxidative stress" (Hai *et al.*, 1997). Saleha Banu (2001) studied the genotoxic effect of monocrotophos and showed that the pesticide induced DNA strand breaks in *T. mossambica* in vivo. The genotoxic potential of methyl parathion and phosphamidon, two commercial formulations of organophosphorus pesticides, was evaluated through induction of sister chromatid exchanges (SCE) and chromosome aberrations in fish gill tissues (Das and John, 1999).

Phosphamidon appears to affect the principal cells of caput epididymidis indirectly through its toxic effect on the Leydig cells and the clear cells of the cauda epididymidis appear to be directly vulnerable to the toxic action of the pesticide (Akbarsha and Sivasamy, 1998). In vitro administration of chlorpyrifos-ethyl resulted in the induction of erythrocyte lipid peroxidation and significant changes in antioxidant enzyme activities (Gultekin *et al.*, 2000). Chlorpyrifos also interferes with brain development, in part by multiple alterations in the activity of transcription factors involved in the basic machinery of cell replication and differentiation (Crumpton *et al.*, 2000)

The long term and repeated administration of novel phosphorothionate caused significant increase of Acid (AcP) and Alkaline Phosphatases (AkP) in serum and kidney (AcP), whereas, these enzymes simultaneously decreased significantly in liver, kidney (female rat AkP) and lung tissues in both male and female rats after 45 and 90 days of treatment (Rahman *et al.*, 2000). The acute toxicity of two organophosphorus pesticides, diazinon and malathion to *Cyprinus carpio* and *Barilius vagra* was revealed from the study of (Alam and Maughan, 1993). Elumalai *et al.*, (1999) investigated the impact of monocrotophos on protein and carbohydrate metabolism in different tissues of albino rats and showed that the protein content

decreased in muscle and kidney after treatment with monocrotophos. Due to their reputation for low environmental persistence, OP pesticides are often used indiscriminately resulting in detrimental exposure to humans and other nontarget species. Poovala *et al.*, (1998) hypothesized, that oxidative stress may play a role in the pathogenesis of acephate (AT)-induced acute tubular necrosis and renal dysfunction observed in cases of AT overdoses. A gradual decrease in muscle glycogen and an increase in lactate contents were observed in rogor exposed Indian catfish (Begum and Vijayaraghavan, 1999)

There is great concern about the use of pesticides for agriculture, silviculture, and public health protection (FAO, 1986). Sublethal levels of pesticides may accumulate in fish from the water or food. As by their nature, these diverse groups of toxic chemicals are often persistent in the environment and leach into the aquatic ecosystems, effects of pesticides and their breakdown products on fish need to be thoroughly investigated. Pesticides may act strongly on fish populations by indirectly affecting food chains. The Government of India has laid special emphasis on the adoption of Integrated Pest Management (IPM) strategies with a view to reduce the load of pesticide application in the crop eco-system by restoring to need-based application based on ETL (economic threshold level) of the target pest species. The major thrust thus lies in decreasing the pesticide load by substituting with readily degradable or 'soft' pesticides such as the organophosphates, carbamates, pyrethroids, etc. Even among these newer compounds the time has come to review and identify among them compounds, which are relatively environment friendly (REF) and fit into an IPM approach. At the same time, the wide spread use of organophosphate pesticides in agriculture and forestry conservation programs has prompted the need for evaluation of the hazards of such materials to wildlife and also non-target organisms.

Though the acute and sub acute toxicities of certain pesticides primarily the chlorinated hydrocarbon, then the organophosphate pesticides, have been investigated in several species of fishes, studies on the sublethal concentrations of organophosphorous toxicants are meagre. Two organophosphate pesticides, phosphamidon (Dimecron) and Quinalphos are selected for present toxicity study as they are most commonly used for management of insect pest of field crop and tea plantation in India especially in the state, West Bengal

General properties of Phosphamidon and Quinalphos

PHOSPHAMIDON

COMMON NAME	PHOSPHAMIDON
CHEMICAL NAME	2 - CHLORO - 2 - DIETHYLCARBAMOYL - 1 - METHYLVINYL DIMETHYL PHOSPHATE.
EMPIRICAL FORMULA	$C_{10}H_{19}CL\ N\ O_5P$
MOLECULAR WEIGHT	299.69
PHYSICAL STATE	REDDISH BROWN VISCOUS LIQUID
SOLUBILITY IN WATER	IT IS MISCIBLE IN WATER.
SOLUBILITY IN ORGANIC SOLVENTS	MISCIBLE IN MOST ORGANIC SOLVENTS EXCEPT SATURATED PARAFFINS. ITS SOLUBILITY IN HEXANE IS 3.2 % AT 25 ^{0}C.

QUINALPHOS

COMMON NAME	QUINALPHOS
CHEMICAL NAME	O,O DIETHYL O - 2 - QUINOXALINYL PHOSPHOROTHIOATE
EMPIRICAL FORMULA	$C_{12}H_{15}N_2O_3P\ S$
MOLECULAR WEIGHT	298.3
PURITY PERCENTAGE	70 % MINIMUM
PHYSICAL STATE	THE PURE PRODUCT IS COLOURLESS CRYSTALLINE SOLID.
SOLUBILITY IN WATER	IT IS MISCIBLE IN WATER.
SOLUBILITY IN ORGANIC SOLVENTS	SOLUBLE IN METHANOL, ETHANOL, ETHER, KETONES, AROMATIC SOLVENTS.

Materials and Methods

ANIMALS AND CHEMICALS

ANIMALS

Test fish

Air breathing fish, *C. punctatus* (Bloch) weighing 30-32 g and with mean body length of 12-15 cm were collected from a commercial fish farm. Fish were brought to the laboratory and acclimatized for 2 weeks prior to experimentation. Dechlorinated tap water was used throughout the course of the experiments. The physico-chemical characteristics of the test water were: temperature 26.5±1.0°C; pH 7.5; hardness 79 mg/l (as $CaCO_3$); alkalinity 87 mg/l (as $CaCO_3$) and dissolved oxygen concentration (D. O.) 6.5 mg/l. The LC_{50} value was determined in the laboratory as per the methods of Reish and Oshida (1987) starting with range finding tests to acute toxicity trials. The 96-h LC_{50} was found to be 18 mg/l for dimecron and 25 mg/l for quinalphos.

CHEMICALS

Dimecron

Dimecron, which is a trade name of Phosphamidon (O, O-dimethyl-O- (2-chloro-2-diethyl carbamoyl-1-methyl-vinyl) phosphate). Dimecron, marketed by Hindusthan Ciba-Geigy Limited, India was used throughout the study. For each experiment the required concentrations were prepared from fresh stock solutions.

Quinalphos

Quinalphos, which is a trade name of Basuquin 25 EC (O, O- diethyl O- quinoxalin –2- yl phosphorothiate) marketed by Hindusthan Ciba-Geigy Limited, India was used throughout the study. For each experiment the required concentrations were prepared from fresh stock solutions.

TOXICITY

Sub lethal toxicity

Fishes were fed regularly with dried shrimp powder and a minimum quantity of antibiotics to free from any ailments. The water in the aquaria was changed every 24 hr. to maintain a constant concentration of dimecron (phosphamidon) and quinalphos during the period of exposure. Aeration was provided to each aquarium to prevent hypoxic conditions. Two Sub lethal concentrations of dimecron (1.8 mg/l and 0.36 mg/l) and quinalphos (2.5 mg/l and 0.50

mg/l) were added to the medium (tap water) of aquaria. About 25-30 acclimatized fish were introduced in each aquarium. Simultaneously, an aquarium was set up for the study of control animals. A minimum of five fish was sacrificed each time from treated as well as control aquaria. The analysis was performed at 10, 20 and 30 days of exposure.

STUDY SCHEDULE

OUTDOOR STUDY SCHEDULE

Water quality

Chronic tests were conducted in outdoor earthen vats (circumference 64 cm, mean depth 32 cm.) each holding 60 liters of borehole water and 3.5 kg uncontaminated soil. Fifteen fish (*Channa punctatus* of approximately 12-15 cm. in length and 30-32 gms. in weight) per vat were stocked. Each test had three replicates all accompanied by controls. Fish (*Channa punctatus*) exposed to sub lethal concentrations of dimecron and quinalphos based on acute toxicity data (Reish and Oshida, 1987). The concentrations were used low to high @ 0.36 mg/l to 1.8 mg/l and @ 0.50 mg/l to 2.5 mg/l respectively. Water quality such as pH, alkalinity, dissolved oxygen (DO), free carbon dioxide (CO_2), hardness and water temperature were analysed following APHA (1995) at 10 day intervals. Plankton samples were collected at 10 day intervals by filtering surface water through plankton net (bolting silk no, 25). The collected samples were fixed in 5% formalin on the spot and carefully brought in the laboratory for analysis. Counting was done following standard method (APHA, 1995). Temperature (maximum and minimum), atmospheric pressure, relative humidity and rainfall were also noted during the study.

INDOOR STUDY SCHEDULE

Air breathing fish, *Channa punctatus* (Bloch) were obtained from a commercial fish farm and acclimatized to laboratory conditions for at least two weeks before the experiments commenced. *Channa punctatus* of approximately 12-15 cm. in length and 25-32 gms. in weight were chosen as experimental animals. The fishes were kept in glass aquaria of about 100 litres capacity. Dechlorinated tap water was used throughout the course of the

experiments. The physico-chemical characteristics of the test water temperature 26.9 $\pm 1^0$ C; pH 7.5; hardness 79 mg/l (as $CaCO_3$); alkalinity 87 mg/l (as $CaCO_3$) and dissolved oxygen concentration 6.5 mg/l. The LC_{50} value was determined in the laboratory as per the method of Reish and Oshida (1987) starting with range finding tests to acute toxicity trials. The 96-h LC_{50} was found to be 18 mg/l for dimecron and 25 mg/l for quinalphos. One-tenth and 1/50 th of the 96-h LC_{50}, i.e. 1.8 mg/l and 0.36 mg/l and 2.5 mg/l and 0.50 mg/l were selected for sub lethal test trials.

Fishes were fed regularly with dried shrimp powder and a minimum quantity of antibiotics to free from any ailments. The water in the aquaria was changed every 24 hr. to maintain a constant concentration of dimecron (phosphamidon) and quinalphos during the period of exposure. Aeration was provided to each aquarium to prevent hypoxic conditions. Two Sub lethal concentrations of dimecron and quinalphos were added to the medium (tap water) of aquaria. About 25-30 acclimatized fish were introduced in each aquarium. Simultaneously, an aquarium was set up for the study of control animals. A minimum of five fish was sacrificed each time from treated as well as control aquaria. All the experiments were repeated three times for each biochemical estimation. The analysis was performed at 10, 20 and 30 days of exposure. The liver, kidney and muscle were removed immediately and frozen until required (not more than 1 hr.) for biochemical and enzymological analysis.

DETERMINATION OF BODY WEIGHT AND ORGAN WEIGHT

Body weight of individual fish was determined gravimetrically with the help of sensitive weighing balance. Organ weight was taken after taking the total organ of five fish at a time on a thin slide or watch glass, as the may be, and its wet weight was determined by deducting the tare weight.

BEHAVIORAL STUDY

The behavioural changes of exposed and control fish were observed regularly at eight hours intervals throughout the experiment.

STUDY OF PROTEINS

QUANTITATIVE STUDY (LIVER, KIDNEY, AND MUSCLE)

Total quantity of protein was estimated following the method of Lowry *et al.*, (1951). Weighed individual tissue of fish was homogenised in 0.1 N sodium hydroxide and centrifuged at 3000 g for 15 minutes. The supernatant was collected and quantitatively transferred in a neutral glass test tube. This served as the aliquot.

A definite volume of the stock solution was taken in a glass test tube and diluted with 0.1 N sodium hydroxide to make a final volume of 1 ml. Then 5 ml of reagent C (prepared by mixing reagent A and B at ratio of 1: 50) was added. After 5 minutes, 0.5 ml of reagent D was added to the sample and mixed thoroughly. An analysis blank with 1 ml of 0.1 N sodium hydroxide, 5 ml of reagent C and 0.5 ml of reagent D was run simultaneously. This served as control. All the preparations were kept in the dark for thirty minutes at room temperature. After the stipulated period, the color intensity of the solutions was recorded in a spectrophotometer (Milton Roy Co., USA) at 750 nm. The concentration of the protein in the sample was estimated from the standard curve prepared in an identical way with a known concentration of analytical grade bovine serum albumin (Sigma, USA).

Reagent A: A solution of 0.5 % copper sulphate and 1.0% potassium sodium tartrate prepared freshly and stored in colour bottle.

Reagent B: This consists of 2.0 % sodium carbonate in 0.1 N sodium hydroxide (w/v) and was always prepared fresh when used.

Reagent C: This was prepared by mixing 50 ml of fresh reagent B with 1 ml of reagent A. This mixture was vigorously shaken.

Reagent D: It is Folin phenol reagent. This consists of 100 g of sodium tungstate, 25 g of sodium molybdate, 700 ml glass distilled water, 50 ml 85% phosphoric acid and 100 ml concentrated hydrochloric acid, boiled under reflux for 10 hours followed by the addition of 150 g lithium sulphate, 50 ml of water and few drops of bromine. The mixture was boiled for nearly 15 minutes to remove the excess of bromine, cooled, diluted to 1 litre and stored in a

stoppered bottle after filtration. In fresh condition, it is yellowish in colour. It was diluted with double the amount of glass-distilled water, when it was used.

The quantity of protein was estimated on wet weight basis and the value of concentration of protein was expressed as mg/gm of tissue.

QUALITATIVE STUDY (LIVER, KIDNEY AND MUSCLE)

The qualitative analysis of protein from liver, kidney and muscle were done through the separation of the component bands with their molecular weight in the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

PREPARATION OF TISSUE MATERIAL

A definite amount of liver, kidney and muscle tissue were homogenised in 0.9% sodium chloride solution. The homogenate was centrifuged at 3000 g at 4^0C for 15 minutes. The supernatant was collected and kept at 4^0C for subsequent study.

SDS-GEL ELECTROPHORESIS

For the qualitative study of tissue proteins, sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) was done by following the method of Laemmli (1970). Acrylamide, N, N, N', N'-tetramethyl ethylene Diamine, Tris- buffer, Sodium dodecyl sulphate, Coomassie Brilliant Blue R-250 etc. were purchased from Sigma Chemical Co., USA. 10.0 % separating gel and 5.0 % stacking gels were made. Before loading onto the gel slots, proteins were extracted from the tissues by homogenizing with cold normal saline and the supernatants were isolated by centrifuging at 3000 g for 15 minutes. The supernatant was then mixed with equal volume of sample buffer (1:1) and the content was then boiled for 3 min in a boiling water bath. Before loading proteins, the slots were washed with electrode buffer and then blotted with blotting paper. About 0.02 ml to 0.03 ml of protein sample buffer mixture containing about 100-125 μg of protein was loaded into each slot. The gel apparatus was then connected with the power pack. Electrophoresis was performed at 4^0C. 100 V was applied first and when the dye marker crossed the stacking gel area voltage was increased to

150 V and constant current was applied. After 3-4 hours, when the tracking dye reached near the end of the gel, apparatus was disconnected from the power pack, gel was removed from the glass plates, fixed in 20 % TCA solution with constant rocking movement for 20 minutes and then kept in Coomassie Brilliant Blue R-250 staining solution overnight. On the next day, gel was removed from the staining solution and placed in destaining solution until the bands became clearly visible. The gels were then stored in 6% acetic acid for future studies.

REAGENTS PREPARED

i) 4 X Separating gel buffer

16.95gm Tris (1.5 M)

90 ml distilled Water

pH adjusted to 8.8 with HCl

4 ml 10 % SDS

Volume brought upto 100 ml. with distilled water.

ii) 10 X Electrode buffer

30.25gm Tris (0.25 M)

144 gm glycine

850 ml distilled Water

pH adjusted to 8.3 with HCl

100 ml 10 % SDS

Volume brought upto 1000 ml. with distilled water.

iii) 2X Stacking gel buffer

3 gm Tris (0.25 M)

90 ml distilled Water

pH adjusted to 6.8 with HCl

2 ml 10 % SDS

Volume brought upto 100 ml. with distilled water.

iv) 2X Sample buffer

0.15 gm Tris (0.125 M)

4 ml distilled Water

pH adjusted to 6.8 with HCl

1 ml glycerol

20 mg Bromophenol blue

0.4 ml β- mercaptoethanol

Volume brought upto 10 ml. with distilled water.

v) Fixing Solution

20 gm Trichloroacetic acid

50 ml distilled water

Volume brought upto 100 ml with distilled water.

vi) Staining Solution

200 mg Coomassie Brilliant Blue R-250

50 ml methanol

10 ml glacial acetic acid

40 ml distilled Water.

vii) Destaining Solution

50 ml methanol

10 ml glacial acetic acid

40 ml distilled Water.

viii) Fixing Solution

6% acetic acid

PREPARATION OF GEL

30 % Acrylamide: Bisacrylamide (28:2) solution was prepared first in distilled water. For 10 % gel 20 ml of Acrylamide: Bisacrylamide stock 15 ml 4 X separating gel buffer and 50 ml distilled water were added. After that 0.1-ml ammonium persulphate solution and 0.03 ml (30 µl) of TEMED was added. This mixture was gently poured in the already set glass plates and a thin layer of water was made over the gel solution to make sharp, flat gel surface. After 30-40 minutes the upper unpolymerized liquid was poured out and stacking gel solution (5%) was prepared by adding 1 ml 30 % Acrylamide: Bisacrylamide stock, 4 ml water, 5 ml 2 X stacking gel buffer, 0.01 ml ammonium persulphate and 5 µl TEMED. The mixture was then quickly poured on the polymerized separating gel. The slot former was also quickly placed on

the stacking gel and the mixture was then allowed to polymerize. It took 3-5 minutes to polymerize. The gel plates used were 10x10 cm. with 0.5-mm spacers.

The Standard molecular weight proteins were purchased from Sigma Chemical Co., USA.

The molecular weight calculated from the Rf values of the Standard run under the same conditions. The gel percentage used was 10 %.

NUCLEIC ACIDS

EXTRACTION OF NUCLEIC ACIDS

The tissues were dissected out, kept in an ice bath. For determination of nucleic acids, the method of Cherry (1962) was followed. Definite amount of tissue was taken for homogenisation with cold methanol. The tissue was centrifuged at 3000 g for 20 minutes in cold in a centrifuge (SCR 20BA, Hitachi Himac centrifuge, Japan). The supernatant was discarded and to the precipitate 0.02M perchloric acid was added. After waiting for 5 minutes the content was centrifuged at 3000 g for 20 minutes. The supernatant was discarded and to the precipitate ethanol: ether (1:1) was added and incubated at 50^0 C in a water bath for 30 minutes. Again it was centrifuged and the supernatant was discarded. To the precipitate known amount of 5% perchloric acid was added and incubated overnight in cold. On the next day the tissue was centrifuged and here the supernatant was collected. From this supernatant the DNA and RNA concentration were estimated by the diphenylamine and orcinol reactions respectively as described by Plummer (1988).

ESTIMATION OF DEOXYRIBONUCLEIC ACID (DNA)

The DNA was estimated through diphenylamine reaction. Diphenylamine reagent was prepared by mixing 1 g of DPA, 100 ml of glacial acetic acid and 2.5 ml of concentrated sulphuric acid (36N). From the stock solution prepared previously a measured amount was taken in a test tube and the volume was made 1 ml by the addition of 5% PCA 5 ml of DPA reagent was further added to the tube. An analysis blank with 1 ml 5% PCA and 5 ml DPA was prepared. All the tubes were kept in a boiling water bath for 10 minutes. It was cooled,

and the intensity of blue colour was determined at 595 nm in a spectrophotometer (Milton Roy Co., USA). The concentration of DNA was determined from the standard curve and the value expressed as mg/g.

Composition of diphenylamine Reagent (DPA): Diphenylamine reagent was prepared by mixing 1 g DPA, 100 ml glacial acetic acid and 2.5 ml concentrated sulphuric acid.

ESTIMATION OF RIBONUCLEIC ACID (RNA)

RNA was estimated through orcinol reagent. It was prepared by mixing 3.5 ml of solution A with 100 ml of solution B. A measured amount of the same stock solution (as prepared) was taken in a test tube and final volume of 2 ml was made with 5% PCA. 3 ml of orcinol reagent (freshly prepared) was further added to it. An analysis blank with 2 ml of 5% PCA and 3 ml of orcinol reagent was also prepared. Both the control and experimental tubes were heated for 20 minutes in a boiling water bath and brought to room temperature and the intensity of green color was estimated in a spectrophotometer (Milton Roy Co., USA) at 665 nm against a blank. The concentration of RNA was determined by the standard curve and the value was expressed as mg/g.

Composition of orcinol reagent: It was prepared by mixing 3.5 ml of solution A with 100 ml of solution B.

Solution A: This consisted of 6% orcinol.

Solution B: It was prepared by dissolving 100 mg ferric chloride in 100 ml concentrated HCl.

ENZYMES

EXTRACTION OF PHOSPHATASES

A definite amount of liver, kidney and muscle of fish was taken and homogenised in 0.9% sodium chloride. The homogenate was centrifuged at 4000 g for 10 minutes at 4^0C. The supernatant was quantitatively collected and used for further analysis.

ESTIMATION OF ACID PHOSPHATASE

Acid phosphatase enzyme activity was determined by the method of Walter and Schutt (1974). For the estimation of acid phosphatase, 1 ml acid buffer (pH 4.8) was taken in two separate test tubes. In one of the tubes, marked as control, 2ml 0.1 N sodium hydroxide was added prior to the addition of 0.2-ml enzyme extract. Both the tubes were prewarmed at 25^0C & then 0.2-ml enzyme extract was added in each tube and incubated for 30 minutes in a 25^0C water bath. At the end of stipulated period, the reaction in the experimental tubes was stopped by the addition of 2 ml of 0.1 N sodium hydroxide. The amount of 4-nitrophenol in the medium was estimated by measuring the yellow colour at 405 nm in a spectrophotometer (Milton Roy Co., USA) against a blank. Activity of the enzyme was determined in terms of the protein present per mg of the tissue sample.

Composition of Acid buffer: This was prepared by mixing 0.41 g citric acid, 1.125 g sodium citrate, 0.203 g 4-nitrophenyl phosphate and 100 ml glass distilled water.

ESTIMATION OF ALKALINE PHOSPHATASE

Alkaline phosphatase enzyme activity was determined by the method of Walter and Schutt (1974). For the estimation of alkaline phosphatase 2 ml alkaline buffer (pH 9.8) was taken in two separate test tubes. In one of the tubes, marked as control, 10 ml 0.05 N sodium hydroxide was added. Both the tubes were prewarmed at 25^0C prior to the addition of enzyme extract. 0.05 ml cold enzyme extract was added in both the tubes and incubated for 30 minutes in a 25^0C water bath. At the end of stipulated period, the reaction in the experimental tubes was stopped by the addition of 10 ml of 0.05 N sodium hydroxide. The amount of 4-nitrophenol in the medium was estimated by measuring the yellow colour at 405 nm in a

spectrophotometer (Milton Roy Co., USA) against appropriate blank. Activity of the enzyme was determined in terms of the protein present per mg of the tissue sample.

Composition of Alkaline buffer: This was prepared by mixing 1.052 gms diethanolamine, 8.0 ml of 0.1 N HCl, 46.4 mg 4-nitrophenyl phosphate in 85 ml double distilled water, pH adjusted to 9.8 by addition of 0.1 N HCl. Volume made upto 100 ml with distilled water.

TISSUE PREPARATION FOR LPO, GSH AND CYTOCHROME P-450

The liver, kidney and muscle were quickly isolated after fish were sacrificed. A definite amount of tissue was homogenised with ice-cold 1.15% KCl (10 % w/v) followed by centrifugation at 7000 g for 15 min. The supernatant was then centrifuged at 100000 g for 60 min in a Sorvall ultracentrifuge at 4^0 C. The firmly packed microsomal pellet was washed twice and resuspended in 1.15 % KCl to make 10-20 % suspension calculated from the original weight of tissue.

ESTIMATION OF LIPID PEROXIDATION

Lipid peroxidation was determined from the homogenate by the method of Ohkawa *et al.*, (1979). To 0.2 ml homogenate 0.2 ml 8.1% (w/v) Sodium dodecyl sulphate, 1.5 ml 20 % acetic acid, adjusted to pH 3.5 with 20 % (w/v) sodium acetate and 1.5 ml 0.8 % (w/v) thiobarbituric acid was added. The mixture was diluted to 4 ml with water and then heated for 60 min in a boiling water bath. Then it was cooled to room temperature and 1 ml water followed by 5.0 ml of a mixture of n- butanol and pyridine (15: 1 v/v) was added. The mixture was shaken vigorously and centrifuged at 1500 g for 15 min. The absorbance of the organic layer was measured at 532 nm. The results were expressed as nmol of malondialdehyde (MDA) formed/g wet tissue.

ESTIMATION OF REDUCED GLUTATHIONE

The reduced glutathione (GSH) in liver, kidney and muscle was determined by the method of Ellman (1959). 1-ml cytosol was mixed with 1 ml 4 % (w/v) sulphosalicylic acid and the mixture was centrifuged at 1500 g for 15 min. The supernatant was allowed to react with 0.1

mM 5, 5'- dithio-bis-2-nitrobenzoic acid. The solution was then kept at room temperature for 10 min and read at 412 nm. The result was expressed as m mole GSH/g wet tissue.

ESTIMATION OF CYTOCHROME-P-450

The microsomal pellet obtained after ultra centrifugation was resuspended in isotonic KCl solution and centrifuged as above. The twice washed microsome was finally suspended in isotonic KCl to make 10 –20 % solution and Cyt-p-450 content was assayed according to the method of Omura and Sato (1964) on an Aminco DW-2a UV/Vis spectrophotometer at 520 nm. The result was expressed as n mole/mg microsomal protein.

Protein was estimated from the cytosolic and microsomal fractions by the method of Lowry *et al.*, (1951) using bovine serum as a standard.

STATISTICAL ANALYSIS

The student's t-test and "ANOVA" (One Way) were conducted for testing significance at 5% and 1% level respectively between data of control and treated series at different fixation intervals. Data were analysed statistically using analysis of variance (ANOVA) in MS-EXCEL programme.

Chapter-I

Introduction

The degradation of aquatic system is a worldwide phenomenon originated from the intense population and from the corresponding increase in industrial activities as well as agricultural practices. Further, the inland aquatic resources are highly threatened due to indiscriminate dumping of industrial wastes, domestic sewage, agricultural run-off and soil erosion (Jhingran, 1991). In case of lentic water bodies (ponds, lakes and reservoirs) the impacts of aquatic pollution are more extensive due to the low water residence periods and to the physical structure of these systems. The various types of pesticides are extensively and indiscriminately used in agricultural fields to control insect pests for higher crop yield and this has increased the incidence of environmental pollution by pesticides. Moreover, most of the water bodies are susceptible to chemical contamination from other agricultural activities such as accidental spills, drainage from washing and cleaning of spray equipment and pesticide containers, drift from spraying operations.

These pesticides find their way to the aquatic ecosystem through different routes (Konar, 1975, Basak and Konar, 1975) where they adversely affect aquatic life. Different groups of pesticides such as organophosphate, chlorinated hydrocarbon and carbamate are used in crop fields. Due to their lower persistence in the environment (Edwards, 1966; Hill and Wright, 1978), organophosphates are used judiciously to control a wide variety of agricultural pests as well as ectoparasites in fish in aquaculture. Organophosphate, especially dimecron (Phosphamidon), is maximally used in paddy fields of West Bengal, India. However, the indiscriminate use of these pesticides in agriculture and public health operations has increased the scope of ecological imbalance and thus many non-target organisms have become victims. Among the aqua fauna, fish form an important group due to their commercial value. Therefore, it becomes a matter of great concern when aquatic pollution due to pesticides is discussed.

From adjoining crop fields pesticides are drained into ponds (Konar *et al.*, 1997), resulting in depletion in fish yields. Biomagnification may also threaten the human food chain, especially where fish are affected. Since majority of these pesticides are highly toxic to fish and other aquatic life (Konar, 1977, Mani and Konar, 1985, Pal and Konar, 1985), we have tremendous

responsibility to use them wisely to keep the purity of aquatic environment. The plankton community on which whole aquatic population depends directly or indirectly is very much sensitive to the environment and any stress in this environment leads to shift in plankton abundance and composition. In biomonitoring study, this phenomenon of shifting plankton population is used for proper assessment of water pollution in addition to physico-chemical monitoring.

This air breathing fish, *C. punctatus*, inhabiting the accumulated water in the paddy field besides ponds, are the worst sufferers of residual effect of pesticides (though lower persistent) as revealed from some earlier records of investigation (Akhtar, 1984; Sun *et al.*, 1999; Hassan *et al.*, 1993; Carr *et al.*, 1997). Pesticides have been recognized as environmental pollutants of potential toxicological concern to fishes (Konar, 1981; Rao *et al.*, 1986). Pesticides are known to alter their behaviour pattern (Anderson, 1971), growth and nutritional value (Arunachalam *et al.*, 1980; Yaganobano and Tariq, 1981) reproductive potential (Johnson, 1967), and physiology (Baskaran 1980; Natarajan, 1981). A number of workers have investigated the toxicity, uptake and tissue distribution of pesticides in a number of fishes (Anderson and Defoe, 1980; Guiney and Peterson, 1980; Tilak *et al.*, 1980; Annes, 1975; Eisler, 1969; Saxena *et al.*, 1997).

Fish accumulate these xenobiotic compounds through their gills. Organophosphates are powerful neurotoxic chemicals as they inhibit acetylcholinesterase (AChE) (Casida, 1964; Coppage and Mathews, 1975; Kabeer Ahamed and Rao, 1980; Klaverkamp and Hobden, 1980). The rate of oxygen uptake of the bivalve depressed by the sub lethal levels of dimecron (Kulkarni and Keshavan, 1989). The toxicity of dimecron has been studied in relation to some pathological and histochemical changes of fresh water fish (Sastry and Malik, 1979).

The effect of pesticides on muscle protein has been studied by many workers (Wild, 1975; Shanmugam, 1977; Ganesan *et al.*, 1980; Panigrahi and Mishra, 1980; Murty and Devi, 1982). Sub lethal concentrations of certain pesticides bring about biochemical changes in the liver, muscle, brain and kidney of fish (Mukhopadhyay and Dehadrai, 1980; Sastry and

Sharma, 1981). The qualitative variations in serum protein pattern are also recorded in the fish *Sarotherodon mossambicus* (Peters) exposed to sub lethal concentrations of DDT, malathion (Ramalingam and Ramalingam, 1982). Malathion, an organophosphate pesticide, showed profound effect on the protein pattern of *Heteropneustes fossilis* (Kumar *et al.*, 1995). Variations in DNA and RNA contents in muscle of fish exposed to various pollutants have been noted by Mustafa (1977), Narayan Ram and Satyanesan (1986); Mohapatra and Noble (1992), Das (1998).

A variety of enzymes and other proteins are produced by organisms in response to xenobiotic exposures. Changes induced by pesticides on acid phosphatase (ACP) and alkaline phosphatase (ALP) of serum, liver, muscle, kidney and brain in different species of fish have been recorded by Sastry and Malik (1979), Borah and Yadav, 1996, Sastry and Sharma (1981), Khillare and Wagh (1988) and by Das and Mukherjee (2000). Alkaline phosphatase activity of *Sarotherodon mossambicus* showed a rise in various tissues following exposure to sumithion (Koundinya and Ramamurthi, 1982). Dichlorvos induced differential alterations in lipid levels and lipid peroxidation (LPO) in various regions of the fish brain and spinal cord was recorded by Vadhva and Hasan (1986). Organophosphate alters the levels of antioxidant enzymes of carp and catfish and induces changes characteristic of " oxidative stress" (Hai *et al.*, 1997). Changes induced by various toxicants on the activity of hepatic cytochrome P450 (cyt P450) in different species of fish have been recorded by Fent and Stegeman (1993), Forlin and Celander (1993), Norrgren (1993). Cytochrome P450 (CYP45) and stress proteins (HSP70) levels were significantly elevated in crustaceans for several days following heptachlor exposure (Synder and Mulder, 2001). Cd^{+2} intoxication resulted in a significant decrease of total cytochrome P450, microsomal protein, CYP2E1 activity (Alexandros *et al.*, 1994).

Biological indices based on plankton community are used for evaluation of water quality of fresh water body. Only a meagre information is available on variations of plankton community with respect to physico-chemical variations. Keeping this in view, the present investigation was done to assess the pollutional hazards of phosphamidon on aquatic ecosystem. Not much information, however, is available on the alterations of body weight,

relative organ weight due to organophosphate pesticides in the teleost fish. The available work in this field is that of (Undeger *et al.*, 2000) in rat model and that of (Ghosh and Medda, 1999) in chick model. Studies on the behavioral toxicology are meagre. Earlier report in this view was done by (Radhaiah and Jayantha Rao, 1988) on *Tilapia mossambica* exposed to fenvalerate. Pyrithroids are reported to affect the general locomotor patterns of fish (Bradbury *et al.*, 1985). The organophosphate pesticide exposed female green house-planting workers were characterized by longer reaction times and reduced motor steadiness compared to the unexposed workers (Bazylewicz-Walczak *et al.*, 1999). In India, most rural people consume air breathing fish especially *Channa punctatus* for its high protein content and low price. Thus, it is necessary to study the deleterious effects of phosphamidon on the body weight, relative organ weight along with behaviour of this fish.

The nucleus of the present study was to record our observations on the gel electrophoretic protein profiles, and the protein, DNA and RNA contents in liver, muscle and kidney of experimental fish, *Channa punctatus*, which forms an important food item of most rural people in India. Though a good number of literatures are available on the toxicity of pesticides in fishes, studies on the effects of sub lethal concentrations of toxicants on the enzymological changes are meagre. Biomarkers are important tools in the detection of various stresses such as pesticides, metals and other environmental pollutants in aquatic species. Hence, in the present study a modest attempt has also been made to evaluate the effect of sub lethal effects of phosphamidon on various biomarker enzymatic levels such as acid phosphatase (ACP), alkaline phosphatase (ALP), lipid peroxidation (LPO), reduced glutathione (GSH) and cytochrome p450 (cyt P450) in liver, muscle, kidney of a freshwater air breathing teleost fish, *Channa punctatus*, that forms an important food item of most rural people in India.

Results

Water quality:

Temperature (maximum and minimum), relative humidity and rainfall were noted during the study (Table 1). A gradual increase in free carbon dioxide and decrease in DO content occurred in waters exposed to 0.36 to 1.8 mg/l of phosphamidon and most significant ($p < 0.05$) at 1.8 mg/l, which was reflected in the Table-2, Fig: 2 & 1. Water temperature, (Table-2, Fig: 6) color and odour were not affected significantly. Total alkalinity was significantly ($p < 0.05$) reduced at 0.9 and 1.8 mg/l (Table-2, Fig: 3). Hardness of water gradually reduced, but not significant ($p > 0.05$) which varied from 226 -183 (Table-2, Fig. 4). The differences in pH of exposed waters were negligible (Table-2, Fig: 5).

Phytoplankton (PP) population was always greater than that of zooplankton (ZP) population in control and exposed waters. Total count of Phytoplanktons was reduced significantly ($p < 0.05$) with increasing concentration of the pesticide though at 0.36 mg/l, it showed an abrupt reduction to below 50% of the control. On the other hand, there was a linear reduction in number of Zooplanktons with the increase of phosphamidon concentrations though result was significant ($p < 0.05$) only at 0.9 and 1.8 mg/l (Table-3, Fig.7 & 8)

Behavioral responses

There was no marked increase in the swimming activity, no excitement and hyperventilation of the fishes immediately after they were transferred to sub lethal concentrations (0.36 mg/l and 1.8 mg/l) of dimecron. Higher concentration-exposed fish showed abnormality after 10 days onwards and lower concentration-exposed fish showed the same after 15 days onwards. Marked increase in swimming activity with darting movement was observed in T1 group after 20 days.

Erratic swimming along with loss of touch sensation and loss of balance (to some extent) was also observed in T2 group after15 days onwards. After 28 days, in fishes of T2 group surface ward movement and spasms including jerky movement was observed. Besides, laboured respiration, lack of desire to take food and aggressive behaviour also found.

Table 1. Atmospheric parameters recorded during test period.

Month	Temperature (°C) maximum	minimum	Relative Humidity %	Rainfall (mm)
January	26.8	13.1	65.7	0.00
February	27.4	15.2	62.4	0.00
March	31.2	17.8	64.3	0.71
April	35.6	23.9	70.3	4.25
May	37.4	25.3	73.4	6.17
June	39.7	25.8	77.1	4.68
July	34.7	24.5	79.0	12.66
August	33.6	26.1	82.1	7.28
September	32.3	25.5	75.3	5.62
October	30.9	20.7	67.4	2.36
November	27.1	16.3	66.2	0.00
December	20.2	11.5	61.6	0.00

Table 2. Influence of phosphamidon on water quality parameters dissolved oxygen (DO), free carbon dioxide (CO_2), total alkalinity, hardness, pH and temperature. Asterisk (*) indicate statistical significance at $p< 0.05$ over control value.

Concentration Treatment	Dose (mg/l)	DO (mg/l)	CO_2 (mg/l)	Alkalinity (mg/l)	Hardness (mg/l)	pH	Temperature (°C)
T1	0	9.78	1.88	196.75	226	7.29	24.42
T2	0.36	9.32	1.92	181.02	212	7.62	24.52
T3	0.45	8.64	2.06	174.25	204	7.64	24.66
T4	0.60	8.19	2.16	172.91	196	7.68	24.68
T5	0.90	7.96	2.22	168.82*	190	7.70	24.72
T6	1.8	6.92*	2.44*	157.70*	183	7.72	24.82

Table 3. Influence of phosphamidon on the phytoplankton (PP, number/liter), and zooplankton (ZP, number/liter). Asterisk (*) indicate statistical significance at $p< 0.05$ over control value.

Concentration Treatment	Dose ((mg/l))	PP (no./liter)	ZP (no./liter)
T1	0	92.02	29.2
T2	0.36	31.25*	25.4
T3	0.45	29.00*	22.3
T4	0.60	27.00*	20.1
T5	0.90	24.02*	15.0*
T6	1.8	22.00*	12.0*

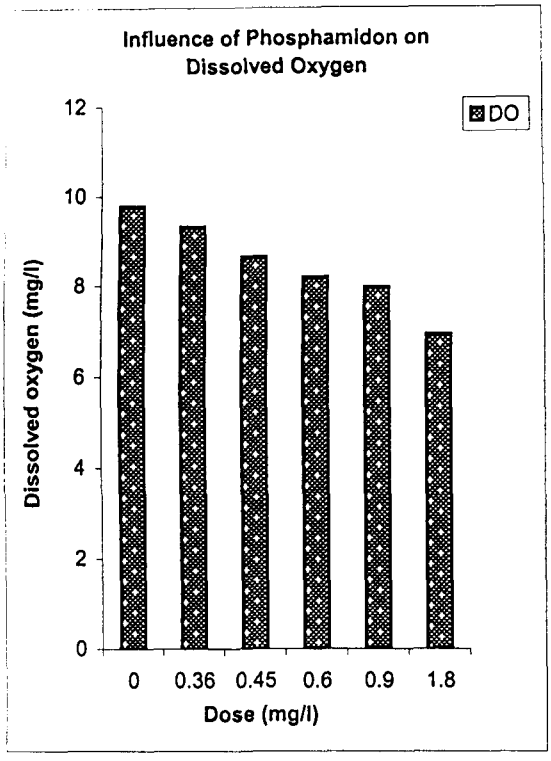


Fig:1

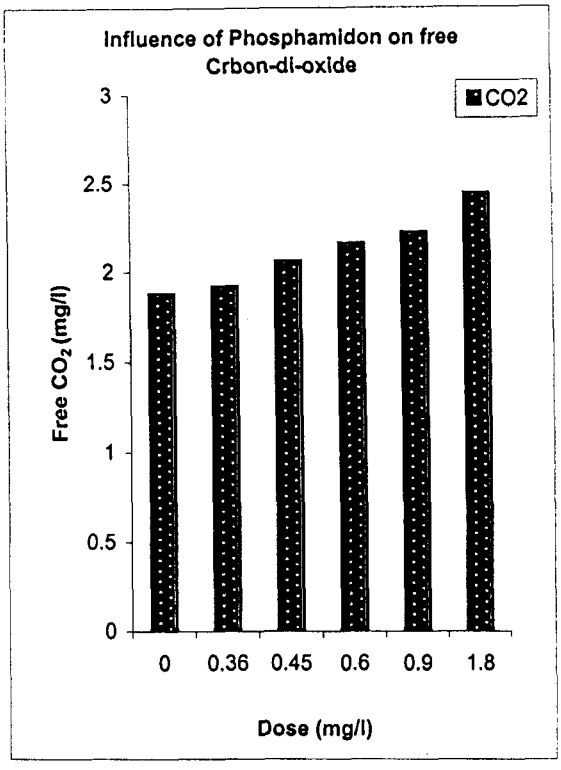


Fig:2

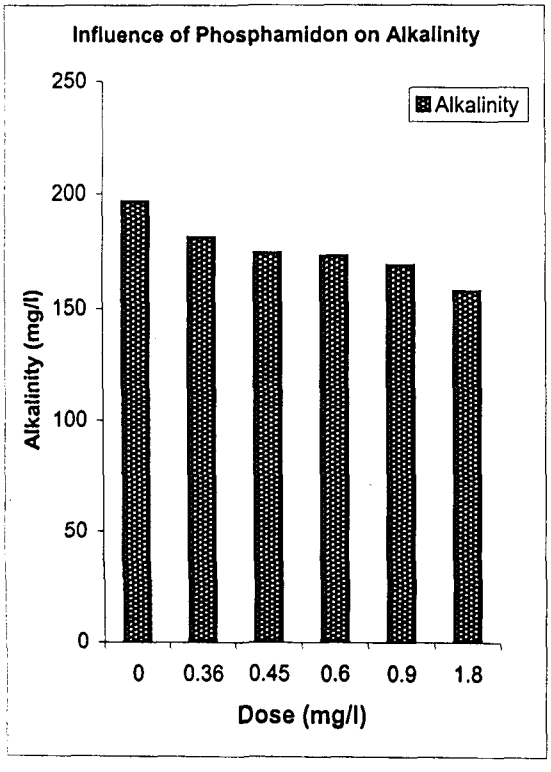
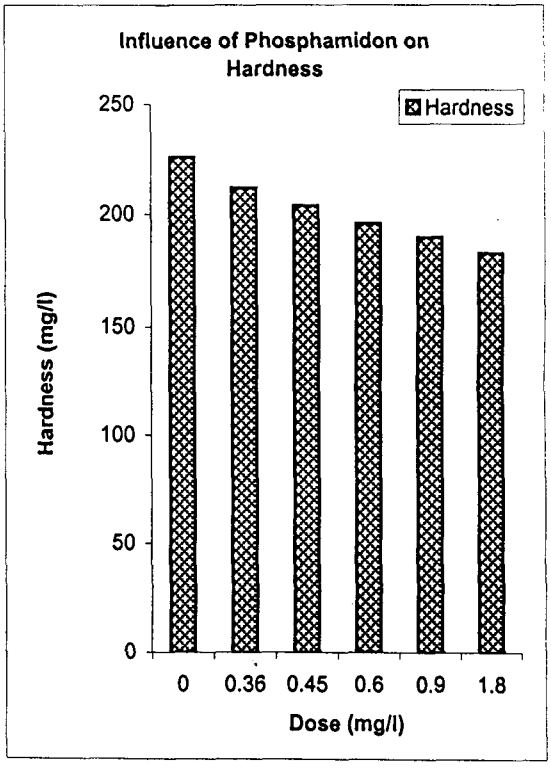


Fig : 3



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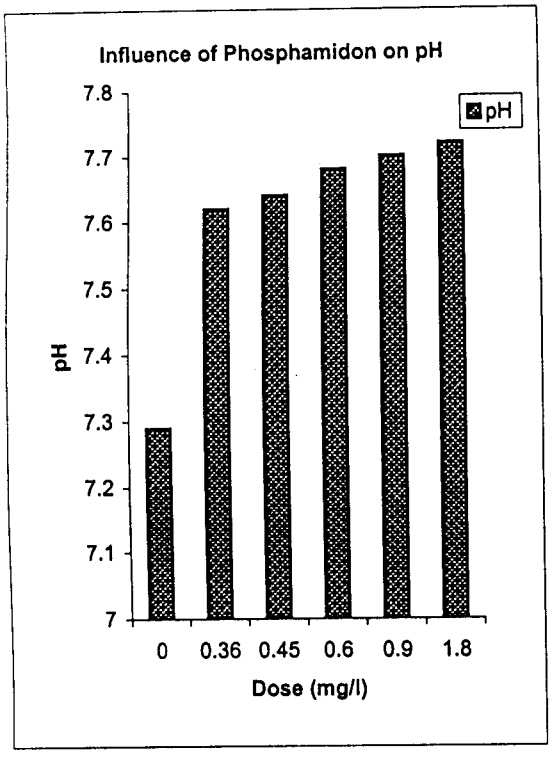

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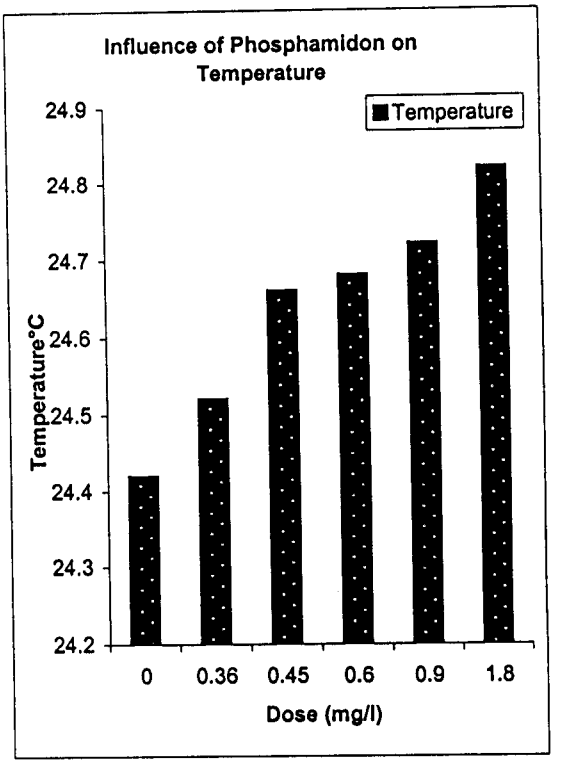

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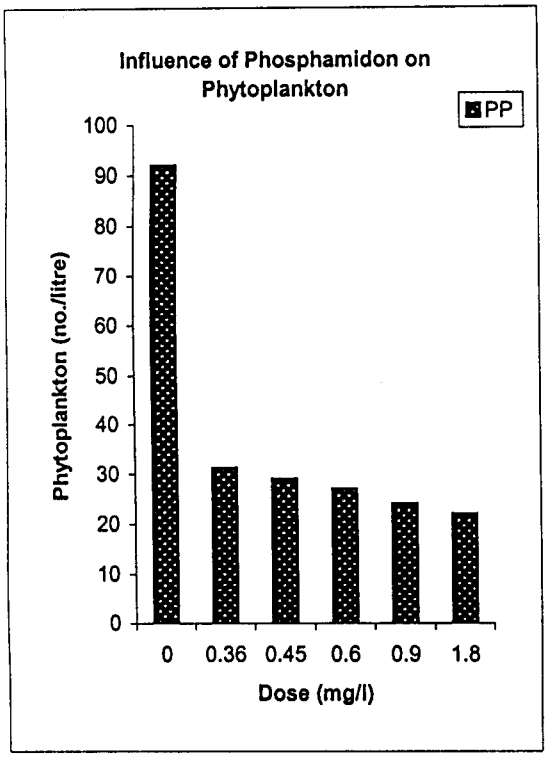

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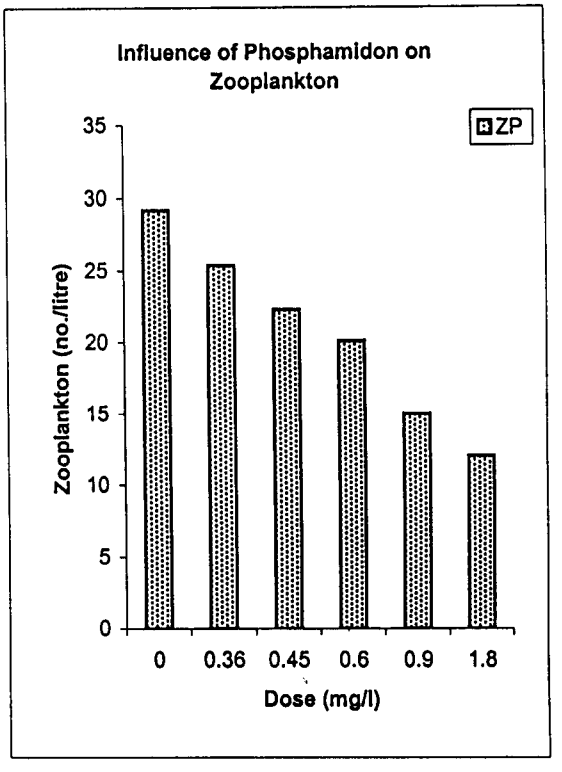


Fig:8

Body Weight

The mean body weight of the fish exposed to dimecron started decreasing in comparison to unexposed control from 10 days and upto 30 days. The same trend was observed in both the treated series. The decrease was maximum on 30 days in T2 group (i.e. @ 1.8 mg/l) when compared with the control animal. The decrease in body weight is statistically significant ($p < 0.05$) (Table-4, Fig: 9).

Organ Weight (Liver, Kidney)

Table-5, Fig: 10 and 11 shows that mean organ weight of liver and kidney does not remain constant throughout the days under study. This is applicable for both control and treated animals. The weight of liver and kidney increased remarkably from the controls as determined for different days, though the increase in liver weight at 0.36 mg/l of 10 days exposure was not significant. The maximum increase for both the organs on 30 days in T2 group (i.e. @ 1.8 mg/l). On statistical analysis it is found that the differences in weight between control and treated animals are Statistically significant ($p < 0.05$).

Organosomatic Indices (OSI)

Somatic indices for liver and kidney have been presented in Table-6 and 7, respectively. The data clearly indicates that dimecron caused a significant increase in the organ weights in all the exposure except liver weight at lower dose of 10 days exposure. This is clearly revealed from the HSI and RSI. The OSI were calculated by multiplying 100 to the ratio of organ weight to body weight.

Table 4. Distribution pattern of Body Weight (gm) of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l)	T2 (1.8 mg/l)
10	31.9±1.21	27.3±1.02*	26.4±1.01*
20	32.0±1.22	25.3±1.01*	24.2±0.98*
30	32.3±1.26	23.2±0.97*	22.4±0.86*

Table 5. Distribution pattern of Organ Weight (gm) of *Channa punctatus* at different exposures of Phosphamidon

Days	Organ	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	Liver	0.220±0.007	0.226±0.006*	0.262±0.001*
20	Liver	0.225±0.008	0.255±0.005*	0.272±0.009*
30	Liver	0.228±0.009	0.256±0.002*	0.288±0.007*
10	Kidney	0.140±0.002	0.163±0.004*	0.172±0.001*
20	Kidney	0.142±0.003	0.173±0.002*	0.183±0.004*
30	Kidney	0.145±0.005	0.179±0.001*	0.192±0.003*

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

Table 6. Hepatosomatic Index (HSI) of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.689	0.827	0.992
20	0.703	1.007	1.123
30	0.705	1.103	1.285

Table 7. Renosomatic Index (RSI) of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.438	0.619	0.651
20	0.443	0.683	0.756
30	0.448	0.771	0.857

Organosomatic Index= organ weight X 100/ body weight

No. of Fishes in all cases (15)

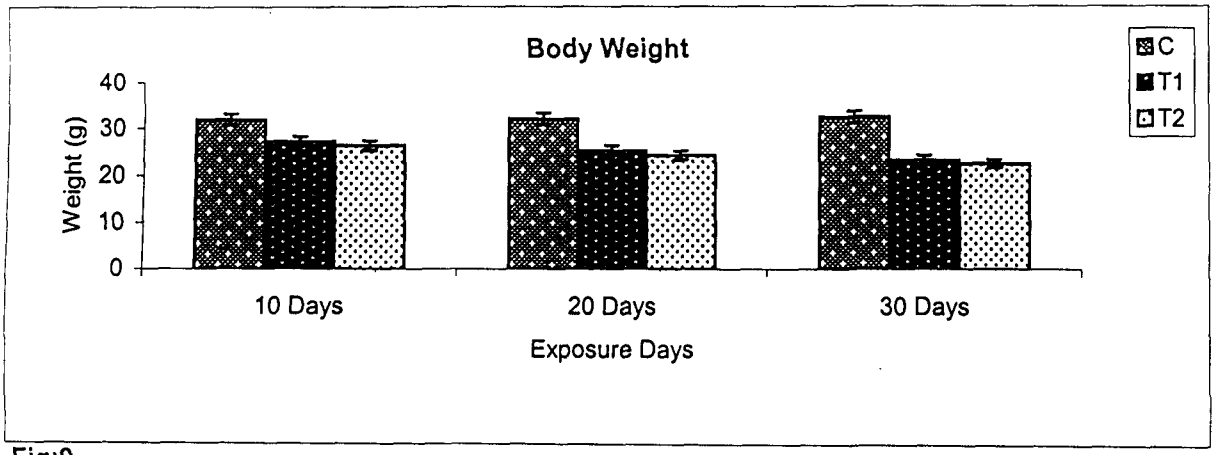


Fig:9

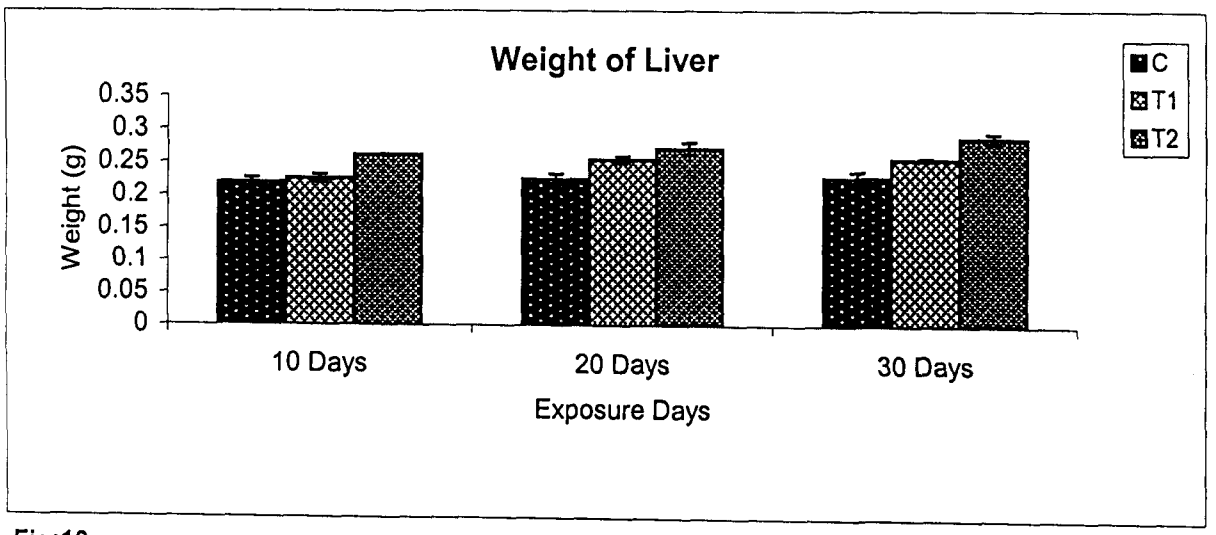


Fig:10

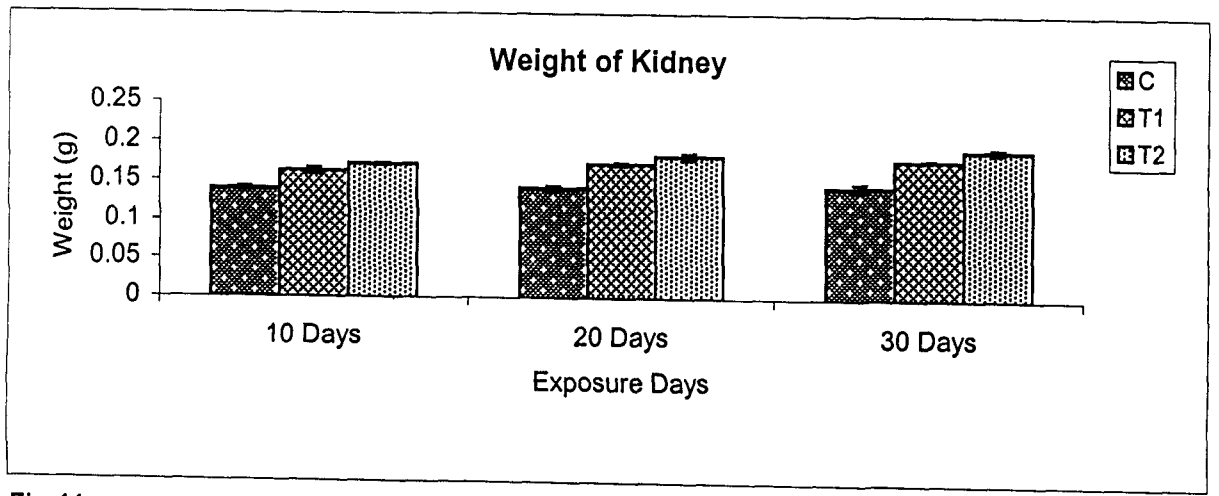


Fig:11

C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)

Quantitative estimation of total Protein, DNA and RNA

The concentration of protein in liver in the exposed animals reduced significantly in both T1 and T2 groups at 10, 20 and 30 days except in T1 group of 10 days exposure. The reduction in liver protein in T2 group was statistically significant ($p < 0.01$) in comparison to control animal on 20^{th} and 30^{th} day (Table 8, Fig. 12). Similarly, DNA concentration in liver decreased gradually in T1 and T2 groups at all the exposure periods though the reduction of DNA was not significant in T1 group on 10^{th} day (Table 9, Fig. 13;). On the other hand RNA content in liver tissue was decreased significantly ($p<0.01$) in both 0.36mg/l and 1.8mg/l exposures on all three occasions in comparison to control groups (Table 10, Fig.14).

The muscle protein in the exposed animals reduced at the above two concentrations, were however, not significantly low in comparison to the unexposed control on the 10 th day at 0.36 mg/l exposure. The same trend was found in the reduction of muscle DNA though the RNA of muscle tissue decrease significantly ($p<0.01$) in T1 and T2 groups at all the exposure periods (Table 11-13, Figs. 15-17).

Compare to the control groups the total protein content in kidney of T2 series showed a significant ($p<0.01$) reduction at all the three exposure periods the DNA of kidney tissue decreased significantly ($p<0.01$) at 0.36 mg/l and 1.8 mg/l exposure on 30 th days and at 1.8mg/l on 20 th days. The reduction of RNA in kidney tissue at above two concentrations were significant ($p<0.01$) on all the exposure days (Table 14-16, Figs. 18-20).

Qualitative analysis of protein band profiles

The gel electrophoretic protein profiles on 30^{th} day in liver, muscle and kidney of the experimental fishes have been presented in photographs 21- 23 (including marker, "M denote for marker in the photographs"). A critical analysis of the band comparison would reveal that certain bands present in unexposed control groups were found to be missing and a few unknown protein bands originated in treated fishes (i.e. @ 1.8 mg/l of dimecron exposure).

Table 8. Total Protein (mg/g) content in Liver of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	16 ±1.2	15.8 ±0.3ns	14.2 ±0.3*
20	16.7± 0.9	15.5± 0.3*	14.0± 1.2**
30	17.0± 1.1	15.3± 0.2*	13.5 ±1.3**

Table 9. Total DNA (mg/g) content in Liver of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	30.2±5.2	28.5± 4.8ns	26.2 ±3.1*
20	32.4± 4	27.5 ±4.7*	25.8 ±3.9**
30	34.4± 3	25.8± 3.9**	24.4± 3.1**

Table 10. Total RNA (mg/g) content in Liver of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	126.5± 11.4	89.7 ±6.2**	79.3 ±9.5**
20	128± 9.2	88.6 ±5.4**	72.4 ±8.4**
30	130.5 ±8.4	86.4 ±4.9**	70.2 ±7.2**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

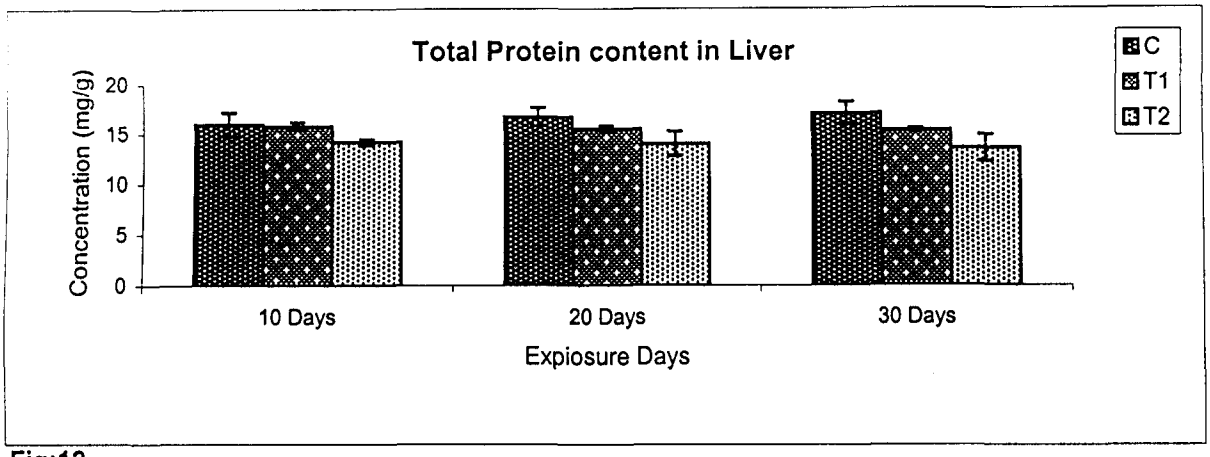


Fig:12

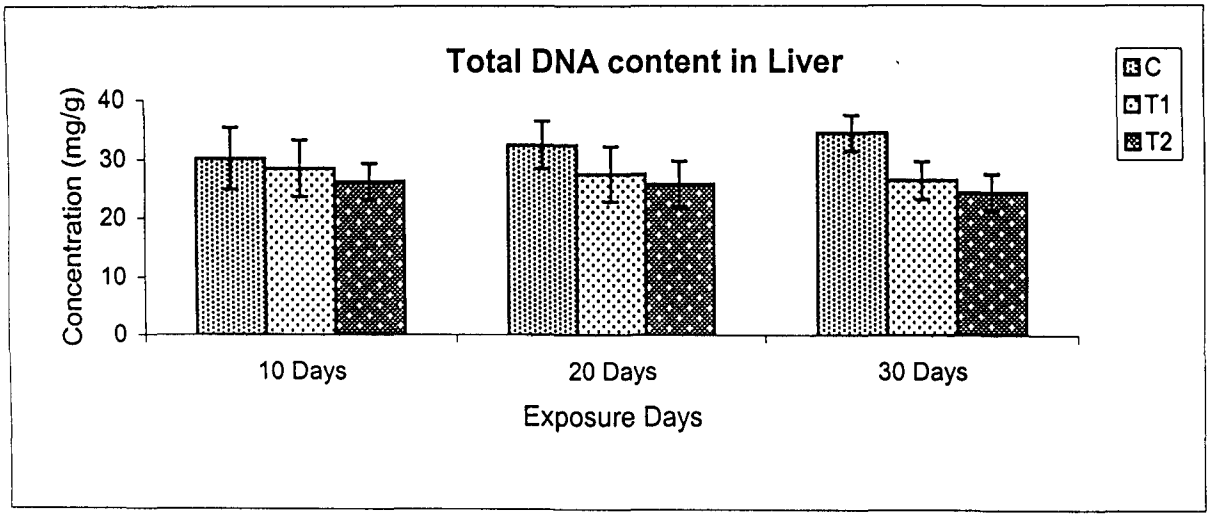


Fig:13

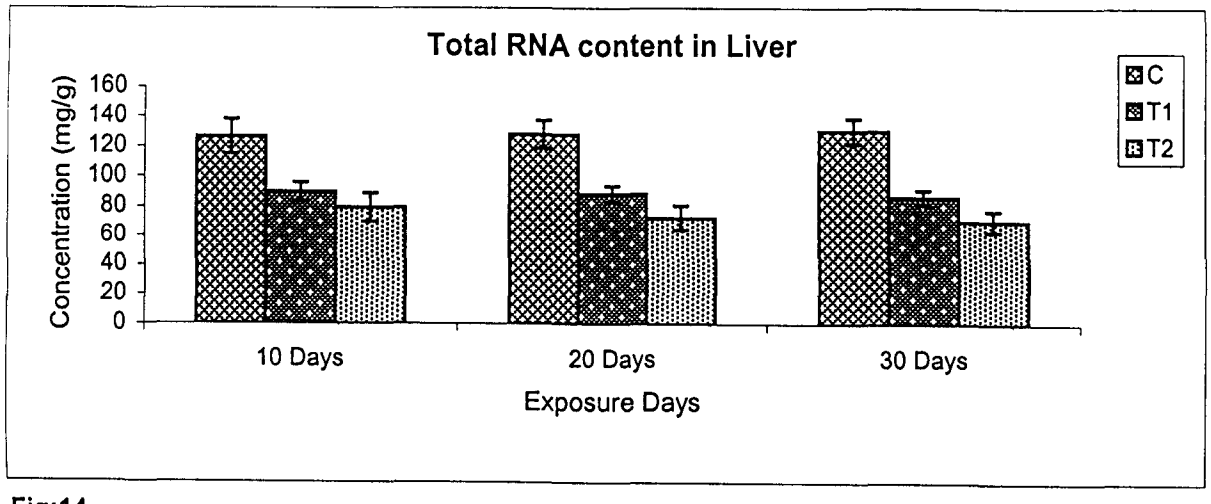


Fig:14

C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)

Table 11. Total Protein (mg/g) content in Muscle of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	19.0± 1.3	18.2± 0.9ns	16.2 ± 0.32*
20	20.0± 1.0	17.8 ± 0.4*	14.7 ± 1.3**
30	20.5 ± 1.2	15.2 ± 0.32**	12.6± 0.28**

Table 12. Total DNA (mg/g) content in Muscle of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	32.2± 5.4	30.1± 5.1ns	25.9 ±3.2*
20	34.6 ±5.1	28.4 ±4.9*	24.2 ±3.8**
30	35.9 ±4	27.4 ±4.1**	22.3± 3.7**

Table 13. Total RNA (mg/g) content in Muscle of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	128.2± 12.2	87.4 ± 8.5**	85.2± 6.1**
20	129.1± 9.8	86.2± 5.6**	82.8 ± 5.7**
30	130.2 ± 7.8	84.1± 4.9**	80.3 ± 6.1**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

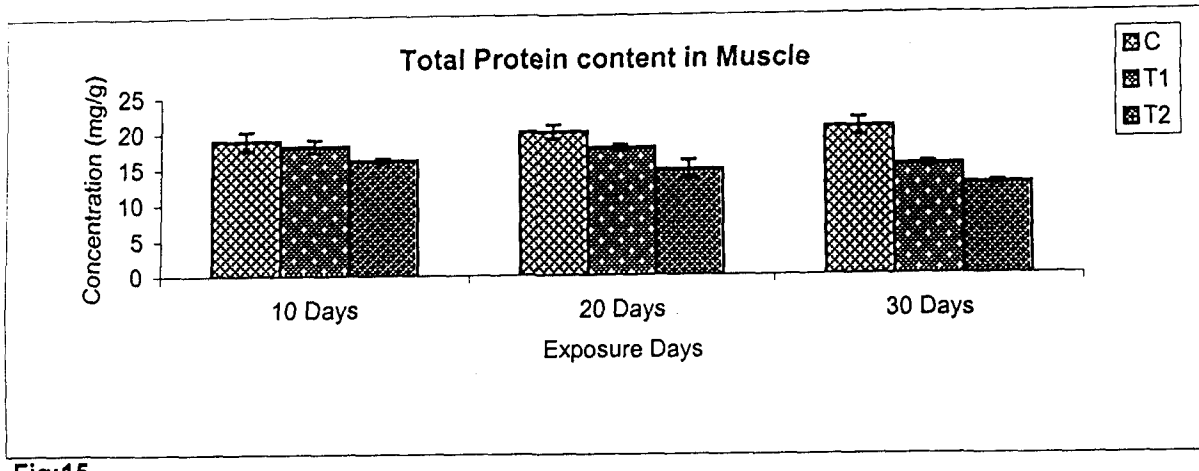
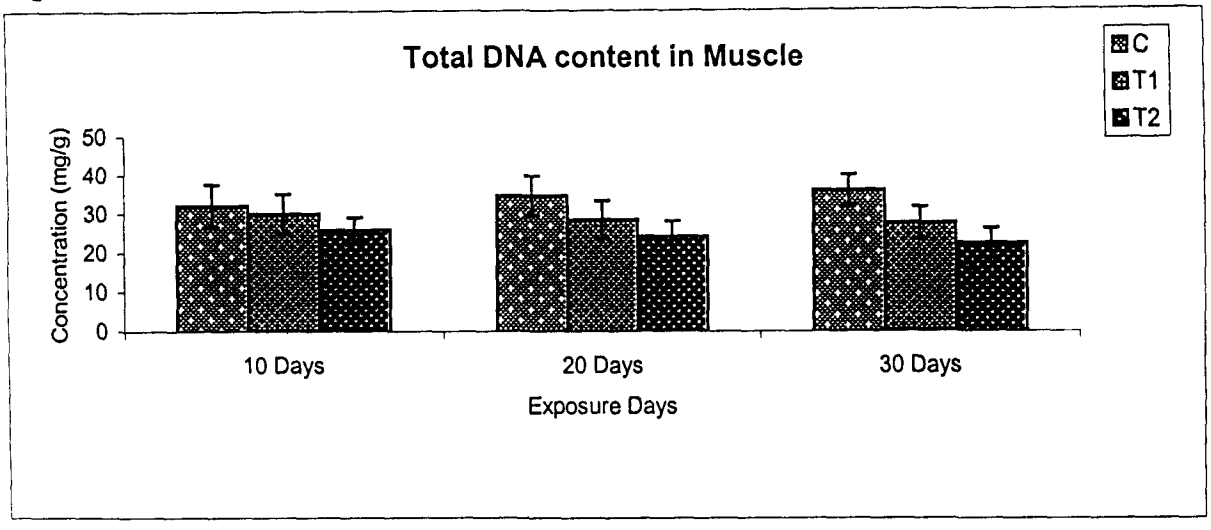


Fig:15



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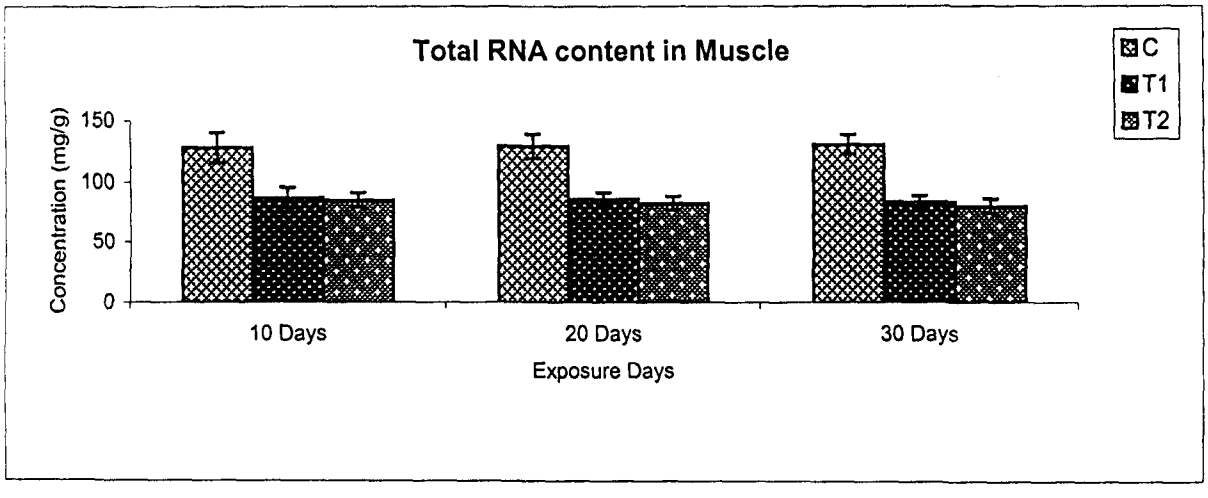


Fig:17

C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)

Table 14. Total Protein (mg/g) content in Kidney of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	12 ±1.1	11.5±0.8ns	9.8 ±0.22**
20	12.9± 2.2	10.5± 0.4*	9.6 ±1.2**
30	14.1 ±2.9	10.1 ±0.22**	9.2 ±1.3**

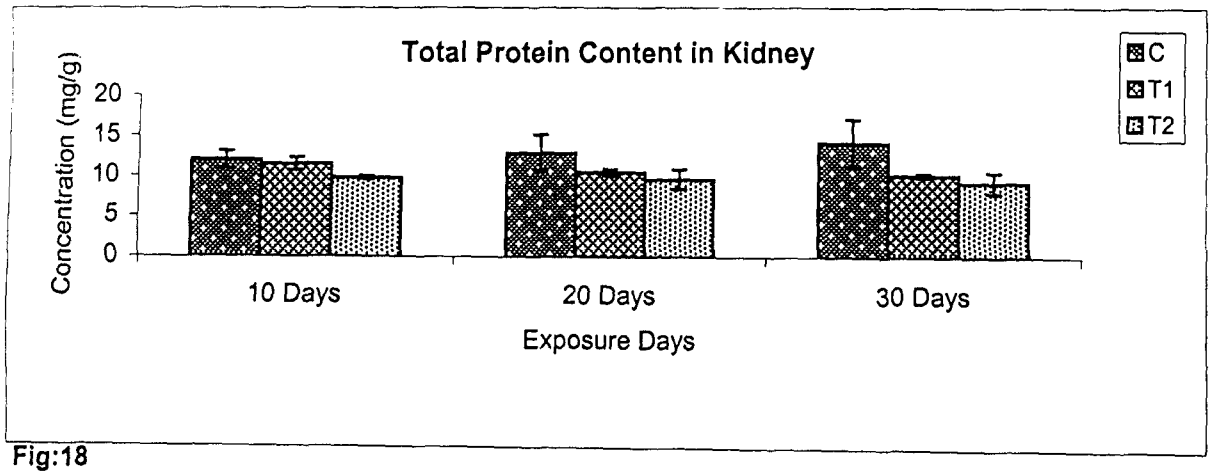
Table 15. Total DNA (mg/g) content in Kidney of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	29.4± 4.8	28.1± 4.2ns	23.8 ±2.2*
20	30.1 ±4.1	26.2±3.9*	23.4± 3.7**
30	31.2 ±4.4	25.3 ±3.1**	22.7 ±2.9**

Table 16. Total RNA (mg/g) content in Kidney of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	112.4 ±11.2	92.4 ±5.9**	81.2 ±7.5**
20	114.3± 5.9	85.3 ±5.4**	72.6 ±6.9**
30	115.1 ±6.2	84.3 ±5.4**	70.3 ±6.2**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**



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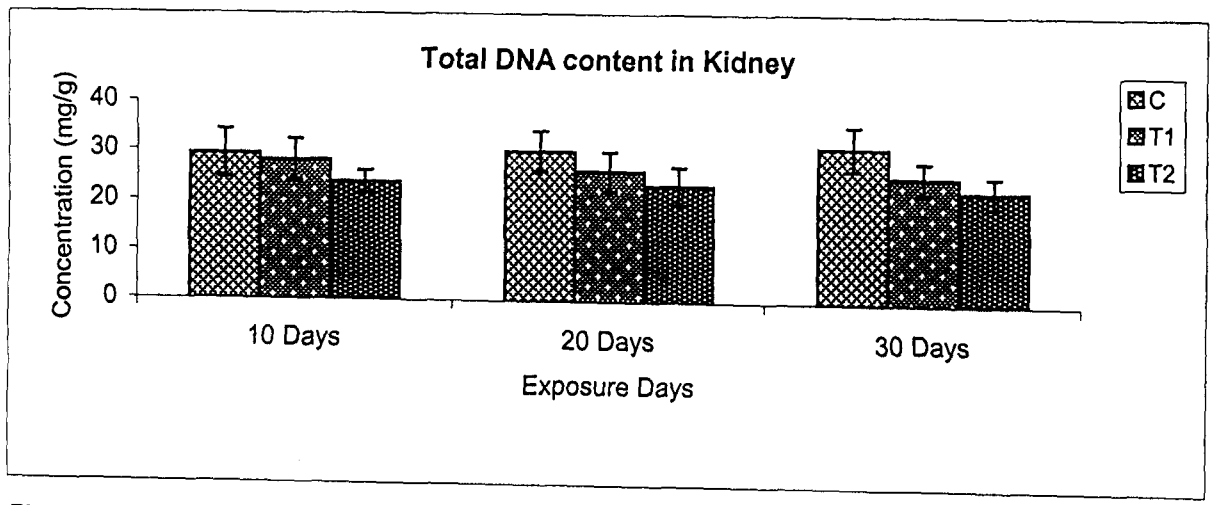

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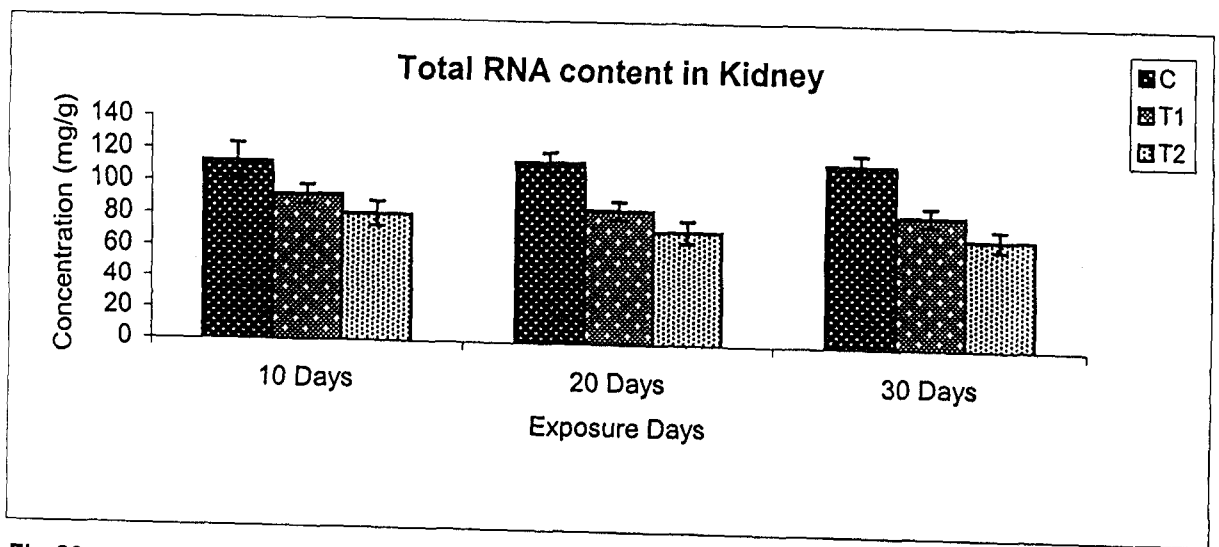
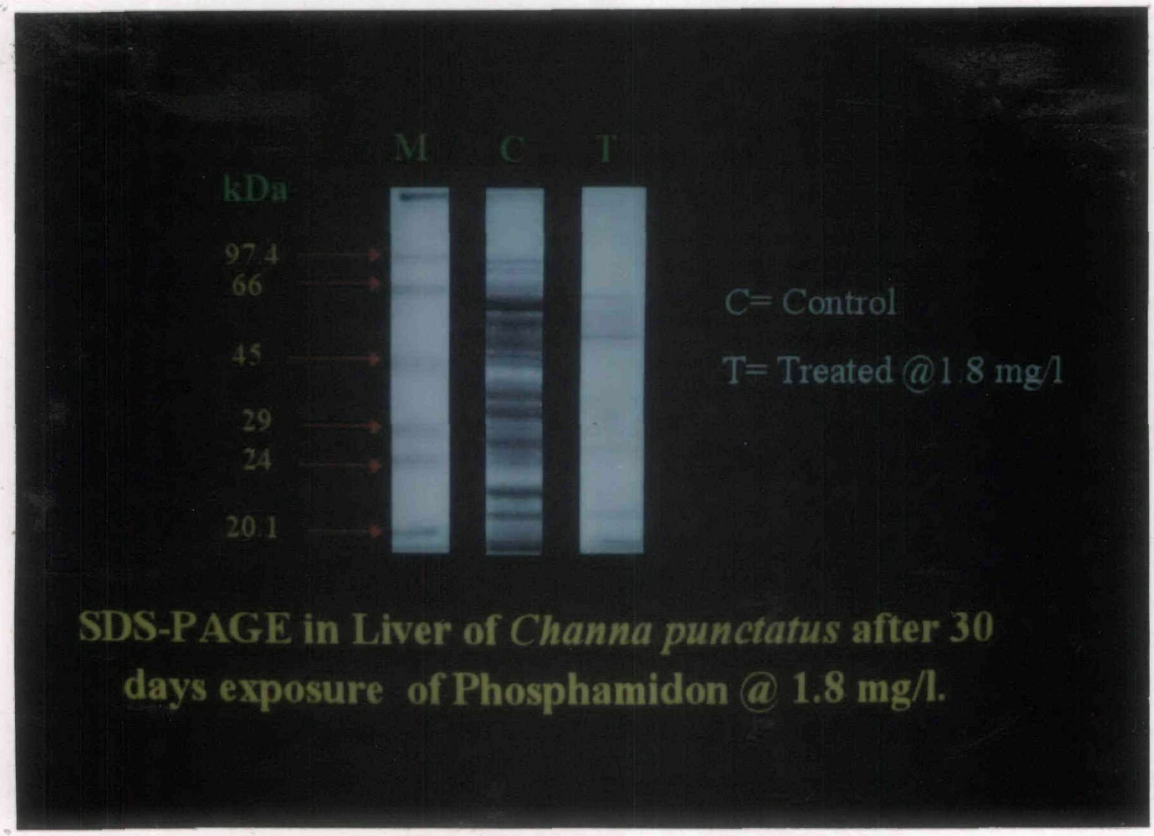
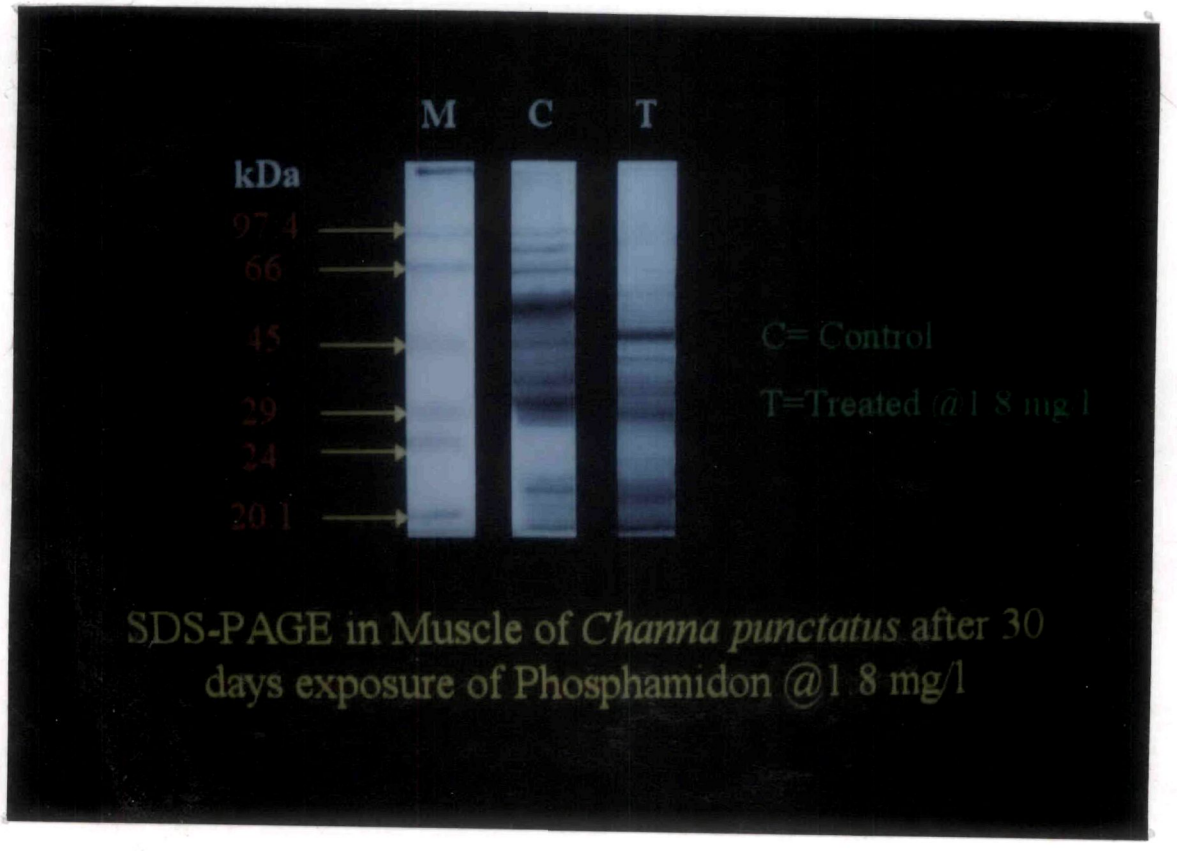


Fig:20

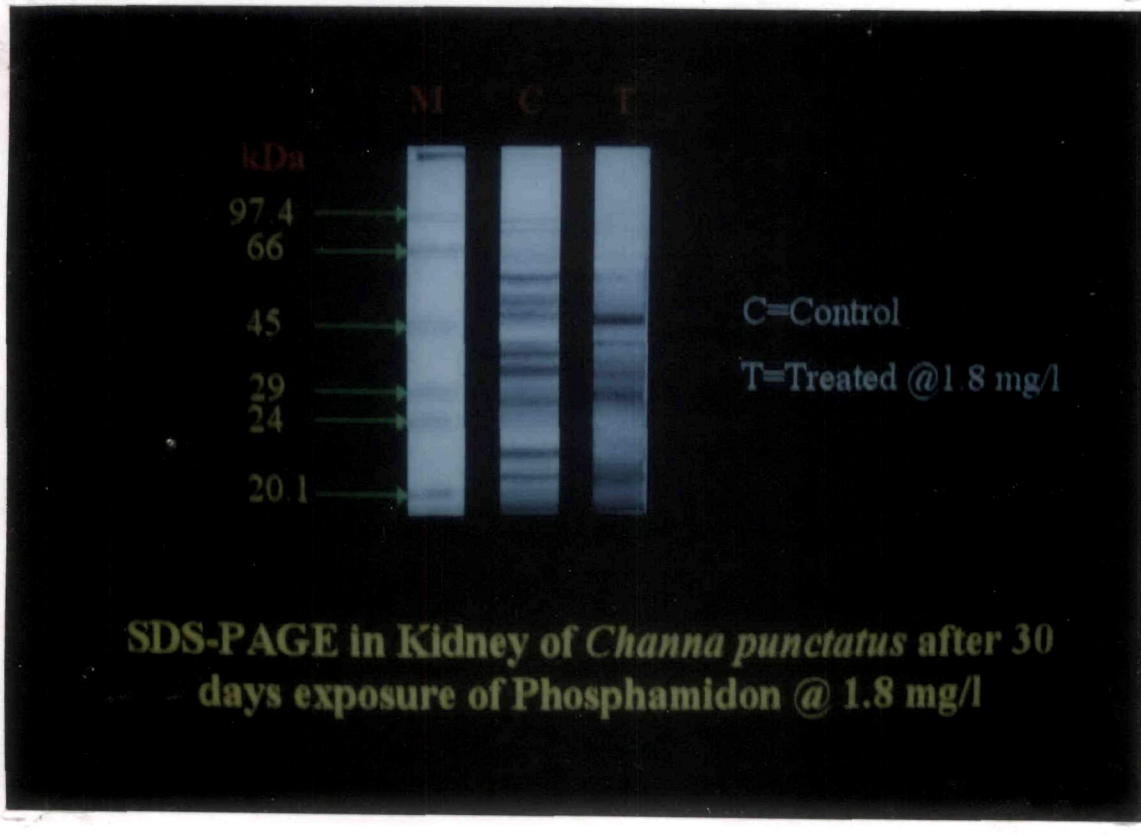
C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)



SDS-PAGE in Liver of *Channa punctatus* after 30 days exposure of Phosphamidon @ 1.8 mg/l.



SDS-PAGE in Muscle of *Channa punctatus* after 30 days exposure of Phosphamidon @1.8 mg/l



SDS-PAGE in Kidney of *Channa punctatus* after 30 days exposure of Phosphamidon @ 1.8 mg/l

Acid phosphatase and Alkaline phosphatase activities

Among the enzyme activities, ACP activity was significantly ($p<0.01$) elevated in liver, muscle and kidney exposed to both sublethal concentrations (0.36 mg/l and 1.8 mg/l) at all the exposure periods though the elevation was significant at 5% level in kidney tissue exposed to 0.36 mg/l concentration at 10 days exposure period (Table 17, 22, 27, Figs. 24, 29, 34). Similarly significant ($p<0.01$) elevation of ALP activity was noticed in liver, muscle exposed to both concentrations at all the treatment periods. In case of kidney tissue, though significant elevation of ALP was also found but not always at 1% level. The maximum increase was observed in liver and muscle tissue compared to kidney (Table 18, 23, 28, Figs. 25, 30, 35).

Lipid peroxidation and glutathione levels

The concentration of malondialdehyde (MDA), an indicator of lipid peroxidation, was observed to increase in liver, muscle and kidney of dimecron exposed groups, which has been summarized in the (Table 19, 24, 29, Figs. 26, 31, 36). The activity of lipid peroxidation was, in decreasing order, in liver, kidney and muscle, and was significant in both the concentrations at all the treatment periods. On the other hand, the maximum percentage of inhibition of GSH was recorded in liver tissue followed by muscle and then kidney, at both the concentrations in all the treated fishes (Table 20, 25, 30, Figs. 27, 32, 37).

Cytochrome P450 activity

The cyt P450 level (nmol/mg microsomal protein) of various groups, both treated and controls have been summarized in (Table 21, 26, 31, Figs. 28, 33, 38). Data showed that cyt P450 activity in liver, muscle and kidney was significantly ($p<0.01$) induced in both the concentrations over 20 and 30 days. There was a linear increase in the activity in liver, muscle and kidney tissues (in muscle more pronounced) along with the lapse of time.

Table 17. Activity of the acid phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation) in liver of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.320 ±0.011	1.42 ±0.21**	1.48 ±0.15**
20	0.321± 0.008	1.78± 0.23**	2.43± 0.13**
30	0.325 ±0.009	1.82 ±0.28**	2.46 ±0.16**

Table 18. Activity of the alkaline phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation) in liver of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.932 ±0.46	3.32 ±1.28**	3.79± 0.70**
20	0.978± 0.281	3.82 ±1.32**	3.95± 0.90**
30	0.958 ±0.231	4.02 ±1.39**	4.22± 1.02**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

Table 19. Lipid peroxidation (n mole MDA/g) level in liver of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	338.10± 6.26	510.02 ±18.01**	522.89 ±19.81**
20	356.05 ±8.20	526.26± 18.80**	542.02 ±19.70**
30	362.04 ±7.56	556.03 ±19.95**	562.07 ±20.01**

Table 20. Reduced glutathione (m mole/g) level in liver of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.231± 0.004	0.112± 0.007*	0.109± 0.009*
20	0.232 ±0.005	0.0992 ±0.007**	0.0872 ±0.006**
30	0.236± 0.005	0.0792± 0.004**	0.0752± 0.006**

Table 21. Cytochrome p450 (m mole/mg) level in liver of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.455± 0.006	0.475 ±0.007**	0.625± 0.009**
20	0.458 ±0.009	0.515± 0.008**	0.695 ±0.007**
30	0.459± 0.01	0.575± 0.04**	0.702± 0.02**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

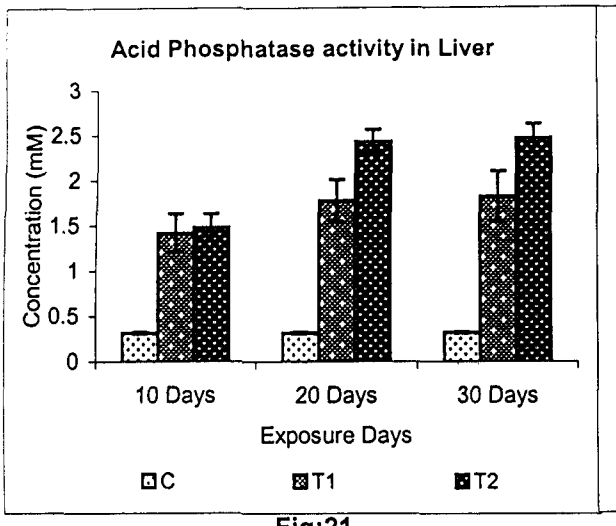


Fig:21

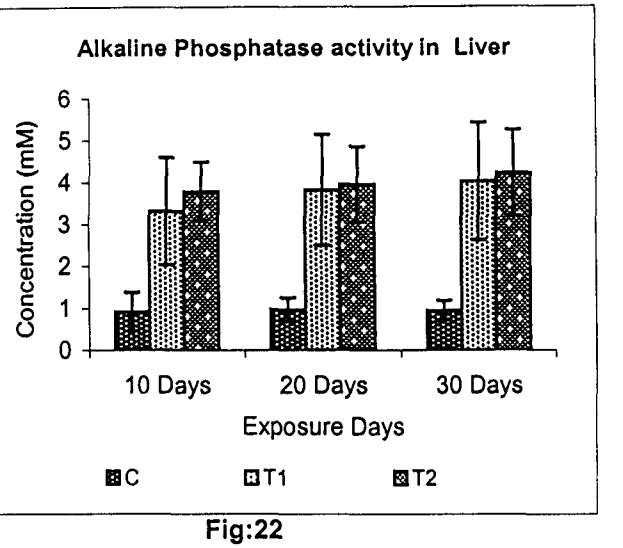
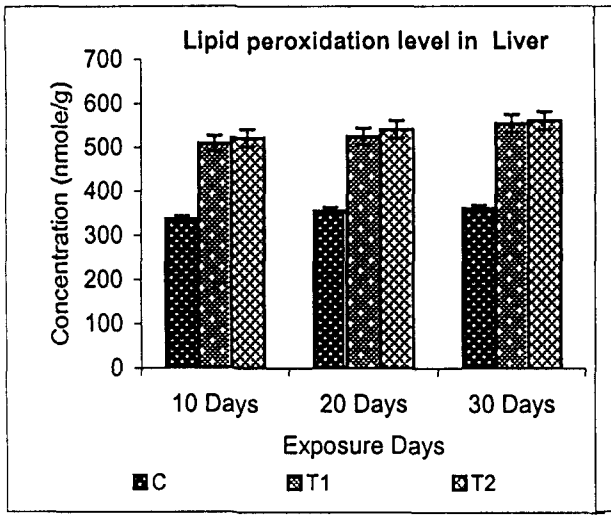


Fig:22



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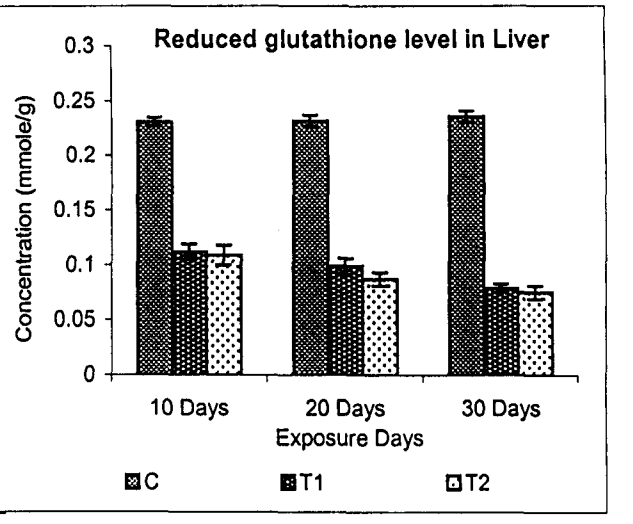


Fig:24

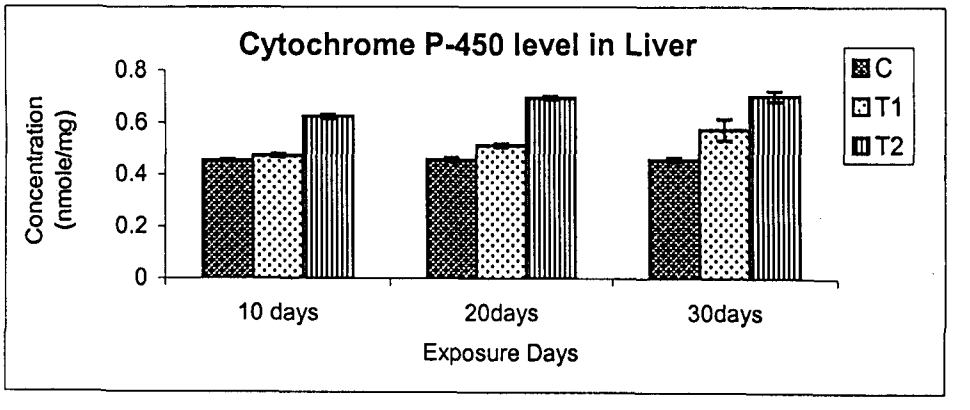


Fig:25 C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)

Table 22. Activity of the acid phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation in muscle of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.294 ±0.007	1.002± 0.023**	1.32± 0.03**
20	0.292± 0.008	2.12± 0.025**	2.32 ±0.023**
30	0.294 ±0.009	2.39 ±0.031**	2.48 ±0.034**

Table 23. Activity of the alkaline phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation in muscle of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.371± 0.011	1.44± 0.03**	1.84± 0.05**
20	0.363± 0.006	2.92 ±0.039**	3.01± 0.042**
30	0.372 ±0.007	3.05± 0.044**	3.10± 0.052**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

Table 24. Lipid peroxidation (n mole MDA/g) level in muscle of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	629.20± 5.28	782.17± 18.28*	812.11 ±20.61*
20	626.20 ±7.40	792.17 ±18.38*	821.02± 18.36*
30	630.31 ±8.02	812.32± 17.92**	830.34±19.72**

Table 25. Reduced glutathione (m mole/g) level in muscle of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.413± 0.009	0.259± 0.005**	0.225± 0.003**
20	0.425 ±0.005	0.243± 0.006**	0.215± 0.006**
30	0.427 ±0.007	0.232± 0.005**	0.212± 0.004**

Table 26. Cytochrome p450 (m mole/mg) level in muscle of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.452 ±0.009	0.512± 0.008*	0.610 ±0.006**
20	0.454 ±0.01	0.598 ±0.007**	0.682± 0.005**
30	0.455 ±0.012	0.604± 0.006**	0.712 ±0.004**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

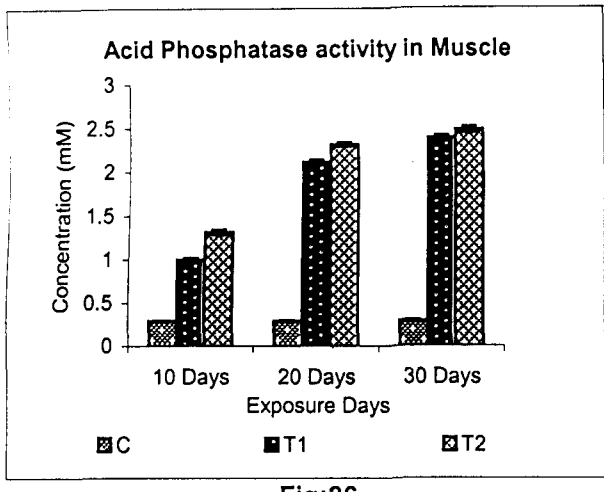


Fig:26

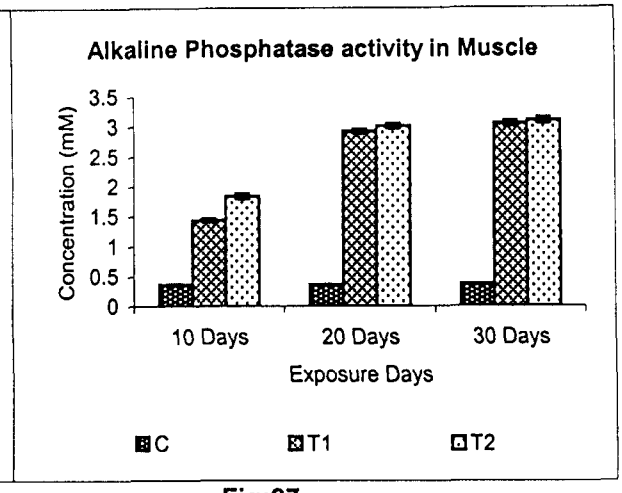
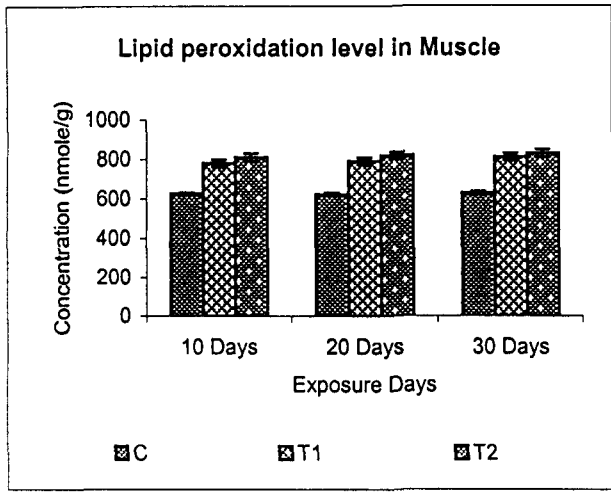


Fig:27



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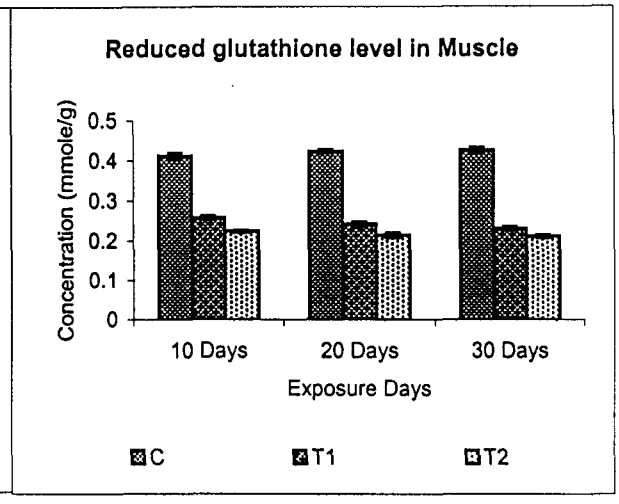


Fig:29

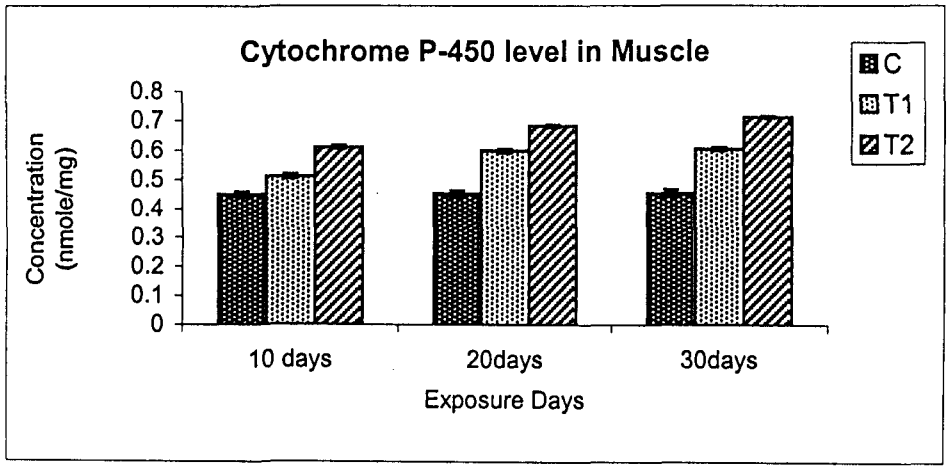


Fig:30 C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)

Table 27. Activity of the acid phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation in kidney of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.179± 0.011	0.286 ±0.030*	0.302± 0.032**
20	0.175± 0.012	0.312± 0.036**	0.322±0.042**
30	0.178 ±0.014	0.324 ±0.044**	0.328± 0.052**

Table 28. Activity of the alkaline phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation in kidney of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.312 ±0.045	0.373± 0.086*	0.399 ±0.066*
20	0.314 ±0.046	0.401 ±0.072**	0.422± 0.081**
30	0.315± 0.049	0.432 ±0.082**	0.455± 0.091**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

Table 29. Lipid peroxidation (n mole MDA/g) level in kidney of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	323.70 ±6.28	403.39±8.10*	418.78± 8.12*
20	326.81± 6.78	428.68 ±9.14**	432.72± 10.12**
30	328.82± 6.92	444.62± 10.34**	452.52 ±10.92**

Table 30. Reduced glutathione (m mole/g) level in kidney of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.245± 0.007	0.220 ±0.011*	0.196± 0.012*
20	0.246 ±0.008	0.188± 0.015**	0.162 ±0.012**
30	0.244± 0.007	0.152± 0.014**	0.140± 0.012**

Table 31. Cytochrome p450 (m mole/mg) level in kidney of *Channa punctatus* at different exposures of Phosphamidon

Days	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	0.352± 0.009	0.412± 0.011*	0.474 ±0.012*
20	0.354 ±0.010	0.454 ±0.015**	0.493 ±0.014**
30	0.355± 0.016	0.462 ±0.024**	0.506± 0.026**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

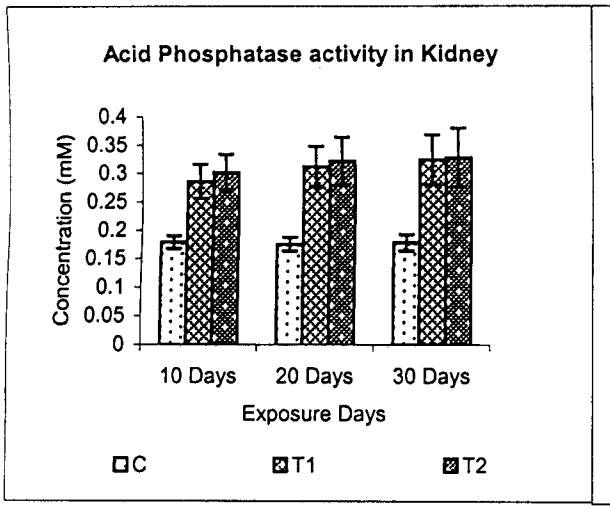


Fig:31

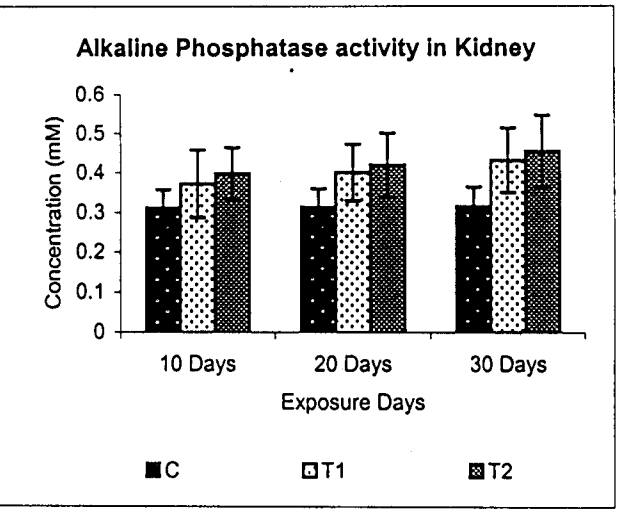


Fig:32

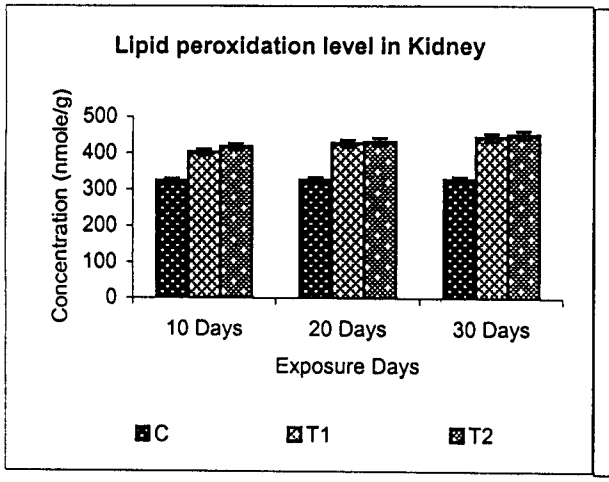


Fig:33

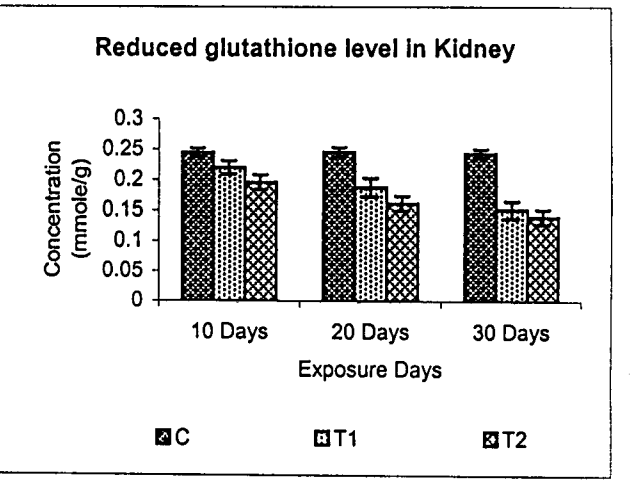


Fig:34

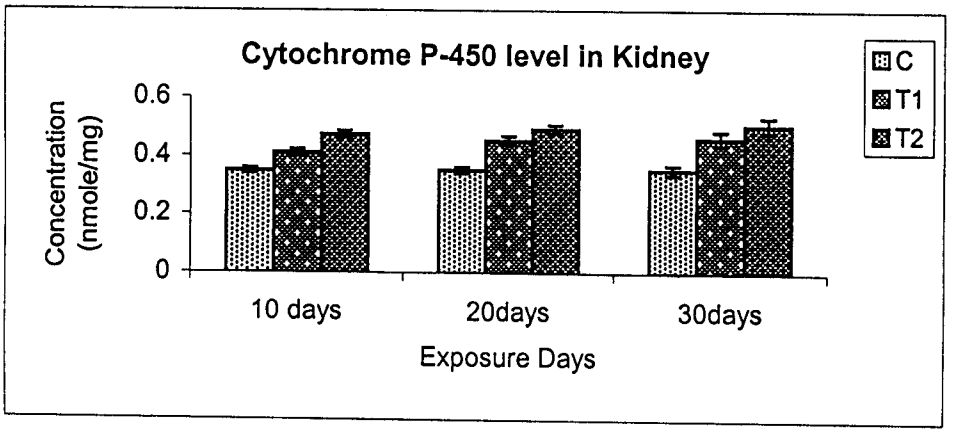


Fig:35 C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)

Discussion

The present study indicates, that phosphamidon induces great pollutional hazards and its contamination of aquatic ecosystem at the concentration tested must be prevented for better prospects of fisheries. The lower values of DO in pesticide-exposed water indicated the stress condition of the aquatic system. Though phosphamidon had no significant influence on hardness and temperature, the reduction of water alkalinity and elevation of free carbon dioxide due to the exposure of phosphamidon will be a major factor in reducing significantly the yield of fish. High free CO_2 concentration reduces the capacity of blood to transport O_2, which in turn keeps the fish under stress also. pH is very important in regulating respiration and enzyme system (Odum, 1977). The little fluctuation of pH was observed in the present study though not significant. Studies by Muirhead-Thomson (1971) have attested the importance of physico-chemical factor of water, which greatly influence the pesticide impact on fresh waters.

Ratio of phytoplankton and zooplankton population play a decisive role in the growth and overall production of fishes especially which feed on plankton. Even survival of fish is impossible if this food is wiped off or contaminated. In the present investigation it was also noticed that the rate of phytoplankton reduced gradually. Significant reduction in zooplankton population occurred in all exposed water and the maximum reduction was at 0.9 and 1.8 mg/l. This is due to the high sensitivity of zooplankton to this pesticide, which in turn will hamper the growth of fishes especially carp fishes in India as zooplankton is the main source of food and is related with the growth of rohu, mrigal and other carps (Chakraborty and Jana, 1991, Jhingran and Pullin, 1985). Population of phytoplankton was lowered suggesting that the tested concentrations of phosphamidon was also phytotoxic. The reduction of phytoplankton in exposed water was always greater than that of zooplankton. It was due to more confinedness of phytoplankton to the surface water for photosynthesis as, solar radiation is more available to the surface water.

The water used for aquaculture will not give desired production unless the prevailing water quality parameters are not optimal for the organisms under culture. A gross effect upon any major factor or group of organism produces changes in the entire ecosystem. Therefore, there is need also for judicious use of another organophosphate pesticide, phosphamidon so that,

the natural resources of waters particularly, fish fauna and their food organisms may be protected for human benefit.

Although toxic effects of organophosphate pesticides in respect to biochemical parameters in teleost fish had been reported (Hai *et al.*, 1997; Das and Mukherjee, 2000), no detailed study had earlier been made on the gain of weight of different individual tissue in contrast with the loss of body weight as a whole along with altered behavioral response. Further, the extent to which even sub-lethal concentration of phosphamidon at minimum exposure could affect the tissue weight had not also been properly assessed. From the results presented above, it would be quite evident that even sub-lethal concentration of phosphamidon brought about striking loss in weight of the individual fish at three successive intervals observed up to 30 days. It is also related with the dose and with the time. Enzymatic pathways may be altered in response to toxicant exposure in order to maintain homeostasis. The changes could involve a shift in an anabolic process (eg. protein synthesis for tissue repair) or a catabolic process (eg. use of lipid stores to meet energy demands created by stressor). Hence, loss of body weight in present experiment suggests that phosphamidon has prominent effect on metabolic processes and is fairly in agreement with the reports of Cavanagh (1964).

An increase in HSI results following the exposure of fish to phosphamidon. This is due to the attempt the fish is making to adapt to the presence of the toxicant. The organism attempts to increase the effectiveness of the liver to detoxify the substance by increasing the volume of the liver. This is done by either increasing the number of cells in the liver (hyperplasia) or by increasing the size of each newly produced liver cell (hypertrophy). As the detoxifying mechanism of not only liver but also kidney is very sharp, the increase in the RSI observed might be due to the hyperactivity of the kidney under the toxic influence of the insecticides. It is fairly in agreement with the reports of (Sarin and Saxena, 1982).

The behavioral study was also a remarkable feature of this study. Erratic swimming, loss of balance and loss of touch sensation in phosphamidon exposed fish is due to failure of nervous system. As is known, acetylcholinesterase (AChE) plays an important role in the transmission of nerve impulses (Stryer, 1995). Organophosphates are powerful neurotoxic

chemicals as they inhibit acetylcholinesterase (Coppage and Mathews, 1975; Kabeer Ahmed and Rao, 1980; Rath and Mishra, 1981). This enzyme's main function in the nervous system is to break down the neurotransmitter acetylcholine. When AChE is inhibited by Organophosphate pesticides, it cannot perform this breakdown function and acetylcholine accumulates. Acetylcholine accumulation increases nerve impulse transmission and leads to nerve exhaustion and, ultimately, failure of the nervous system. When the nervous system fails, muscles do not receive the electrical input they require to move. The respiratory muscles are the most critical muscle group affected, and respiratory paralysis may often the immediate cause of stress. Respiratory abnormality could be due to hypoxia caused by inadequate tissue use of oxygen (Guyton and Hall, 2000).

Hence this study is an indicative of the hazardous effect of phosphamidon even in a lesser dose in the fishes as reflected in changes in the body weight, relative organ weight and behavioral response.

Accumulation of Organophosphate compounds (Hassan *et al.* 1993) could drastically affect the metabolic as well as functional activities of those tissues, which in turn, could drastically lead to reduction in enzymatic activities or functional activities. This could explain the diminished total protein content in different tissues as a result of phosphamidon exposure. Incidentally, the biochemical and cellular effects of long-term exposure to organophosphate compound have been well documented earlier (Mukhopadhyay and Dehadrai, 1980, Narayan Ram and Satyanesan, 1986, Hai *et al*; 1997). The significant reduction of DNA content was noticed in case of 10, 20 and 30 days exposure to 0.36 and 1.8 mg/l of phosphamidon in the present study. Decrease in RNA content in muscle tissue of *Labeo rohita* exposed to a sub lethal concentration of malathion was also reported by Das and Mukherjee (1997). The decrease in RNA content of muscle and other tissues following sub lethal exposure of phosphamidon in the present study may be attributed to the anorexia especially the pathological condition of anorexia nervosa developed during the test period.

In view of the significant correlation of RNA and protein, a deficient synthesis of any type of RNA should have its reflection in corresponding failure of protein synthesis, as seen in the

present study. Possibility of lesion of m-RNA functional capacity for such failure cannot be ignored (Bruin, 1976). Further, protein synthesis is dependent on DNA synthesis (Balis, 1968) phosphamidon might have blocked the synthesis of DNA and consequently the synthesis of DNA directed RNA formation and hence, the resultant reduction of proteins. It is clear from this study that decrease of RNA, DNA level over the control indicates the reduction in the synthesis of protein. As stated earlier, the loss in total protein and diminished RNA content would point to the failure of protein synthesizing machinery of the cell. The precise mechanism of protein synthesis in both lower and higher forms of organisms is under genetic control and is well documented (Cooper, 1997; Lewin, 1997). Therefore, cytotoxicity brought in due to phosphamidon exposure must have led to derangement of this machinery which otherwise functions with a high degree of fidelity. Thus, incomplete or faulty expressions of certain genes regulating the metabolic activities of these organs could be a real possibility.

In our present study on the gel electrophoretic protein band profiles of the experimental and control fish, a critical analysis of the data and band comparison revealed that certain bands present in control fish were found to be missing and a few unknown protein bands originated. This is in agreement with the malathion induced qualitative and quantitative change in plasma protein of another air breathing fish, *Heteropneustes fossilis* (Bloch) (Kumar *et al.*, 1995). It may be inferred from our studies that some of the genes involved in the synthesis were switched off resulting in the disappearance of some of the proteins. The appearance of some new proteins may be due to the switching on of some genes.

According to abnormal protein hypothesis, regulation of stress protein synthesis is closely linked to the extent of protein damage. Stress proteins are a group of proteins whose synthesis is induced by a wide variety of physical conditions and chemical agents: heavy metals, xenobiotics, oxidative stress, anoxia, salinity stress, teratogens, and hepatocarcinogens. Some of these proteins are believed to play a role in protecting the cell from damage that can result from environmental perturbations. Others are involved in the regulation of various genes. Stress results in a dramatic redirection of metabolism; a suite of stress proteins is rapidly synthesized and production of cellular proteins is repressed.

(Adams, 1990; Arthur, 1990; Robert *et al.*, 1992 and WHO, 1993). The list of inducers of stress proteins that are of major environmental concern includes trace metals, pesticides, teratogens, and UV irradiation (Sanders, 1993). In the fathead minnow, the stress response is induced in a tissue specific manner by elevated temperatures, arsenites, chromate, lindane, and contact organophosphate diazinon (Dyer *et al.*, 1991, 1993a,b). In the present study the changes in protein sub-fractions and concomitant changes in DNA and RNA contents would not only confirm the protein loss (due to phosphamidon exposure) in a more powerful and precise manner, but would again strongly support the cellular protective response against organophosphate toxicity through possible expression of certain genes encoding stress proteins though characterization of those proteins need to be studied.

Biomarkers are indicators that can be used to assess the effects of environmental perturbations. Biomarkers must have the ability to be used in a broad range of organisms when exposed to a wide variety of stress conditions in the environment. Biomarkers must correlate with decreased physiological function and survival of the organism. The increased level of acid phosphatase in the present study may be explained with the fact that phosphamidon toxicity causes disruption of lysosomal membranes because biocides are known to produce cytotoxic action and changes in membrane fragility (Vijayendra Babu and Vasudev, 1984). Phosphamidon might cause liver damage, which in turn lead to the release of acid phosphatase. The increased lysosomal enzymatic activity was accompanied by a decrease in RNA and protein content (Shah, 1980). This could be due to adverse effect of organophosphate compounds on the lysosomal membrane, which release nucleases proteases affecting RNA and protein metabolism. Similarly, alkaline phosphatase activity in the present study registers a rise in liver, muscle and kidney due to exposure of phosphamidon. Similar observations were made by Koundinya and Ramamurthi (1982); Mukhopadhyay and Dehadrai (1980) in liver, kidney, brain, gill and intestine of fish exposed to organophosphorus pesticide. Alkaline phosphatase activity is stimulated in hyperglycemia condition (Latner, 1975). Thus, increased activity of alkaline phosphatase in the present study is related with the breakdown of glycogen and induction of a condition of hyperglycemia. In order to combat the stress arising out of phosphamidon exposure, demand of energy is very likely supplied by increased phosphatase activity.

It is established beyond doubt that free radicals in tissues and cells can damage DNA, proteins, carbohydrates and lipids. These potentially deleterious reactions are at least partly controlled by antioxidants capable of scavenging free radicals. It is widely believed that a proper balance between free radicals and antioxidants is essential for the health of an organism. In aerobic life, oxidative stress arises from both endogenous and exogenous sources. Despite antioxidant defense mechanisms, cell damage from oxygen free radical (OFR) is ubiquitous. Free radicals are atoms or molecules with one or more unpaired electrons. The reactive radicals responsible for tissue damage are generally short-lived species that are generated in situ (Freeman and Crapo, 1982). Free radicals are produced in normal or pathological cell metabolism, from xenobiotics or through ionizing radiation. An important feature of free radicals reaction with non-radicals is that they result in new radicals, which leads to chain reactions (Halliwell and Gutteridge, 1984). Electron acceptors such as molecular oxygen reacts easily with free radicals, to become radical themselves, the OFR, and thus become the primary mediators of cellular free radical reactions. Free radicals can also destroy the unsaturated bonds in membrane lipids, leading to severe cell damage, or they can also free their extra energy to nucleic acids and subsequently damage DNA and cause mutations. Cellular receptor functions can be altered too by the free radicals, e.g., by reacting with carbohydrates (Machlin and Bendich, 1987).

The reaction of OFR with biomolecules gives rise to organic radicals that can propagate the oxidative damage. Thus, the peroxidation of membrane lipids to organic peroxyl radicals initiates a chain reaction that may explain many membrane mediated effects of OFR (Freeman and Crapo, 1982; Halliwell and Gutteridge, 1984).

Oxygen free radicals, especially ·OH attacks the fatty acid side chains of the membrane phospholipids, preferentially the polyunsaturated fatty acid (PUFA), such as arachidonic acid.

Lipid peroxidation is at present considered as one of the elementary mechanism for cell damage. During cell injury and carcinogenic process, free radicals are generated which attack cell membrane to bring about lipid peroxidation of the PUFA. Free radical attack on PUFA

resulted in the formation of lipid radicals initiating lipid peroxidation (Schoneich *et al.*, 1989) followed by severe pathological changes such as triglyceride accumulation, polyribosomal disaggregation, depression of protein synthesis, cell membrane breakdown and eventual cell death (Halliwell and Gutteridge, 1984; Roy *et al.*, 1991).

Lipid peroxidation occurs as a consequence of environmental stressors including extreme temperature, light or exposure to xenobiotic compounds. Elevated concentrations of malondialdehyde are an indication that lipid peroxidation has occurred. Vadhva and Hasan (1986) showed that the rate of lipid peroxidation level was significantly increased in all the regions of the central nervous system of fish due to the effect of dichlorovos organophosphate compound. Organophosphate compound also affects to reduce the glutathione level and produce oxidative stress of carp and catfish (Hai *et al*; 1997). The results of the present study indicated, that lipid peroxidation in case of phosphamidon exposed fish was increased significantly in the different tissues such as liver, muscle and kidney in comparison to normal control. This may be due to peroxidation of the primary substrates i. e., the PUFA (Rana *et al.*, 1994).

On the other hand the level decreased in case of reduced glutathione. All these changes are indications of cytotoxicity. GSH concentration of the host of carcinogen control animal had appreciably less GSH level due to intracellular GSH depletion because of highly reactive metabolites and free radicals. The reduced GSH status in case of carcinogen treated animal may also be due to irreversible loss by oxidative stress or by conjugation. For e.g., GSH consumption by oxidative stress are reported for Wilson's disease (Summer and Eisenberg, 1985) or GSH consumption by conjugation has been described for acute alcohol intoxication (Videla and Guerri, 1990), pulmonary fibrosis and liver dysfunction. In all these cases the GSH depletion may aggravate the underlying disease and enhance the adverse effect of a chemical by making symptoms visible below certain threshold of protection by GSH. Thus we can assume that the much reduced GSH level in T1 and T2 group i.e. phosphamidon exposed animals may be due to loss of GSH by oxidative stress or by conjugation. The oxidative metabolism has been greatly impaired in both liver and muscle as revealed from the studies on lipid peroxidation and reduced glutathione level. Phosphamidon actually generated

a reduction in cellular glutathione content, which has rendered the cells more susceptible to damage by OFR. So the changes observed in the enzymatic studies are also very significant. Present studies on increased lipid peroxidation and concomitant decrease in reduced glutathione level indicated that organophosphate compound caused oxidative stress. An enzymatic study also indicates the cytotoxicity and cellular malfunctioning in different tissues. From our present investigation it can be inferred that organophosphate compound can produce cytotoxicity as well as oxidative stress.

The cytochrome P-450 induction serve as a highly sensitive indicator of an organism's toxic burden, or the extent to which it has been exposed to chemical inducers in the environment. Hepatic P450 content and its dependent monooxygenases were induced following quinalphos treatment to rat (Dwibedi *et. al.*, 1998) and the cyt P450 –mediated ROS formation was also reported (Bondy, 1994). An elevated value of cyt P450 in exposed groups in this study may be due to bioactivation mechanism in liver and other extra hepatic tissues of *Channa punctatus*, though we have noticed a direct correlation between increase in cyt P450 level and increase in MDA level in liver tissue only. Similar observation has been made already by various workers under different stress conditions (Singh and Rao, 1993; Kawashima *et. al.*, 1994; Montoliu *et. al.*, 1994). This study also suggests that the cyt P450 –mediated oxidative biotransformation of dimecron may produce reactive electrophilic intermediates and / or ROI that may be responsible for induction of lipid peroxidation. From our present investigation it can be concluded that dimecron even in a lesser dose, is a strong candidate for producing cytotoxicity as well as oxidative stress in the fishes as reflected in changes of the enzymatic levels and ROI and / or oxyradicals may be involved in the toxicity of dimecron.

This study on various biomarker enzymatic levels was conducted to understand the intricacies of the pathophysiological alterations that take place in the fish during exposure to sub lethal concentrations of an organophosphate (phosphamidon), which usually eludes visual observations and hence this study is indicative of the hazardous effect of phosphamidon even in a lesser dose in the fishes as reflected in changes in the enzymatic levels.

Chapter-II

Introduction

Pollution is the general term associated with unfavourable alterations on the ecology, resulting in deleterious effects on human health and resources. It is an insidious and growing process, which manifests itself only when the outflow of effluents exceeds the capacity of the receiving ecosystem and upsets the balance in natural environment. Growing industrial activity imposes a serious burden of bioresistant organic chemicals on the environment. Further, the domestic wastes and industrial effluents are being indiscriminately discharged in the rivers, large water bodies and adjacent fields. This chemical is toxic to organisms. This aquatic pollution now has become a serious problem both from the point of view of public health and aquaculture (Saha and Konar, 1984; Ghosh and Bagchi, 1979).

Pollution of the aquatic environment, originate from atmospheric inputs, land drainage and runoff of pesticides and seepage through land as in the case of ground water. The giant stride made in industrial and agricultural sectors led to the dumping of a large number of pollutants into the aquatic environment. Among these pollutants, pesticides, pose a threat of becoming a menace to public health. The major reason for the particular sensitivity of aquatic systems to pollution influences may lie in the structure of their foods chains. Compared with land systems, the relatively small biomass in aquatic environments generally occurs in a greater variety of trophic levels, whereby accumulation of xenobiotic and poisonous substances can be enhanced. According to Matsumura (1985), the rate of bioaccumulation in aquatic environments generally appears to be higher than that in terrestrial environments, which might be due to the lipophilic nature of the persistent insecticides. Since fish is at the top of the aquatic food chain, fish contaminated with pesticides might influence the levels of pesticides in human body (Kulshrestha, 1991). A significant number of quinalphos exposed subjects had altered plantar and ankle reflexes. Higher nervous functions such as memory, learning and vigilance were also found to be affected in these subjects (Srivastava *et al.*, 2000).

Kilgore and Li (1976), Edwards (1977), and Kalra and Chawla (1981) have indicated the various routes by which pesticides can reach the aquatic environment such as rivers, lakes, ponds, and oceans. The varieties of pesticides used indiscriminately in view of better crop production, find their way to adjoining aquatic medium and poses great threat to the precious

aquatic life (Konar, 1975; Hoffman, 1960; Cope, 1966; Edwards, 1977). Since majority of these pesticides are highly toxic to fish and other aquatic life (Konar, 1977; Mani and Konar, 1985; Pal and Konar, 1985), we have tremendous responsibility to use them wisely to keep the purity of aquatic environment.

Voluminous literature is available on the effects of pesticides on fish. Apart from the lethal effects of pesticides, the sub-lethal effects have been responsible for indirect effects such as disturbance of population dynamics, changed food habits and reproductive behaviour. Cases of vertebral fractures and symptoms of vertebral and spinal deformation in fish due to certain pesticides have been reported by Koeman (1979). Attri (1981) mentioned that sub-lethal concentrations of pesticides cause reproductive abnormalities. Kaur and Toor (1977) reported several deformities in fertilized eggs of *Cyprinus carpio* communis exposed to sub-lethal concentrations of diazinon, malathion, fenitrothion, and phosphamidon. Srivastava and Srivastava (1990) reported deformation of the skull and in Indian catfish to sub-lethal concentrations of malathion. Higher level of pesticide residues was noted in carnivorous fishes (Kaphalia *et al.*, 1986; Kulshrestha *et al.*, 1989). Several workers have investigated the toxicity, uptake and tissue distribution, and haematological changes of pesticides in the fish (Tilak *et al.* 1980; Alam and Maughan, 1993; Abidi and Srivastava 1988; Omoregie *et al.* 1990; Kumar and Nelson 1997; Das, 1998). Organophosphate compound quinalphos affects testicular steroidogenesis in *Clarias batrachus* (Bagchi *et al.*, 1990). Dimethoate alters protein metabolism of muscle tissue in the same fish. (Begum and Vijayaraghavan, 1996).

A considerable amount of literature is available on the ill effects of organophosphorus insecticides on a number of fishes and other aquatic animals. Most of the information available concerns bioassay tests (Anees, 1975; Lingaraja and Venugopalon, 1978; Dubale and Shah, 1979; Dubale and Awasthi, 1981), biochemical alterations (Meteleve, 1972; Thomas and Murthy, 1976; Shah, 1980; Dubale and Awasthi, 1982, Ghosh, 1989), haematological abnormalities (Metelev, 1972; Anees, 1978b). Metabolic disturbances leading to carbohydrate metabolism in the liver due to hepatotoxic agents such as pesticides have been reported by several workers (Carevic and Fiser Herman, 1962; Piccaluga *et al.*, 1965; Rozengart *et al.*, 1971). There are reports on the changes on serum protein (Abidi,

1990; Gill *et al.*, 1990); blood glucose level (Bhattacharya *et al.*, 1987; Ghosh, 1989); hemoglobin percentage (Sastry *et al.* 1982; Pandey *et al.* 1980).

The long term and repeated administration of a novel phosphorothionate caused significant increase of AcP and AkP in serum and kidney (AcP), whereas these enzymes simultaneously decreased significantly in liver, kidney (female rat AkP) and lung tissues in both male and female rats after 45 and 90 days of treatment (Rahaman *et al.*, 2000). The sub lethal effects of the organophosphate pesticide, quinalphos on some biochemical parameters of muscle, brain, liver and kidney of the Indian major carp, *Labeo rohita* was studied by Das and Mukherjee (2000a). The muscle protein and RNA levels decreased whereas DNA levels and acid phosphatase were elevated. Similarly, alkaline phosphatase was depleted in exposed groups of fishes. Das and Mukherjee (2000b) also studied the sub lethal effects of quinalphos on some blood parameters of same carp fish and reported the reduction of serum protein level, Hb% and total erythrocyte count (TEC) in exposed fingerlings. Quinalphos toxicity on enzymes of different tissues of *Channa punctatus* (Bloch) was also reported by Gupta *et al.*, (2000). Joshi and Mukhopadhyay (1990) studied the toxicity of quinalphos along with endosulfan to different stages of *Panaeus monodon.*

Damage to hepatic parenchymal tissue has been the most frequently reported pathological effects in fishes exposed to various chemical agents (Couch, 1975; Johnson, 1968; McKim *et al.*, 1974;Tucker and Leitzke, 1979). The primary characteristics of this response include vacuolation of parenchymal cells and increased degenerative changes of hepatocytes that result in focal or zonal necrosis. These observations support the contention that, as in mammals, the fish is susceptible to damage from a variety of toxicants. Current interest in awareness of the role of the fish liver in mediating processes of biotransformation and elimination of xenobiotic compounds, as well as observations of toxicant- induced liver damage, have provided an impetus for further study of the comparative toxicology of this organ system in these poikilothermic vertebrates. Processes mediating hepatotoxic responses in fishes have been studied only superficially and consequently, are poorly understood.

The toxicity of nuvan have been studied in both freshwater as well as marine fishes (Ghosh and Chatterjee, 1989; Thain *et al.*, 1990). Inhibition of brain cholinesterase in fish exposed to pesticides in food or water has been reported (Weiss, 1959; Holland and Lowe, 1966; Post

and Leasure, 1979; Coppage and Mathews, 1975; Gantverg and Perevoznikeov, 1984; Fernandez *et al.*, 1996). A variety of ATPase have also been found to be sensitive to pesticides when tested *in vitro* (Koch, 1969; Farlane, 1981). Dwivedi *et al.*, (1998) reported the induction of hepatic P450 content and its dependent monooxygenases in quinalphos (QP) treated groups of rat. The hepatic antioxidant defense system, comprising catalase, glutathione (GSH) reductase, superoxide dismutase (SOD) and GSH peroxidase, was also significantly increased in QP treated rats, while in the brain only catalase was increased and GSH reductase decreased. Gultekin *et al.*, (2000) studied the toxic effects of organophosphate pesticide *in vitro*. Administration of chlorpyrifos-ethyl (CE) resulted in the induction of erythrocyte lipid peroxidation and significant changes in antioxidant enzyme activities, suggesting that ROS and/or free radicals may be involved in the toxic effects of CE.

The stress of this chapter, the need for measuring sub lethal effects of organophosphate compound, Quinalphos, reflects the current intensive effort to provide habitats for aquatic life where it can not only survive, but thrive. This change in concern from survival to well being reflects the advancement that has occurred in the field of aquatic toxicology over the past 10-20 years, a change that has greatly increased the work needed in aquatic toxicology. As organophosphates are widely used to control ectoparasites in fish, zero can not be the most desirable concentration in the water after application, so the researchers must therefore elucidate the full range of effects of organophosphates on aquatic life especially fishes.

Quinalphos is extensively applied in paddy fields, as well as, tea plantation for pest eradication in India, it is pertinent to study its hazardous effect on the aquatic system as it is assumed that the residue might affect the fish. So in this thesis, study was undertaken to assess the pollutional hazards of quinalphos, an organophosphorus compound on fish including aquatic ecosystem as a whole.

Results

Water quality

Temperature (maximum and minimum), atmospheric pressure, relative humidity and rainfall were noted during the study (Table 32). A gradual increase in free carbon dioxide and decrease in D. O. content occurred in waters exposed to 0.5 to 2.5 mg/l of quinalphos and most significant ($p < 0.05$) at 2.5 mg/l, which was reflected in the Table-33, Fig: 40 & 39 respectively. Water temperature increased gradually and most significant at 2.5 mg/l (Table-33, Fig: 44) though color and odour were not affected. Total alkalinity and hardness of water gradually reduced and most significant ($p < 0.05$) at 2.5 mg/l concentration of quinalphos, which was reflected in the Table-33, Fig: 41 & 42 respectively. The difference in pH of exposed waters in comparison to unexposed control was significant ($p < 0.05$) at 0.125 and 2.5 mg/l concentrations. (Table-33, Fig: 43).

Phytoplankton (PP) population was always greater than that of zooplankton (ZP) population in control and exposed waters. Total count of Phytoplanktons was reduced gradually with increasing concentrations of the pesticide and the reduction was significant ($p < 0.05$) at 0.83, 0.125 and 2.5 mg/l (Table 34, fig.45). There was a linear reduction in number of Zooplanktons with the increase of quinalphos concentrations though result was significant ($p < 0.05$) only at 0.125 and 2.5 mg/l. (Table-34, fig. 46)

Body Weight

The mean body weight of the exposed fishes although decreased at 0.5 and 2.5 mg/l concentrations, were however, not significantly low in comparison to the unexposed control (Table 35, fig. 47) on the 10^{th} and 20^{th} days. But the reduction of mean body weight at the above two concentrations was significant ($p < 0.05$) on the 30^{th} day.

Table 32. Atmospheric parameters recorded during test period.

Month	Temperature (°C) maximum	minimum	Relative Humidity %	Rainfall (mm)
January	25.8	13.2	65.9	0.00
February	27.3	14.2	63.4	0.00
March	31.0	17.6	64.8	0.73
April	34.6	23.7	70.1	4.29
May	37.2	26.3	74.4	6.28
June	38.7	25.7	77.8	4.72
July	34.1	24.3	79.0	13.69
August	33.2	25.1	82.8	7.29
September	32.2	24.5	74.3	5.78
October	30.6	20.9	67.3	2.56
November	26.1	16.1	65.2	0.00
December	20.1	10.5	61.9	0.00

Table 33. Influence of Quinalphos on water quality parameters dissolved oxygen (DO), free carbon dioxide (CO_2), total alkalinity, hardness, pH and temperature. Asterisk (*) indicate statistical significance at $p< 0.05$ over control value.

Concentration	Dose	DO	CO_2	Alkalinity	Hardness	pH	Temperature
Treatment	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)		(°C)
T1	0	10.27	1.98	198.75	229	7.50	24.92
T2	0.50	10.15	2.01	197.05	228	7.71	24.98
T3	0.625	9.78	2.22	196.15	227	7.75	25
T4	0.83	9.18	2.32	195.20	226.92	7.79	25.22
T5	0.125	9.09	2.42	194.3	225	7.81*	25.52
T6	2.5	8.29*	2.92	192.12*	222*	7.82*	25.80*

Table 34. Influence of Quinalphos on the phytoplankton (PP, number/liter), and zooplankton (ZP, number/liter). Asterisk (*) indicate statistical significance at $p< 0.05$ over control value.

Concentration	Dose	PP	ZP
Treatment	((mg/l))	(no./liter)	(no./liter)
T1	0	80.21	30.21
T2	0.50	60.64	28.82
T3	0.625	58.28	27.54
T4	0.83	56.42*	26.12
T5	0.125	54.20*	25.82*
T6	2.5	49.78*	22.78*

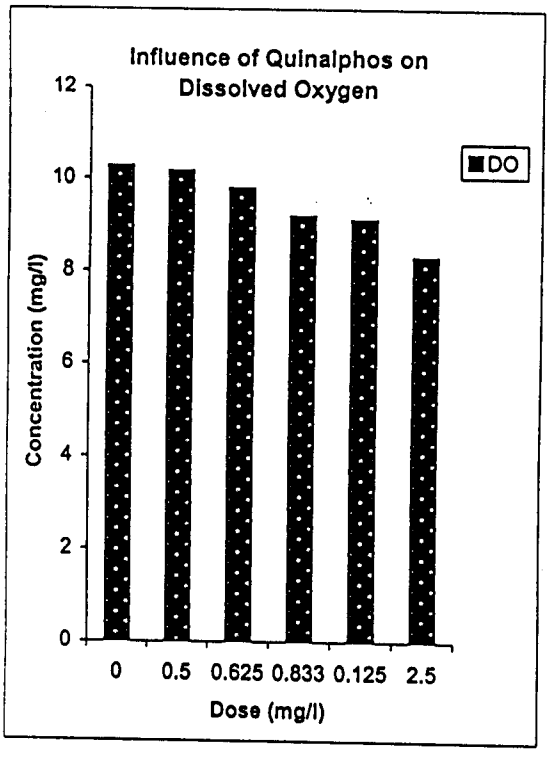
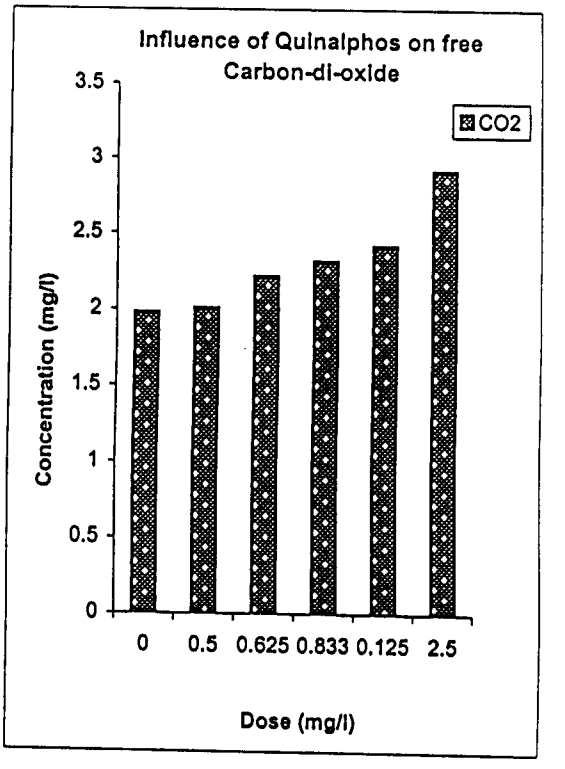


Fig:39



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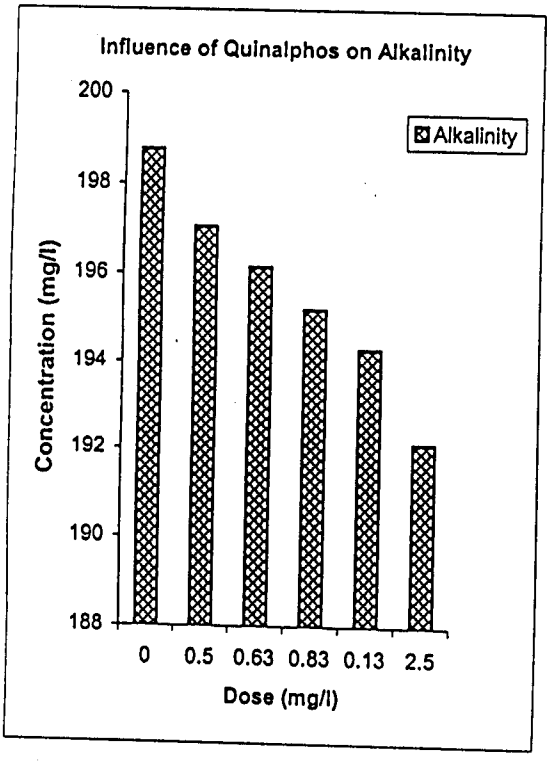

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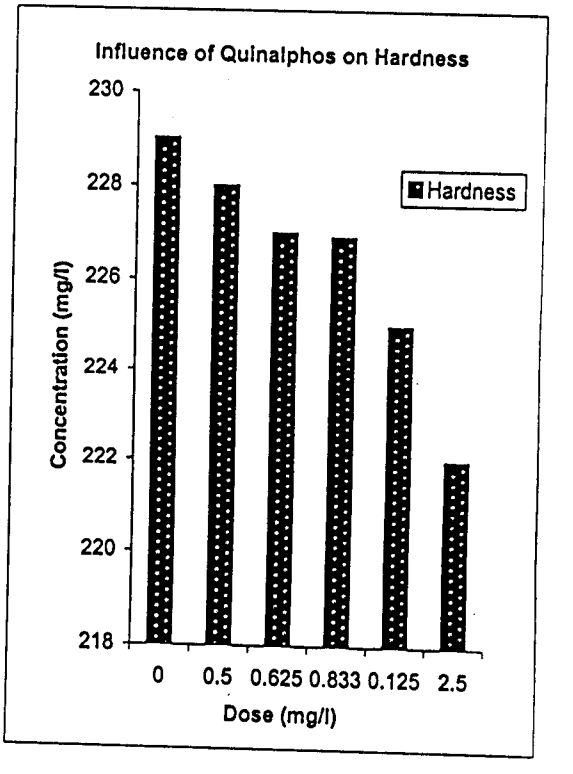

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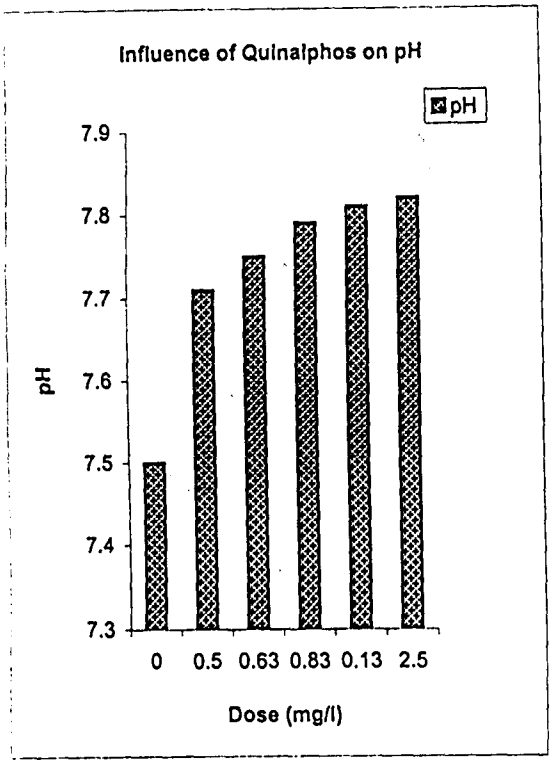

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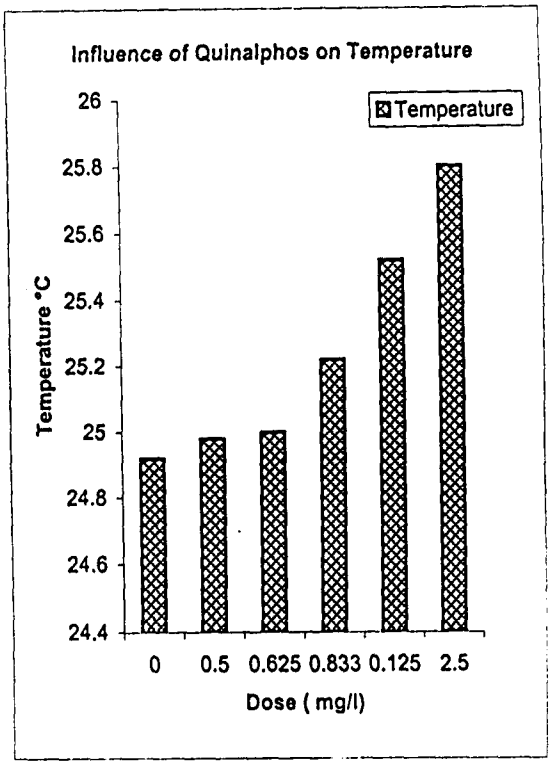

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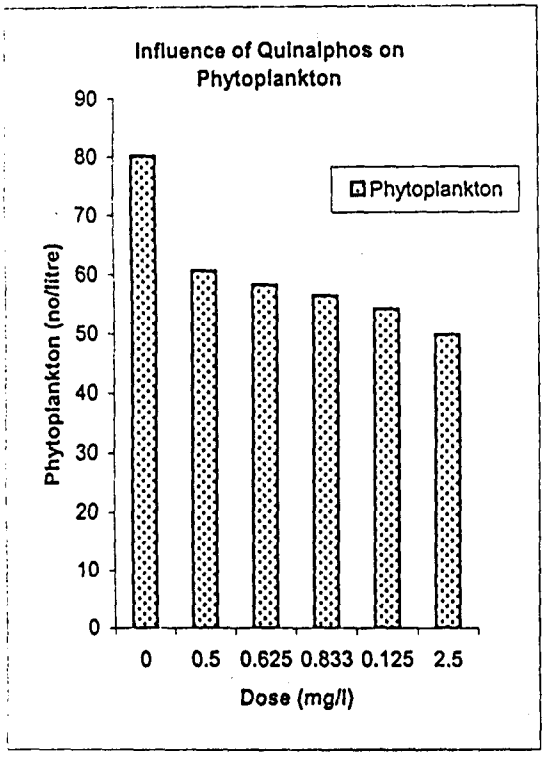


Fig:45

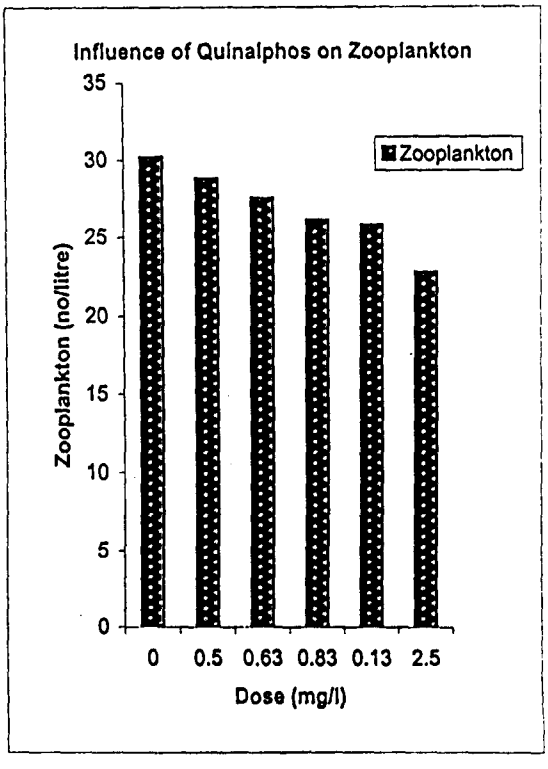


Fig:46

Table 35. Distribution pattern of Body Weight (gm) of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	25.83± 0.012	25.92± 0.016	25.84 ±0.015
T1	25.83 ±0.012 ^{ns}	25.72 ±0.015 ^{ns}	22.12 ±0.011*
T2	24.92 ±0.015 ^{ns}	23.81± 0.014 ^{ns}	18.82± 0.014**

Table 36. Distribution pattern of Organ Weight (gm) of *Channa punctatus* at different exposures of Quinalphos

Days	Organ	Control	T1 (0.36 mg/l	T2 (1.8 mg/l)
10	Liver	0.228±0.008	0.229± 0.006 ^{ns}	0.234 ±0.005 ^{ns}
20	Liver	0.227± 0.007	0.230± 0.004 ^{ns}	0.235± 0.007*
30	Liver	0.225± 0.009	0.236 ±0.008*	0.242 ±0.009**
10	Kidney	0.162± 0.005	0.165± 0.006 ^{ns}	0.167 ±0.007 ^{ns}
20	Kidney	0.163± 0.006	0.170± 0.004 ^{ns}	0.175± 0.008*
30	Kidney	0.161 ±0.007	0.176± 0.005*	0.180 ±0.009**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
***= $p<0.05$**
****= $p<0.01$**

Table 37. Hepatosomatic Index (HSI) of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.882	0.875	0.870
T1 (0.50mg/l)	0.886 ^{ns}	0.894 ^{ns}	1.06*
T2 (2.5 mg/l)	0.898 ^{ns}	0.986*	1.28**

Table: 38. Renosomatic Index (RSI) of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.627	0.628	0.623
T1 (0.50mg/l)	0.638 ^{ns}	0.660 ^{ns}	0.795 *
T2 (2.5 mg/l)	0.670 ^{ns}	0.734 *	0.956 **

Organosomatic Index= organ weight X 100/ body weight

No. of Fishes in all cases (15)

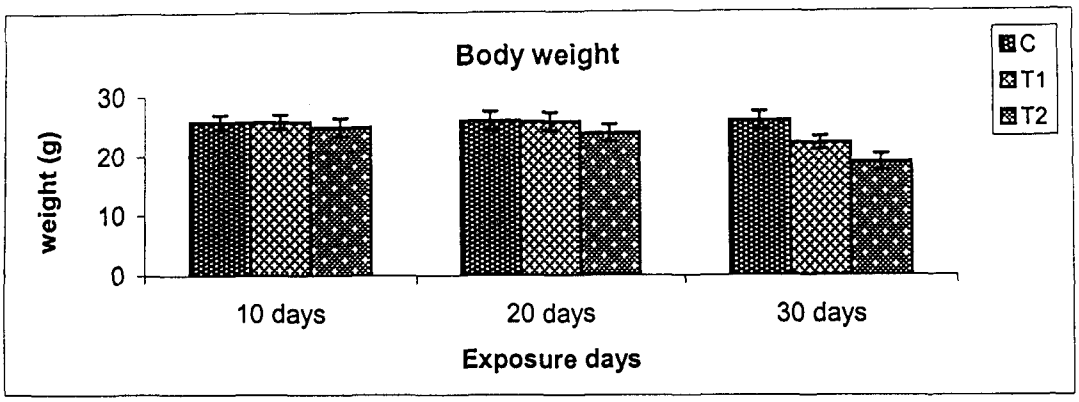


Fig:47

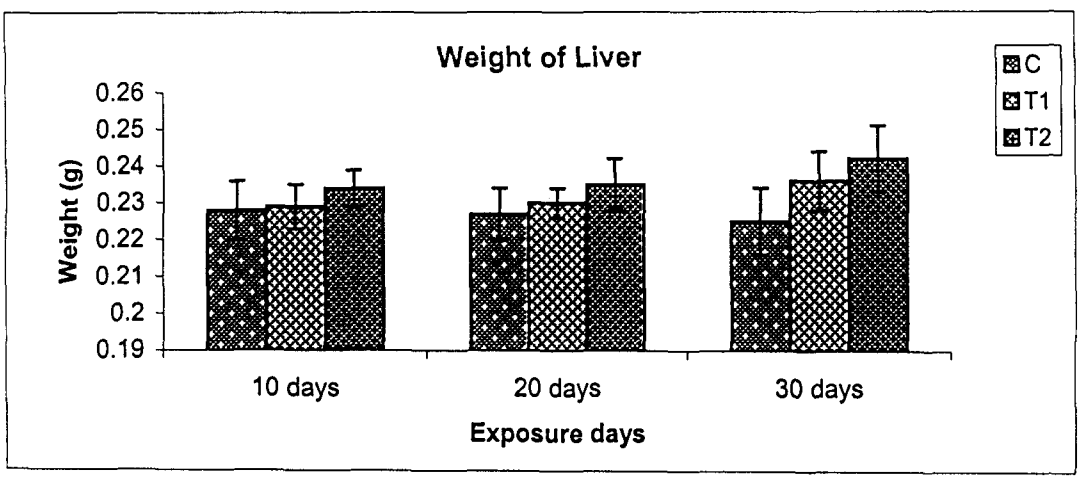


Fig:48

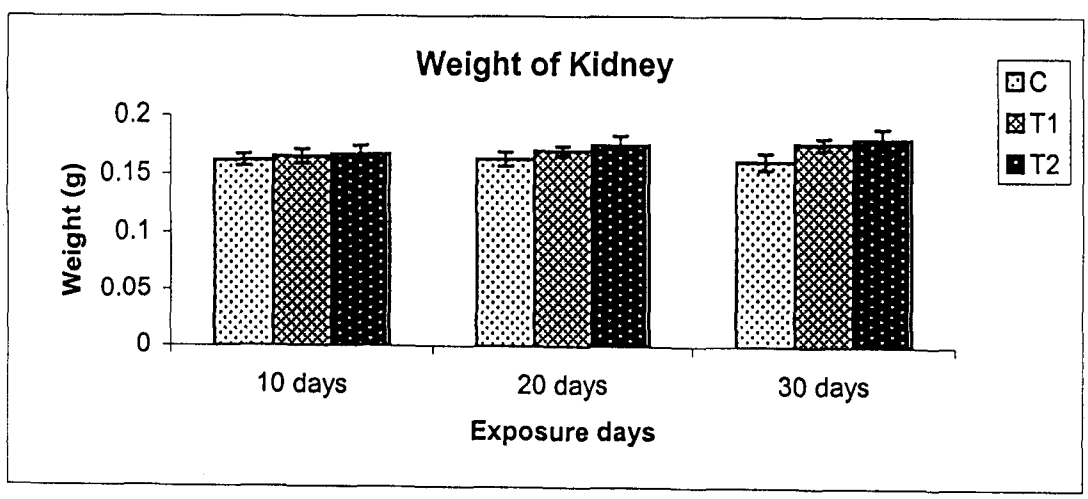


Fig:49

C= Control
T1= Quinalphos exposed (@ 0.50 mg/l)
T2 = Quinalphos exposed (@ 2.5 mg/l)

Organ Weight

Table-36 and Fig: 48, 49 show mean organ weight of liver and kidney does not remain constant throughout the days under study. This is applicable for both control and treated animals. The weight of liver and kidney of exposed fishes although increased at both the concentrations, were however, not significantly high in comparison to unexposed control (Table 36 and fig. 48, 49) on the 10^{th} day. But the increase in liver and kidney weight were significant ($p < 0.05$) at 2.5 mg/l concentrations on the 20^{th} day and at 0.05 mg/l concentration on the 30^{th} day. The maximum increase in organ weight was noticed at 2.5 mg/l concentration on the 30^{th} day, which was statistically significant ($p < 0.01$).

Organosomatic Indices (OSI)

Somatic indices for liver and kidney have been presented in Table-37 and 38, respectively. The data clearly indicates that quinalphos caused a significant increase in the organ weights in all the exposure except 10 days exposure. This is clearly revealed from the HSI and RSI, which were most significant ($p < 0.01$) at 2.5 mg/l concentration on 30^{th} day. The OSI were calculated by multiplying 100 to the ratio of organ weight to body weight.

Behavioral responses

There was no marked increase in the swimming activity, no excitement and hyperventilation of the fishes immediately after they were transferred to sub lethal concentrations (0.5 mg/l and 2.5 mg/l) of quinalphos. Higher concentration-exposed fishes showed abnormality after 14 days onwards and lower concentration-exposed fish showed the same after 22 days onwards. Marked increase in swimming activity with darting movement was observed in T1 group after 27 days onwards. Erratic swimming along with loss of touch sensation and loss of balance (to some extent) was also observed in T2 group after 18 days onwards. After 28

days, in fishes of T2 group surface ward movement was observed. Besides these laboured respiration, lack of desire to take food and aggressive behaviour also found.

Quantitative estimation of Protein, DNA and RNA

The concentration of protein in liver, kidney and muscle in the exposed animals although reduced in T1 and T2 groups in 10, 20 and 30 days but not always significant. The reduction in liver protein at both cases in T1 and T2 were statistically significant ($p < 0.05$) and ($p < 0.01$) in comparison to control animal on 20^{th} day and 30^{th} day respectively though the result was not statistically significant on 10^{th} day (Table 39 and fig. 50). On the other hand, muscle and kidney protein were reduced significantly only on 30^{th} day exposure. The reduction in muscle protein was significant ($p < 0.05$) and ($p < 0.01$) in T1 and T2 groups respectively on the 30^{th} day (Table 42, Fig. 53). But the kidney protein was reduced significantly ($p < 0.01$) at both the groups on the same days exposure (Table 45, Fig. 56).

Similarly, DNA and RNA concentration in liver, kidney and muscle decreased significantly in T1 and T2 groups only at 20 and 30 days exposures (Table 40, 41, 43, 44, 46, 47 and fig.51, 52, 54, 55, 57, 58). After 20 days exposure, the DNA and RNA content of all the tissues were reduced significantly ($p < 0.05$) only in T2 groups. But on 30^{th} day after exposure, the reduction of DNA and RNA content of all the tissues were significant ($p < 0.05$) and ($p < 0.01$) on T1 and T2 groups respectively.

Qualitative analysis of protein band profiles

The gel electrophoretic protein profiles on 30^{th} day in liver, muscle and kidney of the experimental fishes have been presented in photographs 59, 60 and 61 (including marker "M denote for marker in the photographs"). A critical analysis of the band comparison would reveal that certain bands present in unexposed control groups were found to be missing and a few unknown protein bands originated in treated fishes (i.e. @ 2.5mg/l of quinalphos exposure).

Table 39. Total Protein (mg/g) content in Liver of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	16.2 ±1.28	16.4 ±1.29	16.3 ±1.21
T1 (0.50mg/l)	16.0 ±1.19^{ns}	15.8 ±1.09*	15.0±1.11**
T2 (2.5 mg/l)	15.9± 1.19^{ns}	15.6 ±1.24*	14.6 ±1.22**

Table 40. Total DNA (mg/g) content in Liver of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	32.4 ±5.6	32.2 ±5.4	32.1 ±5.2
T1 (0.50mg/l)	30.8 ±4.8 ^{ns}	29.7 ±4.6 ns	24.8 ±3.8*
T2 (2.5 mg/l)	30.4 ±4.7 ^{ns}	25.3 ±4.3*	18.6 ±4.9**

Table 41. Total RNA (mg/g) content in Liver of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	130.2± 12.3	130.4 ±11.9	129.8 ±12.2
T1 (0.50mg/l)	129.3± 11.8 ^{ns}	127.4 ±12.6 ^{ns}	120.8 ±11.9 *
T2 (2.5 mg/l)	128.4 ±11.6 ^{ns}	122.8 ±9.8 *	116.7 ±8.9 **

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
*= $p<0.05$
**= $p<0.01$

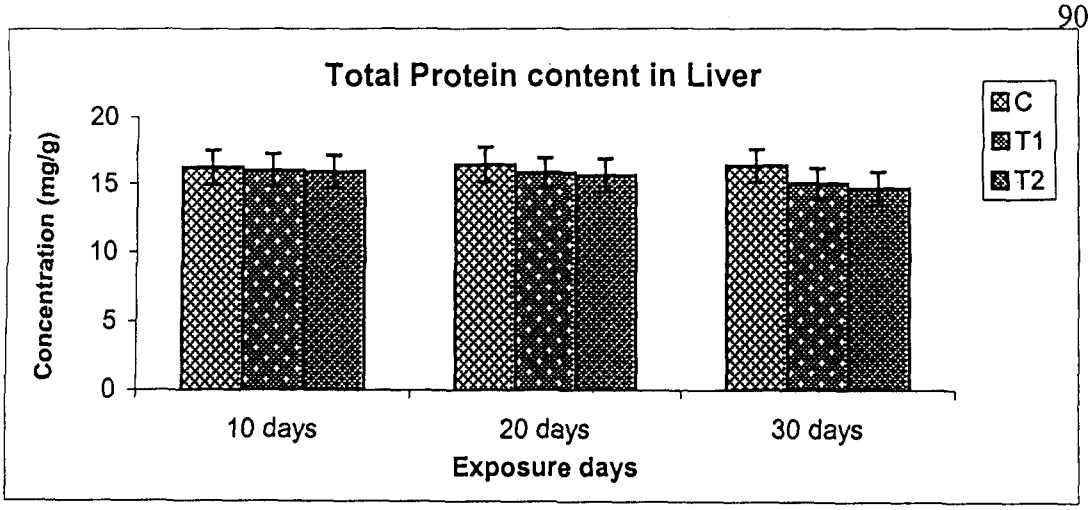


Fig:50

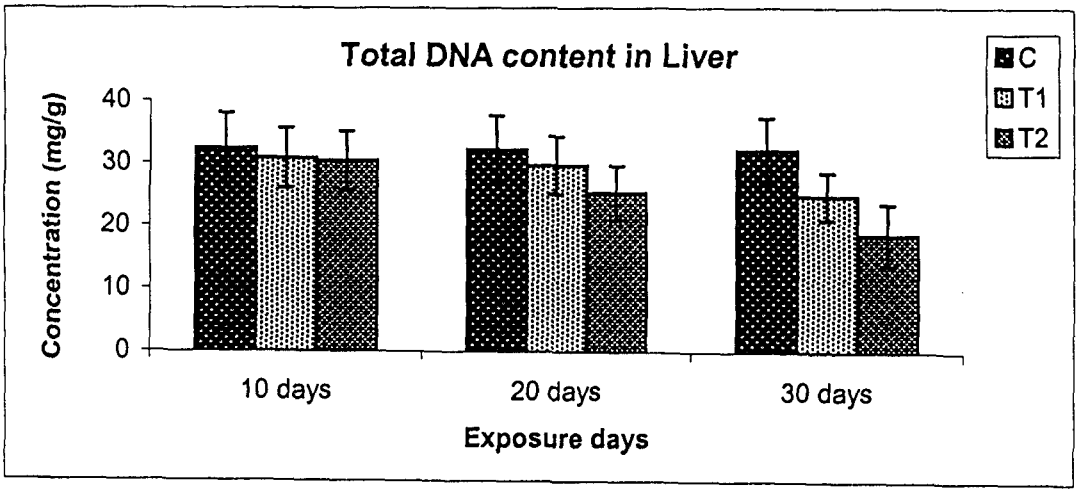


Fig:51

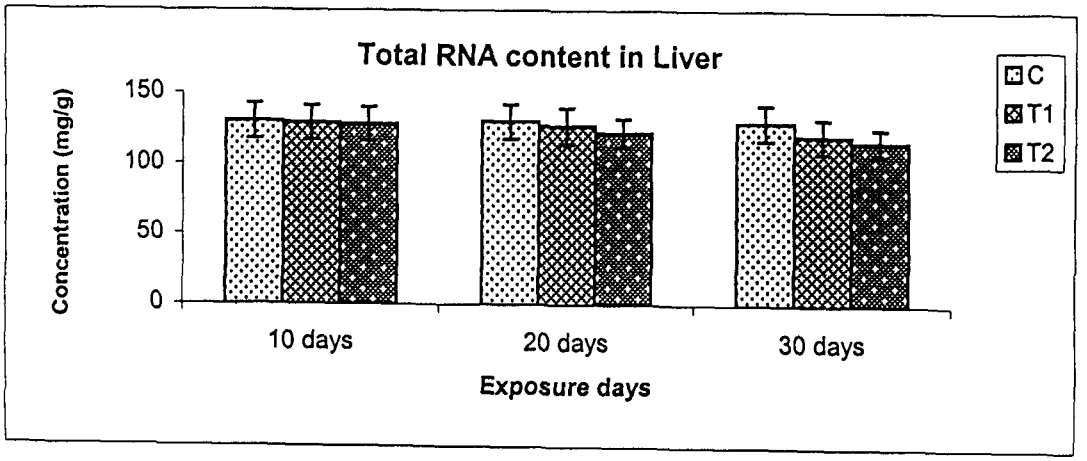


Fig:52

C= Control
T1= Quinalphos exposed (@ 0.50 mg/l)
T2 = Quinalphos exposed (@ 2.5 mg/l)

Table 42. Total Protein (mg/g) content in Muscle of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	19.8± 2.28	19.7 ±2.27	19.5 ±2.25
T1 (0.50mg/l)	18.8± 1.98ns	17.6± 1.92ns	12.8 ±1.01*
T2 (2.5 mg/l)	18.7 ±1.96 ns	16.6 ±1.92ns	10.2 ±1.12**

Table 43. Total DNA (mg/g) content in Muscle of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	33.4 ±5.7	33.2± 5.5	33.1 ±5.3
T1 (0.50mg/l)	31.8 ±4.9ns	30.7 ±4.7ns	25.8 ±3.9*
T2 (2.5 mg/l)	31.4 ±4.7 ns	26.3 ±4.3*	19.6 ±4.9**

Table 44. Total RNA (mg/g) content in Muscle of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	129.2± 11.3	129.4 ±11.8	128.8 ±12.1
T1 (0.50mg/l)	128.3 ±11.8 ns	126.4 ±12.5ns	119.8 ±11.8*
T2 (2.5 mg/l)	127.4 ±11.5 ns	121.8± 9.8*	115.7 ±8.9**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
*= $p<0.05$
**= $p<0.01$

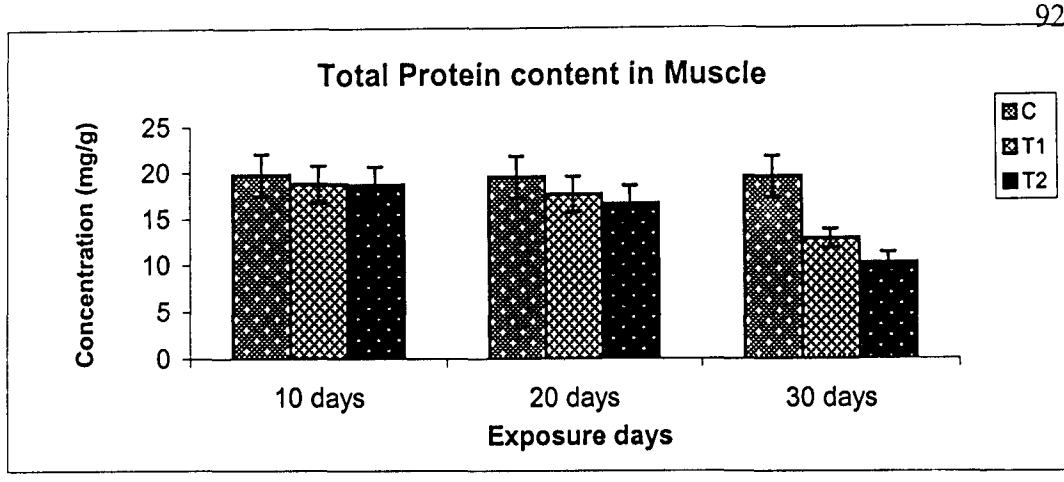
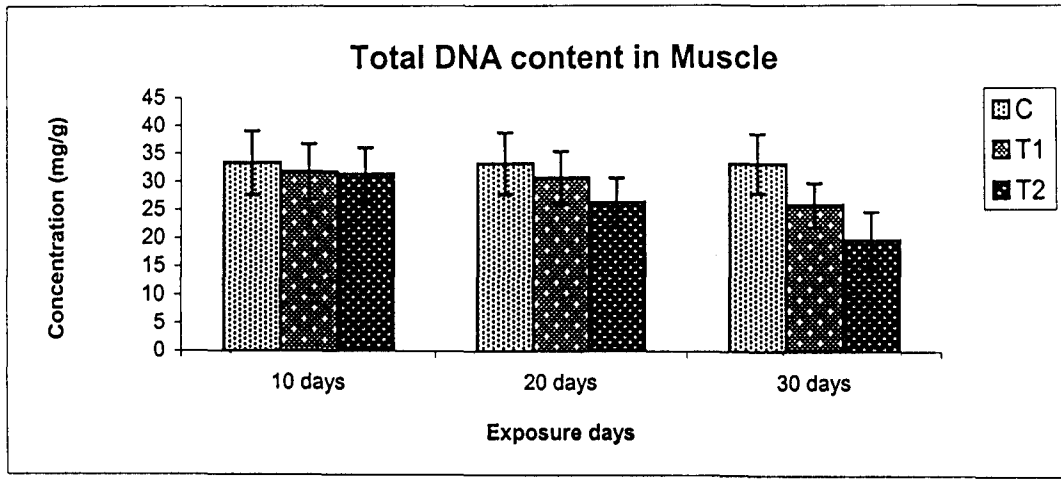


Fig:53



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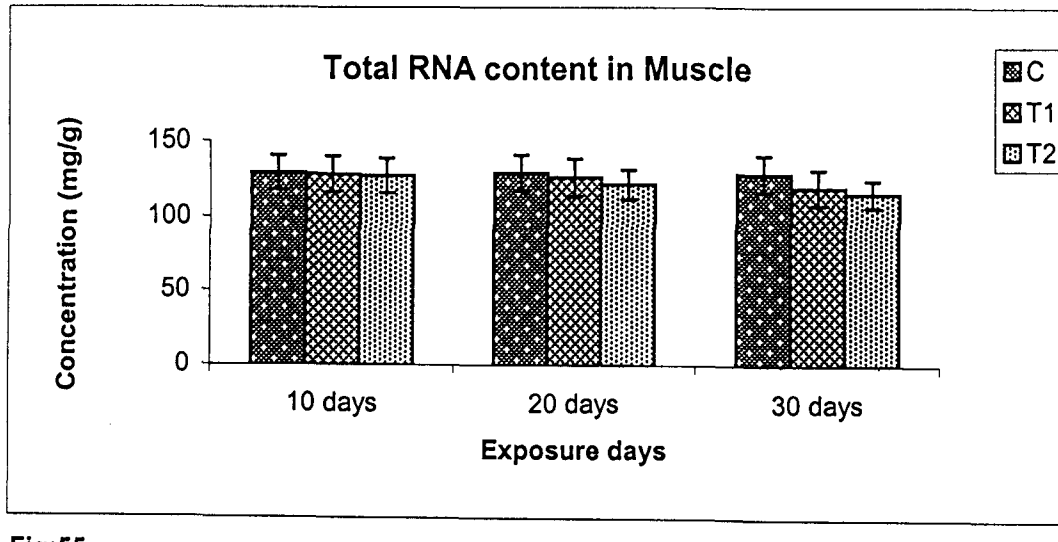


Fig:55

C= Control
T1= Quinalphos exposed (@ 0.50 mg/l)
T2 = Quinalphos exposed (@ 2.5 mg/l)

Table 45. Total Protein (mg/g) content in Kidney of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	14.8 ± 2.29	14.7 ± 2.28	14.5 2.26
T1 (0.50mg/l)	13.8 ± 1.99ns	12.6 ± 1.93ns	7.8 ± 1.03**
T2 (2.5 mg/l)	13.7 ± 1.96ns	11.6 ± 1.92ns	5.2 ± 1.14**

Table 46. Total DNA (mg/g) content in Kidney of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	28.4 ± 5.6	28.2 ± 5.4	28.1 ± 5.2
T1 (0.50mg/l)	26.8 ± 4.8ns	25.7 ± 4.6ns	20.8 ± 3.8*
T2 (2.5 mg/l)	26.4± 4.6ns	21.3 ± 4.2*	14.6 ± 4.8**

Table 47. Total RNA (mg/g) content in Kidney of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	119.2 ±9.3	119.4± 9.8	118.8 ±10.1
T1 (0.50mg/l)	118.3 ±9.8 ns	116.4 ±10.5ns	109.8 ±9.8*
T2 (2.5 mg/l)	117.4 ±9.5ns	111.8 ±7.8*	105.7 ±6.9**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
*= $p<0.05$
**= $p<0.01$

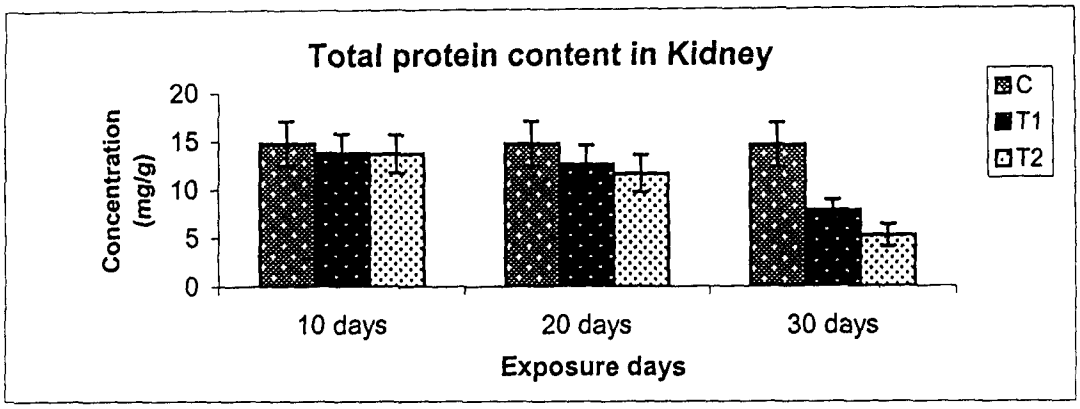


Fig:56

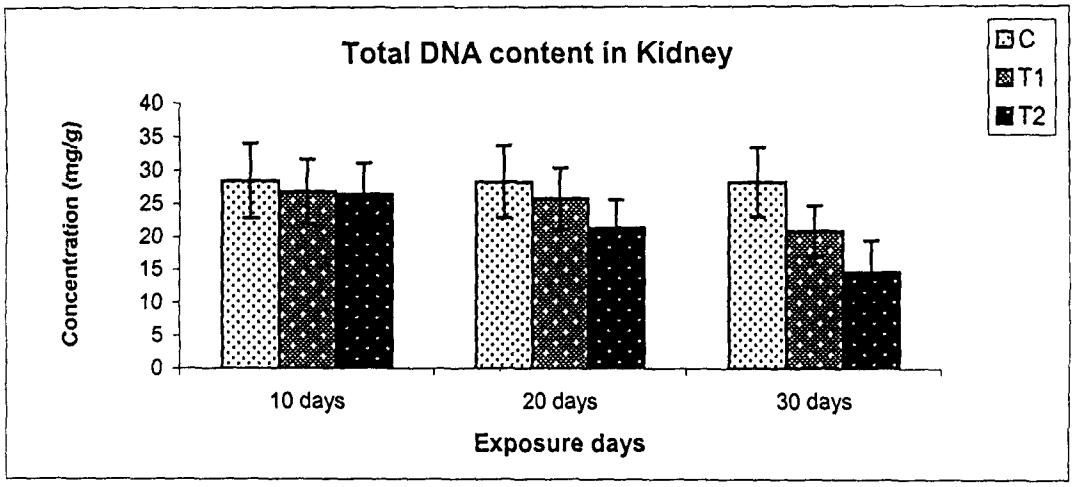


Fig:57

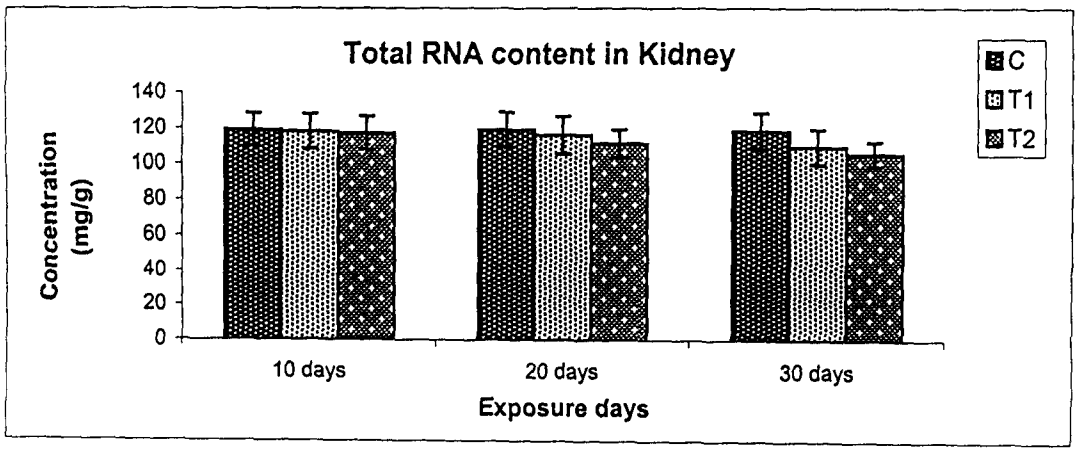
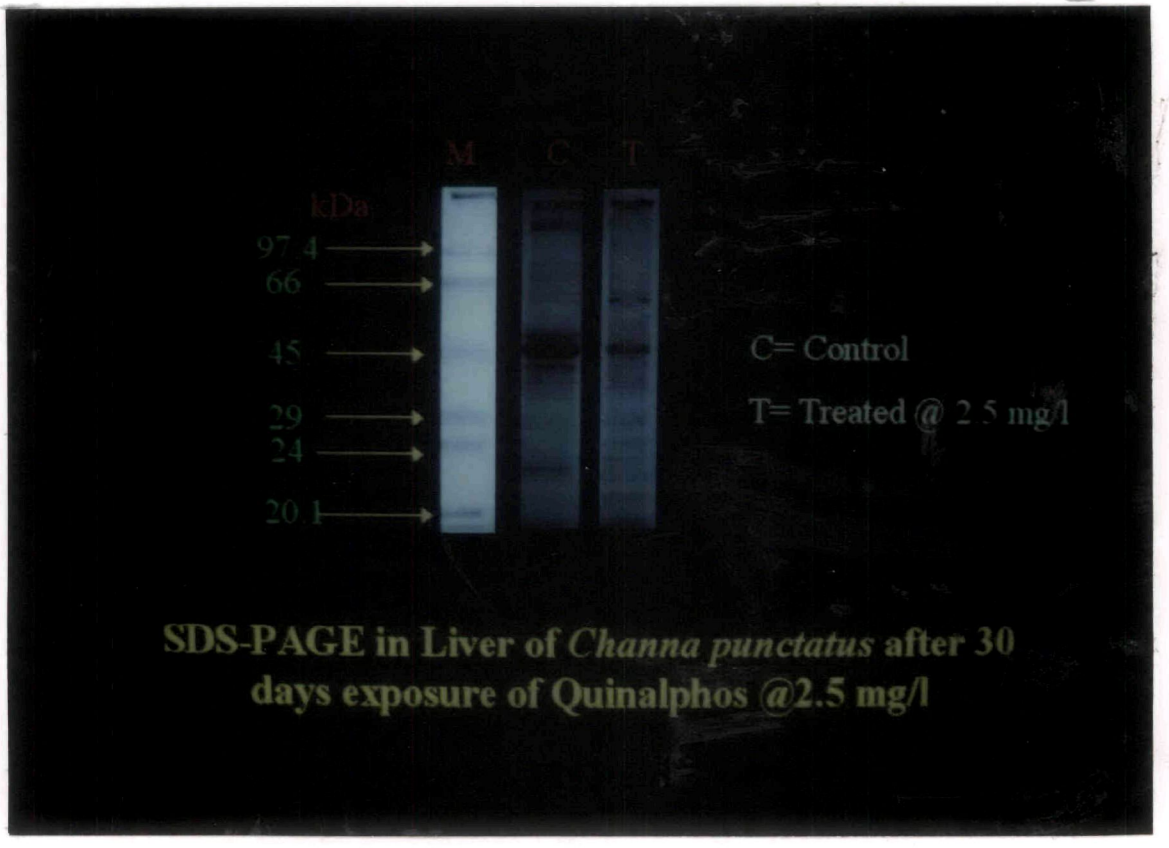
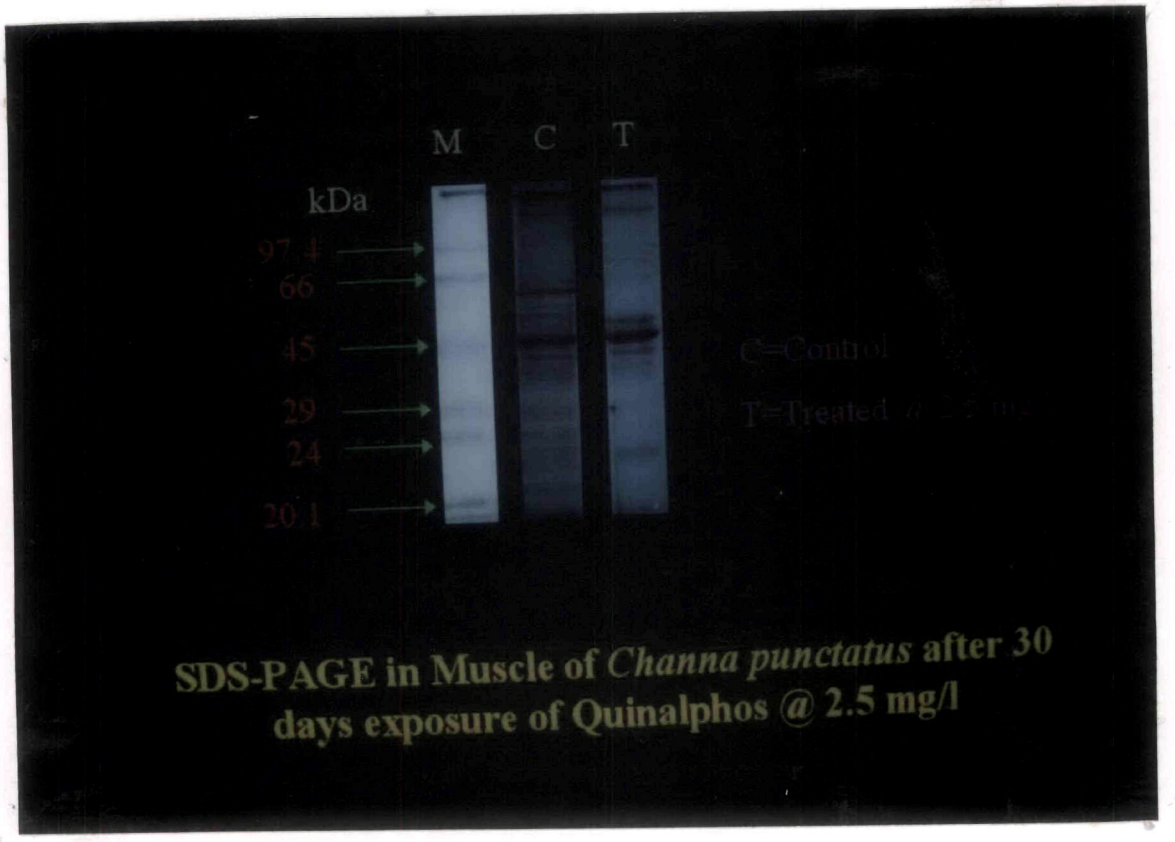


Fig:58

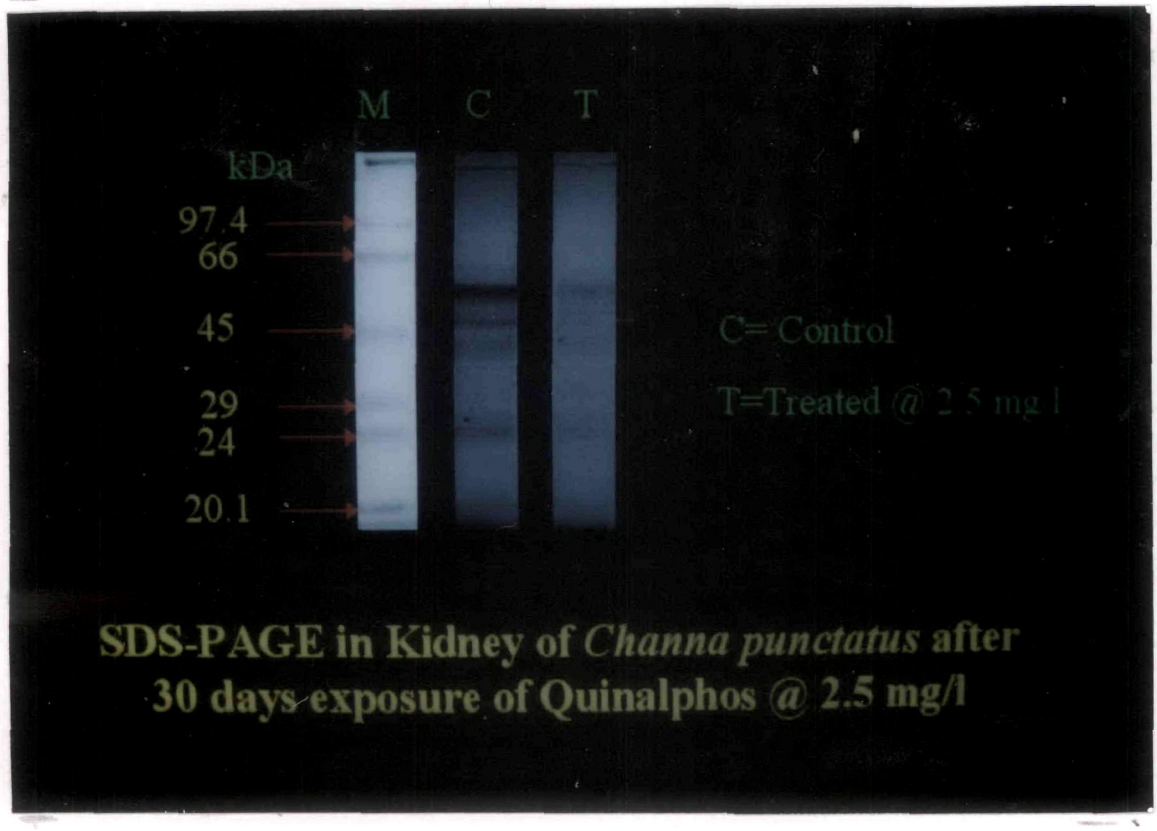
C= Control
T1= Quinalphos exposed (@ 0.50 mg/l)
T2 = Quinalphos exposed (@ 2.5 mg/l)



SDS-PAGE in Liver of *Channa punctatus* after 30 days exposure of Quinalphos @2.5 mg/l



SDS-PAGE in Muscle of *Channa punctatus* after 30 days exposure of Quinalphos @ 2.5 mg/l



SDS-PAGE in Kidney of *Channa punctatus* after 30 days exposure of Quinalphos @ 2.5 mg/l

Acid phosphatase and Alkaline phosphatase activities

The activity levels of ACP and ALP in liver, kidney and muscle at different exposure periods have been depicted in Figure (62, 63, 67, 68, 72, and 73) and the actual data summarized in Table- 48, 49, 53, 54, 58 and 59. From these it would be revealed that both ACP and ALP were activated in the quinalphos-exposed fishes and that there was a linear increase in the activity in liver, kidney and muscle (more pronounced in kidney) along with the lapse of time though the result was not significant on 10^{th} day. The maximum rise in ACP and ALP level was noticed at both the concentrations on 30^{th} day, which was statistically significant ($p < 0.01$).

Lipid Peroxidation and reduced glutathione levels

The concentration of malondialdehyde (MDA), an indicator of lipid peroxidation, was observed to increase in the quinalphos-exposed groups in liver, kidney and muscle, which has been depicted in Figure: 64, 74 and 69 and Table- 50, 60 and 55. The level of GSH in liver, kidney and muscle, on the other hand, was reduced to a significant extent in the quinalphos-exposed groups though the result was not significant on 10^{th} day (Table. 51, 61, 56 and Figure 65, 75, 70). The maximum reduction of GSH was recorded at 30 days post-exposure in 2.5mg/l, which was statistically significant ($p < 0.01$).

Cytochrome P450 activity

The cyt P450 level (nmol/mg microsomal protein) of various groups, both treated and controls have been summarized in (Table 52, 57, 62; Figs. 66, 71, 76). Data showed that cyt P450 activity in liver, muscle and kidney was significantly ($p<0.01$) induced at 2.5 mg/l concentration on 30 days exposure. There was a linear increase in the activity in liver, muscle and kidney tissues along with the lapse of time except at 10 days exposure in liver tissue.

Table 48. Activity of the acid phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation) in liver of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.343 ±0.009	0.342± 0.007	0.341 ±0.006
T1 (0.50mg/l)	0.398 ±0.007ns	0.402 ±0.008*	0.422 ±0.009**
T2 (2.5 mg/l)	0.399 ±0.006ns	0.419 ±0.009*	0.498 ±0.008**

Table 49. Activity of the alkaline phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation) in liver of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.940± 0.011	0.941 ±0.012	0.940 ±0.011
T1 (0.50mg/l)	0.968± 0.014 ns	1.002 ±0.016*	1.315 ±0.017**
T2 (2.5 mg/l)	0.970± 0.015 ns	1.215± 0.018*	1.418± 0.019**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
*= $p<0.05$
**= $p<0.01$

Table 50. Lipid peroxidation (n mole MDA/g) level in liver of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	340 ±8.8	340.2 ±8.6	341.8± 8.7
T1 (0.50mg/l)	346 ±7.8 ns	352 ±8.6*	375± 9.7**
T2 (2.5 mg/l)	347 ±8.7 ns	364 ±9.8*	395 ±9.9**

Table 51. Reduced glutathione (m mole/g) level in liver of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.235± 0.002	0.236 ±0.004	0.238 ±0.006
T1 (0.50mg/l)	0.232 ±0.003 ns	0.229 ±0.004 ns	0.219 ±0.006*
T2 (2.5 mg/l)	0.231 ±0.0031 ns	0.222 ±0.005*	0.197± 0.006**

Table 52. Cytochrome p450 (n mole/mg) level in liver of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.458± 0.012	0.459±0.011	0.460 ±0.010
T1 (0.50mg/l)	0.502 ±0.008 *	0.532±0.007*	0.566 ±0.009**
T2 (2.5 mg/l)	0.570±0.007 *	0.588 ±0.009 *	0.602 ±0.012**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
*= $p<0.05$
**= $p<0.01$

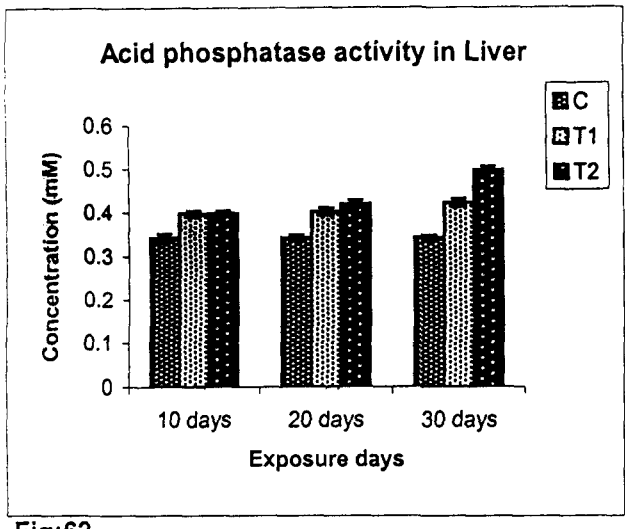


Fig:62

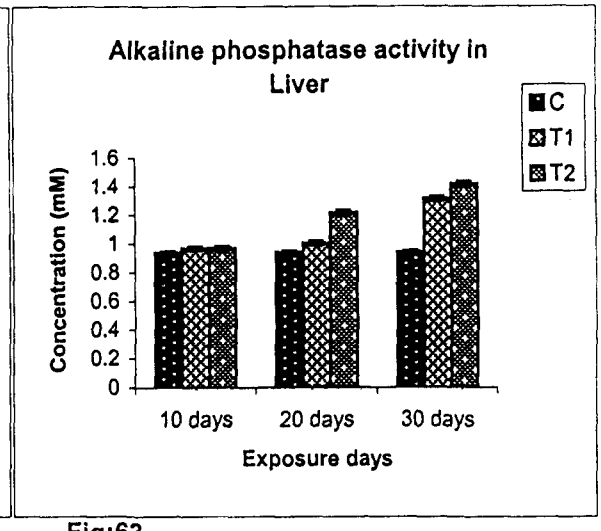


Fig:63

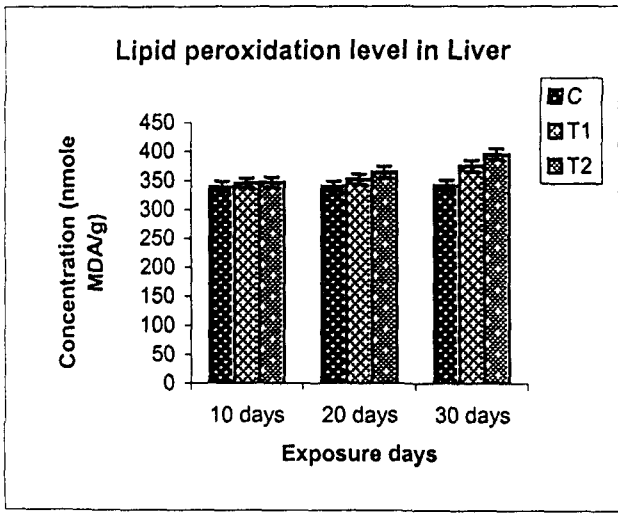


Fig:64

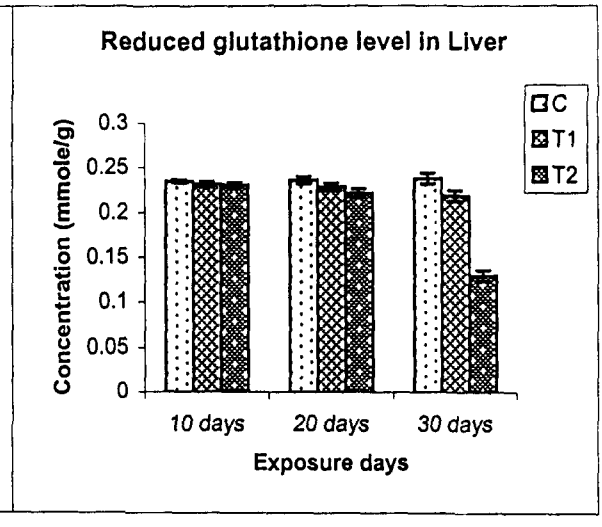


Fig:65

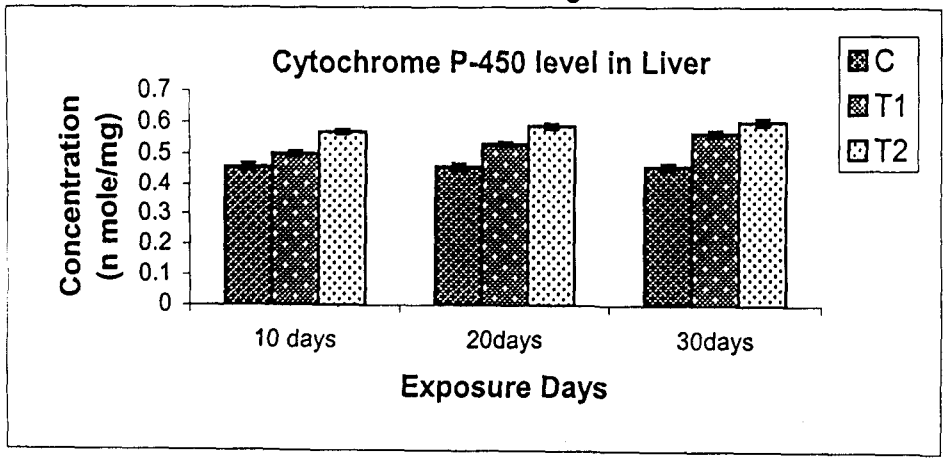


Fig:66

C= Control
T1= Quinalphos exposed (@ 0.50 mg/l)
T2 = Quinalphos exposed (@ 2.5 mg/l)

Table 53. Activity of the acid phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation) in muscle of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.341± 0.007	0.340 ± 0.005	0.339 ± 0.004
T1 (0.50mg/l)	0.396 ± 0.005ns	0.400 ± 0.007*	0.420 ± 0.007**
T2 (2.5 mg/l)	0.397 ± 0.004ns	0.417 ± 0.007*	0.496 ± 0.006**

Table 54. Activity of the alkaline phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation) in muscle of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.941 ±0.012	0.942 ±0.013	0.941 ±0.012
T1 (0.50mg/l)	0.969 ±0.015ns	1.003 ±0.017*	1.316 ±0.018**
T2 (2.5 mg/l)	0.971 ±0.016ns	1.216 ±0.019**	1.419± 0.019**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
*= $p<0.05$
**= $p<0.01$

Table 55. Lipid peroxidation (n mole MDA/g) level in muscle of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	345 ±13.8	345.2 ±13.6	346.8 ±13.7
T1 (0.50mg/l)	351± 12.8 ns	357 ±13.6*	380 ±14.7**
T2 (2.5 mg/l)	352 ±13.7ns	369 ±14.8*	400 ±14.9**

Table 56. Reduced glutathione (m mole/g) level in muscle of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.230 ±0.003	0.231± 0.005	0.231 ±0.007
T1 (0.50mg/l)	0.227 ±0.004ns	0.224 ±0.004ns	0.214 ±0.007*
T2 (2.5 mg/l)	0.226± 0.004ns	0.217 ±0.006*	0.192 ±0.007**

Table 57. Cytochrome p450 (n mole/mg) level in muscle of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.460± 0.014	0.461 ± 0.013	0.462 ± 0.012
T1 (0.50mg/l)	0.514 ± 0.010 *	0.544 ± 0.009*	0.578± 0.011*
T2 (2.5 mg/l)	0.592± 0.009 *	0.603 ± 0.010*	0.614± 0.014**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
*= $p<0.05$
**= $p<0.01$

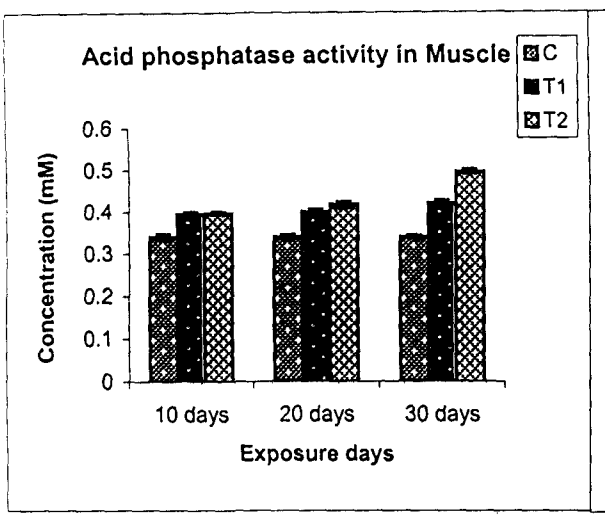


Fig:67

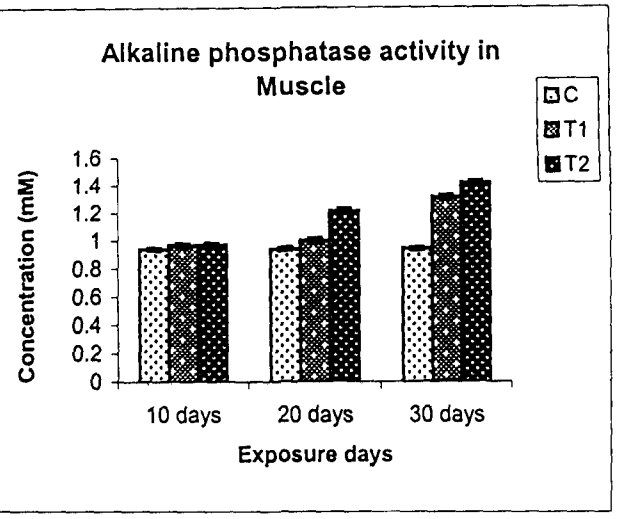


Fig:68

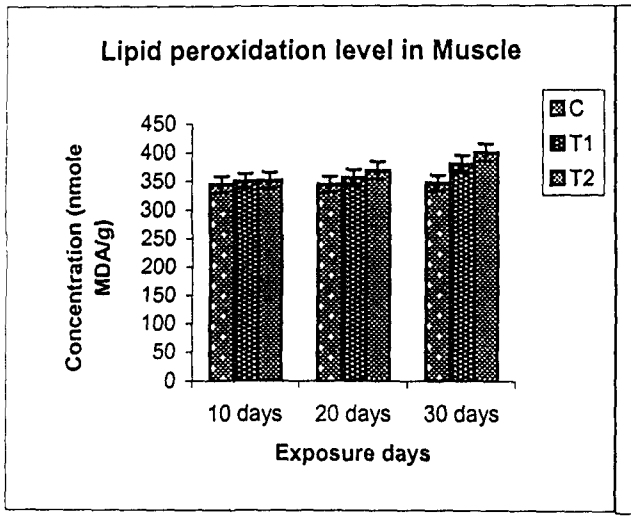


Fig:69

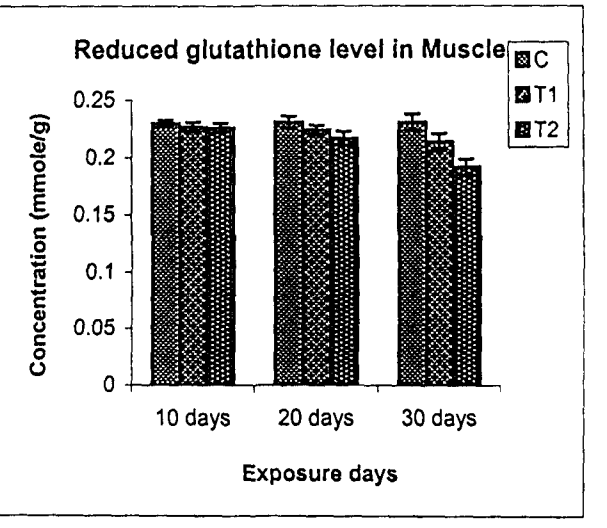


Fig:70

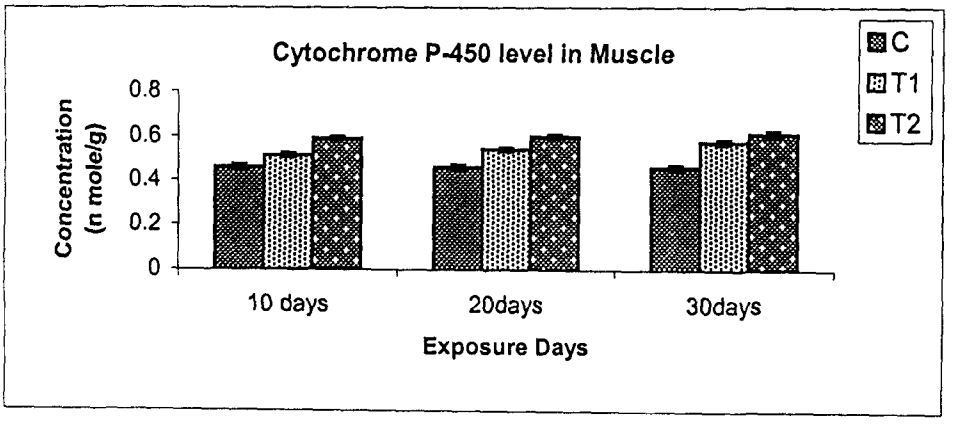


Fig:71

C= Control
T1= Quinalphos exposed (@ 0.50 mg/l)
T2 = Quinalphos exposed (@ 2.5 mg/l)

Table 58. Activity of the acid phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation) in kidney of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.331 ±0.008	0.330± 0.006	0.329 ±0.005
T1 (0.50mg/l)	0.386 ±0.006ns	0.390 ±0.008*	0.410 ±0.008**
T2 (2.5 mg/l)	0.387± 0.005ns	0.407 ±0.008*	0.486 ±0.007**

Table 59. Activity of the alkaline phosphatase (expressed in mM of phenol liberated per 100 mg of protein at 25°C after 30 min of incubation) in kidney of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.341± 0.011	0.342 ±0.012	0.341± 0.013
T1 (0.50mg/l)	0.369± 0.014 ns	0.430 ±0.016*	0.763± 0.017**
T2 (2.5 mg/l)	0.371± 0.015ns	0.616 ±0.018*	0.819 ±0.018**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
*= $p<0.05$
**= $p<0.01$

Table 60. Lipid peroxidation (n mole MDA/g) level in kidney of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	325± 12.8	325.2 ±12.6	326.8 ±12.7
T1 (0.50mg/l)	331± 11.8 ns	337± 12.6*	370 ±13.7**
T2 (2.5 mg/l)	332± 13.7ns	349± 13.8*	380 ±13.9**

Table 61. Reduced glutathione (m mole/g) level in kidney of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.225± 0.003	0.226± 0.005	0.226± 0.007
T1 (0.50mg/l)	0.222 ±0.004ns	0.219 ±0.004ns	0.209± 0.007*
T2 (2.5 mg/l)	0.221± 0.004ns	0.212 ±0.006*	0.187 ±0.007**

Table 62. Cytochrome p450 (n mole/mg) level in kidney of *Channa punctatus* at different exposures of Quinalphos

Dose	10 days	20 days	30 days
C	0.359 ±0.011	0.360 ±0.011	0.361 ±0.012
T1 (0.50mg/l)	0.414 ±0.008 *	0.454 ±0.007*	0.468 ±0.009*
T2 (2.5 mg/l)	0.482 ±0.009 *	0.493± 0.008*	0.504 ±0.012**

Data are represented as mean ± SD (Standard Deviation)
No. of Fishes in all cases (15)
ns = Non significant
*= $p<0.05$
**= $p<0.01$

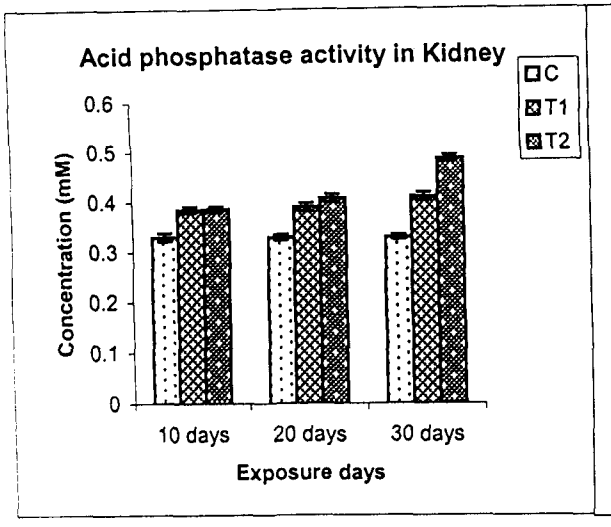


Fig:72

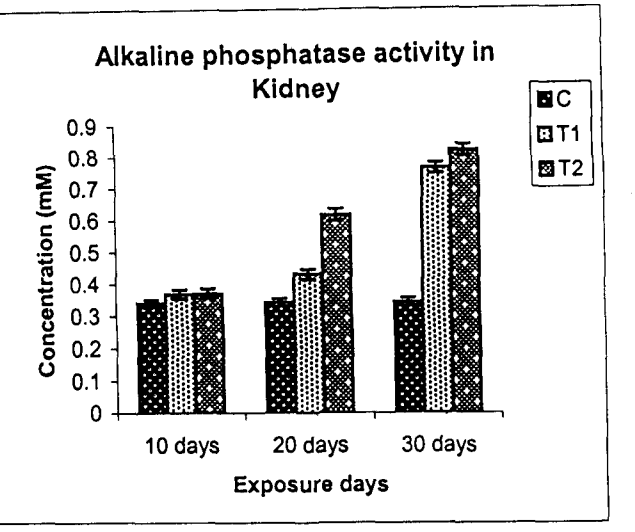


Fig:73

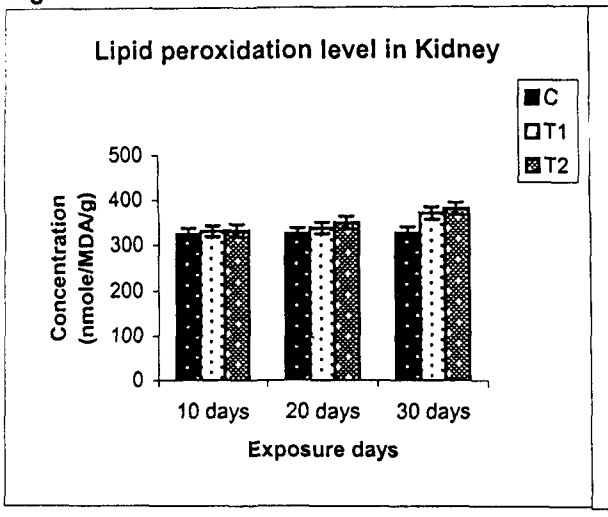


Fig:74

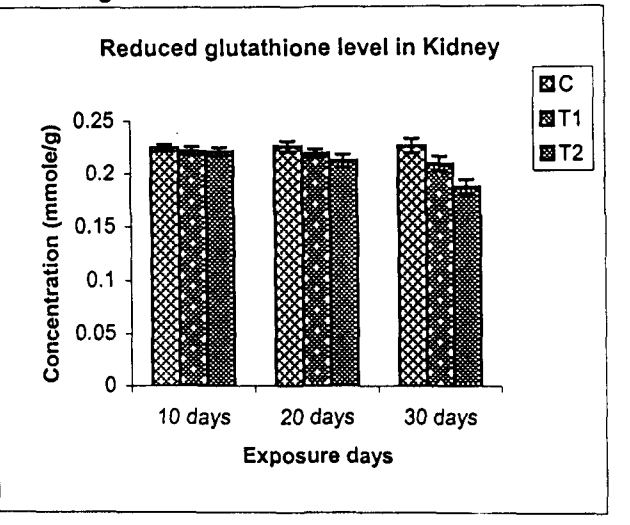


Fig:75

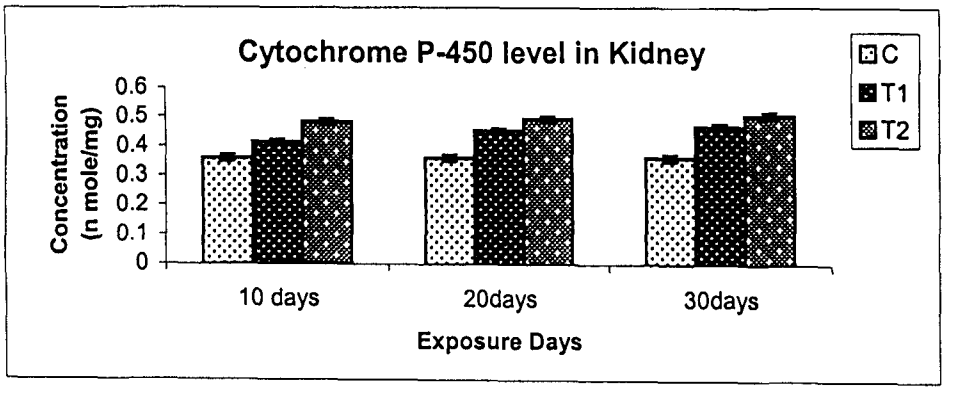


Fig:76

C= Control
T1= Quinalphos exposed (@ 0.50 mg/l)
T2 = Quinalphos exposed (@ 2.5 mg/l)

Discussion

Quinalphos like dimecron causes serious pollutional hazards and its contamination of aquatic ecosystem at the concentration tested must be prevented for sustainable aquaculture. It is also a neurotoxic organophosphate pesticide though less potent than dimecron. There is much evidence that contamination of this pesticide in the environment has caused serious intoxication in human beings also. The organs that are most affected critically are the liver, kidney and muscle in case of fish. The lower values of DO in pesticide exposed water indicated the stress condition of the aquatic system. Since quinalphos showed significant influence on pH, hardness and water alkalinity and free carbon dioxide, like dimecron it also will be a major factor in reducing significantly the yield of fish. Other study (Muirhead-Thomson, 1971) have attested the importance of physical and chemical factor of water, which greatly influence the pesticide impact on fresh waters.

Planktons form an important trophic level in an aquatic ecosystem. Survival of fish is impossible if their food is wiped off or contaminated. In the present investigation it was also noticed that the rate of phytoplankton reduced gradually. Significant reduction in zooplankton population occurred in all exposed water and the maximum reduction was at 0.9 and 1.8 mg/l. This is due to the high sensitivity of zooplankton to this pesticide. Population of phytoplankton was offered suggesting that the tested concentrations of quinalphos were phytotoxic. As interaction of all these parameters contributes to the production of fishes in ponds and in natural waters, any disorder in these parameters may reduce the fish yield ultimately. A gross effect upon any major factor or group of organism produces changes in the entire ecosystem. Therefore, there is need for rational use of quinalphos so that the natural resources of waters, particularly fish fauna and their food organisms may be protected for human benefit.

No detailed study had earlier been made on the gain of weight of different individual tissue in contrast with the loss of body weight as a whole along with altered behavioral response in quinalphos exposed fishes. Further, the extent to which even sub-lethal concentration of quinalphos at minimum exposure could affect the tissue weight had not also been properly assessed.

Quinalpnhos intoxication has led to a decrease in body weight. This might be due to a general decline in the metabolic activity in these animals. This decrease is noticed at three successive intervals observed up to 30 days. It is also related with the dose and with the time. Enzymatic pathways may be altered in response to toxicant exposure in order to maintain homeostasis. Hence, loss of body weight in present experiment suggests that quinalphos like other potent organophosphate compounds has prominent effect on metabolic processes (Carevic and Fiser Herman, 1962; Piccaluga *et al.*, 1965; Rozengart *et al.*, 1971; Begum and Vijayaraghavan, 1996). An increase in HSI results following the exposure of fish to quinalphos. This is due to the attempt the fish is making to adapt to the presence of the toxicant. As liver is the first organ to face any foreign molecule that is carried through portal circulation and is subjected to more damage (Couch, 1975; Johnson, 1968; McKim *et al.*, 1974;Tucker and Leitzke, 1979). Liver is the chief detoxifying organ and is thus adversely affected by toxicants. The organism attempts to increase the effectiveness of the liver to detoxify the substance by increasing the volume of the liver. This is done by either increasing the number of cells in the liver (hyperplasia) or by increasing the size of each newly produced liver cell (hypertrophy). As the detoxifying mechanism of not only liver but also kidney is very sharp, the increase in the RSI observed might be due to the hyperactivity of the kidney under the toxic influence of the insecticides.

Though Dwivedi *et al.* (1998) reported that albino rats treated orally with quinalphos (0.52 and 1.04 mg/kg body weight) for 60 days showed a significant decrease in body along with brain and liver weights. The behavioral study was also a remarkable feature of this experiment. As is known, acetylcholinesterase plays an important role in the transmission of nerve impulses (Stryer, 1995) and Organophosphates are powerful neurotoxic chemicals as they inhibit acetylcholinesterase (Post and Leasure, 1979; Gantverg and Perevoznikeov, 1984; Fernandez *et al.*, 1996). Erratic swimming, loss of balance and loss of touch sensation in quinalphos exposed fish is due to impairment of nerve impulse transmission. Respiratory abnormality could be due to hypoxia caused by inadequate tissue use of oxygen (Guyton and Hall, 2000). Hence this study is an indicative of the hazardous effect of quinalphos even in a

lesser dose in the fishes as reflected in changes in the body weight, relative organ weight and behavioral response.

The protein content is seriously affected in all the organs. It has decreased to an appreciable extent in all the organs studied. A dose dependent decrease was observed. Accumulation of Organophosphate compounds (Hassan *et al.* 1993) could drastically affect the metabolic as well as functional activities of those tissues, which in turn, could drastically lead to reduction in enzymatic activities or functional activities. This could explain the diminished total protein content in different tissues as a result of quinalphos exposure. Incidentally, the biochemical and cellular effects of long-term exposure to organophosphate compound have been well documented earlier (Das and Mukherjee, 2000 a b; Metelev, 1972; Thomas and Murthy, 1976; Shah, 1980; Dubale and Awasthi, 1982).

Quinalphos exposure has also reduced the DNA and RNA content in liver, muscle and kidney. The reduction in the concentration of RNA indicates inhibition of transcriptional activity and ultimately the transcription of protein is inhibited.

In view of the significant correlation of RNA and protein, a deficient synthesis of any type of RNA should have its reflection in corresponding failure of protein synthesis, as seen in the present study. Possibility of lesion of m-RNA functional capacity for such failure cannot be ignored (Bruin, 1976). Further, protein synthesis is dependent on DNA synthesis (Balis, 1968) quinalphos might have blocked the synthesis of DNA and consequently the synthesis of DNA directed RNA formation and hence, the resultant reduction of proteins. It is clear from this study that decrease of RNA, DNA level over the control indicates the reduction in the synthesis of protein. As stated earlier, the loss in total protein and diminished RNA content would point to the failure of protein synthesizing machinery of the cell. The precise mechanism of protein synthesis in both lower and higher forms of organisms is under genetic control and is well documented (Cooper, 1997; Lewin, 1997). Therefore, cytotoxicity brought in due to quinalphos exposure must have led to derangement of this machinery which otherwise functions with a high degree of fidelity. Thus, incomplete or faulty

expressions of certain genes regulating the metabolic activities of these organs could be a real possibility.

Data on protein patterns in PAGE revealed that quinalphos treatment caused disappearance of many proteins, although a few proteins have also been observed to be appearing. Here also some new proteins appeared. Thus quinalphos exposure has markedly inhibited the synthesis of proteins, although some indications have also taken place for the synthesis of new proteins. It may be inferred from our studies that some of the genes involved in the synthesis were switched off resulting in the disappearance of some of the proteins. The appearance of some new proteins may be due to the switching on of some genes. As it is known protein is synthesized basically through an elaborate mechanism by transcription of specific parts of DNA to form various types of RNA (mRNA, tRNA, rRNA), which interact with specific amino acids. The amino acids are attached with one another in a definite sequence to produce a certain type of protein (polypeptide). Therefore, any degradation or denaturation of protein would be reflected in gel electrophoretic band profiles and likely to be reflected in the DNA and RNA contents as well.

The changes observed in the activities of the enzymes are also very significant. Extensive toxicological studies have now established that increase in lipid peroxidation, alkaline and acid phosphatase activities along with decreasing level of glutathione denote cytotoxicity and hepatocellular dysfunction (Srivastava and Pandey, 1982; Comporti, 1985; Deboyser *et al.*, 1989; Banerjee *et al.*, 1993; Plaa *et al.*, 1991; Tomokuni, 1970). The increased level of acid phosphatase in the present study may be explained with the fact that quinalphos toxicity causes disruption of lysosomal membranes because biocides are known to produce cytotoxic action and changes in membrane fragility (Vijayendra Babu and Vasudev, 1984). Quinalphos might cause liver damage, which in turn lead to the release of acid phosphatase. The increased lysosomal enzymatic activity was accompanied by a decrease in RNA and protein content (Shah, 1980). This could be due to adverse effect of organophosphate compounds on the lysosomal membrane, which release nucleases proteases affecting RNA and protein metabolism. Increased activity of alkaline phosphatase in the present study is related with the breakdown of glycogen and induction of a condition of hyperglycemia. In order to combat

the stress arising out of quinalphos exposure demand of energy is very likely supplied by increased phosphatase activity.

The results of the present study showed that the level of lipid peroxidation was elevated significantly in liver, muscle and kidney following quinalphos exposure. On the other hand the level decreased in case of reduced glutathione. All these changes are indications of cytotoxicity. The oxidative metabolism has been greatly impaired in both liver and muscle as revealed from the studies on lipid peroxidation and reduced glutathione level. Quinalphos actually generated a reduction in cellular glutathione content, which has rendered the cells more susceptible to damage by oxygen free radical. So the changes observed in the enzymatic studies are also very significant. Our studies on increased lipid peroxidation and concomitant decrease in reduced glutathione level indicated that organophosphate compound caused oxidative stress.

CytP-450 related enzymes play an important role in the detoxification of many drugs, chemical carcinogens and other toxic agents, they are also responsible for catalyzing the metabolic activation of some substrates to highly reactive free radical, alkylating or arylating intermediates, which then react with critical cellular macromolecules to initiate toxic and carcinogenic events (Guengerich and Shimada, 1991). An elevated level of cyt P450 was observed in the quinalphos exposed groups. There are various supporting reports showing induction of CytP-450 during toxicant exposures (Dwibedi *et. al.*, 1998; Bondy, 1994). The induction in the present study may be due to bioactivation mechanism of quinalphos in liver and other extra hepatic tissues of *Channa punctatus*. This study also suggests that toxicity of quinalphos possibly due to formation of reactive oxygen intermediates (ROI) not for parent compound.

From the present discussion it is quite evident that the mechanism of quinalphos toxicity is complex like other potent organophosphate pesticide dimecron and not clearly understood. Although the vast number of possible interactions makes it difficult to pinpoint the main molecular causes of toxic effects, its hazardous effect on non target organism fish, particularly in areas prone to quinalphos contamination is a matter of great concern.

Conclusion

Both dimecron (phosphamidon) and quinalphos contamination are seriously affecting the aquatic ecosystem as a whole especially, commercially important aquatic fauna, fish which is not the target species in view of pesticide application. In the present thesis, an attempt has been made to assess the toxicity induced by these pesticides in different organs. Both these substances have been found to cause injury to different tissues, although the degree of damage varies in different organs.

The toxicity has been reflected in both water quality and biochemical parameters investigated in the present study. It has been observed that dimecron has caused much more hazards in aquatic system than quinalphos. The changes observed in the biochemical parameters are also very significant. Our studies on increased lipid peroxidation and concomitant decrease in glutathione level indicates that both dimecron and quinalphos are cytotoxic and also caused oxidative stress. Elevated cyt P450 level is an indication of cyt P450 –mediated oxidative bioactivation of dimecron and quinalphos. From our present investigation it can be concluded that dimecron and quinalphos even in a lesser dose, is a strong candidate for producing cytotoxicity as well as oxidative stress in the fishes as reflected in changes of the enzymatic levels and ROI and / or oxyradicals may be involved in the toxicity of dimecron.

Stress proteins are a group of proteins whose synthesis is induced by a wide variety of physical conditions and chemical agents: heavy metals, xenobiotics, oxidative stress, anoxia, salinity stress, teratogens, and hepatocarcinogens. Some of these proteins are believed to play a role in protecting the cell from damage that can result from environmental perturbations. Others are involved in the regulation of various genes. Stress results in a dramatic redirection of metabolism; a suite of stress proteins is rapidly synthesized and production of cellular proteins is repressed. This contention can explain the results obtained in this investigation in the light of the ability of the potent organophosphate compounds, dimecron and quinalphos to induce stress. In the present study, the changes in protein sub-fractions and concomitant changes in DNA and RNA contents would not only confirm the protein loss (due to dimecron and quinalphos exposure) in a more powerful and precise manner, but would again strongly support the cellular protective response against organophosphate toxicity through possible expression of certain genes encoding stress proteins though characterization of those proteins need to be studied.

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