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.

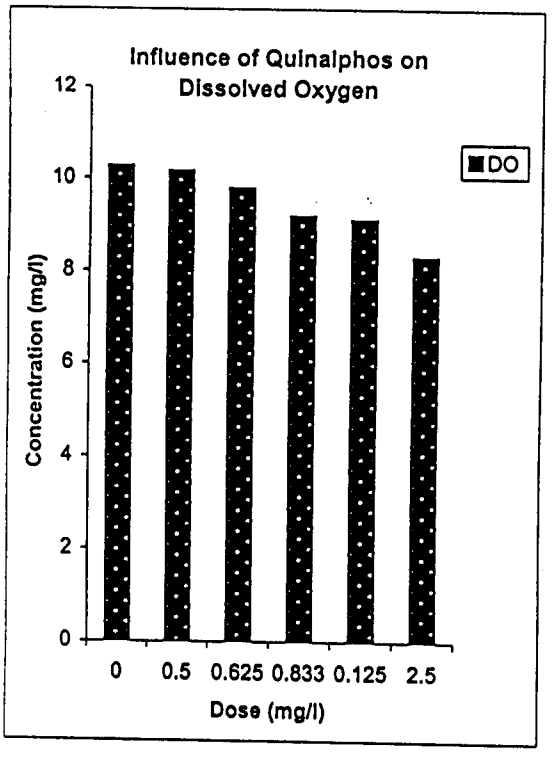
Month	Temperature (°C) maximum	minimum	Relative Humidity %	Rainfall (mm)
January	25.8	13.2	65.9	0.00
February	27.3	14.2	63.4	0.00
March	31.0	17.6	64.8	0.73
April	34.6	23.7	70.1	4.29
May	37.2	26.3	74.4	6.28
June	38.7	25.7	77.8	4.72
July	34.1	24.3	79.0	13.69
August	33.2	25.1	82.8	7.29
September	32.2	24.5	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*



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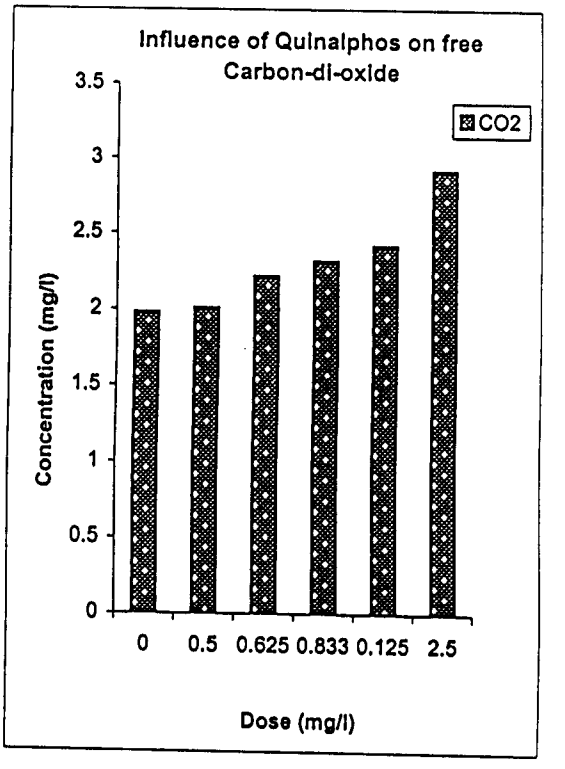

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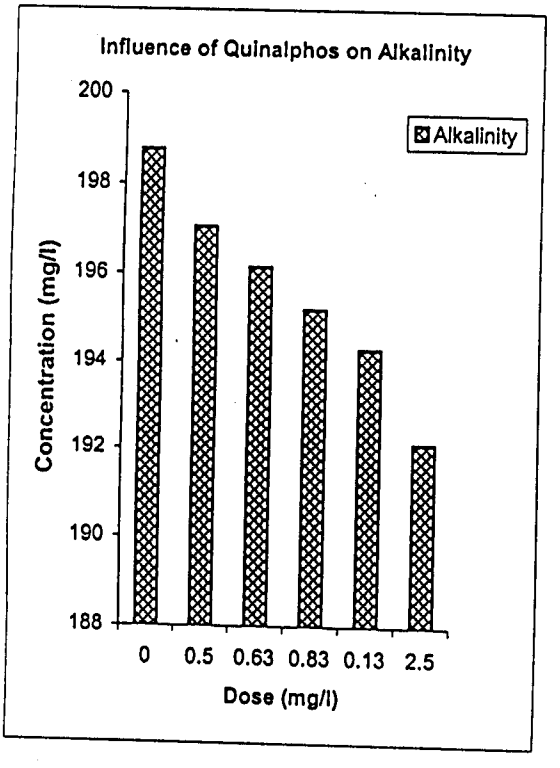

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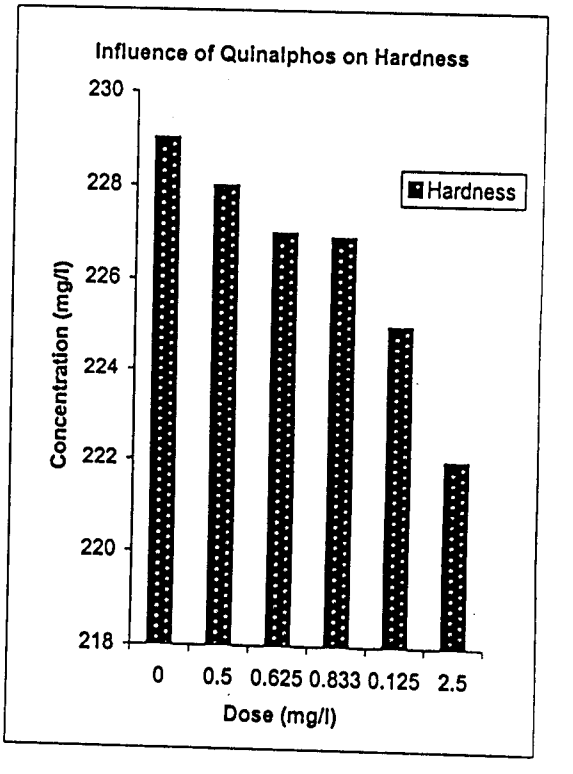

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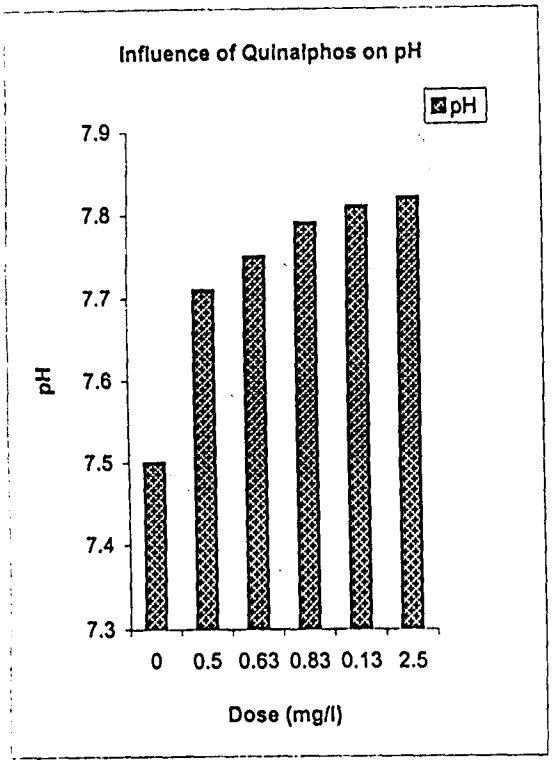

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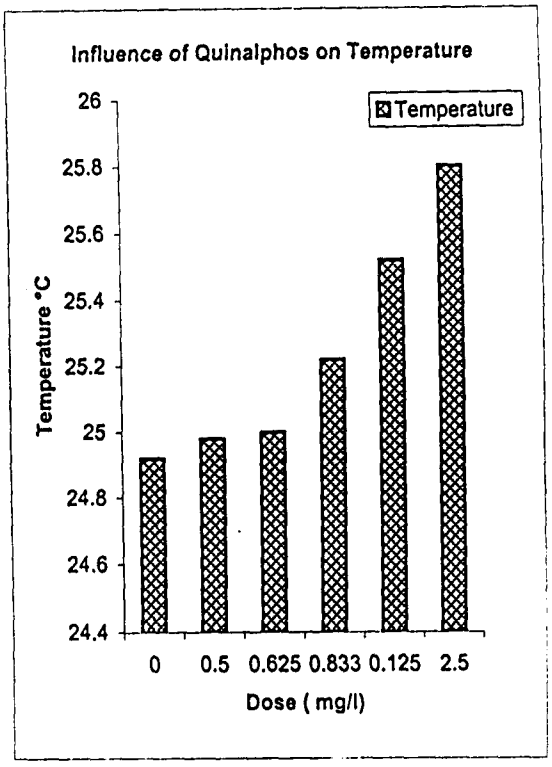

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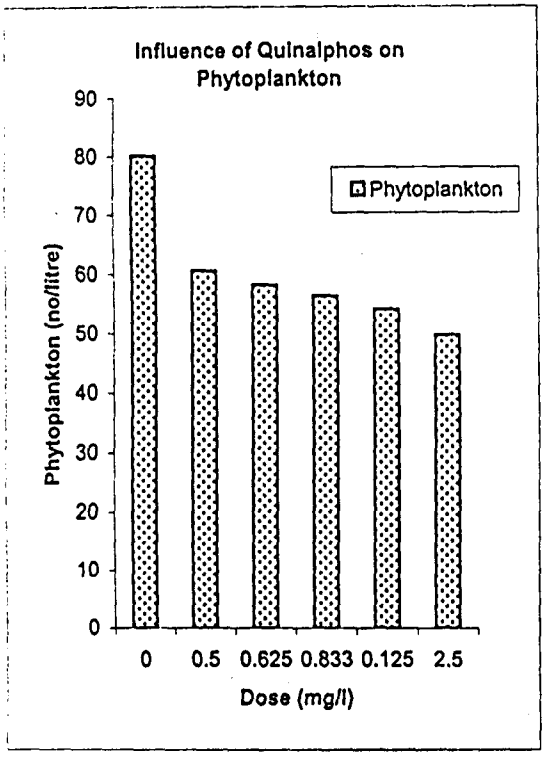

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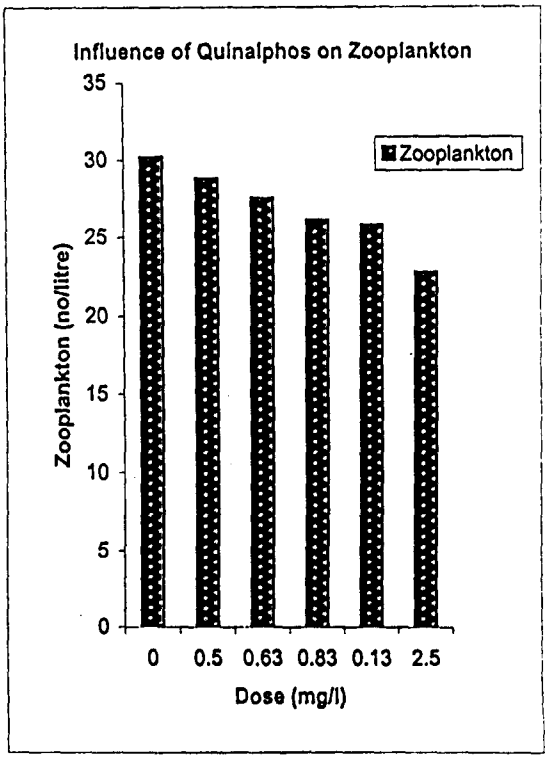


Fig:46

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)

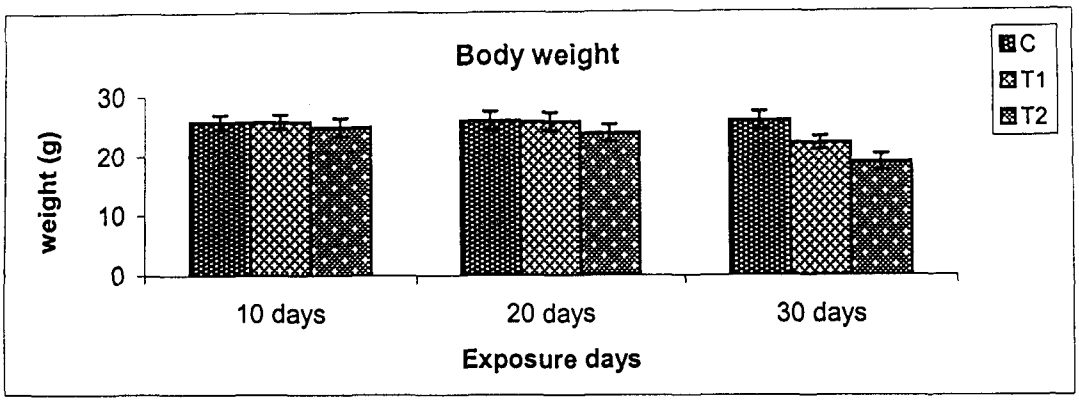
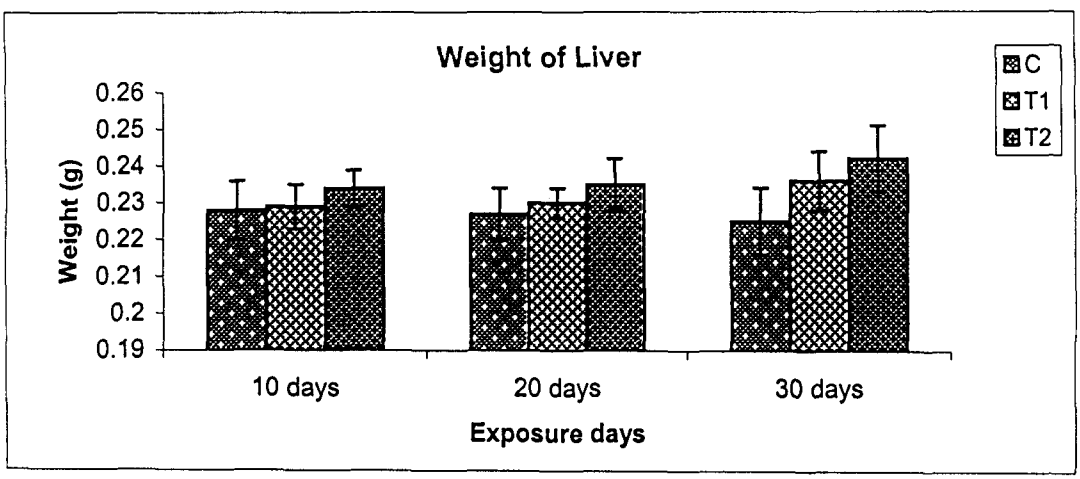


Fig:47



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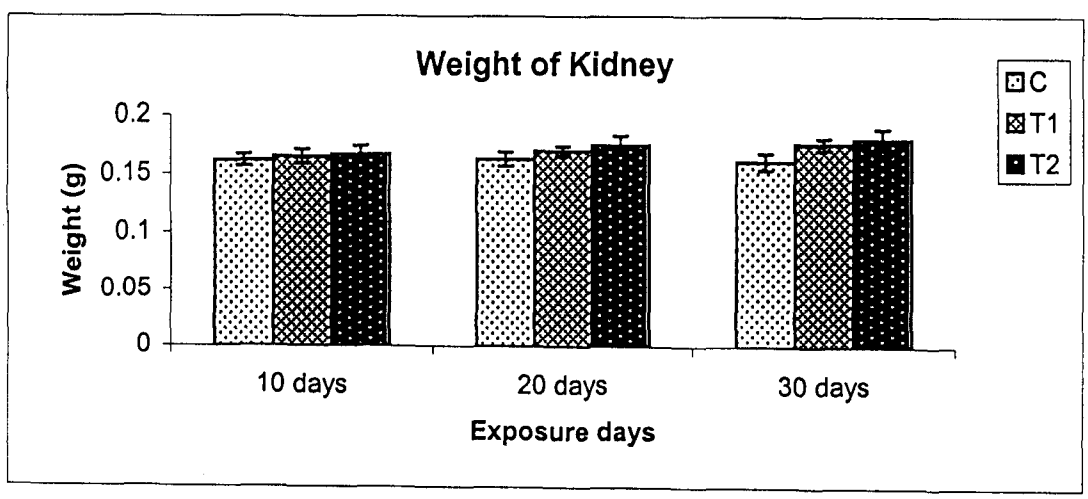


Fig:49

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
*= $p<0.05$
**= $p<0.01$

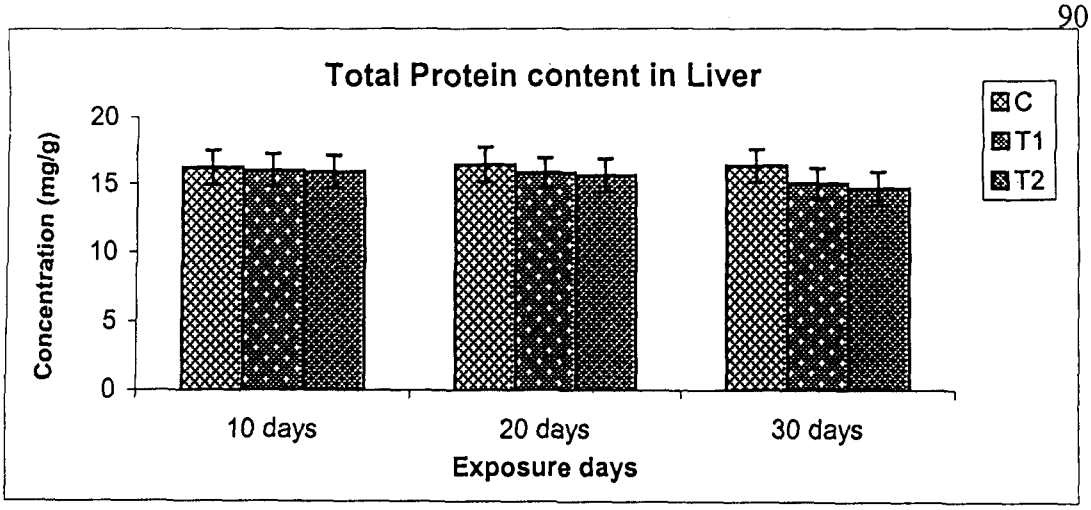
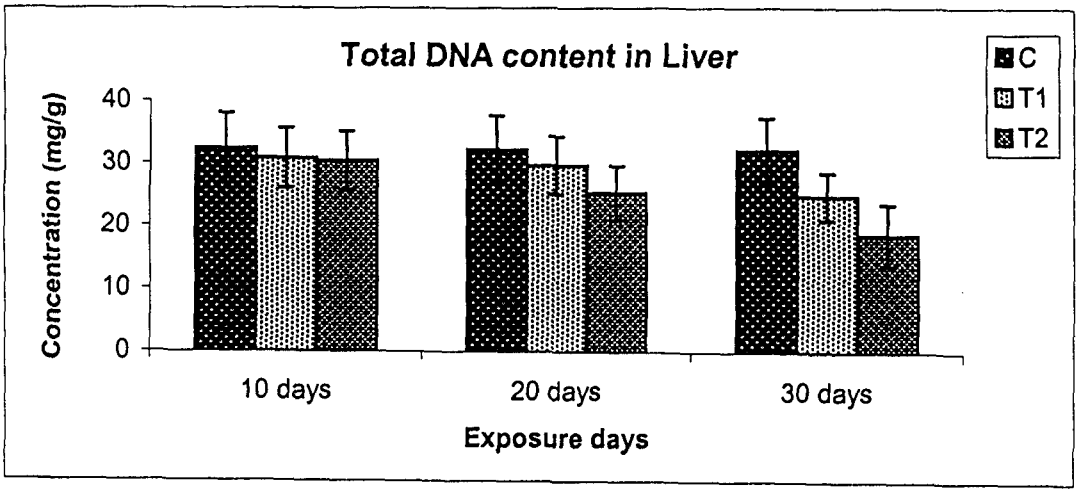


Fig:50



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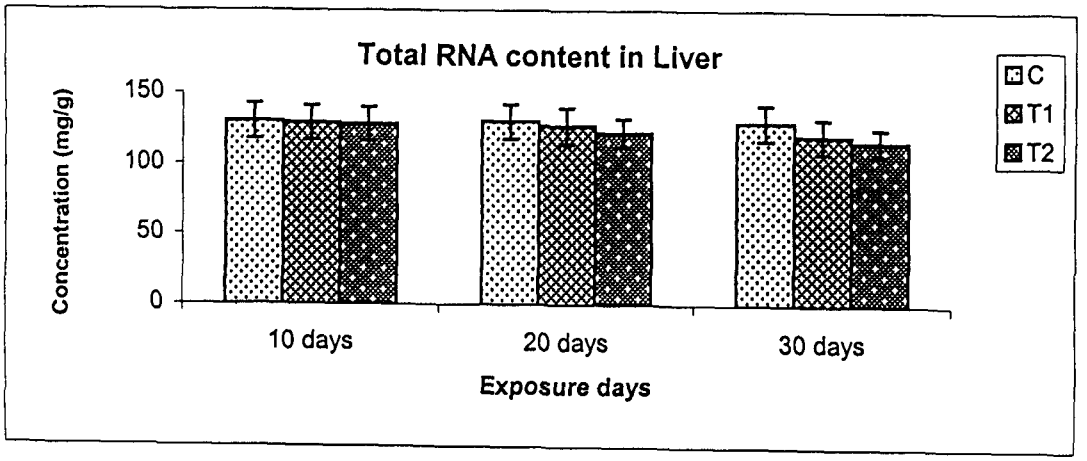


Fig:52

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
*= $p<0.05$
**= $p<0.01$

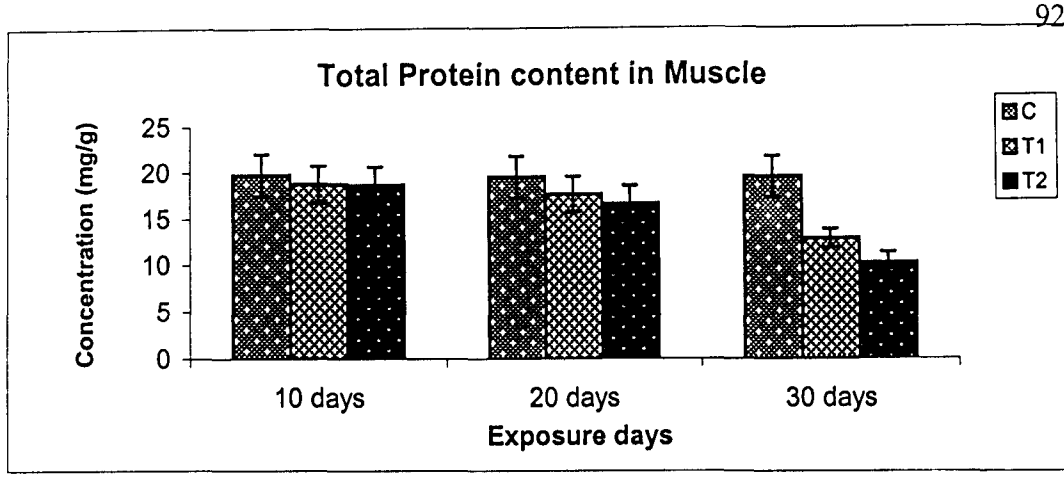


Fig:53

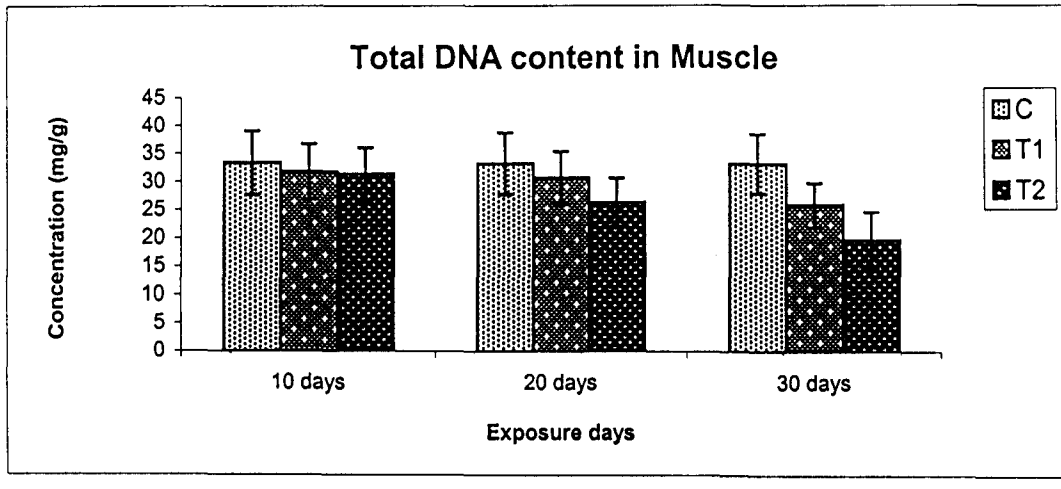


Fig:54

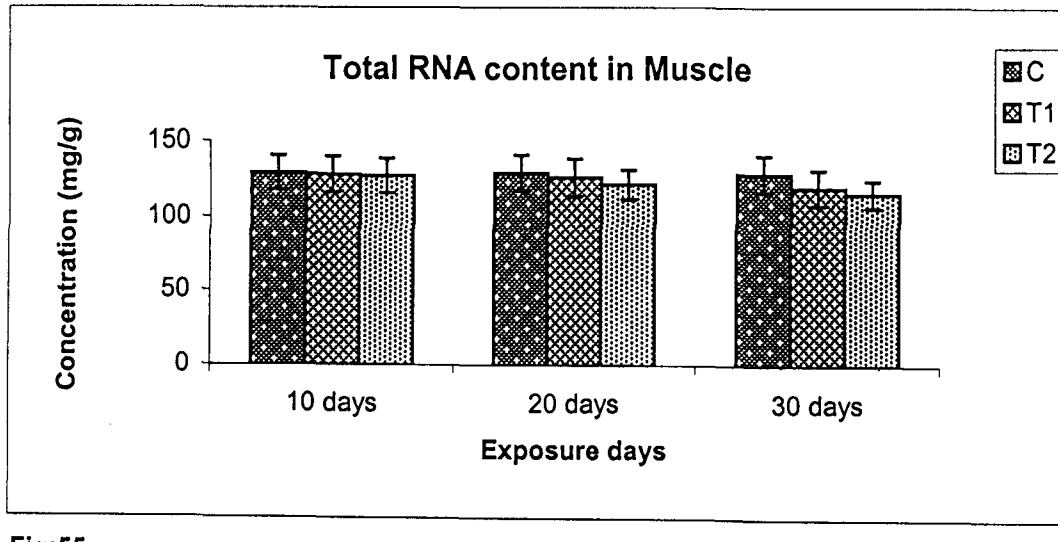


Fig:55

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
*= $p<0.05$
**= $p<0.01$

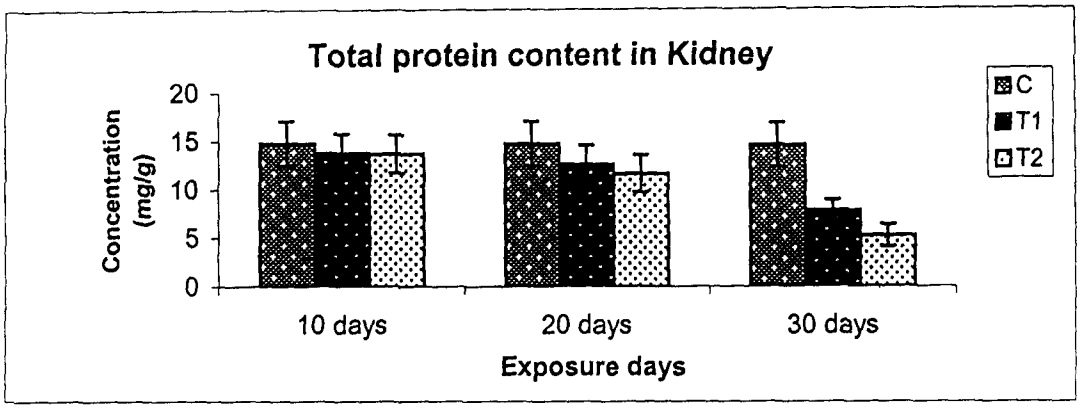


Fig:56

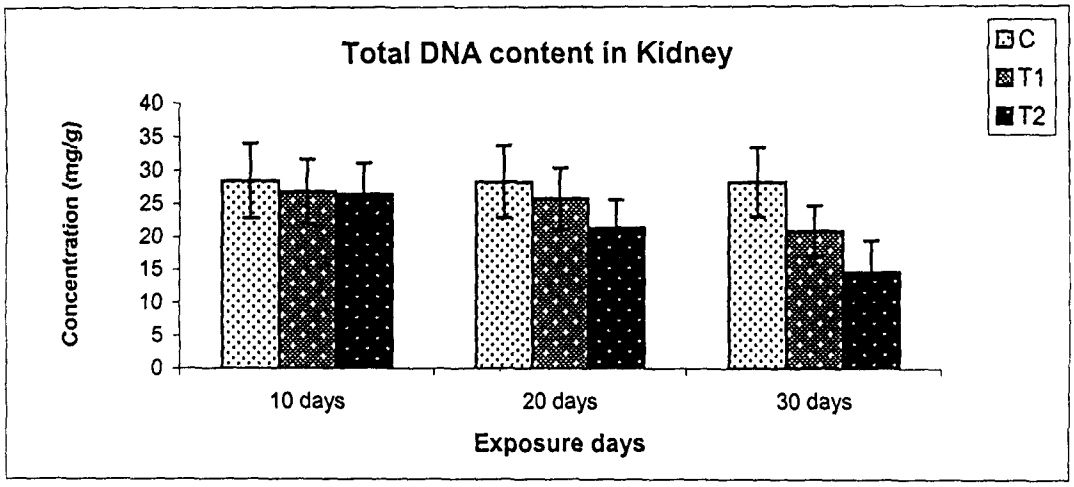


Fig:57

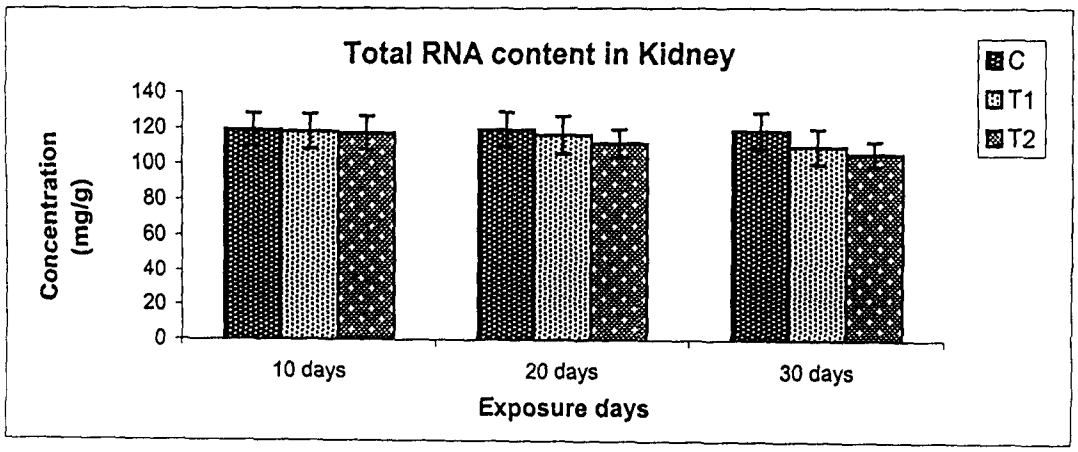
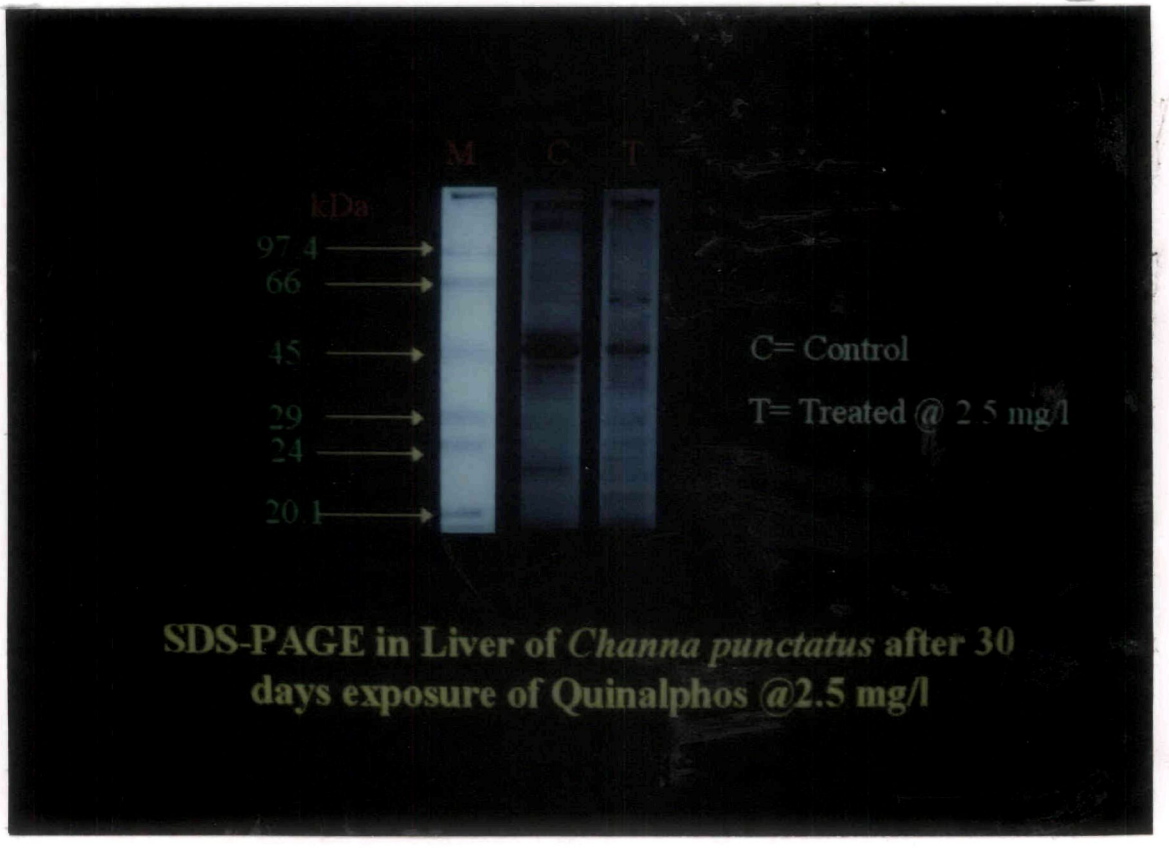
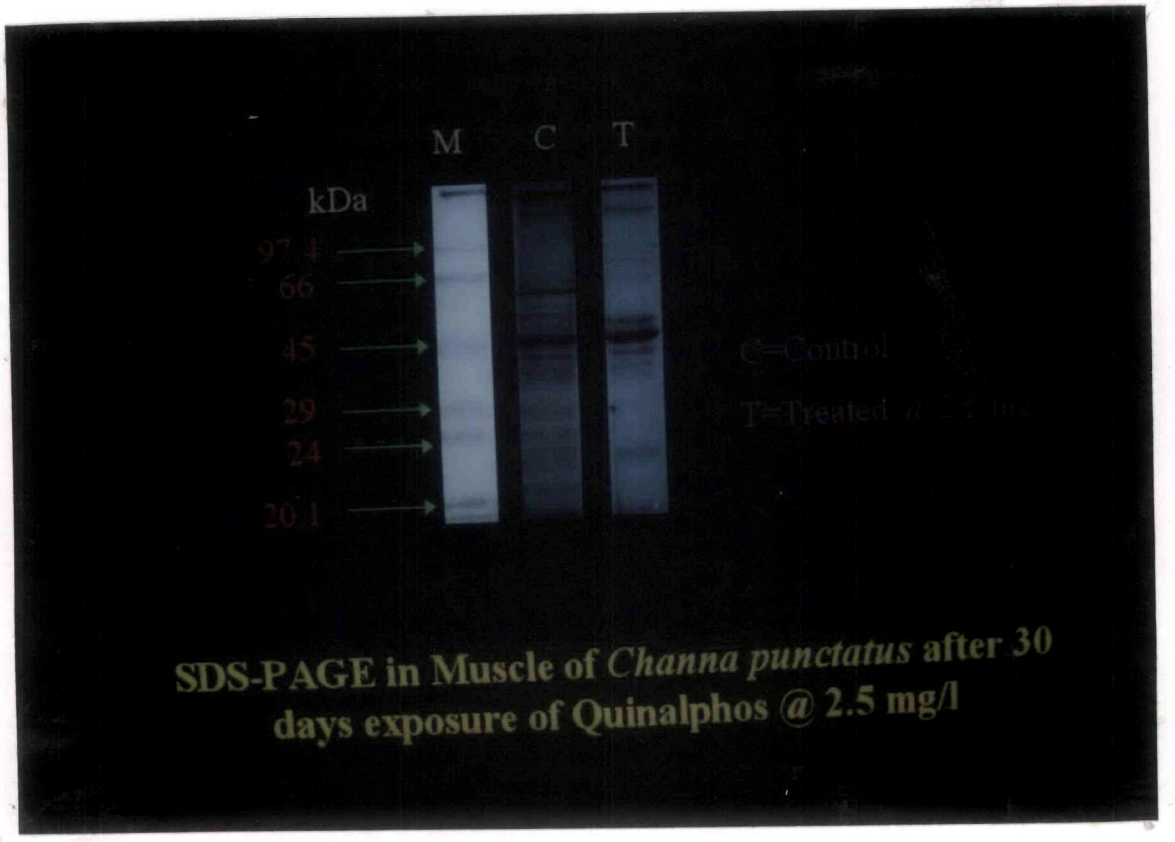


Fig:58

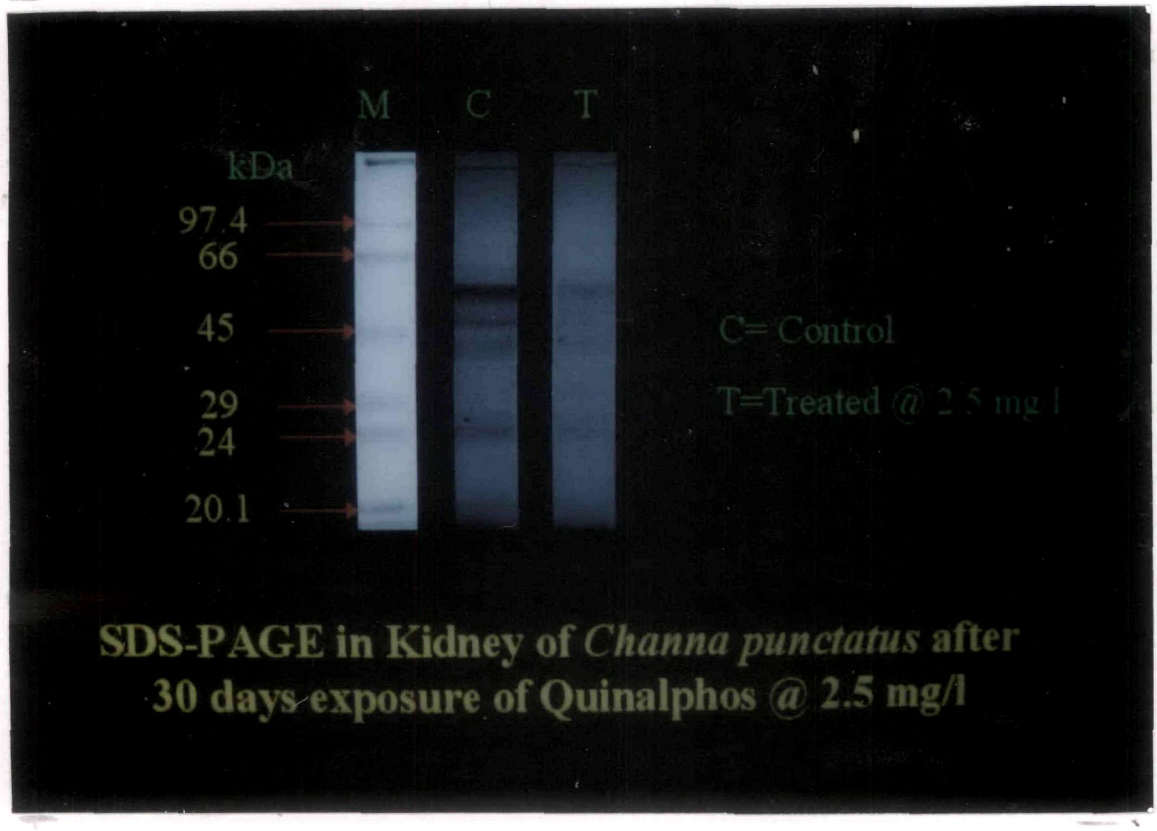
C= Control
T1= Quinalphos exposed (@ 0.50 mg/l)
T2 = Quinalphos exposed (@ 2.5 mg/l)



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
*= $p<0.05$
**= $p<0.01$

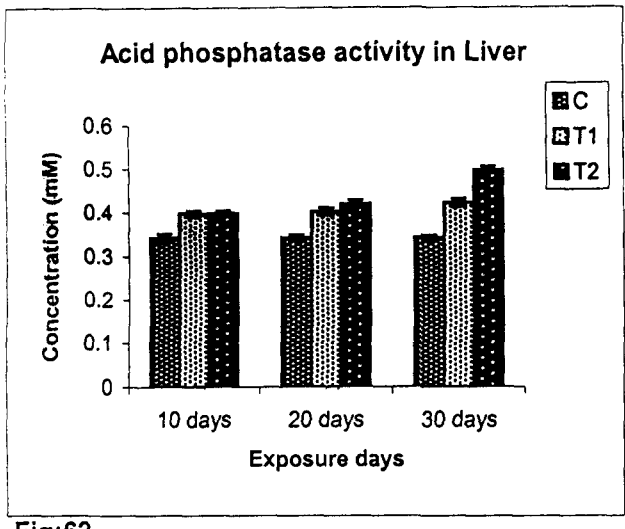


Fig:62

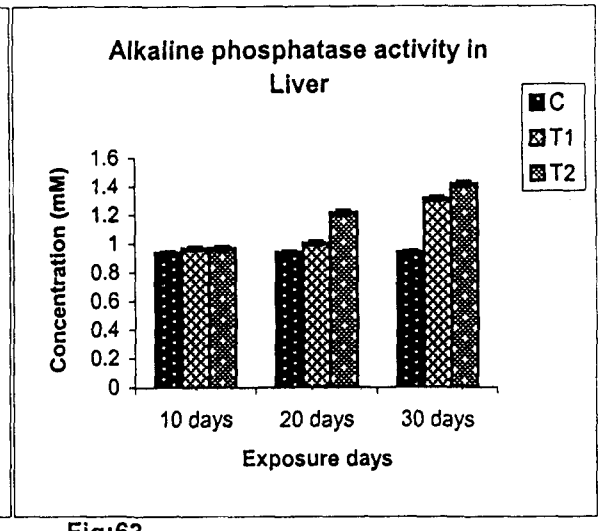


Fig:63

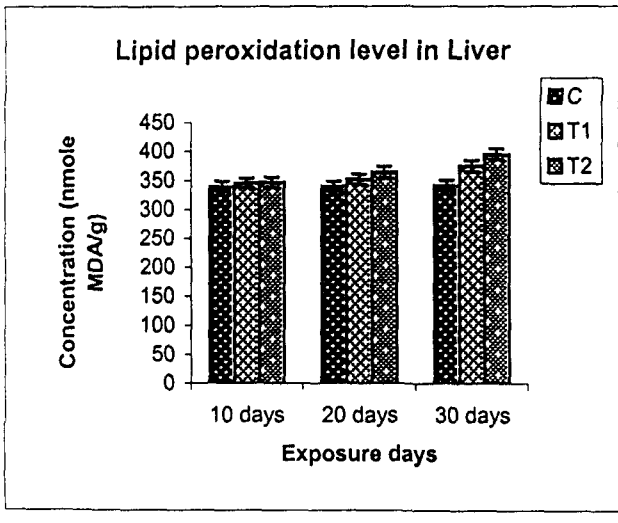


Fig:64

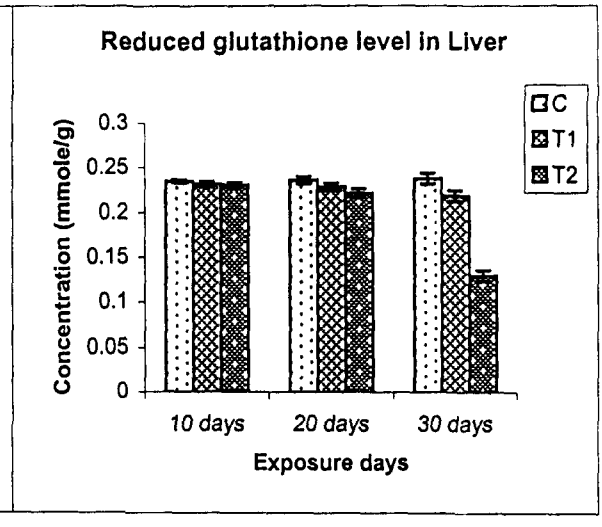


Fig:65

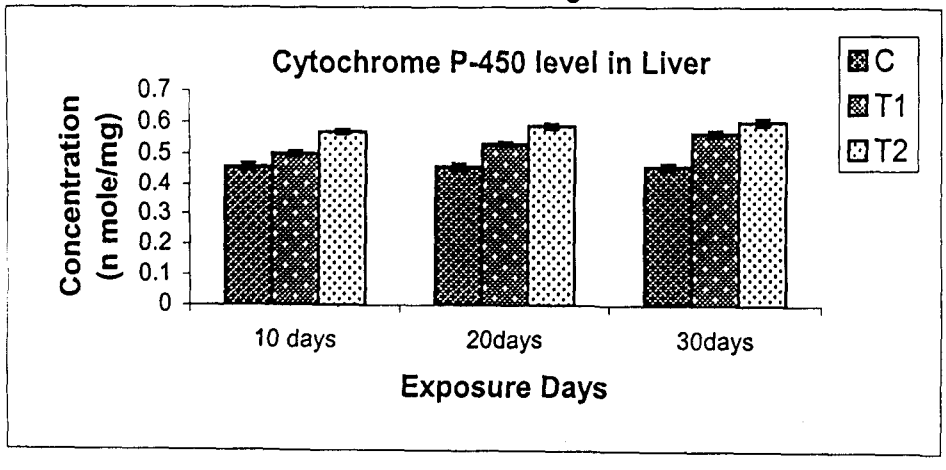


Fig:66

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
*= $p<0.05$
**= $p<0.01$

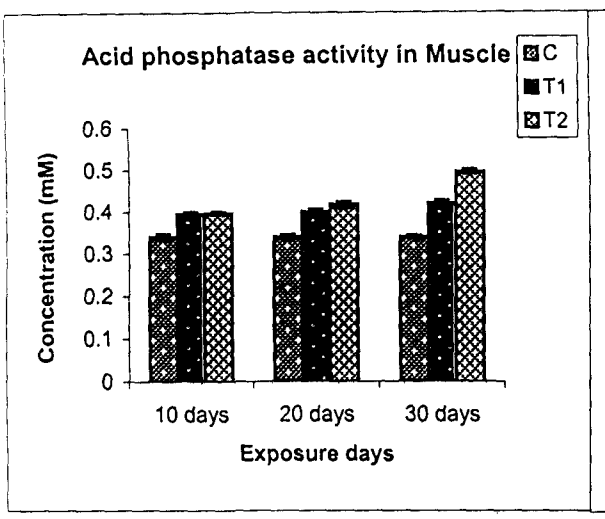


Fig:67

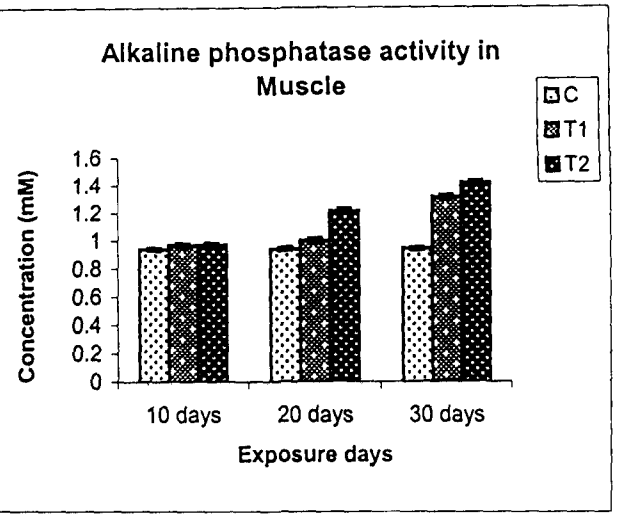


Fig:68

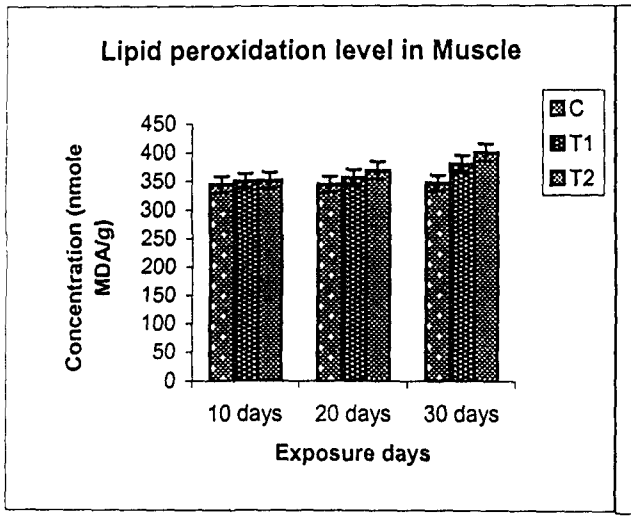


Fig:69

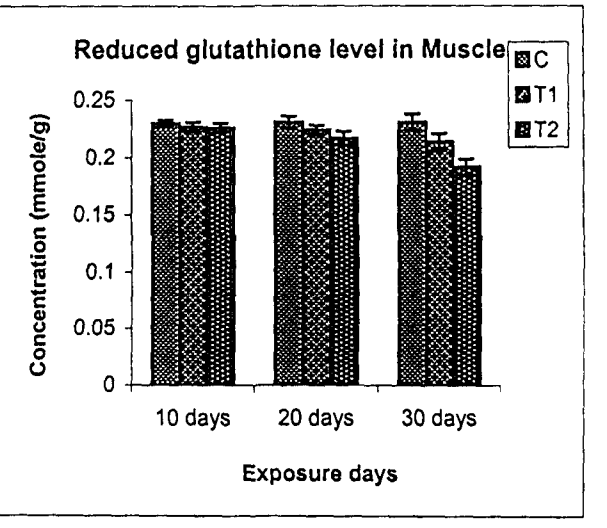


Fig:70

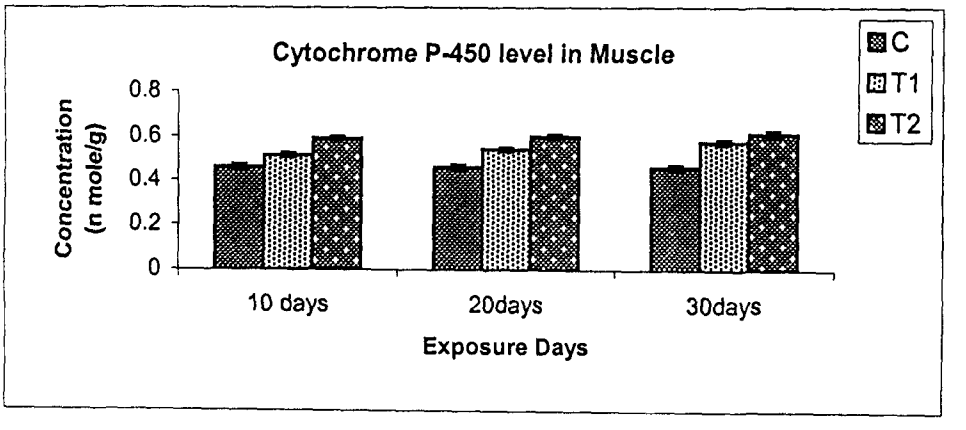


Fig:71

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
*= $p<0.05$
**= $p<0.01$

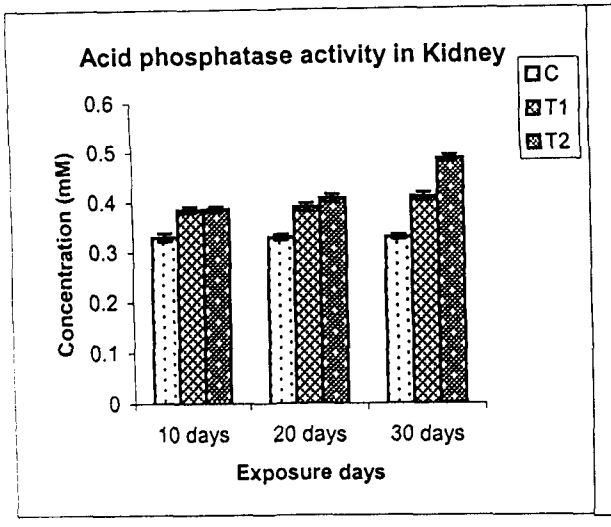


Fig:72

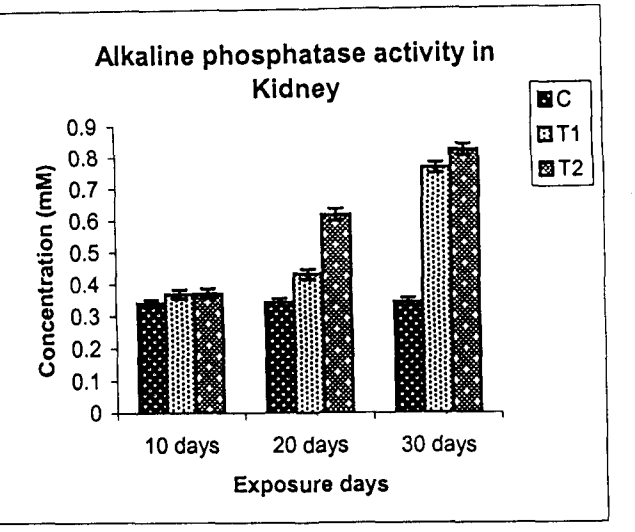


Fig:73

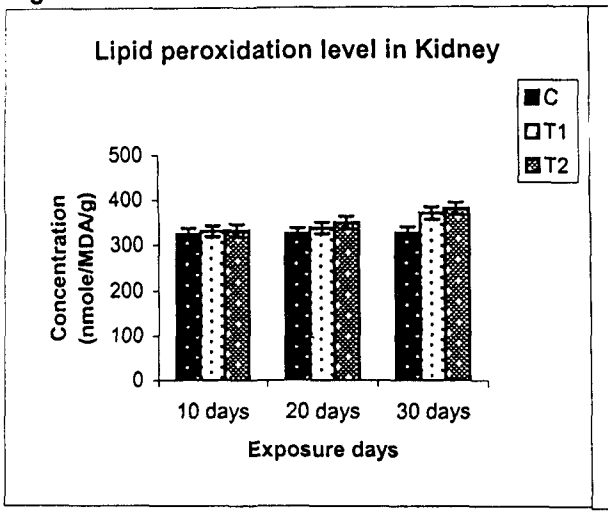


Fig:74

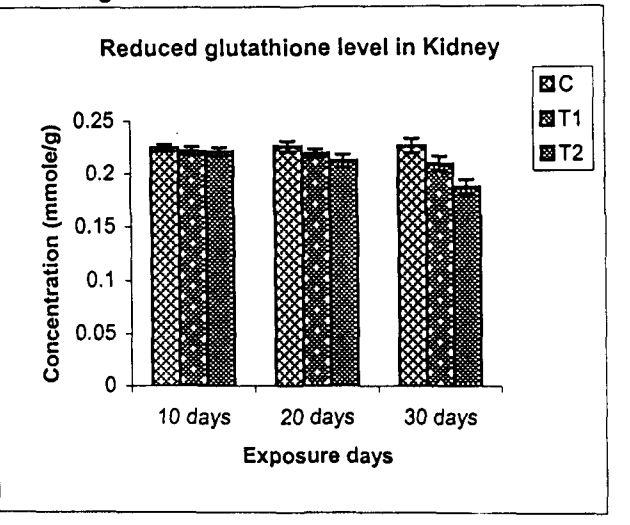


Fig:75

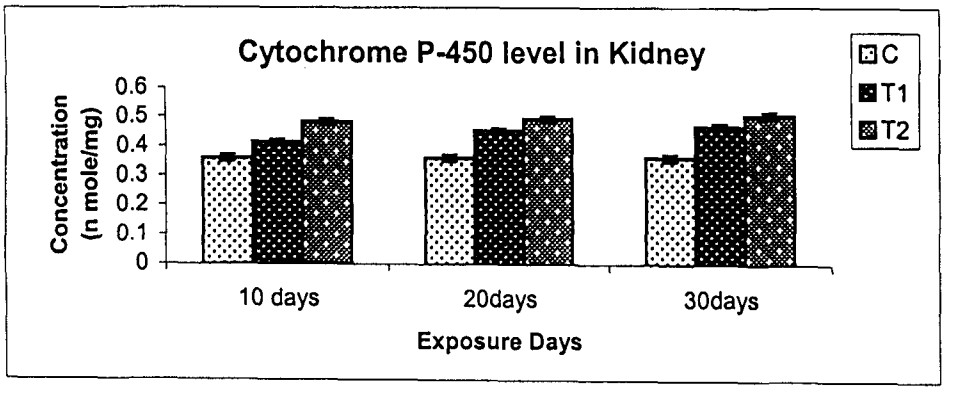


Fig:76

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.