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*

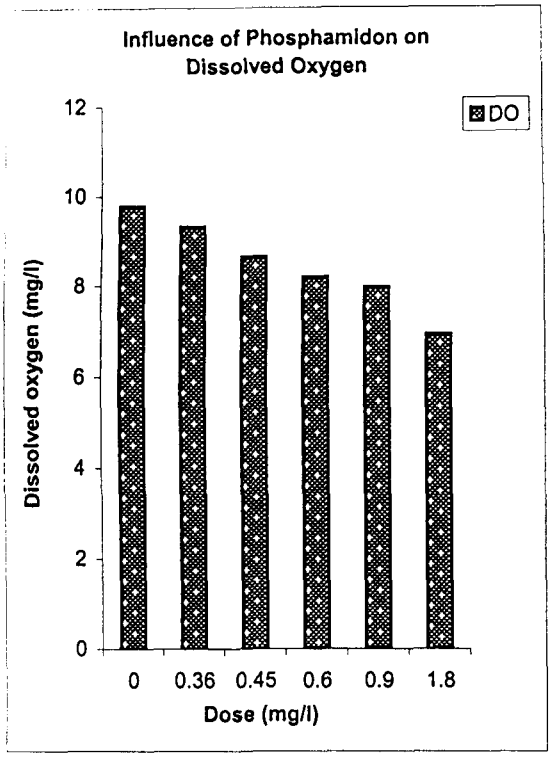


Fig:1

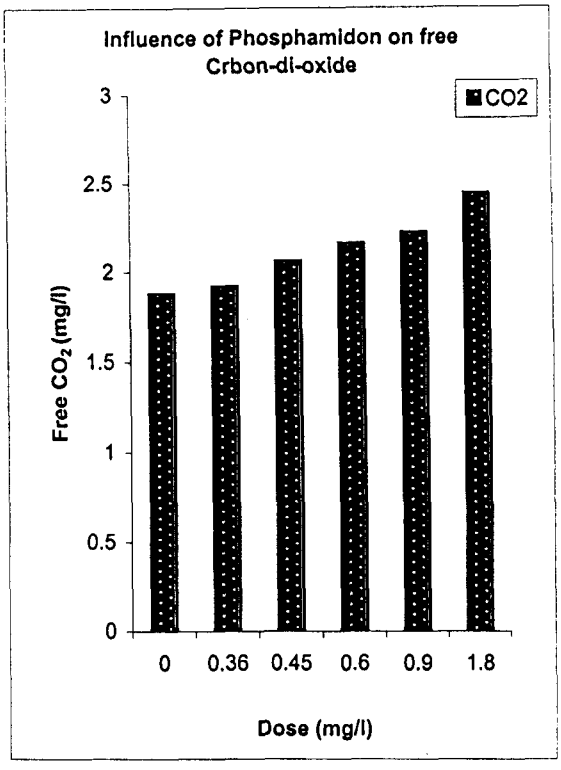
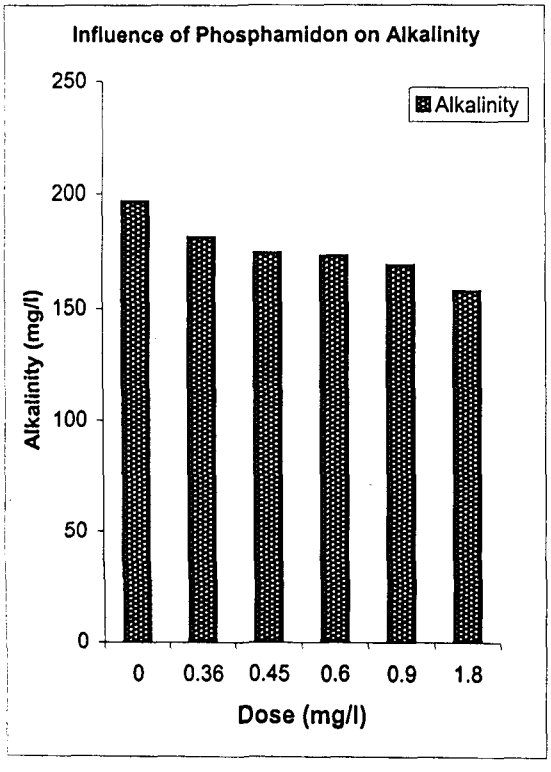


Fig:2



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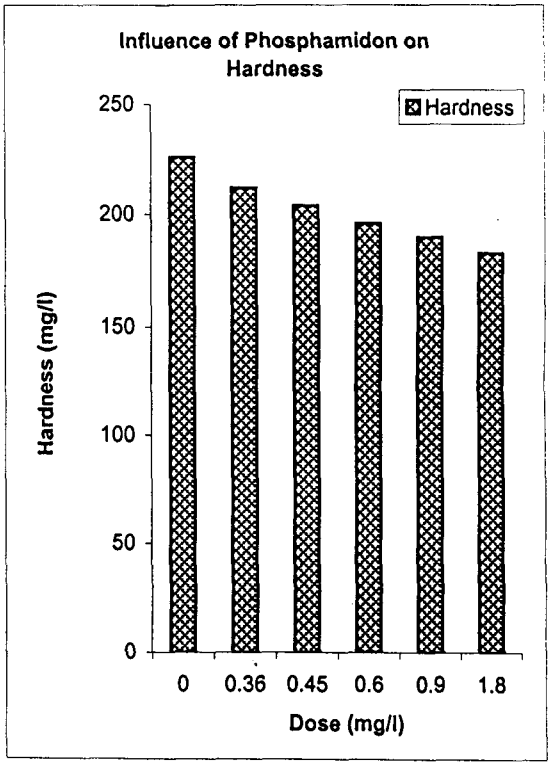

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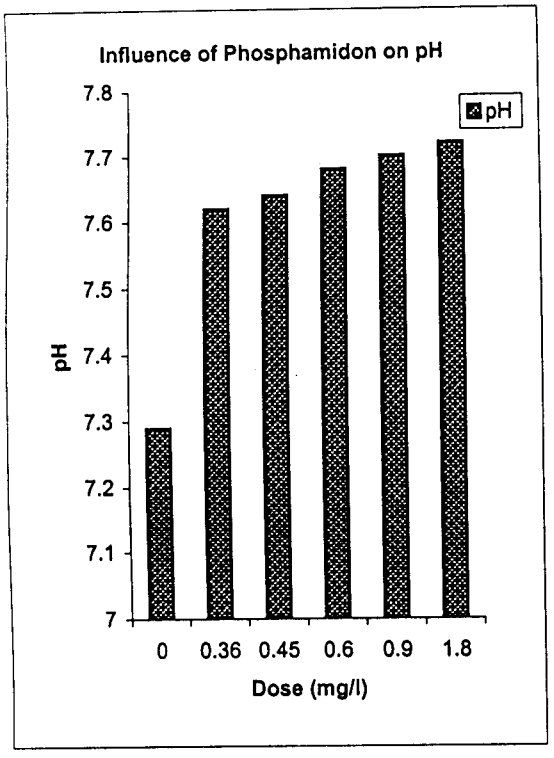

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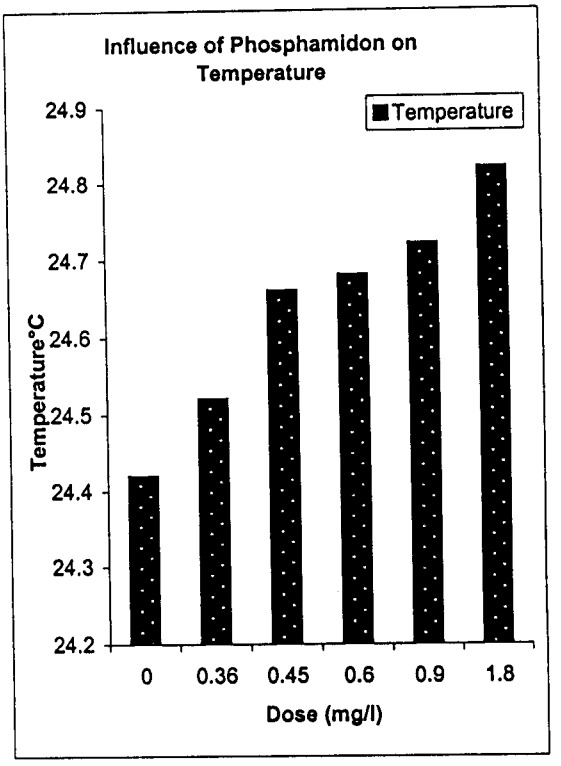

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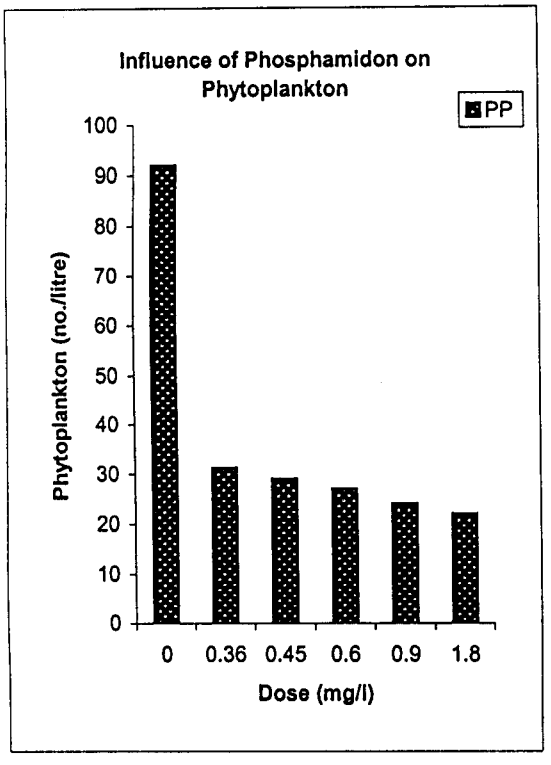

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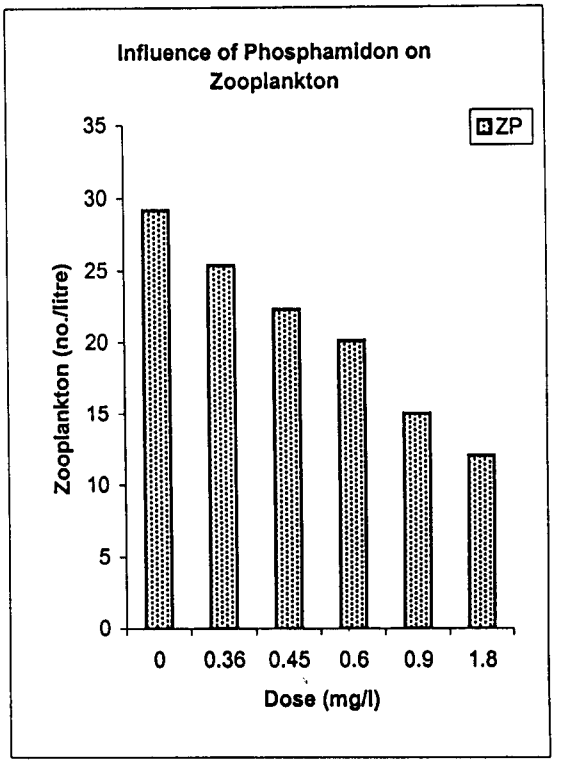


Fig:8

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)

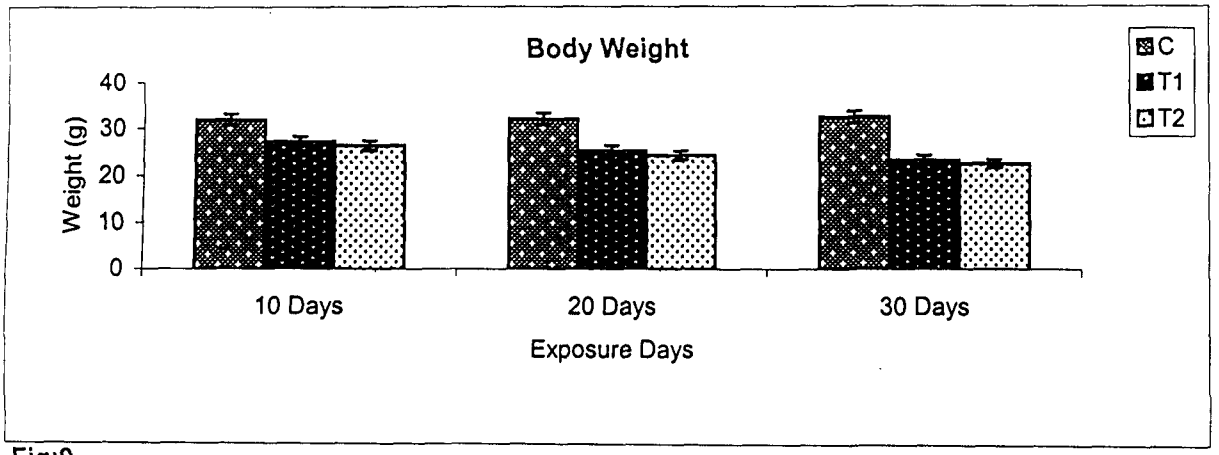


Fig:9

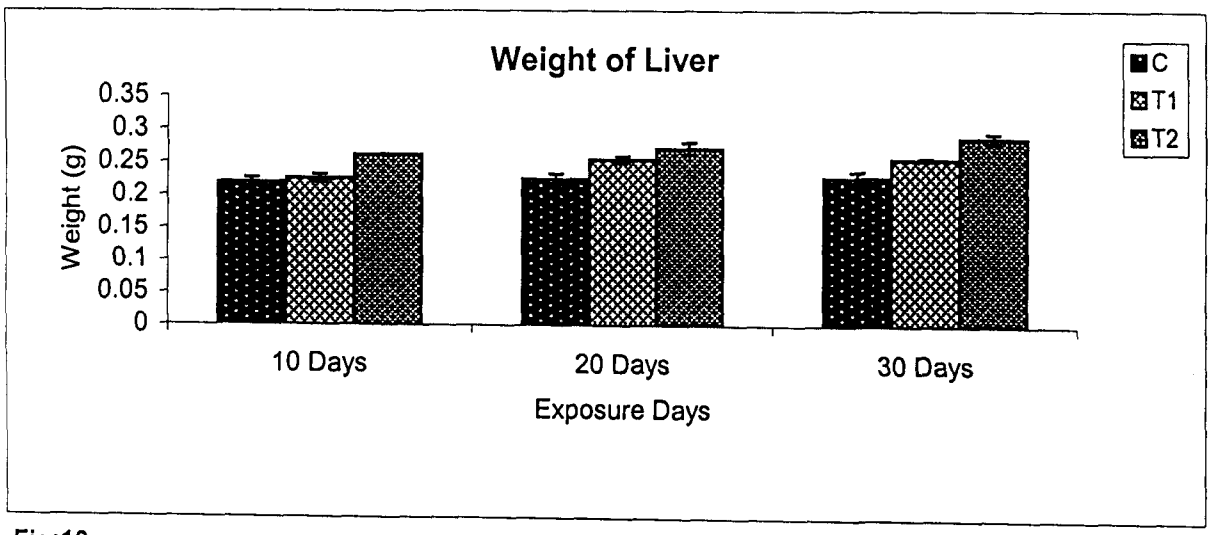


Fig:10

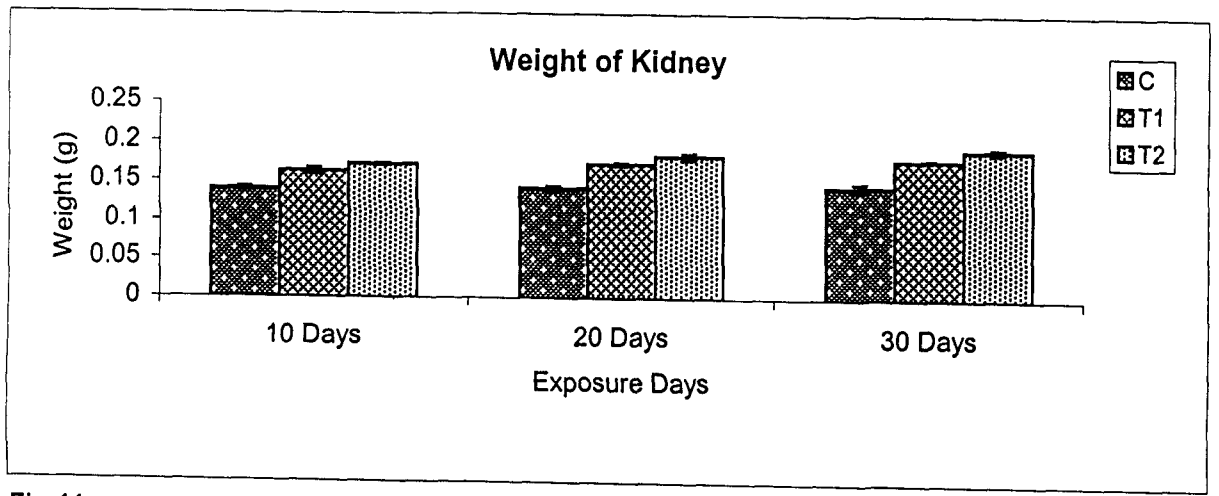


Fig:11

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**

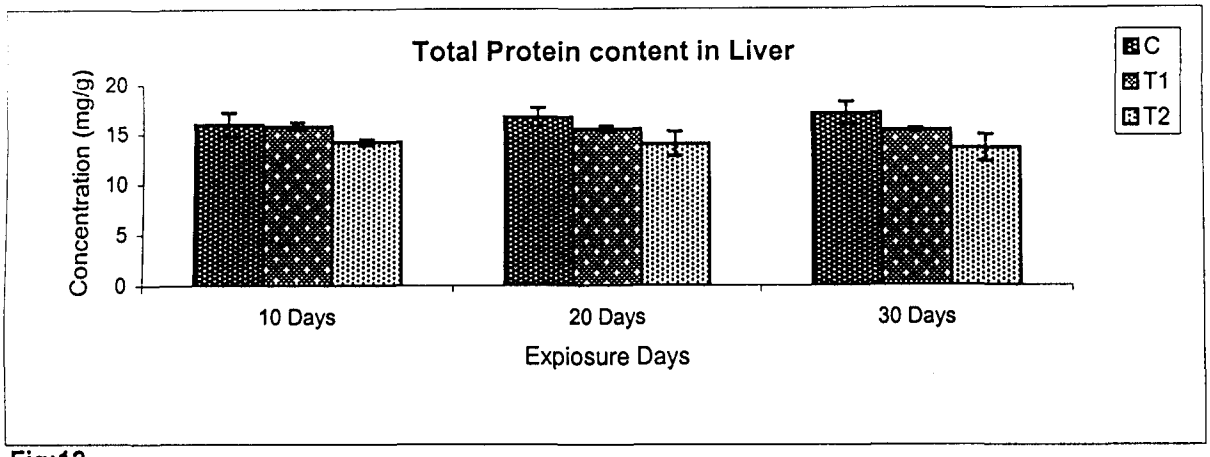
Table 9. Total DNA (mg/g) content in Liver of *Channa punctatus* at different exposures of Phosphamidon

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



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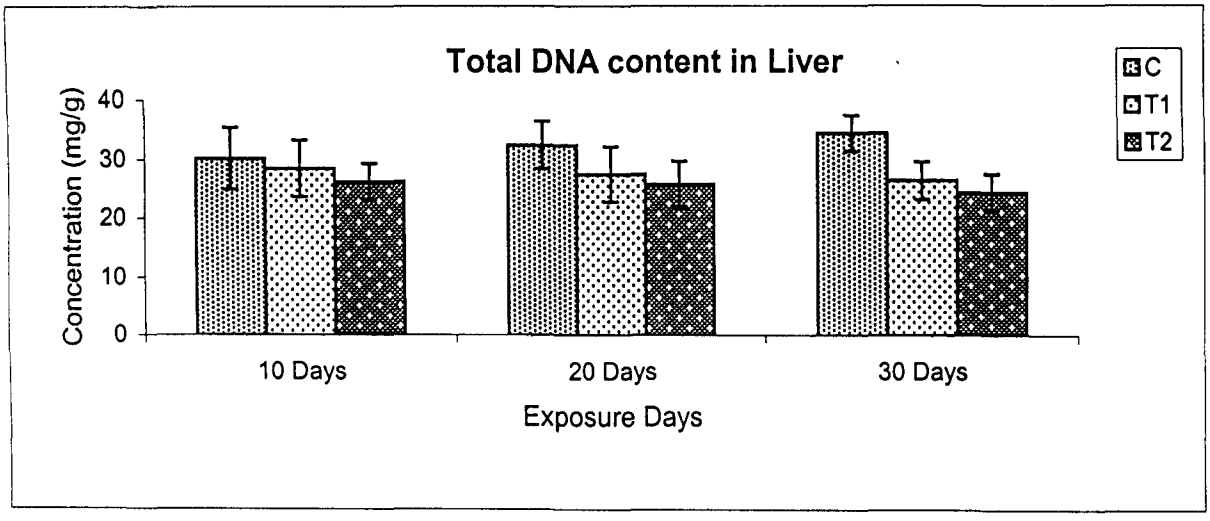

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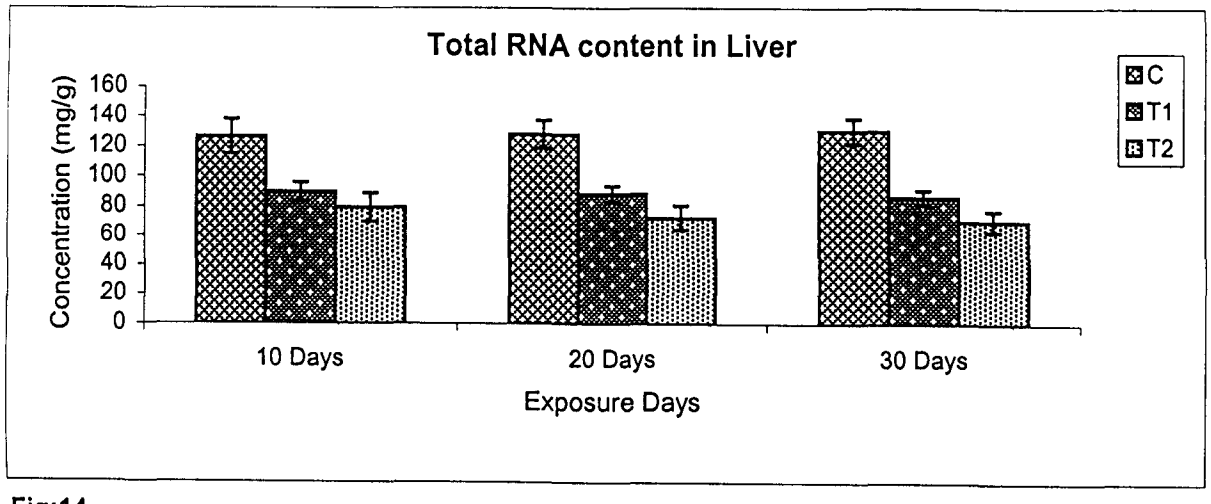


Fig:14

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

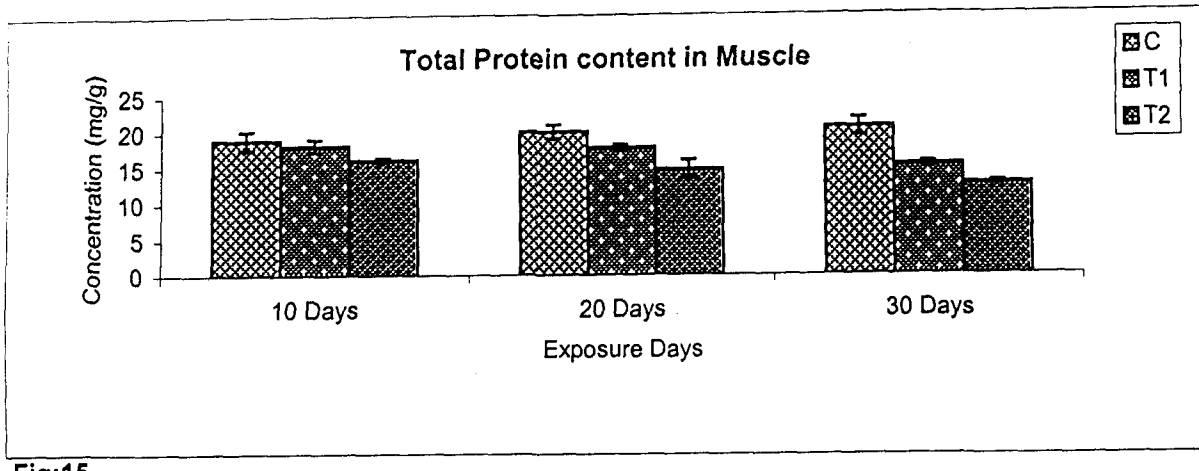


Fig:15

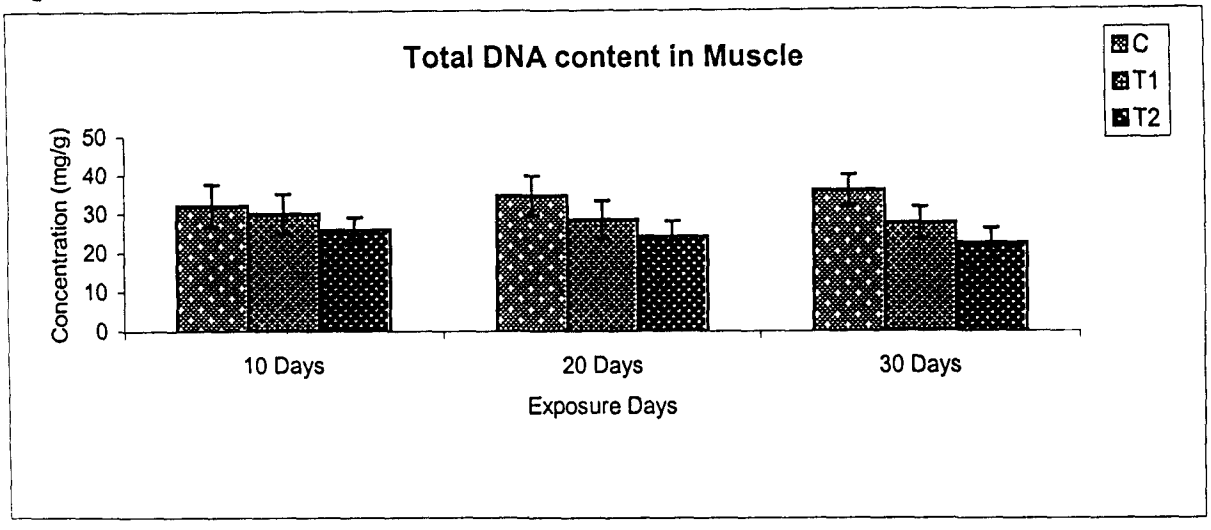


Fig:16

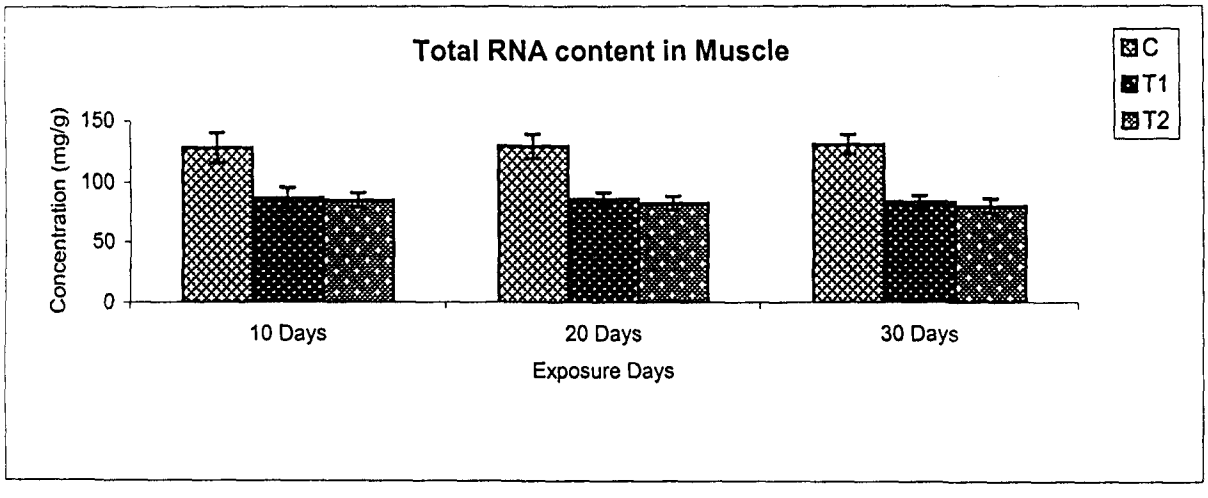


Fig:17

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

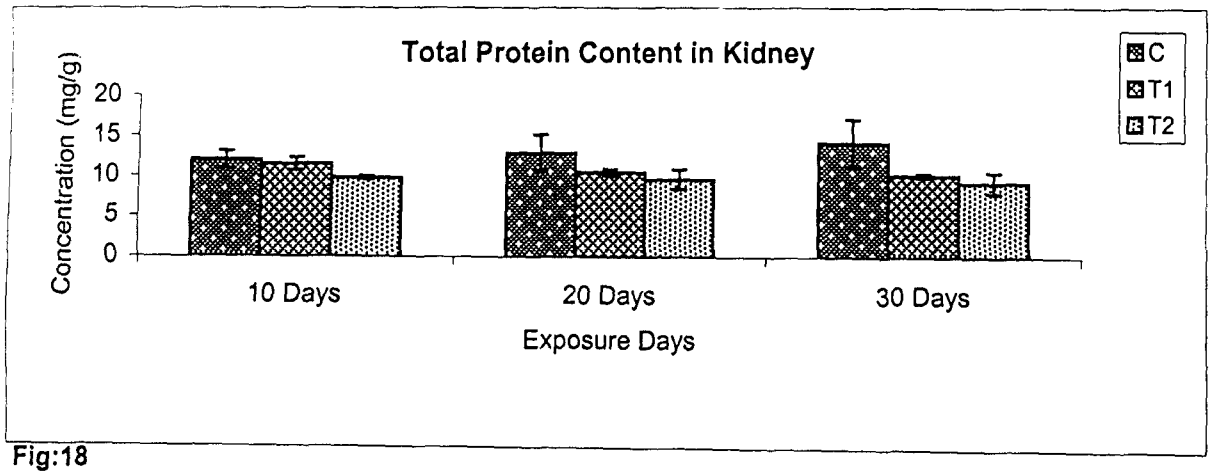


Fig:18

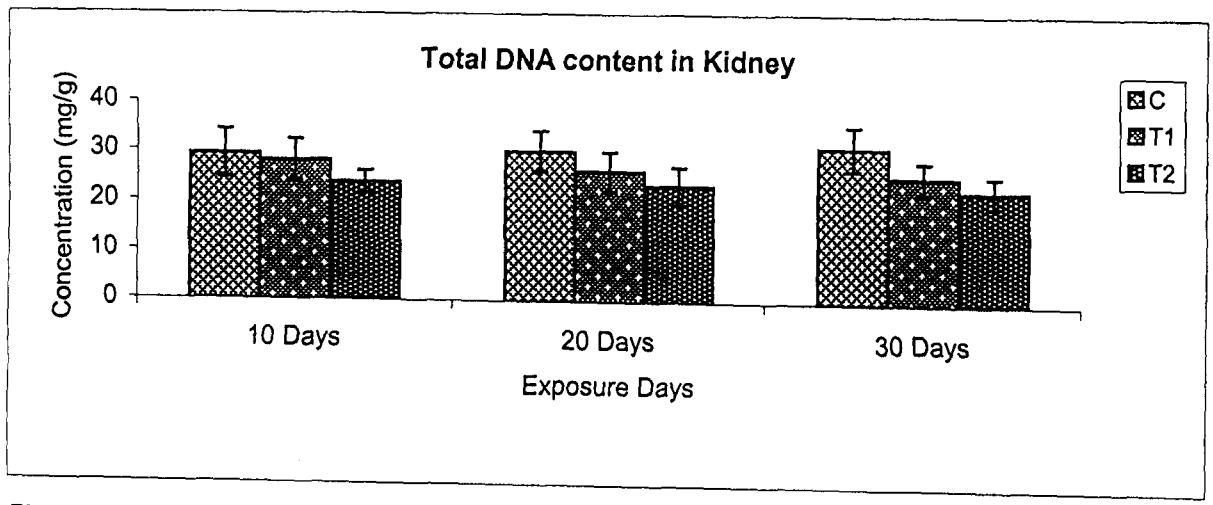


Fig:19

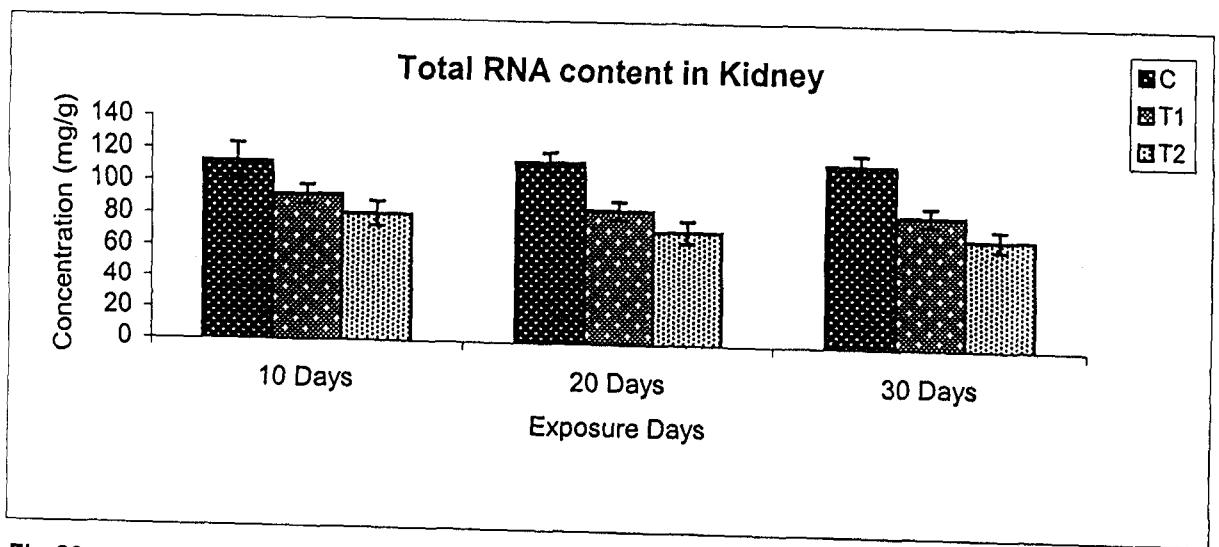
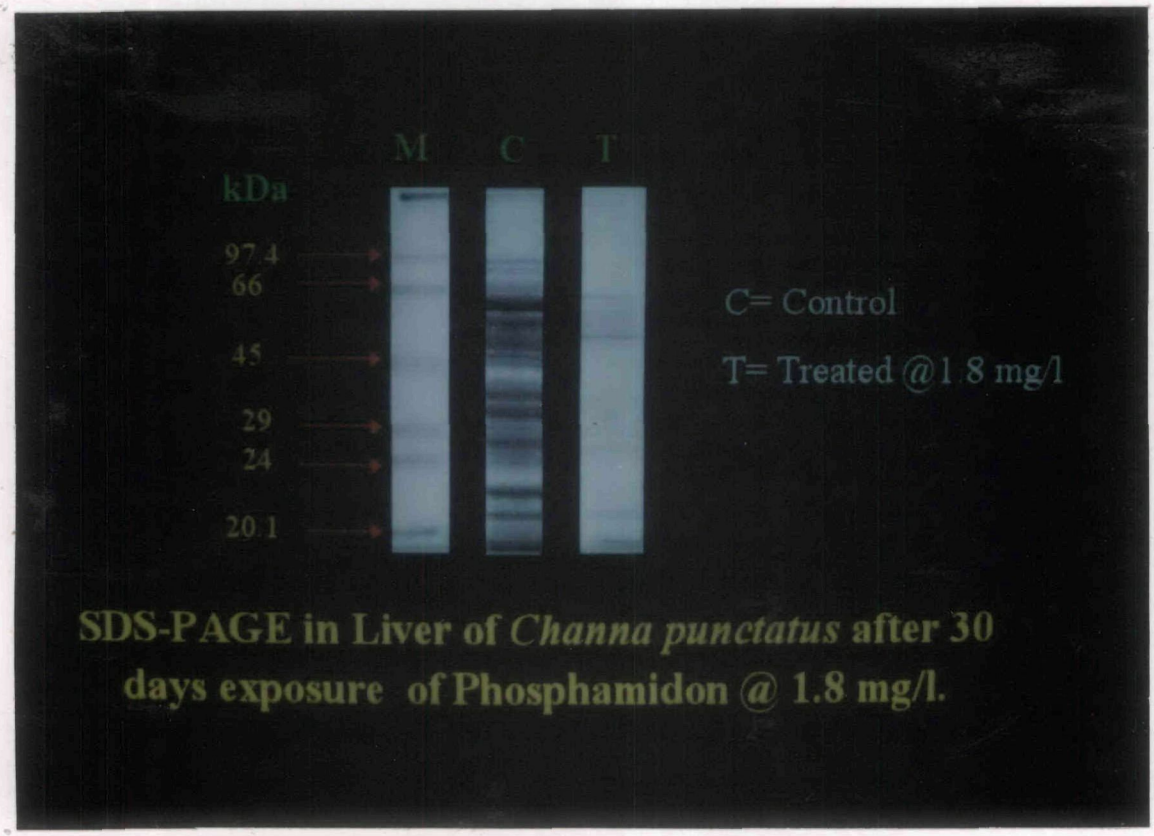
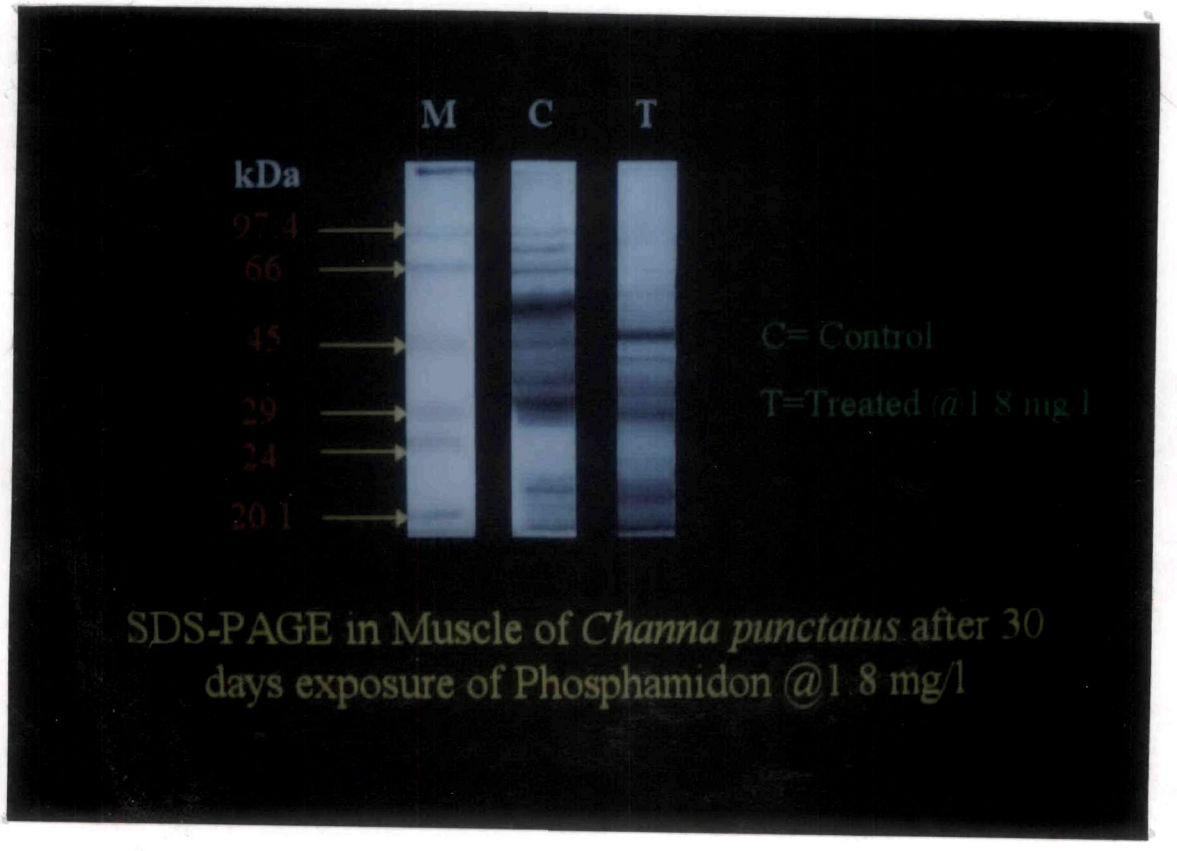


Fig:20

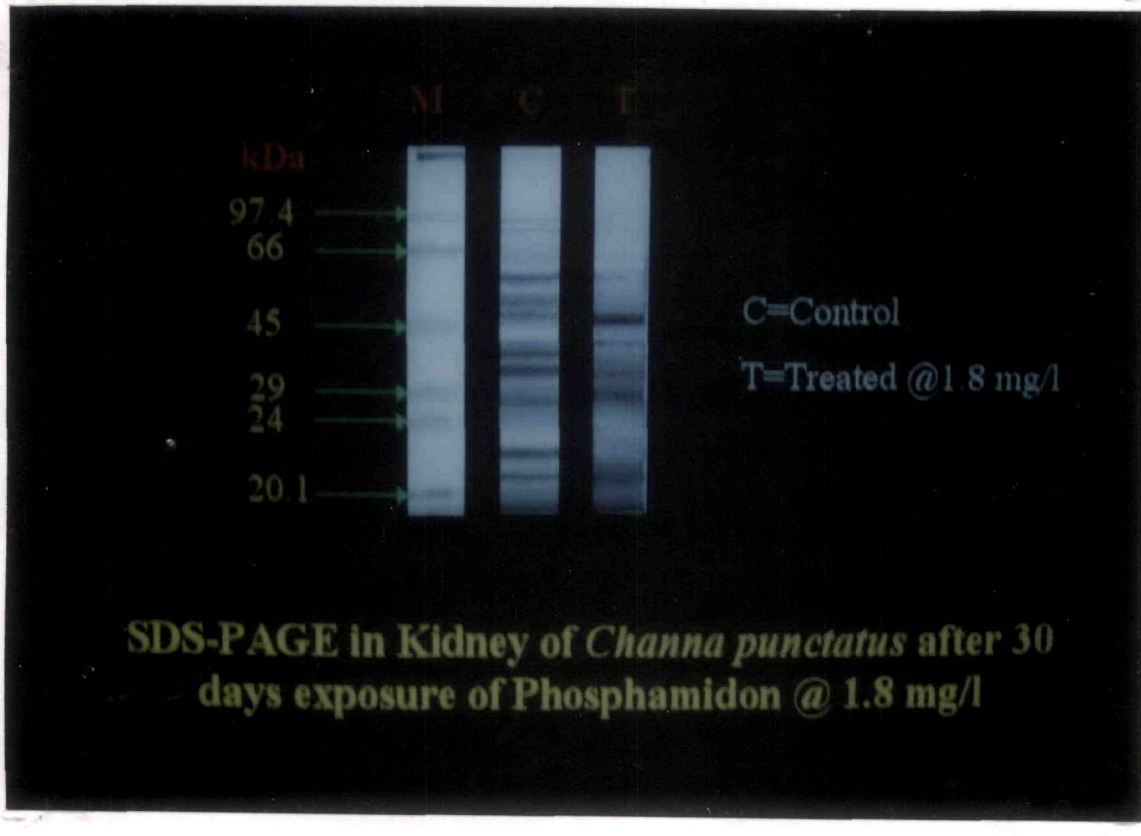
C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)



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

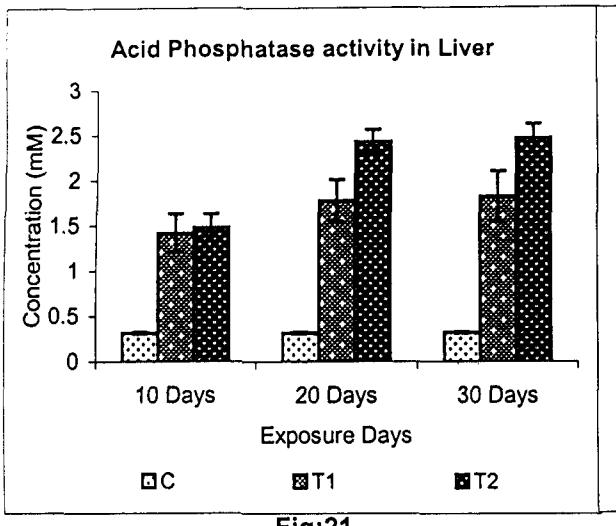
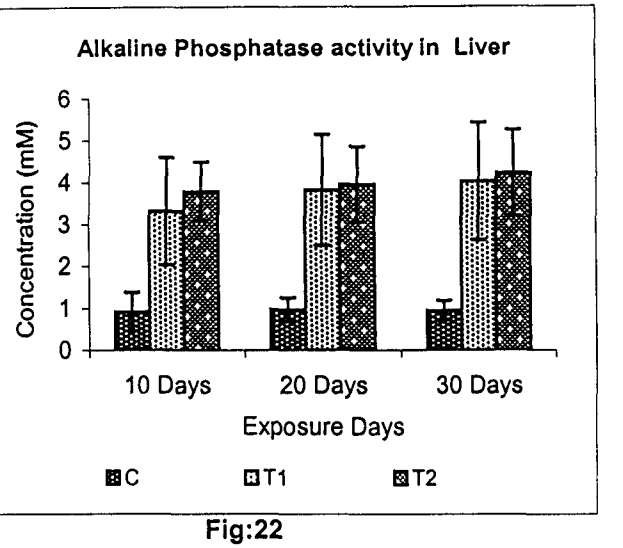


Fig:21



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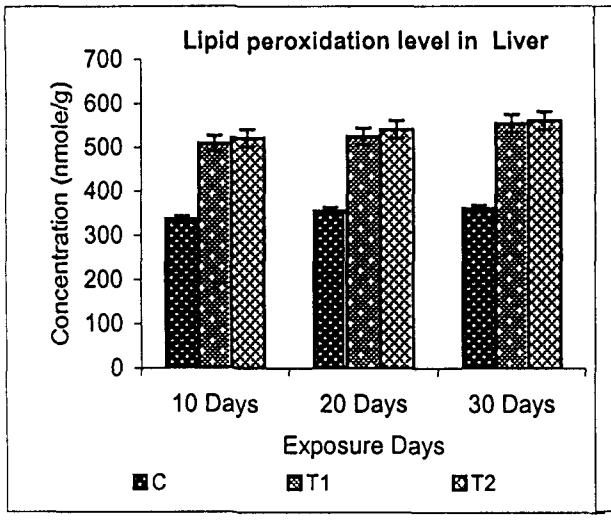
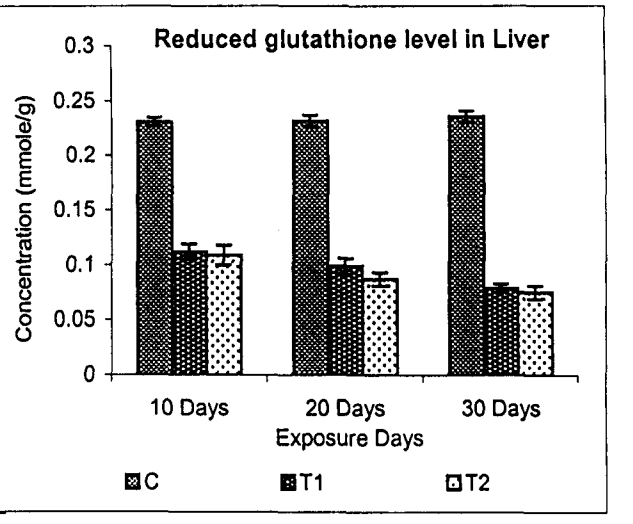


Fig:23



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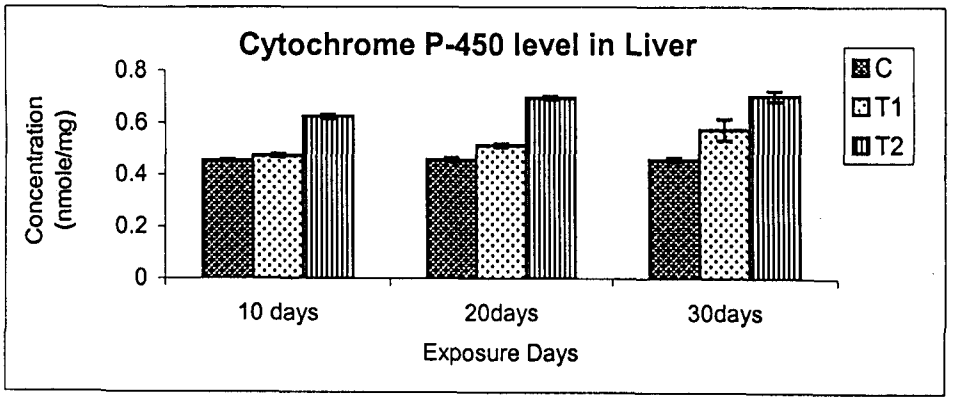


Fig:25 C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)

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

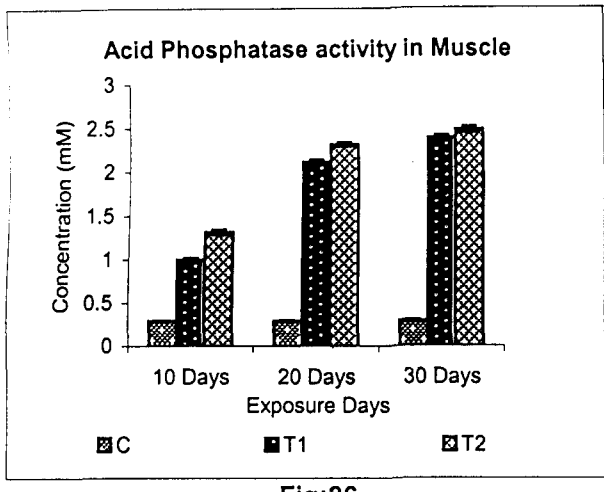


Fig:26

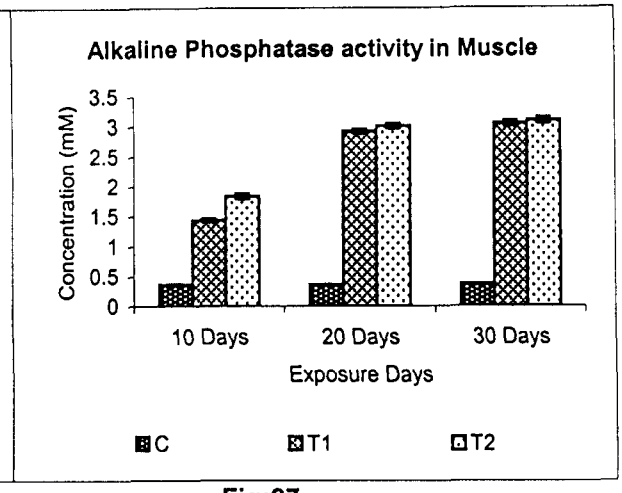
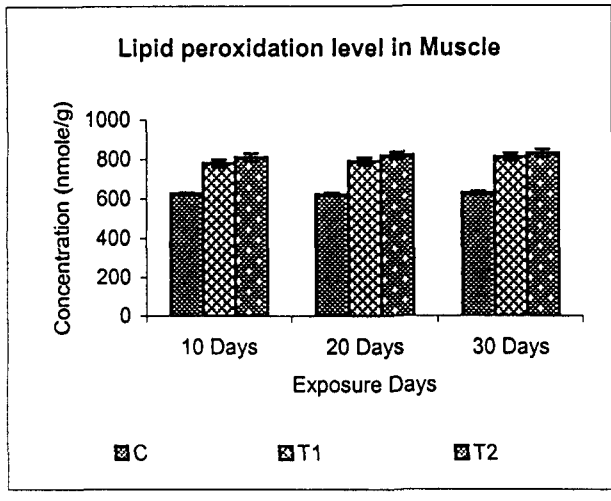


Fig:27



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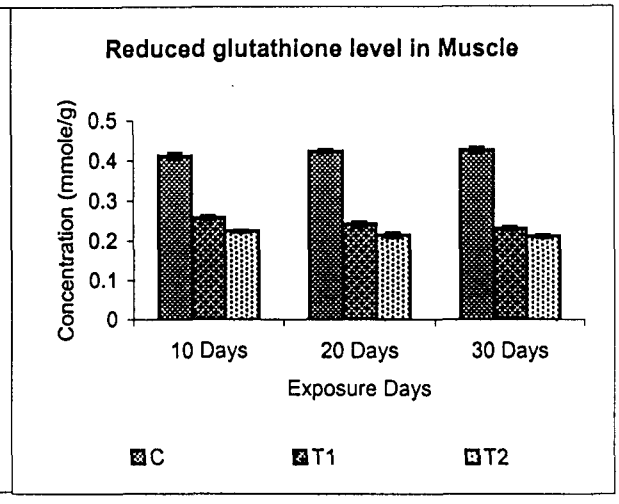

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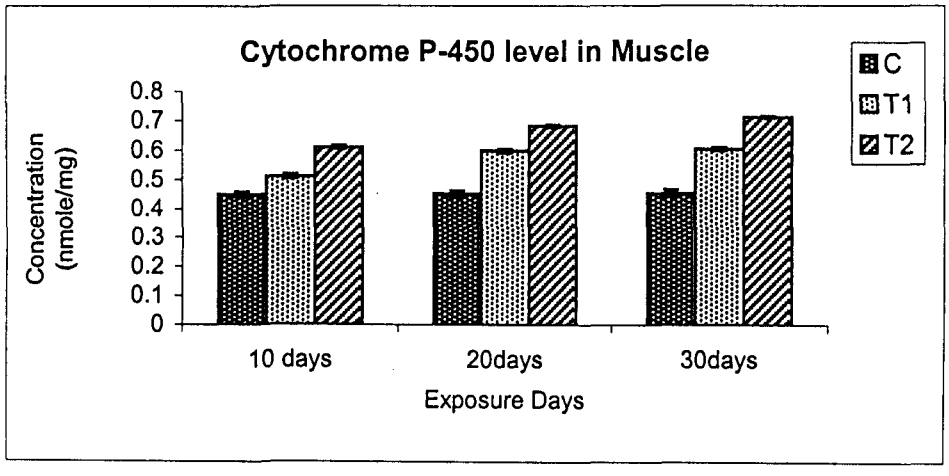


Fig:30 C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)

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

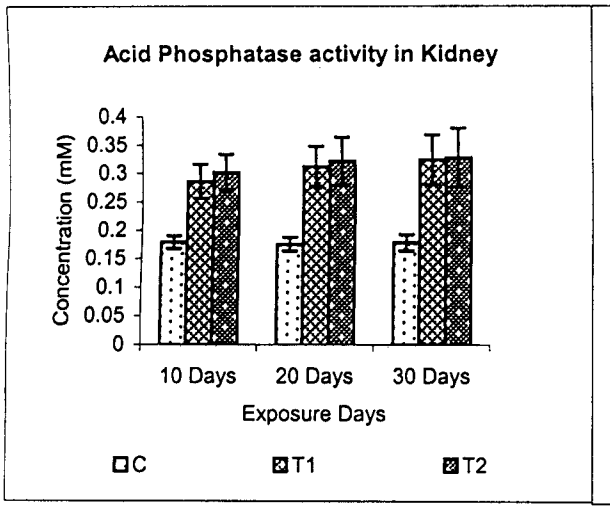


Fig:31

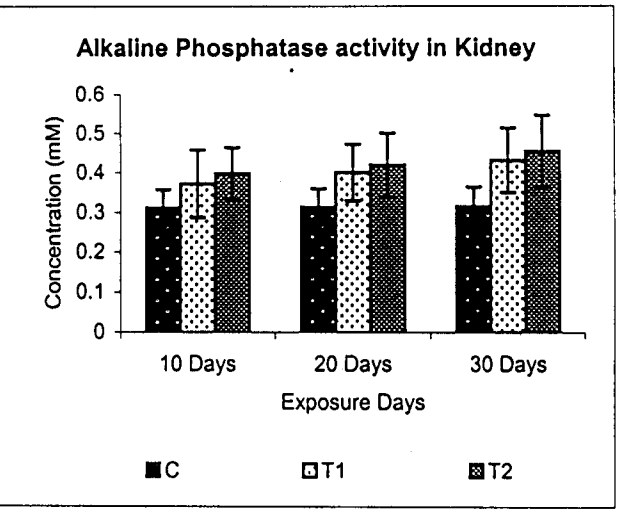
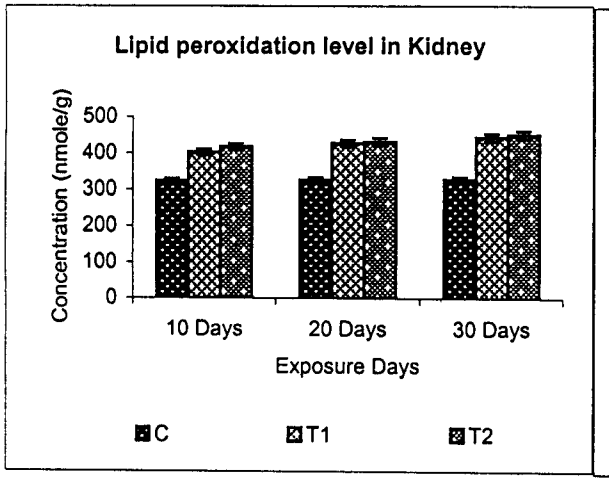


Fig:32



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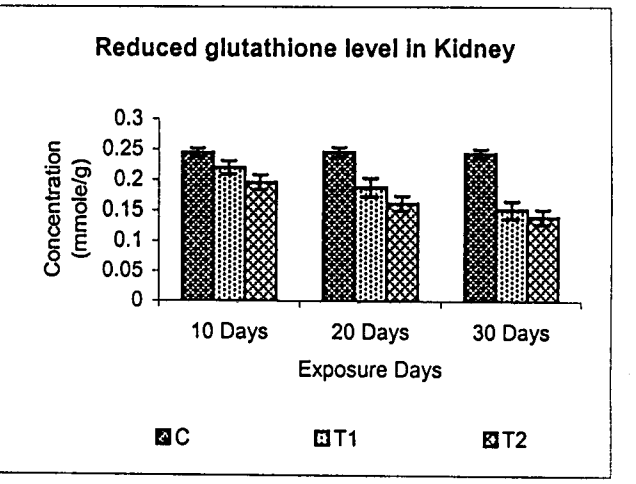

Fig:34

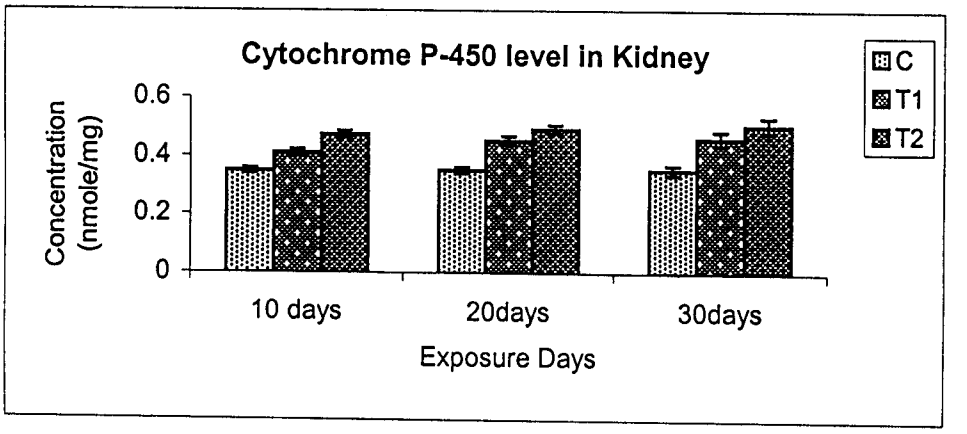

Fig:35 C= Control
T1= Phosphamidon exposed (@ 0.36 mg/l)
T2= Phosphamidon exposed (@ 1.8 mg/l)

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.