EFFECT OF SUBLETHAL CONCENTRATIONS OF PHOSPHAMIDON ON ACID AND ALKALINE PHOSPHATASE ACTIVITY IN THE TISSUES OF A FRESH WATER TELEOST, *Oreochromis mossambicus*.

By

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INTRODUCTION

Our well-being is dependent on the well-being of the environment. In recent years, the environmental quality deteriorates and we face the problem of environmental pollution. Water, the most vital resource for all kind of life on the planet, is adversely affected by human activities on land, air or water. The pollution of surface and ground water is increasing more rapidly than our solutions to the water crisis. The continual growth of cities together with the rapid expansion of industry is overwhelming the ability of most rivers to carry off wastes (John J.Fagan 1974).

The major rivers of India such as the Ganges, Hoogly, Cauvery, Bhavani and others have been reported as polluted by various agents like industrial effluents, sewage water, pesticides and other waste materials (Sath and Bhaskaran 1950, Ray and David 1966, George et.al., 1965, Sreenivasan and Sounder Raj 1967).

Pesticide chemicals are one of the important sources of pollution (Cremlyn 1978). Water quality of major river system is getting rapidly degraded due to massive pesticide residues because of the extensive use of pesticides in pest control programs. (Varshney 1983) with the modernization of agricultural operations and the rapid growth of industrial activity, there has been much increase in the manufacture and utilization of insecticides which ultimately find their way into the rivers, lakes and ponds (Ridgway et al., 1978).

The major source of environmental contamination by pesticides, is the deposits resulting from the application of these chemicals to control agricultural pests. Only one per cent of the pesticide hit the target and the remaining drift into the environment, enter the food-web and thus affect the balance of the ecosystem (Suman Cherian 1991).

The available reports indicate that our environment is not free from pesticides, and infact the level of contamination may be even higher, than in many other parts of the world (Edwards 1973).

Admittedly pesticides are mutagenic, carcinogenic and teratogenic. (Narender Sivaswamy 1991) Pesticides thus pose a major health hazard and threat to life if not handled carefully. Inspite of all these known effects the use of pesticides have been on an increase in the past few decades. Hence it is important to know the impact of pesticides on water pollution and to find ways and means for the safe use and control of pesticides.

Pesticides reach water bodies either by direct application or indirectly and accidentally. Addition of these chemicals to control weeds and algal blooms in ponds and streams, to control insects which breed in water,(Sumancherian 1991) to control mosquitoes in their larval stage, or accidental spraying would result in the direct introduction of pesticides. Indirect sources include runoff from treated areas, spray drifts, waste discharges by pesticide producing industries. Among the above sources, aerial and large scale spraying, and runoff waters from agricultural fields are the important contributions of pesticide pollution in fresh water system (Charles Revelle 1979).

When a pesticide is used for the treatment of crops atleast some part of it will inevitably finds its way into the water and there comes in contact with the fish which lives there. The irrigation runoff is found to contain many organo--phosphorus insecticides, in such concentration as to kill fishes. (Kodam and De 1978)

In reality, the greatest hazards of pesticides are to the aquatic organisms, which seem to be much more susceptible to them and also concentrate them in their tissues more readily than do terrestrial organisms. Toxic pesticides in the water bodies accumulate in the tissues of fishes and after accumulation are converted into 'living bomb' which poses a serious challenge to human life. As the fishes are being connected in the food chain, ultimately human beings will be affected. Therefore the knowledge of toxicity of pesticides to aquatic life is very crucial. Taking this point into consideration the impact of pesticides is analysed in the aquatic ecosystem. In our country, the aquatic fauna serves as a food source and it is highly essential to know the impact of water pollution on these organisms.

The organophosphates and carbamates are much less persistant compared to organochlorine pesticides. (Cremlyn 1978).

Organophosphorus compounds developed as an alternative to chlorinated hydrocarbons are broad spectrum biocides and are much more poisonous than the banned DDT that they have replaced (Janathan Turk 1985).

Organophosphorus compounds are often recommended for their quick chemical and microbial degradation property. Even pesticides that degrade rapidly are not without serious drawbacks. Many organophosphate insecticides are extremely toxic and non-selective. Places where they are applied are left nearly devoid of insects including the natural enemies of the intended targets. Since the chemicals disappear rapidly, fields are extremely vulnerable to new attacks by insects from adjacent areas. Pests always multiply rapidly than their predators. In addition, insects resistant to the pesticides emerge rapidly. The typical response is to apply heavier doses of the same pesticide at shorter intervals. These compounds may need to be applied several times during a season to control pests. Hence there are more chances for these organophophorus compounds to contaminate our environment. (The First Annual Report of the Council of Environmental Quality 1970).

Organophosphorus pesticides are becoming unavoidable in the agricultural field and the practice of indiscriminate usage against pests contaminate the aquatic environment. These pollutants in the aquatic medium enter into fish through food, gill and body surface. The organophosphorus pesticides being lipophilic easily cross the blood brain barrier and alter the enzyme systems (Devaraj 1991).

The organophosphorus pesticides are well known Anti-cholinesterases interfering with synaptic transmission of nerve impulses and are known as nerve poisons. (Anjum 1990), Miny Samuel 1989, Coppage 1972. The combination of organo-phosphorus compound and cholinesterase is a fairly stable one and is not broken down immediately by the body. For this reason, several non-toxic doses in a short period of time can produce symptoms of poisoning (Charles Revelle 1979).

Commercial grade pesticide Dimecron 85% S.L. is used in the present study. As it is essential to measure fish toxicity with commercial grade pesticides rather than with technical grade, because in agriculture only the commercial grade is being widely used through which the major part of the pesticides get into the aquatic environment. (Alabaster 1969) The commercial preparation of phosphamidon (Dimecron 85% S.L.) used for the present study is a water soluble wide spectrum organophosphorus insecticide cum acaricide. It is a systemic insecticide, effective against sucking, chewing and mining insects, and also against mites. It is extensively used in agriculture, mainly in paddy fields also in forest pest management as a substitute for DDT to control defoliating spruce bud worm in fir forests. As it is widely used in paddy fields it may affect synchronous system of paddy cum fish culture by increasing fish mortality. (Chakrabarty and Banerjee 1989).

The fish selected for the present study is Oreochromis mossambicus, which is formerly called as Tilapia mossambicuus, a fresh water euryhaline teleost (Peters) of cichlidae family. This is a commercially important fish. Dougless and Irwin (1962) pointed out that fishes utilised as test animal in toxicity bioassays should be neither too resistant nor too sensitive. On the basis of this criteria Oreochromis mossambicus is a suitable fish for bioassay studies. Potts and Foster (1967) reported Oreochromis mossambicus, as a more suitable and prolific fish for laboratory experiments. Many Indian research workers namely (Mathai et al., 1989, Radhaiah et al., 1987, Joshi and Desai 1981 used Oreochromis mossambicus for bioassay studies.

Acute bioassy tests have been used to determine the actual impact of various pesticides on aquatic life. The concentration at which, the pesticide is lethal to 50% of the test animals in a medium (such as water) is considered as median tolerance limit. These LC 50 values are the indicators of the toxicity, the pesticide may have on human beings.

One of the marked effect of pesticide poisoning (acute) is death. However quite often the amount of pesticide concentration in the environment may not be enough to kill the organisms. But these sublethal concentrations often cause changes in the biochemistry of the organism. Biochemical changes induced by pesticide stress lead to disturbance in metabolism, inhibition or elevation of important enzymes, retardation of growth and premature death. Therefore it is essential to know the chornic effect of pesticide on aquatic environment.

Organophosphorus pesticide induced alterations in the enzyme activities may have diagnostic significance in the evaluation of adverse health effects of toxic substances. The enzyme analysed in the present study was Acid and Alkaline phosphatase, the enzymes of lysosomal origin which helps in autolysis of the cell after its death. Phosphatases are concerned with carbohydrate metabolism (Miller and Crane 1961) oxidative phosphorylation (Goodman and Rothstein 1957) as well as growth and differentiation (Barker and Alexander 1958). Moreover phosphatases are highly sensitive to various stress conditions. Hence inducement or depression of phosphatases activity of fish may successfully be used in pollution monitoring programmes of aquatic environments.

The main organs, affected by toxicants are Liver and Gill. Since Liver is the first organ to face any foreign molecule that is carried through portal circulation, it is subjected to more damage. Fishes have to pass large amount of water for the purpose of respiration and as such the respiratory surface of gills get exposed more and more to toxic compound dissolved in water. Hence the effect of pesticides on these tissues, are worth investigating.

Hence, laboratory tests were conducted to investigate the effect of commercial grade phosphamidon on the activities of acid and alkaline phosphatase, in liver and gill of a fresh water teleost Oreochromis mossambicus challenged with sublethal concentrations.

Review of Literature

REVIEW OF LITERATURE

Industrialised agriculture more popularly known as green revolution agriculture has prompted an extra ordinary use of dangerous chemicals in the form of pesticides. Due to their widespread distribution and toxic nature, pesticides may have a serious impact on the aquatic environment and exert adverse effects on the associated organisms.

Recently a great deal of attention has been paid to evaluate the hazardous effects of Organophosphorus compounds on the physiology of many non-target organisms particularly fishes.

Hazards due to the use of organophosphorus pesticides on aquatic organisms were reviewed by many authors. (Toor and Kaur 1974, Eaton and John 1970, Srivastava et. al., 1977) Johnson (1968) studied about various pesticides toxicity, their effects on fishes and some of the principal toxicological generatives. Knowledge on these aspects are needed to assess the effect of different concentration of different kinds of pesticides on various fishes. Bhatia, (1971) analysed the toxicity of some insecticides on Cirrhinus mrioala (Hamilton). Poisoning with monocrotophos, an organophosphorus pesticide was analysed by Simpson, 1969. Acute toxicity tests on fish lebisturreticularis using the waste waters from pesticides manufacturing industry was studied by Gajghate *et. al.*, 1988. Effect of pesticides on the survival rate of aquatic organisms was found by various authors after conducting toxicity tests (Verma *et. al.*, 1977, Handerson *et. al.*, (1959), George *et. al.*, 1965.

Effect of pesticides mixed in different ratios to the fresh water *Lareo Rohita* was analysed by Tilak and Jonardhana Rao (1991). Comparative evaluation of pesticide induced toxicities were studied and reported by many scientists (Sherakar and Kulkarni 1988, Tejendra S.Gill and Jaishree Pande 1991, Sambasiva Rao *et. al.*, 1988, Jogan *et. al.*, 1989. The behavioural responses of fishes to pesticide was reported by Bakthavathsalam *et. al.*, 1982. Shanmugavel *et. al.*, 1988 reported that sublethal concentrations of phosphomidon and methyl parathion significantly affected the rates of feeding, absorption, metabolism, conversion rate of *Oreochromis mossambicus*.

Influence of temperature on toxicity of several pesticides to *Melanopsis dufouri* was studied by Almus and Ferrando 1988. Influence of pH and temperature on the toxicity, accumulation and degrdation of parathion in aquatic systesms were analysed by Lydy *et. al.*, (1990). Sparks (1982) observed the increase in toxicity due to the increase in temperature. Decrease in toxicity of pesticide to fish due to the high salinity of water was reported by Ferrando and Andreumoliner, (1991). Decrease in the toxicity with an increase in the size of the fish was reported by many scientists. (Olson and Marketing 1973, Deshmukh and Marathe 1980, Patwardhan and Gaikwad 1991. Reports are available regarding the toxic effect of pesticides on Carbohydrate, lipid and protein content in some fresh water fishes. Depletion of tissue protein in fishes exposed to organophosphorus pesticides were reported by many authors (Joshi Urmila et.al.,1988, Bakthavathasalm 1987, Bashamohideen et.al.,1988.) Effect of pesticides on some lipid parameters were analysed by many research workers. (Srinivasulu Reddy and Ramana Rao 1988, Ghosh and Chatterjee (1989). Pesticide induced Dysfunction in Carbohydrate metabolism in fishes were worked out by Verma et al 1983, Ghosh 1987, Bakthavathasalam and Srinivasa Reddy 1985. Changes in nucleic acid metabolism of fishes exposed to organophosphorus pesticides was studied by (Rath and Misra 1980, Tayyaba et.al.,1981.

Attemps have been made to study the effect of pesticides on haematology. Effect of organophosphorus pesticides on haematological parameters of fish was analysed by many scientists (Dalela et.al.,1981, Chakrabarty Papia Banerjee 1988, Verma et al 1982, Khalid and Muhammed Yaqub 1977).

Respiratory distress is one of the symptoms of organophorus toxicity. Changes in respiratory potential of fish due to pesticides was analysed by many authors. (Pandey et.al.,1976, Sambasiva Rao et.al.,1987, Chanchal and Kumar

1990, Gopalakrishna Reddy and Gomathy 1977.

The effect of pesticides on various tissues of fish has attracted the attention of many authors. Biochemical changes in the liver and kidney of a cat fish was analysed by Dubale and Aswath 1982. Hepatic pathology in fish due to insecticides was studied by many scientists (Anees 1978), Dubala and Sha 1979, Mathur 1962, 65, 76, King 1962). S.Rao et.al.,(1980) reported the drastic effect of Malathion on the gills of Tilapia mossambica. Morphological and biochemical alterations on liver, kidney and gills of fishes by pesticides was analysed by Rojik et.al.,1983. Further, it was reported that acute and chronic treatment of pesticide cause biochemical alterations in organs involved in detoxification mechanisms (Dikshit et.al.,1975 and Sastry and Sharma 1979).

Enzyme studies have been the subject of recent interest influence of pesticides and various pollutants on enzymes have been widely studied especially in aquatic organisms.

Recently, Ramana Rao et.al.,(1991) studied about the inhibition and recovery of selected target enzyme activities in tissues of penacid prawn Metapenaeus monoceros (Fabricus) exposed to different insecticides. Effect of lethal concentrations of metasystox on selected oxidative enzymes, tissue respiration and histology of gills of the fresh water air breathing fish Channa striatus was analysed by Natarajan 1981. Invitroinhibition of fish brain ATP ase activity by cyclodiene insecticides and related compounds was observed by Yap et al 1975. An increase in the levels of NAD dependent glutamate dehyrogenase, glutamine systhetase, arginase, AMP deaminase and adenosine deaminase activities of Penaeus indicus due to phosphomidon and methyl parathion were reported by Srinivasulu Reddy and Ramana Rao 1988. Tissue specific alterations of aminotrasnferases and total ATP ase in the fish Tilapia mossambicus under methyl parathion impact was analysed by Sivaprasada Rao and Ramana Rao 1984 Tandon and Ajaidubey 1983 reported elevated activity of fructose-1-6diphosphate aldolase in liver, brain, and gill of the fresh water fish Clarias batrachus on exposure to malathion and dimecron. Effects of DDT and malathion on tissue SDH and LDH isoenzymes of Sarotherodon mossambicus was studied by Ramalingam 1985. Invitro inhibition of brain Ca^{2+} ATP ase by monocrotophos, diemethoate, diazinon and DDT in Tilapia messambica was reported by Anjum and Siddiqui 1990.

The organophosphorus pesticides are well known anticholinesterases interfering with synaptic transmission of nerve impulse. Inhibition of acetyl cholinesterase due to organophosphorus pesticides was reported by many scientists (Ansari and Kumar 1984, Rath and Misra 1981, Sahib and Rao

1980). Blockage of acetyl choline synthesis in organophosphorus poisoning was studied by Hopff 1984. Acetyl Cholinesterase activity in the tissues of the common crop *Cyprinus carpio* subjected to sublethal concentration of malathion was analysed by Bashamahideen and Saibala 1989. Effect of methidathion on distribution of molecular forms of acetylcholinesterase in carp as revealed by density gradient centri fugation was observed by Nemcsok *et. al*.,1990. Inhibition of acetylcholinesterase due to dischlorvos residues was reported by Horsberg *et. al*.,1989. Relationship between AchE and Mao in brain regions of *Oreochromis mossambicus* exposed to phosalone was studied recently by Devaraj *et. al*., 1991. Inhibition of brain acetyl cholinesterase in the sheep head minnow *Cyprinodon variagatus* due to Diazinon toxicity was reported by Goodman *et. al*.,1979.

Acid and Alkaline phosphatase alterations in the fish *Oreochromis nilotica* on exposure to methyl parathion was analysed by Sarbadhikary and Sur 1991. Inhibition of Acetylcholinesterase and phosphatase in the nervous tissue of Zebra fish *Brachydanio rerio* was observed by Ansari *et. al*.,1987. Effect of viscose rayan factory effluent on phosphatase activity of water crab *Paratelphusa hydrodomus* was studied by Santhi and Aruchami 1983. Increased levels of acid and alkaline phosphatase activities in liver and gill of infected *Channa punctatus* following starvation was reported by Rai *et. al*.,1984.

An increase in alakaline phosphatase activity in liver and muscle of fish exposed to thiodon, roger, at lethal concentrations and a decrease at sublethal concentration of both the pesticides was reported by Verma *et.al*.,1982. Toxic impact of Aldrin on acid and alkaline phosphatase activity of penaeid prawn *Metapenaeus monoceros* was studied recently by Srinivasulu Reddy *et.al*.,1991. Reddy *et.al*.,(1984) observed the invivo subacute physiological stress induced by sumithion on the hepatopancreatic ACP activity in the fresh water crab. Phosphatase activity in the Hepatopancreas and the larval Digenean parasites of *Lymnaea luteola* was studied by Venkata Ramakrishna 1981. Histochemical studies on the effect of roger and thiodon on the activity of ACP in liver, muscle, kidney of *Channa gachua* was studied by Dalela *et.al*.,1978.

Elevation in ACP activity due to phosphamidon was observed by Reddy and Rao 1988. Elevation of Hepatic acid phosphatase on exposure to phosphamidon was reported by Thomas and Murthy (1976). Inhibition of Alkaline phosphatase in liver and intestine and elevation in the activity of acid phosphatase in liver of a fresh water fish *Channa punctatus* on exposure to Dimecron was observed by Sastry and Malik 1979.

Increase in acid and alkaline phosphatase activities in liver and kidney of a fresh water fish *Tilapia mossambica* due to chronic effect of monocrotophos was observed by Joshi and Desai 1981. Recently Tajendra. S. Gill *et al*.,(1990) studied about the acutely sublethal concentration of phosphamidon in the resy barb, *Puntius conchonius* and observed decreased hepatic AcP and AlP activities but stimulated these enzymes in the kidney, liver, gill, ovaries and gut. Thus it was reported that results of these two studies dealing with OP-induced modulation of ACP and ALP are only partial agreement.

After reviewing the literature in the field of pesticide toxicity to non-target aquatic organisms mainly fishes, the present study was so designed as to elucidate the effect of acutely sublethal and chronically sublethal concentrations of phosphamidonon on acid and alkaline phosphatase activities in liver and gill of a fresh water teleost, *Oreochromis mossambicus* was attempted.

Materials and Methods

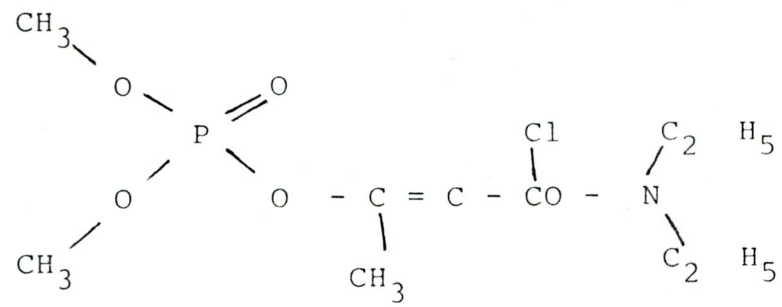
MATERIALS AND METHODS

The fish selected for the present study was a common fresh water teleost, Oreochromis mossambicus. The fish being selected because of its easy availability, resistance to disease, adaptability to laboratory conditions, capacity to thrive in low oxygen budget, ability to tolerate very high salinities, and above all its varying degrees of sensitivity to toxic substances. Further, it is a most successful and prolific fish in a wide variety of external media (Potts et.al. 1967).

The fishes of uniform length of 10-12 cm and weight of 8-15 gms of both ses were collected from the local ponds in and around Coimbatore, Tamil Nadu during the months from February to April. After their capture and transport in plastic buckets to the laboratory, the fishes were acclimatized to laboratory conditions in a cement tank of 250 litres capacity for a period of ten days and were fed daily with groundnut oil cake powder and rice bran, mixed in the ratio of 1:3. The medium was not aerated and the water was changed daily. Fishes were carefully handled with a net to minimise disturbance and handling effect.

The borewell water from the laboratory tap was used for holding experimental fishes. The same water was used for all bioassay tests to avoid variations of water characteristics. The pH (7.5) was determined by pH meter and the temperature was also noted (30 ± 1°C). The dissolved oxygen content (2.814 ppm) was estimated by Winkler's method.

The pesticide used in the study was phosphamidon. Its trade name is Dimecron, its chemical formula is (2-Chloro -2-diethyl carbonyl-1-methyl-vinyl-dimethyl phosphate) its struuctural formula is



and it is manufactured by Hindustan Ciba Geigy Ltd., Commercial product was used in the present study which is bright violet in colour. Phosphamidon is a wide spectrum organophosphorus insecticide cum acaricide and is extensively used in agriculture.

Bioassay:

The toxicity tests were conducted in accordance with the method recommended by sprague 1969. Healthy fishes of the same size were selected for experimental studies and the fishes were kept in starvation for 24 hours prior to their use in the experiments to clear off the alimentary canal from any food materials and were not fed during bioassay studies to avoid any change in the toxicity of pesticide by excretory products.

Dimecron was dissolved in tap water and the desired concentrations were prepared. After the period of acclimatization, fishes were transferred invidivually to glass containers of one litre capacity. LC_{50} is that concentration of a substance in a medium, (such as water) which is lethal to 50% of the test animals.

Preliminary tests were carried out to find the median tolerance limit (LC_{50}) of fish. The concentration at which 50% survival/mortality occured was taken as the mediam lethal concentration. Six fishes were exposed to each concentration of pesticide. Each experiment was accompanied by a control having the same number of fishes without the pesticide. Three replicates were there for each concentration. Dead fishes were removed immediately from experimental containers. After each test the containers were washed with commercial detergent and kept in hot sun.

LC_{50} for 24 and 72 hours of exposure to pesticide were found out for <u>Oreochromis mossambicus</u> at room temperature. The LC_{50} values were calculated.

Enzyme - Assay:

LC_{50} values for 24 and 72 hours were found out. From this suitable sublethal concentrations were determined. The chronically sublethal concentration was taken, which is 1/10th of 24 hours LC_{50} concentration, in which there is no observed death of the fish for a longer time (above 15 days). The fishes were sacrificed after 7,14 days of exposure.

In the case of acutely sublethal concentration fishes were exposed to 6 ppm (72 houurs median tolerance limit) and after 24 and 48 hours, six surviving fishes were sacrificed and tissues were dissected out.

The pesticide medium was also changed daily by mixing the required amount of phosphamidon in water. This was done in order to maintain the medium concentration during the exposure period.

Liver and gill from six surviving fishes were dissected out, pooled and quickly weighed. Tissues were homogenised immediately in cold 0.25 M sucrose solution. The homogenates were centrifuged in a high speed centrifuge at 1200 rpm for 10 minutes, and clear supernatent was used for the estimation of enzyme activities.

Activity of the enzymes Acid and alkaline phosphatase were estimated colorimetrically following the method of King and Armstrong as described by Varley (1975) using the substrate disodium phenyl phosphate.

Results

RESULTS

The spectacular spurt in pesticide use has resulted in their widespread distribution in the environment and has been shown the exert deleterious effects on the biological system, particularly posing much danger to fishes and other aquatic life. Hence the study of bioassay with fish as a responsive animal has gained increasing importance in determining the effects of any pollutant on aquatic life. The LC_{50} values were provided intested organisms on experimental conditions, but it is worth-while to mention that LC_{50} values cannot be considerd safe for long exposure, because continued action of less toxic or non toxic chemicals may eliminate fish species indirectly.

25 ppm concentration of Dimecron 85% S.L. causes Lethal toxicity in Oreochromis mossambicus within 24 hours of exposure. 9ppm concentration of Dimecron 85% S.L. causes lethal toxicity in Oreochromis mossambicus within 72 hours of exposure. LC_{50} values for 24 and 72 hours were shown in Fig-1, 2 and Table 1, 2. LC_{50} value for 24 hours was found to be 15 ppm and LC_{50} for 72 hours was found to be 6 ppm.

Behavioural Responses:

Behavioural responses may be used as indicators of pollution.

The fishes exhibited varied behavioural response according to the test concentration. At higher concentrations, the pesticide exposed fish showed erratic swimming movements and appeared to be in distresesOpercuular movements were quicker. Restlessness was noticed often as the fishes come to the surface of the water to gulp atmospheric air directly and even tried to jump out of the containers. It was observed that the entire body of the fish turned dark.

The loss of equilibrium and sense of balance were exhibited by their irregular vertical revolving movements when the fish completely losts its sense of balance it rolled itself and the entire activity became very slow and started floating on the surface, then the fish sank to the bottom of the container and died.

No mortality and changes in the behaviour were observed in control. The normal behaviour noticed in these fishes clearly indicate the impact of Dimecron.

Enzyme Modulation:

Results obtained in the present study revealed an appreciable effect of phosphamidon on the enzymes Acid phosphatase and Alkaline phosphatase.

Acid Phosphatase Activity:

On exposure to chronically sublethal concentration of phosphamidon, there was a slight increase in Acid phosphatase activity in the liver on the 7th day of exposure.

At the 14th day, there was an appreciable increae in the level of Acid Phosphatase. In gill, a slightly different pattern of activity was obtained. There was a significant increase on the 7th day of exposure but at 14 days of treatment, the level of Acid phosphatase returned to normal.

In acutely sublethal concentration, the liver Acid phosphatase showed the same response that were obtained with chronically sublethal concentration. There was a slight increase after 24 hours and an pronounced increase after 48 hours of exposure. In gill, Acid Phosphatase activity was sharply raised after 24 hours and 48 hours of exposure. The Acid Phosphatase modulations were shown in Table 3,4 and Fig 3.4.

Alkaline Phosphatase Activity:

In chronically sublethal concentration of phosphamidon Alkaline Phosphatase activity was significantly inhibited in liver on the 7th day of exposure which returned to normal at 14 days phosphamidon treatment. In gill there was a significant increase in Alkaline Phosphatase activity after 7 days of exposure which came down to normal by the 14th day.

In acutely sublethal concentration, Liver Alkaline Phosphatase activity decreased during 24 hours and 48 hours exposure. Gills of subacutely intoxicated fish showed an enhanced Alkaline Phosphatase activity after 24 and 48 hours of exposure to phosphamidon.

The control values of individual enzymes remained more or less stable throughout the experimental period, and therefore the mean control value for each parameter was used for the purpose of comparison with values obtained from pesti cide exposed fish. The control values were obtained as 100% for individual enzymes.

Alkaline Phosphatase modulations were shown in Table 3,4 Fig.3,4.

Disscussion

DISCUSSION

In modern day agriculture and farm management pesticides farm an important component. Organophosphorus compounds are developed as an alternative to organochlorine compounds, Their indiscriminate use has increased the pollutional hazards posing much danger to fishes. Phosphamidon an organophosphorus pesticide, is one of the versatile pesticides extensively used in agricultural preparations all over the world. It is systemic insecticide cum acaricide and it has a wide spectrum of action.

Organophosphorus pesticides though may be degraded, do not get totally eliminated at once and so their degradation does not necessarily mean the end of hazard. Instead these may produce an equally or even more toxic metabolic products. (Suman Cherian 1991) pesticides reach water bodies and cause cellular damage leading to the death of many aquatic organisms. (Datta and Dikshit 1973).

Earlier, scientists reported that phosphamidon is of relatively low toxicity to fish as compared to other organophosphorus pesticides. Toor and Kaur (1974) compared the toxicity of some selected pesticides and reported phosphamidon as the least toxic pesticide and fenitrothion as the most toxic pesticide.

Comparative evaluation of some organophosphorus pesti cide toxicity to the fish was analysed by Sherakar and

Kulkarni and it was reported that, phosphamidon is less toxic compared to other organophosphorus pesticides and the order of pesticides based on toxicity was found to be methyl parathion > malathion > disyston > phosphamidon. Effect of different organophosphorus insecticides on Tilapia fish was reviewed by many research workers and it was reported that phosphamidon is comparitively less toxic to fishes.

The results of the present investigation is in accordance with the above findings. The present experiment reveals that the LC_{50} values of the Oreochromis mossambicus were 15 ppm at 24 hours of exposure, 6 ppm at 72 hours of exposure.

Comparision of LC_{50} values obtained in this study, with those reported earlier, is difficult, because variations in the toxicity may result from difference in the test conditions and species-specificity of the test chemical, pesticide toxicity is affected by a variety of factors including temperature. Temperature and toxicity are positively correlated for most organophosphates in that toxicity increases as temperature increases (Sparks et. al.,1982). The size of the test organisms has an immense importance in acute toxicity studies. Larger organisms are found to be more resistant than the smaller ones. The toxicity of pesticide decreases with increase in size of the test animal (patwardhan and Gaikwad 1991), pH, salinity, waterhardness and dissolved oxygen content of water also influence the toxicity of a compound. The pesticide toxicity is reduced in highest salinity of water (Ferrando and Andreu Moliner, 1991) The exposure time is found to have a significant effect on the LC_{50} value. LC_{50} value decreases with the increase of time of exposure or in other words, the resistance of the fish decreases with the exposure time. (Macek et. al.,1969, Pimental 1971.) In the present study also exposure of Oreochromis mossambicus for 24 and 72 hours reveal fact that the increase in exposure period decreases the LC_{50} value.

The pesticide treated fishes became restless and appeared to be in distress, showed erratic movements finally lapse its balance, similar behavioural response due to pesticide exposure was also reported by many other research workers (Carter 1971, Bakthavathsalam 1972).

Enzyme Modulations:

Biochemical toxicity of phosphamidon is clearly reflected in the altered enzyme activities in the fish Oreochromis mossambicus.

The phosphatases are active at specific pH and are usually termed phosphomonoesterases. Phosphamidon poisoning in the fish under report altered the activities of both acid and alkaline phosphatase.

Acid Phosphatase Activity:

Fishes exposed to acutely and chromically sublethal concentrations of phosphamidon showed an increase in acid phosphatase activity in liver and gills. The results of the present investigation concerned with ACP activity is in coincidence with the reports of Thomas and Murthy 1976, Reddy and Rao 1978, Srivastava et. al.,1975, who have also reported increased acid phosphatase activity in liver induced by the action of varieties of chemical agents including pesti cides.

Acid phosphatase is a lysosomal enzyme and the rise in its activity is probably related to the cellular damage (Dubale and Shah 1929). Eller, (1971) has reported on liver damage after insecticide treatment. Increased permeability of liposomal membrane was noted by Maderia and Maderia (1929) after insecticide treatment. Wilson et. al.,(1970) reported stimulation of acid phosphatase activity due to tissue damage

Increased Acid Phosphatase activity related to tissue damage was reported by Bryc (1973). Joshi and Desai (1981) have observed cellular necrosis and simultaneous increase in Acid Phosphatase activity in the liver of *Tilapia mossambica* after monocrotophos intoxication.

From the present study it is clear that the increased Acid phosphatase activity in the liver and gills after phos- phamidon exposure may represent a response through increased utilization of enzymes from lysosemal damage leading to hepatic necrosis. Hence the increase in the enzyme activity is related to the strong toxic effect of the pesticide, probably resulting in cell damage, the rupture of cellular and lysosomal membrane followed by increased utilization of lysosomal enzymes leading to cellular necrosis.

Alkaline Phosphatase Activity:

Fishes exposed to phosphamidon, at subacute and chromically sublethal concentrations, led to the decrease in Alakaline Phosphatase activity of liver, and this decrease in Alakaline Phosphatase activity may be due to tissue damage resulting in an overall insufficient compensatory enzyme production. Inhibition of alkaline phosphatase after pesticide treatment was also reported by Rashatwar and Ilyas(1984) and they attributed it to be due to the decreased synthesis of enzyme, or due to the direct effect of the pesticide. Decrease in alkaline phosphatase activity in brain was observed by Ansari et. al.,1987 and they also suggested that, it may be due to direct effect of pesticide since, like acetylcholinesterase, alkaline phosphatase also has serine residue at its active site (Mahendra and Agarwal (1983) and organophosphorus compounds are generally inhibitors of serine containing anionic sites of the enzymes (Bell et. al.,1970). It may also act on the anionic sites of Alkaline Phosphatase thus causing inhibition.

The gills showed raised Alkaline Phosphatase activity The high Alkaline Phosphatase activity of gills observed in the present study may be due to the increase in active uptake of ions through gills. Mullainathan (1982), reported that gills form a major site of accumulation of foreign substances. Rai et. al.,(1984) have also reported enhanced Alkaline Phosphatase activity in gills and attributed it may be due to increase in active uptake of ions through gills The present results are in collaboration with the results of Tejendra S.Gill et.al.,1990.

In the present study, gill Acid Phosphatase activity and Alkaline Phosphatase activity of liver and gills returned to normal, after 14 days of phosphamidon exposure. Dubale and Shah (1979) have reported a recovery from histopathological lesions in the fish Channa punctatus, after Cadmium Nitrate intoxication and reported that the observed recovery can be related to the reduction in biological toxicity of the pesticide due to detoxification mechanism in the tissues.

It is therefore clear from the present investigation, that phosphamidon has serious impact on the enzyme profile of the fish Oreochromis mossambicus.

The possible reasons include elevation of Acid Phosphatase in liver and gills. Inhibition of liver AlP activity and elevation of alkaline phosphatase in gill.

From the foregoing discussion, it is clear that phosphamidon in both acutely sublethal and chronically sub-lethal concentrations, has adverse effects on the Acid and Alkaline phosphatase activities of the fish *Oreochromis mossambicus.*

PERCENTAGE MORTALITY OF *OREOCHROMIS MOSSAMBICUS* AT DIFFERENT CONCENTRATIONS OF PHOSPHAMIDON AT 24 HOUR OF EXPOSURE

Concentration (ppm)	Log concentration	Number of fishes exposed	Number of fishes dead	Percentage mortality
1	0.0000	6	0	0
5	0.6990	6	0	0
10	1.0000	6	1	16.7
15	1.1761	6	3	50
20	1.3010	6	5	83.3
25	1.3979	6	6	100
30	1.4771	6	6	100

Table 1

PERCENTAGE MORTALITY OF <u>OREOCHROMIS MOSSAMBICUS</u> AT DIFFERENT CONCENTRATIONS OF PHOSPHAMIDON AT 72HR OF EXPOSURE

Concentration	Log concentration	Number of fishes exposed	Number of fishes dead	Percentage mortality
2	0.3010	6	0	0
3	0.4771	6	0	0
4	0.6021	6	1	16.7
5	0.6990	6	2	33.33
6	0.7782	6	3	50
7	0.8451	6	4	66.7
8	0.9031	6	5	83.3
9	0.9542	6	6	100
10	1.0000	6	6	100

Table 2

EFFECT OF PHOSPHAMIDON ON ACID AND ALKALINE PHOSPHATASE ACTIVITY IN THE LIVER OF <u>ORECHORMIS MOSSAMBICUS</u>

(ACTIVITIES ARE EXPRESSED IN KA UNITS)

Enzyme	Control	Exposure period in			
		Acutely Sublethal Conc		Chronically sublethal conc.	
		24 Hr.	48 Hr	7days	14 days
Acid Phosphatase	27.29 + 0.20	29.27 ± 0.28	36.14 ± 1.42	29.07 + .93	50.32 ± 1.41
% Change		+ 7.25	+ 32.43	+ 6.52	+ 84.4
Alkaline phosphatase	31.87 + 0.62	17.77 ±0.50	26.53 ± 0.66	17.72 ± 0.45	29.74 ± 0.37
% Change		- 44.24	- 16.76	- 44.40	- 6.68

Each value is the Mean + S.D. of 4 individual observations.

Table 3

EFFECT OF PHOSPHAMIDON ON ACID AND ALKALINE PHOSPHATASE ACTIVITY IN THE GILLS OF *OREOCHROMIS MOSSAMBICUS*

(ACTIVITIES ARE EXPRESSED IN KA UNITS)

Enzyme	Control	Exposure period in			
		Acutely Sublethal Conc.		Chronically Sublethal conc.	
		24 hr	48 hr	7 days	14 days
Acid Phosphatase	14.17 + 1.50	22.59 ± 0.82	24.64 ± 1.07	23.57 ± 0.71	15.75 ± 1.32
% change		+ 59.42	+ 73.89	+ 66.34	+ 11.15
Alkaline Phosphatase	23.72 + 0.72	28.69 ± 0.46	44.23 ± 0.34	32.68 ± 0.38	24.88 ± 0.44
% change		+ 20.95	+ 86.47	+ 37.77	+ 4.89

Each value is the Mean + S.D. of 4 individual observations.

Table 4

ESTIMATION OF Lc_{50} VALUE AT 24 hrs.

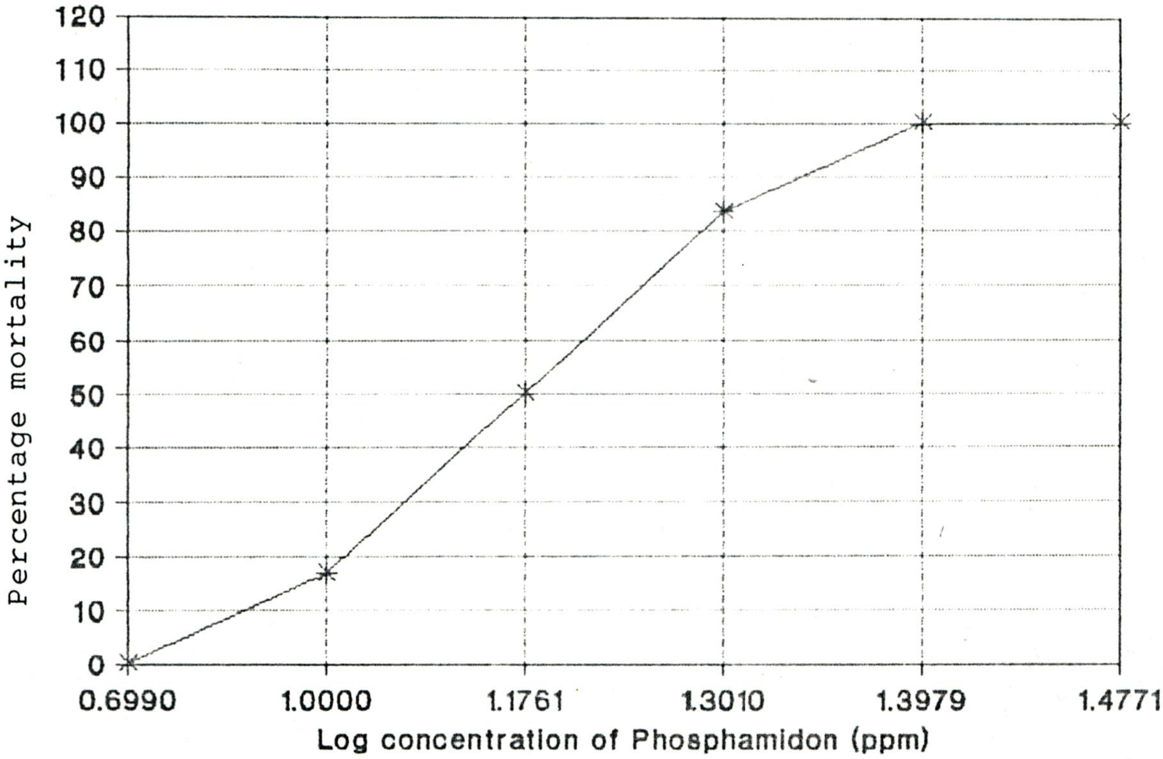


FIGURE -1

ESTIMATION OF Lc_{50} VALUE AT 72 hrs.

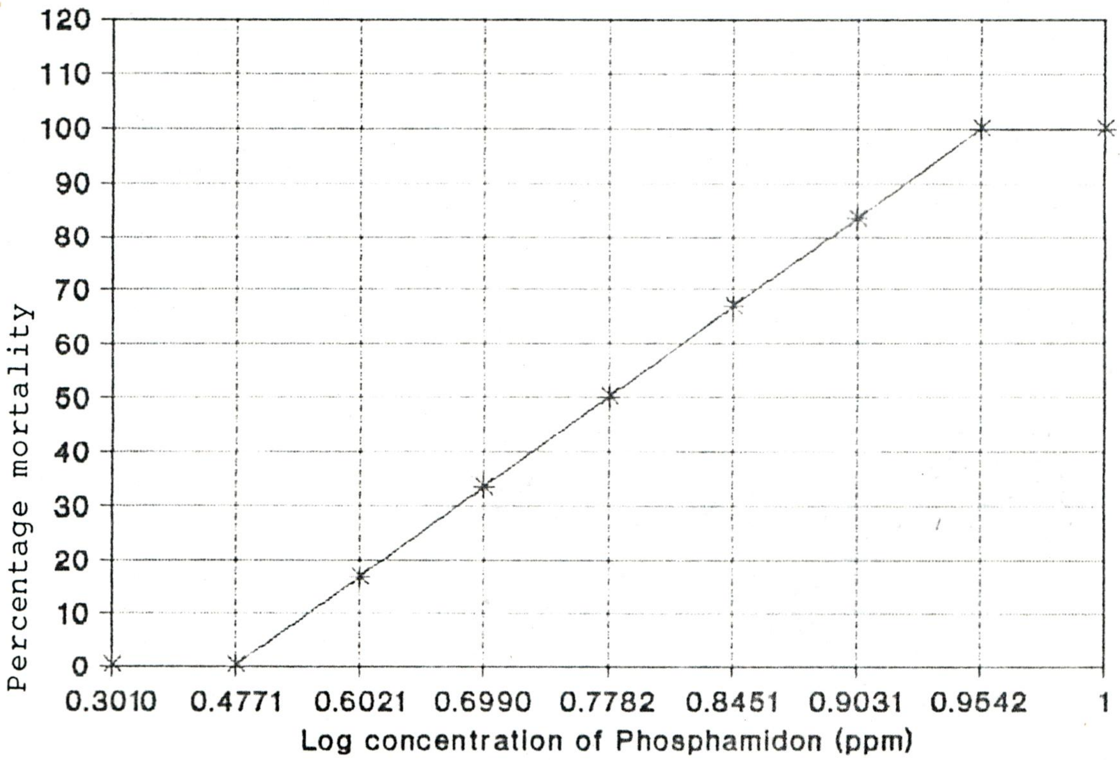


FIGURE - 2

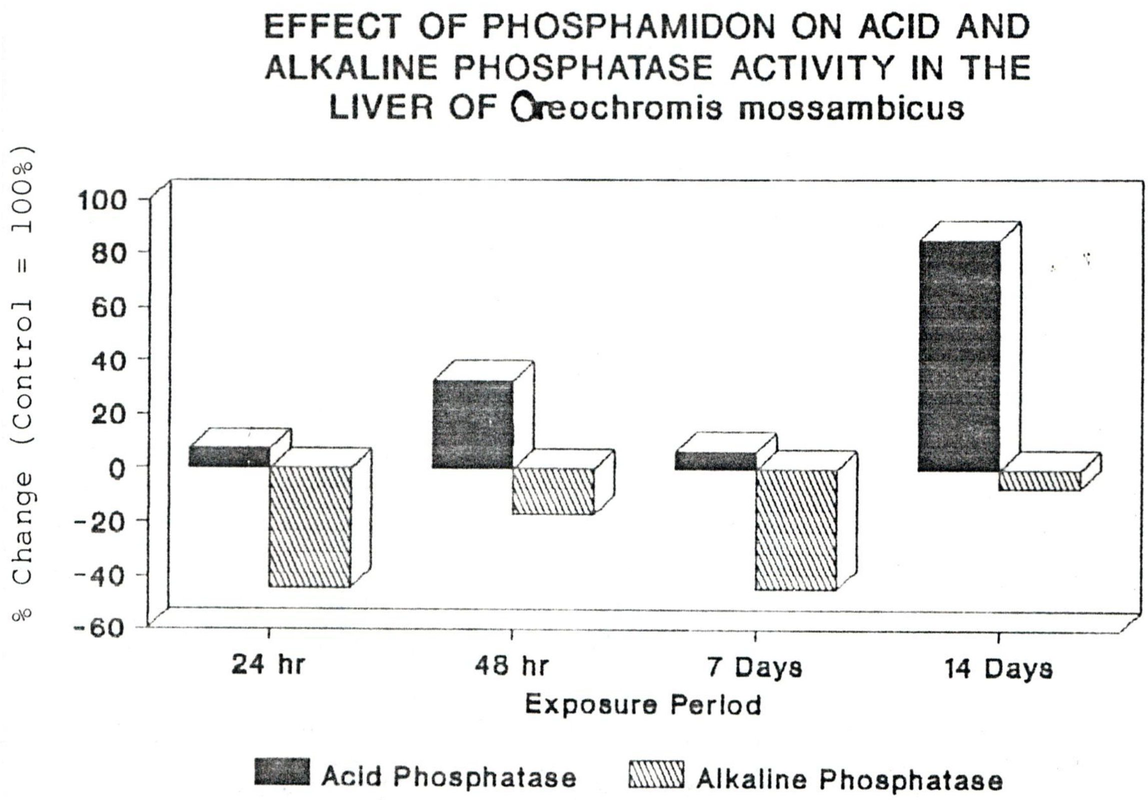

EFFECT OF PHOSPHAMIDON ON ACID AND
ALKALINE PHOSPHATASE ACTIVITY IN THE
LIVER OF Oreochromis mossambicus
% Change (Control = 100%)
100
80
60
40
20
0
-20
-40
-60
24 hr
48 hr
7 Days
14 Days
Exposure Period
Acid Phosphatase
Alkaline Phosphatase

FIGURE - 3

EFFECT OF PHOSPHAMIDON ON ACID AND ALKALINE PHOSPHATASE AC TIVITY IN THE GILLS OF *Oreochromis mossambicus*

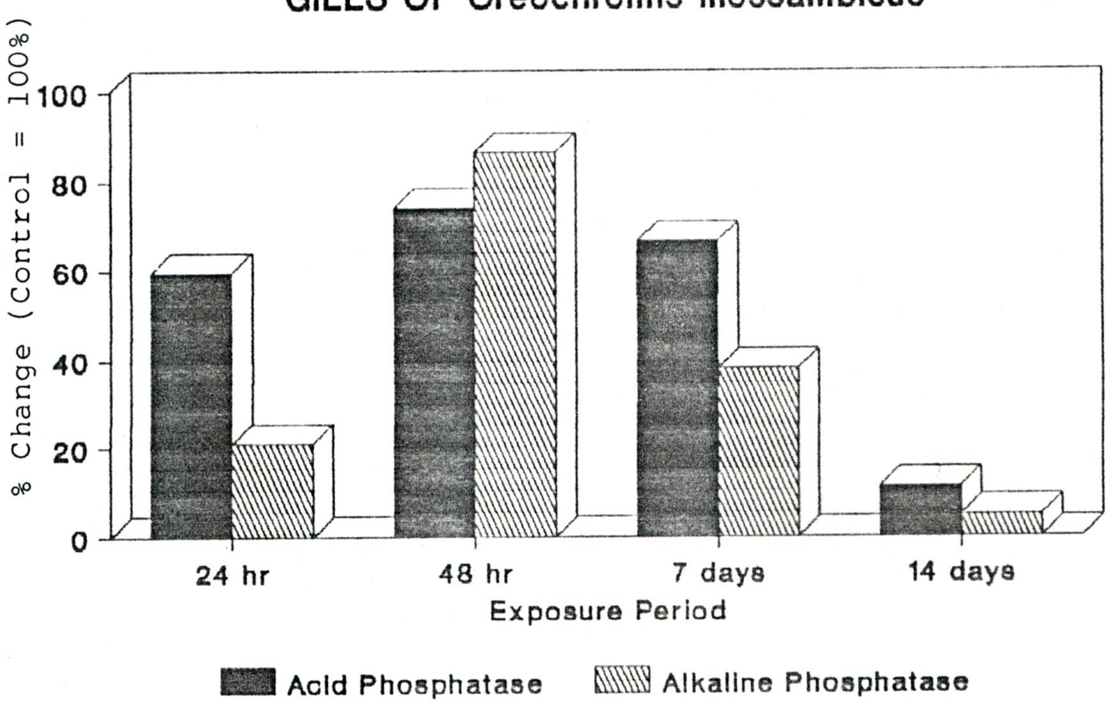


FIGURE - 4

Summary

SUMMARY

The effect of widely used commercial grade organophosphate pesticide Dimecron 85% S.L. on Oreochromis mossambicus was studied, by conducting acute bioassay studies. The LC_{50} values of 24 and 72 hours of exposure to phosphamidon were 15 ppm and 6 ppm respectively.

Changes in behavioural response of fish due to pesticide stress was observed. Fishes were exposed to sublethal concentrations of phosphamidon and tissues were dissected out and acid and alkaline phosphatase activities were analysed.

Increased Acid phosphatase activity of liver was correlated with tissue damage and increased acid and alkaline phosphatase activity in gills could be correlated with the possible increase in the active uptake of ions and tissue damage. Decrease in Alakaline Phosphatase activity in liver was assumed to be due to tissue damage resulting in an overall insufficient compensatory enzyme production.

From the present study, it is clear that phosphamidon in both acutely sublethal and chronically sublethal concentrations seriously affects the enzyme profile of the fish Oreochromis mossambicus.

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