TOXICITY OF PARAQUAT (GRAMOXONE) TO *Oreochromis niloticus*

BY

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D E D I C A T I O N

TO THE ALMIGHTY GOD

and

MY BELOVED HUSBAND

MR. J.A. BABATUNDE

D E C L A R A T I O N

I hereby declare that this thesis is my own work and has not been presented in any form for another qualification at any other University or Institution. Information derived from the published or unpublished works of others has been duely acknowledged in this work.

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C E R T I F I C A T I O N

This thesis entitled "Toxicity of paraquat (Gramoxone) to Oreochromis niloticus meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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of gill damage and thus impairment of osmoregulation.

Histological damage was observed on the gills at concentrations 11.20mgl^{-1} ,12.00mgl^{-1},14.20mgl^{-1} , at 96-h test and 1.02mgl^{-1}, 1.40mgl^{-1} and 2.00mgl^{-1} for O. niloticus at the long term test. Pesticide - related changes included, hyperplasia of epithelial cells of lamellae, thrombosed blood vessels, oedema in gills of fish exposed to sublethal concentrations of paraquat, atrophy and total disruption of gill structures in gills of fish exposed to acute concentrations of paraquat which led probably to impairment of osmoregulation amd opercular ventilation.

The Liver also showed toxicosis, necrosis and vacuolation of hepatocytes. The pesticide - related changes in brain, included Oedema, inflammation of neuronal cells, glial scarring etc. The extent of damage is dose - dependent. The damage to the brain tissue might be responsible for abnormal behaviour of the fish.

Opercular ventilation rate showed decrease with increase in exposure period at the beginning of bioassays and an increase towards the end. A statistically significant difference ($P<0.05$) was observed in the opercular ventilation rate of control group of fish compared to the ones exposed to paraquat. This could have been a result of stress as well as gill damage.

There is evidence that indiscriminate discharge of

effluents containing paraquat or usage of paraquat for agricultural purpose which might be washed into streams and rivers by rain water, or usage to control hazardous water plant can have severe harmful effect on the ecosystem leading to the destruction of our fish stock and perhaps other forms of life.

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CHAPTER ONE

I N T R O D U C T I O N

Fish provides one major source of animal protein the world over and especially in Nigeria. It is the chief source of animal protein in poultry ration (ONI, 1979). Fish meal has also been known to be a good source of other essential nutrients of poultry feed such as mineral elements (Ca, mg, Na etc), vitamins (A,B,D and E) and bone meal. It is already a common practice among poultry farmers to supplement poultry ration with smoked and dried fish of all sorts (ONI, 1979).

The importance of fish as a source of protein in human diets cannot be over emphasized. According to Abiagom (1980), the total demand projection for fish by 1985 in Nigeria will be 2.82 million metric tonnes. Out of this, it is estimated that only 687,446 metric tonnes may be produced from the available resources (study group on fisheries, 1981). Matton (1982) observed that Nigeria consumed about 1.5 million metric tonne of fish in 1980 out of which about 900,000 metric tonnes of frozen fish were imported (Ologunmeta, 1985).

By his projection, more than 2 million metric tonnes would be demanded by 1985, and out of this figure about 1.4 million metric tonnes would have to be imported. Olaniawo (1983) also indicated that the 1983 percaput fish consumption figure of 5.17kg in Nigeria was far below the planned average figure of 10kg envisaged by 1985. Fish consumed in Nigeria is either

as a run off from agricultural farm lands as well as through spraying in water bodies such as ponds and rivers to eradicate weeds such as water hyacinth. Although there are definite advantages in the use of this pesticide, particularly in area of crop production, the extensive use has resulted in large reduction in fish production, not only in capture fisheries sector but also in aquaculture. The indiscriminate use of pesticides has been identified as a cause for many previously unexplained fish kills in fish farms in other parts of the world (Oloruntuyi et. al, 1992).

Thus the improvement of the physico-chemical properties of the water bodies is vital to the enhancement of fish production and success of government's agricultural programme on self-suffiency and reliance in fish food supply (Ologunmeta, 1985).

This study is therefore designed with the following objectives:

a. Determining the LC_{50} of paraquat and its acute effects on some physiological parameters vital to the survival of Oreochromis niloticus.

b. Determining the effects of sublethal concentrations (chronic effects) of paraquat in our aquatic environment on some physiological parameters vital to the survival of Oreochromis niloticus.

c. Examining histopathological effects of paraquat in tissues.

d. Examining haematological effects of paraquat on

Oreochromis niloticus blood.

e. Examining paraquat's effects on growth, behaviour and respiratory system of *Oreochromis niloticus*

CHAPTER TWO

2.0 LITERATURE REVIEW

Since the beginning of history, mankind has had to compete with more than 1 million other species for the earth's goods which are essential for survival. Many insects, rodents and birds found man's crops to their liking, and his dwellings provided shelters not only for man but also for a host of his competitors. As man continued to modify his surrounding, the competition became more intense, and organized patterns of "pest control" evolved (McEwen and Stephenson, 1979). At first these efforts were minimal: scarecrows to discourage birds, hand removal etc. With technological advancement and greater menance of pests, there was development of more advanced ways of dealing with the insect and plant pests. These included use of arsenic, use of oils and salt for weed control and copper and mercury compounds for control of certain plant diseases.

According to McEwen and Stephenson (1979), developments of pesticides were slow when viewed from the modern era. By 1939, only 30 were registered for use in the United States. Application techniques were limited largely to small sprayers and dusters, and applying of pesticides was time-consuming and the acreage of crops treated being relatively small. Under these circumstances, the environmental impact of pesticides was small and restricted to the immediate area of application. World war II placed a strain on Western Civilization to an

unprecedented magnitude with restriction in availability of imported pesticides and pest control became more difficult. This led to research to find replacement for pesticides which are no longer available because of the war. DDT (1,1,1-trichloro-2-2-bis (parachlorophenyl) ethane) was the first of the "new" insecticides. It had been synthesized as part of a doctoral study by Zeidler in 1874, but its insecticidal properties were not recognized until 1939, when Miller, searching for something to control clothes moths repeated Zeidler's synthesis (McEwen and Stephenson, 1979).

The rapid developments in insecticides were paralleled by new discoveries of compounds effective for plant disease and weed control. In weed control, the search was escalated by rising labour costs that made hand-weeding too expensive, and the pioneering discoveries of 2,4-D and atrazine provided selective herbicides that contributed immeasurably to the mechanization of crop production and the efficiency of labour input. Water hyacinth Eichornia crassipes which was first recorded in Nigeria in September 1984 (Kusemiju, 1992) has been subjected to a vigorous control by governments (Akinyemiju and Imevbore, 1990) and estuarine communities (Adekoya, 1987). However, mechanical control first used had limited success (Adekoya and Ekpo, 1992). Chemical method's pilot scheme conducted yielded good result. This could lead to more intensive use of pesticides for aquatic weed control nationwide.

CHEMISTRY OF PARAQUAT

Diquat and paraquat are two heterocyclic cationic compounds that are referred to as either bipyridinium or quaternary ammonium compounds because of the positively charged "four bonded" nitrogens in their structure. Brian *et al.*, (1958) first disclosed the herbicidal properties of this family of compounds in 1958. Both diquat and paraquat act as nonselective contact herbicides with essentially no activity in the soil (McEwen and Stephenson, 1979). Diquat and paraquat are potent inhibitors of photosynthesis in plants. Both compounds are highly water-soluble and are absorbed by weeds (Way *et al*, 1979). Levels of these herbicides rapidly decrease within 4 to 14 days of treatment (Earnest, 1971). This may occur due to uptake by weeds and adsorption to suspended soil particles or bottom mud (Calderbank, 1970).

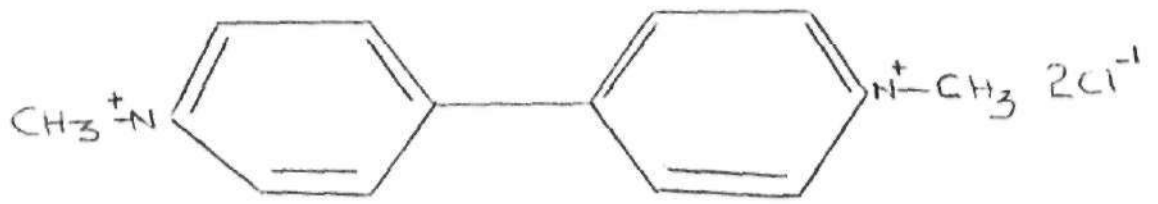
Dawood *et al.* (1965) reported that paraquat was used in Egypt to control aquatic weeds in irrigation canals, drainage ditches and marshes where it caused mortality of snails Bulinus truncatus, Biomphalaria alexandria and Lymnaea calliad. In New Zealand, treatment of a small river with 2.0ppm paraquat reduced amphipods to 5% of the pre-treatment level in 30 minutes exposure (Burnet, 1972).

Paraquat is available commercially as a salt of dichloride.

PARAQUAT OR

1,1-DIMETHYL-4,4-Bipyridinium

(Cation) dichloride.

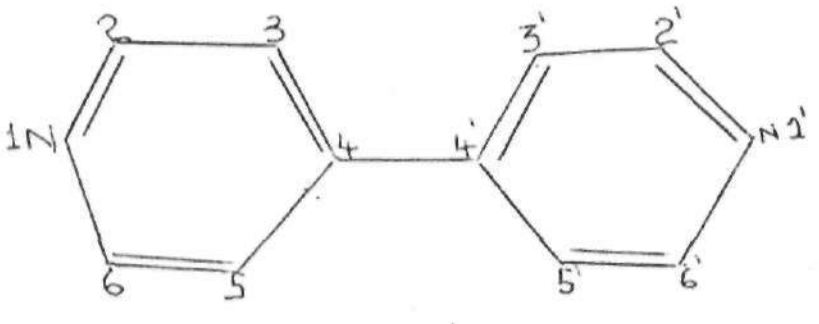


Paraquat salts had been known since 1882 (Weideland Russo, 1882) and had been used as oxidation reduction indicators under the name methyl viologen since 1932 (Michaelis, 1932). The bipyridinium herbicides are water-soluble salts. They rapidly desiccate all green plant tissue with which they come in contact. They are substantially inactivated on reaching the soil by being adsorbed on clay mineral constituents. They have no residual activity (Summers, 1980). They are effective as herbicides at low concentrations of about 1/8 - 2lb/acre (0.14-2.2kg/hectare). This combination of properties led to their world-wide use in a variety of weed control situations. These include weed control in orchards, plantation crops and forests, in weed control before crop sowing or before crop emergence, in pasture renovation, in pre-harvest desiccation, in total weed control and in aquatic weed control.

The principal formulations of diquat and paraquat available to farmers consist of concentrated aqueous solutions of the herbicides containing about 20-25% of the active ingredient in addition to additives such as wetting agents and

corrosion inhibitors. These concentrates are diluted in the field before being applied. The paraquat concentrate is known under the registered trade mark of Gramoxone.

The usual starting material for the synthesis of paraquat is the isomeric bipyridine, 4,4-bipyridine, which is solid at ambient temperature (4,4^1-bipyridine melts at 114°C and distils



at 305°C). The 4,4^1-bipyridine obtained is then purified from its isomers by distillation, crystallization and continuous extraction into an organic solvent such as trichloroethylene (imperial chemical industries LTD, 1963). The 4,4-bipyridine is treated in water with methyl chloride under pressure. This gives an aqueous solution of paraquat dichloride or 1,1^1-dimethyl-4,4-bipyridinium di-chloride.

Homer *et al.* (1960) put forth the theory that the herbicidal activity of the type shown by diquat and paraquat is due to the ability of the salts to be reduced to free radicals in plants. It was also discovered that the one electron

salts of 4,4'-bipyridines resulted in the salts interferring with aspects of electron flow in the photosynthetic mechanism in plants thus giving rise to their herbicidal and desiccating activity (Summers, 1980).

It is mostly applied pre - emergence. It is an important annual weed and grasses controller. It is also used on many fruits: almond, apples, apricots; potato, sugarcane, sorghum, as cotton defoliant, peppers, soyabeans tomatoes, rubber trees, palm and other tropical crops for weed control.

It is immediately inactivated when in contact with the soil, thereby building up no residues in soil. It is rapid acting and non-volatile- It can be translocated but most activity is by local absorption. Paraquat's active ingredient is 270g paraquat dichloride, (200g) paraquat (Thompson, 1971).

2.2 SOURCES OF PESTICIDES IN WATER

2.2.1 INTENTIONAL APPLICATION

McEwen and Stephenson (1979) reported that pesticides are used to control weeds on farmlands and algal blooms in ponds and streams; to control insects such as mosquitoes, black flies, and biting midges that breed in water, to control lamprey which is predaceous on useful fish species, and to remove trash fish or diseased fish from ponds prior to restocking. Each of these procedures results in contamination of surface waters, the extent of contamination and its duration being a function of conditions in the aquatic environment and

the nature of the pesticide involved.

2.2.2 UNINTENTIONAL APPLICATION

Water is also contaminated in the same way as soils through pesticide drift during application and as atmospheric fallout on rain and dust. Water may be contaminated through soil erosion, industrial effluent, sewage and occasionally by spill into or adjacent to water courses (McEwen and Stephenson, 1979).

2.2.3 PERSISTENCE OF PESTICIDES IN WATER

McEwen and Stephenson (1979) explained that the persistence of pesticides in water depends on a number of factors. These factors include nature of pesticide, that is whether they are persistent in water or not. Summers (1980) reported that Diquat is more easily degraded than paraquat since aqueous paraquat solutions are degraded only very slowly, if at all, in sunlight. However it is evident that paraquat and diquat are broken down by irradiation, with diquat being more susceptible to degradation than paraquat.

Other factors include nature of water, PH, temperature, aquatic life and amount of suspended organic and inorganic materials. Since many pesticides degrade by hydrolysis, it might be assumed that pH of natural water would play an important role in their persistence. Laboratory studies on fenitrothion indicate it is quite stable under slightly acid to

slightly alkaline conditions and is stable for 45 days in tap water at pH 7.0. However, its persistence in natural waters is only a few days (Sundaram, 1973). Paraquat tends to be more stable in water of pH 10-12 (Summers, 1980). Paraquat usually dissipates quickly from natural water systems. The disappearance is largely due to their adsorption by sediments and suspended materials and by absorption and uptake by aquatic plants and algae (Summers, 1980).

McEwen and Stephenson (1979) also explained that increase in temperature increases the rate of chemical reaction and the rate of volatization of pesticides. Thus, higher temperatures tend to reduce persistence of volatile pesticides.

2.3 SELECTION OF THE TEST ORGANISM

It has been suggested that economically important local fish species be used in toxicity assay (Doudroff et al., 1[illegible]1). Oreochromis niloticus of the family Cichlidae and genus Oreochromis, is a fish of high commercial value and is very common in Nigeria freshwaters. Eyo (1975) reported it to be of high nutritive value. It is also reported to be hardy, being able to acclimatise to stressful conditions. Reed (1967) in his studies of fish and fisheries of Northern Nigeria gave the report as contributed by John Burchard that where the Cichlids occur, they are usually a dominant element in the aquatic fauna. They also feature prominently in many fisheries. They are used extensively for fish culture because of their great

adaptability, high fecundity and rapid growth rate. Burchard (1967) reported that certain species of Oreochromis, in particular, are important in fisheries planning in Nigeria and in many other tropical regions where animal protein is at a premium.

Cichlids are one of the very few groups which extend parental care to the young for a considerable time after hatching. They are reported to have brought this family life to a degree of perfection not seen in other fishes. The elaborate behaviour this involves has made them favourite objects for the study of animal social life, and stocks of cichlid fish are said to be kept in behaviour laboratories in many parts of the world (Reed and Burchard, 1967). Oreochromis are basically vegetarian, Pelmatochromis are bottom grazers or detritus feeders and Hemichromis are predators. Oreochromis niloticus can be recognised at a glance by the characteristic pattern of dark and light bands crossing the caudal fin. The body is rather elongated and usually shows a number of rather narrow bands on the back. O. niloticus inhabits rivers and lakes in Asia minor East Africa, and the Chad, Niger and Senegal basins in West Africa. In Nigeria its natural occurrence is limited, to River Niger and Lake Chad but it has been transplanted to other parts of the country for pond culture (Reed and Burchard, 1967). O. niloticus is one of the largest Tilapia, reaching the considerable length of about 50cm. It is a typical mouthbrooder, in which the female takes

care of the eggs and later takes sole charge of guarding and rearing the fry. Purdom (1993) corroborated the findings of Reed by reporting that Oreochromis are hardy, easy to breed and show good growth rates. They are also attractive in market in terms of having good flavour and a good flesh texture. They are important as a source of protein in underdeveloped countries particularly as they are mainly Omnivorous with distinct leanings towards vegetarianism.

Madu (1984) also reported that it can be kept in captivity and it grows to table size within a short period and with minimum management. It has a high tolerance of poor water quality and spawns easily. It is resistant to many diseases and it can be handled easily. It has good consumer acceptability. All these factors make it an excellent fish in aquaculture. Therefore on the basis of availability, commercial and ecological importance, Oreochromis niloticus has been chosen for this study.

2.4 PARAQUAT HAZARD TO MAN

Paraquat is normally immobilized almost immediately by absorption unto the soil particles, from which it is not released and it is then broken down by bacterial decomposition which is of advantage if carefully applied to its target (Mellanby, 1980). However it has disadvantages and dangers when wrongly used. First, it is very poisonous to man if accidentally drunk in undiluted form, and has caused death in

man when exposed through dermal route (Delarrad, 1969), and through oral route (Broad-hurst et al., 1968). Its action is particularly unpleasant, causing asphyxiation by damaging the lungs which are choked by the proliferation of the epithelial tissues. Eye irritation is also caused by exposure to low volume (and therefore concentrated spray). Secondly, the chemical is much less rapidly immobilized in wet vegetation, when it is sprayed unto weedy stubble after rain, wild animals, particularly hares which are more susceptible than rabbit or cattle have often been killed (Mellanby, 1980).

2.4.1 EFFECTS OF HERBICIDES IN WATER AND ON DIFFERENT FISH SPECIES

The use of the bipyridinium herbicides in aquatic weed control, as well as the possible pollution of rivers, irrigation canals, ponds and reservoirs by herbicide run-off from agriculture has prompted extensive investigations into the toxicity of diquat and paraquat to fresh water fish and other aquatic fauna. The toxicity of the herbicides to fish is frequently expressed as the median tolerance limit (TLM) or LC_{50}. These limits refer to the concentration of the herbicide that produces 50% mortality after being in contact with the fish for a specified period of time (Summers, 1980).

Herbicides are more toxic to fish in soft water than in hardwater and the paraquat commercial formulation, Gramoxone, containing wetting agents is more toxic than the formulation without wetting agent, at least on species that had been

examined e.g. Blue gills (Lepomis macrochirus) Brown trout (Salmo trutta), Roach (Rutilus rutilus), Minnows, etc. (Summers, 1980).

Paraquat is very rapidly absorbed by the weeds, reaching concentrations in the plant tissue well above that in the surrounding water (Newman, 1966, Way, 1971), so that very low concentration (from 0.1ppm - 1.5ppm) in the water will give effective control. Residue levels in water decline very rapidly due to uptake by the weeds and adsorption to suspended soil particles at mud bottom (Newman, 1971, Calderbank, 197.,. The rate of disappearance of this herbicide from water is thus rather variable and depends on movement of the water, density of weeds presence of mud and suspended salt and sunlight. Usually, residues decline to 0.01ppm or below within 4-14 days of treatment (Way, 1971, Newman, 1971, Calderbank, 1970, Earnest, 1971).

Austin (1963) and Calderbank (1970) also reported that there is a wide safety margin between the concentration of paraquat which is toxic to fish in 96-h immersion tests and that which is needed for aquatic weed control.

Paraquat, during laboratory tests indicated low toxicity to fish (trout) but when field trials were carried out, unforseen events occurred which are of considerable ecological interest (Water Pollution Research 1966, 1967; Summers, 1980). A lake, 17,850m^2 in area containing trout, roach and perch was treated with paraquat at 0.5ppm. Within five days, the minimum

daily oxygen content of the water had fallen from about 110% saturation to 4%. By that time, aquatic weeds had begun to disintegrate and all captive trout were dead. Captive perch still survived. The interpretation of these events was that the lethal effect on trout was caused entirely by the indirect effect of the herbicide destroying the plants and inhibiting photosynthesis, respiration continued, and oxidation of the organic matter produced by the disintegrating plant material led to severe oxygen depletion (Summers, 1980).

Carter (1971) demonstrated that paraquat is not accumulated in fish kept in solution of this herbicide for extended periods. Thus, Rainbow trout exposed to 1ppm paraquat for 16 days had a whole body level of 0.5-0.6ppm of the herbicide. The residues were confined to the skin, gills, gut and other organs of the fish. No residue, however, could be detected in the muscle tissues. On returning the fish to the freshwater, the residues steadily disappeared. The work of Carter (1971) corroborated those of Cope (1965) and Howe and Wright (1965) who reported residues of bipyridinium herbicides (Diquat and paraquat) in exposed fish.

In pools treated with 1-3ppm of diquat, residues in fish reached peak levels 10 days after treatment and disappeared after 6-12 weeks. The maximum residue found in adult blue-gills was 0.5ppm from 1ppm treatment and 1.45ppm from the 3ppm treatment (Summers, 1980). These results, in which the residues were determined by chemical assay, agree generally

with the result using radiolabelled (C^{14}) diquat reported by Beasly et al. (1965, 1966). Radio-activity in most organs increased with time, but no accumulation occurred above the external concentration of the chemical.

Daniel and Gage (1966) reported that on oral administration of the bipyridinium herbicides to rats, diquat, and to a lesser extent, paraquat, were substantially metabolised but little metabolism of the herbicides occurred after subcutaneous administration. Calderbank and Slade (1976), however, suggested that paraquat is less metabolized after oral administration in contrast to the report by Daniel and Gage (1966).

2.4.2 EFFECT OF DURATION OF EXPOSURE

Mohammed (1995) reported that the period for which the LC_{50} is determined is usually of considerable importance, values normally being much lower after 96-hours, than after 24-hours. The ratio between such values is however very variable for different pesticides. Karz (1961), determining LC_{50} values for periods of 24, 48, 72 and 96 hours in static test, using four species of fish and thirteen insecticides in general, found only relatively small increase in susceptibility from 24 to 96 hours, with Rainbow trout showing no change at all with DDT and methoxychlor, and almost none with lindane. Pickering et al. (1962) found that there was only a small decrease in the LC_{50} value from 24 to 96 hours in assays for of a number of

value from 24 to 96 hours in assays for of a number of Organophosphorus insecticide formulations. TEPP (bis-0,0-diethylphosphoric anhydride), which is easily hydrolysed, caused no decrease. Metathion, parathion, Demeson and EPN only caused a slight decrease. However Trichlorphon and metasytor caused more significant decreases. Chlorinated insecticides, in general are more stable than organophosphorus compounds and show a greater difference in toxicity between 24-hour and 96-hours tests.

2.4.3 EFFECT OF SIZE AND SPECIES OF TEST FISH

Larger fish will remove proportionately more of pesticides than smaller fish (Mohammed, 1995). Most investigations according to Mohammed (1995), suggest that susceptibility decreases with increase in size. Pickering et al. (1962) found that larger fish were less susceptible in static bioassays, and Mount (1962) reported 96-hour LC^{50} values of 0.27ug/L and 0.47ug/L endrine respectively, using 60mm long guppies and blunt nose minnows. However, Cope (1969) reported in contrast to earlier studies, that DDT showed no size dependence in static bioassay over the size range 0.5g to 40g. In haematological studies in white fish, it was reported by McKnight (1965), that differences were observed in the various values of haemoglobin and hematocrits between small and large fish, males and females and for fish taken in summer and winter.

that, the effectiveness of the continuous flow system employed could account for a lower severity of damages done to some organs like livers and kidneys of the exposed fish when compared with reports of other workers in static bioassays.

2.4.5 EFFECT OF TEMPERATURE

The effect of temperature on the toxicity of a large number of chemicals to fish is well documented. The toxicity of a number of pesticides at different temperature was studied by Macek et al. (1969) using bluegills and rainbow trout, in static tests.

Generally, toxicity increased with temperature but there were some exceptions (Mohammed, 1995). With Methoxychlor, the susceptibility of both species of fish decreased with increasing temperature. Bluegills were unaffected by temperature in tests with lindane and azinphos methyl. After 96-hour tests, the effect of temperature was less than in 24-hour tests, except with trifluralin on rainbow trout. Cope (1965) found that with bluegills the effect of temperature on the toxicity of trifluralin was considerable, although less in 96-hour tests than in 24-hour test. At 7.2°C, the 24-hour and 96-hour LC_{50} values were 1.30 and 0.28mgl^{-1} respectively but at 29.4°C the corresponding values were 0.010 and 0.0084mgl^{-1}

Respiration and hence the absorption of pesticide via gills, will be more rapid at higher temperatures, the oxygen demand being greater but oxygen solubility less (Mohammed,

1995). However, as Macek and his colleagues indicated, in the static tests, the pesticide available is limited, and after 96hours the effect of temperature could be expected to be less than 24hours.

Toxicity has in some instances been found to increase with decreasing temperature. Cope (1969) gave data for the 48 hour LC_{50} of DDT for blue gills, showing that the value at 45°F (7.2°C) was 0.0024mg/L and at 85°F (29.4°C) was 0.064 mg/L. In 96 hours the values were 0.0016 and 0.0056mg/L respectively.

Murad et al. (1990) in their studies on haematological response of Carassium auratus L. to increased temperature in a long term assay, discovered that increasing temperature was associated with modest reduction in the haemoglobin and haematocrit levels of control animals. Similar changes have been reported in a number of species (Houston, 1980) and may be linked to Viscocity reduction and metabolic cost of cardiac work. In treated animals, both variables increased. By day 50, analysis of variance, (ANOVA), revealed no significant differences at the 0.01 level between the two groups.

Mohammed (1995) reported that increased metabolism in O.niloticus and oxygen uptake resulted in lower dissolved oxygen concentration and the accumulation of higher concentrations of waste products, thus increasing susceptibility.

2.4.6 EFFECT ON BEHAVIOUR AND PHYSICAL CHANGES

Walsh and Ribelin (1975) in his report showed more of appetite depression in fish exposed to pesticides such as Carbaryl, malathion, endosulfan, dieldrin, 2,4-D etc. while only DDT stimulated appetite in the fish. Other changes include disturbance of equilibrium, alteration of posture, increase in response to stimuli, increase and in some cases, decreased activities, alteration of pigmentation and alteration of water depth selection.

Following exposure of the indian cat fish (Heteropneustes fossilis Bloch) to heptachlor in an acute toxicity test, the fish were reported to show hyperactivity characterised by rapid pectoral and opercular movements, erratic swimming or darting and gradual loss of equilibrium, followed by sluggishness and death (Srivasta and Mishra, 1987). It was also further reported by the authors that prior to death, the fish swam laterally or lay at the bottom of the test container belly up. Mount (1962) attributed the behavior in blunt-nose minnows poisoned with endrin to disorder of the central nervous systems commencing with rapid, jerky movement of the body and fins, an increase in ventilation rate, and in sensitivity to external stimuli. The fish then moved to the surface, swimming slowly and sometimes backwards and convulsion followed which eventually ended in death.

2.4.7 HISTOPATHOLOGICAL EFFECTS

Earnest (1971) observed no abnormalities in the eyes, skin or muscles of green sunfish exposed to 1ppm Paraquat in water. On histological examination, occasional necrotic spots were observed in the liver and some abnormalities were noticed in the Pancreas. Oloruntuyi et al. (1992) in his 96-h bioassays observed extensive hyperplasia in the gill filaments and epithelial slouching in O. niloticus when exposed to paraquat. Omoregie and Ufodike (1991) reported that damage done to gills of O. niloticus exposed to actellic 25EC resulted in respiratory impairment of the fish. The gills were the most damaged organ by the pesticide.

Studies on different pesticides in a 30day-sublethal tests on different fish as reported by Walsh and Ribelin (1975) gave abnormalities which include hepatoma of the liver, nuclear hypertrophy and cytoplasmic vacuolization of the liver. Others included hyperemia, peterchia or discoloured area, congestion of the liver sinusoids and veins and periportal necrosis. Thiyagarajah and Grizzle (1985) reported hepatic necrosis of liver in the fish Rivulus marmoralis exposed to diethylnitrosamine for 6 weeks at sublethal concentration. Chinabut et al. (1987) reported that fatty degeneration in the liver was the histopathological change commonly observed in Cyprinus carpio exposed to sublethal concentrations of dipterex.

Walsh and Ribelin (1975) reported precipitate in kidney

tubules and vacuolization of distal tubules when lake trout and cohosalmon were exposed to several pesticides. Other effects include degeneration of distal tubules and tubular epithelium, disintegration of convoluted tubules, yellow pigment in tubules and vacuolated cells in glomeruli. Thiyagarah and Grizzle (1985) reported Oedema of kidney and gills.

Walsh and Ribelin (1975) reported vascular congestion in blue gill trout brain exposed to phenoxy acetate herbicides. In lake trout and Coho Salmon, when exposed to several pesticides, cerebral hyperemia or haemorrhage of brain was observed during necropsy and microscopic examination. Vacuolation of optic tectum or lateral geniculate was also observed during necropsy. Erosion of the premaxillary region and haemorrhage of base of dorsal fin was also observed on the skin during necropsy (Walsh and Ribelin, 1975).

2.4.8 EFFECT ON HAEMATOLOGY

Mclay and Vars (1931) first demonstrated that alteration in blood and damage to haemopoietic tissue in fish can be associated with pathological conditions related to water-borne pollutant. Halsband and Halsband (1963), while studying the threshold effects of various contaminants, noted that the blood was affected prior to any other visible changes in fish.

Haematocrit measurements reported by Allison et al. (1964) showed no difference between exposed and control cut throat trout and Gilderhus (1966) found the same with the exposure of blue gills to sodium arsenite. Other workers, however, have

noted a significant reduction in haematocrit values. Andrews et al. (1966) observed an increase in haematocrit values of blue gills exposed to 0.05mg/L of heptachlor for four hours, but they returned to normal after 28 days. Mount and Putnicki (1966), investigating a fish-kill due to endrin, found tissue abnormalities in the fish kidneys, where large vacuolated cells were seen in glomeruli while haematocrit values were 50% of normal. Eisler (1967) also found that both methoxychlor and methyl parathion produced this effect on Northern puffers.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The method employed in ths study is based on the recommendation for test of acute toxicity of pollutents to fish described by Sprague (1973) ASTM (1977), standard methods for examination of water and waste (APHA, 1985).

3.1 SELECTION OF TEST ORGANISM

It has been recommended (Doudoroff et al., 1951) that economically important local fish species be used in toxicity assays. Oreochromis niloticus of the family Cichlidae and genus Oreochromis are fishes of high commercial and nutritive value. They are very common in Nigerian freshwaters. The Nigerian government has of recent focused her attention on the protein needs of the increasing population by encouraging both small and large scale fish farmers Oreochromis niloticus is one of the most common fishes which can be kept in captivity and grow to table size within a short time and with minimum management and as such could occupy a prominent position in this effort.

3.1.1 COLLECTION AND MAINTENANCE OF THE FISH

Fingerlings of O. niloticus of weight range of 6.97-772g and mean weight of 7.35±0.53g were obtained from the Ahmadu Bello University dam. The fish were transported to the laboratory in an ice-box containing sufficient water from the

dam which is the natural habitat of the fish. In the laboratory, the dam water was gradually replaced with dechlorinated tap water over a period of 24hours during which the aquarium tanks were aerated. After twenty-four hours, the water was then changed to pure dechlorinated laboratory tap water. The fingerlings were acclimated at temperature range of 21.5°C - 24°C for two weeks prior to the commencement of the assay. The laboratory was lit by flourescent lamps but the natural daylight photoperiod of 12/12 was maintained.

The fish were fed daily during acclimatization on a feed formulated based on the the recommendation of the Kainji Lake Research Institute (Sado, 1986). Feeding stopped 24 hours prior to the commencement of the acute toxicity assay and during the assay. They were fed twice daily during the long term assay.

3.1.2 PREPARATION OF FORMULATED DIET

The artificial diet used for this study consisted of a mixture of groundnut cake, corn meal, fish meal and vitamin premix in the proportious shown in Table 2. Ingredients made up of 7kg, 1.5kg and 1.5kg of corn, fish meal and groundnut cake respectively were ground finely and mixed together with 2 litres of water into a thick paste. Cooked starch was added as a binder and all ingredients were cooked at low heat for 20 minutes for effective mixing. The paste was then spread into trays, allowed to set and cut into small cubes of uniform size.

The cubes were then sun-dried. The dry cubes were put in a water-proof container and kept in a dry cool place. The proximate composition of the feed is shown in Table 2.

The fish were fed to satiation once daily during the period of acclimation.

3.1.3 EXPOSURE OF FISH TO PARAQUAT

During the assay, eight, 30.5cm x 30.5cm x 92.5cm of glass aquaria were used and repicated subsequently. The fish were exposed to 7 concentrations of the toxicant and the control. Each of the concentrations was replicated. Water was renewed partially thrice a week using dechlorinated tap water. Dechlorination of tap water was by allowing water to stand for a week with accasional agitation. Water was aerated during holding and acclimation but no aeration was carried out during the assay. Renewal of water was by siphoning three quarters of the fish water and the feacal materials using a rubber hose. The remaining fish water was then topped with fresh dechlorinated water during acclimation.

Change of test water in sublethal test was achieved by freshly preparing fresh test water in duplicate tanks and when the water in test - tanks had drained to a level barely covered the fish, the newly prepared test water (which had stood for at least 30 minutes after the introduction of paraquat for complete and even mixing through diffusion) was introduced into the appropriate test aquaria.

The temperature, PH, dissolved oxygen, hardness and conductivity were determined daily during the 96-h acute toxicity assay and once a week during the sub-lethal test. Standard Methods (APHA 1985) were used. The physico-chemical composition of the water is presented in Table 3.

TABLE 1: PERCENTAGE COMPOSITION BY WEIGHT OF THE FORMULATED DIET FED TO O. niloticus EXPOSED TO PARAQUAT

CONSTITUENTS	CONCENTRATION %
Corn	69%
Fish Meal	15%
Groundnut Cake	15%
Vitamin Premix	1%
TOTAL	100%

Vitamin Premix

Vitamin	A (retinol)	8000 I.U
"	E (51-tocophnol acetate) m	60 I.U
"	D_2 (calciferol)	10,00 I.U
"	K (Menadione)	
Thiamine hydrochloride (B_1)		4mg
Riboflavin (B_2)		8mg
Panto themic acid		12mg
Nictinic acid (niacin)-		50mg
Folic acid (Folacin)		10mg
Pyridoxine hydrochloride (B_6)		6mg
choline chlride		200mg
Cobalamin (B_{12})		12mg

--1 vitamin premix was prepared by lebsure, RHM, Agriculture (South Ltd; Poolo, Dorset, BH 15 111L (Registered office) England.

I.U = International units.

TABLE 2: PROXIMATE COMPOSITION AND MINERAL CONCENTRATION OF THE DIET FED TO O. niloticus EXPOSED TO PARAQUAT IN WATER

COMPONENTS	RANGE	PERCENTAGE OF DRY WEIGTH
Protein	25.58-2616	25.87±0.47
LIPID	28.8 -28.9	28.85±0.11
Carbohydrate	38.91-40.20	39.56±0.91
Potassium	0.67-0.69	0.68±0.04
Sodium	0.21-0.23	0.22±0.011

TABLE 3: PHYSICO - CHEMICAL PARAMETERS

	RANGE	MEAN
Temperature (°C)	21.9-24	22.7±0.77
Dissolved Oxygen (mgl^{-1})	5.2-6.1	5.7±0.72
CoductivityUm/tos	3.5-5.5X10^2	4.5±0.14
Hardness (mgl^{-1})	20-35	28.0±0.83
Alkalinity (mgl^{-1})	14-15	15.0±0.07

3.2 SELECTION OF PARAQUAT CONCENTRATIONS

The literature on both acute and sub-lethal toxicity of paraquat to tropical fish species are limited, therefore a series of exploratory tests (Pilot assays) were carried out to determine the range of concentrations to be used in the definitive tests. The concentrations of pesticide used to determine LC_{50} were 9.60mgl^{-1}, 10.40mgl^{-1}, 11.20mgl^{-1}, 12.00mgl^{-1}, 14.20mgl^{-1}, 15.20mgl^{-1} and 16.00mgl^{-1}. Apha (1980) recommended a wide range of concentrations, usually in a logarithmic ratio such as 0.01, 0.10, 1.00, 10.00 and 100%. However Stephan (1977) explained that in many toxicity tests with aquatic organisms, the measures, and sometimes, the intended toxicant concentrations do not fall exactly in a geometric series or any other series either by design or accident as experienced in this bioassay.

3.3 EXPERIMENTAL DESIGN

3.3.1 Acute Toxicity Assay:- In this test, the LC_{50} or the concentration of the toxicant that would kill 50% of the populations, is the accepted measure of the toxicity to the test organism. Ninety-six hours (96h) is the exposure duration. The acute toxicity most often used is the 96-h median lethal concentration or 96-h LC_{50} (Mayer and Hamelink, 1977; Mohammed, 1995).

The static method of bioassay was used to determine the acute toxicity of paraquat on O. niloticus. Eight-glass aquaria

tanks of size 30.5 x 30.5 x 92.5cm, each containing a different concentration of paraquat and a control tank were used. Appropriate volumes of the stock solution were taken and discharged into 50L of dechlorinated water in each test tank to give the following concentrations, 9.60mgl^{-1}, 10.40mgl^{-1}, 11.20mgl^{-1}, 12.00mgl^{-1}, 14.20mgl^{-1}, 15.20mgl^{-1}, 16.00mgl^{-1} and the last tank without the toxicant representing the control. The mixture was allowed to stand for 30 minutes before introducing test organisms.

3.3.2 RANDOMIZATION

Finney (1964) suggested a formal randomization procedure and cited proven examples of correlation between weight of experimental animals (mice) and the order in which they were caught. With fish, it is difficult not to see why the weakest swimmer in a holding tank might be caught first. If each successive group of 10 fish captured were placed in test tanks in order of concentrations, it might introduce a systematic error in the result. To avoid this, randomization of the fish was carried out as suggested by sprague (1969). The randomness was by distributing the first ten fish from the holding tanks into one of each of the test tanks in random order according to the occurrence of numerals one to ten (1-10) in a prepared table of random numbers to each tank as before. This was continued until all the fish were distributed. To guard against any effect of positions, the test concentrations were

randomly assigned to the tanks.

3.3.3 OBSERVATIONS AND TREATMENT OF DATA

Observations:- Observation of mortality were carried out at intervals of 15, 50, 60minutes, 2, 4, 8, 12, 24, 48, 72, and 96 hours, However records were only kept for 12, 24, 48, 72 and 96hours. During each observation time, fish were prodded gently to see if there was any response and in addition, the cessation of opercular movement also was used as index of death of fish. Fish that did not respond were presumed dead. The dead fish were removed and number recorded. The gills and livers of moribund fish were removed and put in 10% neutral buffered formalin immediately for histopathological examination later.

TREATMENT OF DATA

The percentage mortality in each concentration was determined. The 96-h median lethal concentration (LC_{50}) was calculated using probit analysis (Steele, 1983, Dick and Dixon, 1985).

In the determination of LC_{50}, the percentage mortality to probit scale using the probit table (Finney, 1964; Wardlaw, 1989), and the concentrations were transformed into logarithm ($\log_{10}$). The transformed values of mortality (probit) were then plotted against the $\log_{10}$ of concentrations. A straight - line through the points was fixed by regression analysis as

indicated in the appendix. From the straight line regression curve, the value of X and Y at 50% was read off. This gave the concentration that was lethal to 50% of the fish. i.e LC_{50} for that exposure period. The graph of probit mortality against log-concentrations of challenge suspension is given in Fig 1. It should be noted that probit values for 0% or 100% mortality could not be plotted, since such proportions are indeterminate on the probit scale. However, it was indicated that points were above or below the line as shown with arrowed points (Fig 1). This method agrees with the finding of Wardlaw (1985).

CONFIDENCE LIMIT OF LC_{50}

Having obtained an estimated value of LC_{50} from Fig 1; estimates of the 95% confidence limits were obtained. For this, standard error (S.E) of $\log_{10} LC_{50}$ ($S \log_{10} LC_{50}$) was needed and estimated approximately by the formula

$$S \log_{10} LC_{50} \qquad \frac{1}{b\sqrt{pn \cdot W}}$$

The detailed calculation is presented in Table 7. The 95% confidence limits is established by the equation.

$$\log_{10} (95\%CL) = \log_{10} LC_{50} + 1.96 \ (S \log_{10} LC_{50})$$

(Wardlaw, 1985). The measure of dispersion of the population was by estimate of standard error of the mean of all readings.

3.4 HAEMATOLOGY

Determinations for haemoglobin (Hb), haematocrit (Hct), mean corpuscular haemoglobin concentration. (MCHC) and leucocyte differential counts were carried out on 3 of the surviving fish in each test solution of 9.60mgl^{-1}, 11.20mgl^{-1}, 12.00mgl^{-1} and 14.20 mgl^{-1} and control but none could be carried out on fish in solution of concentration 16.00mgl^{-1} because they had all died by the end of the 96-hour. Histogram was plotted to compare the effect the different concentrations of paraquat had on the blood of test organism. Statistical analysis was carried out to check the significance of the differences. Ventilation rates and tail fin movement rates were graphically represented using histograms.

3.5 LONG - TERM SUBLETHAL BIO-ASSAY EXPOSURE

In each case a fraction of the 96-h LC_{50} was used to determine sublethal concentration range as recommended by Amminikiuthy and Rege (1977), Oladimeji and Ologunmeta (1987) and Mohammed (1995).

In the test with O. niloticus, 1/6, 1/9 and 1/12 of the 96h LC_{50} (12.25mgl^{-1}) were used. These gave nominal concentrations of 2.04mgl^{-1}, 1.36mgl^{-1} and 1.02mgl^{-1} of paraquat. The values were rounded up to 2mgl^{-1}, 1.4mgl^{-1} and 1.02mgl^{-1} respectively.

Eight, 30.5 x 30.5 x 92.5cm aquaria tanks were utilised in each set of the assay. Four tanks were used for test, while

the remaining four were duplicates. The changing was as described for the acute toxicity assay.

Appropriate Volumes of stock solution were discharged into 50litres of dechlorinated water in each of the test tanks except the control which contained only the dilution water.

The tanks were randomly arranged on the working table and left for 30 minutes to allow toxicant to be uniformly distributed in the water. Fish of weight range of 6.77-7.72g and mean weight of 7.35 ± 0.53g were used. The fish were randomly assigned to give a loading of 16 (sixteen fish per test tank). They were fed to satiation once daily on compounded pelletised fish food. The feed was crushed into smaller units before being fed to the fish. Natural photoperiod of 12h light and 12h darkness that prevailed during the period of the assay was used since the room was well lit with sunlight rays streaming into the laboratory.

Opercular ventilation rate and tail fin movement rate per min were observed on four fish per tank marked by the little cut on tail fin once forthnightly.

3.6 GROWTH STUDY

Four tanks with the appropriate toxicant concentrations were set up separately including the control. Ten fish of weight range 3.84 - 7.64g and mean weight of 5.74 ± 2.69g were assigned to each tank randomly. Fish were fed to satiation once daily. Subsequent weighing was done once in two weeks for a period of 8 weeks. Constant monitoring of water parameters.

and changing of test solutions twice weeklywere carried out through-out the test period. Photoperiod of 12h day and 12h night was maintained. Weight gain and percentage cumulative weight gain were worked out at the end of the test.

3.7 HAEMATOLOGY

The fish were exposed to various concentrations of paraquat for 10 weeks. Four fish were removed forthnightly from the sixteen in each test tank, stunned for immobilization instead of anaesthesizing them (Korcock et al. 1988). It was explained that when specimen recovery is not a consideration, the possibility of anaesthetics - induced errors can be avoided by stunning animals before sampling for blood (Korcock et al., 1988). Stunning was effected by a sharp blow at the juncture of Cranium and Spine with a metal wedge which caused irreparable central nervous system damage and immediate paralysis (Korcock et al., 1988).

3.7.1 BLOOD SAMPLING TECHNIQUE

Blaxhall (1972) proposed a method which was adopted in this study. The stunned fish was placed on its side on paper towel, dried with the clean towel and blood was withdrawn by Cardiac puncture using a 2ml sterile plastic syringe and needle. The use of plastic syringes is a necessary precaution with fish blood because contact with glass results in shortened coagulation times (Smith et al., 1952). The site chosen for

the puncture was about half and inch behind the apex of the "V" formed by the gill covers and Isthmus (Klontz and Smith, 1968). The blood drawn into the syringe was decanted into a vial containing 100g/L EDTA (Potassium salt of ethylene diamine tetra-acetic acid). Five milligram of EDTA per cm^3 blood was used (Owolabi, 1995). Hesser (1960) Cautioned that excessive pressure should be avoided when expressing blood through the needle to prevent lysis of the cells. Wintrobe (1962) reported on the use of various anticoagulants and recommended EDTA for human haematology. He opined that heparin had been used satisfactorily as an anti-coagulant by many investigators but it did not prevent clotting as completely as EDTA when mixed with mountain white fish blood. The rate of coagulation of the fish blood was so fast that a little of the EDTA had to be introduced into the syringe and needle.

3.7.2 PREPARATION AND FIXATION OF BLOOD FILM

Smears of the blood sample obtained by cardiac puncture were prepared. The smear was allowed to air dry. It was then immersed in absolute methanol i.e 5g/25L of methanol (Owolabi, 1995) for at least 5 minutes. It was air - dried again (Klontz, 1972). It was stained with Giemsa stain with 1% PH 6.4 phosphate buffer. Leucocyte differential count was then taken from the slide. The micro-photographs of the blood cell of the fish were taken.

3.7.3 HEMATOCRIT (HCT)

To determine the haematocrit, blood samples were collected in heparinized Capillary tubes, the end of the tubes were sealed with plasticine and the tubes spun for 7 minutes in a micro - haematocrit centrifuge at a speed of 12,500ppm. The hematocrit were measured with a microcapillary reader.

3.7.4 HAEMOGLOBIN (Hb)

The cyanomethemoglobin technique was used (Wintrobe, 1962). After thorughly mixing the blood, 0.02cm^3 of blood was placed into 4cm^3 of Drabkin's reagent. The solution was gently mixed by inversion and allowed to stand for at least 10min for full conversion of haemoglobin to cyanomethaemoglobin. The small coagulum formed was removed with a wooden stick. Transmittance was read on a EEL spectrophotometer at a wavelength of 540mm and this reading was converted to haemoglobin Concentration in grams per 100cm^3 by reference to a graph constructed using commercially available cyanomethaemoglobin standards.

3.7.5 LEUCOCYTE DIFFERENTIAL COUNT

Differential Count refers to the difference in the appearance of the blood cells (Hessler, 1960). the count was read on the slide with the aid of microscope and a counter. Blood chart for fish was prepared and used for identification of the different cell types. For the chart, aid was obtained from works done by Klontz (1972) Blaxhall and Daisely (1973 and

Murad et al., (1990).

3.8 HISTOPATHOLOGY

Gills, livers and brains of fish exposed to 1.02mgl^{-1}, 1.4mgl^{-1} and 2.0mgl^{-1} of paraquat for 10 weeks were fixed in 10% neutral buffered formalin for 24hours (Thompson and Hunt, 1966; Alan et al., 1983). They were then washed, dehydrated, infilterated and blocked in paraffin wax of 59-60°C melting point. All sections of 8μ in thickness were cut and stained with haematoxylin and eosin (H&E). These processes followed standard procedures.

3.9 STATISTICAL ANALYSIS

All data for paraquat treated fish were compared with the control using analysis of variance test (ANOVA) and Duncan Multiple Range Test (DMRT) at 95% probability level.

CHAPTER FOUR

4.0 R E S U L T S

4.1 ACUTE TOXICITY TEST

The percentage mortality trend in the toxicity of paraquat to Oreochromis niloticus (Trewavas) followed a linear pattern as as shown in Fig. 1 with the highest percent mortality recorded in concentration 16.00mgL-1. No mortality was recorded in any of the controls (Table 4).

Symptoms of toxicosis indicated that paraquat caused sub-acute effects that change fish behaviour. Such observed behaviour included air gulping, agitated swimming, haemorrhaging of the pectoral fins, blood-shot eyes and irregular and erratic movement. Disorientation in movement was also observed generally before the death of fish. The fish were also observed to be motionless at the bottom of the tanks for 10-15 minutes immediately they were introduced into the tanks, before making any attempt at swimming. Longer time of quiescence was observed in fish exposed to higher concentrations of paraquat.

Other behavioural changes observed included loss of equilibrium and vertical swimming.The reactions were more pronounced in fish tanks of higher toxicant concentrations. There was an increase in the activities of mucus cells which resulted in the production of copious amount of mucus over the body.

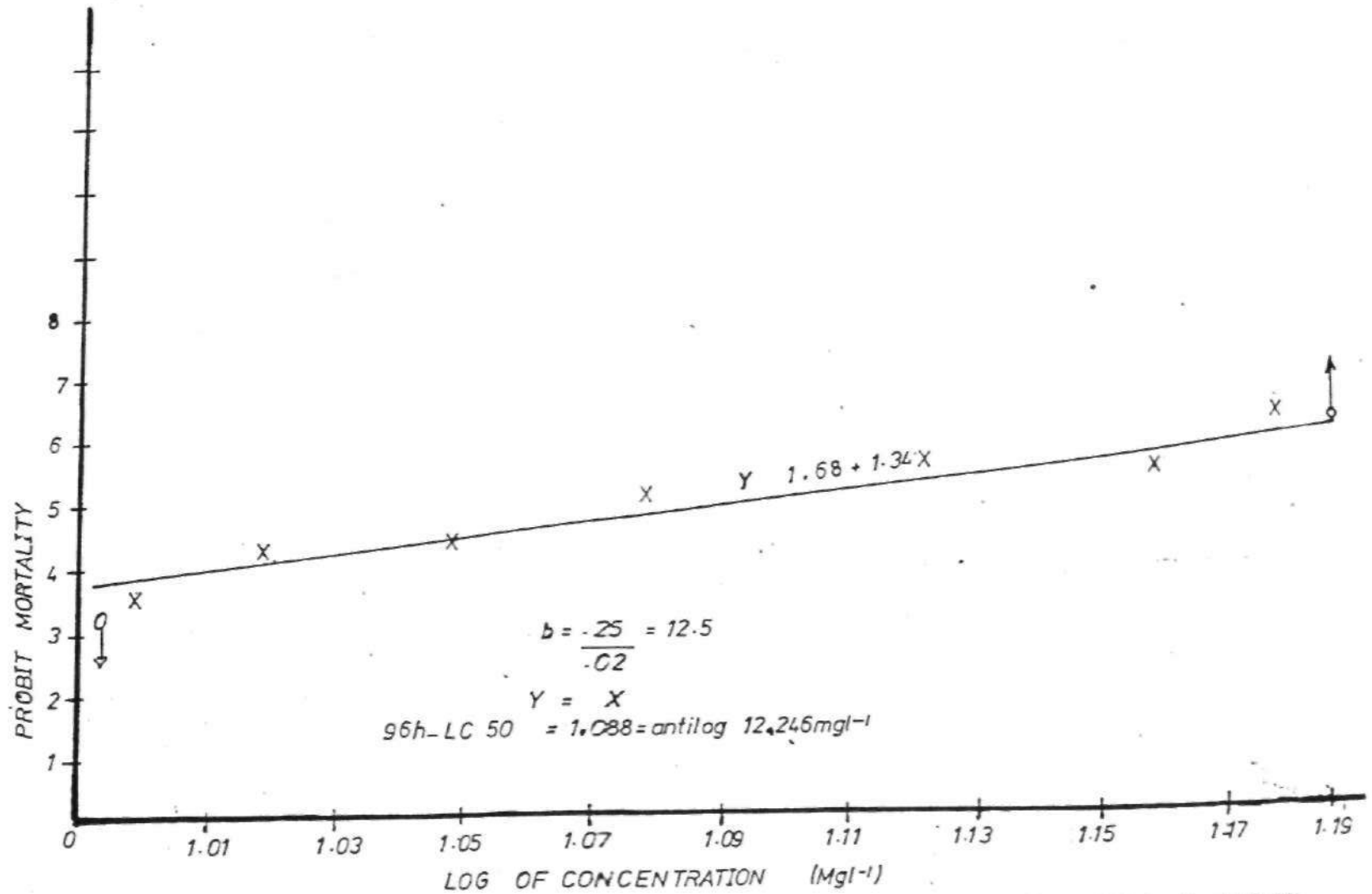


Fig. LINEAR RELATIONSHIP BETWEEN PROBIT MORTALITY (%) AND LOG_{10} CONCENTRATION (mgl^{-1}) OF O. niloticus EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT.

TABLE 4: MORTALITY IN O. niloticus FINGERLINGS EXPOSED TO ACUTE CONCENTRATIONS OF PARAQUAT FOR 96-HOURS

Conc (mgl-1)	0.00		9.60		10.40		11.20		12.00		14.20		15.20		16.00.	
Period of exposure	R1.	R2	R1.	R2	R1.	R2	R1.	R2	R1.	R2	R1.	R2	R1.	R2	R1.	R2
12 HOURS	-	-	-	-	-	-	1	1	-	-	-	-	-	-	2	2
24 "	-	-	-	-	1	1	1	1	1	1	-	-	2	2	2	2
36 "	-	-	-	-	1	1	-	-	-	-	1	1	-	-	2	2
48 "	-	-	-	-	-	-	-	-	-	-	2	1	1	1	4	4
60 "	-	-	-	-	-	-	-	-	-	-	1	2	3	3	-	-
72 "	-	-	-	-	-	-	-	-	-	2	2	2	2	2	-	-
96 "	-	-	1	1	-	-	1	1	3	3	1	1	1	1	-	-
Mortality	0/10	0/10	1/10	1/10	2/10	2/10	3/10	3/10	6/10	6/10	7/10	7/10	9/10	9/10	10/10	10/10

TABLE 5: MORTALITY RATES , PERCENT MORTALITY AND PROBIT KILL IN O. niloticus EXPOSED TO ACUTE CONCENTRATION OF PARAQUAT

Conc mgL^{-1}	$Log._{10}$ of conc (mgl^{-1})	Mean Mortality Rate in Duplicate	Mean% Mortality	%probit
0.00	0.00	0/10	0%	- -
9.60	0.98	1/10	10%	3.72
10.40	1.02	2/10	20%	4.16
11.20	1.05	3/10	30%	4.48
12.00	1.08	6/10	60%	5.25
14.20	1.16	7/10	70%	5.52
15.20	1.18	9/10	90%	6.28
16.00	1.20	10/10	100%	- -

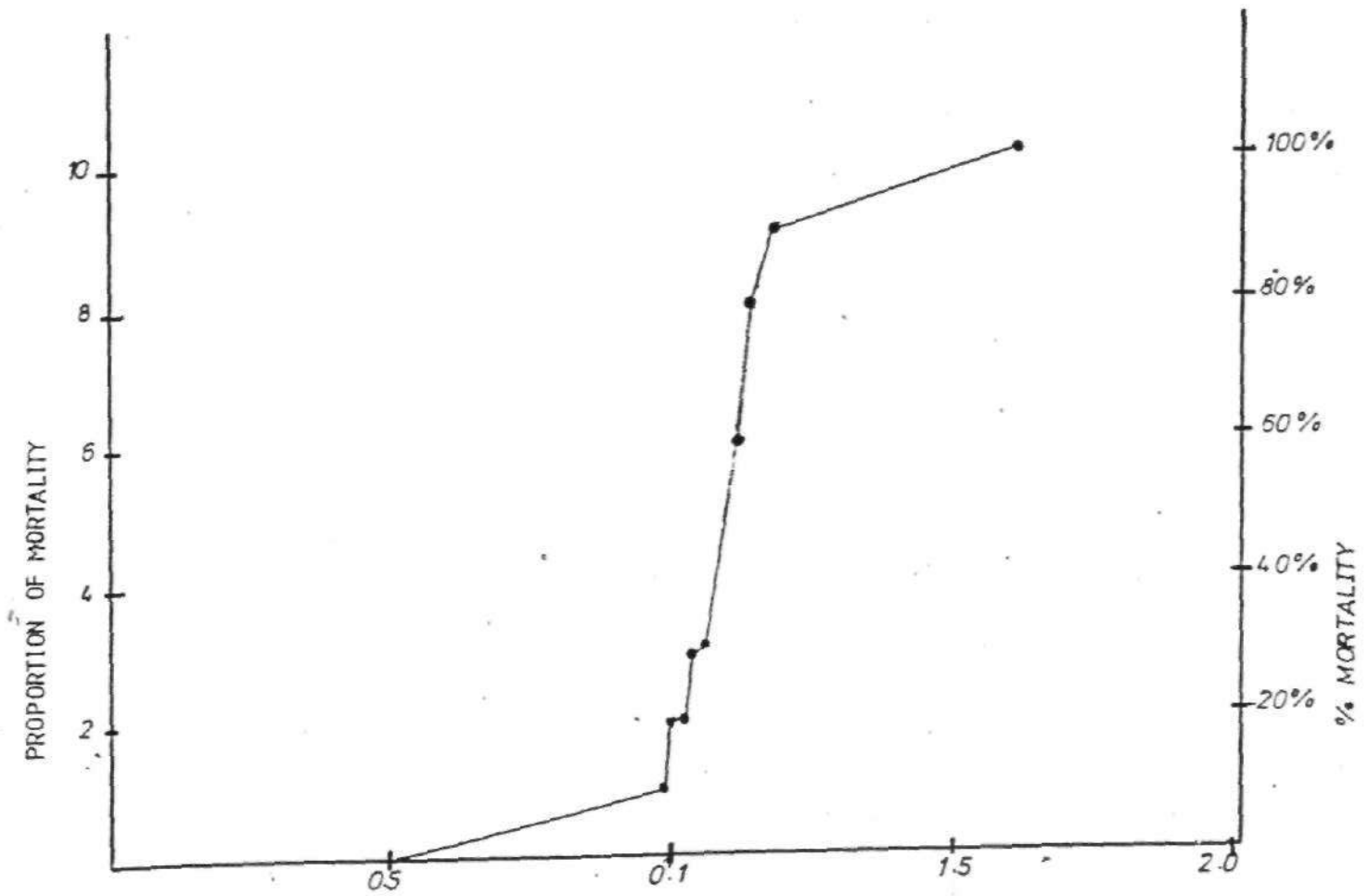


Fig 2: THE SIGMOID DOSE – RESPONSE CURVE OF PROPORTION OF MORTALITY OF *O. niloticus*

TABLE 6: PARAQUAT CONCENTRATION DATA SET OUT FOR CONVENIENCE OF CALCULATING OBSERVED AND EXPECTED PROBITS AND THEIR WEIGHTING

CONC Mgl^{-1}	Mortality Ratio	Mortality Percent (%)	Probit of Observed mortality	expected Probit	Weight(W) Associated with expected Probit(b)
0	0/10	0	-	3.75	0.353
9.60	1/10	10	3.72	3.90	0.405
10.40	2/10	20	4.16	4.20	0.503
11.20	3/10	30	4.48	4.55	0.681
12.00	6/10	60	5.25	4.90	0.634
14.20	7/10	70	5.52	5.90	0.471
15.00	9/10	90	6.28	6.15	0.410
16.00	10/10	100	-	6.35	0.319
					EW3.776
					-
					W 0.472

Wardlaw, A.C. (1989). Practical Statistics for Experimental Biologists. A. Wiley-Interscience Publication 290pp.

TABLE 7: 95% CONFIDNECE LIMIT ANALYSIS

$$S\log_{10} LC_{50} = \sqrt[1]{b\ pnw}$$

b = The slope of the log - dose/probit response line

P = The number of dilutions in the titration inluding the two points (for control and 100% mortality) which are not in each group

n = The number of animals in each group

W = The average weight of the observations

b = 12.5

p = 8

n = 10

W = 0.472.

$$S \log 10\ LC50 = \frac{1}{12.5\sqrt{8x10x0.472}}$$

$$= \frac{1}{12.5\sqrt{37.76}}$$

$$= \frac{1}{12.5 \times 6.145}$$

$$= \frac{1}{76.811}$$

$$= 0.013$$

$$\log 10\ (95\%n\ CL) = \log_{10} LC_{50} \pm 1.96\ (0.013)$$

$$= \log_{10} LC_{50} \pm 0.0265$$

Antilog of both = 12.245 ± 1.06

The LC_{50} and its 95% confidence limits described the distribution of the susceptibility of the individual test organisms Alpha (1980) in standard method, explained that it is recommended that the 95% confidence interval be less than ± 30%. The result of the 95% confidence limit of this study agrees with that recommendation.

The 96-h lethal concentration of paraquat and the 95% confidence limit for the fingerlings of O. niloticus were 12.25 ± 1.06 (Table 7 and Fig. 1).

The results show that concentrations below 9.60mgl^{-1} were not lethal to the fish. Increase in concentration to 10.40mgl^{-1} resulted in 20% mortality. The highest percentage mortality (100%) was recorded at a concentration of 16.00mgl^{-1} at 48 hours.

4.2 OPERCULAR MOVEMENT DURING THE 96-HOUR ACUTE TOXICITY TEST

It was observed that the higher the concentration, the lower the opercular movement during the first 48h while the fish in the control showed a high and relatively constant ventilation rate was maintained throughout the test periods. At 72h and 96h an increase in opercular movement was observed in all the test solutions showing hyper ventilation (Fig. 3b). Statistical analysis showed a significant difference ($P<0.05$) for the 4 days of exposure. Also a highly significant difference was observed in the effect of concentrations on the opercular movement for the 96-hour periods ($P<0.01$)

show a decrease in content with increase concentration. However at 12.00mgl-1, there is a sharp increase in the MCHC value. Lymphocyte (%) decreases slightly with increase in the concentration of the toxicant solution while neutrophils (%) shows no appreciable difference in value. Statistical analysis shows no significant difference (P>0.05) (Appendices 10 and 11). (The data presented in table 8 has been used to plot Fig. 3a).

4.3 HISTOPATHOLOGY

The gills of the remaining fish at 96h show some histological damages as seen in plate VII-IX. With increasing toxicant concentration from 11.20mgl^{-1}, 12.00mgl^{-1} to 14.20mgl^{-1} there is an increase in the disruption of the architecture of the gills. There is congestion of lamellae and total destruction of the filaments at concentration 14.20mgl^{-1}. There is a mild hyperplasia at 11.20mgl^{-1} of paraquat concentration but atrophy is observed only at 12.00 and 14.20mgl^{-1}

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TABLE 8: Mean haemoglobin (g/100ml blood) haematocrit (PCV) and mean corpuscular haemoglobin concentration (MCHC) of O. niloticus exposed to paraquat for 96h (4 days) at 24°C Means ±S.E. = of 3 fish are presented.

Conc (mgl^{-1})	g/100ml Haemoglobin (HB)	Haematocrit (HCT)	MCHC	% L	% N	% M
0	2.95±.35	11.5±2.12	25.8±1.69	92±28	8±2.83	-
9.60	4.4±1.13	18.5±2.12	23.6±3.39	90±11	8±2.83	3±0
12.00	3.4±0.28	4.5±6.36	35.8±0.28	90±0	10±0	-
14.20	Denatured & Coagulated	-	-	-	-	-

Baseline values determined before fish were alloted to the exposure groups were 5.55±0.35, 17.5±0.71, 31.70±0.74, 82±5.66, 14±5.65, for haemoglobin (Hb) haematocrit (Hct, MCHC, Lymphocyte (L) and Neutrophil (N) respectively. Means ± S.E of 3 samples

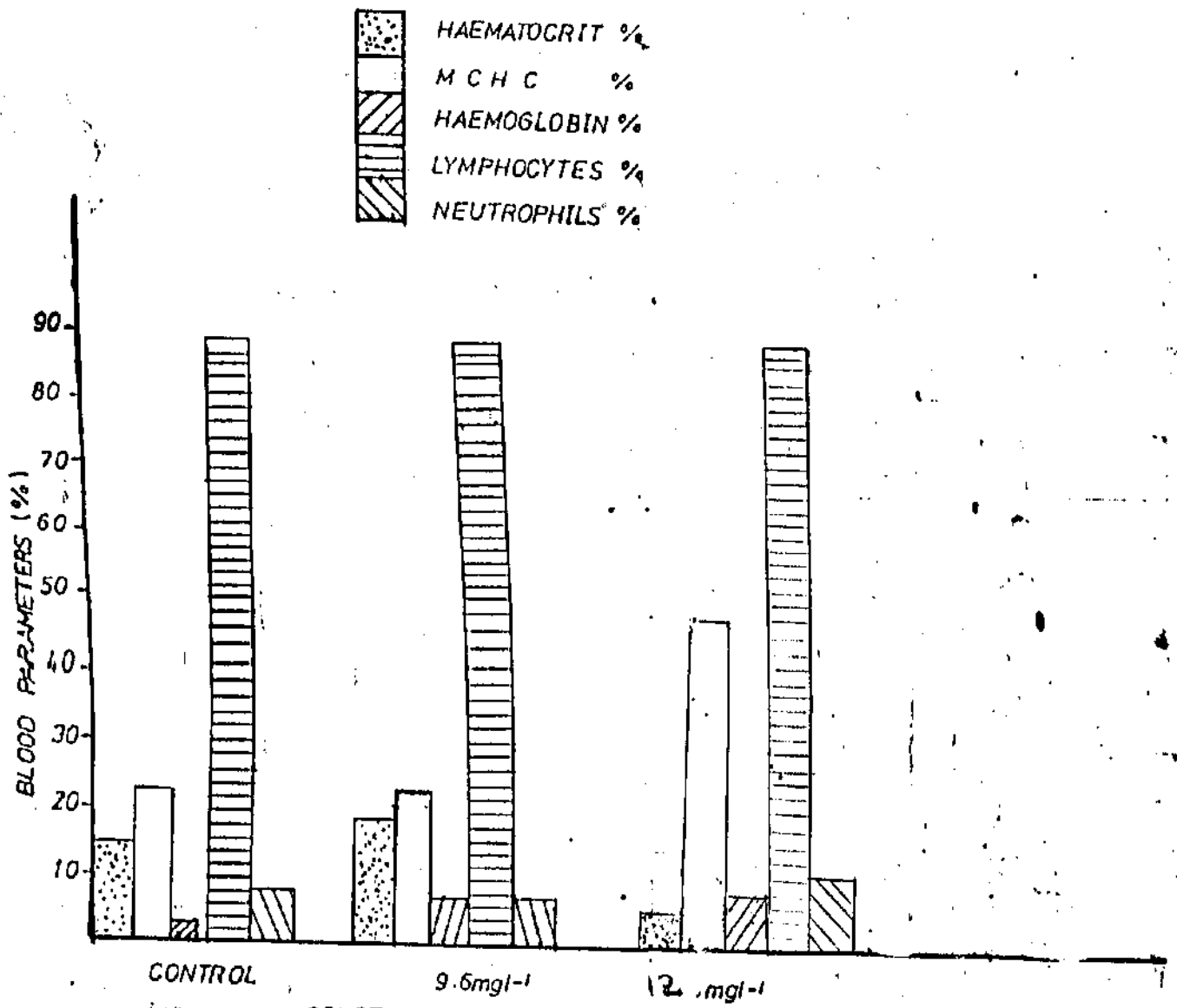


Fig.3a BLOOD PARAMETERS OF *O niloticus* EXPOSED TO PARAQUAT FOR 96 hr

TABLE 9: Opercular movement of O. niloticus exposed to different paraquat concentrations for 96 hours Means ± S.E. of 4 fish samples are presented.

Conc mgl^{-1}	Duration (days)				
	0	1	2	3	4
0	75±4	75±4	95±4	93±1	113±3
9.60	51±1	78±9	93±4	87±15	120±1
10.40	76±6	64±7	95±7	34±4	62±16
11.20	66±4	28±5	19±2	28±9	78±6
12.00	67±18	29±6	24±7	80±13	86±6
14.20	45±10	40±3	74±10	89±2	62±11
15.20	46±2	44±2	32±5	73±14	-

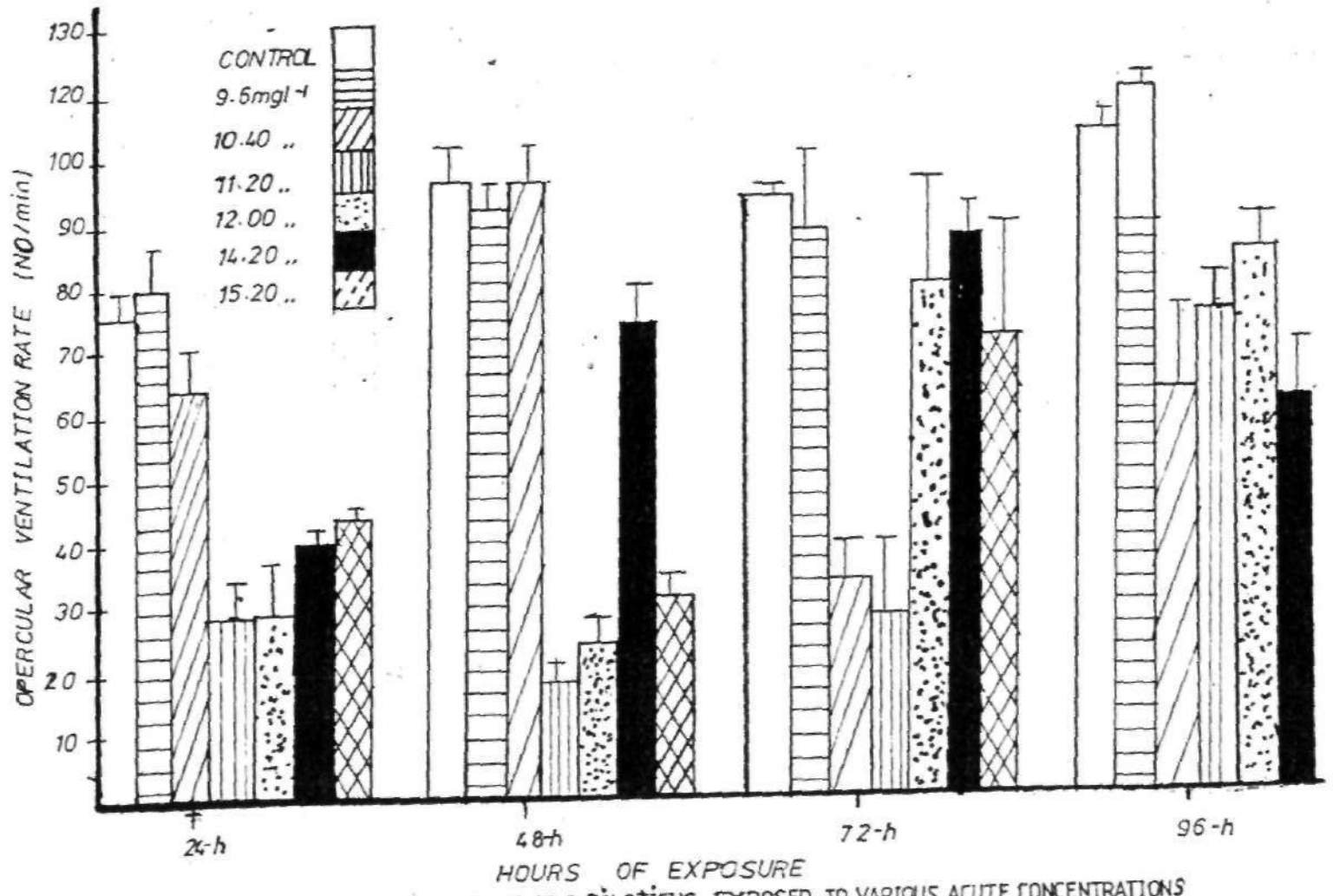


Fig 3 OPERCULAR VENTILATION RATE OF O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT DURING 96-hr BIOASSAY

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TABLE 10: TAIL FIN RATE (rate per minute) of O. niloticus exposed to various acute concentrations of paraquat in 96-hour test. Mean ± S.E of 4 fish are presented.

Conc (mgl^{-1})	PERIODS IN HOURS 0	24	48	72	96
0	33±5	9±0	3±6	10±7	27±11
9.60	69±12	37±5	28±5	54±2	26±6
11.20	62±15	23±4	16±3	2±1	4±5
12.00	9±8	6±3	21±9	12±4	22±4
14.20	7±1	8±4	7±2	1±1	8±2
15.20	6±3	27±11	1±0	6±0	-

4.4 SUB-LETHAL TOXICITY

EFFECT OF PARAQUAT ON GROWTH

The initial mean wet weight of the test fish were 7.64±1.3 for the control and 6.33±0.9, 6.88±0.9, and 3.2±0.7 for the fish exposed to 1.02, 1,40 and 2.0mgl^{-1} of paraquat in water respectively. Growth was estimated from the sum of the individual wet weights of the fish at 2,4, 6 and 8 weeks of exposure to the toxicant and expressed as percent cumulative wet weight gain.

The results show that the cumulative percent wet weight gain in the control group increased from 10.34% at 1 week to 36.13% at 8 weeks (Fig. 5). The growth of fish that were

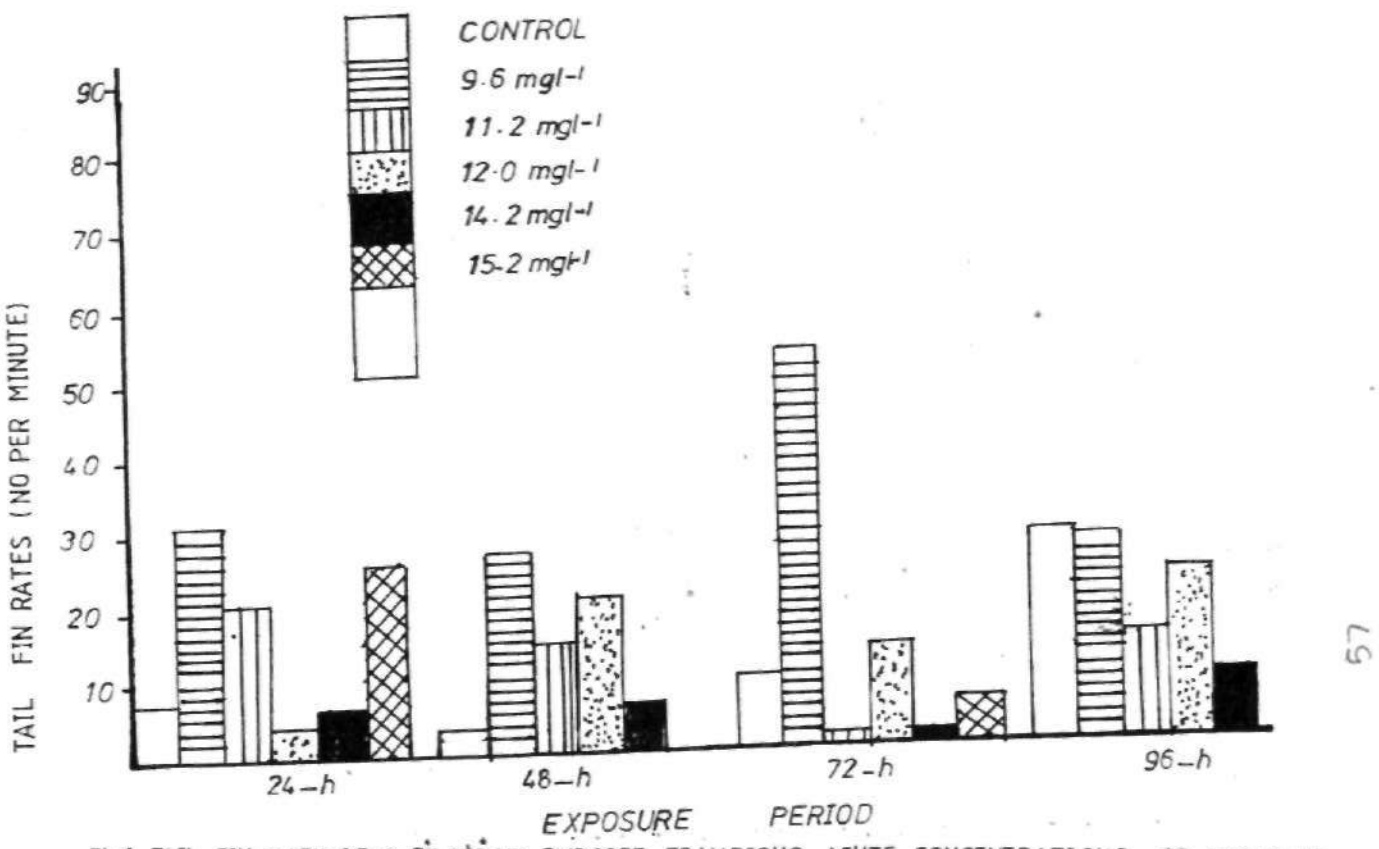


Fig4. TAIL- FIN RATES OF O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT

exposed to 1.02, 1.04 and 2.00mgl-1 increased from 3%, 0.44% and 1.55% at 1 week to 30.81%, 9.59% and 19.81% at 8weeks respectively. A statistically significant difference in growth rate of the treated and control was observed ($P<0.05$).

TABLE 11 Mean weight (g) of O. niloticus exposed to various paraquat concentrations in Sub-lethal toxicity test for a period of 8 weeks. Means± S.E 10 fish are presented.

Exposure Levels (mgl $^{-1}$)	EXPOSURE PERIODS W E E K S				
	0	2	4	6	8
0	7.64±1.3	8.43±2.9	9.48±1.0	10.01±1.0	10.40±3.2
1.02	6.33±0.9	6.52±0.9	7.34±1.0	8.10±1.0	8.28±0.9
1.40	6.88±0.9	6.85±0.9	7.15±0.9	7.33±1.0	7.54±1.0
2.0	3.23±0.7	3.28±0.7	4.24±0.8	3.70±0.7	3.87±0.7

TABLE 12: Weight gain and % Cumulative weight gain in O. niloticus during sub-lethal exposure to paraquat concentrations for a period of 8 weeks.

Exposure Levels (mgl^{-1})	DURATION (WEEKS)			
	2	4	6	8
	0.79	1.84	2.37	2.76
1.00	10.34%	24.04%	31.02%	36.13%
	0.19	1.01	1.77	1.95
1.02	3.0%	15.96%	27.96%	30.81%
	0.03	0.27	0.45	0.66
1.40	0.44%	3.92%	6.54%	9.59%
	0.05	1.01	0.47	0.64
2.00	1.54%	31.27%	14.55%	19.81%

4.5 EFFECT OF PARAQUAT ON OPERCULAR VENTILATION IN SUB-LETHAL TEST

The results show that there was not much difference throughout the 8 weeks in the ventilation rate of the control fish. However there was a higher opercular ventilation rate on the first 2 weeks which was subsequently reduced towards the 8 weeks in the control(Fig. 6). Statistically there is significant difference within the treatments in terms of week, with week 1 control fish showing hyper-ventilation and week 4

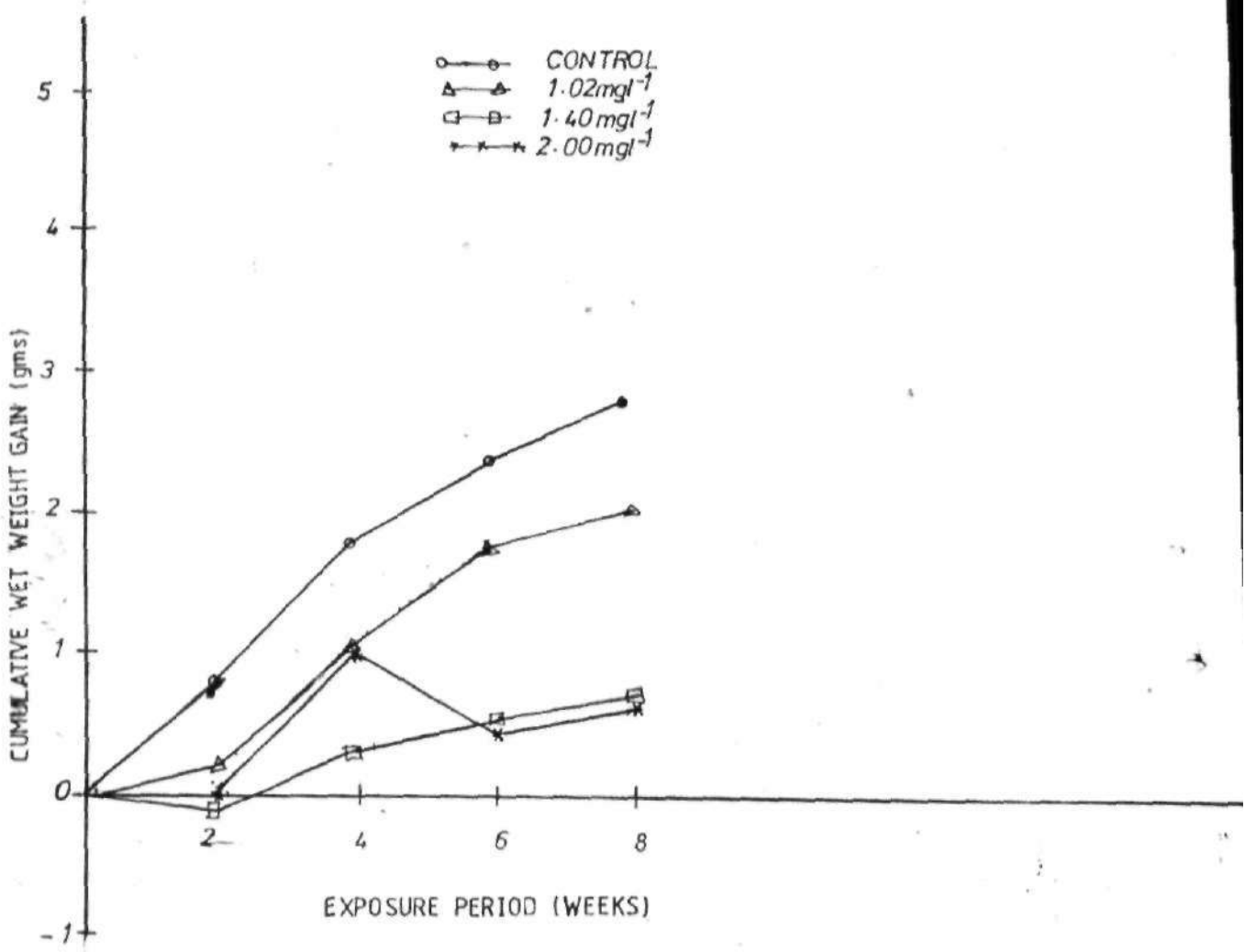


Fig 5 *CUMULATIVE WET WEIGHT GAIN OF* *O. niloticus* EXPOSED TO VARIOUS CONCENTRATIONS OF PARAQUAT FOR 8WEEKS AT 25°C

fish showing the least value in ventilation rate. The control fish exhibited a significantly higher ($P<0.05$) mean value for ventilation rate as compared to fish in other test solutions. Toxicant solutions of 1.02, 1.40, and 2.00 mgl^{-1} showed decrease in ventilation rate of fish for the 6 weeks of exposure period and an increase on the 8th week. However, there was no significant difference statistically in ventilation rate of fish exposed to different concentrations of paraquat ($P>0.05$) (Appendix 15).

TABLE 13: Opercular ventilation Rate (No./min) of O. niloticus exposed to Sublethal concentrations of paraquat for 8 weeks. Means ± S.E of 4 fish are presented.

Exposure Levels (mgl^{-1})	EXPOSURE PERIODS WEEKS				
	0	2	4	6	8
0.00	110±7	111±2	110±4	110±7	102±9
1.02	115±7	92±8	78±4	77±3	78±8
1.40	116±4	108±8	76±4	68±5	88±
2.0	111±4	107±4	85±2	79±7	88±5

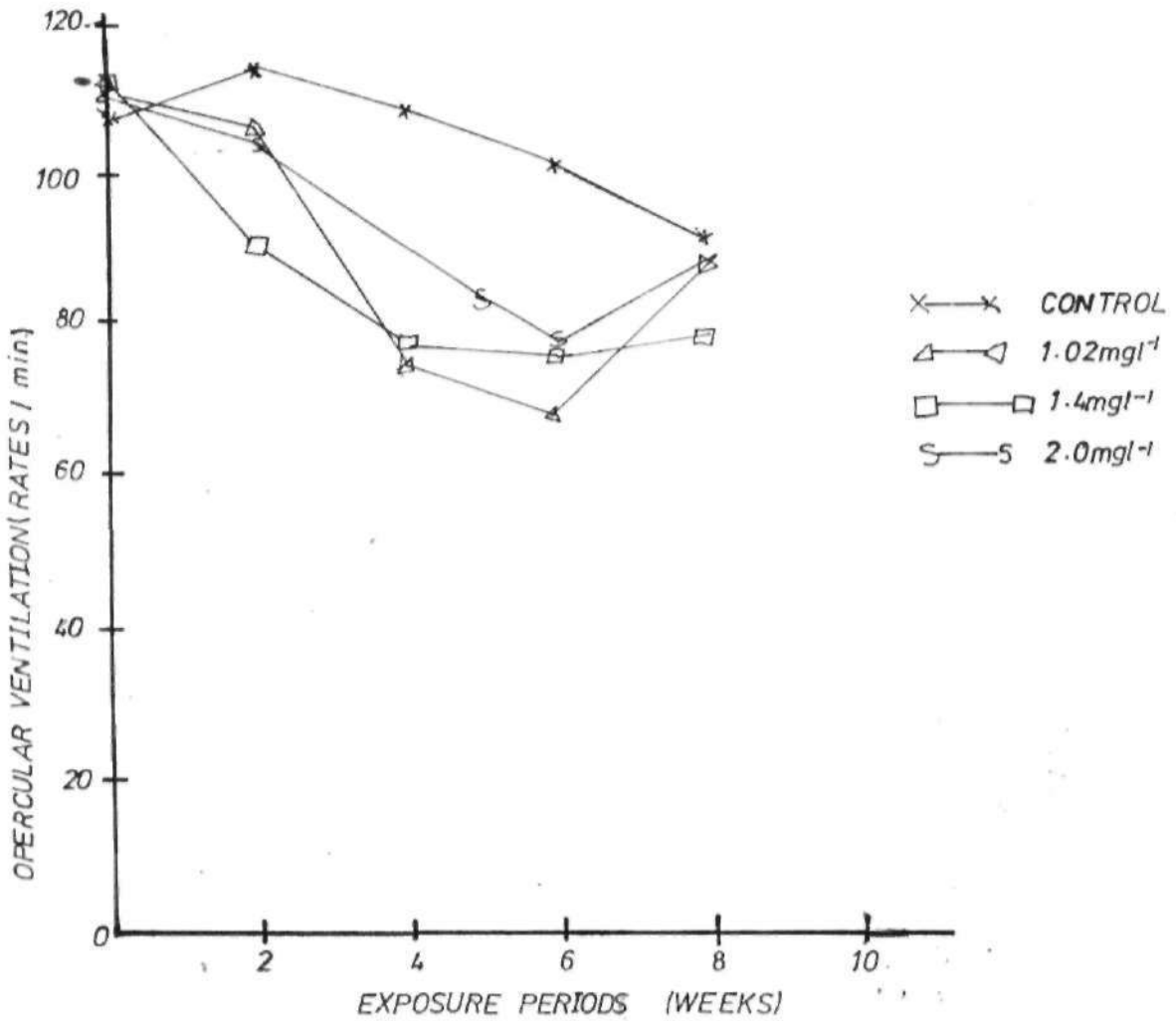
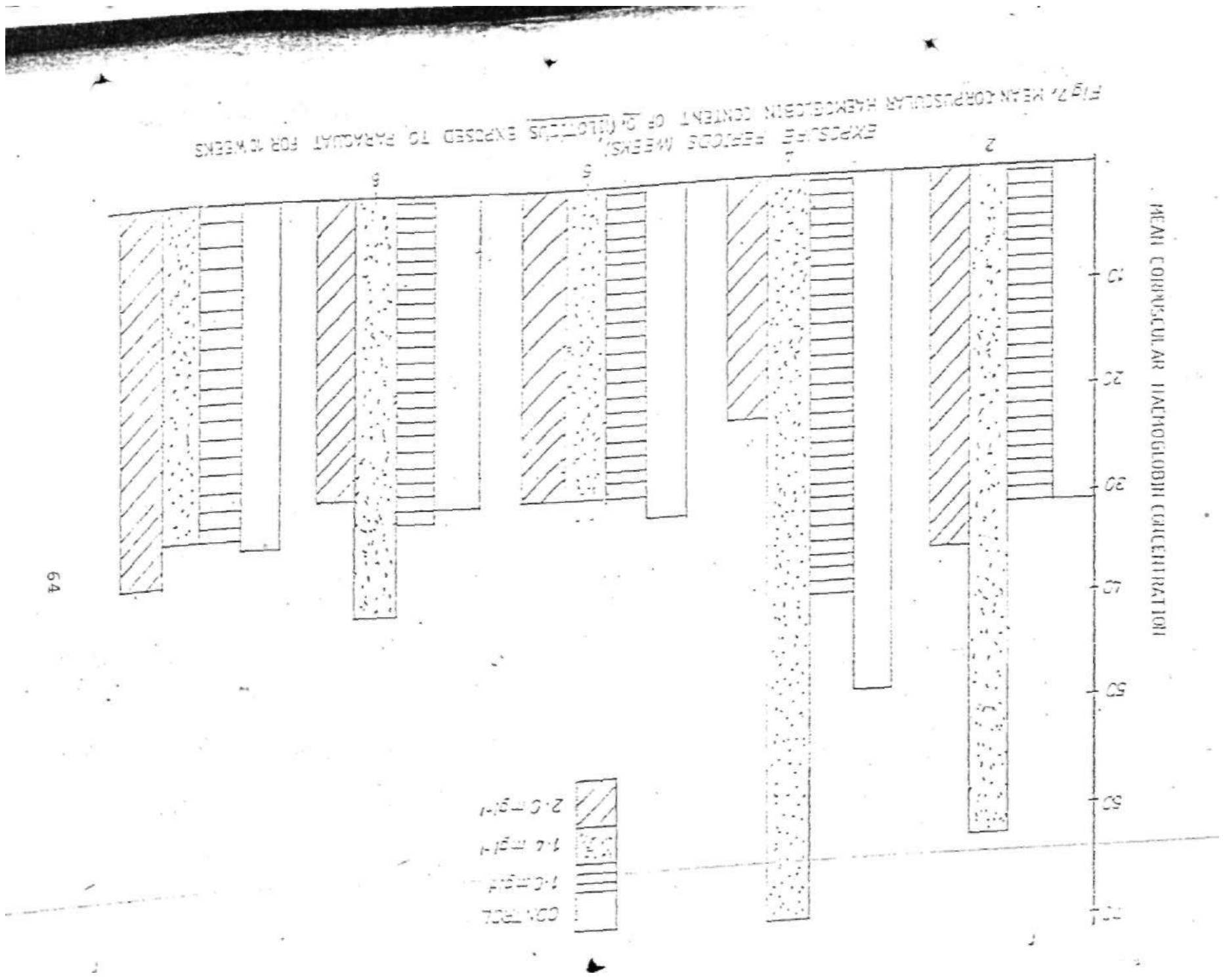


Fig. 6 OPERCULAR VENTILATION RATE OF O.niloticus EXPOSED TO SUBLETHAL CONCENTRATIONS OF PARAQUAT FOR 8 WEEKS

TABLE 14: Mean haemoglobin (g/100ml blood), haematocrit and mean corpuscular haemoglobin concentration (MCHC) of O. niloticus exposed to sub-lethal concentrations of paraquat for 8 weeks at 22.7oC ± 0.8. Means ±S.E of 4 fish are presented.

Conc mgl^{-1}	WEEKS OF EXPOSURE 2	4	6	8	10
			HAEMATOCRIT %		
0.00	14.5±0.71	13.0±1.41	11.5±0.71	14.5±0.71	11±0
1.02	12.0±1.41	9.5±0.70	9.5±0.7	14.0±1.41	-
1.40	7.5±0.71	0±0.0	9.0±0.0	9.5±0.70	-
2.00	0±0.0	17.0±1.40	9.5±0.70	12.0±2.80	-
			HAEMOGLOBIN (HB) g/100ml		
0.00	4.67±0.01	4.45±.07	3.6±.4	4.3±0.42	3.72±0.19
1.02	3.59±.01	3.70±.14	2.75±.7	3.6±0.57	3.26±0.07
1.40	4.35±.07	7.29±.01	2.7±.07	3.8±0.28	3.30±0.14
2.00	3.67±.07	3.58±.04	2.7+.07	3.4±0.28	3.65±0.07
			MCHC (HB/Hct) %		
0.00	32.7±0.07	48.8±0.71	32.7±.01	29.65±1.5	32.70±0.07
1.02	32.7±0.07	40.0±0.35	30.0±.35	26.15±2.19	32.00±0.41
1.40	62.9±0.41	73.0±0.35	30.0±.41	40.±0.0	32.00±0.00
2.00	36.0±0	22.5±0.00	30.0±.07	28.85±450	36.00±3.50

Base line values determined before fish were alloted to the exposure groups were 5.55±0.35, 17.5±0.71, 31.70±0.74, 82±5.66, 14±5.65, for haemoglobin (HB) heamatocrit (Hct) MCHC, Lymphocyte (L) and Neutrophil (N) respectively. Means ±S.E of 4 fish are represented.


MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION
EXPOSURE PERIODS (WEEKS)
2
4
6
8
Fig 7. MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION

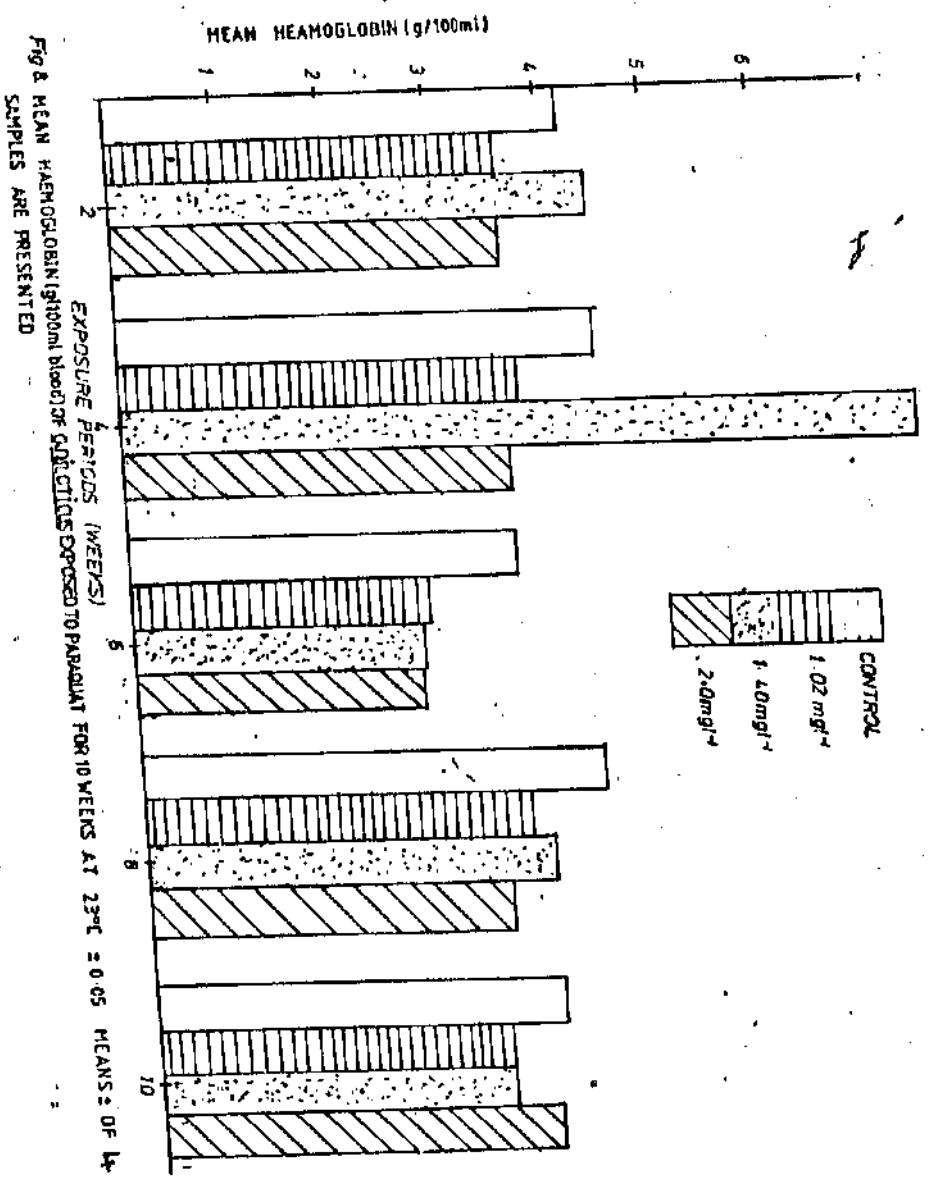


Fig 8 MEAN HAEMOGLOBIN (g/100ml blood) OF GOLDFISH EXPOSED TO PARAQUAT FOR 10 WEEKS AT 23°C ± 0·5 MEANS ± OF 4 SAMPLES ARE PRESENTED

65

Fig. 6. Mean haematocrit of 5 pulses exposed to various concentrations of methacholine for 10 weeks (5 weeks exposure periods)

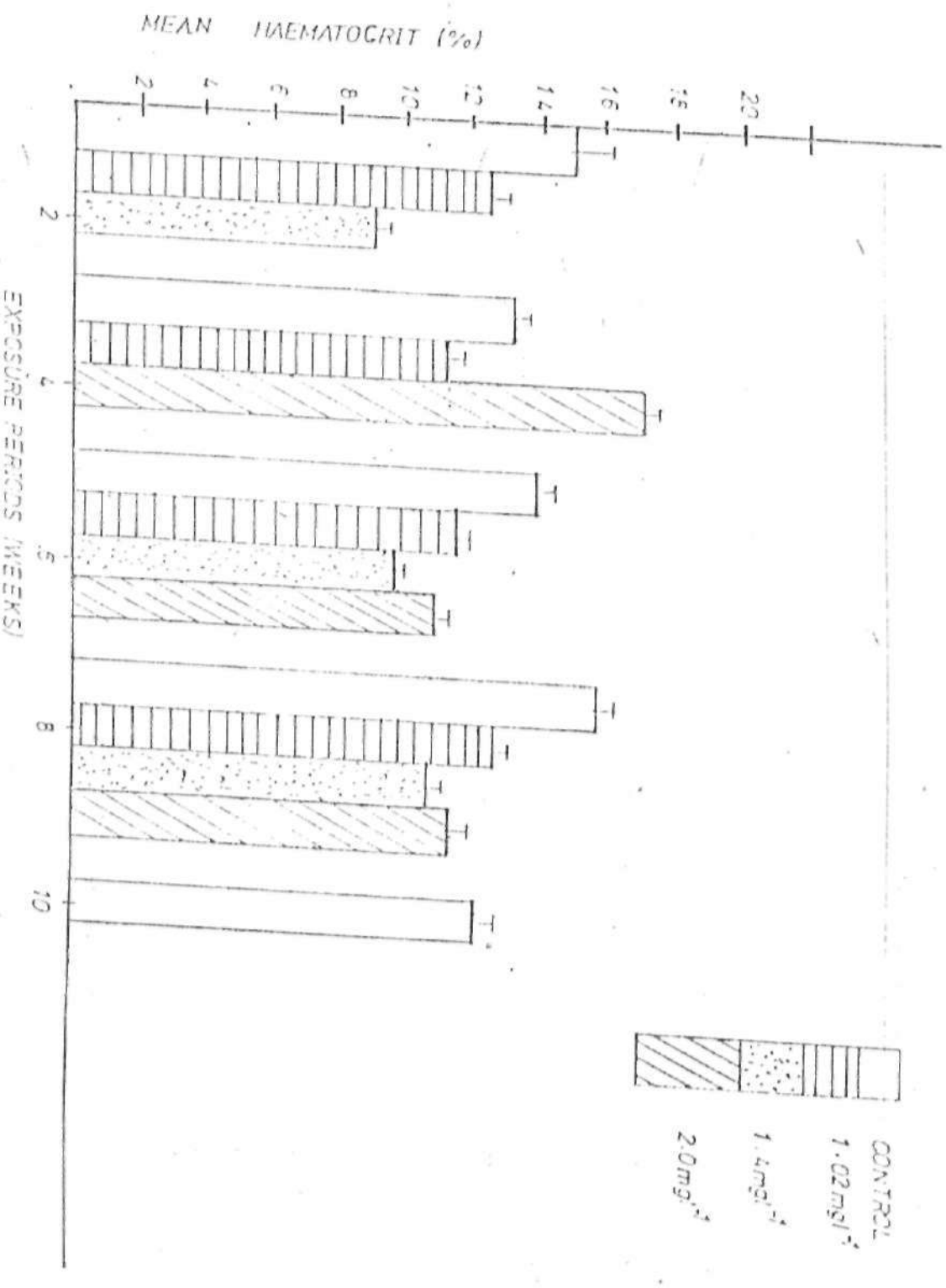


TABLE 15: WBC (Leucocyte) differential blood count (%) for blood of O. niloticus exposed to sublethal concentration of paraquat for 10 weeks. Means ± S.E. of 4 fish are presented.

Conc	WEEKS OF EXPOSURE				
mgl^{-1}	2	4	6	8	10
		LYMPHOCYTES			
0.00	83±0.35	90±0.07	90±0.41	77±1.4	88±0
1.02	88±0.71	86±0.41	89±0.07	89±0.71	-
1.40	84±0.07	0.0	91±0.07	96±0.0	-
2.00	0	89±0.71	88±.35	99±4.0	-
		NEUTROPHILS (GRANULOCYTES)%			
0.00	16±.71	10±.41	10±0.10	22±2.8	-
1.02	12±.07	14±1.4	9±1.4	10±0.7	-
1.40	16±.35	0.0	9±.35	4±0.0	-
2.00	0.0	11±0	12±.07	14±1.4	-

(-) or (0) refers to senile or completely destroyed blood thus no values for the blood parameters

4.6 EFFECT OF PARAQUAT ON HAEMATOLOGICAL PARAMETERS IN SUBLETHAL TEST

Haemoglobin and haematocrit of the control fish did not deviate much from the base line values throughout the test period (Table 14). The haematocrit of the control decreased slightly with time from an initial 15% to 12% at the 6th week of exposure, after which value increased to 14.5%. Haematocrit of O. niloticus exposed to different concentrations of paraquat showed a dose-dependent relationship in which there was decrease in haematocrit value with increase in concentration. However at week 4, blood of fish exposed to paraquat concentration of $1.40mgl^{-1}$ showed total denaturation of blood cells. The same phenomenon was seen in the highest toxicant concentration ($2.0mgl^{-1}$) at week 2. Statistical analysis shows a significant difference in both the within and between treatment. Thus a dose and period dependent effect are shown (Appendix 4).

HAEMOGLOBIN (g/100ml)

The haemoglobin concentration of O. niloticus exposed to various sublethal concentrations of paraquat are presented in table 14. The results show that after 2 weeks of exposure there was a decrease in haemoglobin value with increase in concentration of the toxicant. Haemoglobin concentration in blood of fish exposed to toxicant concentration of $1.40mgl^{-1}$ was close to that of control. The same pattern was observed after

6 weeks of exposure, indicating a dose-dependent decrease in haemoglobin concentration. Analysis of variance shows a significant difference in various haemoglobin Values of the fish exposed to different sublethal concentrations (ANOVA and DMRT<.05) (Appendix 5). The haemoglobin content of the fish blood decreased with increase in exposure duration till 6 weeks, followed by an increase in haemoglobin value by the 8th week. There is a significant difference between control and other toxicant concentration Values.

The mean corpuscular haemoglobin concentration Values of O. niloticus exposed to various paraquat concentration, are presented in Table 14. The baseline mean value is 31.70% which shows no significant difference from that of control throughout the duration of the experiment except at 2 weeks when the value increased only to return to baseline value thereafter. values of fish exposed to Toxicant concentration of $2.0mgl^{-1}$ showed a decrease right from the beginning but later towards the end an increase in value. Fish exposed to $1.40mgl^{-1}$ paraquat showed the greatest variation in values. Statistical analysis shows a significant difference ($P<0.05$) (Appendix 7).

The Lymphocyte (%) of O. niloticus exposed to various Concentrations of paraquat are presented in Table 15. The results show that at the start of the experiment, the lymphocyte of the control fish was 83%. The value increased with increase in concentration as well as increased in exposure period most especially for $1.40mgl^{-1}$ and $2.0mgl^{-1}$. Anova and

DMRT showed significant difference between the treatment groups (Appendix 8). The % neutrophils of O. niloticus exposed to various concentrations of paraquat are presented in Table 15. The mean neutrophilic value of control does not deviate from that of basal value. There is a decrease in percentage neutrophil with increase in concentration of toxicant solution. Blood neutrophil of fish exposed to the highest toxicant concentration of 2.00mgl^{-1}, showed an increase at 8th and 10th weeks. Analysis of variance is presented in Appendix 7. It shows that there is a significant difference between the various concentration (ANOVA & DMRT $P<0.05$) There was denaturing of the blood at concentration 2.00mgl^{-1} at 2 weeks. The same effect was observed at week 4 in toxicant concentration of 1.40mgl^{-1}. At 10 weeks fish in all the toxicant concentrations showed the same conditions with no neutrophil or lymphocyte Values recorded. This test was extended to 10 weeks because of the observation of high incidence of death at 8 weeks with all environmental and water parameters being constant as before to see if death was due to environmental factors or effect of toxicant on fish blood.

A statistically significant difference in neutrophil count was observed (Appendix 7) between 2 and 4 weeks. There was an insignificant increase in the mean values after 6 and 8 weeks of exposure. Toxicant concentrations of 1.02mgl^{-1} and 1.40mgl^{-1} caused a decrease in % neutrophil with increase in exposure period while toxicant concentration 2.00mgl^{-1} induced an

gills of fish exposed to toxicant concentration of 1.4mgl^{-1} showed marked disruption of the architecture of the filament and the lamellae with more marked congestion and lamellar hyperplasia (Plates Vi, XIa and b). The gills of fish exposed to toxicant concentration 2.0mgl^{-1} showed atrophy (reduction in size) of both the filament and their lamellae in some instances to about a third of their normal size (Plates XIIa and b).

In the 96-hour test, toxicant concentrations of 11.2mgl^{-1}, 12mgl^{-1} and 13.6mgl^{-1} caused a progressive disruption of the gill filaments and lamellae. There was also reduction in size, the most severe being the gills of O. niloticus in toxicant concentrations 13.6mgl^{-1} (Plate VII - IX).

LIVER

Liver cells are well demarcated from neighbouring cells in the control (Plate XIII). The liver of fish exposed to toxicant concentration 1.02mgl^{-1} showed crowding of liver cells with plumb lining sinusoidal cells (Plate XIV). Plate XV shows the liver of fish exposed to paraquat concentration of 1.4mgl^{-1}. The architecture of liver is fairly preserved. It showed cytoplasmic Vacuolation and individual cell necrosis while the liver of fish exposed to 12.0mgl^{-1} toxicant concentration showed large scale cytoplasmic Vacuolations. There was disruption of hepatocyte architecture with about 80% of the liver cells showing severe necrosis (plate XVI).

PLATES CAPTION

Plate I — Blood of control fish shows well demarcated cytoplasm and nucleus. x100 Arrow N shows a neutrophil

Plate II — Blood of O. niloticus exposed to 12mgl^{-1} of paraquat at 96-h acute toxicity test (Leishman stain) x100 shows completely denatured cells.

Plate III — Blood of O. niloticus exposed to 1.02mgl^{-1} of paraquat at 6 weeks. Shows few senile (destroyed) erythrocytes (Arrow S) x100

Plate IV — Blood of O. niloticus exposed to 1.4mgl^{-1} of paraquat at 6 weeks. Shows highly haemolysed blood cells. (Arrow S) x100.

Plate V — Blood of O. niloticus exposed to 2.0mgl^{-1} of paraquat at 6 weeks. Shows few destroyed cells (Arrow S) x100

PLATE I

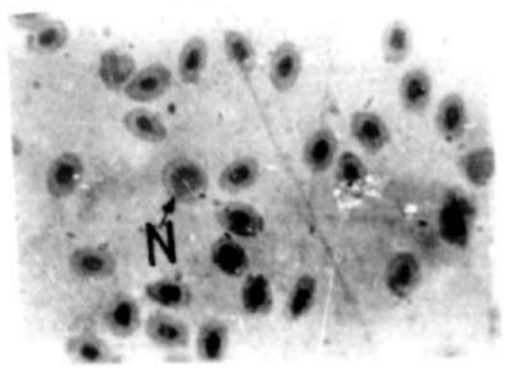
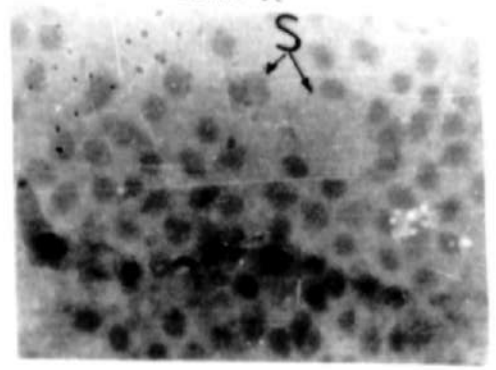


PLATE II



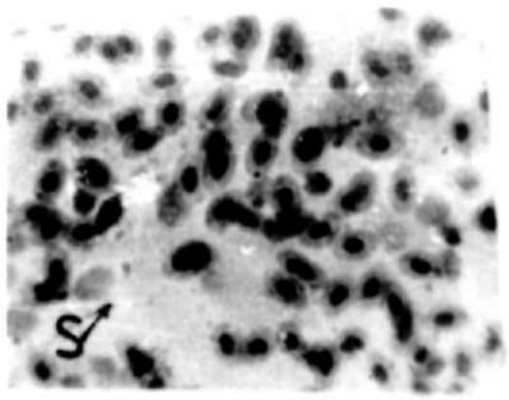


PLATE III

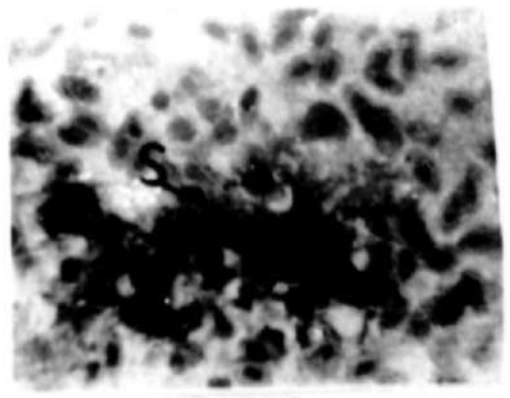


PLATE IV

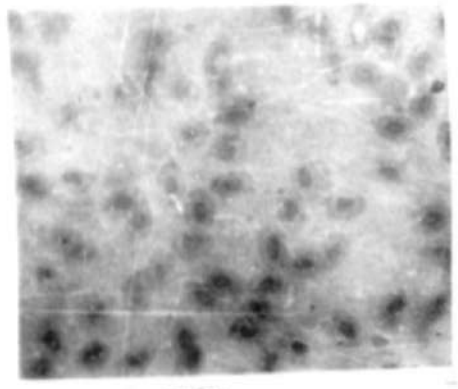


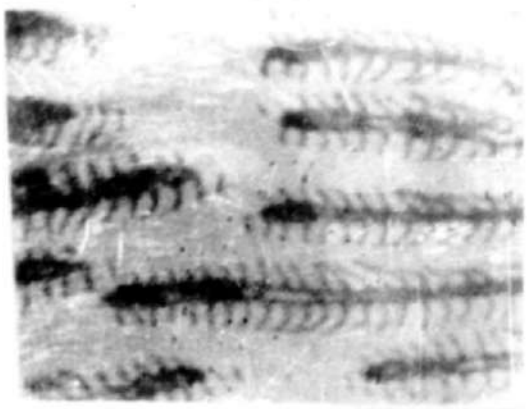
PLATE V

PLATES CAPTION

Plate VI — Shows the gills of control fish with fairly regularly spaced filament and lamellae. x100

Plate VII-IX — Shows the gills of O. niloticus exposed to 11.20, 12.00 and 14.20mgl^{-1} of paraquat at 96-h in acute toxicity test. Note the increase in the disruption of the architecture of the lamellae and filaments at the highest concentration. There is a little hyperplasia at 11.20mgl^{-1} of toxicant concentration but only atrophy is noted at 12.00 and 14.20mgl^{-1}. Plate VIIa= x100; b=x400 VIII=x100; IX=x100.

PLATE VI



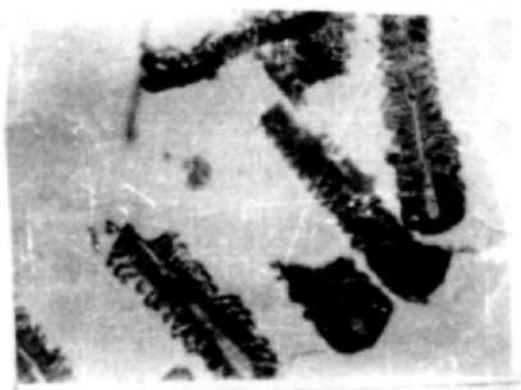


PLATE VII a



PLATE VII b



PLATE VIII

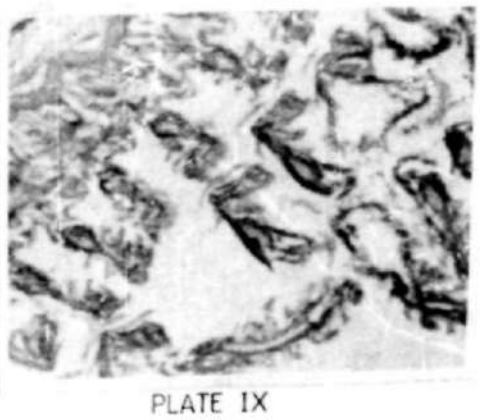


PLATE IX

PLATES CAPTION

Plates Xa & Xb Show gills of O. niloticus exposed to 1.02mg^{-1} of paraquat at 10 weeks showing congestion (expansion) of the filament core and bulbous expansion of the tips of the lamellar as a result of congestion (Arrow B) (H&E) a = x100, b = x400.

Plates XIa & b Gills of O. niloticus exposed to 1.4mgl^{-1} of paraquat at 10 weeks show marked disruption of the architecture of the filament and the lamellar with more marked congestion and hyperplasia.

Note: There is Oedematous expansion of the core of the filament as well. (Arrow C) a = x100; b = x100 (H&E).

Plates XIIa & b The gills of O. niloticus exposed to 2.0mgl^{-1} of paraquat for 10 weeks showing atrophy (reduction in size) of both the filament and their lamellar in some instances to about a third of their normal size. (H&E) a = x100, b = x400. (Arrow E)

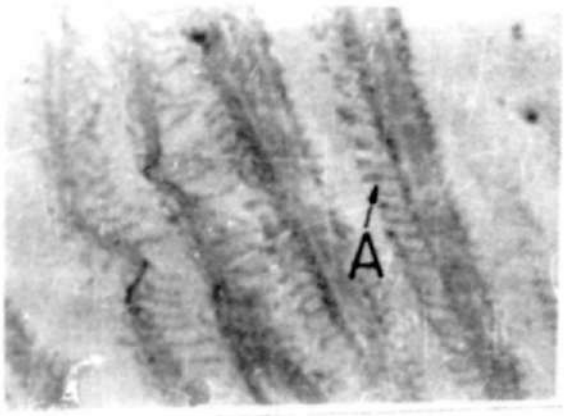


PLATE Xa

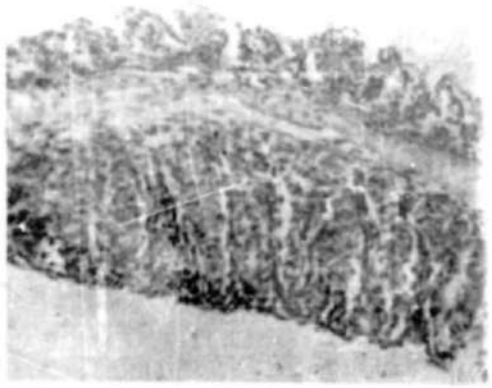


PLATE Xb

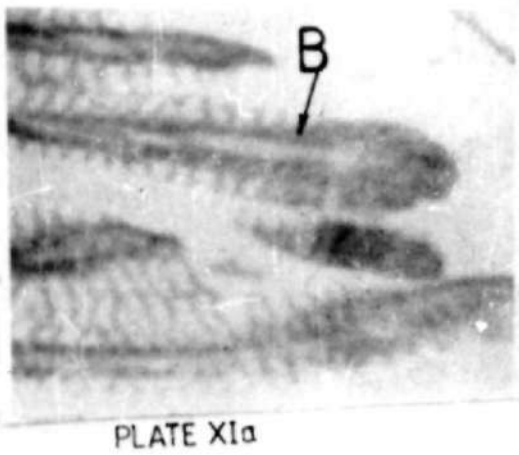


PLATE XIa



PLATE XIb



PLATE XIIa

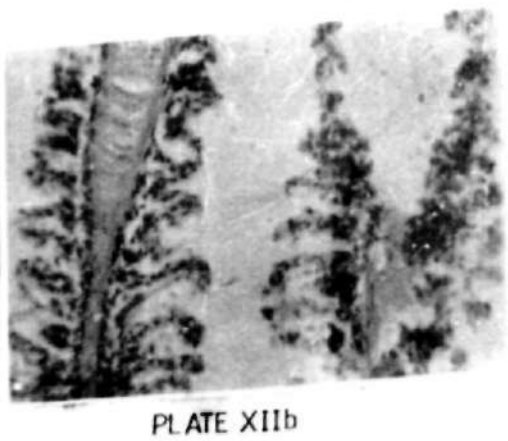


PLATE XIIb

PLATES CAPTION

Plate XIII — Shows control fish liver parenchyma and the central part of pancreatic acinar tissue (Arrow D) (H&E) x400.

Plate XIV — Liver of O. niloticus exposed to 1.02mgl^{-1} of paraquat for 10 weeks. It shows crowding of liver cells with plump lining sinusoidal cells (arrow E) shows lining sinusoidal cell (acting to injury) (H&E) x400.

Plate XV — Liver of O. niloticus exposed to 1.40mgl^{-1} of paraquat for 10 weeks. It shows cytoplasmic vacuolation (arrow F) shows vacuoles and arrow - individual cell necrosis). (H&E) x400.

Plate XVI — Liver of O. niloticus exposed to 2.0mgl^{-1} of paraquat for 10 weeks. It shows large scale cytoplasmic vacuolations (H) and liver cell necrosis. I (Necrosis is indicated by small nucleus therefore death of cell) (H&E) x400.



PLATE XIII

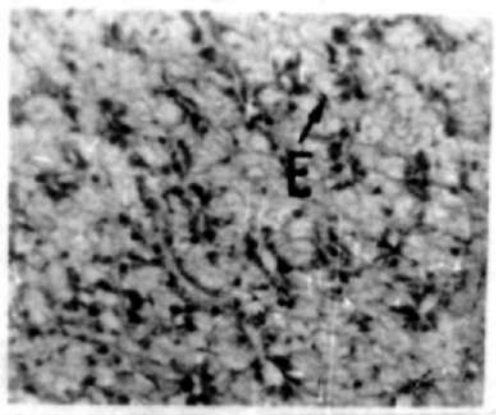


PLATE XIV

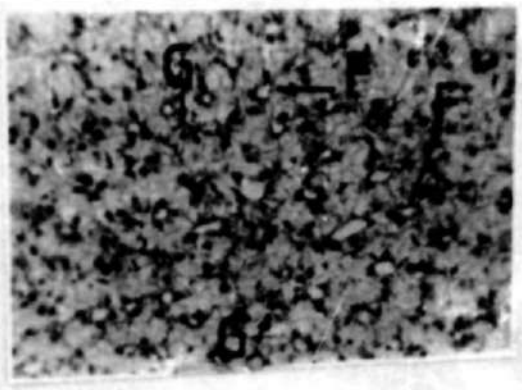


PLATE XV

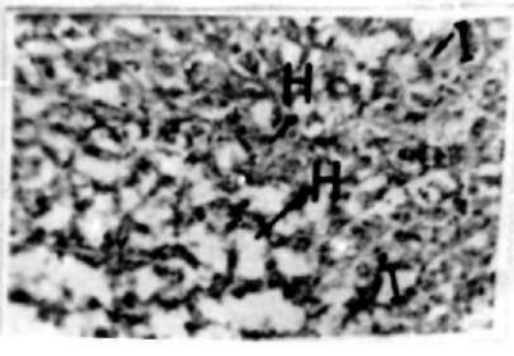


PLATE XVI

PLATES CAPTION

Plate XVII — Normal brain of control O. niloticus (H&E) x100 It shows preservation of general architecture of the brain. T/S Optic Lobe of Mid-brain.

Plate XVIIIa — Brain of fish exposed to 1.02mgl^{-1} of paraquat for 10 weeks. (H&E) x100.

Plate XVIIIb — Brain of fish exposed to 1.02mgl^{-1} of paraquat for 10 weeks. (H&E) x1000. It shows slight increase in the number of glial cells (Arrow J).

Plate XIX — (H&E x1000) Brain of fish exposed 1.4mgl^{-1} of paraquat for 10 weeks. It shows Oedema of the brain indicated by more glial cells (Arrow K).

Plate XX — (H&E) x1000 Shows brain of fish exposed to 2.0mgl^{-1} of paraquat for 10 weeks. There is increase number of Lymphocytes and some polymorphs (indicating encephalitis) (arrow L) and microvacuolar change) (arrow M) indicating degenerative changes. There are more glial cells indicating most severe injury.



PLATE XVII

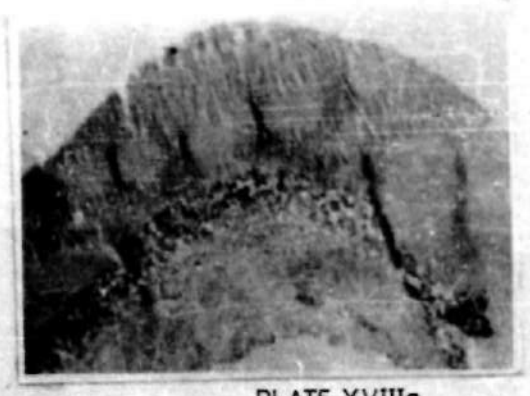


PLATE XVIIIa



PLATE XVIIIb

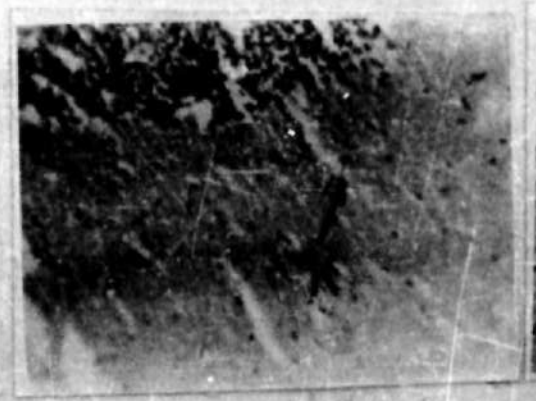


PLATE XIX



PLATE XX

CHAPTER FIVE

5.0 DISCUSSION

5.1 Acute Toxicity Test

Results obtained for acute bioassay revealed that 96h LC_{50} for O. niloticus exposed to paraquat was 12.25mgl^{-1} with a 95% confidence level of ±1.96 (Fig. 1, Table 7). This was calculated using probit analysis according to WardLaw (1985). Expressing the results in log-probit transformation was first used for fish by Fry et al. (1946) for testing lethal temperatures and had since been extensively used for assays of pollutants and toxicants.

These results reveal increase in mortality with increase in paraquat concentrations. The calculated safe concentration for the paraquat was 1.25mgl^{-1} ± 0.13mgl^{-1}. This was derived by multiplying the 96-hour LC_{50} with an application factor of between 0.1 to 0.01, (Koesoemadinate, 1980) depending on the persistence of the pollutant. However 1.25mgl was discovered to be toxic to o. niloticus at 22.7± 0.77°C in the longterm test. This could be interpreted that paraquat is highly toxic to O. niloticus fingerlings in the tropic and thus the application factor to get the safe level should be lower. Oloruntuyi et al. (1992) reported 99.0ppm and 90ppm as LC_{50} values for Round up and paraquat respectively on C. gariepinus at a temperature range of 24.5 - 26.5C. This might indicate that C. gariepinus is more tolerant of paraquat. This might be

due to its possession of additional 'respiratory trees' which aid in the absorption and storage of oxygen. In situations where O. niloticus would have succumbed due to damage to the gills, Clarias might survive such concentration. Morgan (1976) suggested from his data on "safe" levels of diazinon in freshwater streams that there are, for some species at least, less than one-fiftieth (1/50) of the LC_{50} determined on the basis of a 7-day exposure. Nehring (1966) reported that Diquat and paraquat have a low level of toxicity to perch (Perca fluviatilis) and roach (Rutilus rutilus). The median survival period of rainbow trout to 1ppm diquat has been calculated to be 10-15 days (Howe and Wright, 1965). The 96-hour mean toxic levels of paraquat and diquat to guppies were found to be 11.5ppm and 50ppm respectively and to Raspora trilineata, they were found to be 7ppm and 29ppm (Kamwing and Furtado, 1977). These values for paraquat are close to the value obtained in this study.

EFFECT OF PARAQUAT ON OPERCULAR VENTILATION RATE AND TAIL FIN MOVEMENT

Decrease in Opercular ventilation was observed at 24-hours for the high toxicant concentration, then a subsequent increase from 48th to the 96-hour of exposure period. Thus this indicates hyperventilation. Indices of opercular ventilation have been reported to be a strong indicator of stress when fish are in unfavourable environments (Sprague, 1973 and Bulter,

1974). This indicates the damage being caused by paraquat on the gills, the fish, thus increased its ventilation rates in an attempt to take more oxygen. It was also observed that at the first introduction of fish into the toxicant solutions, the fish tended to shut up the mouths and opercular movement was hardly discernable most especially at very high toxicant concentration. This might be due to the odious effect on the fish and also an attempt to acclimatise to the environment. The photomicrograph of the gills at high toxicant concentrations of 11.20, 12.00 and 14.20mgl^{-1}, show remarkable structural disruption of the gills in which case the lamellae got shrunken to almost 1/3 of the normal size. There was total disintegration of the structure of gills at 14.4mgl^{-1}. This coroborates the result obtained in the opercular movement showing that damage to the gills actually might have induced the hyperventilation which led to fatigue and finally death of the fish. This finding agrees with the results obtained by Omoregie et al. (1991) in the acute toxicity test of Actellic 25EC on O. niloticus. Edochiem et al. (1990) in acute toxicity test of gammalin 20 to O. niloticus reported that the opercular ventilation rate and tail fin beat followed the same pattern in which there was an initial sharp increase being directly proportional to the toxicant concentrations, then a drop from 48 hours to the end. This suggests that fish exposed to the toxicants tended to exhibit avoidance syndrome. The tail fin beat fast as the fish moved to escape from toxic area and

increase opercular metabolic rate. However in this study though, the tail fin movement and opercular movement showed similar pattern, there was decrease in movement of both with increase in toxicant concentration at the start of bioassay and subsequent increase in the first 48 hours till the 96 hours. The increase in tail movement might be due to avoidance syndrome however, since the whole medium was toxic, the attempt to escape made fish to become fatigued, hence the subsequent drop in tail fin and opercular movement rates. The combined effects of the fatigue and direct toxic effect of paraquat on the body tissues ultimately led to death. Fest and Schmidt (1973) and O'Brien et al. (1974) associated the action of gammalin 20 on O. niloticus with the central nervous system. However, the role played by stress in the exposed fish has been shown to be important, which makes physiological activities a good test index for toxicants.

Long term effect on the opercular ventilation rate indicated a constancy in the control throughout the 8 weeks. However the period dependent effect exhibited (decrease in ventilation rate with increase in exposure period) in the higher concentration which might be due to stress because of the long exposure and also to the effects on some tissues most especially the gills. Reddy and Gomathy (1977) reported that at lethal concentration ($0.0004mgl^{-1}$), of thiodon, increase in oxygen consumption by about 48% was observed, whereas sublethal dose ($0.0001mgl^{-1}$) depressed the oxygen about 46%. Bengeri et

al. (1984) reported that the pesticide dimethyl parathion gradually reduced the oxygen consumption of the fish Labeo roluta. Pal and Konar (1987) showed that methyl parathion at 3.0ppm significantly reduced the opercular ventilation rate (112 per minute) and at 5ppm it reduced to 95 per minute. These agree with this study in which the opercular rate decreased with increase in the concentration of toxicant.

5.3 BEHAVIOUR AND SURVIVAL

Behaviour and reactions of fish exposed to acute concentrations, included lack of balance, agitated swimming, air gulping and period of quiscence and death. Annune et al. (1991) reported a similar reaction in acute toxicity of zinc to fingerlings of C. lazera and O. niloticus. Sublethal concentration did not show much visible difference in behaviour from those for the control. However, histopathological report showed that the brain was affected. Anees (1975) is of the opinion that behavioural reactions of C. punctatus against acute concentration of organophosphate insecticides, which showed hypersensitivity were not detected under sub-lethal and chronic concentration. Apparently, the fish remained healthy and there was only a little deviation from the normal opercular activity. Normal swimming behaviour was unaffected.

Hyper-activity is a primary principal sign of nervous system failure due to pesticide poisoning (Malsumra, 1975) which affects through physiological and biochemical activities.

Pal and Konar (1987) discovered some behavoural abnormalities caused by sublethal concentration of methyl parathion on fish. These included irregular and erratic movement with jerks, partial jerks of the body, burst swimming etc. Coppage (1972) and Rao et al. (1982) explained that methyl parathion inhibited the activity of acetylcholinesterasses with the corresponding increase of acetylcholine content of different tissues of fish which affects the functioning of nervous impulses. This might possibly be the case of paraquat. Summers (1980) explained that nervous symptoms and respiratory difficulties occur with diquat and paraquat with doses near the LD_{50} values.

5.4 EFFECT OF GROWTH

In this study, the cumulative wet weight gain showed that growth was reduced significantly ($P<0.05$) probably due to the reduction of feeding rate at high concentrations of paraquat. This was very evident from the food always left in the aquaria with the highest toxicant concentration of $2.0mgl^{-1}$. Many authors reported that the growth of fish was reduced by aldrin (0.400 and 0.044ppm). Malathion (0.400 and 0.156ppm, 14-17%) and metasystox (2.0, 1.85 and 0.93ppm, 18-37%) Par and Konarr, 1987). It was advocated very early that "growth should be routinely measured in all chronic experiments since it is "easy" (Sprague, 1971). However, Sprague went on to say that it (growth) is not always a sensitive indicator of toxicity. Rosenthaland and Alderdice (1976) however submitted that it is

apparent that growth is a reflection of food availability, consumption and utilization efficiency. It is also a reflection of organism's stage of development and maturation as well as its size. Thus it is a critical aspect of an organism's existence and, more significantly, of a population's persistence. Woltering (1983) explained that several inherent difficulties are observed in growth response and these include temporary delay of growth early on in the exposure period, but nonetheless, equal size attainment by the end of the test. A similar observation was made at highest concentration of exposure ($2.00mgl^{-1}$) in this study by week 8. Other difficulties come from chemical induced growth stimulation (Mount, 1962, 1968: Pickering, 1968) and the influence of density on those fish surviving the exposure (Woltering, 1983). Density-dependent competition for food and space often results in aggressive behaviour and can lead to variation in the growth of individuals (growth dispensation or size-hierarchy effect).

Ecological reasons for reduction in growth included, increase in susceptibility to predation and disease, reduced ability to obtain food and to compete for food and for suitable habitats, delayed maturation and reproduction (Woltering, 1983).

5.5 HAEMATOLOGICAL EFFECT

Haematological characteristics have been widely used in clinical diagnosis in man. The application of haematological

techniques are valuable for fishery biologists in assessing fish health (Blaxhall, 1972) and monitoring stress (Solvio and Oikari, 1976). Eisler (1967) demonstrated by studies on the blood of Northern puffers that changes in fish blood prior to onset of more striking physiological and morphological changes can be indicative of unfavourable aquatic environment. Anees (1975) noted from his work on haematological abnormalities in C. punctatus exposed to sub-lethal and chronic levels of organophosphorus insecticides that the blood was affected prior to any other visible changes in fish. Haemoglobin concentration, red blood cell counts and haematocrit are particularly recommended as tests which could be carried out on routine basis in fish hatcheries as a check on the health of fish (Anderson and Klontz, 1965). The results of this study show that O. niloticus exposed to - various acute concentrations of paraquat were put under stress, as evidenced by a dose - dependent reduction in haematocrit value, haemoglobin content, MCHC, and % neutrophil as compared to its baseline values or control. The acute effect on the blood was further confirmed by the photomicrograph of the blood cells at 96-hour. The blood cells were completely rendered senile with reduced size and indistinguishable cytoplasmic content. This could be due to increased fragility or rate of destruction of circulating erythrocytes (McLeay, 1973). Leucocytic count showed no significant changes. In the sub-lethal toxicity test of paraquat to O. niloticus, the significant decrease in the

haemoglobin, haematocrit and MCHC with increase in concentrations and in exposure periods observed could be due to an inhibition of erythrocyte production, an increase in the erythrocyte destruction or hemodilution (McLeay, 1973). This may indicate that the herbicide induced an effect similar to the production of anaemia. Similar results have been reported by Eisler (1967) for fishes exposed to methyl parathion.

Eisler and Edmunds (1966) reported that erythropaenia (deficiency in number of red blood cells) was reflected by the reduced haemoglobin content and haematocrit as well as in ESR (Erythrocyte sedimentation rate). He further reported that Anaemia generally causes an increase in ESR possibly owing to an increase in gamma-globulins, as reported in northern puffers following endrin toxicosis. Srivastava (1969) showed that hypocoagulability of whole blood in the fish exposed to heptachlor was due to thrombocytopaenia; that is, blood coagulation was retarded in the fish as thrombocyte counts decreased. However, in the present study, higher coagulability (Hyper coagulability) as well as high haemolytic action in blood samples of fish exposed to high toxicant concentrations were recorded. McLeay (1973) on his work on fish exposed to pulp mill effluent observed a significant decrease in the RBC count and haematocrit which was attributed to possibility of inhibition of erythrocyte production and increase in the rate of erythrocyte destruction or hemodilution. Wedemeyer and Yasatake (1977), suggested that decrease in these parameters

could be due to hemodilution due to impaired Osmoregulation. Mount and Putnicki (1966) and Zambariborsch and Biu (1977) were of the opinion that marked decrease in total number of erythrocytes, leucocytes and thrombocytes after exposure of Heteropneustes fossilis to heptachlor was unlikely to be due to hemodilution because of hyper choroanemia occuring at 48-hour and 96-hour of test period. These changes, according to them, clearly indicated blood dyscrasia (a developmental disorder of the blood) induced by the heptachlor in the fish which interferred with haemopoiesis and/or alteration of cell membranes by hydrolysis of acetylcholine in the body fluid. Musa (1993) reported a decrease in haematocrit, haemoglobin content and RBC count in C. gariepinus exposed to malachite green (Oxalate). The decrease was ssuggested to be possibly due to the effect of the chemical on the membrane ATpase, the glycolytic enzymes in the erythrocytes and glutathione. In this study, with the histopathological results on the gills in which there was marked destruction especially at high toxicant concentrations, decrease in haemoglobin, haematocrit and MCHC could be attributed to hemodilution due to impaired osmoregulation as consequence of gill damage.

Conroy (1972) stated that Lymphocytes constitute the majority of white blood cells present in the peripheral blood of apparently normal salmonids. In this study, lymphocytes had the highest percentage compared to neutrophil and monocyte. However there was slight increase with increase in

concentration of toxicant to which they were exposed and periods, which possibly indicates chronic effect. Neutrophils increased with increased concentration but decreased with the periods.

Grizzle (1977) observed neutrophilia in Ictaturus punctatus 1-3 days after exposure to 0.1mgl^{-1} malachite green. The large increase in neutrophils was the most drastic change. Weineb (1958) and Yokoyama (1960) explained that neutrophils respond to inflammatory conditions followisng turpentine injection. This neutrophil is an indicator of acute effect. There was no indication of inflammatory response in O. niloticus exposed to paraquat in this study. It was observed that fish exposed to toxicant concentration of 1.4mgl^{-1} showed marked effect of toxicant on the fish right from the beginning. At week 6 there was an increase in number of destroyed of the red blood cells by haemolysis as seen in the photomicrograph (plate IV) but gradual acclaimation could be observed in the blood cells at toxicant concentration of 2.0mgl^{-1} with less haemolysed cells. By the 10th week haemolysis had taken place in all treament groups except the control. This could be due to stress response on the blood cells of the fish.

5.6 HISTOPATHOLOGY

In the acute toxicity test, the gills of exposed fish showed marked damage to the filament lamellae and mutilation of the gill rakers. Similar reports on gill damages in Cyprinus carpio exposed to dipterex were documented by Chinabut et al. (1987). Exposure of Rivulus marmoratus to diethyl nitrosamine by Thiyagarajah and Grizzle (1985) and C. gariepinus to inorganic fertilizer by Ufodike Onusiriuka (1990) caused similar damages. However Walsh and Ribelin (1975) were of the view that such changes are of limited diagnostic value since they could be observed in bacterial and nutritional gill disease and even in apparently healthy Coho Salmon and lake trout directly taken from hatchery raceways. In another study, Walsh and Ribelin (1975) distinguished autolytic change from the lamellar Oedema by the presence of proteinaceous Oedema fluid in those fish manifesting the latter condition in which 80% of the cells were necrotic.

In the sublethal assay of this study, the nature of damage to the gills was more of Oedema, hypertrophy of cells, than atrophy seen at acute toxicity test.

In this study, O. niloticus kept in various sublethal concentrations of paraquat, showed liver cell vacuolation which increased with increase concentration. The greatest effect was observed at 2.0mgl^{-1} where 80% of the cells were necrotic, hepatocytic architecture disrupted and of cellular details of pancreatic acinar lost. Wood (1965) found no liver changes in

spot fish exposed to sublethal concentrations of endrin in flowing sea water. However, in spot, surviving near-lethal concentration (0.075ppb) exposures, local necrosis and inflammation in the liver as well as loss of glycogen and lipids were reported. Rainbow trout surviving exposure to 0.26ppb endrin in tanks (LC_{50} 7 days = 0.26ppb) has severe, nonspecific degenerative liver lesions (Walsh and Ribelin, 1975). Eller (1971) believed that the above degenerative changes in the liver suggested nutritional deficiency enhanced by endrin exposure. Omoregie and Ufodike (1991) reported reduced Vacuolation in fingerlings of O. niloticus exposed to at 1.00mgl^{-1} of Actellic 25EC and attributed it to Fatty degeneration. In this report, increased vacuolation may be attributed probably to abnormal accumulation of lipid in the liver; while necrosis of liver cells might be due to the inability of the fish to regenerate new liver cells as a result of constant flooding of the herbicide. Necrosis might also be due to the excessive work required by the fish liver to get rid of the insecticide during the process of detoxification. This is supported by Omoregie and Ufodike's findings (1991).

BRAIN

In this study, fish exposed to paraquat showed pathological change in the brain tissue ranging from moderate increase in number of glial cells to vacuolation of neuronal cells, Meningitis and encephalitis. All these were dose-dependent. Matthiessen and Roberts (1982) reported some effects on the brain of fish exposed to endosulfan insecticide

during tsetse fly control operation as suggested by behavioural changes. The histological changes in Tilapia rendalli brain included encephalitis with the optic tectum and infilteration of Oesinophilic granule cells. These pathological changes in the brain of the fish clearly show that endosulfan has neurotoxic effects for soon after spraying, brain lesions were sufficient to cause behavioural changes such as temporary hyperactive and unco-ordinated movement. Such behavioural changes were observed in O. niloticus in this report and this corroborated the histopathological report which indicated damages to the cells of the brain. Walsh and Ribelin (1975) observed hyperemia of varying degrees in the brains of some Coho Salmon and lake trout exposed to several pesticides. In fish exposed to 2,4-D the change was sufficiently striking to suggest that it might have diagnostic value. The latter observation agrees with the findings of cope, et al. (1971) which maintained that the presence of eosinophilic globules which appeared to be plugging blood vessels was suggested as the mechanism of vascular stasis in blue gills exposed to 2,4-D. Similar globules were reported in blue gills exposed to methoxychlor and in redear sunfish (Lepomis microlophus) exposed to the herbicide Hydrothol 191 (Frear, 1969).

WATER QUALITY PARAMETERS

The water quality results did not differ significantly ($P<0.05$) with the various treatments and exposed periods. Where slight differences occurred, they were within the suggested tolerance ranges (Viveen et al., 1985).

SUMMARY AND CONCLUSION

From the chronic toxicity assay, it is clear that paraquat concentation as low as 1.02mgl^{-1} is harmful to fish, particularly O. niloticus.

From the study, O. niloticus exposed to 1.02 - 2.0mgl^{-1} paraquat in water for 8 weeks still put on some growth, though not as much as the control. However, the death of some individuals with obvious symptoms of paraquat toxicosis, and the degenerative changes in the liver, gills and brain of the surviving fish suggested that, had the exposure period been longer progressively more fish would have died.

Haematological findings also show that sublethal concentrations of paraquat in the aquatic environment may not necessarily result in outright mortality of aquatic organisms. However, they have significant effects which result in several physiological dysfunction as earlier reported by Edwards (1970) and Omoregie et al., (1990). A dose-dependent impairment in the erythrocyte, haemoglobin, haematocrit and MCHC was confirmed. The blood cells were observed to be so vunerable that they easily underwent haemolysis as soon as drawn from the fish body.

There is evidence that indiscriminate discharge of effluents containing paraquat or usage of paraquat for agricultural purpose which might be washed into streams and rivers by rainwater, or usage to control hazardous water plants such as water hyacinth can have severe harmful effects on the

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There is evidence that indiscriminate discharge of effluents containing paraquat or usage of paraquat for agricultural purpose which might be washed into streams and rivers by rainwater, or usage to control hazardous water plants such as water hyacinth can have severe harmful effects on the

ecosystem leading to the destruction of our fish stock and perhaps of other forms of life. If the government's goal of self-reliance in food, particularly protein production is to be achieved, attempts should be made to monitor and control the usage of paraquat and reduce its use to the barest minimum required for effective weed control. Insecticide-producing industries should be encouraged to look into the possibility of reducing the potency to non - target organisms yet still maintaining its effectiveness. Alternatively, research into the discovery of new effective but less potent agro-chemical or herbicides should be encouraged.

Galex is known to be a newer formula but not much work has been reported on its toxicity to life. Research work could be done in future on this.

Whichever approach we adopt to protect the environment, whether by reducing the dose or substituting newer, effective but less persistent pesticides, the ultimate objective is to sustain the ecosystem. In line with this goal the Director General of the Federal Environmental Protection Agency (FEPA) stressed that what should be of concern to the governments and Corporate bodies is the level of toxic waste and the emphasis on the Environmental Impact Assessment (EIA) and the initiation and implementation of policies that would ensure proper management of the environment (Aina, 1996).

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APPENDIX 1.

TABLE I. Transformation of Percentages to Probits

%	0·0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9	1	2	3	4	5
0	—	1·0906	2·1218	2·2622	2·3478	2·4242	2·4879	2·5427	2·5911	2·6344					
1	2·6737	2·7091	2·7420	2·7728	2·8027	2·8299	2·8556	2·8799	2·9031	2·9251					
2	2·9463	2·9665	2·9859	3·0046	3·0226	3·0400	3·0568	3·0732	3·0890	3·1043	For more detail see values for 90–100				
3	3·1192	3·1337	3·1478	3·1616	3·1750	3·1881	3·2009	3·2134	3·2256	3·2376					
4	3·2493	3·2608	3·2721	3·2831	3·2940	3·3046	3·3151	3·3253	3·3354	3·3454					
5	3·3551	3·3649	3·3742	3·3836	3·3928	4·4018	3·4107	3·4195	3·4282	3·4368	0	18	27	36	45
6	3·4452	3·4536	3·4618	3·4699	3·4780	3·4860	3·4937	3·5015	3·5091	3·5167	8	10	24	32	40
7	3·5242	3·5318	3·5393	3·5468	3·5542	3·5615	3·5688	3·5760	3·5831	3·5902	7	14	21	28	36
8	3·5972	3·6040	3·6108	3·6174	3·6243	3·6308	3·6375	3·6442	3·6508	3·6574	6	13	19	26	32
9	3·6639	3·6704	3·6770	3·6835	3·6899	3·6964	3·7028	3·7092	3·7156	3·7219	6	12	18	24	30
10	3·7281	3·7344	3·7406	3·7467	3·7528	3·7589	3·7650	3·7710	3·7770	3·7829	6	11	17	22	28
11	3·7888	3·7947	3·8005	3·8063	3·8121	3·8179	3·8236	3·8293	3·8349	3·8405	6	10	16	21	26
12	3·8461	3·8516	3·8572	3·8627	3·8682	3·8737	3·8792	3·8846	3·8900	3·8954	6	10	15	20	24
13	3·9008	3·9061	3·9114	3·9167	3·9220	3·9272	3·9324	3·9376	3·9428	3·9479	5	9	14	18	23
14	3·9530	3·9581	3·9632	3·9683	3·9733	3·9783	3·9834	3·9884	3·9934	3·9983	4	9	13	18	22
15	4·0033	4·0082	4·0131	4·0180	4·0228	4·0277	4·0325	4·0373	4·0421	4·0469	4	8	13	17	21
16	4·0516	4·0563	4·0611	4·0658	4·0705	4·0752	4·0799	4·0845	4·0892	4·0938	4	8	12	16	20
17	4·0984	4·1030	4·1076	4·1122	4·1167	4·1213	4·1258	4·1303	4·1348	4·1393	4	8	12	16	19
18	4·1437	4·1482	4·1526	4·1571	4·1615	4·1659	4·1703	4·1747	4·1791	4·1834	4	8	11	15	19
19	4·1878	4·1921	4·1964	4·2007	4·2050	4·2093	4·2135	4·2178	4·2220	4·2262	4	7	11	15	18
20	4·2304	4·2346	4·2388	4·2429	4·2471	4·2512	4·2553	4·2594	4·2635	4·2676	4	7	11	14	18
21	4·2717	4·2757	4·2798	4·2838	4·2879	4·2919	4·2959	4·2999	4·3039	4·3079	3	7	10	14	17
22	4·3118	4·3158	4·3197	4·3236	4·3275	4·3314	4·3353	4·3392	4·3431	4·3469	3	7	10	13	17
23	4·3508	4·3546	4·3584	4·3622	4·3660	4·3698	4·3736	4·3774	4·3812	4·3849	3	7	10	13	16
24	4·3887	4·3924	4·3961	4·3998	4·4035	4·4072	4·4109	4·4146	4·4183	4·4220	3	6	10	13	16
25	4·4256	4·4293	4·4329	4·4365	4·4401	4·4437	4·4473	4·4509	4·4545	4·4581	3	6	9	12	16
26	4·4617	4·4653	4·4688	4·4724	4·4759	4·4795	4·4830	4·4865	4·4901	4·4936	3	6	9	12	15
27	4·4972	4·5007	4·5042	4·5077	4·5112	4·5147	4·5182	4·5217	4·5252	4·5287	3	6	9	12	15
28	4·5322	4·5357	4·5392	4·5427	4·5462	4·5497	4·5532	4·5567	4·5602	4·5637	3	6	9	12	15
29	4·5672	4·5707	4·5743	4·5778	4·5813	4·5849	4·5884	4·5920	4·5955	4·5991	3	6	9	12	15
30	4·6026	4·6062	4·6098	4·6134	4·6170	4·6206	4·6242	4·6278	4·6315	4·6351	3	6	9	12	15
31	4·6387	4·6424	4·6460	4·6497	4·6534	4·6571	4·6608	4·6645	4·6682	4·6719	3	6	9	12	15
32	4·6757	4·6794	4·6832	4·6870	4·6907	4·6945	4·6983	4·7021	4·7060	4·7098	3	6	9	12	15
33	4·7137	4·7175	4·7214	4·7253	4·7292	4·7331	4·7370	4·7410	4·7449	4·7489	3	6	9	12	15
34	4·7529	4·7569	4·7609	4·7649	4·7690	4·7730	4·7771	4·7812	4·7853	4·7894	3	6	9	12	15
35	4·7936	4·7977	4·8019	4·8061	4·8103	4·8145	4·8187	4·8230	4·8272	4·8315	3	6	9	12	15
36	4·8358	4·8401	4·8445	4·8489	4·8533	4·8577	4·8622	4·8667	4·8712	4·8757	3	6	9	12	15
37	4·8802	4·8848	4·8894	4·8941	4·8988	4·9035	4·9083	4·9131	4·9179	4·9228	3	6	9	12	15
38	4·9278	4·9328	4·9379	4·9430	4·9482	4·9534	4·9586	4·9639	4·9693	4·9747	3	6	9	12	15
39	4·9803	4·9860	4·9917	4·9976	5·0035	5·0095	5·0156	5·0218	5·0281	5·0345	3	6	9	12	15
40	5·0410	5·0477	5·0545	5·0615	5·0686	5·0759	5·0833	5·0909	5·0987	5·1066	3	6	9	12	15
41	5·1148	5·1231	5·1317	5·1404	5·1494	5·1586	5·1680	5·1777	5·1876	5·1978	3	6	9	12	15
42	5·2082	5·2189	5·2300	5·2414	5·2531	5·2653	5·2778	5·2908	5·3042	5·3181	3	6	9	12	15
43	5·3324	5·3472	5·3624	5·3782	5·3945	5·4113	5·4288	5·4469	5·4657	5·4852	3	6	9	12	15
44	5·5055	5·5267	5·5488	5·5718	5·5958	5·6209	5·6471	5·6746	5·7035	5·7338	3	6	9	12	15
45	5·7658	5·7995	5·8351	5·8728	5·9128	5·9554	6·0008	6·0494	6·1015	6·1578	4	7	11	15	19
46	6·2187	6·2854	6·3588	6·4401	6·5312	6·6341	6·7514	6·8869	7·0465	7·2400	5	9	14	18	23
47	7·4878	7·8000	8·2000	8·8000	9·8000	11·8000	15·8000	25·8000	50·8000	100·8000	10	20	30	40	50
48	7·4878	7·8000	8·2000	8·8000	9·8000	11·8000	15·8000	25·8000	50·8000	100·8000	10	20	30	40	50
49	7·4878	7·8000	8·2000	8·8000	9·8000	11·8000	15·8000	25·8000	50·8000	100·8000	10	20	30	40	50
50	7·4878	7·8000	8·2000	8·8000	9·8000	11·8000	15·8000	25·8000	50·8000	100·8000	10	20	30	40	50

	0·0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
60	5·2533	5·2559	5·2585	5·2611	5·2637	5·2663	5·2689	5·2715	5·2741	5·2767
61	5·2793	5·2819	5·2845	5·2871	5·2898	5·2924	5·2950	5·2976	5·3002	5·3029
62	5·3055	5·3081	5·3107	5·3134	5·3160	5·3186	5·3213	5·3239	5·3266	5·3292
63	5·3319	5·3345	5·3372	5·3398	5·3425	5·3451	5·3478	5·3505	5·3531	5·3558
64	5·3585	5·3611	5·3638	5·3665	5·3692	5·3719	5·3745	5·3772	5·3799	5·3826
65	5·3853	5·3880	5·3907	5·3934	5·3961	5·3989	5·4016	5·4043	5·4070	5·4097
66	5·4125	5·4152	5·4179	5·4207	5·4234	5·4261	5·4289	5·4316	5·4344	5·4371
67	5·4399	5·4427	5·4454	5·4482	5·4510	5·4538	5·4565	5·4593	5·4621	5·4649
68	5·4677	5·4705	5·4733	5·4761	5·4789	5·4817	5·4845	5·4874	5·4902	5·4930
69	5·4959	5·4987	5·5015	5·5044	5·5072	5·5101	5·5129	5·5158	5·5187	5·5215
70	5·5244	5·5273	5·5302	5·5330	5·5359	5·5388	5·5417	5·5446	5·5476	5·5505
71	5·5534	5·5563	5·5592	5·5622	5·5651	5·5681	5·5710	5·5740	5·5769	5·5799
72	5·5828	5·5858	5·5888	5·5918	5·5948	5·5978	5·6008	5·6038	5·6068	5·6098
73	5·6128	5·6158	5·6188	5·6219	5·6250	5·6280	5·6311	5·6341	5·6372	5·6403
74	5·64[illegible]	5·6464	5·6495	5·6526	5·6557	5·6588	5·6620	5·6651	5·6682	5·6713
75	5·6745	5·6776	5·6808	5·6840	5·6871	5·6903	5·6935	5·6967	5·6999	5·7031
76	5·7063	5·7095	5·7128	5·7160	5·7192	5·7225	5·7257	5·7290	5·7323	5·7356
77	5·7388	5·7421	5·7454	5·7488	5·7521	5·7554	5·7589	5·7621	5·7655	5·7688
78	5·7722	5·7756	5·7790	5·7824	5·7858	5·7892	5·7928	5·7961	5·7995	5·8030
79	5·8064	5·8099	5·8134	5·8169	5·8204	5·8239	5·8274	5·8310	5·8345	5·8381
80	5·8416	5·8452	5·8488	5·8524	5·8560	5·8596	5·8633	5·8669	5·8705	5·8742
81	5·8779	5·8816	5·8853	5·8890	5·8927	5·8965	5·9002	5·9040	5·9078	5·9116
82	5·9154	5·9192	5·9230	5·9269	5·9307	5·9346	5·9385	5·9424	5·9463	5·9502
83	5·9542	5·9581	5·9621	5·9661	5·9701	5·9741	5·9782	5·9822	5·9863	5·9904
84	5·9945	5·9986	6·0027	6·0069	6·0110	6·0152	6·0194	6·0237	6·0279	6·0322
85	6·0364	6·0407	6·0450	6·0494	6·0537	6·0581	6·0625	6·0669	6·0714	6·0758
86	6·0803	6·0848	6·0893	6·0939	6·0985	6·1031	6·1077	6·1123	6·1170	6·1217
87	6·1264	6·1311	6·1359	6·1407	6·1455	6·1503	6·1552	6·1601	6·1650	6·1700
88	6·1750	6·1800	6·1850	6·1901	6·1952	6·2004	6·2055	6·2107	6·2160	6·2212
89	6·2265	6·2319	6·2372	6·2426	6·2481	6·2536	6·2591	6·2646	6·2702	6·2759

	1	2	3	4	5
60	3	6	8	10	13
61	3	6	8	10	13
62	3	6	8	11	13
63	3	6	8	11	13
64	3	6	8	11	13
65	3	6	8	11	14
66	3	6	8	11	14
67	3	6	8	11	14
68	3	6	8	11	14
69	3	6	9	11	14
70	3	6	9	12	14
71	3	6	9	12	15
72	3	6	9	12	15
73	3	6	9	12	15
74	3	6	9	12	15
75	3	6	10	13	16
76	3	7	10	13	16
77	3	7	10	13	17
78	3	7	10	14	17
79	4	7	11	14	18
80	4	7	11	14	18
81	4	7	11	15	19
82	4	8	12	15	19
83	4	8	12	16	20
84	4	8	13	17	21
85	4	9	13	18	22
86	5	9	14	18	23
87	5	10	15	19	24
88	5	10	15	21	26
89	5	11	16	22	27

	0·0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
90	6·2816	6·2873	6·2930	6·2988	6·3047	6·3106	6·3165	6·3225	6·3285	6·3346
91	6·3408	6·3469	6·3532	6·3595	6·3658	6·3722	6·3787	6·3852	6·3917	6·3984
92	6·4051	6·4118	6·4187	6·4255	6·4325	6·4395	6·4466	6·4538	6·4611	6·4684
93	6·4758	6·4833	6·4909	6·4985	6·5063	6·5141	6·5220	6·5301	6·5382	6·5464
94	6·5548	6·5632	6·5718	6·5805	6·5893	6·5982	6·6072	6·6164	6·6258	6·6353
95	6·6449	6·6546	6·6645	6·6747	6·6849	6·6954	6·7060	6·7169	6·7279	6·7392
96	97	98	99	100	102	103	104	106	108	110
97	6·7507	6·7624	6·7744	6·7866	6·7991	6·8119	6·8250	6·8384	6·8522	6·8663
	117	120	122	125	128	131	134	138	141	145
	6·8808	6·8957	6·9110	6·9268	6·9431	6·9600	6·9774	6·9954	7·0141	7·0335
	149	153	158	163	168	174	180	187	194	202

	0·00	0·01	0·02	0·03	0·04	0·05	0·06	0·07	0·08	0·09
98·0	7·0537	7·0558	7·0579	7·0600	7·0621	7·0642	7·0663	7·0684	7·0706	7·0727
98·1	7·0749	7·0770	7·0792	7·0814	7·0836	7·0858	7·0880	7·0902	7·0924	7·0947
98·2	7·0969	7·0992	7·1015	7·1038	7·1061	7·1084	7·1107	7·1130	7·1154	7·1177
98·3	7·1201	7·1224	7·1248	7·1272	7·1297	7·1321	7·1345	7·1370	7·1394	7·1419
98·4	7·1444	7·1469	7·1494	7·1520	7·1545	7·1571	7·1598	7·1622	7·1648	7·1676
98·5	7·1701	7·1727	7·1754	7·1781	7·1808	7·1835	7·1862	7·1890	7·1917	7·1945
98·6	7·1973	7·2001	7·2029	7·2058	7·2086	7·2115	7·2144	7·2173	7·2203	7·2232
98·7	7·2262	7·2292	7·2322	7·2353	7·2383	7·2414	7·2446	7·2478	7·2509	7·2539
98·8	7·2571	7·2603	7·2636	7·2669	7·2701	7·2734	7·2768	7·2801	7·2835	7·2869
98·9	7·2904	7·2938	7·2973	7·3009	7·3044	7·3080	7·3116	7·3152	7·3189	7·3226
99·0	7·3263	7·3301	7·3339	7·3378	7·3416	7·3455	7·3495	7·3535	7·3575	7·3616
99·1	7·3656	7·3699	7·3738	7·3781	7·3824	7·3867	7·3911	7·3954	7·3999	7·4044
99·2	7·4089	7·4135	7·4181	7·4228	7·4276	7·4324	7·4372	7·4422	7·4471	7·4522
99·3	7·4573	7·4624	7·4677	7·4730	7·4783	7·4838	7·4893	7·4949	7·5006	7·5063
99·4	7·5121	7·5181	7·5241	7·5302	7·5364	7·5427	7·5491	7·5556	7·5622	7·5689
99·5	7·5758	7·5828	7·5899	7·5972	7·6045	7·6121	7·6197	7·6276	7·6356	7·6437
99·6	7·6521	7·6606	7·6693	7·6783	7·6874	7·6968	7·7065	7·7164	7·7266	7·7370
99·7	7·7478	7·7589	7·7703	7·7822	7·7944	7·8070	7·8202	7·8338	7·8479	7·8627
99·8	7·8782	7·8943	7·9112	7·9290	7·9478	7·9677	7·9889	8·0116	8·0361	8·0618
99·9	8·0902	8·1214	8·1559	8·1947	8·2389	8·2905	8·3528	8·4316	8·5401	8·7190

	1	2	3	4	5
90	6	12	18	24	29
91	6	13	19	25	32
92	7	14	21	28	35
93	8	16	24	31	39
94	9	18	27	36	45
95					
96					
97					

APPENDIX 2

(a) Expected probit is obtained by extrapolating upwards from each dose value on the absussa (X-axis) to intersect the fitted straight line on graph 1. The point of intersection is thend read across horizontally to the probit scale to get the expected probit.

(b) Weight (W) associated with expected probit was calculated thus:-

Observed	Expected probit using weighing
	3.80 = 0.370
3.75	3.70 = 0.336
	0.10 = 0.034
	if 0.10 = 0.034
	. . 0.05 = $\frac{0.34 \times .05}{.10}$
	= 0.017
	0.3361 0.017
	= 0.353
	NOTE: 0.05 is obtained from 3.75 - 3.70 = 0.05
4.55	4.60 = 0.601
	$\underline{4.50}$ = $\underline{0.581}$
	0.10 = 0.020

OBSERVED PROBIT	EXPECTED PROBIT
	4.55 - 4.50 = 0.05
	IF 0.10 = 0.020
	.. 0.05 = $\frac{0.20 \times .05}{10}$
	= 0.1
	45.55 = 0.581 + 0.1
	= 0.681
6.15	6.1 = 0.450
	$\frac{6.1}{0.1}$ = $\frac{0.370}{0.080}$
	.05 = 0.40
	0.15 = 0.450 - 0.040
	= 0.410
6.35	6.30 = 0.336
	6.40 = $\underline{0.302}$
	0.10 = 0.034
	0.05 = $\frac{0.034 \times .05}{.1}$
	= 0.0.017
	6.35 = 0.336 - 0.017
	= 0.319
3.90	0.405
4.20	0.503
4.90	0.634
5.90	0.471

APPENDIX 3

REGRESION LINE $(EZ)^2$

X	Y	XY	X^2	$\bar{Y}$	$\bar{X}$	$(EX)^2$
0.98	3.72	3.65	0.96	4.90	1.08	41.86
1.02	4.16	4.24	1.04			
1.05	4.48	4.70	1.103			
1.08	5.25	5.67	1.166			
1.16	5.52	6.40	1.345			
1.10	6.28	7.41	1.392			
6.47	29.41	32.07	7.01			

$$b = \frac{E\,XY - (EX)\,(EY)\,/n}{E\,(X^2 - (EX)^2\,/n}$$

$$= \frac{32.07 - (1.47)\,(29.41)\,/6}{7.01 - \frac{(6.47)^2}{6}}$$

$$= \frac{32.07 - (6.47)\,(4.90)}{7.01 - 6.98}$$

$$= \frac{0.37}{0.03}$$

$$= 12$$

$$a = y - bx$$
$$= 4.90 - 12\,(1.08).$$
$$= 4.90 - 12.96$$
$$= -8\,.06$$

$$Y = a + bx$$

$$x = 1.$$

$$Y = 8.06 + 12 \times 1$$
$$= 3.94$$

$$x = 1.16$$

$$Y = 8.06 + (12)\,(1.16)$$
$$= 8.06 + 13.92$$
$$= 5.86$$

APPENDIX 4

ANOVA TABLE FOR HAEMATOCRIT (PCV)% OF O NILOTICUS

EXPOSED TO PARAQUAT FOR 1 - 10 WEEKS AT 23°C

EXPOSURE PERIOD (WEEKS)

SOURCE OF VARIAN	DF	SS	MS	F-RATIO
Model	20	1200.50	60.025	57.31
Error	19	19.90	1.047	
Corrected Total	39	1220.40		

R - Square	C.V	Root MSE	V1 mean
0.983694	11.76334	1.023410	8.700

TABLE OF MEANS FOR HAEMATOCRIT BETWEEN TREATMENT
(Duncan's multiple Range Test for variable)

Treatment	conc	mean	Duncan's grouping
A X	3	5.20	D
A X	4	7.70	C
A X	2	9.00	B
A X	1	12.90	A

means with the same letter are not significantly different.
Alpha = 0.05 df = 19 MSE 1.047368

Source	DF	SS	MS	F	PR F
Reps	1	0.10	0.10	0.10	0.7607
Conc	3	309.80	103.27	98.60	0.001
Weeks	4	421.15	105.29	100.63	0.001
(Error) CONC VS WEEKS	12	469.45	39.12	37.35	

Duncan's multiple Range Test for Variable

Treatment	Week	Mean	Duncan grouping
A X	5	2.75	A
A X	1	8.50	B
A X	2	9.88	B
A X	3	9.88	C
A X	4	12.50	D

APPENDIX 5

ANOVA TABLE FOR HAEMOGLOBIN (g/100m) () OF O. NILOTICUS

EXPOSED TO SUB-LETHAL CONCENTRATIONS OF PARAQUAT FOR

10 WEEKS AT 22.7°C + 0.08

SV	DF	SS	MS	F	Pr
Reps (R-1)	1	0.0330	0.0330	0.94	NS 1
CONC (C-1)	3	6.9608	2.320	66.31	
WEEKS (w-1)	4	14.456	3.614	103.29	
CONC VS WEEKS (W-1) (c-1)	12	15.0786	1.257	35.91	
ERROR	19	0.664			
	39	37.1937			

TABLE OF MEANS FOR HAEMOLGLOBIN (g/100ml)
WITHIN TREATMENT (WEEKS)

Alpha = 0.05 df = 19 MSE = 0.03498

Number 0 Mean	2	3	4	5
Critical Range	0.195	0.205	0.212	0.216

Treatment	Weeks	mean	Duncan grouping	N
A X	2	2.956	E	
A X	4	3.463	D	
A X	1	3.786	C	
A X	3	4.068	B	
A X	2	4.754	A	

Means with the same letter are not significantly different.

TABLE OF MEANS FOR HAEMOLGLOBIN

BETWEEN TREATMENT (CONC)

Treatment	conc	mean	Duncan grouping	N
B X	2	3.379	B	
B X	4	3.405	B	
B X	1	4.139	A	
B X	3	4.298	A	

Alpha = 0.05 df = 19 MSE = 0.034989

Number 0 Mean	2	3	4
Critical Range	0.175	0.184	0.190

APPENDIX 6

ANOVA TABLE FOR MEAN CORPOSCULAR HAEMOGLOBIN CONCENTRATION (MCHC) OF O. NILOTICUS EXPOSED TO PARAQUAT FOR 10 WEEKS AT

22.7°C + 0.80 COMPARING CONC AND WEEKS

SV	DF	SS	MS	F
REPS	1	1.616	1.616	0.06
CONC	3	310805.834	103101.945	3661.13
WEEKS	4	226543.280	56635.820	2001.42
CONC VS WEEKS	12	774685.984	64557.165	2281.35
ERROR	19	537.660		
	---	--------		
	39	1312574.720		

TABLE FOR MEANS OF MCHC (%) WITHIN TREATMENT (WEEKS)

Treatment	Weeks	mean	Duncan grouping	N
B X	3	29.93	D	
B X	4	31.78	D	
B X	1	121.63	C	
B X	3	190.17	B	
B X	2	207.02	A	

Alpha = 0.05 df = 19 MSE = 28.298

Number 0 Mean	2	3	4	5
Critical Range	5.559	5.837	6.029	6.140

Means with the same letter are not significantly different.

APPEDIX 6 Cont'd

TABLE FOR MEANS OF MCHC BETWEEN TREATMENT (CONC)

WITHIN TREATMENT (WEEKS)

Alpha = 0.05 df = 19 MSE 28.298

Number of Means	2	3	4
Critical Range	4.972	5.221	5.393

Treatment	conc	mean	Duncan grouping
B X	2	30.91	C
B X	1	33.39	C
B X	4	162.11	B
B X	3	238.02	A

Means with the same letter are not significantly different.

APPENDIX 7

ANOVA TABLE FOR PERCENTAGE NEUTROPHILS OF O. NILOTICUS EXPOSED TO VARIOUS SUBLETHAL CONCENTRATIONS OF PARAQUAT FOR 10 WEEKS (B)

SV	DF	SS	MS	F	Pr F
REPS	1	2.50	2.50	3.06	0.0961
CONC	3	560.40	186.80	228.98	0.0001
WEEKS	4	333.85	83.46	102.31	0.0001
CONC VS WEEKS	12	799.35	66.61	81.65	0.0001
ERROR	19	15.50	0.816		

TABLE FOR MEANS OF PERCENTAGE NUETROPHILS WITHIN TREATMENT (WEEKS)

Treatment	Weeks	mean	Duncan grouping	N
B X	5	3.125	D	
B X	2	8.75	B	
B X	4	9.375	BC	
B X	3	10.250	B	
B X	1	11.50	A	

Alpha = 0.05 df = 19 MSE = 0.815789

Number 0 Mean	2	3	4	5
Critical Range	0.944	0.991	1.024	1.043

Means with the same letter are not significantly different.

TABLE FOR MEANS OF % NEUTROPHILS BETWEEN TREATMENT (CONC = A)

Alpha = 0.05 df = 19 MSE = 0.815787

Number of Means = 2 34

Critical Range = 0.844 0.886 0.916

Treatment	Weeks	mean	Duncan's grouping
A X	4	4.900	D
A X	3	5.900	C
A X	2	9.100	B
A X	1	14.50	A

APPENDIX 8

ANOVA TABLE FOR PERCENTAGE OF LYMPHOCYTES O. NILOTICUS EXPOSED TO VARIOUS SUBLETHAL CONCENTRATIONS OF PARAQUAT FOR 10 WEEKS

SV	DF	SS	MS	F	Pr F
REPS	1	2.50	2.50	4.13	0.0563
CONC	3	7613.30	2237.77	3697.18	0.0001
WEEKS	4	24911.60	6227.90	10289.57	0.0001
CONC VS WEEKS	12	28346.20	2362.18	3902.74	0.0001
ERROR (c-1)(w-1)	19	15.50	0.6053		
CORRECTED TOTAL	39	59985.10			

TABLE OF MEANS OF PERCENTAGE LYMPHOCYTES WITHIN TREATMENT (WEEKS)

Treatment	Weeks	mean	Duncan grouping
B X	5	22.00	D
B X	1	64.13	C
B X	2	66.63	B
B X	3	90.13	A
B X	4	90.38	A

Alpha = 0.05 df = 19 MSE = 0.605263

Number of Mean	2	3	4	5
Critical Range	0.813	0.054	0.882	0.898

Means with the same letter are not significantly different.

TABLE OF MEANS OF PERCENTAGE LYMPHOCYTE BETWEEN TREATMENTS

(CONCENTRATIONS)

Treatment	conc	mean	Duncan grouping
A X	3	54.40	D
A X	4	55.40	C
A X	2	70.70	B
A X	1	86.10	A

Alpha = 0.05 df = 19 MSE = 0.605263

Number 0 Mean	2	3	4
Critical Range	0.727	0.764	0.789

Means with the same letter are not significantly different.

APPENDIX 9

ANOVA TABLE FOR % HAEMATOCRIT OF O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT FOR 96 HOURS

SV	DF	SS	MS	F
TREATMENT (A)	4	52.46	130.85	16.56
ERROR	5	39.5	7.9	
TOTAL	9	562.9		

TABLE FOR MEANS FOR % HAEMATOCRIT (PCV) EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT OF 96 HOURS.

Treatment	CONC	mean	Duncan Grouping	N
A X	1	0.00	D	
A X	3	0.00	D	
A X	2	4.5	C	
A X	5	11.5	B	
A X	4	18.5	A	

APPENDIX 10

ANOVA TABLE FOR % HAEMOGLOBIN O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT FOR 96 HOURS

SV	DF	SS	MS	F
REPS	1	2.40	2.40	1.98 NS
TREATMENT (A)	4	23.36	5.84	4.83 NS
ERROR	4	4.84	1.21	
TOTAL	9	30.60		

TABLE FOR MEANS FOR % HAEMATOCRIT OF O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT FOR 96 - HOURS.

Treatment	CONC	mean	Duncan grouping	N
A X	1	0.00	A	
A X	3	1.60	A	
A X	5	2.95	A	
A X	2	3.40	A	
A X	4	4.40	A	

APPENDIX 11

ANOVA TABLE FOR MCHC OF O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT FOR 96 HOURS

SV	DF	SS	MS	F	
TREATMENT	4	65,570.18	16,392.5	0.79	NS
RESIDUAL	5	103,382.08	20,676.4		
(ERROR)	9	168,952.26			
	9	19,269.5	4816.95		

TABLE FOR MEANS OF MCHC OF O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT FOR 96 HOURS.

Treatment	CONC	mean	Duncan grouping	N
A	X	1	0.00	A	
A	X	3	16.00	A	
A	X	2	23.60	A	
A	X	4	25.80	A	
A	X	5	35.80	A	

APPENDIX 12

ANOVA TABLE FOR % HAEMOGLOBIN O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT FOR 96 HOURS

SV	DF	SS	MS	F
TREATMENT	4	19,261.0	4815.25	2,832.5
RESIDUAL (ERROR)	5	8.5	1.7	
	9	19,269.5	4816.95	

TABLE FOR MEANS FOR % LYUMPHOCYTES O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT OF 96 - HOURS.

Treatment	CONC	mean	Duncan grouping	N
A X	1	0.00	B	
A X	3	0.00	B	
A X	2	90.00	A	
A X	4	90.50	A	
A X	5	92.00	A	

APPENDIX 13

ANOVA TABLE FOR % NEUTROPHILS OF O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT FOR 96 HOURS

SV	*DF*	*SS*	*MS*	*F*
TREATMENT	4	185.6	46.4	14.5
ERROR	5	10	3.2	

TABLE FOR MEANS FOR % NEUTROPHILS OF O. NILOTICUS EXPOSED TO VARIOUS ACUTE CONCENTRATIONS OF PARAQUAT OF 96 HOURS.

Treatment	CONC	mean	Duncan Grouping	N
A X	1	0	BC	
A X	3	0	B	
A X	5	8	A	
A X	4	8	A	
A X	2	10	A	

APPENDIX 14

ANOVA TABLE OF OPERCULUM MOVEMENT (MIXIMUM/MIN) OF O NILOTICUS EXPOSED TO DIFFERENT PARAQUAT CONCENTRATION FOR 96 HOURS AT 22.5°C

SV	DF	SS	MS	F	Pr F
MODEL	37	110311.657	2981.396	9.53	0.0001
REPS	3	268.514	89.505	0.29	
PERIOD(DAY)	4	6314.643	1578.661	5.04	
CONC	6	47321.043	7886.841	25.20	
PERIOD VS					
CONC	24	56407.457	2350.310	7.51	
ERROR	102	31925.486	312.995		
TOTAL	139	142237.143			

TABLE FOR MEANS OF OPERCULUM MOVEMENT WITHIN TREATMENT

Alpha =0.05 df = 102 MSE = 312.995

Number of Means	2	3	4	5
	9.39	9.88	10.19	10.42

Treatment (a)		Period	Mean	Duncan Grouping
A	X	2	50.964	B
A	X	1	60.357	AB
A	X	3	61.214	A
A	X	4	69.036	A
A	X	5	69.143	A

APPENDIX 14 CONT'D

TABLE FOR MEANS OF OPERCULUM MOVEMENT BETWEEN TREATMENT

No of Means	2	3	4	5	6	7
Critical Range	11.11	11.69	12.06	12.33	12.57	12.76

Treatment (a)	Conc	Mean	Duncan Grouping
B X	7	38.85	E
B X	4	43.65	DE
B X	5	52.30	CD
B X	6	58.10	BC
B X	5	66.00	B
B X	2	85.70	A
B X	1	90.40	A

APPEDIX 15 CONT'D

TABLE FOR MEAN OF OPERCULAR MOVEMENT OF O. NILOTICUS EXPOSED TO VARIOUS PARAQUAT CONCENTRATION FOR 8 WEEKS (BETWEEN TREATMENT)

Alpha = 0.05 df = 57 MSE = 123 - 249

Number of Mean	2	3	4
Critical Range	7.034	3.396	7.635

Treatment	CONC	mean	Duncan's grouping
B X	2	88.90	B
B X	3	91.00	B
B X	4	93.90	B
B X	1	101.85	A

Means with the same letter are not significantly different.