TOXIC EFFECTS OF SUBLETHAL CONCENTRATIONS OF LEAD ON *Tilapia nilotica*(Linnaeus)

BY

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THESIS APPROVAL

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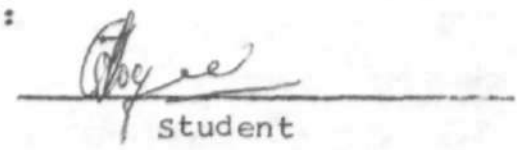
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DEDICATION

This research study is dedicated to the affectionate memory of my beloved mother Madam Esan Ologunmeta.

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ABSTRACT,

Toxic effects of sublethal concentrations of lead was observed on fingerling Tilapia nilotica(L) of size range 12-14.5cm total length, caught from Maska and Maigana fish farms, located at kilometer 145 and 25 along Zaria - Funtua and Zaria - Jos roads in Kaduna State of Nigeria respectively.

Effects of lead on the growth, haemoglobin, haematocrit, blood serum protein, red cell morphology, liver and skeletal muscle glycogen and liver histology were observed in fish exposed to lead concentrations of O.o76, 0.163 and O.326 mgL^{-1}(76, 163 and 326 ugl^{-1} respectively) for a period of six weeks in a static assay. The experiment was replicated once and the pooled values presented. Residues of lead in test fish were also • determined with Atomic Absorption Spectrophotometer.

The study indicate that sublethal concentrations of lead caused a dose-dependent inpairment in the growth of Tilapia nilotica. The percent weight increase after six weeks were 27.73, 27.11, 23.38 and 20.00 for control, 0.076, 0.163 and O.326 mgL^{-1} group respectively. The growth rates in the lead-exposed groups were not significantly ($p > 0.05$) different from that for the control. Growth impairment is attributed to lead interference with energy metabolism in cells mitochondria The values for heamoglobin, haematocrit blood serum protein and liver glycogen in fish exposed to the various concentrations of lead dropped considerably slightly below those for the control. No effect of lead on skeletal muscle glycogen was however observed.

'Blacktail' and 'blackhead' lesions were observed in 40% of fish exposed to 0.163 mgL^{-1} lead after five and six weeks respectively. This was attributed to a probable neurological lead effect or biochemical metabolic inhibition. This observation is in agreement with the occurence of blacktail in lead concentration 0.12 mgL^{-1} for rainbow trout Salmo gairdueri as reported by Davies et al(l976), Hodson et al(l978), (1982), and Alen et al (1983).

Tilapia nilotica accumulated lead concentration up to a level of 8.75 ugg^{-1} tissue after exposure to 0.326 mgL" lead for six weeks.

The livers of test fish exposed to all lead concentrations used in this study degenerated on histological observation, due to the preapitation of the hepatocytes cytoplasm and disintegration of blood vessels.

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ABBREVIATIONS

CHAPTER 3

Hb	---	Haemoglobin
Hct	---	Haematocrit
MCHC	---	Mean corpuscular haemoglobin concentration
BSP	---	Blood serum protein
LG	---	Liver glycogen
SMG	---	Skeletal muscle glycogen

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CHAPTER ONE

INTRODUCTION

Fish provides one major source of animal protein the world over, and especially in Nigeria. It is infact the chief source of animal protein in poultry ration (Oni 1979). According to Oni (1979), the main reason for the high cost of poultry feeds in Nigeria is that the major components, maize (corn), fishmeal, bloodmeal, bone and vitamins are imported into the country. Corn and fish are the major (sometimes the only) sources of protein in poultry feeds. Fishmeal 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.

Fish as a source of protein in human diets cannot be overemphasized. 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 tonnes of fish in 1980 out of which about 900,000 metric tonnes of

frozen fish were imported. 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 per caput fish consumption figure of 5.17 kilograms in Nigeria was far below the planned average figure of 10 kilograms envisaged by 1985.

This fish is either obtained locally through artisinal fishing from fresh water bodies or obtained from trawl fishing from marine water bodies. While efforts are being directed towards increasing fish production by government agencies, in an attempt to meet the demand, many factors are subverting the efforts. Among the many factors that are negating the efforts is pollution. Literature review further pointed out that many of the water bodies are being reduced to depots for poisonous chemical pollutants which kill the fish and other aquatic organisms. One major pollutant in these water bodies are metals. The identified potentially toxic metals are lead, zinc, copper, arsenic, antimony, mercury, berylliuin, bariuin, cadmium, nickel, and selenium (Luckey and Venogopal 1977). Other toxic metals to aquatic life ϕf absorbed in excessive amounts include cobalt, and to some extent manganese.

The improvement of physicochemical properties of the water bodies is vital to the enhancement of fish production and success of government's agricultural program on self reliance and sufficiency in food supply.

This study is therefore designed with the following objectives;

(a) determining the effects of sublethal concentrations of lead in our aquatic environment on some physiological parameters vital to the survival of Tilapia nilotica,

(b) examining histopathological effects of lead in tissues,

(c) providing information on the accumulation and distribution of lead in Tilapia which is of commerical importance in Nigeria.

Sources of Metals to Aquatic Environment.

The sources of these metals pollutants to our aquatic ecosystem have been documented (Waldron and Stoifen 1974, Goyer and Mehlman 1977, Lucky and Venugopal 1977, Ellgaard and Rudner 1982, Oladimeji 1983). The aquatic environment may be naturally contaminated by metals (e.g. erosion or seepage from metal-rich surface deposits in mineralized zones, volcanic and thermal-spring activity). In addition, human activities may play a major role (e.g. past and present metalliferous mining, smelting, the manufacture of glass, cement, brick or paper and fossil - fuel combustion that include automobile exhausts). Wastes of human activities like sewage sludge which are often found to contain substantial amount of certain metals may be dumped extensively on agricultural land and water bodies in many countries. Contamination by inorganic

fertilizers may also make a significant contribution.

With the increase in industrialization and technology, the volume and complexicity of heavy metals which find their way into our water bodies also increase. In Nigeria for instance, the smelting and refining of tin in Jos mines have resulted in higher concentration of lead, mercury and zinc in River Dilimi into which the effluents from mines industry are discharged (Akueshi 1980). In Kaduna, The Peugeout Automobiles Nigeria Ltd (PAN), discharge waste water mainly containing paints with heavy metals especially lead and zinc while the Nigeria National Petroleum Company (NNPC) refinery produce waste water with biosludge fines, silt from storm water runoff which contain certain oil, watersand, and silt heavy metals, phenol and cyanide (Clement 1982). In addition to the trace metal contents of effluents from automobile industries, tetraethyllead is used as an anti-knock in gasoline. On combustion; smoke containing lead particles get emitted into the atmosphere. When rain falls, these particles are washed directly into either water bodies or onto the soil from where it leaches into the aquatic environment. Besides a number of our tanneries use sodium arsenate to protect leathers against attack of insects and microbes. This chemical has been banned in developed countries because of its

Carcino-genicity. The effluents from such tanneries containing the chemical later end up in rivers and streams.

Toxicity of Metals to Fish

When heavy metal discharges into either terrestrial or aquatic environment exceed the natural turnover in the ecosystem, it may have some harmful effects on the biota. Some pollutants and effuent discharge into the water bodies act on the gill of the fish, resulting in death by a combination of chemical and physical injuries (Ellis 1937).

Once the metal enters into the circulation of the organism and becomes bound to the various tissue components, its clearance and hence its toxicity is directly related to such physiological factors such as metabolic rate, or other species specific, time dependent and definable functions such as body size and /or the amount of fat in the tissue (adiposity). Ozoh (1979) injected lead concentration up to 600mgL^{-1} into Zebra ciclid fish which according to Ellgaard and Rudner (1982) killed bluegill, the Zebra fish survived and even ovulated. Heavy metals interfere with pigmentation in fish. Ozoh (1980) incubated eggs of Zebra fish Brachydanio rerio in 50 - 72 mgL^{-1} lead acetate for 24 hours. The embryo so obtained suffered

alteration in pigmentation pattern.

The danger of food poisoning from consumption of contaminated sea foods are quite known. In Japan, symptons of "Minamata disease" was reported from consumption of fish contaminated by mercury(Goyer and Nehlman 1977 (eds), Gill and Pant 1981). A number of studies on toxicity of toxic metals to fish have been reported (Davies et al 1976, Ellgaard and Rudner 1982, Oladimeji and offem 1983), as well as biochemical effects(Gill and Pant 1982). Physiological effects include Hodson et al (1982), Alan et al(1983) and histo pathological effects on fish anatomy have also been reported(Kumar and Pant 1981, Elsie et al 1984).

Experts(1985) mentioned that heavy metals like lead, mercury, cadmium and iron may be poisonous to equatic organisms like fish, shrimp, crabs, lobsters in the effect, reducing their availability. The researchers also stressed that certain edible species of fish and other sea animals are leaving Nigerian waters due to lack of food, since small animals and plants on which bigger sea organisms feed have been destroyed by chemical pollutants.

Regardless of the progress made in the treatment and detoxicification of environmental wastes, heavy metals still constitute great health hazards to human and other living organisms. Unlike other classes of pollutants which can be biodegraded and destroyed

dompletely, metals can neither be created nor destroyed. Unfortunately, knowledge and comprehensive information about the chemistry of their formation and concentration is very meagre. Some metals can be transformed to more harmful forms while others can be complexed to stable and less toxic compounds. Moreover, some bound metals in the earth's crust are released by natural and artificial chemical processes into the aquatic environment. It is therefore of necessity to continously monitor both aquatic and terrestrial environments with the purpose of unfolding baseline data by which one could decide whether the extent of pollution has exceeded tolerance or safety levels.

Lead

Lead is a grey, ductile, malleable metal with a bright luster that is more than eleven times as dense as water. It is present everywhere in our environment as trace constituent of rocks, soil, water, and in fact, of plant and animal life. According to Nriagu (1978), the total amount of lead in the earth's crust and soil may be about 3.8×10^{20} and 4.8×10^{15} g respectively. Utilisation of lead by man started as far back as 3000 B.C. (Ellgaard and Rudner 1981), and with the rise in industrialization and technology, the use has increased. Robinson (1978) indicated that automobile and construction industries are presently the main consumers of lead. As substantial amount of lead are

smelted and burnt as lead alkyls daily in the increasing construction automobile industries, it may be expected that our biota will be exposed to more lead in its various life-support systems than even before.

Two major groups of lead compounds exist, inorganic and organic. The inorganic lead compounds occur both as occupational and general environmental hazards. The organic lead compounds occur exclusively as occupational hazards. In this study, only the effect of the first group will be considered.

Lead is readily complexed in natural waters, the degree of which largely depends upon the alkalinity (bi-carbonate - carbonate concentration) of the water into which it is released (Davies et al 1976). Lead has been found to be more toxic to fish in soft water than in hard water. A partial explanation for this is that lead is partially tied-up or complexed into non-toxic chemical species in hard natural water (Davies et al 1976).

Lead may be distributed among all three possible states of heavy metals (particulate, colloidal and dissolved forms), though it is the dissolved fraction that is directly toxic to fish (Davies et al 1976).

The problem confronting field men and toxicologist that are monitoring heavy metal pollutants which are

completed or partially tied-up in natural waters is in identifying and analysing that fraction of such heavy metal that is toxic. If this has not been accomplished, realistic water quality standard will be difficult to establish.

Lead Toxicity to Vertebrates Other Than Fish

A number of previous studies on the toxic effect of lead on vertebrates in general have been carried out. These have been reviewed by More and Goldberg (1980). The authors noted that patient who died of lead poisoning showed thinness of blood and pale tissues on post mortem examination.

Lead affect the heme synthesis path way of man. This may be noticeable in the occurence of excess porphyrin in the urine of a patient intoxicated with lead, due to the action of lead upon haemoglobin synthesis in the bone marrow. Excessive urinary excretion of δ - aminolevulinic acid has also been linked to lead intoxication. Anemia resulting from lead poisoning is also known. This may be due to depression of heme biosynthesis, disturbance of globin synthesis and hemolysis of red blood cells(More and Goldberg 1980). According to the authors, lead interferes with sodium-potassium dependent Adenosin triphosphate(ATP) enzymatic activity in the red cell. As a consequence, red cells incubated with high concentration of lead leak large quantities of potassium,

resulting in an increased mechanical fragility of the red blood cell and consequent lysis. At the same time, there is little change in the imput of Sodium concentration in lead poisoned erythrocytes, associated with potassium - mediated shrinkage of the cell.

Symptoms of nervous system lead poisoning ranging from ataxia, to stupor, coma and convulsions have been observed in vertebrates. Lead has been implicated as causing brain damage (encephalopatty) in mammals (Davies et al 1976), although the toxic effect of lead on the central nervous system is little understood. Luckey and Venugopal (1977), reported the carcinogenic, teratogenic and embryocidal effects of lead to mammals.

Dogs are known to develop plumbism as a result of chewing objects covered with lead - based paint or even eating metalic lead (Waldron and Stofen 1974). Abortion have been reported in ewes grazing in areas around lead-mine and the lethal dose of lead is lower in pregnant than non-pregnant ewes (Waldron and Stofen 1974).

Toxicity of Lead to Fish

A number of studies have been carried out of the acute toxicity of lead to fish (Pickering and Henderson 1966, Davies et al 1976, Hodson et al 1978, Ellgaard and Rudner 1982, Oladimeji and Offem 1983). Pickering and Henderson (1966) reported 96hr LC-50 of

442mgL^{-1} as lead chloride for the bluegill sun fish Lepomis macrochirus. This observation was consistent with the value of approximately 400 mgL^{-1} 96-hr LC50 of lead acetate reported for same fish by Ellgaard and Rudner (1982). Davies et al (1976) in their research on rainbow trout Salmo gairdneri reported two 96-hr LC50s of 1.32 and 1.47 mgL^{-1} analysed (disolved) lead from introduction of 542 and 471 mgL^{-1} total lead respectively in water of total hardness 353 mgl^{-1} as $CaCo_3$. The authors further observed a 96-hr LC50 of 1.17 mgL^{-1} lead in soft water (28mgL^{-1} as $CaCo_3$) and concluded that the fish had a lethal threshold LC50 of 0.20mgL^{-1} after 14 days exposure to lead in the soft water. Mortality began 5 days after the experiment was started and continued for a total of 14 days before a lethal threshold was reached. Hodson et al (1978), working on the same fish reported 21 - day LC50 of 2.4mgL^{-1} lead in water of total hardness 135 $\pm$ 2mgL^{-1} as $CaCo_3$.

Recently, Oladimeji and Offem (1983) reported 24-hr and 96-hr LC50s of 4.21 and 2.41mgL^{-1} as lead nitrate respectively for Tilapia nilotica in soft water. Acute toxicity of lead to fish has been attributed to destruction of the respiratory epithelium (Dorfman and Whitworth 1969). Although it was reported that lead at level of 400mgL^{-1} was lethal to bluegill (Pickering and Henderson 1966), the tissues contained only 0.1mgL^{-1}.

There are numerous literature on the long-term effect of lead on temperate fish(Drilling and Smith 1926, Dawson 1935, Ellis 1937, Grandall and Goodnight 1983 etc).

Drilling and Snith (1926) found that plaice exposed to 4 mgl^{-1} lead for 156 days had reduced growth. Dawson (1935), reported brownbull heads survived 16 - 183 days in water that contained 27.3 mgl^{-1} lead but became anaemic. Ellis (1937) reported goldfish survived lead concentration of 5.5 mgL^{-1} lead for 61 days. Crandall and Goodnight(1963) also observed that guppies exposed to 1.24 and 3.12 mgl^{-1} lead for up to 129 days had histological damages, retarded gonadal development and reduced growth. Chronic exposure to sublethal concentrations of lead are known to affect haemopoiesis in fish resulting in decreased haematocrit, decreased haemoglobin concentrations and deformed erythrocytes (Wong et al 1978).

When the rainbow trout Salmo gairdneri or brook trout Salvelinus fontinalis were continuously exposed to chronically toxic concentrations of water borne lead, starting at the egg stage, they developed the following abnormaities;

(a) "Black tail", a darkening of the caudal area behind the dorsal fin, usually followed by spinal curvature

(b) elevated concentration of lead in tissues and

(c) inhibition of the erythrocyte enzyme, δ- amino-levulinic acid dehydratase (ALA-D) (Davies et al 1976, Holcombe et al 1976, Hodson et al 1978, 1982). Davies et al (1976) suggested that there is possible inhibitory effect of lead on the biochemical metabolism of vitamin C or tryptophan. This is because nutritional deficiencies of ascobic acid (vitamin C) has been reported to cause acute spinal curvature in trout (Davies et al 1976). Hodson et al (1982) observed no obvious relationship between wet weight and whole-body lead concentrations, blood lead concentrations and erythrocyte ALA-D activities of lake trout Salvelinus namaycush. And in contrast, Hodson et al (1982) observed that the whole-body lead concentration of rainbow smelt Osmerus nordax was negatively correlated to wet weight. Lead concentrations in the blood and opercular bone and the percentage inhibition of ALA-D increased with time in all sizes of rainbow trout exposed to $0.1 mgL^{-1}$ lead for 8 days (Hodson et al 1982). Ellgaard and Rudner (1982), did not observe any dramatic changes in locomotor activities when blue gill, sun fish Lepomis macrochirus are exposed to concentrations of lead which approach toxic levels.

Alan et al (1983) reported that there was no obvious histological effects on rainbow trout Salmo gairdneri exposed to sublethal concentration of $0.1 mgL^{-1}$ lead for a period of 32 weeks. According to the authors, some lesions noted in the kidney of the lead-exposed trout due to the presence of hyaline droplets, were also observed in the

control fish. The authors also noted that the blacktail effect on rainbow trout were only observed in waterborne and not in the lead-fed experiment.

While reports on the effect of lead and other heavy metals on various physiological changes of fishes of the temperate regions are readily available, studies dealing with the effect of sublethal concentration of lead on the same parameters on tropical fish and in Nigeria in particular are few. Clement (1982) noted that only little published work is available on the toxicity of pollutants to tropical fish or the effect of pollutants on tropical stream ecology; however, many examples of reduction in fish catches which could have been due to accumulation of pollutants over the years have been cited by fishermen. Oladimeji (1983) similarly observed that very little has been done in Nigeria to estimate the hazard of trace heavy metals on aquatic life.

Since early detection of specific physiological and other abnormalities provide an indication of exposure prior to manifestation of any gross damage, the measurement of haematological, biochemical,

physiological and histopathological changes in the fish under exposure to the toxicant, may be used to predict effects upon chronic exposure (Gill and Part 1981). The present study is therefore undertaken to measure the aforementioned changes (haematological, biochemical, physiological and histopathological) in lead - intoxicated Tilpia nilotica (L) with a view to furthering our understanding of the toxic effect of lead.

CHAPTER TWO

MATERIALS AND METHODS

Maintenance of the Fish:

The fish used for this study were fingerling Tilapia nilotica (Linnaous) which were obtained from Maigana fish farm located at about kilometer 25 along Zaria - Jos road and Maska fish farm located at about kilometer 45 along Zaria - Funtua road. These fish farms are jointly owned by Federal and Kaduna State governments. It was not possible to obtain fish of uniform size; fish size ranged between 12-14.5cm(total length) and average weight of 39.83 ± 0.91g were used. Fish were collected from the farms in early morning when ambient temperature was not so high as to stress them. They were transported in polytene bags containing oxygenated pond water.

Upon arrival at the laboratory, the fish were held at 23.3 - 25^{0}c in two 130 litre plastic aquaria before commencement of assay. The aquaria were aerated and situated in an airconditioned room. The fish were fed daily on formulated experimental diet of soyabean - ricebran - fish meal mixture during acclimatisation, compared to cotton seed meal fed upon in the farm. The fish were apparently healthy and less than 1% mortality occured during the holding period, which lasted for about two weeks prior to commencement of the experiments.

Exposure of Fish to Lead:

The experimental tanks used were large glass aquaria of size 30.5 x 30.5 x 92.5 cm (60 litre capacity). Eight tanks were used altogether. Four served as test tanks while the remaining four served for changing test water. The changing of test-water was achieved by draining test tanks to a level that just barely covered the fish and fish quickly transfered with a scoop net to duplicate test tanks containing freshly prepared test water. The room housing experiment was lit with flourescent light which maintained a photoperiod of 12 hours of light daily. The aeration period of 2hrs per day of Nagendra and Katre (1979) was considered insufficient for this assay as test fish used were bigger. Test water was aerated continuously and changed thrice weekly. No antagonistic or synergistic relationship between water oxygenation and lead toxicity to fish has ever been reported.

Due to shortage of test tanks, the experiment and replicate were carried out one after the other. All data were pooled to obtain a single overall result.

The laboratory was supplied with Zaria City tap water and stored in 1000 litre capacity reservoir supported by a concrete plat-form outside the experimental room (laboratory). Water was delivered to the experimental room through water pipes after it has been dechlorinated according to Standard Methods

(1980) using solar energy and continual agitation with plastic rod. The water was also aerated inside the reservoir by means of an air pump for not less than 48hrs before use.

The chemistry of the water over the period during which experiments were carried out is presented in Table 1. Water alkalinity, total hardness, residual chlorine and dissolved oxygen were determined according to Standard Methods (1980). Chloride content of water was determined by Mohr method according to Fodeke et al (1983). Water PH and temperature were read with PH metre and celcius thermometer respectively.

Preparation of Diet:

The artificial diet used for this study has been used elswhere (Cruz 1975, Oladimeji and Offem 1983). The diet consisted of a mixture of rice bran, soyabean and Fish meal. Ita (1981), Ita and Orobor (1983) used guinea corn and maize bran supplemented with chicken layers concentrate in the ratio of 3:1 and small quantity of fish meal powder to grow Tilapia zilli in Cages. The soyabean was boiled, oven dried at 60^{oc} and ground to powder. The rice bran was also ground to powder. Unused fresh fish were eviscerated, oven dried at 60^{oc} and ground to form the fish meal powder. Appropriate quantities of rice bran, soyabean and fish meal were measured to make the powered mixture (Table 2). A Small quantity of vitamin premix was added. 3 parts of powered mixture was added to 6 parts by weight of hot distilled water and 1 part

Table 1: Chemical characteristics of dechlorinated treated water as determined by standard and other methods.

Constituent	Concentration
Water temperature	22.30 - 25^{oc}
P.H.	7 - 7.3
Alkalinity	20 - 25mg $CaCo_3L^{-1}$
Total Hardness	63 - 78mg $CaCo_33L^{-1}$
Chloride	0.01mgL^{-1}
Residual Chlorine	Not detectable
Dissolved Oxygen	4 - 7.1mgL^{-1}

Table 2: Percent Composition by weight of powdered mixture used for the diet of T. nilotica exposed to lead.

Constitutent	Concentration
Rice bran	54%
Soyabean	35%
Fish meal	10%
Vitamin premix	1%
Total	100%

• Vitamin Premix [1]	
Vitamin A (retinol)	8000 I.U
Vitamin E (& -tocophnol acelate)	60 I.U
Vitamin D_2 (Calciferol)	1000 I.U
Vitamin K (Menadione)	
Thiamine hydrochloride (B_1)	4mg
Riboflavin (B_2)	8mg
Panto themic acid	12mg
Nicotinic acid (niacin)-	50mg
Folic acid (Folacin)	10mg
Pyridoxine hydrochloride (B_6)	6mg
Choline chloride	200mg
Cobalamin (B_{12})	12mg

[1] Vitamin premix was prepared by Lebsure, RHM, Agriculture (South) Ltd; Poole, Dorset, BH 15 111L (Registered office) England.

of gelatin powder as binding agent. The resulting mixture was poured into a shallow tray, put in the fridge to set and over-dried to constant weight at 60^{oc}. This was then cut or crumbled into small pellets which were used.

The percent proximate composition and mineral concentration of the feed is presented in Table 3. The crude protein was analysed by Macro-Kjeddahl's method (AOAC 1975). This method does not measure the protein content directly, but measures the total nitrogenous matter present in the feed sample. 0.5g of the sample is first digested with a mixture of concentrated sulphuric acid, anhydrous sodium sulphate and anlydrons copper sulphate. The sodium sulphate raises the temperature of the reaction while the copper sulphate catalyses it. The digested sample is cooled and distilled with 45% sodium hydroxide solution(11.25N NaoH) to release ammonia(NH_3) from the sample. The ammonia is collected over a standard sulphuric acid containing an indicator(screen methyl red indicator). The standard acid is then titrated with dilute sodium hydroxide to an end point. The experiment was done in triplicate and the mean obtained. A blank containing the same amount of concentrated sulphuric acid, plus anhydrous sodium sulphate and copper sulphate minus the sample was carried out simultaneously with sample reactions. The total percentage of Nitrogen in the samples was calculated using the equation below:

Table 3: Proximate composition and mineral concentration of the diet fed to *T.nilotica* exposed to lead in water.

Components	Percentage of dry weight.
Protein	27.00
Lipid	20.60
Carbohydrate	48.30
Total organic matter	95.90
Total ash	4.10
Potassium	0.38
Sodium	0.07
Magnesium	0.09
Calcium	0.95
Phosphorous	0.18

$$\frac{0.014\ (B-A)\ m \times 100}{W}$$ where

B = Average volume of the Standard sodium hydroxide used in neutralizing the acid used for the blank determination

A = Average volume of the Standard sodium hydroxide used in neutralizing the acid used for the sample extract.

M = molarity of the Standard sodium hydroxide

W = weight in grams of the feed sample used

0.014 = mass of Nitrogen per ml of solution. The crude protein content of the sample was calculated by multiplying the total Nitrogen content by 6.25 which is a constant factor of conversion for the protein.

Lipid in feed sample was analysed by soxhlet extraction set as used by Efuna (1984). Three clean thimbles were labelled and their weights (w_1) recorded. 1.0g of the sample was weighed into each of the thembles and their weights (w_2) recorded. The thimbles were plugged with fat-free cotton wool to stop spillage during the extraction and then dropped into the extraction chambers. 200mls of Diethyl ether/methanol mixture in ratio of 1:1 was poured into 500 ml round bottom distilling flasks. The soxhlet extractors were arranged on a heating mantle set at 80^{oc} for 8hrs. At the end of the extraction, the fat-free contents were removed from the extraction chambers, dried in an oven set at 50^{oc} for 24hrs and later cooled in dessicator. The thimbles and the fat-free contents were reweighed (w_3) and recorded. The amount of

lipid extracted was obtained thus:

W_1 = wt of empty thimble

W_2 = wt of empty thimble plus sample before extraction

W_3 = " " " " plus sample after extraction.

Therefore Wt. of lipid extracted = $W_2 - W_3$

Percentage of lipid extracted = $\frac{W_2 - W_3 \times 100}{W_2 - W_1}$

The mean of values from the three thimbles were used.

Carbohydrate in the feed was determined by the difference methods of Watts (1957). Mineral concentrations were determined using Flame photometry, Colorimetry and (A.A.S.) (A.O.A.C 1975).

Protein requirement is the most important aspect of fish nutrition (Ita and Orobor 1983). Therefore the technique for feed formulation is aimed at attaining an adequate quantity of crude protein levels first, before the energy levels. Carnivorous or predatory fish require a higher protein level in a feed than herbivorous fish (Ita and Orobor 1983). Tilapia nilotica (L) is a herbivore, plankton feeder and Ominivore (John et al 1972). Kavalec (Undated) proposed 18% protein level in the feed for middle quality Tilapia nilotica and 28 - 32% protein for predatory fish.

Selection of Test Species and Duration of Studies:

The nutritive value of Tilapia nilotica (L) has been studied by a number of researchers (Eyo 1975, Rakiya 1977, Ayeni 1979). It is a fish of commerical

importance which is very common in Nigerian fresh water bodies. It's selection for this study is connected with its commercial value. Besides, the fish has been used in previous acute toxicity studies(Oladimeji and offem 1983). An understanding of sublethal lead dosage on the same species will expand our knowledge of the toxic effects of lead. The fish was exposed to lead for a long term because of the paucity of literature on chronic toxicity of metal particularly to tropical fish species. The fish were exposed to lead for a period of 6 weeks.

Selection of Concentrations of Lead in Water:

Justification for sublethal concentrations used in the literature is scarce, sublethal concentrations to which the fish were used in this study were therefore arrived at on the basis of the available information about acute toxicity values of previous studies.

The sublethal concentrations thus selected were 1/7 ($0.344 mgl^{-1}$), 1/14($0.172 mgl^{-1}$) and 1/28($0.086 mgL^{-1}$) of the estimated 96hr LC50. This is in accord with the recommendation of Amminukuthy and Rege(1977). In addition, four fish of size ranging from 6 - 8 cm

were exposed to higher lead concentration of 0.48 mgL^{-1}) (1/5 of LC50 96hr) as trial assay before the start of actual experiments, with a view to observe the extent of toxicity of sublethal concentration to test fish. The dissolved lead in test water as measured by Atomic Absorption Spectrophotometer is presented in table 4.

Preparation of Lead Concentration:

5000 mgL^{-1} of lead stock solution was prepared by diluting 7.990g(5 x1.598g) lead nitrate to 1 litre with distilled water in volumetric flask.

Mol. wt. of Lead nitrate = 331.2,

At. wt. of Lead = 207.21,

therefore 1g of lead = $\frac{231.20}{207.21}$ = = 1.598g of Lead nitrate

Appropriate volume of lead stock solution was diluted to mark in test tanks with dechlorinated water supplied from the reservoir by water pipe to make concentrations 0.344, 0172 and 0.086 mgL^{-1} of lead of three treatments. Dechlorinated water served as control.

Experimental Design:

At the beginning of each experiment, 52 Tilapia nilotica (L) of weights ranging from 30.89 to 54.17g were selected and randomly distributed in each of the treatment tanks and control at the rate of 13 fish per tank. At the end of the sampling period of 6 weeks, one fish left per test tanks having been exposed to lead

Table 4

Lead concentration in test water as determined by Atomic Absorption Sectro photometer model Hitachi 170-30

Sample no.	Introduced nominal lead concentration in test water(mgl^{-1})	Determined lead concentration in test water(mgl^{-1})
1	0.086	0.076
2	0.172	0.163
3	0.344	0.326
Control	0.000	0.000

for a period of 6 weeks and that in the control were processed for histopathological examination of the liver.

Growth Study:

After selection and random distribution of test fish to the test tanks, three days later the fish were anaesthetized in MS 222 (Triaine methane sulphonate) using a concentration of 1/15000 (Blaxhal and Deisley 1973). Fish were tagged through the musculature anterior to the dorsal fin(Adeney and Mathiessen 1979) with fingerling tags[1]. They were blotted dry and weighed to the nearest hundredth of a gram. The Standard and total lengths were taken. Test fish were weighed weekly. In all cases fish were fed to satiation once daily.

Haemotology:

Two fish were randomly sampled weekly from each test tank. These two fish were removed, anaesthetized and weighed. Blood samples were collected in heparinized microcapillary and sampling tubes from the caudal artery after severing the caudal peduncle.

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1 Fingerling fish tags, 5mm x 3mm. Floy Tag Manufacturing Ltd., 4616 Union Bay Place N.E. Seattle, Washington 98105.

In both experiments, the two fish sampled per test tank at sampling periods were used for blood haematocrit and haemoglobin; and for liver and skeletal muscle glycogen determination. Total blood protein and tissue residual lead were determined in the second experiment. The morphology of the red blood cells was also observed using light microscopy.

Packed Cell Volume (PVC):

To measure haematocrit, blood samples were collected in heparinized microcapillary tubes and the head sealed in a flame. The head temperature was cooled in ice water to prevent anoxia and cellular swelling (Hodson et al 1978). The tubes were centrifuged with haematocrit centrifuge. Haematocrit were measured with a microcapillary reader and the mean haematocrit for each fish was used in all subsequent calculations.

Total Plasma Protein:

The total blood protein was determined using the blood plasma siphoned out from the microcapillary tubes and read on the Godberg's Refractometer model 10400 A T.S. Readings of total protein level were obtained by direct scale reading at 20^{oc} with a unit of gml 100ml.

Haemoglobin:

To measure haemoglobin, appropriate blood dilutions were prepared in isoton (R) 11 (Azide free balanced electrolyte solution) according to the instruction

manual accompanying the haemoglobinometer. Values were read on the haemoglobinometer model HGBR 6279.

Red Cell Morphology:

The method of Alan et al (1983) was employed for red cell morphology. Smears were obtained from every blood sample collected from each sampled fish per test tanks. The smear was allowed to dry by waving the slide in air, fixed in absolute alcohol for 3 - 5 minutes and stained in Giemsa stain for 30 minutes, washed and dried.

Glycogen Determination:

The method of Sam et al (1950), modified by Hassid (1957) was employed. This method was also used by Gill and Pant (1981); Murty and Priyamuda (1982) and found to be accurate. Fish were dissected to expose the liver. 90mg of liver and skeletal muscle were dissected out and put in preweighed separate test tubes. The tubesand contents were then reweighed and the weight of sample determined by difference. One ml of 30% KOH was added to each test-tube and samples digested for 20 minutes in water bath containing boiling water. The digest was cooled in cold H_2O. Appropriate dilution of liver digest was made and 5ml of final dilution introduced into a fresh text tube. 10 ml of 0.2% anthrone reagent was added, boiled for 10 minutes in a water bath, cooled and absorbance read at 620 nm in Corning Colorimeter 253.

In the case of skeletal muscle digest, 1.25ml of 95% alcohol was added after cooling stirred with glass rod washed in 60% alcohol, and the whole content brought to boiling for a few minutes in water bath. The whole content was cooled and centri fuged at 3000 r.p.m for 15 minutes. The supernatant was decanted and test tube drained on filter paper for few minutes. 5ml of distilled water was added to the precipitate and 10ml of 0.2% anthrone reagent added. The rest procedure was carried out as for liver sample. 5ml glucose solution of 20 $\mu g l^{-1}$ was used as Standard. Glycogen in samples was calculated from the equation.

$$\frac{100 \times U}{1.11 \times S}$$ where

U = the optical density of the unknown test solution

S = the optical density of the glucose

1.11 = the factor determined by Morris (1948) for the conversion of glucose to glycogen.

three replicate analysis were made per sample and mean absorbance values used in subsequent calculations. Glycogen is expressed as milligram per gram wet weight of the tissue.

Lead Residue in Tissues:

Fish residual lead concentration was determined according to Hodson et al (1978) and Hussana (1983). Each sampled fish carcass was placed in an acid-washed, preweighed dry large crucible and the wet weight of whole sample recorded. The samples were dried at 80^{oc}

for 48 hrs and transfered to a muffle furnace. The temperature was maintained at 460^{oc} for 24 hrs. The ash was cooled in a desicator. 1gm of ash was weighed out into an acid - washed pre-weighed test tube to obtain ashweight by difference. 5ml of concentrated hydrochloric acid was added and heated for 15 minutes in boiling water bath. 1ml of concentrated Nitric acid was added. The resulting solution was again boiled in a water bath and evaporated almost to dryness. Distilled water was then added and the solution was filtered and transfered quantitatively into 50ml volumetric flask and made up to mark with distilled water. Lead in samples was read in Atomic Absorption Spectrophotometer(AAS) model Perkin-Elmer 400.

Lead Determination by Atomic Absorption Spectrophotometry:

The lead standard. Stock solution contained $100mgL^{-1}$ lead. This was obtained by disolving 0.1598g lead mitrate in 20ml of nitric acid (1%V/V) and diluting to 1 liter with distilled water. Working standard of concentration ranging from o to $5mgL^{-1}$ were prepared from the stock solution and used to construct a calibration curve(Fig 1).

(a) Warelength of determination = 283.3m
(b) Lamp = Electrodless discharge lamp
(c) Flame = Air Acetylene(C_2H_2).
(d) Suitable range = 0 to 5 mgL^{-1}
(e) Potential interference = None
(f) Detection limit = Not $1mgL^{-1}$

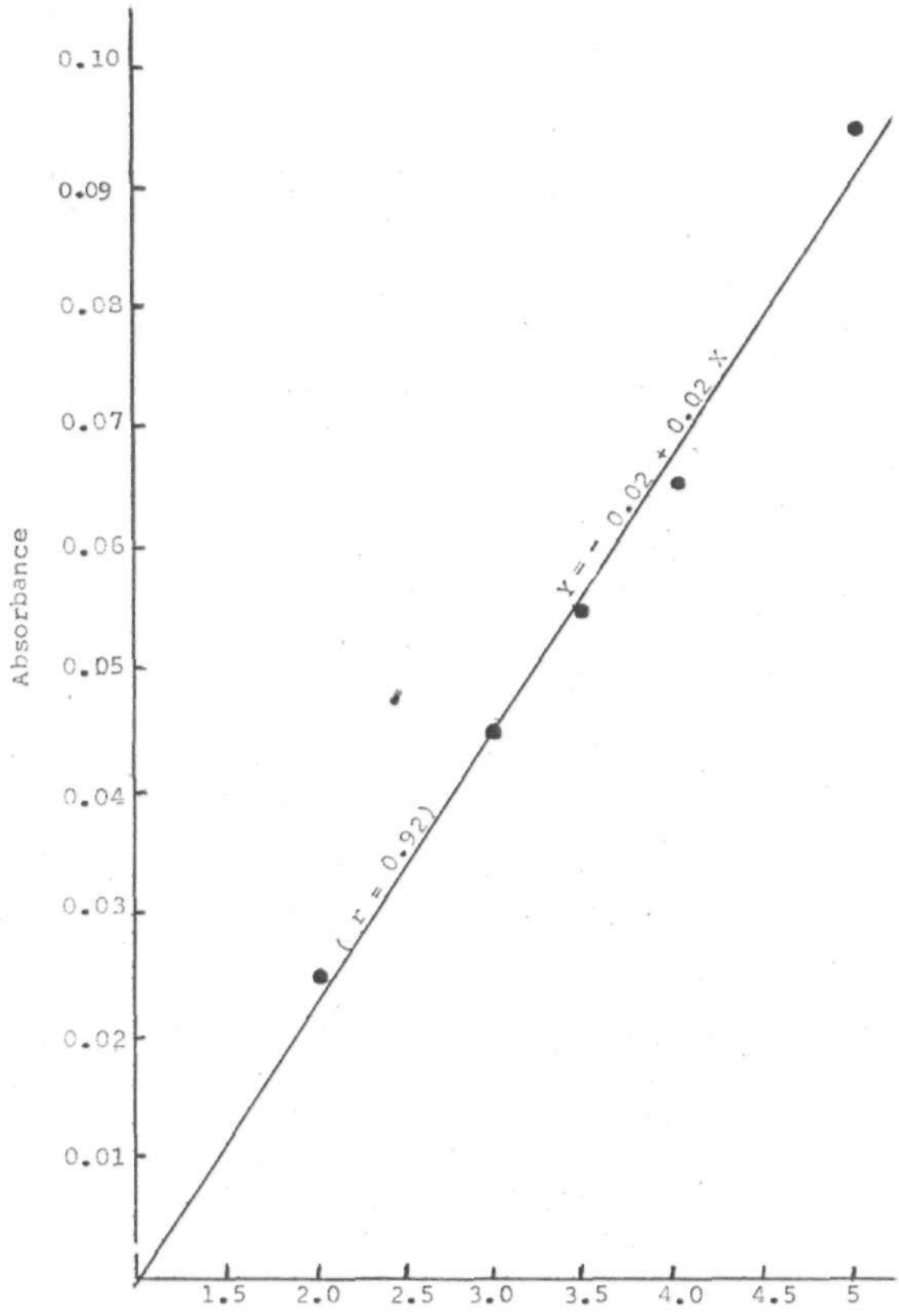
Histo-pathological Study:

Fish for histo-pathological study were sampled at the end of the experiment. Only samples from the second experiment were processed. Fish were killed by a blow on the head and the abdomen opened to expose the viscera. The livers were carefully dissected and freed of adhering tissue. These were fixed in formol saline (100ml of 10% formalin + .9gNacl) (Thompson and Hunt 1966, Alan et al 1983); washed, dehydrated, infiltrated and blocked in paraffin wax of 58^{oc} - 60^{oc} melting point. Sections that were 8µm in thickness were cut and stained with Haematoxylin and Eosin (H&E).

Statistical analysis:

All data for lead-treated fish were compared with the control using student t-test at 95% probability level.

Fig 1



Standard curve for residual tissue lead determination

CHAPTER THREE

R E S U L T S

Effect of lead on growth:

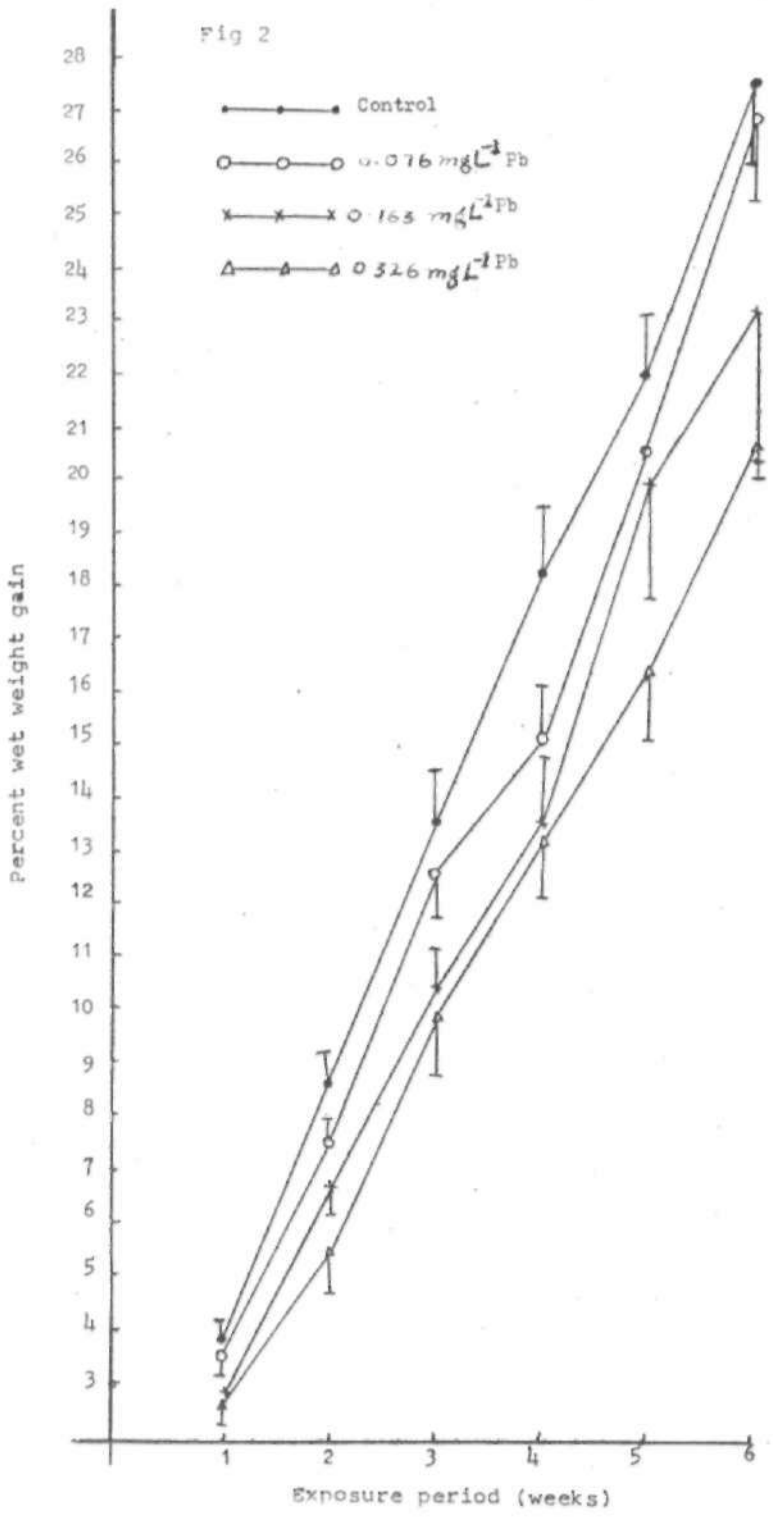
The initial mean wet weight of the test fish were 38.77 ± 1.05 for the control and 40.78 ± 1.43, [illegible] 41.86 ± 1.39 and 37.89 ± 1.26 for the 0.076, 0.163 and 0.326 mgL^{-1} of lead in water respectively. Growth was estimated from the sum of the individual wet weights of the remaining fish at 1, 2, 3, 4, 5 and 6 weeks and expressed as percent wet weight gain.

The result shows that the percent wet weights gain in the control group increased from 3.82% at 1 week to 27.73% at 6 weeks (Fig 2). The growth of fish that were exposed to 0.076, 0.163 and 0.326 increased from 3.47%, 2.78% and 2.70% at 1 week to 27.11%, 23.38% and 20% at 6 weeks respectively. The result indicates an insignificant ($P > 0.05$) decrease in weight gain of fish exposed to lead compared to the control group. Generally, the weight gain in fish exposed to 0.326 mgL^{-1} group was lower compared to 0.163 mgL^{-1} lead and much lower than 0.076 mgL^{-1} group indicating a dose-dependent effect.

Effect of lead on haemotological parameters:

Haemoglobin and haematocrit for the control group did not deviate much from the baseline values throughout the test period (Table 5). The haematocrit

Fig 2


Control
0.076 mgL⁻¹ Pb
0.165 mgL⁻¹ Pb
0.326 mgL⁻¹ Pb
Percent wet weight gain
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
1
2
3
4
5
6
Exposure period (weeks)

decreased slightly with time from an initial 24.18 to 22% after 4 weeks of exposure, after which values started returning to baseline value.

Haematocrit of fish exposed to 0.076 mgL^{-1} lead did not deviate from the control value up to 3 weeks. The levels in the fish from this group analysed at subsequent time pericds fell but were not significantly different from the control group. Haematrocrit of fish exposed to 0.163 and 0.326 mgL^{-1} lead were consistently lower than the control group throughout the duration of study although the difference is not significant ($P > 0.05$).

The haemoglobin of the blood of fish exposed to 0.076 mgL^{-1} rose only slightly above the control up to 3 weeks of exposure; thereafter values dropped progressively below the control, with a maximum fall at 6 weeks to 6.95 g/100ml blood. Similarly blood haemoglobin of fish, exposed to 0.163 and 0.326 mgL^{-1} rose above control values at 1 and 2 weeks respectively after which subsequent values declined.

The baseline mean corpuscular haemoglobin concentration (MCHC) was 33.14%. The control did not show any significant difference from the baseline value throughout the duration of experiment (Table 5). However, the MCHC of all lead.- exposed fish were on the decline after 2 weeks of exposure, reaching the lowest value after 6 weeks exposure. The values after

Table 5:

Mean haemoglobin(g/100ml blood), haematocrit and mean corpuscular haemoglobin concentration (MCHC) of Tilapia nilotica (L) exposed to lead for 1 to 6 weeks at 23^{oc}. Means ± S.E. of 4 samples (2 for each experiment 1 and 2) pooled for both parameters are presented.

Dose of Lead in MgL^{-1}	Weeks of Exposure					
	1	2	3	4	5	6
	Haemoglobin (Hb)					
0	7.78±0.13	7.85±0.10	7.94±0.16	7.86±0.05	7.44±0.03	7.93±0.23
0.076	7.93±0.15	8.00±0.40	8.15±0.12	7.18±0.06	7.25±0.08	6.95±0.19
0.163	7.96±0.22	7.60±0.23	7.28±0.18	7.53±0.30	7.28±0.54	6.50±0.30
0.326	7.60±0.29	8.10±0.32	7.50±0.32	7.25±0.34	6.90±0.09	6.00±0.41
	Haematocrit (Hct)					
0	24.18±0.12	23.00±0.91	23.25±0.25	22.00±0.25	22.00±0.32	22.88±1.53
0.076	24.00±0.46	23.75±1.45	24.25±0.60	21.25±0.60	20.45±0.45	22.75±0.88
0.163	22.40±0.68	22.50±0.46	22.60±0.91	21.25±0.32	22.50±1.19	21.63±0.38
0.326	22.38±0.47	22.50±0.20	22.25±1.16	21.75±0.52	21.00±0.74	21.16±0.54
	MCHC -- ^{Hb}/Hct X 100					
0	32.16±0.52	34.31±1.54	34.14±0.37	35.74±0.47	33.80±0.42	34.96±1.61
0.076	33.02±0.13	33.67±0.53	33.62±0.32	33.85±1.05	35.49±0.61	30.62±0.58
0.163	35.57±0.49	33.77±0.67	32.43±2.05	35.43±1.12	32.23±0.75	30.02±0.93
0.326	34.18±1.85	35.98±1.20	33.78±0.76	33.30±1.03	32.94±0.72	28.56±1.70

Baseline values determined before fish were alloted to the exposure groups where 24.48 ± 0.40, 8.11 ± 0.09 and 33.14 ± 0.20 for Hct, Hb and MCHC respectively. Means ± S.E. of 4 samples.

6 weeks were 30.62%, 30.02% and 28.56% for 0.076, 0.163 and 0.326 mgL^{-1} lead - exposed groups respectively.

Blood cells of fish exposed to lead did not reveal any morphological or histological alteration from the control group (Plate Ia- Id).

Blood serum protein for the control and for fish exposed to lead tended to increase with time over the baseline value of 6.45g/100ml blood (Table 6). This could be due to the protein content of the experimental diet. A dose dependent decrease of blood serum protein of treated groups compared to the control was recorded. In all cases, the differences were not significant ($P > 0.05$).

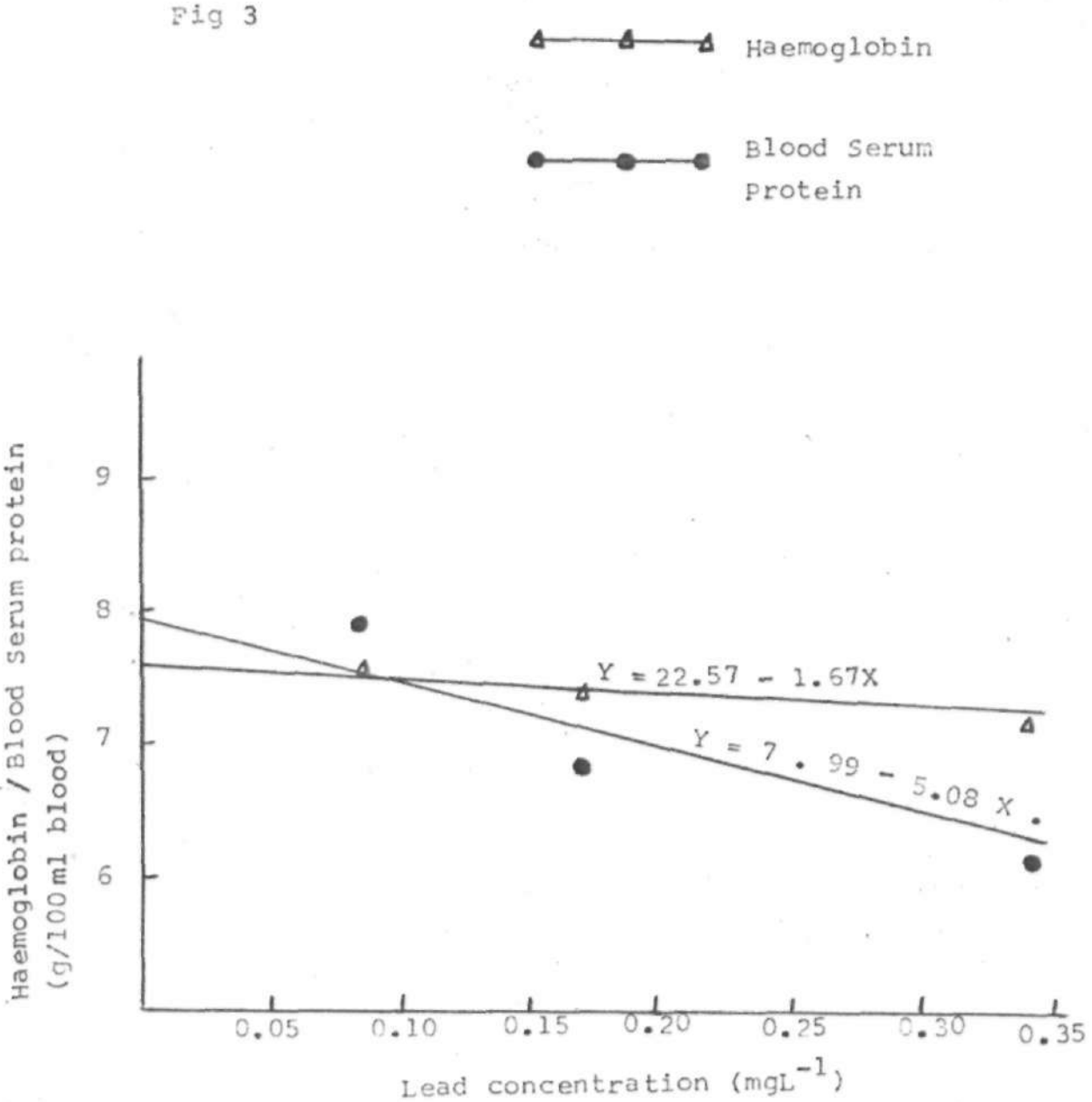
Regression analysis indicated a low negative correlation coefficient of haematocrit and haemoglobin with lead concentration (Fig 3, 4 and Table 7), while the same for MCHC and blood serum protein were - 0.67 and - 0.80 respectively.

One fish from the 0.326 mgL^{-1} lead showed sign of imminent death after 12 days of exposure. It swam erratically and lost equilibrium. The fish was removed and the blood parameter determined.

Effect of lead on tissue glycogen:

Liver glycogen levels of control group were not significantly ($P > 0.05$) different from one another as evidenced in the relatively uniform peaks recorded

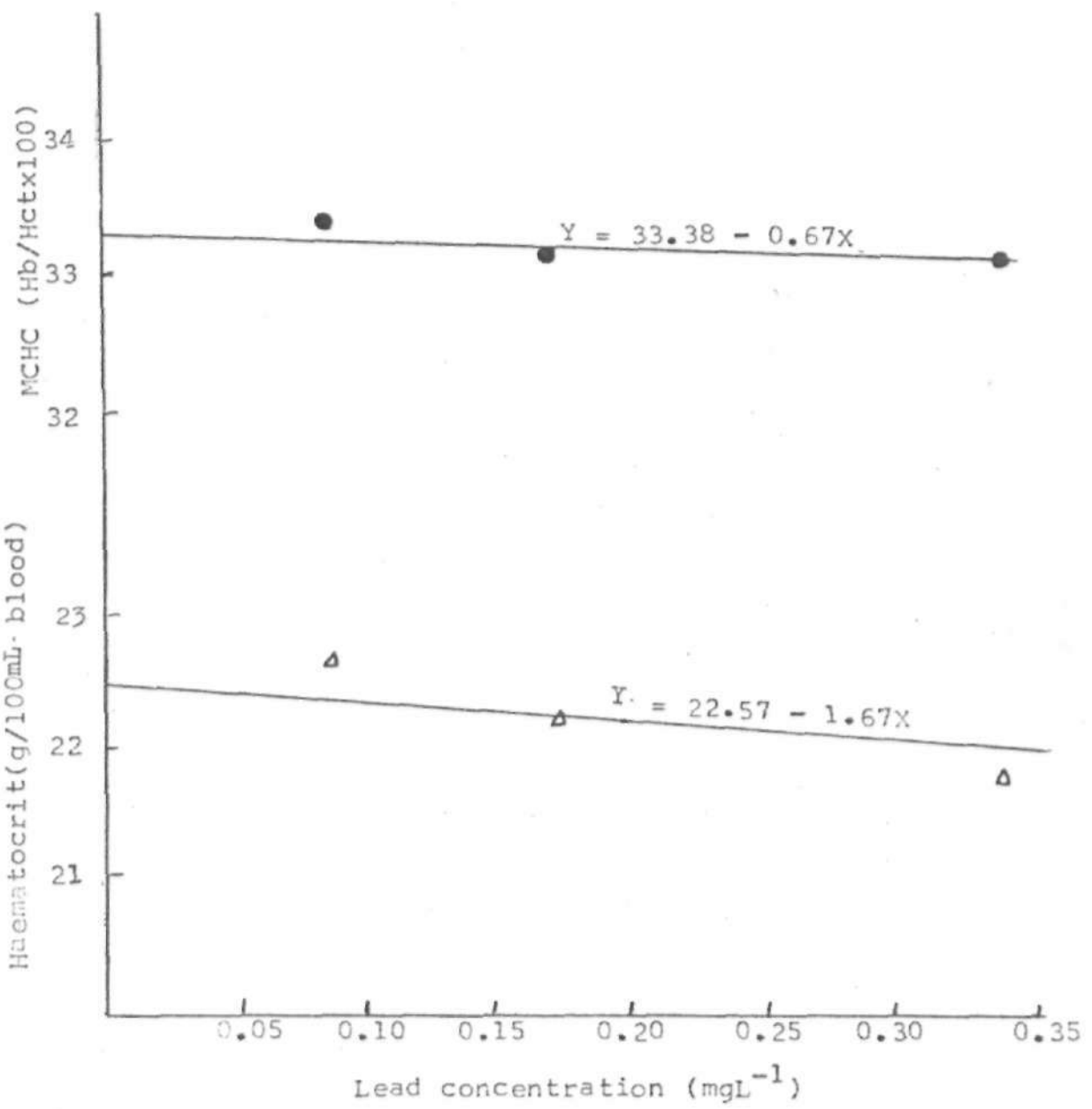
Fig 3



Regression lines of Haemoglobin and Blood serum protein for Tilapia nilotica (L) exposed to 0.076, 0.163 and 0.326 mgl^{-1} of lead

(Means of each treatment presented)

Fig 4



Haematocrit and Mean Corpuscular Haemoglobin Concentration of Tilapia nilotica (L) exposed to varying lead treatments. The lines of best-fit are defined by the equations shown on them (Each point represents means of 24 fish)

Table 6:

Blood Serum Protein of Tilapia nilotica (L) exposed to lead for varying time periods at 23°C. Means ± S.E. of 2 samples are presented.

Dose of lead in MgL^{-1}	Weeks of Explosure					
	1	2	3	4	5	6
0	6.50±0	8.65±2.35	6.26±0.45	5.10±0.60	10.50±0	8.95±2.66
0.076	6.05±0.05	6.40±1.10	6.80±0.60	9.55±0.45	9.00±1.20	9.75±2.56
0.163	4.95±0.25	7.00±1.50	7.30±2.11	6.40±2.61	7.70±0.30	7.70±0
0.326	3.95±0.15	5.25±0.75	6.75±0.05	4.95±1.45	6.95±1.25	9.05±0.65

Baseline value of Blood Serum Protein determined before fish were alloted to the varying lead treatment was 6.45 ± 0.07. Means ± S.E. of 4 samples.

Table 7

Correlation and significant level of probability of haemoglobin, haematocrit, blood serum protein, MCHC; liver and skeletal muscle glycogen of Tilapia nilotica (L) exposed to lead. Values for means of each treatment presented.

Exposure group	Correlation coefficient(r)	Coefficient of determination	Significant level of probability	Remark
Hb	-0.48	0.23	$P > 0.05$	•
Hct	-0.51	0.26	$P > 0.05$	•
MCHC	-0.67	0.45	$P > 0.05$	•
BSP	-0.80	0.64	$P > 0.05$	• •
LG	-0.86	0.75	$P > 0.05$	• •
SMG	0.14	0.02	$P >> 0.05$	•

• Relationship between the two variables is not meaningful.

• •Relationship between the two variables as explained by the regression line is meaningful. Variation in one variable could be used to predict the other, although the r value is insignificant ($P > 0.05$) due to small sample point used in the plot. However, r^2 value is higher than baseline significant level (0.55 - 0.6), thereby making the variation predictable.

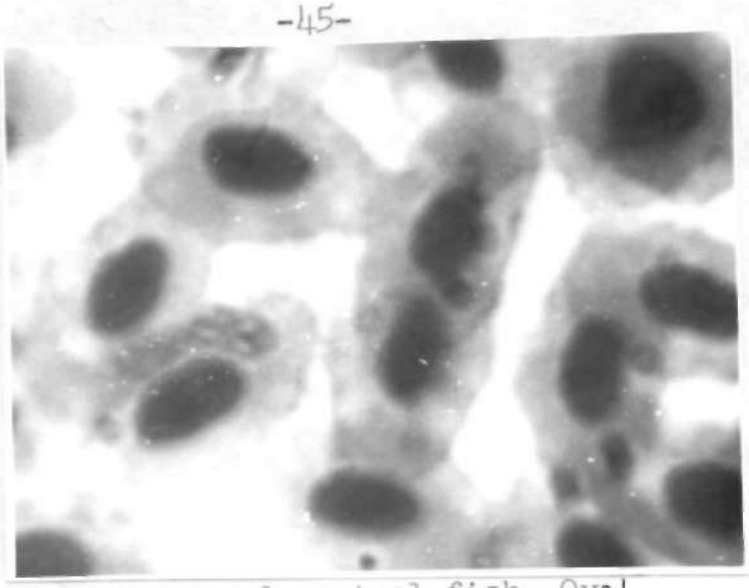


Plate Ia: Blood cells of control fish. Oval shape of erythrocytes is prominent. Giemsa stain, x 1000.

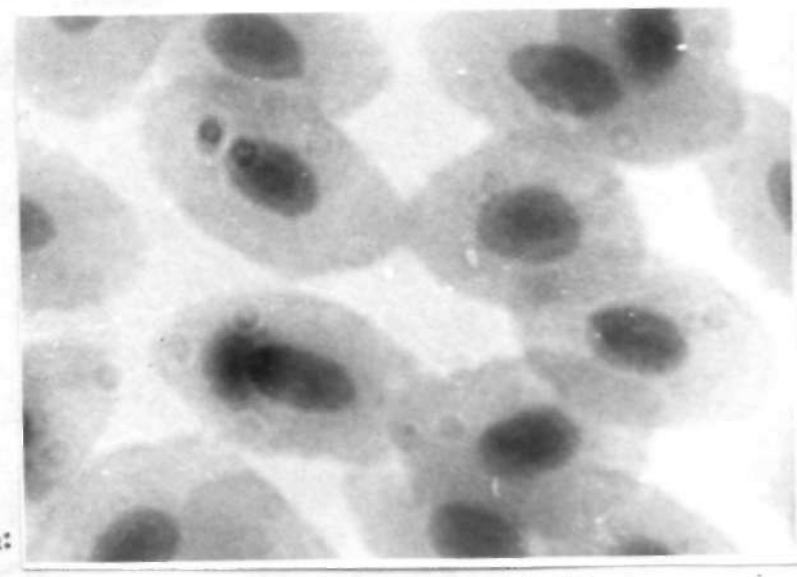


Plate Ib: Blood cells of fish exposed to 0.076mgL^{-1} lead after 6 weeks exposure. Giemsa stain, x 1000

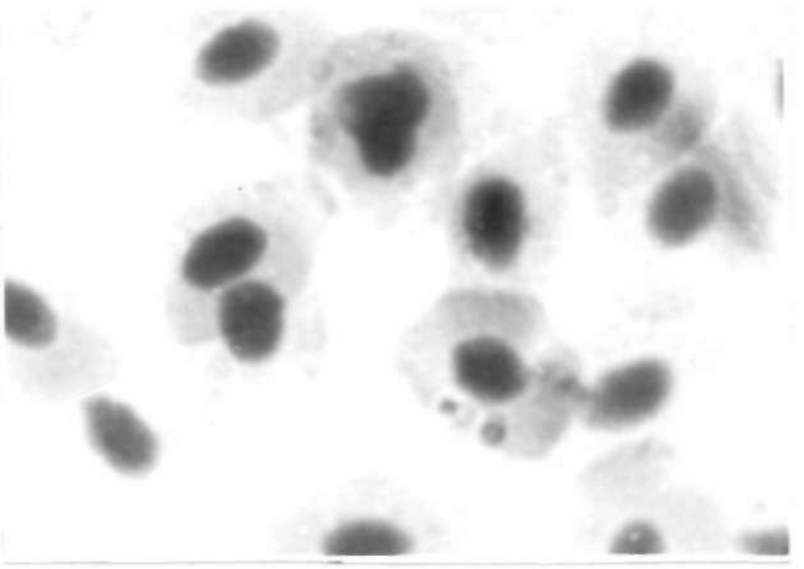


Plate IC : Blood cells of 0.163 mgL^{-1} Pb exposed fish after 6 weeks. Giemsa stain, x 1000.

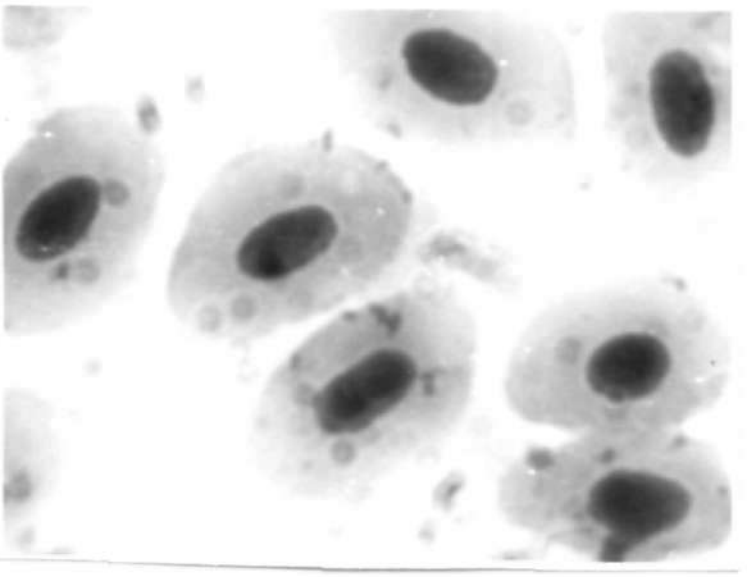


Plate 1d: Blood cells of 0.326 mgL^{-1} Pb group. Erythrocytes nuclei and cytoplasm are prominent Giemsa Stain, x 1000.

during sampling period (Fig 5).

Glycogen levels of fish exposed to 0.076 mgL^{-1} lead fell after 1 week of exposure compared to the control. There was a rise over the control after 2 weeks exposure. However, glycogen values of subsequent time periods were lower than those for the control group. Similarly, glycogen levels of fish exposed to 0.163 mgL^{-1} lead initially fell after 1 week, rose again over the control group at 2 weeks exposure. Thereafter, glycogen values obtained declined comparatively with the control group. The glycogen levels of fish exposed to 0.326 mgL^{-1} lead were consistently lower than the control and fish exposed to 0.076 and 0.163 mgL^{-1} lead during the study period. In all cases, the increase or decrease was insignificant ($P > 0.05$). Generally the result of the liver glycogen value indicate a dose - dependent decrease effect of waterborne lead on Tilapia as well as highly negative correlation (r) value (-0.86) between glycogen level and lead concentration (Fig 6 and Table 7).

The relationship between the glycogen levels in muscles of fish exposed to lead, the duration of exposure and the lead concentration in the water (level of exposure) does not show any clear trend. The attainment of maximum or minimum glycogen peaks were not dose - dependent as compared to the liver (Fig 7). A correlation plot between the glycogen level and lead

Fig 5:

Liver glycogen (mgg^{-1} tissue) in Tilapia nilotica (L) exposed to various concentration of lead. Values plotted represent means of 4 pooled samples.

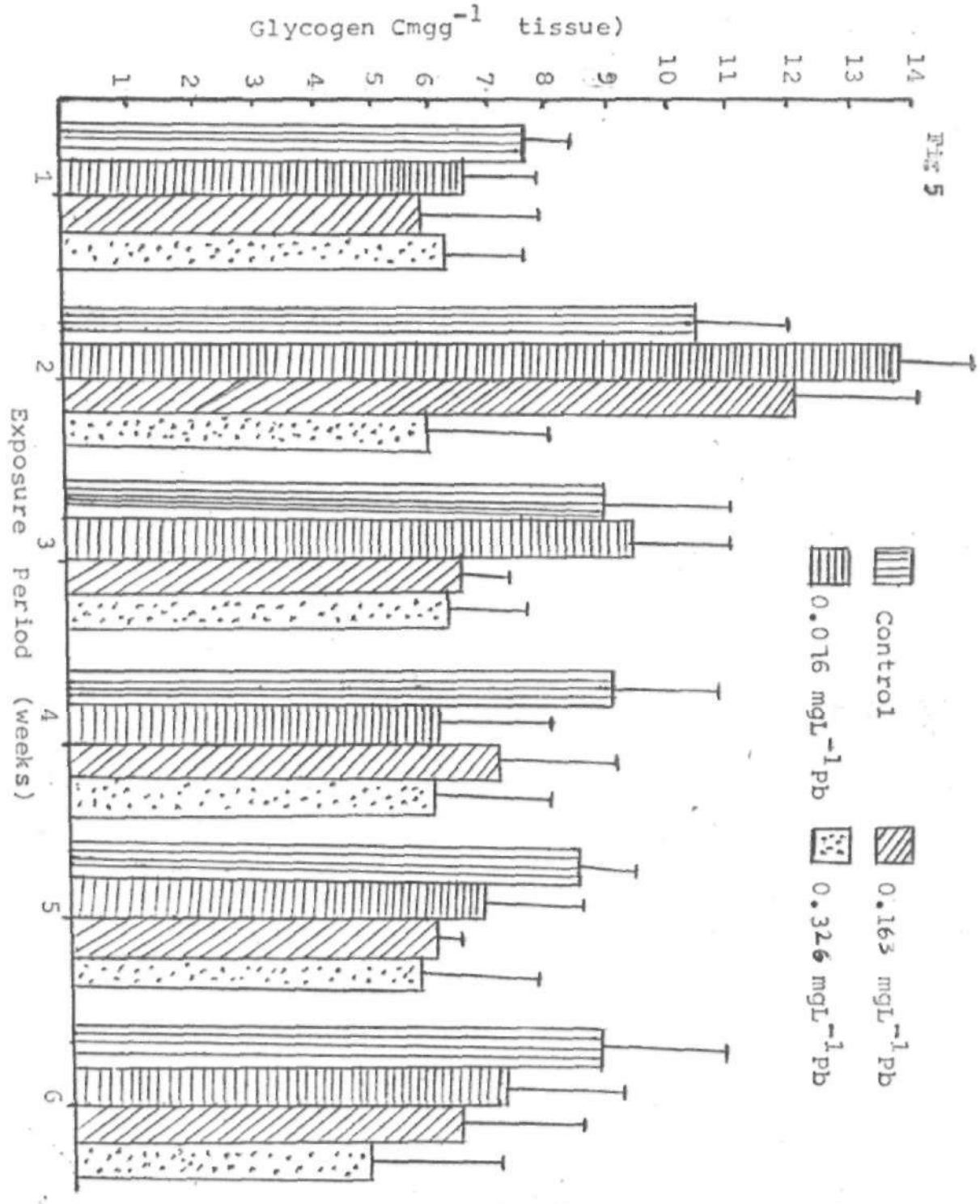
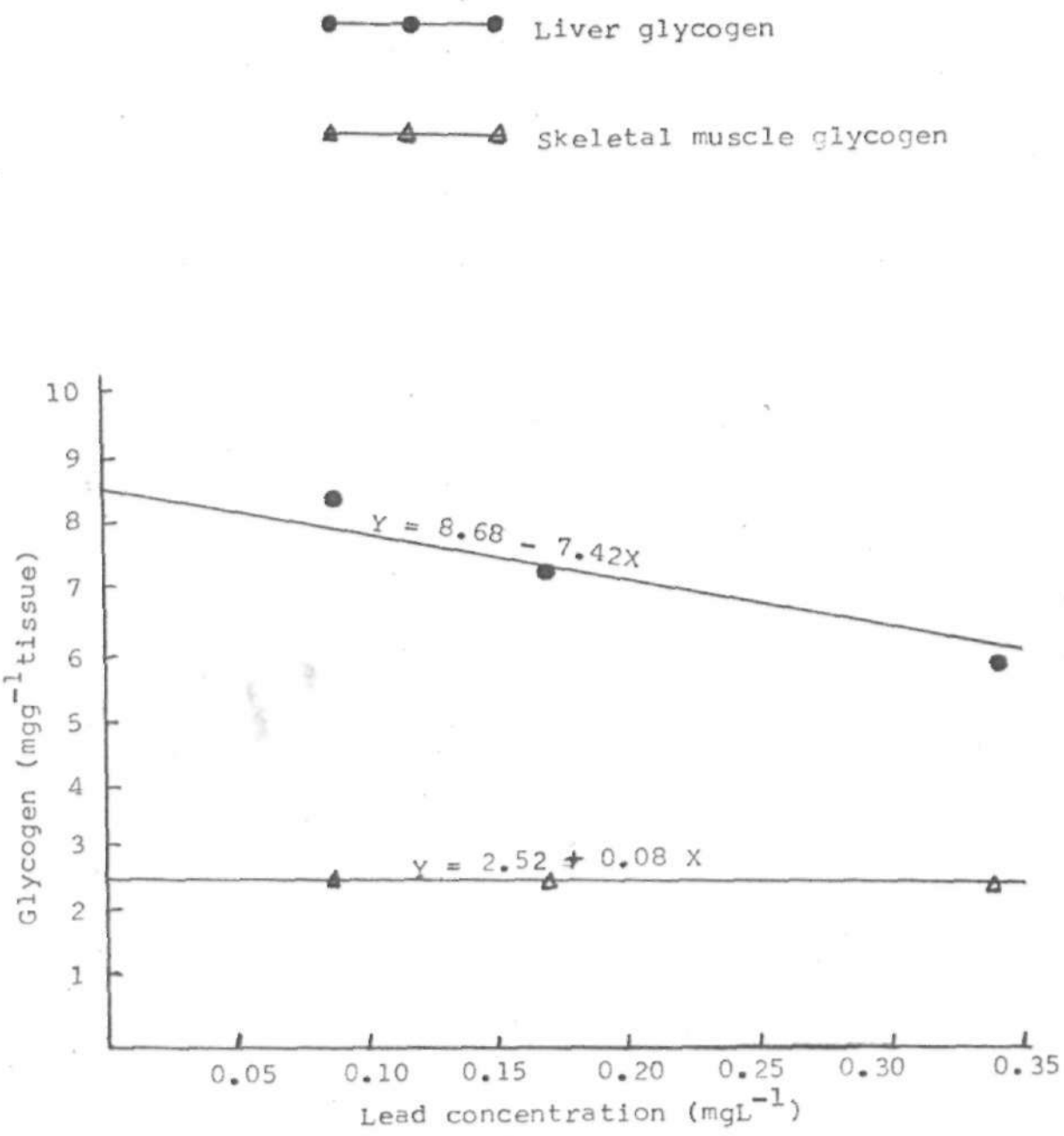

Glycogen Cmgg^{-1} tissue)
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Exposure Period (weeks)
1 2 3 4 5 6
Control
0.076 mgL^{-1}Pb
0.163 mgL^{-1}Pb
0.326 mgL^{-1}Pb

Fig 5

Fig 6



Liver and skeletal muscle glycogen for Tilapia nilotica (L) exposed to various concentrations of lead. The lines of best-fit are defined by the equations shown on them (Each point represents means of 24 fish)

Fig 7

Skeletal muscle glycogen (mgg^{-1} tissue) in *Tilapia nilotica* (L) exposed to various concentration of lead in water. Means of 4 pooled samples are plotted.

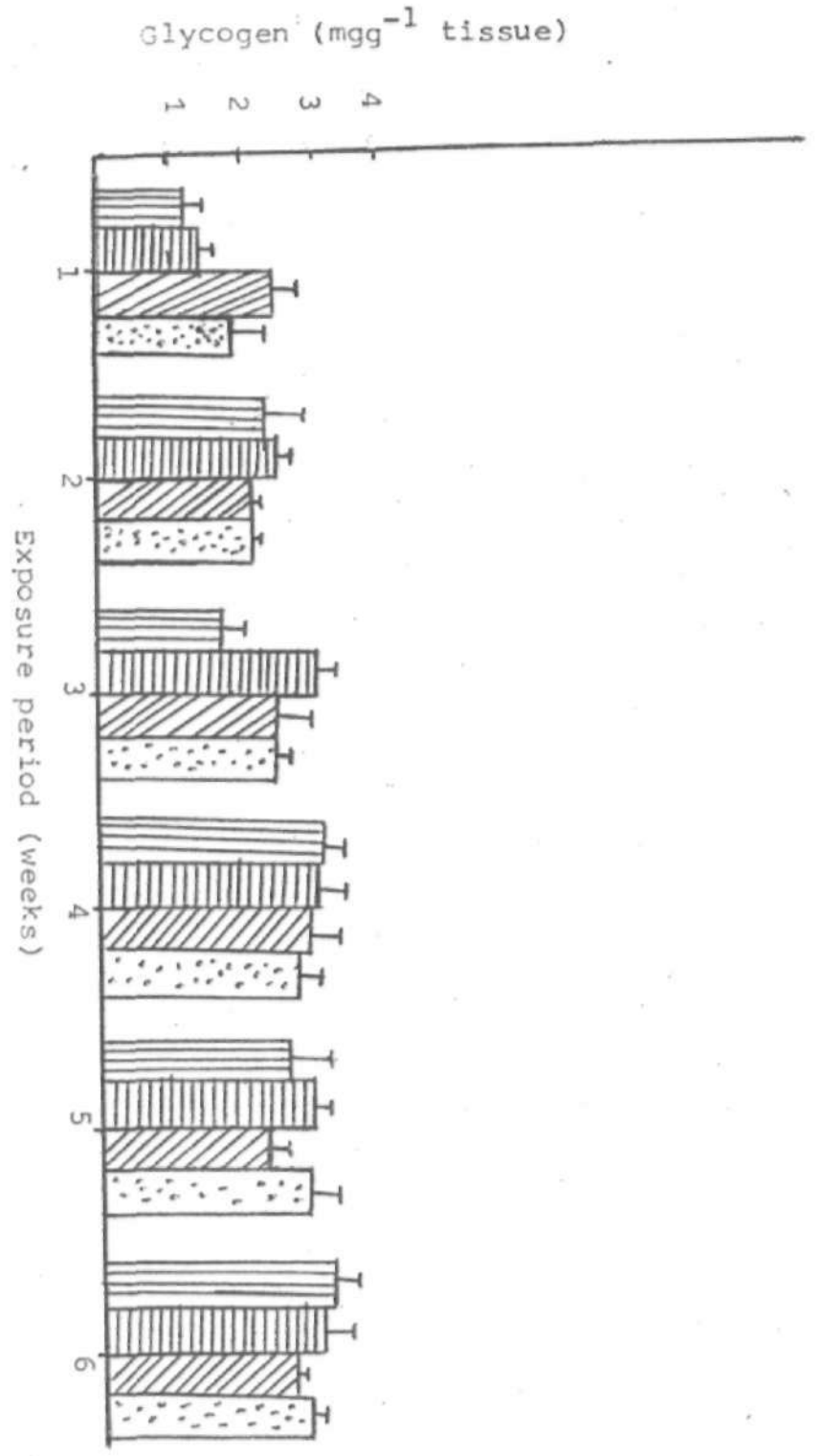

Glycogen (mgg^{-1} tissue)
1
2
3
4
Exposure period (weeks)
1
2
3
4
5
6
Control
0.163 mgL^{-1} Pb
0.C76 mgL^{-1} Pb
0.326 mgL^{-1} Pb

Fig 7

concentration showed a low value (r = 0.14)(Table 7) and therefore no meaningful relationship could be derived. There was not much difference in the glycogen of lead exposed fish and the control. The glycogen of control fish increased from an initial value of 1.32 $mg_{,}^{-1}$ tissue at 1 week to 3.22mgg^{-1} tissue after 6 weeks. Levels in muscle of fish exposed to 0.076mgL^{-1} lead increased from an initial value of 1.38 mgg^{-1} at 1 week to 2.96 mgg^{-1} after 3 weeks. Thereafter, levels of glycogen obtained were not different. Muscle glycogen for 0.163 mgL^{-1} lead group showed a similar increase from the initial 2.50 mgg^{-1} tissue at 1 week to 2.90 mgg^{-1} at 4 weeks. Other values were 2.36 and 2.57 for 5 and 6 weeks exposure periods respectively. Glycogen of 0.326 mgL^{-1} lead-treated group increased to 2.90 mgg^{-1} tissue after 5 weeks and declined to 2.83 mgg^{-1} tissue after 6 weeks of exposure. Generally, glycogen content of skeletal muscle of test fish tended to increase with exposure time probably due to their being held in captivity.

Abnormalities induced by lead:

"Blacktail", the darkening of skin from the dorsal to caudal fin and terminating in a sharp line underneath the dorsal fin was noted in fish exposed to 0.163 mgL^{-1} lead after 5 week exposure period (plate IIa). The lesion affected 40% (2 of 5) of fish left in test tank during the period. During the 6th week; the darkening

of skin extended to the head of the same fish, covering completely the right side of the head including the Opercular bone and the skin surrounding the eyes (Plate IIb).

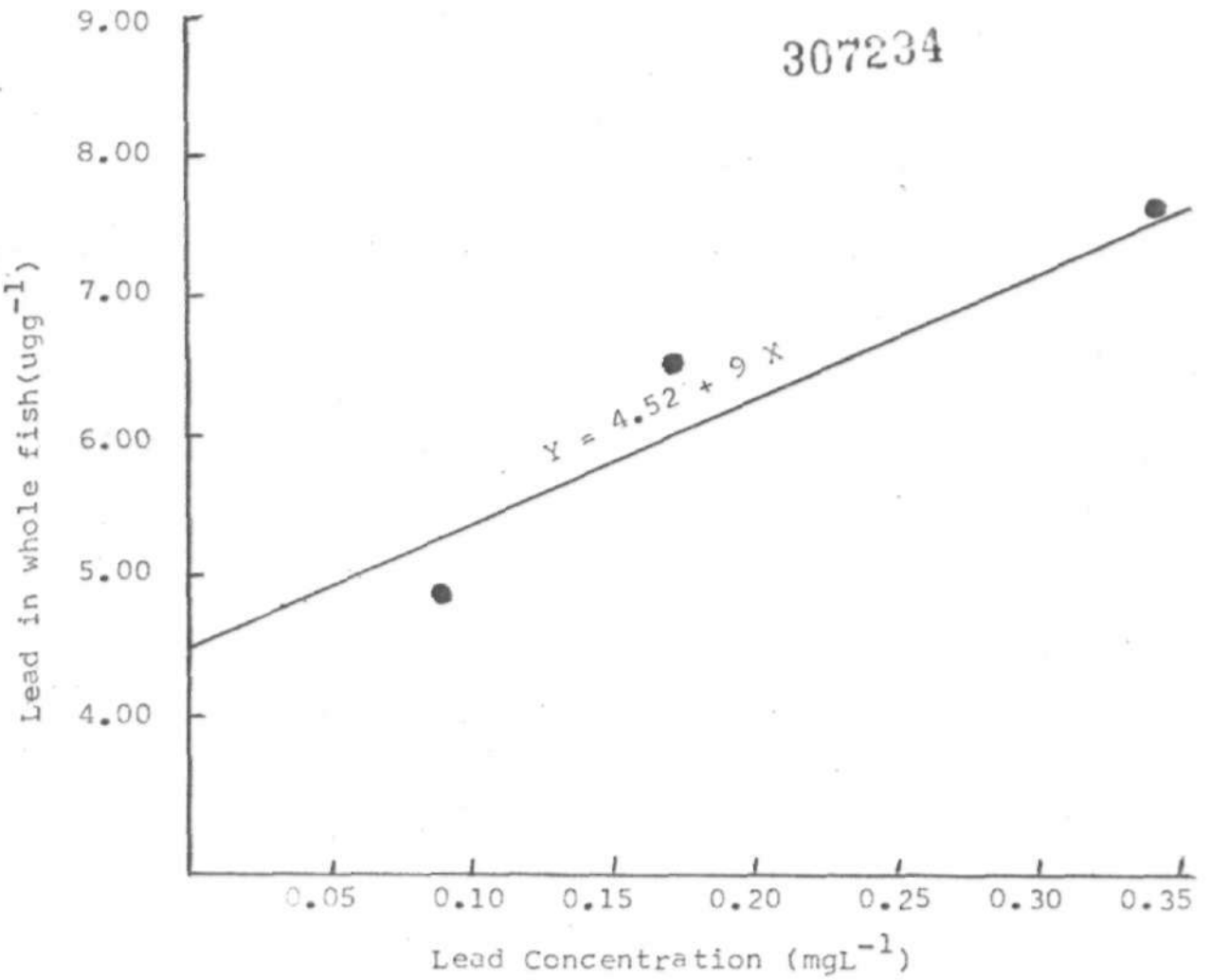
'Spinal curvature' was observed in one of the 4 fish exposed to higher concentration 0.482 mgL^{-1} lead ($\frac{1}{5}$ of 96hr LC50) of the trial assay 4 weeks after commencement of assay (plates IIIa - IIIb). The onset of the spinal curvature subsequently killed the fish as affected fish could not maintain a normal equilibrium in water or move around. In this same assay, 1 other fish died 3 weeks from the start of assay while the 2 fish left survived up to the end of assay of 5 weeks.

Some of the lead - treated fish in both trial and actual experiment exhibited signs of respiratory distress as evidenced by their frequent visits to water surface to gulp air.

Lead residue in tissues:

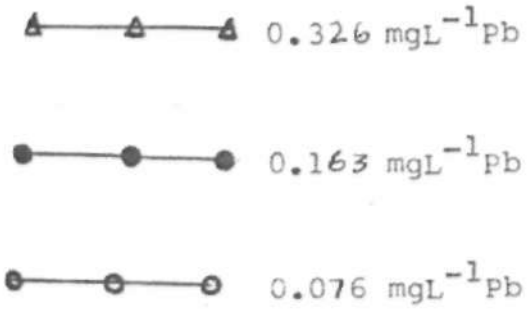
In general, the levels of lead accumulated in the tissue were directly related to the concentration of lead to which the fish were exposed (Table 8). Residual lead in whole fish regressed against lead concentration (Fig 8) indicated a highly positive correlation between the concentration of lead in water and that accumulated by the fish (Table 9). In all lead concentrations, accumulation of lead in tissue increased with exposure period (Fig 9).

Fig 8



Residual lead in Tilapia nilotica (L) exposed to various concentrations of lead (Values plotted represent means for each treatment).

Fig 9



Residues of lead in whole body of Tilapia nilotica (L) exposed to different concentrations of lead in water for varying time periods.

Table 8:

Residue of lead (ugg^{-1} wet wt) Tilapia nilotica (L) whole fish exposed to 0.076, 0.163 and 0.326 mgL^{-1} waterborne lead for 6 weeks at 23^{oc},

Means ± S.E. of 2 samples are presented.

Exposure groups mgl^{-1} Pb	Exposure periods (weeks)					
	1	2	3	4	5	6
0	--	--	--	--	--	--
0.076	3.47±0.48	4.47±0.20	4.96±0.15	5.09±0.41	4.39±0.82	6.05±1.19
0.163	5.33±0.15	5.12±0.55	7.76±0.28	5.69±0.18	7.20±0.20	7.78±0.30
0.326	7.14±1.62	6.99±0.20	7.20±0.39	7.76±0.32	7.80±0.01	8.72±0.78

Table 9

Correlation and significant level of probability of lead in whole body of Tilapia nilctica (L) exposed to different concentrations of lead in water for varying exposure periods.

Exposure group	Correlation coefficient(r)	Coefficient of determination.	Significant level of probability	Remark
0.076	0.71	0.50	P> 0.05	*
0.163	0.71	0.50	P > 0.05	*
0.326	0.91	0.83	P > 0.05	* *
Means of each treat-ment	0.92	0.85	P 0.05	* *

* Variation of graph as explained by regression line is not meaningful or predictable.

** Variation of graph as explained by the regression line is absolutely meaningful and predictable.

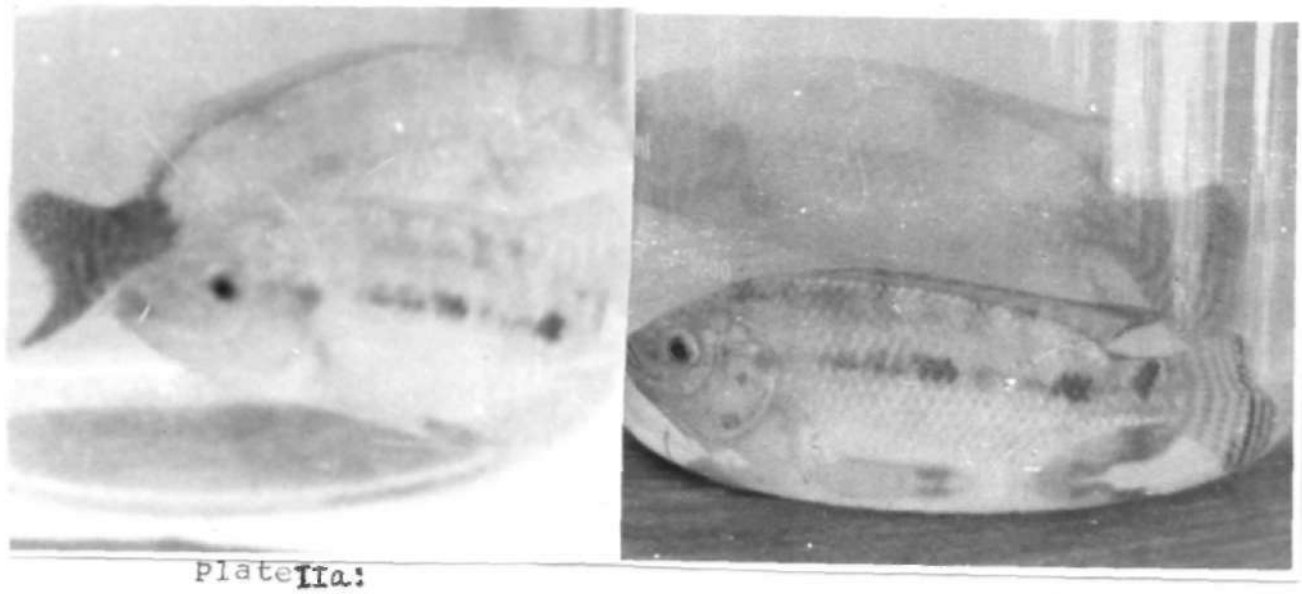


Plate IIa:

Fish inflicted with "blacktail" lesion after exposure to $0.163 mgL^{-1}$ for 5 weeks



Plate IIb:

Fish's head darkened after 6 weeks lead expsure to $0.163 mgL^{-1}$ as compared to unaffected fish from same group.



Plate III(a): Fish suffering from "Spinal Curvature" after exposure to higher lead concentration of 0.480mgL^{-1} for 4 weeks. (Dorsal view).



Plate III(b): Spinal deformity in fish from 0.480 mgL^{-1} Pb after 4 weeks. (side view).

The lead concentration in tissue of fish exposed to 0.076 mgL^{-1} lead increased from an initial value of 3.47 ugg^{-1} tissue after 1 week of exposure to 6.05 ugg^{-1} tissue after 6 weeks. In the group exposed to 0.163 mgl^{-1} lead, concentration reached 7.76 ugg^{-1} tissue after 3 weeks. The levels in the tissue of fish from this group analysed at other time periods were not significantly different P>0.05). The same trend was observed in the 0.326 mgl^{-1} lead group which accumulated 7.14 ugg^{-1} tissue after 1 week of exposure. Subsequently values increased gradually with exposure period.

Effect of lead on the histology of the liver:

Normal liver: The liver of the Tilapia nilotica as observed from the control fish is made up of a continuous mass of hepatic cells with cord like formation. The cells are large in size, polygonal in shape, with more or less centrally placed nucleus and homogenous cytoplasm (Plate IVA). Bile ducts and blood vessels are found scattered among the hepatic mass of these cords (Plates VIA and VIb). Very few hepatic vacuoles were also observed among the liver cells.

Lead-exposed fish lever: The liver of fish exposed to 0.076, 0.163 and 0.326 mgL^{-1} lead showed precipitating or coagulating cytoplasm as evidenced in unclear and less homogenous cytoplasmic morphology when observed under high powered light microscope. The degree of cytoplasmic precipitation increased with increasing

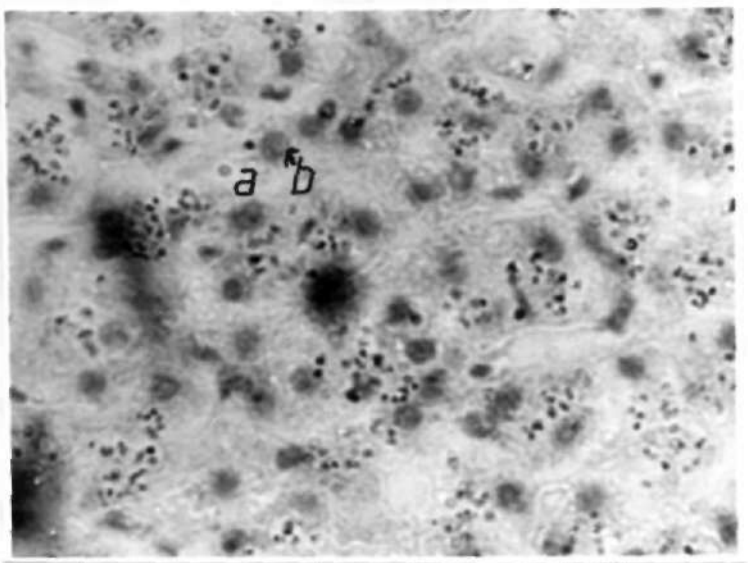


Plate IVA: Hepatocytes of liver from control group(T.S). Nucleus and homogenous cytoplasm are shown. a = cytoplasm, b = Nucleus, Heamatoxylin and eosin stain x400.

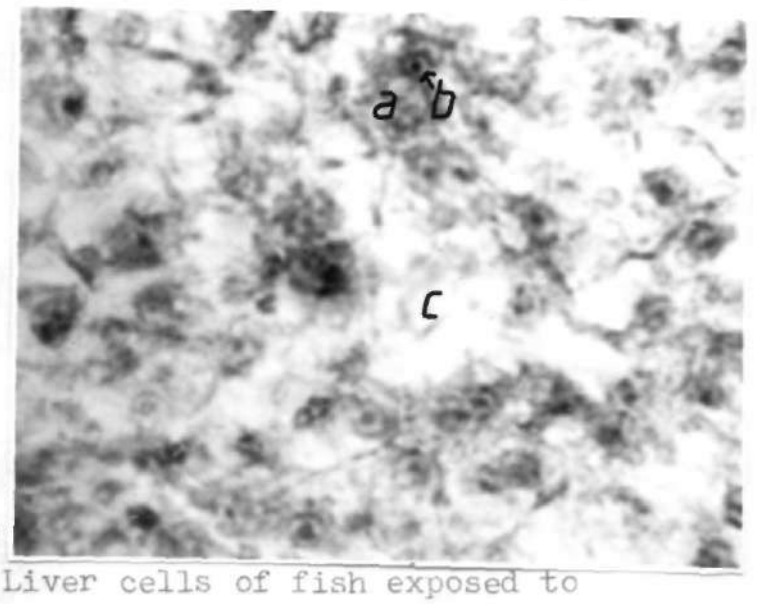
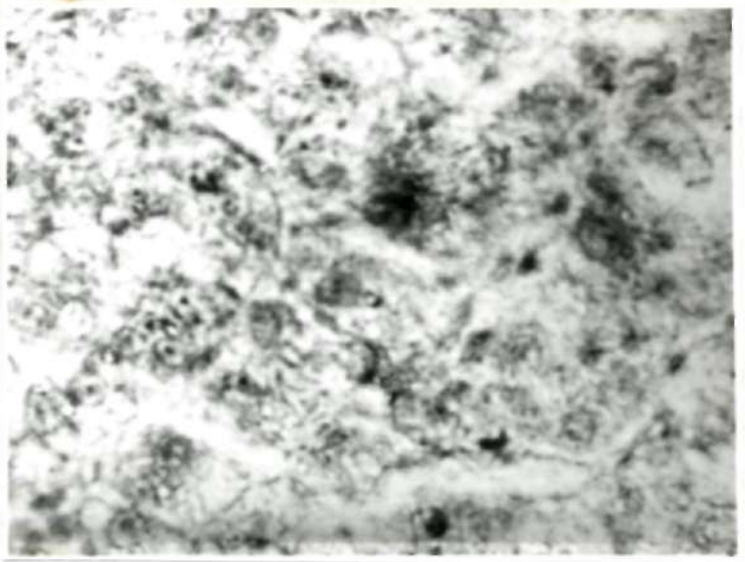


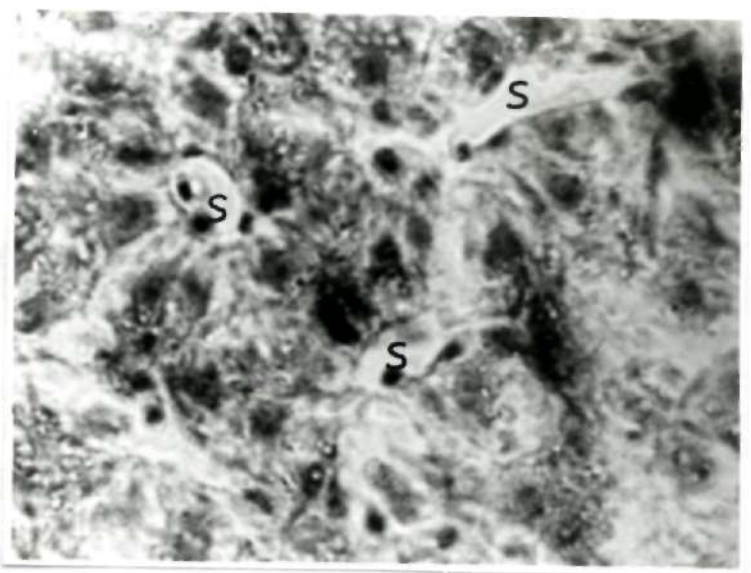
Plate IVB: Liver cells of fish exposed to 0.076 mgL^{-1} lead after 6 weeks. Precipitating cytoplasm (fibrosis), hepatic vocuolarity are indicated. a = Precipitating cytoplasm, b = Nucleus, c = Hepatic vacuole. Heamatoxylin and eosin stain x400(T.S.).

Plate IVC:



Liver cells of fish exposed to 0.163 mgL^{-1} lead after 6 weeks(T.S.) Note the degree of cytoplasmic fibrosis. Heamatoxylin and eosin stain x400.

Plate IVD:



0.326 mgL^{-1} lead-exposed fish liver cells after 6 weeks(T.S). Note increasing rough cytoplasm of hepatocytes and disintegrating blood vessels. S = blood vessel. Heamatoxylin and eosin stain x400.

lead concentration(Plates IVB - IVD). An increasing hepatic vacuolarity was also observed among the hapatocyctes of livers of fish exposed to lead compared to the control(Plates VA - VD). The nuclei of the affected hepatocytes have vacated complete the cytoplasm, while some stayed on the periphery. A few of the blood vessels in the livers of fish exposed to 0.326 mgL^{-1} lead had started disingtegration (Plate IVD).

Also, in the highest lead concentration(0.326 mgL^{-1}), the cytoplasm of the hepatocytes have started complete disintegration leaving a number of large spaces in between the hepatocyte cells nuclei (Plate VIIa). The liver morphology of lead-exposed fish was compared with the liver of Tilapia hilotica that died 2hrs after exposure to 4 mgL^{-1} lead concentration. The liver hepatocytes of the dead fish showed complete disintegration of the hepatocytes cytoplasm with ruptured or dead nuclei(Plate VIIb).

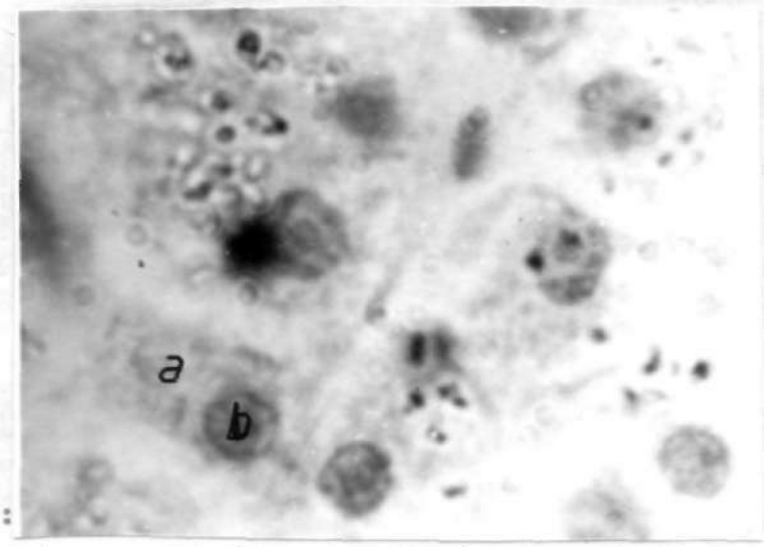


Plate VA: Liver cells(Hepatocytes) of control fish(L.S). Polygonal shaped hepatocytes are prominent. a = Cytoplasm, b = Nucleus. Heamatoxylin and eosin stain x1000.

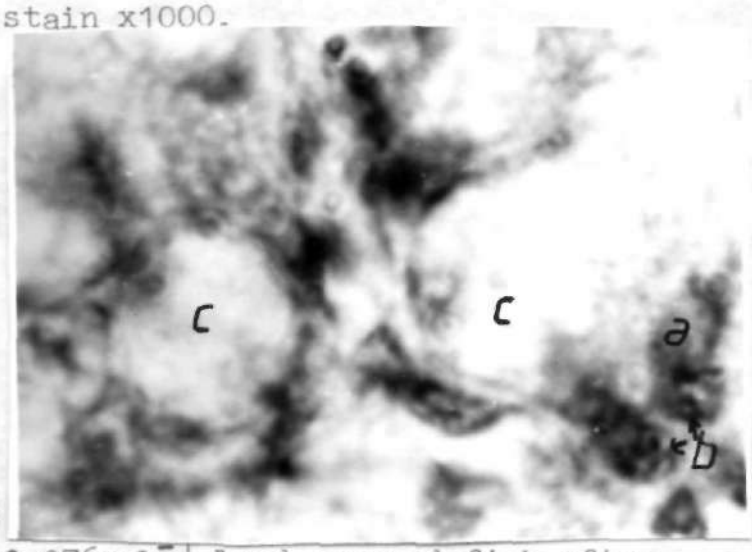


Plate VB: 0.076mgL^{-1} lead exposed fish after 6 weeks. Note some hepatocyte nuclei at the periphery of vacuolated cytoplasm. a = Precipitating cytoplasm, b = Nucleus, c = Vacuole. Heamatoxylin and eosin stain x1000 (L.S).

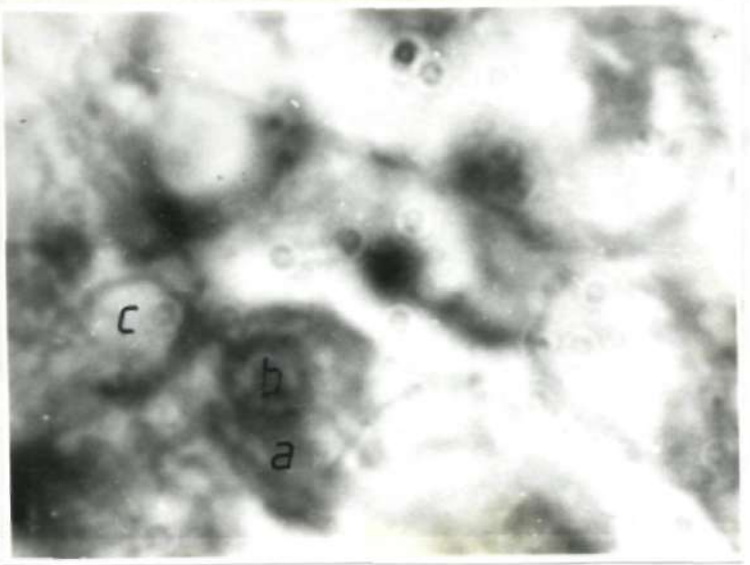


Plate VC: 0.163 mgL^{-1} lead group liver cells after 6 weeks(L.S) = precipitating cytoplasm, b = Nucleus, c = Vacuole. Heamatoxylin and eosin stain x 1000.

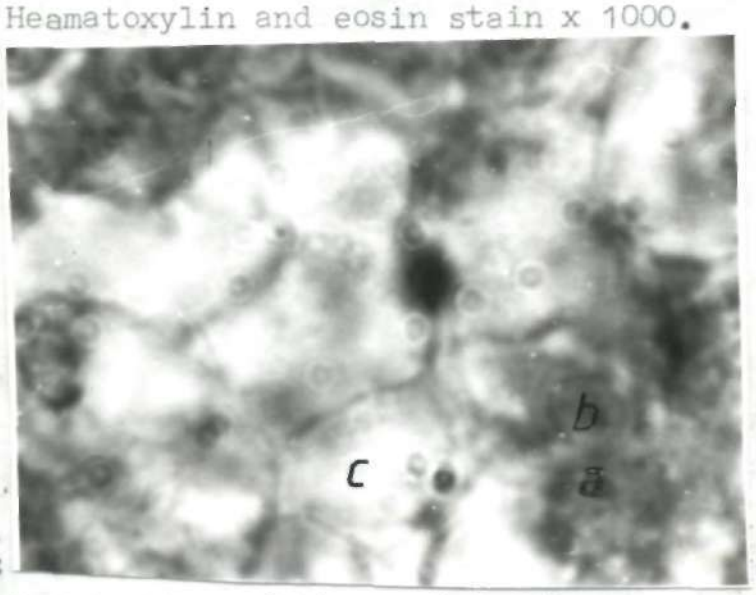


Plate VD: Hepatocytes of liver of 0.326 mgL^{-} lead - exposed fish after 6 weeks(L.S) a = cytoplasm, b = Nucleus, C= Vacuole. Heamatoxylin and eosin stain x1000

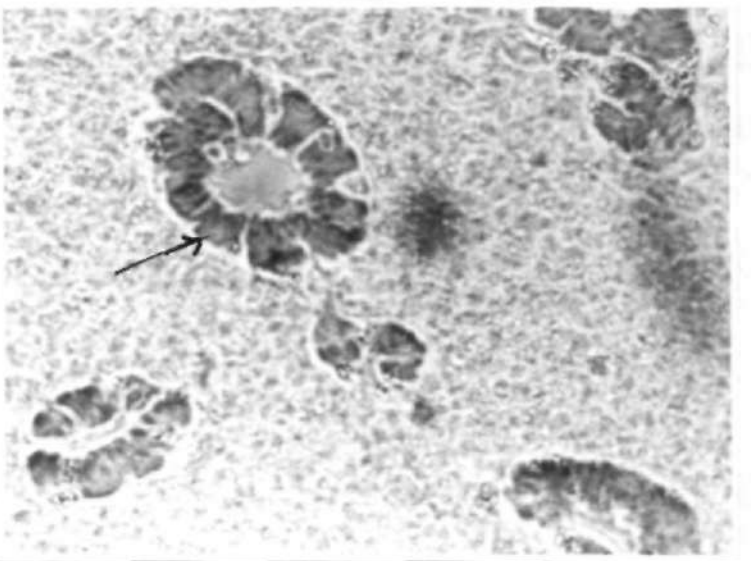


Plate VIa: Bile ducts(arrowed) of liver from control fish (T.S)
Heamatoxylin and eosin stain x1000

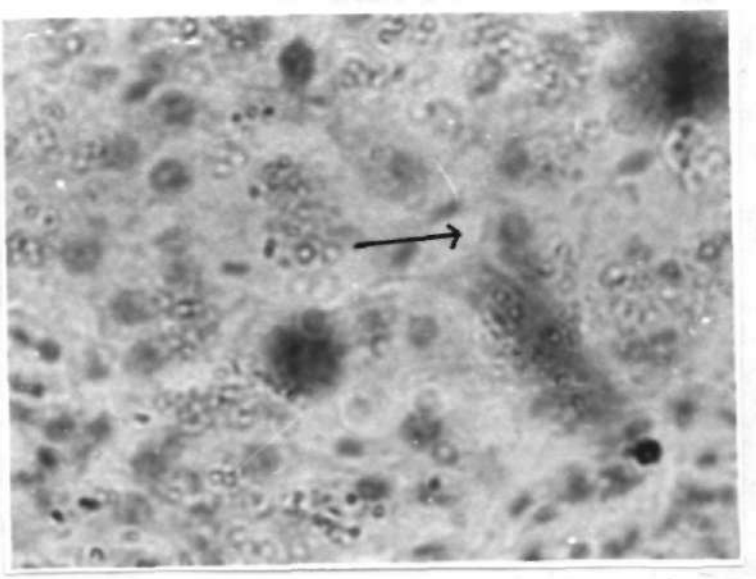


Plate VIb: Blood vessel(arrowed) of the liver (control)
Heamatoxylin and eosin stain x400

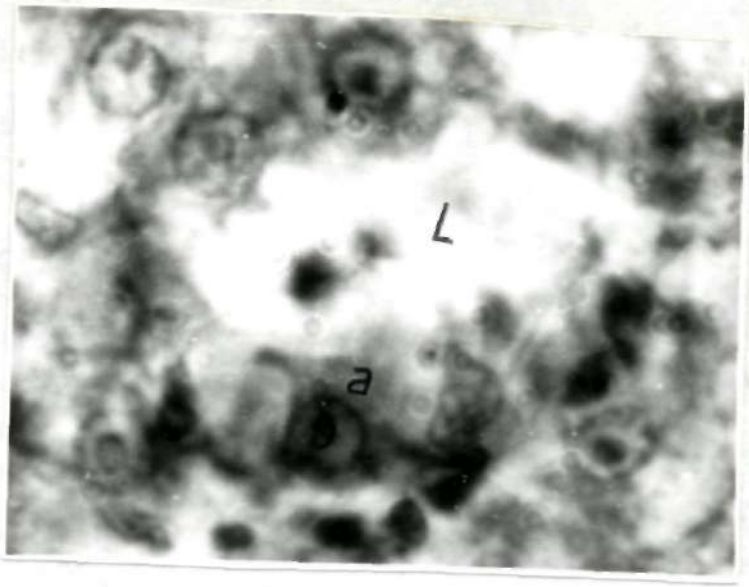


Plate VIIa:

Liver cells of fish exposed to 0.326 mgL^{-1} lead after 6 weeks (T.S.). Note the large space in-between the hepatocytes due to disintegrating cytoplasm. L = Large space, a = Hepatocyte cytoplasm, b = Hepatocyte nucleus. Heamatoxylin and eosin stain x 1000.

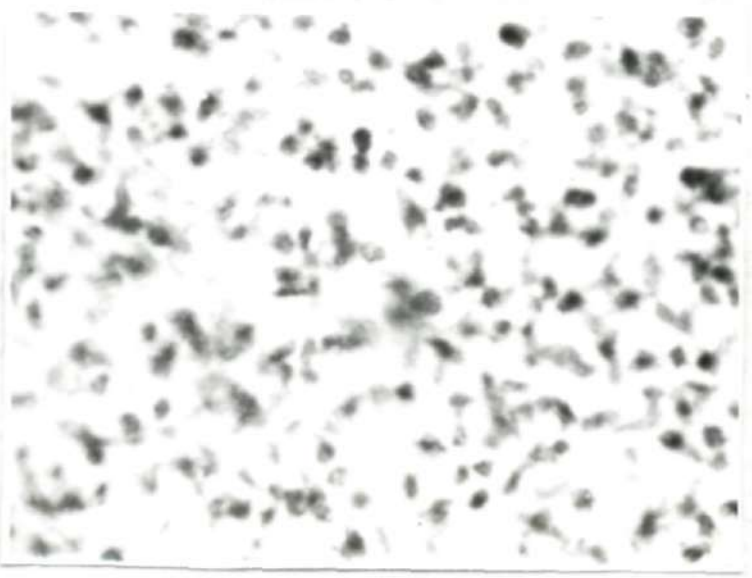


Plate VIIb:

Pyknotic (where nucleus is shrunken and very dark) hepatocytes of liver of fish that died 2hrs after exposre to 4mgL^{-1} lead (T.S.). Note complete disintegration of hepatocyte cytoplasm. Heamatoxylin and eosin Stain x 400

CHAPTER FOUR

DISCUSSION

The growth of Tilapia nilotica (L) exposed to waterborne lead was shown to be reduced at all treatment concentrations. As can be seen from Fig 2 a dose-dependent growth impairement was observed with the most marked effect at the highest concentration of 0.316 mgL^{-1}. The best growth was observed in the control group. A number of studies have demonstrated similar toxic effects, of lead on other fish species. Crandall and Goodnight (1963) observed that guppies, Lebistes reticulatus exposed to 1.24 and 3.12 mgL^{-1} lead for up to 129 days suffered a reduction in growth. Dorfman and Whitworth (1969) showed that lead concentration of 25 mgL^{-1} administered as slug does reduced the growth of brook trout. Davies et al (1976) observed that 20% of rainbow trout Salmo gairdneri exposed to waterborne lead of 55.1 and 61.8 ugL^{-1} respectively in two bioassays in soft water were severely paralysed and unable to feed; a significant difference in size existed between the paralysed and non-paralysed fish.

The reduced growth may have been due to the shortage of energy resulting from lead interference with the energy-yielding metabolic processes. The mode of action of lead is ibhibition of mitochondria respiration through interference with respiratory

enzymes and iron transport (Bull 1980). Quantitatively, the major capacity for conservation of energy derived from oxidation of physiological substrates in aerobic cells is found in mitochondria, and several in vitro studies have clearly demonstrated the ability of isolated mitochondria to accumulate pb^{++}(Bull 1980). Since no high energy will be generated during the lead inhibition, shortage of energy may be created in the tissues. Besides, the reduction in growth may be attributed to low assimilation of consumed food from the fish gut. Similar toxic effects of metals have been reported. Stokes and Fromm (1965) reported that glucose transport was reduced in the gut cells of trout exposed to Chromate ions, while oxygen consumption and phosphate metabolism were altered in mitochondria of blue gill exposed to cadmium or zinc (Hultirum 1971). A reduction in glucose transport could appreciably decrease the tissue glucose level. One of the effect of lead may be inhibition of glucose entrance into the epithelial cells; suggesting that lead pathogenesis may originate with impaired nutrient absorption. Absorption is fundamental to all process of cell (Stokes and Fromm 1965), and if it fails, other processes fail.

The utilization of energy for other physiological functions affect the availability of energy for growth. In the first instance, the energy an organism obtains from its food is made available to sustain reproduction

and other essential activities vital to the survival of such organism. It is after these functions have been taken care of that growth can occur.

The results of the present study indicate that Tilapia nilotica exposed to waterborne lead suffered a decrease in haemoglobin level that bear an inverse relationship with duration of exposure period. The data also show that the mean corpuscular haemoglobin concentration (MCHC) of fish exposed to lead was on the decline after two weeks exposure compared to the control group. This suggest that the primary effects of the lead on blood was a reduction in the haemoglobin. There was no difference observed in the morphology of the blood cells of fish exposed to lead compared to the control. This however, does not mean that lead did not affect the blood cells since the blood cell count was not carried out. The decreased haemoglobin could have been due to a decreased number of erythrocytes. Wong et al (1978) have reported a similar reduction in haematocrit, haemoglobin and in addition, deformed erythrocytes in fish exposed to sublethal concentration of lead. Similarly, Hodson et al (1978) noted that waterborne lead caused a reduction in cellular blood iron in rainbow trout. A reduction of cellular haemoglobin would reduce maximum oxygen carrying capacity of the blood, and also stimulate erythropoiesis (Hodson et al 1978). Therefore the growth reduction

observed in this study could be due to a reduced oxygen carrying capacity of the blood resulting in an inefficient utilization of assimilated food.

The results of the present study indicate that waterborne lead caused a decrease in blood serum protein in Tilapia nilotica that was inversely related to dose (Table 6, Fig 7). This may be due to defective protein synthesis in the intoxicated liver, as high levels of metals have been associated with functional changes. A De Bruin (1976) reported low protein synthesis in vetebrate liver due to direct effect of hepatoxicants on Kupfer cells. This finding correlates well with earlier studies on the effects of metal on serum protein levels in fish blood. Christensen et al (1972) and McKim et al (1970) in their study on the biochemical changes in fish blood and early development stages of fish, caused by metal indicated that; the blood serum protein of the brown bull head Ictalurus nebulosus (L) exposed to nominal concentrations of 27 ug and 107 ug of CuII L^{-1}, fell significantly after 30 days exposure but there was no change in 49ug of $CuIIL^{-1}$ group. Also blood serum protein of the brook trout Salvelinus fontinalis exposed to 39 ug and 67.5 ug of $CuIIL^{-1}$ fell significantly at the end of 21 days exposure. Likewise, there was a decrease in blood protein content of brook trout embryo exposed to sublethal concentrations of methyl mercury

(Christensen 1975).

A dose-dependent depletion of liver glycogen was observed in this study probably due to inefficient absorption of soluble glucose from the intestine or break down of liver cells which store or synthesize glycogen. It may also be as a result of activation of enzymatic activities in the liver like action on specific phosphatase of glycogen phosphorylase (A De Bruin 1976). Alterations in carbohydrate metabolites under toxic chemicals have been reported in vertebrates. Mahaffey (1980); reported that, in lead-treated rat, glucose absorption decreased between 10 and 31%, reduction in absorption of amino acids averaged approximately 20%. The report in this study is qualitatively similar to that reported by Gill and Pant (1981) for Puntius conchonius exposed to mercuric nitrate. However, the onset and duration of glycogen depletion are different probably because much lower concentration of toxic metal with longer observation time.were utilized by Gill and Pant (1981). The possibility of biochemical responses to heavy metal intoxication being species specific cannot also be ruled out. Sam Kacew and Singhal (1980) observed that administration of 0.2 or 1 mg/k lead intraperitoneally to male rat for 45 days depleted the liver glycogen level. The low levels of liver glycogen in the lead-exposed groups in this study may be an evidence of biochemical

stress affecting primarily the energy reserves of the fish. The sharp increase in level of glycogen of fish exposed to 0.076 and 0.163 mgL^{-1} lead (Fig 5) over the control on 2 weeks exposure may be an attempt by the livers to conserve glycogen and therefore to restore normalcy. At a later stage, however, the toxicity was so high creating a depletion in the glycogen level.

There was no adverse effect of lead treatment noticable in the skeletal muscle glycogen of fish exposed to lead compared to the control. This could be due to low accumulation of lead into the muscle. Gilderhus (1966) analysed the muscle of bluegills and found arsenic residues in muscle to be relatively low compared to the liver. Goyer and Mahaffey (1972) also observed that substantial amount of body burden lead up to 90% in mammals is found in the bone. Hodson et al (1978) reported low lead accumulation in skeletal muscle of rainbow trout compared to liver, gill, kidney, Opercular bone and the bones. Oladimeji (1980) similarly reported low accumulation of arsenic in the muscle of rainbow trout compared to the liver.

The most prominent feature of this study was the development of "Blacktail" and "Blackhead" in Tilapia nilotica exposed to waterborne lead (Plates IIa and IIb). The occurence of blacktail is the most sensitive criterion for measuring a chronic response of fish to lead (Davies et al 1976). Davies et al (1976) observed that

the abnormality is highly suggested of direct neurological damage to fish. The authors explained that blacktail in fish could be caused by some direct or indirect action of lead on the central nervous system whereby motor nerves which control inervation of the myomere and /or epaxial musculature and sympathetic nerve control of the caudal pigments cells are affected. Besides, Davies et al (1976) noted that the abnormality could be by biochemical inhibition in metabolism of affected fish. Chisolm (1971) made a similar observation that lead causes brain damage (encephalopathy) in mammals, and that the mechanism of such effect on the central nervous system is little understood.

A number of similar studies have reported the incidence of blacktail in chronic effect of waterborne lead to fish species. Davies et al (1976) noted blacktail in rainbow trout at lead concentration of 0.06 mgL^{-1} in hard-water 6 months after the experiment was started. One month later fish exhibited spinal curvature and eroded fins. Davies et al (1976) also reported blacktail in the same fish 1½ months after commencement of assay in soft water at lead concentration of 0.1mgL^{-1}. Spinal curvature (Lordoscoliosis) and eroded caudal fin were prevalent 1 month later. In severe cases of spinal curvature, paralysis and muscular atrophy of the flexed portion of the fish occured. Holcombe et al (1976) reported similar lesion in brook trout Salvelinus

fontinalis exposed to waterborne lead, at concentration of 0.119 mgL^{-1} in water of 44 mgL^{-1} as $CaCo_3$ hardness. Hodson et al (1978) observed the incidence of blacktail in rainbow trout exposed to lead concentration of 0.12 mgL^{-1} for 32 weeks, and in 0.543 mgL^{-1} for 14 weeks in hard water. Alan et al (1983) observed blacktail in 30% (3 of 10) of the remaining rainbow trout exposed to a total lead concentration of 0.12 mgL^{-1} for 30 weeks. The authorsalso noted that following development of blacktail, Spinal curvature was also evident, particularly when the fish was motionless on the tank botton.

Alan et al (1983), explaining the relationship between lead concentration and lead toxicity concluded that waterborne lead had no obvious effect on rainbow trout at concentration below those which cause the development of blacktail and Spinal curvature (0.12mgL^{-1} total lead). Explaining further, Alan et al (1983) stressed that the concentration (0.12mgL^{-1} total lead) compared favourably with the maximum acceptable toxicant concentration (MATC) found by Davies et al (1976). Using the development of blacktails ans spinal curvature as their criterion for harmful lead exposure, Davies et al (1976) determined the MATC for fingerling rainbow trout in hardwater to be between 0.12 and 0.36 mgL^{-1} total lead. Similarly, the MATC of Davies et al (1976) and confirmed by Alan et al (1976), seems to correlate with the lead concentration of 0.163 mgL^{-1} in this study where blacktail occured for Tilapia nilotica (L).

The occurence of blackhead lesion at a later period in the same fish that had blacktail may not be unconnected with probable increasing neurological effect of lead with increasing exposure time.

In this study, lead accumulation in the tissues of Tilapia nilotica increased with increasing time and levels of lead in test water (Fig 8 and 9). This is similar to the observation of Pagenkopt and Neuman (1974) that lead content of bone was higher in fish from a river compared to fish from a hatchery supplied with water with much lower level of lead. The river water contained 2.56 - 2.96 ugL^{-1} lead, twice that of hatchery water. Brown and Chon (1975), Hodson et al (1978) also reported that fish from Baiedu Dare.lake Huron contained only 0.19 ugg^{-1} lead in muscle while those from Toronto Harbour where local lead contamination due to urban runoff water was prevalent contained 1,78 ugg^{-1} .

Lead accumulation in Tilapia nilotica may probably be via the gill, operaular bone and skin. Haider (1964) stated that Chronic lead poisoning in fish results from resorption of the toxicants, apparently through the gills, which gradually causes functional damage to the inner organs. In a similar observation, Hodson et al (1978)explained that the lead accumulation via the gill is a reflection of the direct contact of gills with lead contaminated water. In another explanation,

Hodson pointed out that opercular bone of fish is suitable for monitoring lead exposure and estimating body lead content of fish. The direct contact the skin and scales of test fish have with the lead treated water probably assisted in the accumulation. However, a relatively low concentration may be accumulated due to less affinity of inorganic lead at low concentration with the thiol or Suphydryl groups (component of fish skin and scale) (A De Bruin 1976).

Generally, the levels of lead accumulated in Tilapia nilotica tended to be related to the damages done to the body physiology. This is consistent with earlier reports on lead toxicity to fish. Holcombe et al (1978), Hodson et al (1978) indicated that chronic lead toxicity to rainbow trout increased with tissue lead accumulation.

Report on liver histology of lead-exposed Tilapia nilotica showed an induced hepatopathy at 0.076, 0.163 and 0.326 mgL^{-1} concentrations. The structural impairment of hepatocytes in liver of Tilapia nilotica were more or less same as observed by Kumar and Pant (1981) in Punctus conchonius after copper and zinc poisoning. However, the result in this study does not agree with previous observation of Alan et al (1983), that sublethal lead concentration of 0.1 mgl^{-1} for 32 weeks did not produce any obvious histological effects on the liver and some tissues of rainbow trout.

The disparity may be partially due to the difference in the tolerance capacities of the two species for lead.

The occurence of increased hepatic vacuolarity in the livers of fish exposed to all lead concentration compared to the very few observed in the liver of control fish may be an additional evidence of the toxic effect of lead to the test fish. The occurence of the few hepotocytes vacuolarity in the liver of the control could not be explained since no traces of lead was measured in the control fish or in dechlorinated water that served as control. Alan et al (1983) similarly observed hepátic vacuolarity in the livers of rainbow trout exposed to lead and the control, and concluded that the interpretation of liver changes from increased hepatocytes vacuolarity as a result of lead exposure was unreliable.

The focal necrosis observed in the livers of fish exposed to lead as compared to the control in this study may probably help to explain the reasons for the fall in liver glycogen, blood serum protein and haemoglobin concentration. The lead intoxicated livers may have been incapacitated in the synthesis of adequate protein into the blood stream leading to a depletion. The same may have prevented liver cells from storing glycogen resulting in a fall. Similarly, the haemopoietic tissues that replenishes blood cells; which are located in the periportal area of the liver, in

addition to their presence in the spleen, kidney, intestinal submucosa and the thymus, may have been damaged. This could have resulted in the decreased blood haemoglobin concentration observed in this study.

SUMMARY AND CONCLUSION

From this chronic toxicity assay, it is clear that lead concentration as low as 0.076 mgL^{-1} is harmful to fish, particularly Tilapia nilotica.

Lead did not seem to accumulate as much in tissues of fish to such a high level as some other elements reported in literature. Lead concentration observed in whole fish after 6 weeks of exposure were 70.35, 45.23 and 26.02 times the amount in the water for the 0.076, 0.163 and 0.326 mgL^{-1} respectively. The levels of lead residue accumulation in tissues were directly related to the intensity of damage on blood and tissue histology.

Exposure of fish to lead depressed the blood plasma protein, haemoglobin and haematocrit. The extent of the depressions were directly proportional to the magnitude of the exposure levels. The decreased values of the blood protein and other blood parameters may be an indication of lead interference with protein synthesis and damage to blood cells respectively.

From the study, Tilapia nilotica exposed to 0.076 - 0.326 mgL^{-1} lead in water for 6 weeks still put on some growth, though not as much as the control. However, the death of some individuals with obvious symptoms of lead poisoning, and the degenerative changes in the liver of dead and surviving fish suggested that, had the experiment been maintained for longer exposure period, progressively more fish would have died.

There is evidence that indiscriminate discharge of effluents containing lead into our aquatic ecosystem can have severe harmful effects on the ecosystem leading to the destruction of our fish stock and perhaps of other forms of life. If governments goal of self-reliance in food, particularly protein production is to be achieved, attempts should be made to reduce the discharge of toxic metals into our water bodies.

R E F E R E N C E S

Abiagom, J.D. (1980) "Situation study of fish demand and supply in Nigeria". Paper presented at the National Seminar on Integrated Development of Artisanal and Inshore Fisheries, Ilorin, Nigeria. 14th - 16th April.

A DE Bruin,A (1976) Biochemical Toxicology of environmental agents. Elserior/North Holland Biomedical Press.

Adeney, R.J. and Mathiessen, (1979) Sublethal effects of the organophosphate blackfly larviade, Abate (temephos) on Sarotherodon mossambicus (Peters). Journal of Fish Biology. Vol. 15 pp. 545 - 553.

Akushi, E.U. (1980) Heavy metal profile in the Jos aquatic environment. Part I. Mercuric profile in Dilimi River B.Sc. thesis, University of Jos, Nigeria.

Alan, J.A.S. Geraci J.R. and Hodson P.V. (1983) Histopathological and physiological responses of rainbow trout Salmo gardneri (R) to sublethal levels of lead. Water Research Vol. 17, pp. 1115 - 1118.

Amminikuthy, C.K. and Rege M.S. (1977) Effects of Acute and Chronic Exposure to pesticides, Thiodan E.C. 35 and Agallor "3" on the liver of Window tetra Gymnocorymbus ternetzi (Boulenger). Indian Journal of experimental

Biology

AOAC (1975) (Association for Official Agricultural Chemists) Handbook official methods of analysis 12 Ed. Washington D.C. pp. 186 - 222.

Ayeni, S.O. (1979) Mineralization in Tilapia galilea with reference to Na,k, and Ca, B.Sc. thesis, Department of Biol. Sci. A.B.U., Zaria.

Blaxhal, P.C. and Daisley K.W. (1973) Routine haematological methods for use with fish blood. Journal of Fish Biology. Vol. 5, pp. 771 - 787.

Brown, J.R. and Chow L.Y. (1975) The comparism of Heavy metals in fish samples taken from Baiedu Dore Lake Huron and Toronto Harbor, Lake Ontario. In Hodson, P.V,. Bererley, R.B. and Douglas J.S. (1978) Water Research Vol. 12. pp. 875.

Bull, R.J. (1980) Lead and Energy metabolism. In Singhal R.L. and Thomas J.A. (Eds.). (1980). Lead Toxicity. Urban & Schwarzenberg Balhmore - Munich.

Chizolm, J.J.Jr. (1971) Lead poisoning. Scientific American. Vol. 224 (2) pp. 15 - 23.

Christensen, G.M. (1975) Biochemical effect of methyl mercuric Chloride, Cadmiuin Chloride and lead nitrate on embryo and alevins of the brook trout Salvelinus fontinalis. Toxicology and Applied Pharmacology. Vol. 32 pp. 191.

Biology

AOAC (1975) (Association for Official Agricultural Chemists) Handbook official methods of analysis 12 Ed. Washington D.C. pp. 186 - 222.

Ayeni, S.O. (1979) Mineralization in Tilapia galilea with reference to Na,k, and Ca, B.Sc. thesis, Department of Biol. Sci. A.B.U., Zaria.

Blaxhal, P.C. and Daisley K.W. (1973) Routine haematological methods for use with fish blood. Journal of Fish Biology. Vol. 5, pp. 771 - 787.

Brown, J.R. and Chow L.Y. (1975) The comparism of Heavy metals in fish samples taken from Baiedu Dore Lake Huron and Toronto Harbor, Lake Ontario. In Hodson, P.V,. Bererley, R.B. and Douglas J.S. (1978) Water Research Vol. 12. pp. 875.

Bull, R.J. (1980) Lead and Energy metabolism. In Singhal R.L. and Thomas J.A. (Eds.). (1980). Lead Toxicity. Urban & Schwarzenberg Balhmore - Munich.

Chizolm, J.J.Jr. (1971) Lead poisoning. Scientific American. Vol. 224 (2) pp. 15 - 23.

Christensen, G.M. (1975) Biochemical effect of methyl mercuric Chloride, Cadmiuin Chloride and lead nitrate on embryo and alevins of the brook trout Salvelinus fontinalis. Toxicology and Applied Pharmacology. Vol. 32 pp. 191.

Christensen, G.M. Mi kim, J. and Brungs, W.A. and Hunt E.P. (1972) Changes in the blood of the brow bull head Ictalurus nebulosus (L) following short and long term exposure to Copper (II). Toxicology and Applied Pharmacology. Vol. 23, pp. 417.

Clement, I.B. (1982) A survey of the pollutant load of Kaduna Industrial discharge. M.Sc. Thesis Dept. of Chemistry, A.B.U., Zaria.

Crandal, C.A. and Goodnight. C.J. (1963) The effects of sublethal concentration of several toxicants to its common guppy Lebistes reticulatus. In Dorfman, D. and Whitworth, W.R. (1969). Journal Fish. Res Bd. Canada Vol. 26. pp. 2493.

Cruz, E. (1975) Determination of Nutrient digestibility in various classes of Natural and Purified Feed for Channel Catfish. (A dissertation submitted to the Graduate Faculty of Amburm University in partial fulfilment of the requirement of Degree of Doctor of Philosophy).

Davies, P.H. Goettl, J. B. Jr; Sinley J.R. and Smith N.F. (1976) Acute and chronic toxicity of lead to rainbow trout Salmo gairdneri (R) in hard and soft water. Water Research. Vol.10 pp. 199 - 206.

Dawson, A.B. (1935) The haemopoietic response in the catifish Ameirus nebulosus to Chronic lead poisoning. In Dorfman, D and Whitworth W.R. (1969). Journal Fish.Res.Bd. Canada. Vol. 26 pp. 2493.

Dilling, W.Y. and Smith, W.C. (1926) Experiment on the effect of lead on the growth of Plaice Pleuronectes Platesta. In Dorfman D. and Whitworth W.R. (1969). Journal Fish.Res. Bd. Canada. Vol. 26. pp. 2493.

Dorfman, D. and Whitworth, W.R. (1969) Effects of Fluctuation of lead, temperature and Dissolved Oxygen on the growth of Brook Trout. Journal Fish Res. Bd. Canada. Vol. 26 pp. 2493 - 2501.

Dubale, M.S. and Shah, P. (1979) Histopathologic lesions induced by Malathron in the liver of Channa punclatus. Indian Journal of Experimental Biology. Vol. 17 pp. 693 - 697.

Efuna, C.T. (Mrs.) (1984) The Nutritional value of some processed fish, smoked Clarias Lazera, smoked protopterus annecteus stockfish and Frozen Mackerel. B.Sc. thesis, Dept. of Biol. Sc., A.B.U.. Zaria.

Ellgaard, E.G. and Rudner, T.W. (1982) Lead acetate; Toxicity without effects on the locomotor activity of the bluegill Sunfish Lepomis macrochirus (R). Journal of Fish Biology. Vol. 24, pp. 411 - 415.

Ellis, M. M. (1937). Detection and measurement of stream pollution. In Ozoh. P.T.E. (1983). Proposal P.HD program, Dept. of Biol. Sc. A.B.U., Zaria.

Elsie, M.B.S., Cumbie, P.M, Bauer, T. L, Bell. J.S. & Harban, C.W. (1984). Histopathological, Haematological, Condition - Factor, and Organ weight changes association with Seleniuin Accumulation in Fish from Beleur Lake, North Carolina Archives of Environ. Contam. Toxicol. Vol. 13 pp. 153 - 162.

Experts (1985). Chemical pollution a menace says experts. Daily Times Newspaper, Wed. Aug. 28.

Eyo, A.A. (1975/76). Proximate composition of some commercially important fish in Lake Kainji. Kainji Lake Research Institute Annual Report.

Fodeke, B.A. Dikko J.B., Trywiaski Z. (1983). I.J.M.B. Chemistry Laboratory Manual. pp. 47.

Gilderhus, P.A. (1966). Some effects of sublethal concentration of sodium arsenate in bluegills and the aquatic environment. In Oladimeji A.A. (1980). P. HD. thesis, University of Ottawa.

Gill, T.S. and Pant, J.E. (1981). Effects of sublethal concentration of Mercury in Teleost Puntius conchonius. Biochemical and Haematological responses. Indian Journal of Experimental Biology. Vol. 19 pp. 571.

Goyer, R.A. and Mahaffey, K.R. (1972) Susceptibility to lead toxicity. Environmental Health Perspectives. Vol. 2. pp. 73 - 80.

Goyer, R.A. and Mehlman, M.A. (1977) Toxicity of Trace elements in modern Toxicity. Vol. 2 John Willey and Sons, New York.

Haider, G. (1964) Heavy metal poisoning in Fish I. Demonstration of lead poisoning in rainbow trout Salmo gaidneri R. In Davies, P.H. Goettl, J. P. Jr., Sinley, J.R. and Smith N.F. (1976) Water Research. Vol. 10. pp. 205.

Hassid, W.Z. and Abraham. S. (1957) Chemical procedures for Analysis of Polysacharides I. Determination of Glycogen and Starch. In Methods in Enzymology. Vol. 13 pp. 34. Academic Press, New York.

Hiltbran, R.C. (1971) Effects of cadmium, zinc, Manganese, and calcium on oxygen and phosphate metabolism in bluegill mitochondria. Journal of Water Pollution Control Federation. Vol. 43. pp. 818 - 823.

Hodson, P.V., Blunt, B.R. and Spry. D.J. (1978) Chronic toxicity of water borne and dietary lead to rainbow trout Salmo gairdneri (R) in Lake Ontario water. Water Research Vol. 12. pp. 869 - 878.

Hodson, P.V., Dixon, D.G., Spry, D.J., White, D.M. and Sprague, J.B. (1982) Effects of grow rate and

size of fish on rate of intoxication by waternorne lead. Canadial Journal of Fisheries and Aquatic Science. vol. 39 pp 1245 - 1251.

Holcombe, G.W. Bennl, D.A. Leonard, E.N. and Mckim, J.W. (1976) Longterm effects of lead exposure on the generation of brook trout salvelinus fontinalis Journal Fish Res. Bd Canada Vol. 33 pp1731-1741

Hussana, Y.A.(1983) Automobile related lead content of sediments and Biota of Ponds along Zaria - Kaduna Bye Pass Highway. B.Sc. thesis, Dept. of Biol. Sc. A.B.U. Zaria.

Instruction Manual for Coulter Haemoglobinometer(1977) 9th Ed. Coulter Electronics Limited Coldbarb-omlana. Harpenden Hertford Shire England.

Eta, E.O.(1981) Fish feed preparation for cage culture experiment. Kainji Lake Research Institute (KLRI) KLRI Annual Repot.

Eta, E.O. and Orobor J.I. (1963) Preliminary report of feed formulation techniques for hatchery and pond management in kainji Lake Research Institute (KLRI) KLRI Annual Report

Ita, E.O. and Sade E.K.(1983) Inventory survey of Nigeria Inland waters and their Fisher resources with special refrence to ponds, takes, Reservoirs and major Rivers. Surface area survey and potential yield estimate. Kainji Lake Research Institute Annual Report.

John, E. B., John, H.R. and Williams O.M. (1972) Aquaculture. Willey - interscience. A division of John Willey & Sons Inc. New York.

Kacew, S. and Singhal, R.L. (1980) Aspects of molecular mechanism underlying the biochemical Toxicology of lead. In Singhal. R.L. and Thomas T.A. (Eds.) (1980) Lead Toxocity. Urban & Schwarzenberg Balhomove - Munich.

Kavelec. J. (Undated). Instruction for intensive Fish breeding of Tilapia nilotica F.A.O/UNDP. In Ita E.O. and Orobor J.I. (1983) Kainji Lake Research Institute Annual Report.

Kumar. S. and Pant. S.S. (1981) Histopathologic effect of Acutely toxic levels of Copper and Zinc on gills, livers and kidney of Puntius conchonus (Ham). Indian Journal of Experimental Biology. Vol. 19 pp. 191 - 194.

Luckey, T.D. and Venogopal. B. (1977) Metal Toxicity. Plenum Press New York.

Mahaffey, L.R. (1980) Nutrient lead interaction. In Singhal. R.L. and Thomas, J.A. (Eds). (1980). Lead Toxicity. Urban & Schwarzenberg Balhmore - Munich.

Matton E. (1982) Markets for frozen small pelagic fish F.A.O. Fisheries Technical Paper. No. 221, FI U/T.

Mckim, J.M. and Christensen. G.M. and Hunt. E.P. (1970) Changes in the blood of brook trout

Salvelinus fontinalis after short term and long term exposure to Copper. Journal Fish.Res. Bd. Canada. Vol. 27. oo. 188.

Moore, P.A.M. and Goldberg. (1980) Lead and Heme Bio sythesis. In Singhal. R.L. and Thomas J.A. (Eds.) (1980). Lead Toxicity Urban & Schwarzenberg Balhmore - Munich.

Murty, A.S. and Priyamnada. D. (1982) The effect of Endosulfan and it's isomers on Tissue protein Glycogen, and lipids in the fish Channa Punctata, Pesticide Biochemistry and Physiology. Vol. 17. pp. 280.

Nagendra R. and Katre S. (1979) Studies on Toxicity of Biocides to Cyprinid Forage Fishes: Part I - Effects of sublethal concentrations of Sodium Pentachlorophenate on the Ecophysiology of Puntius ticto (Ham). Indian Journal of Experimental Biology. Vol. 17 pp. 270.

Norberg, G.F. (1978) Factor influencing metabolism and Toxicity of metal. A censensus report Environmental Health Perspective. Vol. 25. pp. 3 - 41.

Nriagu, J.O. (1978) Properties and biogeochemical cycles of lead. In Ellgaard. E.G. and Rudner. T.W. (1982). Journal of Fish Biology. Vol. 21 pp. 411.

Oladimeji, A.A. (1980) Bioaccumulation and Metabolism of Arsenic to rainbow trout Salmo gairdneri

(R) P. HD. Thesis, University of Ottawa.

Oladimeji, A.A. (1983) "Trace metal - A potential threat to our fishery industry". A paper presented at the 3rd Annual Conference of the Fishery Society of Nigeria.

Oladimeji, A.A. and Offem (1983) Toxicity of lead to Tilapia nilotica. Clarias Lazera and Chironomid larva (In preparation).

Olaniawo, A.A. (1983) "Fisheries Manpower Development in the Eighties". Paper presented at the Fisheries Society of Nigeria (FISON) Symposium Maiduguri, February, 23rd.

Oni, K. (1979) Chemical analysis of fish obtained from A.B.U, Dam, M.Sc. thesis Chemistry Department A.B.U., Zaria.

Ozoh, P.T.E. (1979) Studies on intraperitoneal toxicity of lead to Cichlasoma nigrofasciatum (Guenther) development. In Ozoh P.T.E. (1983) Heavy metal pollution (Lead Copper) on Fresh water Fisheries. Propcsal P. HD. program. Dept. of Biological Science, A.B.U., Zaria.

Ozoh, P.T.E. (1980) Effects of lead on pigment pattern formation in Zebra fish. Brachydanio rerio. Bull. Environ. Contam. Toxicol. Vol. 24. pp. 276 - 282.

Ozoh, P.T.E. (1983) Heavy metal pollution (Lead Copper) on Fresh water Fisheries. Proposal P.HD. program. Dept. of Biol. Science A.B.U., Zaria.

Pagenkopt. G.K. and Neuman D.P. (1974). Lead concentration in native trout. Bull. Environ. Contam. Toxicol. Vol. 12 pp. 70 - 75.

Pickering Q.H. and Henderson. C. (1966) The acute toxicity of some heavy metals to different species of warm water Fishes. In Ellgaard E.G. and Rudner, T.W. (1982). Journal of Fish Biology. Vol. 21 pp. 411.

Rakiya, L.M. (1977) An analysis of mineral concentration with respect to size in the bone tissue of fish Tilapia gallilea. B. Sc. thesis Dept. of Biol. Science, A.B.U., Zaria.

Robinson, I.M. (1978) Lead as a factor in the world economy. In Ellgaard. E.G. and Rudner. T.W. Journal of Fish Biology. Vol. 21. pp. 411.

Sam, S., Seymour D. Novic. B. and Edward M. (1950) The estimation of Glycogen with the Anthrone Reagent. Archieves of Biochemistry and Biophysics Vol. 25 pp. 191.

Standard Methods (1980) For the examination of water and Waste water 15th Ed. APHA - ANWA - WPCF.

Stewarte, H.A. Max G., John, A.P. and Christopher Q. (1974) Chemical analysis of Ecological materials: Blackwell Scientific Publication Oxford.

Stiff, M.J. (1971) The chemical states of Copper in polluted fresh water and a Scheme of analysis to differentiate them. Water Research. Vol. 5 pp. 585 - 599.

Stokes, R.M. and From P.O.(1965) Effect of Chromate on glucose transport by the gill of rainbow trout. physiological Zool. Vol. 38, pp. 202-205.

Thompson, S.W. and Hunt. R.D.(1966). Selected Histochemical and Histopathological Methods. cc. Thomas Publisher Spring - Field illinous U.S.A.

Watts. J.C.D.(1957) The chemical composition of West Africa Shad Ethmalosa dorsah. Bull F.M.A.N.

WHO. (1978) Principles and method for evaluating the toxicity of Chemical Part I. Environmental Health Criterion. 6th World Health Organization.

Wong, P.T.S., Silverberg, B.A. Chan. Y.K. and Hodson P.V. (1978) Lead and the aquatic biota. In Ellgaard. E.G. and Rudner. T.W. Journal of Fish Biology. Vol. 21. pp. 411 - 415.

Addendum

Waldron H.A. and Sto ifen(1974) Subclinical lead poisoning. Academic Press London.

APPENDIX I

Test of significant differences between the control and the treated groups:

Test of significance of percent wet weight gain between control and fish exposed to lead was carried out using student t - test.

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{\sum X_1^2 - \frac{(\sum X_1)^2}{N_1} + \sum X_2^2 - \frac{(\sum X_2)^2}{N_2}}{N_1 + N_2 - 2}\left[\frac{1}{N_1} \frac{1}{N_2}\right]}}$$

e.g. Calculation for Week 1:

Control = X_1, 0.344 = X_2

H_o : $X_1 = X_2$

H_1 : $X_1 \neq X_2$

α (Probability level) = 0.05

$\overline{X} = 3.82$ $\overline{X_2} = 2.717$

$\sum X_1^2 = 478.181$ $\sum X_2^2 = 345.552$

$(\sum X_1)^2 = 9878.372$ $(\sum X_2)^2 = 4990.01$

$N_1 = 26$ $N_2 = 26$

$$t = \frac{3.823 - 2.717}{\sqrt{\frac{478.181 - \frac{9878.372}{26} + 345.552 - \frac{4990.010}{26}}{26 + 26 - 2}\left[\frac{1}{26} + \frac{1}{26}\right]}}$$

$$= \frac{1.106}{8.092}$$

$$= 0.137$$

Calculated value = 0.137

Tabulated value = 2.021

Cal.val. $<$. Tab val.

H_o accepted

Statistical inference = Not significant ($P > 0.05$).

APPENDIX II

Correlation and Regression analysis

2.1 e.g. Determination of r for liver glycogen of fish exposed various lead concentrations.

$$r = \frac{N \sum XY - \sum X (\sum Y)}{\sqrt{(N\sum X^2 - (\sum X)^2)\ (N \sum Y^2 - (\sum Y)^2}}$$

X = lead concentration Y = liver glycogen

(Mean of individual treatment)

X	Y	XY
0.086	8.32	0.72
0.172	7.36	1.27
0.344	5.91	2.03
$\bar{X} = 0.20$	$\bar{Y} = 7.20$	$\sum XY = 4.02$
$\sum X = 0.60$	$\sum Y = 21.59$	
$\sum X^2 = 0.16$	$\sum Y^2 = 158.32$	
$(\sum X)^2 = 0.36$	$(\sum Y)^2 = 466.13$	
N = 3	N = 3	

$$r = \frac{3 \times 4.02 - 0.6 \times 21.59}{\sqrt{(3 \times 0.16 - 0.36)\ (3 \times 158.32 - 466.13)}}$$

$$= -0.86$$

Coefficient of determination $r^2 = 0.75$

2.2 Test of significant for r:

H_o: $r = 0$

H_i $r \neq 0$

degree of freedom = N - 2

α (probability level) = 0.05

$$t = \frac{r\sqrt{N-2}}{\sqrt{1-r^2}}$$

$$= \frac{0.86\sqrt{3-2}}{\sqrt{1-0.86^2}}$$

$$= 1.72$$

Calculated value = 1.72

Tabulated value = 12.71

Cal. val < Tab. val.

H_o accepted

Statistical inference = Not significant ($P > 0.05$)

2.3 Regression line:

$$\bar{Y} = a + b\bar{x}$$

$$b = \frac{N\sum xy - (\sum x)(\sum y)}{N\sum x^2 - (\sum x)^2}$$

$$= \frac{3 \times 4.02 - 0.6 \times 21.59}{3 \times 0.16 - 0.36}$$

$$= -7.42$$

$$7.20 = a - 7.42 \times 0.2$$

$$a = 8.68$$

$$\hat{Y} = 8.68 - 7.42x$$

APPENDIX III

Histological method used for processed tissues.

1. Fixed tissues in fixative (formol saline) for 24 hours.
2. Washed material under gentle running water for 2 hours.
3. Dehydrated in 30% alcohol - 2 hours
4. " " 50% " - 2 hours
5. " " 70% " - 2 hours
6. " " 95% " - 2 hours
7. " " Absolute alcohol - 2 hours
8. Dehydrated in 25% (25ml) chloroform/(75) alcohol - 2 hours.
9. Dehydrated in 50% (50ml) chloroform/(50ml) alcohol - 2 hours
10 Dehydrated in 75% (75ml) chloroform/(25ml) alcohol - 2 hours
11 Dehydrated in Pure chloroform - 2 hours
12 " " " " - 2 hours

APPENDIX IV

Staining method

1. Dewaxed sections in Xylene 2 changes for 5 minutes each
2. Hydrated in Absolute alcohol - 2 minutes
3. " " 95% " - 2 minutes
4. " " 70% " - 2 "
5. " " 50% " - 2 "
6. " " 30% " - 2 "
7. Brought to Distilled H_{20} - 2 "
8. Stained in Ehrlich's Haematoxylin - 30 minutes
9. Transfered to tap water - 2 minutes
10 Destained in 70% Acid alcohol - ½ - 1 minutes
11 Washed in 70% Alcohol - 1 minutes
12 Blued in 70% Ammoniated alcohol - 1 minute
13 Washed in 70% Alcohol - 1 minute.
14 Transfered to 50% Alcohol - 1 minute
15 " " 30% " - 1 minute
16 " " distilled H_{20} - 1 minute
17 Counter stained in 1% Eosin in tap water - 2 minutes
18 Washed in tap water - 1 minute
19 Washed in distilled water ½ minute
20 Dehydrated in 30% Alcohol -- 1 minute
21 " " 50% Alcohol -- 1 minute
22 " " 70% " -- 1 minute
23 " " Absolute alcohol -- 1 minute
24 Cleared in dove oil -- 5 minutes

25. Cleared in Xylene -- 2 changes - 5 minutes each

26. Mount in D.P.X using clean coverships

27. Dry shades on luke warm hot-plate.