EFFECTS OF CADMIUM AND ZINC ON FRESHWATER FISH SPECIES, *CLARIAS GARIEPINUS* Burch AND *OREOCHRONIS NILOTICUS* (Trewavas)

BY

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A DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BIOLOGICAL SCIENCES

AHMADU BELLO UNIVERSITY ZARIA

NIGERIA

MAY, 1992

DEDICATION

To my late father, Mr. Annune Akaakii

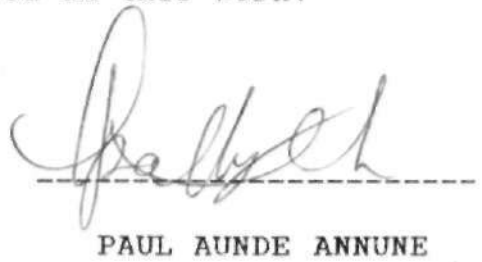
and

My good friend Edna Danboyi.

DECLARATION

I hereby declare that this thesis is my own work and has not been presented in any form for another qualification at any other University or Institution.

Information obtained from the published or unpublished works of others has been duely acknowledged in this work.



PAUL AUNDE ANNUNE

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CERTIFICATION

This thesis entitled "The Effects of Cadmium and Zinc on Freshwater Fish Species, *Clarias gariepinus* Burch and *Oreochromis niloticus* (Trewavas) meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENT

I am very grateful to my major supervisor Dr. T.T. Iyaniwura for his constructive criticisms and suggestions which have made this work a success.

I am also grateful to Prof. A.A. Oladimeji for initiating the work and for the much encouragement and assistance he rendered to make this work a success.

I also thank Dr. S. Ebele for his assistance in the supervision of this work. My thanks also go to Prof. C.G. Vajime through whom I had access to departmental facilities and financial support for this work.

I also wish to thank Mr. J.O.K. Ansah for assisting in the preparation of slides for this work. Above all, my thanks go to God for granting me the strength, wisdom and for sustaining me throughout the time of my carrying out this research.

ABSTRACT

The acute toxicity of zinc, cadmium and zinc/cadmium mixture were determined for fingerlings of *Oreochromis niloticus* and *Clarias gariepinus* in 96h static bioassays. Symptoms of toxicosis in both species indicate that zinc and cadmium cause subacute effects such as altering fish behaviour. These included loss of equilibrium, agitated swimming, air gulping, periods of quiescence and death. *Clarias gariepinus* exposed to lethal concentrations of cadmium also showed swollen abdomen. *Clarias gariepinus* was more susceptible to both metals and the zinc/cadmium mixture. The LC_{50s} of the heavy metals to fingerlings of *C. gariepinus* were 13.25 (12.33-14.24) mgl^{-1}Zn; 10.85 (10.10-11.66) mgl^{-1}Cd; and for zinc/cadmium mixture 14.15 (13.33-15.24) mgl^{-1}Zn and 9.35 (7.69-10.37) mgl^{-1}Cd respectively. For the *O. niloticus*, the LC_{50s} were 24.66 (23.76-25.46) mgl^{-1}Zn; 19.32 (18.38-20.31) mgl^{-1}Cd and for zinc/cadmium mixture 19.92 (18.26-22.99) mgl^{-1}Zn and 12.78 (11.46-13.61) mgl^{-1}Cd respectively. Possible reasons for the difference in susceptibility of these two species to heavy metals are suggested. The toxicity of these heavy metals to freshwater fishes was in the order of zinc/cadmium mixture > cadmium > zinc. For the *Clarias* species the toxicity of zinc reduced from 13.25 mgl^{-1} to 14.15 mgl^{-1} in the Zn/Cd mixture whereas the toxicity of cadmium increased from 10.85 mgl^{-1} to 9.35 mgl^{-1}. In the *Oreochromis* species both zinc and cadmium were found to be more toxic in mixture than when applied individually. Proposed mechanisms for metal-metal interactions are discussed in an attempt to explain the present results.

Effects of lethal concentrations of zinc and cadmium on the structure of gill filaments and liver cells of freshwater fishes (*O. niloticus* and *C. gariepinus*) were investigated. Structural damages caused by lethal concentrations of zinc and cadmium on gill filaments of these fishes include epithelial proliferation, detachment along central axis of the filament, epithelial disarray, partial and complete blanketing of the secondary lamellae. Defects examined on the liver cells include vacuolation of hepatocytes, ruptured blood vessels and fibrosis.

Oreochromis niloticus and *C. gariepinus* were subjected to sublethal concentrations of zinc and cadmium and investigations were made on the effects of these metals on the blood parameters of fish. Decreases of haematocrit, haemoglobin, MCH and MCV; and increases of RBCC and WBCC of *C. gariepinus* exposed to sublethal concentration of zinc were observed. For the *O. niloticus* exposed to sublethal concentration of zinc there was decreases in haematocrit, RBCC, WBCC and increases in MCV, MCH and MCHC. The *O. niloticus* exposed to sublethal concentration of cadmium showed decreases of RBCC, haematocrit and increases of WBCC, MCV, MCH and MCHC. Decreases of haematocrit, haemoglobin concentration, MCV, MCH and increases of RBCC, WBCC and MCHC of *C. gariepinus* exposed to sublethal concentration of cadmium were observed.

Bioaccumulations of zinc and cadmium in body tissues of *C. gariepinus* and *O. niloticus* were investigated in three separate static experiments for eight weeks. The *Clarias* species accumulated zinc in the order of Liver > gut > gills > whole fish > skin > muscles. Cadmium residue in both species was in the

order of liver > gut > gills > skin > muscles > whole fish. The *Clarias* species absorbed and concentrated more cadmium in its body tissues than the *Oreochromis* species. Suggestions were made to ascertain the difference in accumulation of cadmium by these freshwater fishes. The *Clarias* species also accumulated more zinc than it did for the cadmium. Reasons were given for varied levels of accumulation of heavy metals in fish tissues.

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ABBREVIATIONS

LD_{50} = Median Lethal Dose.

TLm = Median Tolerant Limit.

LC_{50} = Median Lethal Concentration.

MCV = Mean Corposcular Volume.

MCH = Mean Corposcular Haemoglobin.

MCHC = Mean Corposcular Haemoglobin Concentration.

RBCC = Red Blood Cell Count.

WBCC = White Blood Cell Count.

CHAPTER ONE

INTRODUCTION

Fish has been in the diet of man from time immemorial. The increased and continued use of fish arises from the fact that fish provides not only all essential amino acids required by man; it supplements these with polyunsaturated fatty acids not found in other sources of protein, mineral salts and some vitamins (Kent, 1984). Thus weight to weight fish meat is more useful to the human body than beef or any other animal source of protein. Fish is very important to man being the cheapest source of high animal protein (Nikolsky, 1983). According to Olayida *et al.*, (1969) the per-capital consumption of fish per day is higher than that of any other livestock in Nigeria.

Nigeria's domestic fish production hardly meets the local demand and there has been yearly increase in fish importation in order to meet the demand. For example, out of the expected 1 to 1.5 million metric tonnes of fish demand in 1980, only one third of this was met by domestic production (Uboma *et al.*, 1981). Lanier (1981) also reported that approximately 900,000 tonnes of fish consumed in Nigeria in 1980 were imported, the bulk of which was frozen fish from U.S.A., and Japan. Tobor (1985) projected that fish demand in Nigeria would increase from 1,018 million tonnes in 1985 to 2,035 million tonnes in the year 2,000. He also indicated that domestic fish production was only 54 to 60% of the demand.

Inspite of the inability to meet the local demand for fish, certain constraints limit our local fish production. One major constraint is pollution.

Water bodies are the ultimate recipients of wastes generated

by man's industrial, and domestic activities and released into the geosphere. Wastes enter the aquatic environment directly, by point source dumping, or indirectly, by rain or fall-out by surface water run-off and ground-water leachings. These wastes have been known to contain trace and toxic metals which may be poisonous to fish and other aquatic organisms. When pollutants reach levels in excess of the assimilative capacity of receiving water bodies they affect the survival, reproduction, growth and movement of organisms within such environment. This results to changes in distribution and abundance of populations with alteration in the energy budget of the ecosystem.

Venugopa and Lucky (1975) identified potentially toxic metals as lead, zinc, copper, arsenic, antimony, mercury, beryllium, barium, cadmium, chromium, nickel and selenium. Others include cobalt and manganese.

The occurrence of heavy metals in water and their toxicity to fish is largely unexplored in Nigeria. Beetseh (1982) reported abnormally high levels of lead, mercury and cadmium in effluents discharged from a number of industries in Kaduna. Singh *et al.*, (1984) determined the level of lead, nickel and copper in soil vegetation and water samples taken from the sides of some major high ways in all the State Capitals of Nigeria. They reported a good correlation between concentration of metal and daily traffic volume. It is also reported that metal concentration in soil and water samples are generally below permissible thresholds except for nickel. Thomas and Adekanye (1985) reported an increase in the concentration of lead, copper, cadmium and nickel which appears to increase with increase in

pool depth. This observation was reported particularly for certain highways in Kaduna State.

With the industrial revolution and fast growing population, the efforts of removing pollutants from the natural environment have not been able to cope with the increasing amount of waste materials. Consequent to this, lakes, rivers and coastal waters have often been transformed into sewage depots where the natural ecological balance is severely upset and in some cases totally disrupted. In most cases, toxic substances enter the food chain and become concentrated in fish and other aquatic organisms. In coastal regions with high local pollution, heavy metal content in fish is usually high. The pollution present can impair the local fishing industry ((Iyaniwura, 1990; McCarty *et al.*, 1978).

With the Federal Government call for increased fish production and environmental protection, it is important that research be conducted on fish species that are locally available and factors that adversely affect the aquatic environment with a view to enhance productivity. Although there is not enough evidence at the moment that metal pollution poses a serious threat to our fresh water fisheries it could become so in the near future as a result of indiscriminate discharge of urban and industrial effluents into our inland waters, unless efforts are made to prevent it.

The main objective of the current work is to assess the effects of cadmium and zinc on freshwater fish species, *Clarias gariepinus* Burch, and *Oreochromis niloticus* (Trewavas). Doudoroff *et al.*, (1951) suggested the use of economically important local fish species in toxicity studies. *Clarias*

gariepinus and *O. niloticus* are of high commercial value and very common in Nigerian freshwaters. They also have the ability to withstand laboratory conditions. They are of high nutritive value even though the data on the nutritive value of *Clarias gariepinus* have not been documented, that of *Oreochromis* are well documented (Eyo, 1975). Thus *C. gariepinus* and *O. niloticus* were chosen for this study on the bases of their economic importance, abundance and ease of handling in the laboratory.

The acute toxicity of zinc and cadmium to *C. gariepinus* and *O. niloticus* or to most other native fishes in Nigeria has not been reported. Thus this work is aimed at finding out the following:

i Acute toxicities of cadmium and zinc to *C. gariepinus* and *O. niloticus*,

ii Acute toxicities of mixed solutions of cadmium and zinc to *C. gariepinus* and *O. niloticus*. Since heavy metals often occur together in polluted areas, it is of vital importance to study metal-metal interactions in fish.

iii The effects of cadmium and zinc on the gill and liver structures of the study fishes.

iv The cadmium and zinc residue in the various organs of the fish.

v The effects of sublethal concentrations of cadmium and zinc on haematological parameters of the study fishes.

1.2 SOURCES OF HEAVY METALS IN WATER BODIES

Apart from contamination from industrial sources, water bodies may be contaminated naturally. This is because heavy metals naturally occur in the earth-crust as ores. For example, zinc occurs as zinc carbonate or zinc sulphate, Lead as galena (Lead sulphide), iron as hematite (ferric tetraoxide) and copper as covelite (copper sulphide). Erosion from metal-rich superficial deposits, volcanic activity and chemical weathering of igneous and metamorphic rocks may add these metals to aquatic habitats. Mining, agricultural activities, sewage sludge and burning of fossil fuels can result in the release of metals into the aquatic environment (Chizhkov, 1968). The use of pesticides containing metals also contributed to the environmental load of metals.

1.3 USES OF ZINC

A considerable amount of zinc is used in general industry. It is used as a protective coating on other metals, particularly in galvanizing iron and steel. In viscose rayon manufacture zinc is added to the precipitating bath to promote crenulation; it is also used in textile dyeing and printing; it is used in agriculture and animal nutrition where it is used to correct zinc deficiency (Morgan, 1977). Zinc chloride forms double salts with many other metallic halides for example ammonium chloride with which it forms $Zn\ Cl_2\ 2NH_4\ Cl$ and $Zn\ Cl_2\ 3NH_4\ Cl$. These are used widely mainly as fluxing agents. Fused zinc chloride itself dissolves many metallic oxides and this is the basis for which a number of fluxes are used in metallurgy (Morgan, 1977). Zinc

is also used in aqueous solution as a wood preservative either alone or with sodium chromate or phenol. Other uses are in oil refining and in the manufacture of dry batteries. Thus with these uses, zinc has a high potential of contaminating the aquatic environment either by illegal industrial waste disposal or by run-off from agricultural usage.

1.4 USES OF CADMIUM

1.4.1 ELECTROPLATING: A thin layer of cadmium is used in coating metal objects made of steel, iron, copper, brass, and other alloys to prevent corrosion. The cadmium layer provides good solderability and conducts electricity. It is ductile and provides good corrosion resistance to saltwater and alkaline substances.

1.4.2 PIGMENTS: Some cadmium compounds are used as colouring agents in many products such as plastics, coated fabrics, textile, rubber, glass, paints, enamels, ceramic glazes, printing inks and artists' colours. Mixtures of cadmium sulphide produces colours ranging from yellow to red, cadmium selenide produces red while a mixture of cadmium sulphide with zinc sulphide produces yellows.

1.4.3 PLASTIC STABILIZERS: Mixtures of cadmium and Barium when mixed with organic acid anions are used as heat stabilizers in plastics to retard degradation due to elevated temperatures.

1.4.4 **NICKEL - CADMIUM ENERGY CELLS:** Cadmium is used as the anode in nickel - cadmium batteries. It is used as a sealed cell in communication equipments, as motor starters, and electric shavers.

1.4.5 **FERTILIZER:** Cadmium is present in phosphate fertilizers made from phosphate rock. Thus cadmium through these various uses can get into the aquatic system either by illegal industrial waste disposal or by run-off from farm lands (Iyaniwura, 1990). In the aquatic environment, cadmium poses threat to many organisms.

1.5 TAXONOMY, MORPHOLOGY AND DISTRIBUTION OF *Clarias gariepinus*, Burch

Clarias gariepinus belongs to catfishes which fall in the following classification:-

Class - Osteichthyes

Order - Siluriformes

Family - Clariidae

Genus - *Clarias*

Members of the genus *Clarias* include *Clarias gariepinus, C. anguillaris, C. Senegalensis, C. Mossambicus, C. lazera C. submarginatus* and *C. batrachus*. Boulenger (1916) reported 33 species of this genus in Africa. Irvine (1947) reported six species of *Clarias* in Gold Coast (now Ghana) and Reed *et al.*, (1967) reported three species in Northern Nigeria.

In Northern and Central parts of Africa, *C. gariepinus* has been described as *C. lazera*, in the Eastern part as *C.*

Senegalensis, in the Western part as *C. mossambicus* and in the Southern part as *C. gariepinus*. Thus according to Jubb (1963), Pienaar (1968) and others, these species earlier separated should be treated as synonyms.

Clarias species are generally characterised by very long scale less bodies with dorsally-flattered heads covered by long plates. They have wide terminal mouths and four pairs of simple barbels. The eyes are small and found on the anterolateral region of the head. Each of the pectoral fins have strong barbed spines in front. Both the dorsal and anal fins are long and have no spines. The pelvic fins are small and have retained their primitive abdominal position. The caudal fin is a single rounded lobe. Adult males have long conical "anal papilla" a projection from the vent bearing the sexual aperture.

Clarias gariepinus (Plate 1) has 35 to 135 gill rakers on the first branchial arch (Holden and Reed, 1972; Teugels, 1982). The vomerine teeth are granular, forming a crescentic band with or without a posterior median process, its greatest width in the middle is 1.5 to 2.5 times that of the premaxillary band (Teugels up cit). The dorsal fin has 62 to 82 fin rays while the anal fin has 50 to 65 fin rays.

The species of the genus *Clarias* are widely distributed in Africa probably due to their enormous resistance to environmental stress. The West African species include *C. anguillaris, C. gariepinus, C. submarginatus* and *C. senegalensis* (Irvine, 1947; Daget, 1954; Holden and Reed, 1972; Teugels, 1982). *C. gariepinus* occurs widely throughout most of the freshwaters of Africa from Lake Galilee in the Middle East throughout the Nile,



Plate I: External Features of *Clarias gariepinus*

Congo and West African river system (Daget, 1959; Blache, 1965). *C. gariepinus* also occurs in South Africa (Orange River, Natal and Transvaal), Mozambique and Angola and Lake Victoria (Teugels, 1982).

1.6 TAXONOMY, MORPHOLOGY AND DISTRIBUTION OF *Oreochromis niloticus* (Trewaves)

Orechromis niloticus is a teleost fish which fall into the following classification:

Class - Osteichthyes

Order - Perciformes

Family - Cichlidae

Genus - *Oreochromis*

The family cichlidae has four genera, *Tilapia, Hemichromis, Oreochromis* and *Sarotherodon.*

Oreochromis niloticus (= *Tilapia nilotica*) (Plate 2) has white and blue - black vertical bars on the soft part of the dorsal fin and on both the tail and anal fins. The body is dark with darker bands and large cycloid scales. The throat and belly are white except during breeding season when they are deep red. The body is deep and laterally compressed. They have double lateral line; one lateral line starts near the head and extends to the end of the dorsal fin while the second one begins underneath the first one and continues to the tail. The anterior region of the dorsal fin is made up of spiny rays and it is followed by the posterior region which consists of soft rays. *O. niloticus* (Plate 2) DXVII - XVIII, 12-14; AIII, 8-10.

Oreochromis niloticus is widely distributed in Nigerian inland water, Lake, Albert, Uganda and Lake Rudolf in Kenya.

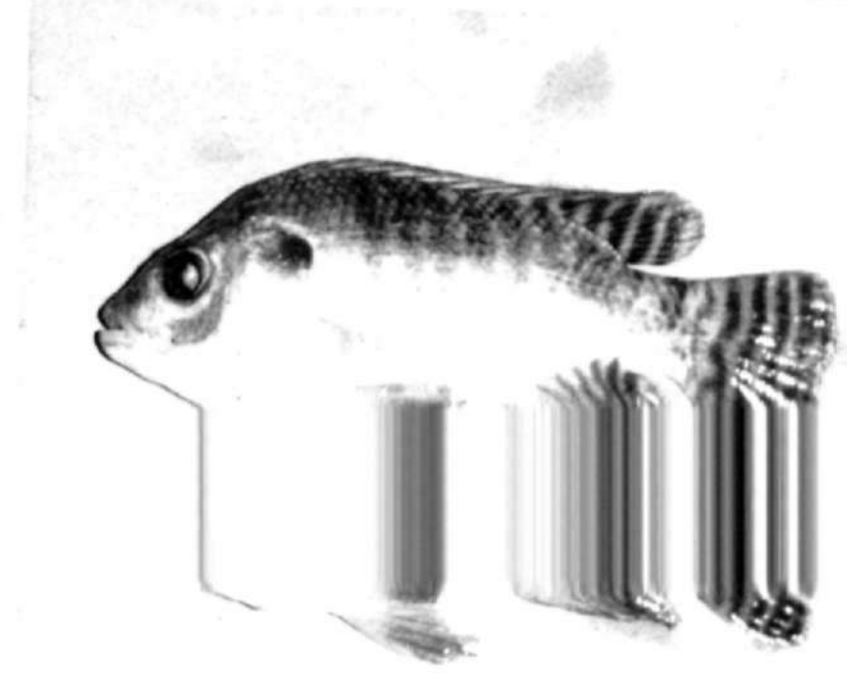


Plate II: External Features of *Oreochromis niloticus*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TOXICITY OF SOME METALS TO FISH

Heavy metals are known to affect the development, growth and survival of the fish as well as biochemical, physiological, histopathological and haematological parameters of fish. Ozoh (1979) reported ovulation stress in *Cichlasoma nigrofasciatum* exposed to lead. Crandall and Goodnight (1963) reported impairment of growth in guppies (*Lebistes reticulatus*) exposed to 1.24 and 3.12 mgl^{-1} Pb for up to 29 days. Oladimeji and Ologunmeta (1987) reported that sublethal concentrations of lead caused a dose-dependent, impairment in growth. They also reported lordosis in *Tilapia nilotica* exposed to 482 μgg^{-1} Pb. Hodson *et al.*, (1978 and 1982) reported lordosis and "blacktail" in fish exposed to lead.

Heavy metals are known to interfere with normal biochemical and physiological mechanisms of fish (Schreck and Lorz, 1978; Waiwood and Beamish, 1978; Anderson and Spear, 1980 a, b; Crespo *et al.*, 1981; Nemcsok and Boross, 1981; Nemcsok and Hughes 1988). Heavy metals produce general stress effects in fishes in increasing catecholamine, blood sugar and cortisol concentrations in blood plasma (Nakono and Tomlinson, 1967; Schreck and Lorz, 1978). According to Nemcsok and Hughes (1988), the adverse effects of copper sulphate on fish are mainly those of tissue necrosis as demonstrated by increased ASAT and ALAT levels in blood plasma; and also inhibition of acetylcholinesterase activity. They also observed an elevated blood glucose. Nemcsok and Hughes (up cit) attributed increased ASAT and ALAT activities

to damage done to the liver as well as other organs such as the gills and kidney. Schreck and Lorz (1978) observed that the kidney of fish exposed to copper showed glomerular atrophy and the gills showed necrosis.

Permanent stress includes impaired γ-globulin information and depressed interferon production which play an important role in the resistance of fishes to various bacterial and viral diseases (Wedemeyer, 1970; Snarski, 1982). Inhibition of AchE activity by CuSo4 is very harmful in fish because the cholinergic system plays a key role in the innervation of the fish heart (Pennec and LeBrass, 1984). Inhibition of AchE would potentiate vagal tone, which may cause some adverse effects in the metabolic processes related to the circulation. In this case, the inhibition of heart function interferes with uptake of oxygen and release of carbondioxide at the gills, which may result in hypoxia at the tissue level (Hughes, 1976).

Exposure of fish to copper affects the catalytic capacity of membranes as a result of interaction of copper with the ligands of proteins (Passow *et al.*, 1961; Luoma, 1983). These led to reduced efficiency of physiological reactions and possibly stimulating a higher production rate of enzymes to generate the amount required to meet physiological demands.

Freshwater fishes are hyperosmoregulators and must counteract the tendency to loose ions and gain water. Ions are selectively taken up at the gill epithelium and retained by the urinary bladder epithelium. Gills serve as primary site of osmoregulation as well as respiration for which reasons they are highly vulnerable to lesions because they are in immediate

contact with aquatic toxicants. Certain heavy metals like Hg, Zn and Cu are known to cause structural gill damage (Skidmore, 1970; Mitrovic, 1972) and interfere with ion transport across gill epithelium (Lewis and Lewis, 1971; Renfro *et al.*, 1973). Both mercuric chloride and methylmercury have been observed to bind to gill tissue where they inhibit sodium uptake in kilifish (Renfro *et al.*, 1973).

An increased cough reflex has been correlated with low levels of copper (Sellers *et al.*, 1975). An elevated cough frequency interferes with oxygen uptake at the gills and reduces the efficiency of oxygen uptake (Davis, 1973) and if continued might cause tissue and respiratory collapse. Damage to the gills, liver, kidney and nervous system of carp has been reported at a concentration of 1,5 ppm copper (Nemcsok *et al.*, 1987). Rojik *et al.*, (1983) have shown that CuSo4 damaged the gills, liver and kidney of carp, silver carp and silure, where the endoplasmic reticulum and mitochondria were damaged. Schreck and Lorz (1978) observed that the kidney of Cu-exposed fish had glomerular atrophy and the gills showed necrosis. Rojik *et al.*, (1983) demonstrated that following CuSo4 treatment, the cytoplasm of secondary lamellar epithelial cells became electron transparent and the cytoplasmic organelles disappeared almost totally.

Changes in blood parameters are often quick responses to environmental or physiological changes and can provide an integrated measure of the physiological status of the fish. Exposure of fish to heavy metals induces changes in haematological parameters due to changes in blood water content

(Tort and Torres, 1988). El-Domiaty, (1987) reported the occurrence of anaemia in *Clarias lazera* after Cu-treatment. He also recorded decreased values in erythrocyte count and haemoglobin and haematocrit and also observed haemolysis in the blood samples. He suggested that copper loosely binds to serum albumin and amino acids and passes readily to erythrocytes where it causes damage. Peters (1967) reported the interference of copper with membrane ATP-ase. Haemolysis may be as a result of membrane ATP-ase Cu-interaction resulting in the breakdown of the sodium pump. Mishra and Srivastava (1980) reported increase in erythrocyte count, haematocrit, thrombocyte count and haemoglobin in *Colisa fasciatus* treated with 3 ppm Cu- after 90 h. They however, observed a decrease in lymphocyte count.

2.2 EFFECTS OF HEAVY METALS ON PLANKTON

Plankton serve as major food for fish, hence their destruction by pollution will adversely affect fish productivity. Plankton and pollutants are ubiquitous in fresh and marine water bodies in all parts of the world. Since they occur together in natural aquatic systems, it is important that potential interactions are understood and when such interactions lead to undesirable effects, that measures are taken to correct them.

Effects of heavy metals on phytoplankton have been reviewed by Davis (1978). Although some metals (Cu, Zn, Co, Mn and Fe) are essential for algal growth and development, these and others (Hg, Cd, Ag, Ni, Pb, Se, Cr and As) may be toxic at concentrations higher than those normally found in natural systems. Although algae generally are not strongly affected by

concentrations of heavy metals in the field, they do accumulate some of them and may transfer them to higher trophic levels. Davies (1973) stated that heavy metals appeared to be accumulated by phytoplankton by ion-exchange processes that involve organic molecules and that the process is governed by the rate of diffusion across cell membrane. Boudou and Ribeyre (1981) demonstrated trophic transfer of mercury from alga, *Chlorella valgaris* to the daphnid, *Daphnia magna*. When the alga was contaminated with mercuric chloride, very little metal was transferred to *D. magna*. However, when contaminated with methyl mercuric chloride and fed to the daphnid, approximately 20% of the mercury on the alga was transferred at 10^{0}C and 60% at 18^{0}C. This shows that large amounts of the methylated form of mercury can be transferred through food chains.

Amiard and Amiared-Triquet (1975) described substantial transfer of cobalt in a food chain that consisted of the diatom, *Navicula* species (producer), the lamellibranch mollusc, *Scrobicularia plana* (Primary consumer) and crab, *Carcinas maenas* (secondary consumer). Amiard-Triquet (1979) also demonstrated trophic transfer of cobalt through a freshwater food chain composed of *Chlorella* species, *Daphnia magna* and the carp, *Cyprinus carpio*. Although the transfer factor between trophic levels was low, uptake by the alga was so great that transfer of cobalt through the chain accounted for more cobalt in the crustacean and fish than did direct uptake from water.

Heavy metals in algae may also affect survival and development of animal larvae. Wilkfors and Ukeles (1982) fed algae contaminated with Cu, Cd and Zn to veliger larvae of the

American oyster, *Crassostrea virginica* and noted poor growth and high mortality of the larvae. Kuiper (1981) exposed natural populations of phytoplankton to mercuric chloride in plastic bags attached to a raft. A single dose of 0.5 $\mu g l^{-1}$ altered the species composition, with a shift to predominance of species of large size, probably because mercury reduced the number of small zooplankton herbivores. At 5 $\mu g l^{-1}$ and above, mercury inhibited growth; the peak in algal biomass was delayed 9 days by 5 $\mu g l^{-1}$ and complete inhibition of growth was caused by 50 $\mu g l^{-1}$. Cell morphology is often affected by heavy metals. Saboski (1977) described the effects of mercury and tin on the frustular ultrastructure of *Nitzschia liebethrutte*. A high rate of abnormal morphology was caused by each metal at 1.5 $\mu g l^{-1}$. Cadmium induced an increase in filament length and a loss of cellular contents in *Anabaena inaequalis* (Stratton and Corke, 1979). The foregoing shows that pollution by heavy metals could decrease rates of primary productivity by algae and that remaining algae would be potentially toxic to grazing animals.

Effects of 21 metals on survival and reproduction of *Daphnia magna* were reported by Biesinger and Christensen (1972). Cadmium, mercury and cobalt were the most toxic to survival (48-h LC_{50}) and reproduction; magnesium, calcium and sodium were least toxic. Weight of *Daphnia magna* was reduced at metal concentrations that permitted survival but impaired reproduction. Sublethal concentrations of cadmium were shown by Griffiths (1980) to cause morphological and ultrastructural abnormalities in *D. magna*. Yan and Strus (1980) also showed that species richness and biomass of crustacean zooplankton in lakes

contaminated with acid waste and heavy metals were strongly reduced in comparison with nearly uncontaminated lakes. Wisely and Blick (1967) tested larvae of seven species with mercury, copper and zinc. The metals caused death of larvae in two hours. Mercury was more toxic to all the species than copper and zinc was least toxic. The bryozoans, *Watersipora cocullata* and *Bugula neritinia* and the tubeworms, *Spirobis lamellosa* and *Galeolaria caepitosa* were most sensitive to each metal. Larvae of the bivalves, *Mytilis edulus* and *Crassostrea commercialis* and the brimeshrimp, *Artemia salina* were relatively insensitive to the three metals.

2.3 OTHER POLLUTANTS OF THE AQUATIC ENVIRONMENT

Crude oil and petroleum products, agrochemicals, pesticides, herbicides and fertilizers are often major sources of aquatic pollution. The effects of oil pollution has been reviewed (Laws, 1981) and pesticides by Iyaniwura (1992).

2.4 TOXICITY OF ZINC AND CADMIUM TO FISH

Zinc is an essential element for plants and animals where it forms a constituent of several enzymes and functions in maintaining cytoplasmic integrity (Weatherley *et al.*, 1980). It is absolutely essential for normal development and growth (Arnac and Lassus, 1985). However, at higher concentrations, zinc exerts noxious effects in fish where it adversely affects the development, growth and survival of fish (Skidmore, 1965; Tuurala and Soivio, 1982). Cadmium is not known to have necessary physiological function.

Cadmium has being found to be toxic to fish (Friberg *et al.*, 1971; Eisler and Gardner, 1973; Tafanelli and Summerfelt, 1975; McCarty *et al.*, 1978; Oronsaye, 1990). Tafanelli and Summerfelt (1975) found the LD_{50} for intraperitoneal injections of cadmium chloride to goldfish as 30; 23; and 20 $mgkg^{-1}$ wet weight at 24, 48 and 96 hours respectively. The maximum dose which they found not to produce mortality was 10 $mgkg^{-1}$. Pickering and Henderson (1966) reported the toxicity of cadmium on fish in both soft and hard water. The median tolerant limit (TLm) values of cadmium for fathead minnows (*Pimephales promelas*) were approximately 1 and 78 mgl^{-1} in soft water and hard water respectively and 7.8 and 88.6 mgl^{-1} in soft and hard water respectively for green sunfish (*Lepomis cyanellus*). In soft water, 24 hrs TLm values of cadmium for blue gills (*Lepomis macrochirus*) goldfish and guppies (*Poecilia reticulata = Lebistes reticulatus*) were 4.6; 3.5 and 3.4 mgl^{-1} respectively. They found 96-hr TLm of cadmium for goldfish as 2.3 mgl^{-1}.

Matthiessen and Brafield (1973) reported lethal zinc concentrations of 0.5-1.0 $mgdm^{-3}$ to sticklebacks within 1-3 days of exposure. Lewis (1978) obtained a 96-hr LC_{50} of 0.79 mgl^{-1} zinc in juvenile longfindace, *Agosia chrysogaster*. Meisner and Hum (1987) reported 96-hr LC_{50s} of zinc to juvenile and subadult rainbow trout, (*Salmo gairdneri*) as 26 and 24 mgl^{-1} respectively.

Zinc is known to adversely affect the development, growth and survival of fish. According to Skidmore (1965) the eggs of zebrafish, *Brachydanio rerio* are more susceptible to zinc than newly hatched larvae and resistance to zinc increases from hatching to 100 days of age. Crandall and Goodnight (1963)

reported that newborn guppies exposed to tap water containing 1.15 mgl^{-1} Zn grew less rapidly than the controls raised in Zn-free water. Brungs (1969) observed significant reduction in egg production at 180 μgl^{-1} Zn. Hatchability and survival have also been affected by sublethal levels of zinc (Benoit and Holcombe, 1978; Cardeihac *et al.*, 1981).

Friberg *et al.*, (1971) showed that cadmium is associated with hypertension, ephysema, renal tubule damage, liver dysfunction and cancer. Eaton (1974) observed that embryos produced by adult bluegills (*Lepomis macrochirus*) maintained in cadmium at 239 μgl^{-1} developed spinal curvature, deformed caudal fins and microcephalia. Histopathological damage to gills which impaired respiration had been reported for fish exposed to cadmium (Eisler, 1971) and Zinc (Lloyd, 1960). Lloyd (up cit) reported cytological breakdown in the gill epithelium of trout exposed to 3 mgl^{-1} Zn. Zinc has also, been found to impair oxygen uptake of rainbow trout (*Salmo gairdneri*) by causing circulatory vasoconstriction by increasing the diffusion distance between the lamellar epithelium and the pillar system (Tuurala and Soivio, 1982).

According to Skidmore and Tovell (1972) the main effect of zinc on the gills is the detachment of the secondary lamellar epithelium from the underlying pillar cells system. This they said increased the diffusion distance from water to blood and also the fusion of the adjacent secondary lamellae, decreased the gill area. With reduction in diffusion capacity the fish is subjected to some form of hypoxia (Hughes, 1973). Histopathological lesions in gill tissue after a Cd-subchronic

exposure have been detected in *Lepomis macrochirus* (Versteeg and Giesy, 1986).

Tort and Torres (1988) reported decrease of haematocrit in dogfish, *Scyliorhinus canicula* following Cd-treatment for 24 h. They attributed this to haemodilution. They objected to the alternative explanation of erythrocyte destruction or haemolysis since RBCC increased. The RBCC elevation they reported could be as a result of blood cell reserve release combined with cell shrinkage, probably due to osmotic alterations of blood by the action of the metal. Spleen concentration after stress has been detected in fish (Abrahamsson and Nilsson, 1975). Cells released from the spleen, which is also an erythropoietic organ (Fange and Johansson-Sjobeck, 1975) would then have a lower MCV value. A similar pattern has been observed in *Cyprinus carpio* after cadmium exposure (Koyama and Ozaki, 1984) in which haematocrit decreased, RBCC maintained and the circulating reticulocytes increased. These indicated a pre-mature release of these cells into the blood stream.

Tort and Torres (1988) observed that measurements of haemoglobin in plasma of dogfish, *Scyliorhinus canicula* exposed to sublethal concentrations of cadmium showed the absence of increased values of extracellular haemoglobin. Leucopenia is a general consequence of stress (Ellis, 1981) and metal pollution (Mishra and Srivastava, 1979; 1980). Nevertheless, leucocytosis has been observed in *Scyliorhinus canicula* after Zinc treatment (Flos *et al.*, 1987). It has been shown that zinc accumulates both in dogfish gill (Crespo *et al.*, 1979) and internal organs (Flos *et al.*, 1979) and induces a depressive effect on tissue

respiration consistent with a general situation of oxygen deficiency in the whole fish leading to death by hypoxia (Burton *et al.*, 1972). Zinc pollution also induced changes in ventilatory and heart physiology (Hughes and Tort, 1985).

Waiwood (1980) attributed increases in haematocrit to the swelling of red blood cells as has been demonstrated for chromium and Zn-treated rainbow trout. Haemolysis of erythrocytes has also been described in rainbow trout exposed to zinc (Kodama *et al.*, 1982). According to Mishra and Srivastava, 1979), 100 ppm of zinc decreased RBCC, haematocrit and leucocyte count, haemoglobin and sedimentation rate increased in *Colisa fasciatus*. Matthiessen (1981) also reported increase in leucocyte count in pike (*Hepsetus odoe*) following stress and endosulfan treatment.

2.5 FACTORS AFFECTING TOXICITY OF CADMIUM AND ZINC

The toxicity of cadmium and zinc is modified by environmental factors such as water hardness, alkalinity, oxygen, carbondioxide, pH, temperature, synergism with other metals and aquatic pollutants such as pesticides from agricultural and public health use (Iyaniwura, 1991, 1992).

2.5.1 HARDNESS

Water hardness is perhaps the most important factor in modifying cadmium and zinc toxicity in natural waters. This is by forming insoluble carbonates or by adsorption on calcium carbonate. This is especially true when waste water flows into natural water body, where the acute toxicity of metals decrease with their bioavailability (Lloyd, 1965; Alabaster and Lloyd,

1980; Brown, 1982). The antagonism of hardness to the toxicity of zinc has been noted by others (Lloyd, 1962; Matthiessen and Brafield, 1977) and shown not to alter the basic phenomenon of toxicity so much as its severity (Skidmore and Tovell, 1972). According to Lloyd (1962) calcium exerts its effect internally and the longer the fish remains in calcium-free water, the more it looses elements from its tissues and hence the shorter its survival time when exposed to acutely toxic zinc concentrations. Magnesium and calcium ions compete with heavy metal ions for active sites in fish tissues, so that this may be a mechanism affecting the lethality of heavy metals by water hardness.

Skidmore (1964) indicated that according to the "permeability" theory, one compound antagonises another by reducing the permeability of cell membranes, and thereby reducing the speed of penetration of the second compound into the tissues. He suggested that this is how alkaline earths exercise their antagonism to the toxicity of zinc solutions. An earlier hypothesis called the "theory of contrary action" (Heilbrunn, 1937) is concerned with the antagonism of calcium to zinc. According to this view, the toxicity of metal ions is due to their tendency to coagulate protoplasm after penetrating cells. Calcium is supposed to inhibit such coagulation.

2.5.2 pH

Exposure of fish to metals and increased H^+ concentrations are associated with excess mucous secretion (Daye and Garside, 1976; Robert *et al.*, 1986). The increased effect of low pH on metal toxicity may be due to stimulated mucous secretions due to

H^+, with the resulting free mucous able to chelate the metals; changes in toxic species of metals with the test pH; and competition inhibition of metal ions by H^+ at gill binding sites (Robert *et al.*, 1986).

2.5.3 TEMPERATURE

It is well established that survival times of fish tend to be shorter at higher temperatures (Lloyd, 1960; Lloyd and Herbert, 1962). Variations in metal toxicity at different temperatures may be an outcome of changes in fish metabolic rate. When metabolism is slowed by cold, survival time in a toxic solution of metal may be increased because of lowered ventilation rate. Acceleration of metabolism by heat may shorten survival time by increasing the accumulation rate of metal in the gills. Other species such as blue gills (*Lepomis macrochirus*) have been shown to have similar reactions to heavy metal and other pollutants (Burton *et al.*, 1972; Iyaniwura, 1992).

2.5.4 OXYGEN

Lloyd (1961) and Lloyd and Herbert, (1962) determined that a decrease in ambient oxygen increased the apparent toxicity of zinc as well as lead, copper and phenols. The effect is however, apparently an artifact of increased water flow rate through the gills in reduced oxygen conditions. This simply enhances the rate of passage of damaging pollutants over the sensitive gill surface in unit time.

2.5.5 SYNERGISM WITH OTHER METALS

Zinc rarely occurs alone as a pollutant but it is usually associated with other metals in ore bodies such as Cu, Pb, Ag and Cd. This can cause synergism. A metabolic synergism between cadmium and zinc may be expected from the chemical similarities of the two metals (Underwood, 1979). The toxicity of cadmium can be increased by appropriate dietary intakes of zinc and the toxicity of zinc can be inhibited by appropriate dietary intake of cadmium (Underwood up cit). Medici and Taylor (1967) demonstrated the mutual antagonism that exists between cadmium and zinc and interactions between cadmium, iron and copper in their studies with flour beetle, *Tribolium confusum*. High levels of zinc in that species were shown to reduce the toxicity of cadmium and additional copper slightly reduced the toxic effects of both cadmium and zinc. The interaction between cadmium and zinc can be explained by competition for protein-binding sites both at the mucosal cell level and in the tissues. This competition for binding sites includes the zinc metalloenzymes.

2.6 METAL INTERACTIONS

The interaction of metals is a process by which metals in their various forms alter either the critical concentration or the toxic effect of a metal under consideration. The term "interaction" is used here to describe the influence of metals on their metabolism and toxicity. Interaction of metals would be classified as follows: Synergism occurs when the effect or response of the combined exposure is greater than additive. This means that one toxicant potentiates the effect of the other.

Antagonism occurs when one toxicant reduces the effect of the other. In the third category, the interaction is said to be additive or substitutive, when the combined effects of two or more toxicants equal that expected when considering the toxicants individually. Possible mechanism of metal-metal interaction include interchange of metal-bound proteins; induction of metal-binding protein; and formation of compound or complexes among metals and metalloids. The various mechanisms of interaction between environmental chemicals and biological systems have been reviewed (Iyaniwura, 1989, 1990).

In interchange of metal-bound proteins, competition for carrier protein may affect the transport of metal ions (Chmielnicka, 1983). The interaction between copper and zinc could be partly explained by competition for binding sites on protein carrier such as albumin in blood plasma (Evans and Hahn, 1974). Copper and Zinc in blood are mainly associated with proteins often as co-factors of enzymes. Copper in blood is mainly bound to the protein ceruloplasma in serum (Chmielnicka, 1983). Zinc in the blood occurs mainly as a factor for enzymes in erythrocytes and the relatively smaller fraction found in serum is mainly bound to albumin. The antagonistic action of zinc on intestinal absorption of copper might be due to the interchange of zinc and copper ions on ligands that mediate the absorption of copper (Cousins and Failla, 1980). The low molecular weight fraction holds the copper and zinc thioneins which may play a major role in the metal interaction (Nordberg, 1979).

In metalloenzymes, the metal ion is an integral part of the

protein structure. ALAD, or 5-aminolevulinate dehydrates (EC 4.2.1.24), is a metalloenzyme - containing zinc. A fall in the activity of ALAD in blood is the most sensitive index of lead exposure (Poitrowski and O'Brien, 1980). Dietary zinc provides protection against the toxicity of lead (Abdulla, 1980). Zinc is strongly antagonistic to inhibition of ALAD by lead as has been shown *invivo* and *invitro* (Abdulla and Haeger-Aronsen, 1979).

In the induction of metal-binding protein, metallothionein (MT) is another possible site of metal-metal interaction. The most potent inducers of thionein synthesis are Zn^{2+}, Cd^{2+} and Hg^{2+} (Kagi and Nordberg, 1979). Although these cations do not form complexes with one another, the retention of any of them is increased by the presence of either of the other two (Chmielnicka, 1983). A likely explanation according to Magos and Webb (1976, 1978) is the depletion of carriers. Changes in the absorption can affect organ distribution of metals.

Besides competition for binding sites, metal-metal interactions may involve induction of metal binding protein synthesis. Metal binding protein modifies the toxicity of many metals. Cd^{2+} and Hg^{2+} will replace Cd^{2+} in the corresponding metallothionein in the liver (Webb, 1972), and kidneys *invivo* (Magos and Webb, 1976). The protection given by Cd^{2+} against a renotoxic dose of Hg^{2+} increases the thionein-bound Hg^{2+} in the kidneys, but the increase in the total uptake is higher, partly because large molecular proteins bind more Hg^{2+} (Magos and Webb, 1976). Zinc can also interchange with cadmium in the thionein in the liver, and cadmium with mercury in the kidneys (Kagi and Nordberg, 1979).

The simplest conceivable interaction mechanism would be a direct chemical reaction leading to the formation of a complex or compound which reduced the biological availability of the elements involved. The formation of such compounds could partly explain the protective effect of selenium against mercury and cadmium. According to Parizek (1978), two types of mechanisms are considered in the interaction between compounds of selenium and those of cadmium and mercury. One is based on a direct chemical reaction between compounds of these elements; the other is based on metabolic changes induced by selenium administration and modifying the dose-effect relationship indirectly without a reaction between selenium and the metallic compound. Selenium alters the binding of cadmium to proteins (Gasiewicz and Smith, 1976, 1978). Competition for extracellular carrier proteins can contribute to the interaction involving transport and this will depend upon dose and the route of administration. The interaction between cadmium and zinc will be examined in the current work to determine the effect of biological interaction on the relative toxicities of the two metals.

2.7 BIOACCUMULATION OF TRACE METALS IN FISH

Heavy metals in trace amounts are normal constituents of aquatic organisms. At high concentrations however, heavy metals are toxic to living organisms so it is important to know by how much their concentration may be increased before effects on aquatic populations can be detected or commercial species become unsuitable as food. The bioaccumulation of trace metals by fish has been extensively studied in the laboratory (Brungs *et al.*,

1973; Ceareley and Coleman, 1974; Eaton, 1974; Middaugh and Rose, 1974; Benoit *et al.*, 1976; Holcombe *et al.*, 1976).

Lande (1977) reported only 0.3 μgg^{-1} Cd and 80 μgg^{-1} Zn (dry weight) in whole fish from a contaminated fjord in Norway. Mathis and Cummings (1973) recorded a maximum of 0.14 μgg^{-1} Cd and 40 μgg^{-1} Zn in muscle tissue of fish from a contaminated river system. Concentrations of cadmium in fish tissues are very variable and do not seem to be under the strict physiological control evident with zinc. Cadmium concentrations do not appear to be correlated to length, weight condition, or age of fish (Murphy *et al.*, 1978). However, Jaahkola *et al.*, (1971) reported concentrations of cadmium to increase in fish muscle and liver tissue as age increased while Anderson *et al.*, (1973) found cadmium content to be negatively correlated to age in fish.

Trace metals can be incorporated into fish along two pathways: absorption across gill surfaces or through the gut tract wall. A relationship between trace metal concentrations in fish and the trophic level of the species has been suggested indicating the importance of the food pathway (Mathis and Cummings, 1973). They also found higher concentrations of zinc in omnivorous fishes than carnivorous ones. Windom *et al.*, (1973) noted that planktivorous marine fishes contained higher concentrations of zinc and cadmium than carnivorous species obtained from the same location.

Available evidence supports the concept of trace metal equilibrium being sought by freshwater fish. Abdullah *et al.*, (1976) found that a gradient of zinc and manganese concentrations in freshwater determined the zinc and manganese concentration in

scales of native salmonid fishes sampled along that concentration gradient. Also, Vancollie and Rousseau (1974) found higher concentrations of trace metals in fish from polluted sites. Lovett *et al.*, (1972) related cadmium concentrations in freshwater fish to background levels of cadmium. Nevertheless, the concentration of trace metals in food has also been shown to play an important role in determining the concentration of trace metals in fish tissue (Hoss, 1964; Pentreath, 1976; Singh and Ferns, 1978).

Essential and metabolically useful trace metals such as zinc (Love, 1970; Bowen, 1972) are concentrated in tissue in excess requirements. Some of this excess perhaps represents a store, particularly in liver and skeletal material. Such stores can be drawn upon in times of shortage or low input of trace metals. Fletcher *et al.*, (1975) measured copper and zinc in the plasma of *Oncorhynchus nerka* during migration to its spawning beds. They observed a 20-50% reduction in these elements in the spawning fish compared with the original pre-spawning condition in the fish. On migration, body stores of trace metals were presumed to be used for gonad ripening.

Beveridge *et al.*, (1985) reported that most metals occurred in highest concentrations in the bone and skin tissues although iron levels were highest in the haemoglobin rich livers. They found muscle tissues to have lowest concentrations of all metals as also reported by Giesy and Wiener (1977). Cadmium has been shown to accumulate in kidney, gills and gut of bluefin tuna, *Thunnus thynnus* (Friedman and Shibko, 1972). Zinc accumulates in the skeletal material rather than in the liver (McDonald *et*

al., 1976).

Nemcsok *et al.*, (1987) found the order of the concentrations of copper taken up by various organs of carp (*Cyprinus carpio*) as skeletal muscle > liver > gills > intestine > kidney > heart > brain. The low CuSo4 accumulation detected in the brain was attributed to correlation with the presence of the blood-brain barrier. The high amount of copper in the gills and liver is related to the fact that the gills play an important role in copper uptake, and the liver in its accumulation and detoxication (Reichenback - Klinke, 1972). In fish, zinc is generally low in muscles and high in gonads (Brooks and Rumsey, 1974; Badsha and Goldspink, 1982). As zinc burdens increase, the levels found in the internal organs also increase although the levels present in the muscles do not increase markedly because of the diluting effect of the muscles (Badsha and Goldspink, 1982).

Lead is known for its specific accumulation in bones, gills and fins of fish (Brooks and Rumsey, 1974). It has low levels of accumulation in the muscles. Cadmium accumulates in the blood system and can not be easily excreted (Hughes *et al*, 1980). It therefore becomes concentrated not only in the kidneys and liver but also in the bones (Badsha and Goldspink, 1982). The present study will consider accumulation of zinc and cadmium in various body tissues as well as the whole fish.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The methods used in the current work were based on recommended methods for tests of acute toxicity of pollutants to fish described by Sprague (1973), American Society of Testing and Materials (ASTM, 1977) and American Public Health Association (1985).

3.1 COLLECTION AND MAINTENANCE OF THE FISHES

Fingerlings of *Oreochromis niloticus* and *Clarias gariepinus* were obtained from Maska Fish Farm on kilometre 45 along Zaria - Funtua road. The fish farm is jointly owned by the Federal Government of Nigeria and the Katsina State Government. An attempt was made to collect fishes of fairly uniform size and of approximately the same age of about five weeks. Due to limited laboratory facilities, fishes were collected at different times for various experiments.

The fishes were conveyed to the laboratory in cool boxes. The fingerlings of *C. gariepinus* averaging 14.75 $\pm$ 0.45cm total length and 18.28 $\pm$ 1.10 gm body weight and *O. niloticus* of total length 10.24 $\pm$ 0.38 cm and body weight 17.83 $\pm$ 1.63 gm were used for this study. The fishes were held in the laboratory in large water bath of 160L capacity at 24.5 - 26.5^{0}C and acclimated for at least two weeks prior to each experiment. The fishes were fed pelleted diet of local food material (See below) throughout the period of the study. A daily photo period of 16:8 h light: dark was maintained during acclimation and tests.

3.2 PREPARATION OF EXPERIMENTAL DIET

The feed was formulated of local food stuffs such as groundnut cake, cassava flour, maize flour, fishmeal and vitamin premix. All these ingredients were grounded into powder by use of a blender and sieved by use of a fine wire mesh sieving apparatus before they were mixed at various percentages by weight (Table 1). The cassava flour served as a major source of carbohydrate as well as a binder (Lovell, 1971). The vitamin premix was used to balance the diet and eliminate problems associated with vitamin deficiencies.

The powdered mixture of the ingredients was placed in a bowl and thoroughly mixed. Boiled water was added to the mixture after which it was turned into a semi-moist paste. This was then put into a pelleting apparatus (icing set) and the pellets were extruded through the nozzle. The pellets were oven-dried at 85^{0}C to prevent moulding and to ensure dense sinking. Dupree and Huner (1984) recommended 85^{0}C to 90^{0}C as the ideal temperatures

Table 1: Percentage Composition by Weight of Local Food Stuffs Used for Experimental Diet

Constituent	Percentage Composition
Groundnut Cake	45
Cassava Flour	20
Maize Flour	24
Fishmeal	10
Vitamin Premix	1
Total	100

for drying dense sinking pellets to ensure starch gelatinization. The prepared diet was stored in sealed polythene bags protected from moisture and extreme heat. Heat and moisture accelerate the destruction of vitamins and oxidation of fats and encourage growth of mould which produces toxic products (National Research Council, 1977; Robinette, 1984). Fish were fed to satiation twice a day during acclimation.

3.3 PHYSICO - CHEMICAL PARAMETERS OF DILUTION WATER

Dechlorinated tap water from Zaria Municipal Water Supply was used. The Physico-chemical parameters of the dilution water were monitored daily. These parameters included temperature, dissolved oxygen, water hardness, total alkalinity and pH. Beside these, the level of zinc, iron, copper and cadmium in the dilution water was determined by use of Atomic Absorption Spectrophometer (AAS, Pye-Unicam SP 192). A Pye-Unicam model 292 pH meter was used for pH determinations.

3.3.1 DETERMINATION OF DISSOLVED OXYGEN

Dissolved oxygen was determined after Standard Method Modification of Winkler's Method (American Public Health Association, 1985). 250 ml of water samples was taken in a B.O.D. bottle and 2ml of manganese sulphate solution added to it. Then 2ml of alkali-azide reagent was added below the surface of the sample. The bottle was cocked with care to expel air-bubble and to discard excess liquid. The bottle was then inverted to mix the content. As the resulting precipitate settled, the bottle was inverted again and then left to settle until about 100ml of clear supernatant was obtained. Then 2ml of

concentrated sulphuric acid was added and the bottle cocked to discard excess liquid. This was then mixed gently by inverting the bottle. 203 ml was taken out from the bottle and put into a conical flask and titrated with 0.025 N of sodium thiosulphate to a pale straw colour. Then 1 ml of starch solution was added and titration continued until the blue colour disappeared to colourless. One ml of 0.025 N sodium thiosulphate is equivalent to one mgl^{-1} of oxygen.

3.3.2 DETERMINATION OF WATER HARDNESS

For the determination of water hardness, E.D.T.A. titration method (American Public Health Association, 1985) was used. Water sample was mixed thoroughly and 25ml taken and diluted to 50ml with distilled water. Then 2ml of buffer solution (0.05N borax, 0.1N sodium hydroxide and 0.025N sodium sulphide) was added and followed by 2 drops of Eriochrome Black indicator. The sample was then titrated with 0.02N sodium ethylenediamine tetra acetate (E.D.T.A.) solution within 5 minutes interval. In the presence of calcium and magnesium, the colour changed from red to blue.

Calculation: The following formula was used for calculating the total hardness in mgl^{-1} $CaCo_3$.

$$\frac{A \times B \times 1000}{\text{ml of sample}} = mgl^{-1}\ CaCo_3$$

where A = ml of titrant

B = mg $CaCo_3$ = 1ml E.D.T.A. titrant i.e. 5ml of standard $CaCo_3$ = 25 mg of $CaCo_3$. 14 mls of titrants were got hence

$$\text{mg } CaCo_3 = \frac{25}{14} = 1.786$$

Therefore the value of B = 1.786.

3.3.3 DETERMINATION OF TOTAL ALKALINITY

For the determination of total alkalinity, standard method (American Public Health Association, 1985) was used. 100 ml of sample was taken into a conical flask and 3 drops of phenolphthalein indicator added to it. As the sample did not turn pink, 3 drops of methyl orange indicator were added. As the sample turned yellow, 0.02N sulphuric acid was added from a burette until the colour changed to orange. The ml of the acid used was then recorded.

Calculation: The following formula was used.

$$\text{Total alkalinity } (\text{mgl}^{-1}\ CaCo_3) = \frac{B \times N \times 50,000}{\text{ml of sample}}$$

where B = ml of titration for sample to reach methyl orange end point. N = normality of acid (0.02N).

3.4 PREPARATION OF ZINC CONCENTRATIONS

Zinc sulphate (analytical grade $ZnSo_4.7H_2O$) was used for preparation of zinc concentrations. Molecular weight of $ZnSo_4.7H_2O$ = 287.5

Atomic weight of Zn^{2+} = 65.4

Therefore 1g of $Zn^{2+} = \frac{287.5}{65.4} \times 1$

$= 4.396g\ ZnSo_4.7H_2O.$

Thus by this proportion, zinc salt was weighed according to the desired nominal concentrations and dissolved in distilled water before being added to the dilution water for various experiments.

3.5 PREPARATION OF CADMIUM CONCENTRATIONS

Cadmium chloride (analytical grade $CdCl_2.21/2H_2O$) was used for preparation of cadmium concentrations.

Molecular weight of $CdCl_2.2½\ H_2O$ = 228.364

Atomic weight of Cd^{2+} = 112.41

Therefore, 1g of $Cd^{2+} = \frac{228.364}{112.41} \times 1$

$= 2.0315g\ CdCl_2.2½\ H_2O.$

Similarly, this proportion was used to weigh out cadmium salt according to the desired nominal concentrations for different experiments. The cadmium salt was dissolved in distilled water before it was added to the dilution water for the experiments.

3.6 PILOTS EXPERIMENTS TO DETERMINE LETHAL CONCENTRATIONS OF ZINC AND CADMIUM

Since literature on both acute and sublethal toxicity of zinc and cadmium to tropical fish species are very limited, pilot studies were carried out to determine the maximum concentration of each of the metals that did not produce death and the minimum concentration that produced 100% death. In between this concentration range, five concentrations of each metal were selected for acute toxicity studies. 30 mgl^{-1} zinc was found to produce 100% mortality in *Oreochromis niloticus* while 16 mgl^{-1} zinc did not cause any death. 20 mgl^{-1} zinc was found to produce 100% mortality among *Clarias gariepinus* while 6 mgl^{-1} zinc did not produce any mortality. Similarly, 24 mgl^{-1} cadmium produced 100% mortality in *O. niloticus* with no mortality observed at 12 mgl^{-1} cadmium. For the *C. gariepinus*, 18 mgl^{-1} cadmium produced

100% mortality with no death recorded at 6 mgl^{-1} cadmium. The varied concentrations chosen for the exposure of *O. niloticus* and *C. gariepinus* for toxicity studies were in accordance with the criteria set by American Society for Testing and Materials (1977). According to these criteria except for control, the concentration of toxicant in each treatment should be at least 60% of that in the next higher one; also, one concentration other than the control which will affect less than 37% of the fish and that which would kill more than 63% should be included in the range of concentrations for the definitive tests.

3.7 EXPERIMENTAL DESIGN

3.7.1 ACUTE TOXICITY ASSAY

Acute 96 - h static bioassays were conducted in the laboratory following the methods by Sprague (1973) and the American Public Health Association (1985) to determine the toxicity of zinc and cadmium in single and mixed solutions to *Oreochromis niloticus* and *Clarias gariepinus*. In each of the bioassay test, 11 glass tanks of size 30.5 x 30.5 x 92.5 cm each containing different concentrations of the toxicant and a control were used. Each concentration had a duplicate. The desired salt concentrations were weighed, dissolved in distilled water and introduced into 50l of dechlorinated and aerated tap water in the glass tanks. The nominal concentrations of zinc used for *O. niloticus* were 0, 20, 22, 24, 26 and 28 mgl^{-1} while 0, 10, 12, 14, 16 and 18 mgl^{-1} were the nominal concentrations of zinc for *C. gariepinus*. The nominal concentrations of cadmium for *O. niloticus* were 0, 16, 18, 20, 22 and 24 mgl^{-1} while for *C.*

gariepinus, 0, 8, 10, 12, 14 and 16 mgl^{-1} were used. The metal concentrations used in the mixtures were simply the concentrations of corresponding salts combined. The mixture of the toxicants and dechlorinated water was allowed to stand for 30 minutes before the introduction of the test fishes.

The fishes were randomly distributed to each glass tank as suggested by Sprague (1969). Each tank contained 10 fish. Each assay was replicated simultaneously and repeated once to determine reproducibility. Aeration of the test water was suspended a few minutes prior to addition of the toxicant and during the tests. The toxicant and test water were renewed after 48 hours during each series of test. The fish were starved 24 hours prior to the start and during each assay test. A daily photoperiod of 16:8 hr light: dark as was used during acclimation was maintained during the test.

All the physico-chemical parameters were determined daily following American Public Health Association (1985). Water samples (25 ml per day) were taken, filtered by use of 0.45 μm filter paper, acidified (0.2% HNO_3) and pooled for four days from each tank for metal analysis following standard atomic absorption spectrophotometric methods.

3.7.2 OBSERVATIONS AND TREATMENT OF DATA

The behaviour and general conditions of the fishes were observed prior to, during and after each bioassay. Symptoms of toxicosis in the fishes were observed, scored in ascending order of severity and recorded (Table II). Time of onset of symptoms and their duration was recorded. Observations of mortality were carried out at intervals of 15,30 minutes; 1, 2, 4, 6, 8, and 12

hours, then two times daily until the end of the 96 - h exposure period. Fish were considered dead when the gill movement stopped and there was no response to gentle prodding. The dead fish were removed and the number recorded. The gills and the liver of the dead fish were removed and put in formo-saline (100 ml of 10% formalin + 0.9g NaCl) for histopathological examination.

For each of the fish species, pooled data from the percentage mortality in each concentration was determined. The 96 - h median lethal concentration (LC_{50}),

Table II Scores of Symptoms of Toxicosis Observed in Poisoned Fishes

Symptoms	Scores
Agitated Swimming	1
Loss of Equilibrium	2
Air gulping	3
Periods of quiescence	4
Death	5

(that is the concentration which is lethal to the average fish and kills 50% of the population), 95% confidence limits and slope functions were calculated using probit analysis (Litchfield and Wilcoxon. 1949; Tallarida and Murray, 1981; Steele, 1983; Dick and Dixon, 1985). In the determination, the percentage mortality was converted to probit scale using the probit table (Finney, 1964) and the concentration was transformed into logarithm (Log).

The transformed values of mortality (probit) were then plotted against the log of concentrations. A straight line through the points was fixed by regression analysis as indicated in the appendix. From the straight line equation ($Y = a + bx$) the value of x when $Y = 5$ was calculated. This gives the concentration that is lethal to 50% of the fish i.e. the LC_{50} for that exposure period.

The combined toxicity of the metal mixtures was estimated by expressing the actual concentration of each toxicant as a proportion of its 96 - h LC_{50} concentration and adding the resultant numbers (Herbert and Vandyke, 1964). If the sum (S) is less than 1, toxicity is more than additive (Synergistic) while values greater than 1 indicate less than additive toxicity (antagonistic). Values of 1 signify additive toxicity.

3.7.3 HISTOPATHOLOGICAL STUDIES

Gills and liver obtained from dead fishes which were exposed to lethal concentrations of the metals were removed and fixed in formo-saline (100 ml of 10% formalin + 0.9g NaCl), (Thompson and Hunt 1966; Alan *et al.*, 1983). The tissues were washed in running tap water to remove traces of formalin. This was followed by dehydration using successive percentages of alcohol (30, 50, 70, 90 and 100%). They were then infiltrated in chloro-alcohol and blocked in paraffin wax of 58°C - 60°C melting point. Samples were embedded in fresh molten wax using embedding moulds. Sections of 8 μm thickness were cut and stained with Haematoxylin and Eosin (H&E). Permanent slides were prepared with these sections and photomicrographs were taken. These were examined and compared with those for the control.

3.7.4 HAEMATOLOGICAL STUDIES

For these studies, fingerlings of *Oreochromis niloticus* and *Clarias gariepinus* were exposed to sublethal concentrations of zinc and cadmium. *Oreochromis niloticus* was exposed to 4.0 mgl^{-1} zinc while *C. gariepinus* was exposed to 2.5 mgl^{-1} zinc. For cadmium treatment, *O. niloticus* was exposed to 4.5 mgl^{-1} cadmium while *C. gariepinus* was exposed to 3.0 mgl^{-1} cadmium. 24 fish of each species were exposed to each of the sublethal concentrations in large water bath of 160l capacity. There was a control for each treatment. The toxicant and the dilution water were renewed at three days interval to maintain the concentration of the metal and the level of dissolved oxygen as well as minimising the level of ammonia concentration of the test water during the experiment. The test water was aerated during the experiment also to maintain the level of dissolved oxygen. Physico-chemical parameters were monitored two times a week. Each of these experiment lasted for six weeks.

3.7.4.1 COLLECTION OF BLOOD SAMPLES

Four fish were randomly sampled from each treatment and control on weekly bases. The caudal artery at the caudal peduncle was punctured. Blood samples were collected by use of microcapillary and sampling tubes treated with an anticoagulant. Blood sample was taken at least 40 seconds after collecting the fish from the water.

3.7.4.2 ANALYSIS OF BLOOD SAMPLES

Blood samples were analysed at least 15 minutes after collection. The parameters analysed for were haematocrit (Ht) or Packed Cell Volume (PCV), haemoglobin (Hb), Red Blood Cell Count (RBCC) and White Blood Cell Count (WBCC). The values for mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the formulae given below:

$$\text{MCV } (\mu m^3) = \frac{\text{Ht (\%)}}{\text{RBCC (cells mm}^{-3})} \times 10$$

$$\text{MCH (pg cell}^{-1}) = \frac{\text{Hb (g 100 ml)}^{-1}}{\text{RBCC (cells mm}^{-3})} \times 10$$

$$\text{MCHC (g 100 ml}^{-1}) = \frac{\text{Hb (g 100 ml)}^{-1}}{\text{Ht (\%)}} \times 10$$

3.7.4.3 HAEMATOCRIT

To measure haematocrit, blood samples were filled in microcapillary tubes and the head sealed with wax and centrifuged by use of microcentrifuge at 1100 r/min for 6 minutes. Haematocrit was measured with a microcapillary reader and the mean haematocrit for each groups of fish was recorded.

3.7.4.4 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. Blood smears from each fish were used and values were read on the haemoglobinometer model HGBR 6279 and recorded.

3.7.4.5 RED BLOOD CELL COUNT

For red blood cell count, the diluting pipette and Toisson's solution (160 ml distilled water + 1g of NaCl + 8g of $NaSo_4$ + 30 ml of glycerol) were used. Blood was taken into the diluting pipette up to 0.5 mark and Toisson;s solution added to make up 10 mark. This was mixed properly by inverting the pipette. The mixture was then dispensed into the already prepared haemacytometer grid and the Red Blood Cells were counted using a light microscope.

3.7.4.6 WHITE BLOOD CELL COUNT

The white blood cell diluting pipette and Türks solution (Trace of gential violet + 100 ml of 1% acetic acid) were used for leucocyte count. Blood was taken up to 0.5 mark in the pipette and Türks solution added to make up to 11 marks. This was mixed by inversion of the pipette and dispensed into the already prepared haemacytometer grid and allowed to wait for 5 minutes. The white blood cells were then counted in the large squares and at the edges under the microscope, and recorded. Statistical analysis was by one-way analysis of variance. In all cases the confidence level was 95%.

3.7.5 ZINC AND CADMIUM RESIDUE IN TISSUES

In the study of accumulation of zinc and cadmium in fish tissues, 1/10 of the 96 - h LC_{50} was used to determine sublethal concentrations as recommended by Amminikuthy and Rege (1977) and used by Oladimeji and Ologunmeta (1987). For zinc treatment, only *Clarias gariepinus* was exposed and 1/10 of the 96 - h $LC_{..}$

(13.25 mgl^{-1} zinc) gave a nominal sublethal concentration as 1.33 mgl^{-1} zinc. With cadmium treatment, 1/10 of the 96 - h LC_{50} (10.85 mgl^{-1} cadmium) gave a nominal concentration of 1.09 mgl^{-1} cadmium for *C. gariepinus*. In the test with *O. niloticus* 1/10 of the 96 - h LC_{50} (19.32 mgl^{-1} cadmium) gave a nominal sublethal value of 1.93 mgl^{-1} cadmium.

Large water baths of 160L capacity each were used for each of the assay. The metal salts were each weighed according to the nominal concentration required and dissolved in distilled water and then introduced in dechlorinated tap water in the water bath. 24 fish of each species were distributed to each water bath. There was a control for each treatment. The fish were fed to satiation once a day on pelleted experimental diet. A daily photo period of 16: 8 h light: dark was maintained during these series of tests. The fish were exposed to various concentrations of zinc and cadmium for eight weeks. Four fish were removed from each treatment at an interval of two weeks. These were blotted dry and weighed before they were dissected to remove various organs (gills, liver, skin, muscles, gut and whole fish).

3.7.5.1 DETERMINATION OF ZINC AND CADMIUM RESIDUE IN FISH

Fish residual zinc and cadmium concentrations were determined according to Hodson *et al.*, (1978). Each dissected fish organ was placed in an acid-washed, pre-weighed dry crucible and the wet weight of the sample recorded. The samples were oven-dried at 60°C for 48 hours. They were then grounded with an acid-washed glass pestle and a mortar until homogenous. The grounded sample was weighed and dry weight recorded. The sample

was then transferred to the murfle furnance and ashed at 450^{0}C for 24 hours until white ash was obtained. The ash was cooled in desicator and put in beaker for digestion.

The ash was dissolved in 70 ml of distilled water according to American Public Health Association (1985) and 5 ml of concentrated nitric acid added to the solution and heated using a hot plate. Heating was maintained to evaporate the solution until the level reduced to 20 ml then 5 ml of concentrated nitric acid was again added and boiled to a clear solution. The solution was then filtered into a 50 ml volumetric flask and the mark was made up with distilled water. The resulting solutions were then analysed for the respective metals by use of Atomic Absorption Spectrophotometer (AAS, Pye-Unicam SP 192). There were three replicates in each treatment. The μgg^{-1} zinc and cadmium residues in the whole fish and the fish tissues were calculated in each replicate. The bioconcentration factors of zinc and cadmium residues in whole fish and various tissues were calculated using the formula $Cf = \frac{C_0}{C_{Wa}}$ (VanLeeuwem *et al.*, 1986; Wicklund *et al.*, 1988).

C_0 = Concentration of metal in tissues

C_{Wa} = Concentration of metal in test water.

Differences in metal residue in various tissues of the fishes were tested with the student's t-test.

CHAPTER FOUR

4.0 RESULTS

4.1 ACUTE TOXICITY TEST

The physico-chemical parameters of the test water fluctuated only slightly (Table III) during the bioassays, but was not thought to have affected fish mortality. The analysed concentrations of zinc for each bioassay were 8.77, 10.36, 12.30, 13.75 and 16.69 ppm compared to nominal values of 10, 12, 14, 16, and 18 mgl^{-1} for *Clarias gariepinus* and 18.08, 20.14, 23.2, 25.68 and 27.52 ppm compared to nominal values of 20, 22, 24, 26, and 28 mgl^{-1} for *Oreochromis niloticus*. The analysed toxicant strength was found to be lower than the toxicant nominal values. Similarly, the analysed concentrations of cadmium for each bioassay were 9.21, 10.87, 13.45, 15.53 and 16.46 ppm compared to the nominal values of 8, 10, 14, and 16 mgl^{-1} for *C. gariepinus* and 16.39, 19.67, 21.67, 22.89 and 24.25 ppm compared to nominal values of 16, 18 20, 22, and 24, mgl^{-1} for *O. niloticus*.

Symptoms of toxicosis indicate that zinc, cadmium and the zinc-cadmium mixture cause subacute effects that alter fish behaviours. Behavioural reactions in order of their appearance were: agitated swimming, loss of equilibrium, air gulping, periods of quiescence and death. These reactions to the toxicants were more pronounced in tanks containing the higher levels of zinc, cadmium and zinc-cadmium mixture. The means score of symptoms in the fish species exposed to various concentrations of the metals are presented in Tables IV a, b, c, d, e, f. The results showed that the means score of the symptoms

Table III: Physico-chemical Parameters of Dilution Water

Parameters	Range	Mean ± Standard Deviation
Temperature (^{0}C)	24.5-26.5	25.5 ± 0.72
Dissolved oxygen (mgl^{-1})	6.15-9.88	7.45 ± 1.185
pH	8.10-9.30	8.65 ± 0.389
Total Hardness (mgl^{-1} CaC_{03})	150.024-157.168	153.396 ± 3.557
Total Alkalinity (mgl^{-1} CaC_{03})	33 - 36	34.5 ± 1.118
Zn (ppm)		0.024
Fe (ppm)		0.01
Cu (ppm)		0.035
Cd (ppm)		0

Table IV a: Means Score of Symptoms in *Oreochromis niloticus* Exposed to Various Concentrations of Zinc n = 8

Conc. (mgl^{-1})	Mean ± Standard Deviation
28	15
26	13.75 ± 2.17
24	12.63 ± 3.28
22	10.88 ± 3.55
20	6 ± 3.67

Table IV b: Means Score of Symptoms in *Clarias gariepinus* Exposed to Various Concentrations of Zinc n = 8

Conc. (mgl^{-1})	Mean ± Standard Deviation
18	15
16	15
14	12.63 ± 3.28
12	11.38 ± 3.08
10	8.38 ± 4.21

Table IV c: Means Score of Symptoms in *Oreochromis niloticus* Exposed to Various Concentrations of Cadmium n = 8

Conc. (mgl^{-1})	Mean ± Standard Deviation
24	15
22	14.38 ± 1.65
20	13.75 ± 2.17
18	10.88 ± 3.55
16	9.75 ± 3.49

Table IV d: Means Score of Symptoms in *Clarias gariepinus* Exposed to Various Concentrations of Cadmium n = 8

Conc. (mgl^{-1})	Mean ± Standard Deviation
16	15
14	15
12	14.38 ± 1.65
10	10.88 ± 3.55
8	8.75 ± 3.42

Table IV e: Means Score of Symptoms in *Oreochromis niloticus* Exposed to Mixed Solutions of Zinc and Cadmium in Various Concentrations Zn^{2+}/Cd^{2+} n = 8

Conc. (mgl^{-1})	Mean ± Standard Deviation
24/20	15
22/18	15
20/16	15
18/14	13.75 ± 2.17
16/12	12.63 ± 3.28

Table IV f: Means Score of Symptoms in *Clarias gariepinus* Exposed to Mixed Solutions of Zinc and Cadmium in Various Concentrations Zn^{2+}/Cd^{2+} n = 8

Conc. (mgl^{-1})	Mean ± Standard Deviation
18/16	15
16/14	15
14/12	15
12/10	13.13 ± 2.42
10/8	10.25 ± 3.19

were dose-dependent for each fish species treated with the heavy metals. For *O. niloticus* exposed to varied concentrations of zinc (Table IV a) the highest means score of symptoms was 15 at 28 mgznl^{-1} followed by 13.75 $\pm$ 2.17 at 26 mgZnl^{-1}. The lowest mean score (6 $\pm$ 3.67) was recorded at 20 mgZnl^{-1}. For *C. gariepinus* exposed to zinc concentrations (Table VI b) the highest means score (15) was recorded for the fish exposed to 18 and 16 (mgznl^{-1}) respectively. The lowest mean score (8.38 $\pm$ 4.21) was recorded for the fish exposed to 10 mgznl^{-1}. In *O. niloticus* exposed to varied concentrations of cadmium, (Table IV c) the highest means scores, 15 and 14.38 $\pm$ 1.65 were recorded at 24 and 22 (mgCdl^{-1}) respectively. The lowest mean score (9.75 $\pm$ 3.49) was recorded at 16 mgCdl^{-1}. In the *C. gariepinus* exposed to cadmium concentrations (Table IV d) the highest means score of symptoms (15; 14.38 $\pm$ 1.65) were recorded for the fish exposed to 16, 14 and 12 (mgCdl^{-1}) respectively. The lowest score (8.75 $\pm$ 3.42) was recorded at 8 mgCdl^{-1}. In Table IV e it is shown that the highest means score (15) of symptoms in *O. niloticus* exposed to mixed salt solutions of zinc/cadmium occurred at 24 mgznl^{-1}/20mgCdl^{-1}; 22 mgznl^{-1}/18 mgCdl^{-1} and 20 mgznl^{-1}/16 mgCdl^{-1} respectively. The lowest means score (12.63 $\pm$ 3.28) was recorded at 16 mgznl^{-1}/12 mgCdl^{-1}. In *C. gariepinus* exposed to mixed salt solutions of zinc/cadmium (Table IV f) the highest mean score (15) was recorded for the fish exposed to 18 mgznl^{-1}/16 mgCdl^{-1}; 16 mgznl^{-1}/14 mgCdl^{-1} and 14 mgznl^{-1}/12 mgCdl^{-1} respectively. The mixed salt solutions of zinc/cadmium appeared to be more toxic to these freshwater fishes (*O. niloticus* and *C. gariepinus*) than the individual metals as reflected in very high means score of

symptoms of fishes exposed to mixed salt solutions. The on-set and duration of these symptoms to each fish species were also dose-dependant. The *O. niloticus* exposed to zinc (28 $mgznl^{-1}$) showed the on-set of symptoms after 4 hrs of exposure whereas those exposed to 26$mgznl^{-1}$ started reacting after 5hrs. The duration of these symptoms in both cases was 5hrs. *Oreochromis niloticus* treated with cadmium (24 $mgCdl^{-1}$) showed the on-set of symptoms after 5hrs of exposure. With this metal, the duration of the symptoms was 6hrs. The *C. gariepinus* exposed to 18 $mgznl^{-1}$ showed the on-set of symptoms after 8 hrs of exposure. The duration for the symptoms was 17 hrs. Similarly, *C. gariepinus* exposed to 16 $mgCdl^{-1}$ showed symptoms of toxicosis after 4 hrs exposure. The duration for these symptoms was 5hrs. In mixed salt solutions of zinc-cadmium, both fish species reacted earlier than when the metals were used individually. At zinc-cadmium concentrations of 28 $mgznl^{-1}$ - 24 $mgCdl^{-1}$, *O. niloticus* showed symptoms of toxicosis after 3 hrs of exposure while *C. gariepinus* exposed to zinc-cadmium mixture of 18 $mgznl^{-1}$ - 16 $mgznl^{-1}$ showed symptoms of toxicosis after 2hrs of exposure. In both fish species treated with the mixed salts, the duration of symptoms was 5hrs.

The *Clarias* species exposed to cadmium also showed swollen abdomen even for the fish exposed to cadmium at 10 mgl^{-1}. Copious accumulation of mucus was observed in the gill filaments and body surfaces of both species exposed to these metals. However, the intensity of mucus production was more pronounced in *C. gariepinus* in which case the body became sloughed off. Traces of blood were observed around the gill coverings of the

dead fishes.

No fish mortality was observed in the controls. The results of acute toxicity tests showing mean percentage mortality of each fish at various concentrations of the respective metals are presented in Table V, VI, VII, and VIII. Those for mixed zinc-cadmium solutions are presented in Tables IX and X respectively. The mean percentage mortality of the fishes to each metal was dose -dependent. Thus for single metal treatments, the highest recorded mean mortality (83.33%) occurred at the highest concentration (28 $mgznl^{-1}$) for *O. niloticus*; 86.66, mean percentage mortality at highest (18. $mgznl^{-1}$) concentration for *C. gariepinus*. For cadmium treatments, the highest mean percentage mortality (80%) was recorded at the highest cadmium ($24\ mgl^{-1}$) concentration for *O. niloticus* (Table VII) while for *C. gariepinus*, the highest mean percentage mortality (96.66) occurred at the highest cadmium ($16\ mgl^{-1}$) concentration (Table VIII). For the mixed salts solutions the highest mean percentage mortality (100%) was recorded at highest ($28\ mgznl^{-1}$ - $24\ mgCdl^{-1}$) concentrations (Table IX) for *O. niloticus*. For the *C. gariepinus* the highest mean percentage mortality (100%) was recorded for both $16\ mgznl^{-1}$ - $14\ mgCdl^{-1}$ and $18\ mgznl^{-1}$ - $16mgCdl^{-1}$ concentrations. The highest mean percentage mortality (100%) for *C. gariepinus* was recorded within 24 hrs of exposure to the highest zinc-cadmium mixture ($18\ mgznl^{-1}$ - $16\ mgCdl^{-1}$) (Table X).

The 96 - h median lethal concentrations (LC_{50s}) of zinc, cadmium and zinc-cadmium mixture to fingerlings of *C. gariepinus* (Table XI) were 13.25, 10.85 and $14.15\ mgznl^{-1}$ - $9.35\ mgCdl^{-1}$ respectively. Cadmium was found to be more toxic to *C.*

Table V: Acute Toxicity of Zinc to Oreochromis niloticus

Zinc Conc. (mgl^{-1})	Mean % Mortality at Various Exposure period			
	24h	48h	72h	96h
0 (control)	0	0	0	0
20	0	0	0	3.33
22	0	6.66	10	16.66
24	13.33	40	50	53.33
26	26.66	46.66	53.33	66.66
28	23.3	56.66	70	83.33

Table VI: Acute Toxicity of Zinc to *Clarias gariepinus*

Zinc Conc. (mgl^{-1})	Mean % Mortality at Various Exposure period			
	24h	48h	72h	96h
0 (control)	0	0	0	0
10	0	0	3.33	20
12	0	3.33	16.66	33.33
14	0	16.66	30	46.66
16	3.33	26.66	63.33	80
18	0	23.33	60	86.66

Table VII: Acute Toxicity of Cadmium to *Oreochromis niloticus*

Cadmium Conc.(mgl^{-1})	Mean % Mortality at Various Exposure period			
	24h	48h	72h	96h
0 (control)	0	0	0	0
16	6.66	13.33	16.66	20
18	16.66	26.66	30	33.33
20	36.66	53.33	56.66	60
22	66.66	73.33	76.66	76.66
24	56.66	66.66	73.33	80

Table VIII: Acute Toxicity of Cadmium to *Clarias gariepinus*

Cadmium Conc.(mgl^{-1})	Mean % Mortality at Various Exposure period			
	24h	48h	72h	96h
0 (control)	0	0	0	0
8	0	0	0	6.66
10	6.66	20	26.66	30
12	3.66	36.66	56.66	75
14	3.33	36.66	80	90
16	30	73.33	83.33	96.66

Table IX: Acute Toxicity of Mixed Zinc and Cadmium Solutions to *Oreochromis niloticus*

Zinc/Cadmium Mean % Mortality at Various Exposure period				
Conc.(mgl^{-1})	24h	48h	72h	96h
0 (control)	0	0	0	0
20/16	3.33	13.33	43.33	53.33
22/18	13.33	30	53.33	63.33
24/20	26.66	70	80	86.66
26/22	33.33	70	86.66	93.33
28/24	46.66	93.33	100	100

Table X: Acute Toxicity of Mixed Zinc and Cadmium Solutions to *Clarias gariepinus*

Zinc/Cadmium Mean % Mortality at Various Exposure period				
Conc.(mgl^{-1})	24h	48h	72h	96h
0 (control)	0	0	0	0
10/8	13.33	20	26.66	26.66
12/10	23.33	36.66	53.33	53.33
14/12	46.66	60	76.66	80
16/14	93.33	96.66	96.66	100
18/16	100	100	100	100

Table XI: Median Lethal Concentrations of Heavy Metals (mgl^{-1}) to Fingerlings of *Clarias gariepinus* and Slope Functions of Response Curves to the Metals

Metal	No. of Fish exposed	96-h LC_{50} at 95% Confidence Limits, (Range)	Slope Function
Zinc	150	13.25 (12.33 - 14.24)	1.33
Cadmium	150	10.85 (10.10 - 11.66)	1.22
Zinc & Cadmium	150	14.15 (13.33 - 15.24) 9.35 (7.69 - 10.37)	1.47 1.73

Table XII: Median Lethal Concentrations of Heavy Metals (mgl^{-1}) to Fingerlings of *Oreochromis niloticus* and Slope Functions of Response Curves to the Metals

Metal	No. of Fish exposed	96-h LC_{50} at 95% Confidence Limits, (Range)	Slope Function
Zinc	150	24.60 (23.76 - 25.46)	1.13
Cadmium	150	19.32 (18.38 - 20.31)	1.25
Zinc & Cadmium	150	19.92 (18.26 - 22.99) 12.78 (11.46 - 13.61)	1.49 1.75

4.2 EFFECTS OF ZINC AND CADMIUM ON HISTOLOGY OF FISH GILLS AND LIVER

The gills of control fishes (Plates III a, b and IV a, b) have normal gill structure consisting of filaments attached to a cartilaginous gill bar with finger-like projections (secondary lamellae) from each side. Each secondary lamellae is embedded by a single layer of epithelial cells known as secondary epithelium. The primary lamella is covered by a mucoid epidermis (primary epithelium) which may contain lymphocytes and eosinophilic granule cells and phagocytic cells. The epithelial cells form a barrier between the fish blood and the surrounding water. The secondary lamellae are supported and separated by pillar cells which are arranged in rows.

There were marked differences between the gills of control fishes (Plates III a, b and IV a, b) and those exposed to varied lethal concentrations of zinc and cadmium. The gills of *Clarias gariepinus* exposed to 12 $mgZnl^{-1}$ (Plate V a); 16 $mgZnl^{-1}$ (Plate V b) and 14 $mgCdl^{-1}$ (Plate VI) all show discernible changes in structure. At these lethal concentrations of zinc and cadmium, proliferation of epithelial cells leading to complete blanketing of the secondary lamellae was observed (Plates V a, b and VII). Also, marked increase in the width of primary lamellae was observed in the gill filaments of *C. gariepinus* exposed to lethal concentrations of zinc (Plate V a, b) and cadmium (Plate VI) respectively. In the *Oreochromis niloticus* also, there was discernible change in the gills of fish exposed to lethal concentrations of zinc and cadmium. Lamellae disarray and epithelial detachment were observed in gill filaments of the fish

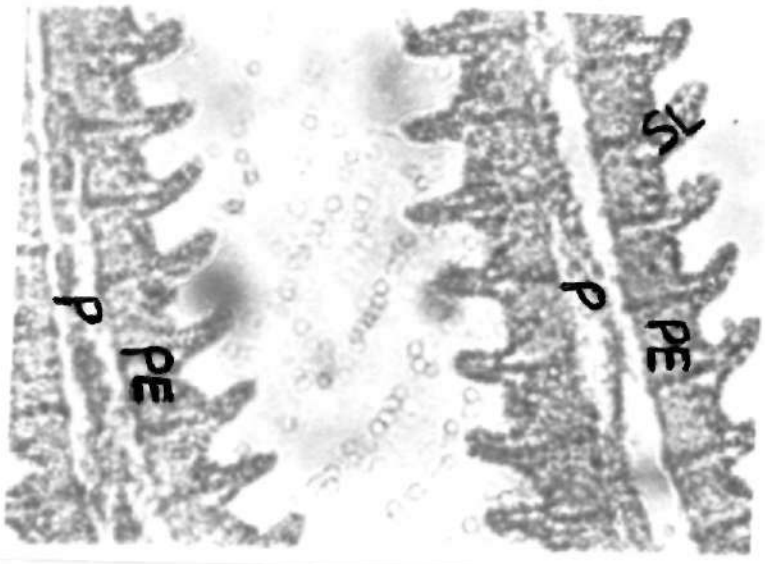


Plate IIIa: Gill filaments of *Clarias gariepinus*; untreated control. S.L, Secondary lamellae; PE, Primary epithelium; P, Primary lamella; note the normal width of the primary lamella. X100.

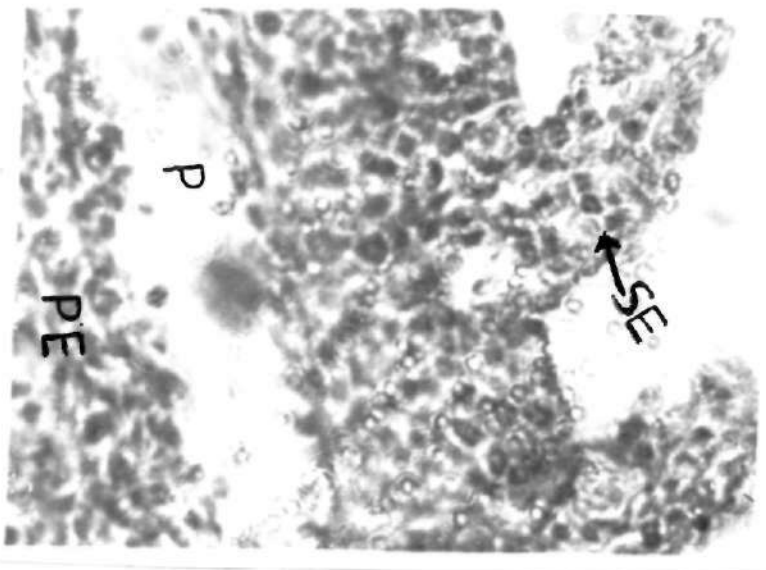


Plate IIIb: Cross-section of gill filament of *C. gariepinus* from control. PE, Primary epithelium; P, Primary lamella; SE, Secondary epithelium embedding the secondary lamellae. X400.

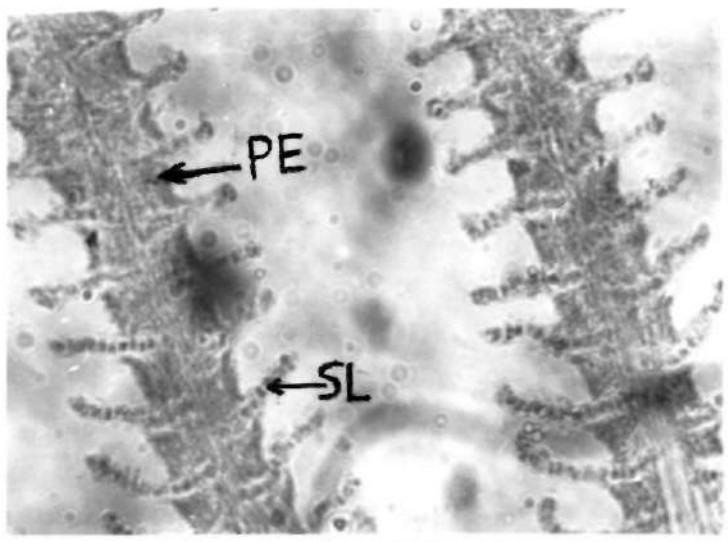


Plate IVa: Gill filaments of *oreochromis niloticus*; untreated control. S.L, Secondary lamellae; PE, Primary epithelium. X100.

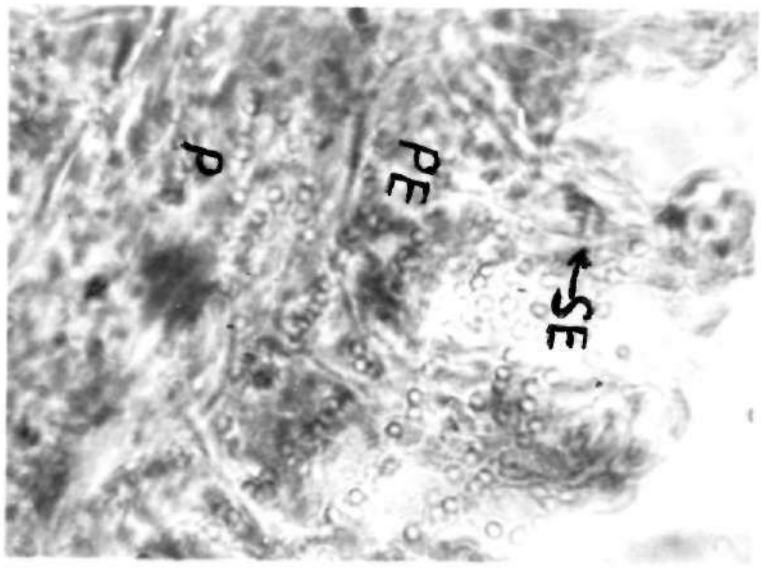


Plate IVb: Cross-section of gill filament of *O. niloticus* from control. PE, Primary epithelium; P, Primary lamella; SE, Secondary epithelium. X400.

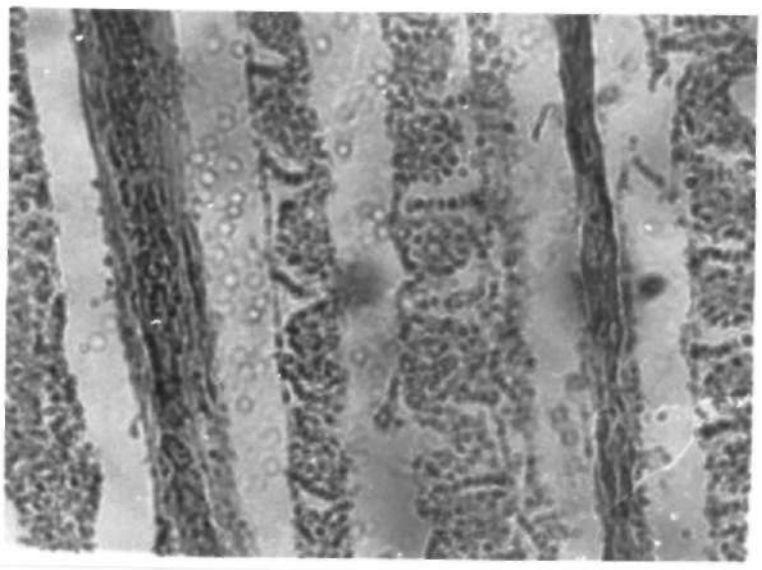


Plate Va: Gill filaments of *C. gariepinus*; exposed to 12 mgl^{-1}Zn. Note the epithelial proliferation that resulted to complete blanketing of the secondary lamellae. Also, marked increase in the width of primary lamella can be observed. X100.

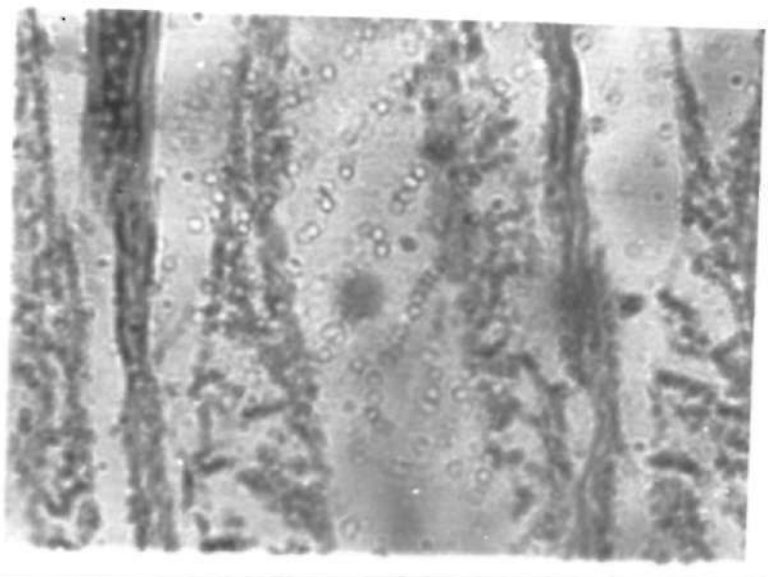


Plate Vb: Gill filaments of *C. gariepinus* exposed to 16 mgl^{-1}Zn. Epithelial cells were disintegrated leading to the fusion of secondary lamellae. X100.

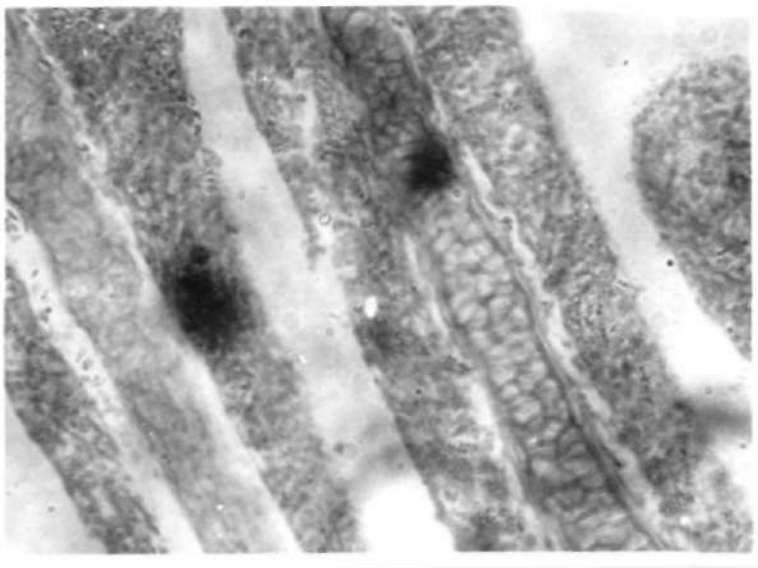


Plate VI: Gill filaments of *C. gariepinus;* exposed to 14 mgl^{-1}Cd. Note the extensive disintegration of epithelial cell and fusion of secondary lamellae (complete blanketing of the lamellae). X100.

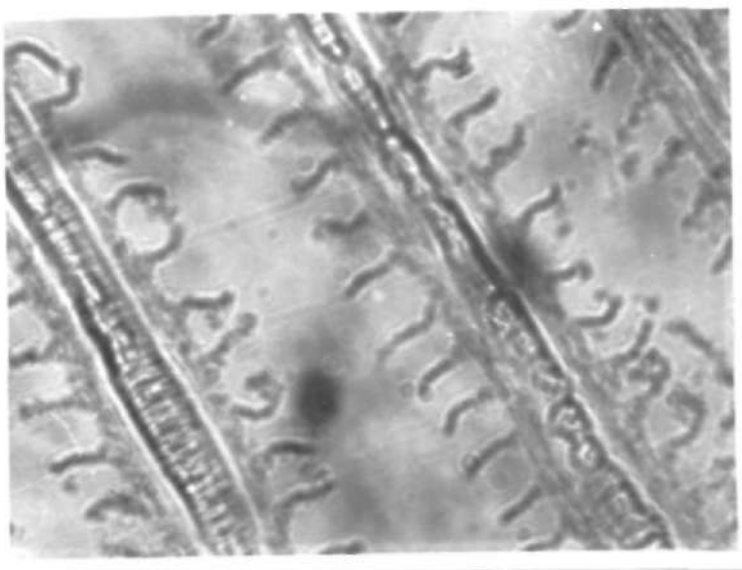


Plate VII: Gill filaments of *O. niloticus* exposed to 26 mgl^{-1}Zn. There was distortion of the secondary lamellae. X100.

exposed to 26 mgZnl^{-1} (Plate VII). The primary lamella was observed to have increased in width unlike that of the control (Plate III a, b). The gills of *O. niloticus* exposed to lethal concentration of cadmium (24 mg Cdl^{-1}) showed epithelial proliferation leading to partial blanketing (Plate VIII).

The livers of the control fish (Plates IX and X) were observed to have hepatic cells with cord-like formation. The cells were polygonal in shape with homogenous cytoplasm. The hepatic cells were observed to have centrally placed nucleus. Bile ducts and blood vessels were found scattered among hepatic cells. Sections of liver from *O. niloticus* exposed to lethal concentrations of zinc (24 mgZnl^{-1}) and cadmium (24 mgCdl^{-1}) are presented in Plates XI and XII respectively; and those of *C. gariepinus* exposed to 12 and 18 mgZnl^{-1} (Plates XIII a, b) and 16 mgCdl^{-1} (Plate XIV). The livers of these metal-exposed fishes showed precipitating cytoplasm (fibrosis) evidence in unclear and less homogenous cytoplasmic morphology. Hepatic vacuolarity was also observed (Plate XI). In *Clarias* species exposed to lethal concentrations of zinc, (Plates XIII a, b) and cadmium (Plate XIV) disintegrated blood vessels and pyknotic hepatocyte (where nucleus is shrunken and very dark) were also observed.

4.3 EFFECTS OF ZINC ON HAEMATOLOGICAL PARAMETERS

No mortality was recorded in any group since the fishes were exposed to sublethal doses of zinc. Table XIII summarises the mean values and standard errors (S.E) of all parameters for each group of *C. gariepinus* exposed to sublethal concentration (0.024 mgl^{-1}) of zinc. There was significant variations in all

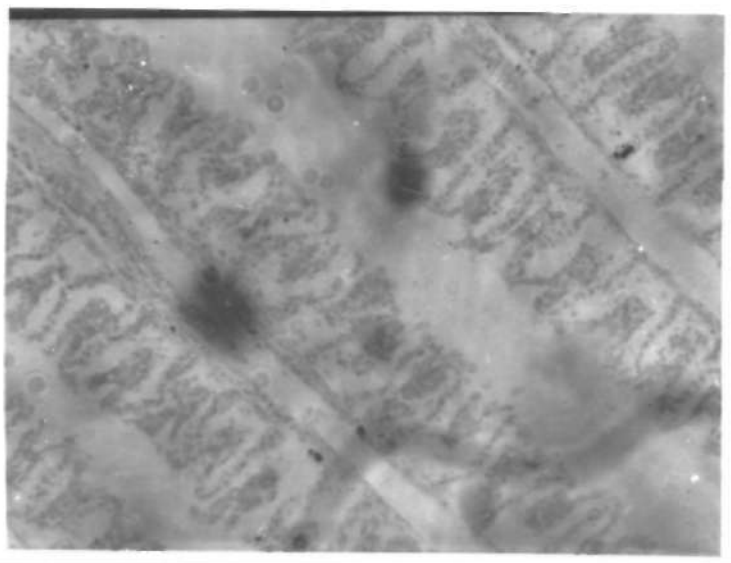


Plate VIII: Gill filaments of *O. niloticus* exposed to 24 mgl^{-1} Cd. Note the disintegration of epithelial cells and partial blanketing of the secondary lamellae). X100.

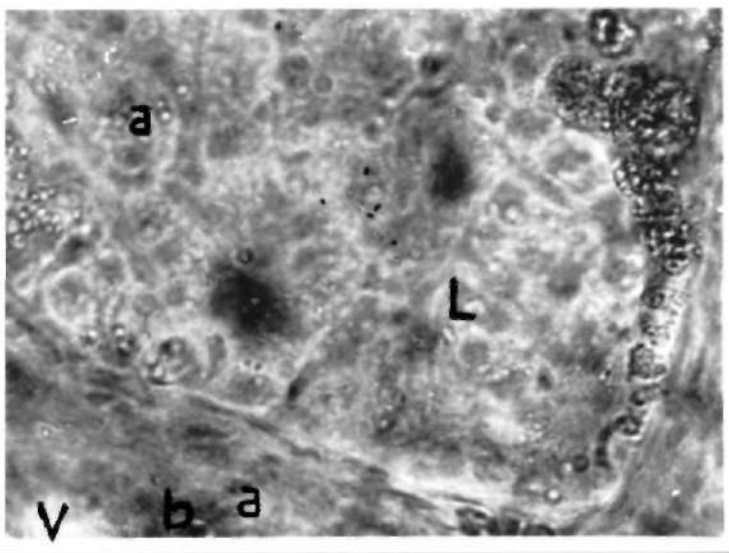


Plate IX: Liver cells (hepatocytes) of *O. niloticus*, untreated control.

L = lumen of central vein filled with red blood cells.

a = cytoplasm, b = Nucleus, v = Vacuole. X400

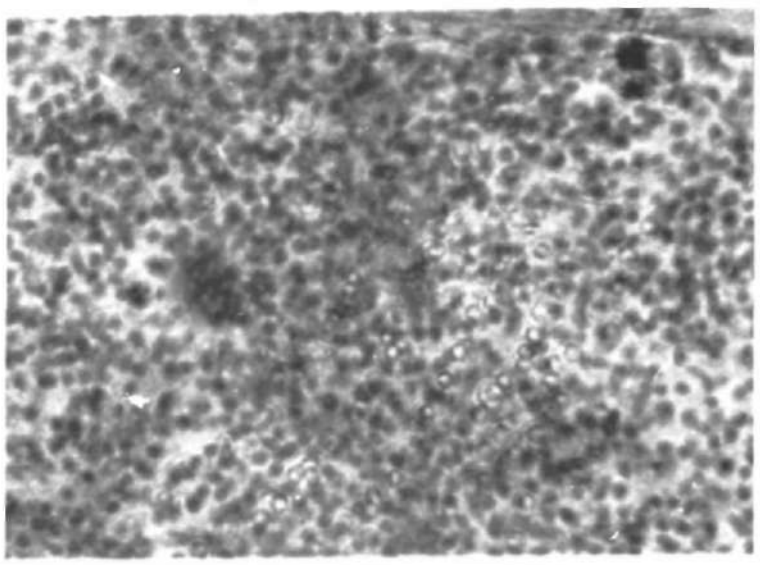


Plate X: Liver cells of *C. gariepinus*, untreated control. Note the cord-like arrays of hepatocytes. The cells have homogenous cytoplasm . X100.

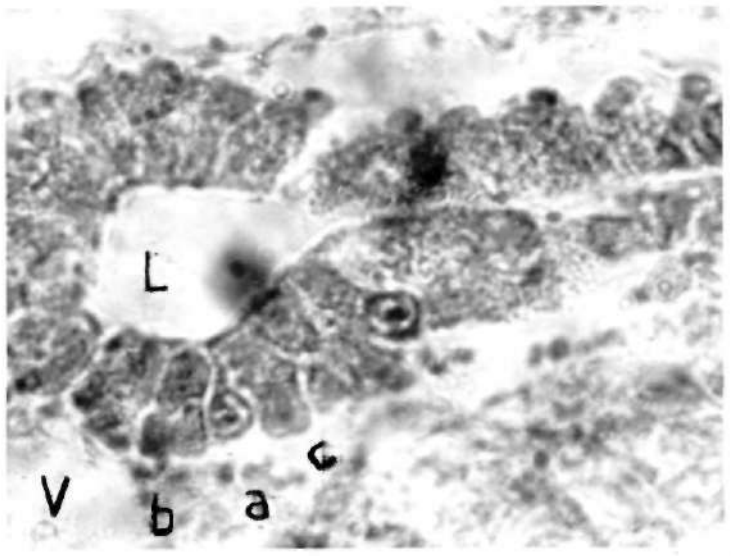


Plate XI: Liver cells of *O. niloticus* exposed 24 mgl^{-1}Zn.

a = Precipitating cytoplasm, b = Nucleus

c = Disintegrated blood vessels.

v = Vacuolation of hepatocytes

L = Lumen of bile duct. X400

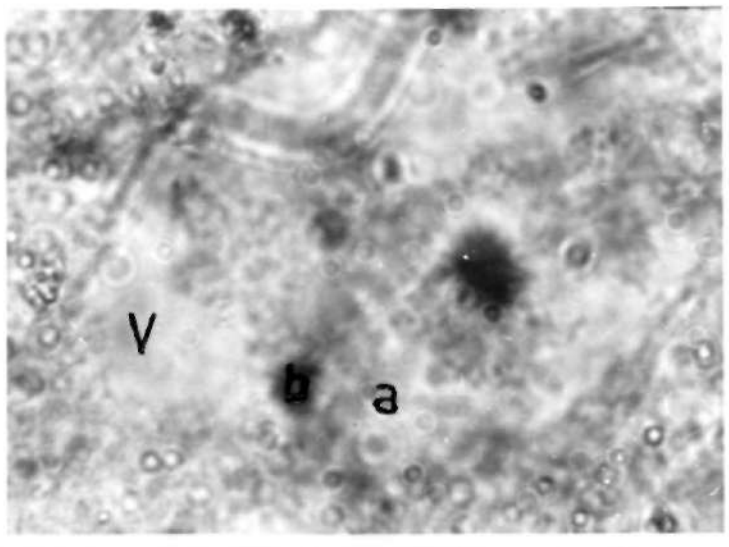


Plate XII: Liver cells of *O. niloticus* exposed to 24 mgl^{-1}Cd, a = Precipitating cytoplasm.
b = Nucleus
v = Vacuolation of hepatocytes. X400

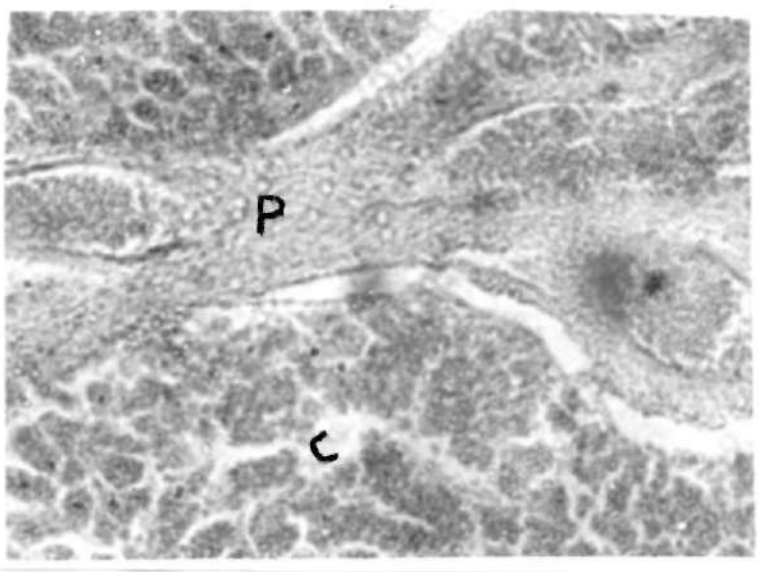


Plate XIIIa: Liver cells of *C. gariepinus* exposed to 12 mgl^{-1}Zn.
c = Disintegrated blood vessels
p = Portal vein with branches. X100

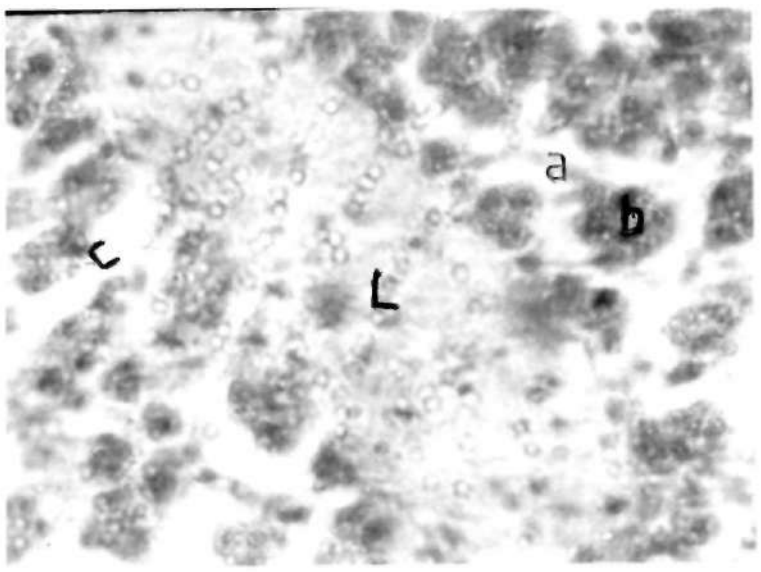


Plate XIIIb: Liver cells of *C. gariepinus* exposed to 18 mgl^{-1}Zn. a = Precipitating cytoplasm, b = Nucleus, c = Disintegrated blood vessels L = Lumen of vein filled with blood cells. X400

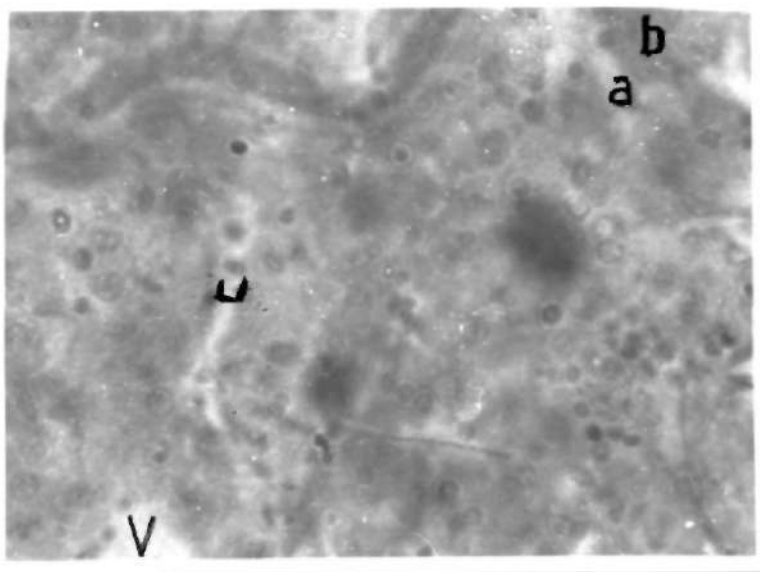


Plate XIV: Liver cells of *C. gariepinus* exposed to 16 mgl^{-1}Cd. a = Precipitating cytoplasm, b = Nucleus c = Disintegrated blood vessels v = Vacuolation of hepatocytes. X400.

Table XIII: The Effects of Zinc on Haematological Parameters in *Clarias gariepinus* Mean ± S.E. for Each Parameter

	Control	Wk1Zn	Wk2Zn	Wk3Zn
Haematocrit (%)	$58.89_{\pm}.93$	$58_{\pm}.47$	$55_{\pm}1.39$	$51.66_{\pm}2.37$
Haemoglobin ($g100\ ml^{-1}$)	$10.16_{\pm}.17$	$6.5_{\pm}.14$	$4.5_{\pm}.07$	8.39
RBCC ($g100\ mm^{-3}$)	$365833_{\pm}11021$	$1452000_{\pm}42723$	$1182000_{\pm}82561$	$618000_{\pm}53116$
WBCC (Cells mm^{-3})	$49436_{\pm}599$	$974860_{\pm}2800$	$833650_{\pm}2049$	$91450_{\pm}5259$
MCV (μm^3)	$1765_{\pm}$ 26	$403_{\pm}$ 42	$473_{\pm}$ 40	$845_{\pm}$ 60
MCH (pg Cell $^{-1}$)	$284_{\pm}$ 11	$54_{\pm}$ 2	$49_{\pm}$ 2	$145_{\pm}$ 5
MCHC ($g100\ ml^{-1}$)	$18.23_{\pm}22$	$11.26_{\pm}.69$	$9.22_{\pm}.72$	$16.47_{\pm}1.34$

Wk4Zn	Wk5Zn	Wk6Zn	Significance*
$35.5_{\pm}.28$	$35_{\pm}1.47$	$27.33_{\pm}1.21$	0.000^{1}
$7.41_{\pm}.04$	$7.6_{\pm}.17$	$8.18_{\pm}.02$	$< 0.05^{2}$
$598666_{\pm}30601$	$808000_{\pm}53116$	$280000_{\pm}40414$	$0.000^{1,2}$
$39900_{\pm}$ 5022	$19450_{\pm}1830$	$36850_{\pm}1968$	$0.000^{1,2}$
$620_{\pm}$ 41	$446_{\pm}46$	$985_{\pm}$ 57	$<0.0348^{1,2}$
$136_{\pm}$ 5	$103_{\pm}3$	$306_{\pm}37$	$0.000^{1,2}$
$20.54_{\pm}.58$	$20.31_{\pm}1.35$	$27.57_{\pm}.78$	$<0.05^{1,2}$

* One - way analysis of Variance

[1] Significant differences between groups

[2] Significant differences between control and Zn-treated groups.

variables, but they followed noncoincident patterns.

Exposure of *C. gariepinus* to zinc for six weeks produced a decrease in haematocrit, Hb, MCH and MCV. The haematocrit decreased significantly ($P < 0.05$) from 58.47 to 27.33% after six weeks of exposure. There was a significant increase ($P < 0.05$) in the values of RBCC during the six weeks of exposure. The values of haemoglobin (Hb) decreased significantly ($P < 0.05$) during the first two weeks of exposure but tended towards recovery during the following weeks. The WBCC values increased significantly ($P < 0.05$) during the first three weeks of exposure but declined during the fourth and the sixth weeks of exposure. The mean corpuscular haemoglobin concentration (MCHC) values decreased significantly ($P < 0.05$) during the first two weeks of exposure but recovered and increased during the fourth to sixth weeks of exposure. The mean corpuscular haemoglobin (MCH) values during the study were lower than the control but significantly ($P < 0.05$) increased during the sixth week of exposure to 306 $\pm$ 37 (pg $Cell^{-1}$) as compared to the control value of 284 $\pm$ 11 (pg $Cell^{-1}$). There was no significant difference ($P > 0.05$) in the values of control of all the parameters during the experiment.

The results of haematological parameters of *O. niloticus* exposed to 10 $mgZnl^{-1}$ are presented in Table XIV. There was significant variations in all the variables though these did not follow any coincident patterns. The haematocrit values decreased significantly during the six weeks of exposure of *O. niloticus* to zinc. The decrease was 14.3 $\pm$ 0.49% in the first week of exposure to 9.0 $\pm$ 0.81% in the sixth week of exposure compared to the control value of 17.78 $\pm$ 0.12%. The values of haemoglobin

Table XIV: The Effects of Zinc on Haematological Parameters in *Oreochromis niloticus* Mean ± S.E. for Each Parameter

	Control	Wk1Zn	Wk2Zn	Wk3Zn
Haematocrit (%)	$17.78_{\pm}.12$	$14.3_{\pm}.49$	$10.33_{\pm}.27$	$8.83_{\pm}.84$
Haemoglobin ($g100\ ml^{-1}$)	$5.7_{\pm}.07$	$6.91_{\pm}.03$	$7.75_{\pm}.14$	$4.5_{\pm}12$
RBCC ($g100\ mm^{-3}$)	$861333_{\pm}13238$	$1003666_{\pm}55714$	$483666_{\pm}38971$	$161666_{\pm}11836$
WBCC (Cells mm^{-3})	$34636_{\pm}109$	$400960_{\pm}2396$	$41750_{\pm}814$	$30416_{\pm}1356$
MCV (μm^3)	$213_{\pm}21$	$158_{\pm}54$	$226_{\pm}60$	$557_{\pm}44$
MCH (pg $Cell^{-1}$)	$77_{\pm}1$	$79_{\pm}2$	$172_{\pm}6$	$286_{\pm}37$
MCHC ($g100\ ml^{-1}$)	$32.01_{\pm}59$	$48.76_{\pm}3.57$	$74.5_{\pm}1.97$	$50.42_{\pm}2.68$

Wk4Zn	Wk5Zn	Wk6Zn	Significance*
$6.66_{\pm}.72$	$10.33_{\pm}1.18$	$9_{\pm}.81$	$<0.05^{2}$
$4.5._{\pm}.12$	$4.46_{\pm}.3$	$4.92_{\pm}.33$	
$130000_{\pm}11547$	$147666_{\pm}7796$	$169666_{\pm}10682$	$<0.05^{2}$
$23973_{\pm}1099$	$19096_{\pm}5262$	$23746_{\pm}1590$	$<0.05^{2}$
$528_{\pm}55$	$701_{\pm}62$	$536_{\pm}54$	
$356_{\pm}59$	$324_{\pm}53$	$295_{\pm}43$	$0.000^{1,2}$
$67.36_{\pm}2.36$	$43.42_{\pm}1.87$	$54.32_{\pm}1.18$	$<0.0001^{1,2}$

* One - way analysis of Variance

[1] Significant differences between groups

[2] Significant differences between control and Zn-treated groups.

increased slightly during the first two weeks of exposure but decreased slightly during the third to the sixth weeks of exposure. The values of haemoglobin obtained from treated *Oreochromis* did not differ significantly ($P > 0.05$) from those of the control. There was significant ($P < 0.05$) increase in RBCC during the first two weeks of exposure but this decreased significantly ($P < 0.05$) in the subsequent weeks to 169666 ± 10682 (Cells mm^{-3}) compared to the control value of 861333 ± 13238 (Cells mm^{-3}). Similarly, the WBCC increased significantly ($P < 0.05$) during the first two weeks of exposure of *O. niloticus* to zinc but decreased significantly ($P < 0.05$) in the subsequent weeks of the experiment. The mean corpuscular volume (MCV) decreased significantly ($P < 0.05$) after the first week of exposure but increased significantly ($P < 0.05$) during the following weeks of the experiment. The MCH values increased significantly ($P < 0.05$) during the experimental period. The values of MCHC also increased significantly ($P < 0.05$) during the experimental period though there were fluctuations in the values, after the third, fourth, fifth and sixth weeks of exposure. The values of the controls in all the parameters did not differ significantly throughout the experimental period.

4.4 EFFECTS OF CADMIUM ON HAEMATOLOGICAL PARAMETERS

There was no mortality in any group of fish exposed to sublethal concentrations of cadmium. Table XV summarises the mean values and standard errors (S.E) of all parameters for each group of *Oreochromis niloticus* exposed to 8 $mgCdl^{-1}$ for six weeks. The table also shows significant differences between

Table XV: The Effects of Cadmium on Haematological Parameters in *Oreochromis niloticus* Mean ± S.E. for Each Parameter

	Control	Wk1Cd	Wk2Cd	Wk3Cd
Haematocrit (%)	$17.78_{\pm}.12$	$20.3_{\pm}1.15$	$16.66_{\pm}2.36$	$10.5_{\pm}.29$
Haemoglobin ($g100\ ml^{-1}$)	$5.7_{\pm}.07$	$7.32_{\pm}.03$	$8_{\pm}.24$	$5.08_{\pm}.14$
RBCC ($g100\ mm^{-3}$)	$861333_{\pm}13238$	$756000_{\pm}24846$	$373666_{\pm}33775$	$139666_{\pm}10682$
WBCC ($Cells\ mm^{-3}$)	$34636_{\pm}109$	$37573_{\pm}547$	$35453_{\pm}537$	$26960_{\pm}823$
MCV (μm^3)	$213_{\pm}21$	$299_{\pm}20$	$455_{\pm}44$	$762_{\pm}59$
MCH ($pg\ Cell^{-1}$)	$77_{\pm}1$	$107_{\pm}26$	$224_{\pm}60$	$374_{\pm}55$
MCHC ($g100\ ml^{-1}$)	$32.01_{\pm}59$	$34.92_{\pm}2.1$	$47.05_{\pm}1.51$	$46.89_{\pm}2.22$

Wk4Cd	Wk5Cd	Wk6Cd	Significance*
$6_{\pm}.58$	$9_{\pm}1.15$	$11.33_{\pm}.88$	$.000^{1}$
$4.77_{\pm}.12$	$4.22_{\pm}.13$	$5.58_{\pm}.17$	
$145666_{\pm}19919$	$157666_{\pm}11836$	$371666_{\pm}20496$	$<0.05^{1,2}$
$15360_{\pm}149$	$24183_{\pm}367$	$45610_{\pm}555$	$<0.05^{1,2}$
$422_{\pm}54$	$575_{\pm}60$	$317_{\pm}58$	
$338_{\pm}45$	$287_{\pm}54$	$172_{\pm}50$	$0.020^{1,2}$
$74.08_{\pm}1.55$	$45.78_{\pm}3.48$	$47.97_{\pm}1.99$	$<0.05^{1,2}$

* One - way analysis of Variance

[1] Significant differences between groups

[2] Significant differences between control and Cd treated groups.

treatments. There were significant variations in all variables, but they followed noncoincident patterns. The haematocrit values increased slightly after the first week of exposure but decreased significantly ($P < 0.05$) during the subsequent weeks of the experiment. Similarly, the values of haemoglobin increased slightly during the first two weeks of exposure, but decreased though not significantly in the following weeks of the experiment. The RBCC decreased significantly ($P < 0.05$) during the six weeks of exposure of *O. niloticus* to cadmium. The WBCC increased significantly ($P < 0.05$) during the first two weeks of exposure, but significantly ($P < 0.05$) decreased in the subsequent weeks until the sixth week of exposure during which a significant ($P < 0.05$) increase was recorded. There was a significant ($P < 0.05$) increase in MCV values during the experimental period. Similarly, the MCH and MCHC values increased significantly ($P < 0.05$) during the six weeks exposure of *O. niloticus* to sublethal concentration of cadmium. However, there were fluctuations in the values of MCH and MCHC within the weeks of exposure. There was no significant ($P > 0.05$) difference in the controls of all the parameters during the experimental period.

The results of haematological parameters of *Clarias gariepinus* exposed to 0.0071 $mgCdl^{-1}$ for six weeks are shown in Table XVI. The table shows the mean values and standard errors (S.E) of all parameters for each group and the significant differences between treatments. There were significant variations in all variables but these did not follow coincidental patterns. The haematocrit values decreased significantly ($P <$

Table XVI: The Effects of Cadmium on Haematological Parameters in *Clarias gariepinus* Mean ± S.E. for Each Parameter

	Control	Wk1Cd	Wk2Cd	Wk3Cd
Haematocrit (%)	$58.89_{\pm}.93$	$57.66_{\pm}.72$	$47_{\pm}1.73$	$36.66_{\pm}2$
Haemoglobin ($g100\ ml^{-1}$)	$10.16_{\pm}.17$	$6.25_{\pm}0.7$	$5_{\pm}0.7$	$8.25_{\pm}.1$
RBCC ($Cells\ mm^{-3}$)	$365833_{\pm}11021$	$1299333_{\pm}23383$	$786333_{\pm}5783$	$720333_{\pm}5783$
WBCC ($Cells\ mm^{-3}$)	$49436_{\pm}599$	$142400_{\pm}1975$	$187383_{\pm}4963$	$92016_{\pm}2795$
MCV (μm^3)	$1765_{\pm}26$	$442_{\pm}61$	$602_{\pm}39$	$514_{\pm}44$
MCH ($pg\ Cell^{-1}$)	$284_{\pm}11$	$58_{\pm}3$	$76_{\pm}2$	$125_{\pm}12$
MCHC ($g100\ ml^{-1}$)	$18.23_{\pm}.22$	$11.75_{\pm}1.99$	$11.71_{\pm}1.41$	$21.56_{\pm}1.85$

Wk4Cd	Wk5Cd	Wk6Cd	Significance*
$23.66_{\pm}1.45$	$18.66_{\pm}.33$	$14.33_{\pm}.88$	$.000^{1,2}$
$8.13_{\pm}.7$	$6.53_{\pm}.13$	$6.58_{\pm}.1$	$<0.05^{2}$
$517333_{\pm}3711$	$524000_{\pm}7023$	$428000_{\pm}3511$	$.000^{1}$
$92016_{\pm}2795$	$105616_{\pm}598$	$117250_{\pm}433$	$.000^{1}$
$469_{\pm}59$	$376_{\pm}56$	$335_{\pm}45$	$<.037^{1,2}$
$168_{\pm}37$	$136_{\pm}44$	$164_{\pm}26$	$<0.0591^{1,2}$
$32.27_{\pm}2.78$	$33.15_{\pm}3.49$	$46.48_{\pm}1.83$	$<0.05^{1,2}$

* One - way analysis of Variance

[1] Significant differences between groups

[2] Significant differences between control and Cd treated groups.

0.05) during the six weeks of exposure. The haemoglobin values decreased significantly ($P < 0.05$) during the first two weeks and then in the fifth and sixth weeks of the experiment. There was a slight decrease in the haemoglobin values during the third and fourth weeks of the experiment. The RBCC and WBCC both increased significantly ($P < 0.05$) though there were fluctuations in these values within the weeks. There was significant ($P < 0.05$) decrease in MCV values during the six weeks of exposure of *C. gariepinus* to cadmium. Similarly, the MCH values significantly ($P < 0.05$) decreased though there was increase in the subsequent weeks, these values were not up to those of the controls. The MCHC values decreased significantly ($P < 0.05$) during the first two weeks of exposure and thereafter increased significantly ($P < 0.05$) in the subsequent weeks of the experiment. The values of controls for each parameters did not differ significantly ($P > 0.05$) during the experimental period.

4.5 ZINC UPTAKE IN TISSUES

The results of the distribution studies are shown in Table XVII a. Zinc rapidly disseminated through the tissues. The results in Table XVII a, show that zinc residue in tissues of *Clarias gariepinus* increased with increase in duration of exposure, (8 > 6 > 4 > 2 weeks). Zinc accumulations in fish tissues were in the order of liver > gut > gills > whole fish > skin > muscles. Zinc residues in fish exposed to 1.325 $mgZnl^{-1}$ were significantly ($P < 0.05$) higher than those of the control. There was a significant ($P < 0.05$) increase in zinc residues accumulated over the 2, 4, 6 and 8 weeks of exposure in all the

Table XVII a: Zinc Residue (μgg^{-1} dry wt.) in Various Tissues of *Clarias gariepinus* Exposed to 1.325 mgZn/l. Means $\pm$ S.E. Presented

Body Tissues	Duration of Exposure (weeks)			
	2	4	6	8
Liver	$45.27_{\pm}3.24$	$80.56_{\pm}2.05$	$204.99_{\pm}3.6$	$282.85_{\pm}2.8$
Gill	$21.44_{\pm}1.7$	$38.52_{\pm}2.3$	$50.05_{\pm}2.1$	$105.62_{\pm}4.2$
Gut	$20.8_{\pm}1.5$	$34.09_{\pm}2.6$	$52.61_{\pm}1.8$	$139.51_{\pm}3.5$
Muscles	$8.37_{\pm}1.05$	$15.42_{\pm}1.3$	$24.25_{\pm}0.5$	$35.88_{\pm}2.4$
Skin	$13.84_{\pm}1.25$	$25.27_{\pm}2.1$	$45.31_{\pm}1.3$	$72.67_{\pm}2.63$
Whole Fish	$16.17_{\pm}1.31$	$28.43_{\pm}1.21$	$44.06_{\pm}0.4$	$84.88_{\pm}2.5$
Control Whole-Fish	$1.35_{\pm}0.46$	$1.1_{\pm}0.58$	$1.45_{\pm}0.3$	$1.36_{\pm}0.23$

Table XVII b: Bioconcentration Factors (BCF_s) of Zinc in Whole Fish and Body Tissues of *Clarias gariepinus* Exposed to 1.325 mgZn/l.

Body Tissues	Duration of Exposure (weeks)			
	2	4	6	8
Liver	34.17	60.8	154.71	213.47
Gill	16.18	29.07	37.77	79.71
Gut	15.7	25.73	39.71	105.29
Muscles	6.32	11.64	18.3	27.08
Skin	10.45	19.07	34.2	54.85
Whole Fish	12.2	21.46	33.25	64.06

body tissues. The levels of zinc in each tissues were significantly ($P < 0.05$) different from each other.

Table XVII b shows the bioconcentration factors (BCF) of zinc in whole fish and body tissues of *C. gariepinus*. The BCF of zinc in fish tissues increased with increase in duration of exposure (8 > 6 > 4 > 2 weeks). The BCF of zinc in tissues of *C. gariepinus* were in the order of Liver > gut > gills > whole fish > skin > muscles. The values of BCF of tissues significantly ($P < 0.05$) differed from each other.

4.6 CADMIUM UPTAKE IN TISSUES

The results from analysis of cadmium accumulation in tissues of *C. gariepinus* and *O. niloticus* are presented in Tables XVIII a and b respectively. Table XVIII a shows that cadmium residues accumulation in whole fish were higher than those of control though these did not differ significantly ($P > 0.05$). Cadmium uptake in body tissues were in the order of the liver > gut > gills > skin > muscles > whole fish. The tissues residues accumulation increased with increase in duration of exposure of *C. gariepinus* to cadmium (8 > 6 > 4 > 2 weeks).

Table XVIII b shows that there was rapid uptake of cadmium in body tissues of *O. niloticus*. The residues accumulation significantly ($P < 0.05$) increased with increase in duration of exposure in liver, gills and gut. There was slight increase in the cadmium accumulation in muscles, skin and whole fish with increase in duration of exposure. The cadmium accumulation in body tissues were in the order of the Liver > gut > gills > skin > whole fish > muscles. There was significant ($P < 0.05$)

Table XVIII a: Cadmium Residue ($\mu g g^{-1}$ dry wt.) in Various Tissues of *Clarias gariepinus* Exposed to 1.09 mgl^{-1} Cadmium Means $\pm$ S.E. Presented

Body Tissues	Duration of Exposure (weeks)			
	2	4	6	8
Liver	$261.39_{\pm}1.2$	$383.37_{\pm}3.4$	$402.55_{\pm}2.8$	$877.9_{\pm}4.54$
Gill	$16.04_{\pm}1.25$	$19.2_{\pm}2.45$	$36.76_{\pm}2.84$	$39.82_{\pm}3.18$
Gut	$94.62_{\pm}1.8$	$110.33_{\pm}2.47$	$165.62_{\pm}4.86$	$201.07_{\pm}5.14$
Muscles	$0.43_{\pm}0.15$	$0.55_{\pm}.22$	$0.81_{\pm}0.29$	$1.62_{\pm}0.38$
Skin	$1.27_{\pm}.83$	$2.54_{\pm}.56$	$3.46_{\pm}.24$	$11.69_{\pm}1.51$
Whole Fish	$0.4_{\pm}.021$	$0.64_{\pm}.05$	$0.92_{\pm}.04$	$1.32_{\pm}0.25$
Control	$0.12_{\pm}0.02$	$0.15_{\pm}0.04$	$0.18_{\pm}0.05$	$0.17_{\pm}0.04$

Table XVIII b: Cadmium Residue ($\mu g g^{-1}$ dry wt.) in Various Tissues of *Oreochromis niloticus* Exposed to 1.93 mgl^{-1} Cadmium Means $\pm$ S.E. Presented

Body Tissues	Duration of Exposure (weeks)			
	2	4	6	8
Liver	$191.63_{\pm}2.26$	$248.11_{\pm}2.5$	$363.89_{\pm}2.05$	$494.6_{\pm}3.41$
Gill	$7.95_{\pm}.21$	$9.9_{\pm}.45$	$23.8_{\pm}2.61$	$27.13_{\pm}.35$
Gut	$37.91_{\pm}1.2$	$45.14_{\pm}2.6$	$52.29_{\pm}1.35$	$95.73_{\pm}1.52$
Muscles	$0.11_{\pm}0.03$	$0.26_{\pm}0.05$	$0.57_{\pm}0.04$	$0.7_{\pm}0.07$
Skin	$0.58_{\pm}0.05$	$0.78_{\pm}0.04$	$1.28_{\pm}0.25$	$3.14_{\pm}0.36$
Whole Fish	$0.04_{\pm}0.01$	$0.61_{\pm}0.03$	$0.72_{\pm}0.05$	$1.24_{\pm}0.11$
Control	$0.42_{\pm}0.12$	$0.46_{\pm}0.11$	$0.5_{\pm}0.15$	$0.48_{\pm}0.12$

difference in the levels of cadmium of each body tissues from the other. The levels of cadmium in the muscles, skin and whole fish of *O. niloticus* were as low as those found in the muscles, skin and whole fish of *C. gariepinus*.

The bioconcentrations factors (BCF) of body tissues of *C. gariepinus* and *O. niloticus* exposed to cadmium are shown in Tables XIX a and b respectively. Table XIX a shows that the BCF of the liver, gills and gut of *C. gariepinus* significantly ($P < 0.05$) increased with increase in duration of exposure while the BCF of muscles, skin and whole fish increased but not significantly ($P > 0.05$) with increase in duration of exposure. The BCF values of tissues of *C. gariepinus* exposed to cadmium were in the order of liver > gut > gills > skin > muscles > whole fish. The BCF of body tissues of *O. niloticus* exposed to cadmium are presented in Table XIX b. Just like the trend observed in BCF of body tissues of *C. gariepinus*, the BCF of liver, gills and gut of *O. niloticus* increased significantly ($P < 0.05$) with increase in duration of exposure while the BCF of muscles, skin and whole fish did not increase significantly ($P > 0.05$) with increase in duration of exposure. The BCF of body tissues of *O. niloticus* exposed to cadmium were in the following order: liver > gut > gills > skin > whole fish > muscles.

Table XIX a: Bioconcentration Factors (BCF_s) of Cadmium in Whole Fish and Body Tissues of *Clarias gariepinus* Exposed to 1.09 mgl^{-1} Cadmium

Body Tissues	Time of Exposure (weeks)			
	2	4	6	8
Liver	99.29	128.55	188.54	256.27
Gill	4.12	5.13	12.33	14.06
Gut	19.64	23.39	27.09	49.6
Muscles	0.06	0.13	0.3	0.36
Skin	0.3	0.4	0.66	1.63
Whole Fish	0.02	0.32	0.37	0.64

Table XIX b: Bioconcentration Factors (BCF_s) of Cadmium in Whole Fish and Body Tissues of *Oreochromis niloticus* Exposed to 1.93 mgl^{-1} Cadmium

Body Tissues	Time of Exposure (weeks)			
	2	4	6	8
Liver	239.8	352.63	369.31	805.41
Gill	14.72	17.61	33.72	36.53
Gut	86.81	101.22	151.94	184.47
Muscles	0.39	0.5	0.74	1.49
Skin	1.17	2.33	3.17	10.72
Whole Fish	0.37	0.59	0.84	1.21

CHAPTER FIVE

5.0 DISCUSSION

5.1 ACUTE TOXICITY STUDIES

The physico-chemical parameters of the test water fluctuated only slightly (Table III) during the bioassays, but did not appear to have affected fish mortality. The analysed values of zinc for each bioassay were 8.77, 10.36, 12.30, 13.75 and 16.69 ppm compared to nominal values of 10, 12, 14, 16, and 18 mgl^{-1} for *Clarias gariepinus* and 18.08, 20.14, 23.2, 25.68 and 27.52 ppm compared to nominal values of 20, 22, 24, 26, and 28 mgl^{-1} for *O. niloticus*. These analysed toxicant values were found to be lower than the toxicant nominal values. This may be attributed to uptake by fish, absorption on the walls of the tanks and loss to evaporation. A similar observation was made by Lewis (1978) who worked on acute toxicity of heavy metals to juvenile longfin dace *Agosia chrysogaster*.

The results show that both species of *C. gariepinus* and *O. niloticus* exposed to lethal concentrations of zinc, cadmium and zinc/cadmium mixture showed alterations in behaviour. Such observed behaviours were agitated swimming, loss of equilibrium, air gulping, period of quiescence and death. Similar observations were made by Lewis (1978) and Annune *et al.*, (1991) who worked on toxicities of heavy metals to different fish species. These reactions to the toxicant were dose-dependent and these were reflected in the means score of symptoms in each of the fish species exposed to the metals. From the means of symptoms of toxicosis, it appears that fingerlings of *Clarias gariepinus* are more susceptible to zinc, cadmium and zinc/cadmium

mixture than fingerlings of *Oreochromis niloticus*. This was demonstrated by the relatively high means score of symptoms of toxicosis of the *Clarias* species exposed to these metals. The highest means score (15) was observed for *Clarias* species exposed to 18 and 16 ($mgZnl^{-1}$) respectively; 15, 14.38 means score were recorded for those exposed to 16, 14, and 12 ($mgCdl^{-1}$) respectively; and for the mixed salt solutions of Zinc/cadmium the highest means score (15) was recorded for the fish exposed to 18 ($mgZnl^{-1}$)/16 ($mgCdl^{-1}$); 16 ($mgZnl^{-1}$)/14 ($mgCdl^{-1}$) and 14 ($mgZnl^{-1}$)/ 12 ($mgCdl^{-1}$) respectively. For the *Oreochromis* species, the highest means score of symptoms (15) was recorded at 28 $mgZnl^{-1}$; 15 and 14.38 means score were recorded for those exposed to 24 and 22 ($mgCdl^{-1}$) respectively; and for the mixed salt solution of zinc/cadmium the highest means score (15) was recorded in the fish exposed to 24 $mgZnl^{-1}$/20 $mgCdl^{1}$; 22 $mgZnl^{-1}$/18 $mgCdl^{1}$ and 20 $mgZnl^{-1}$/16 $mgCdl^{1}$ respectively. The reasons for the difference in susceptibility of these two species to heavy metals will be discussed later. The symptoms of toxicosis also showed that cadmium was more toxic to these freshwater fishes than zinc. This was reflected in the high means score of symptoms of the fishes exposed to these metals. Lack of literature has made it difficult to compare these results with previous works.

The *Clarias* species exposed to lethal concentrations of cadmium showed swollen abdomen. Post-mortem showed swollen gut hence the gut of *Clarias gariepinus* might be a target site for action/absorption of cadmium. However, there was no observation of swollen abdomen in *C. gariepinus* exposed to lethal concentrations of zinc/cadmium mixture. Zinc may have inhibited

the absorption of cadmium in the gut of *C. gariepinus*.

Accumulation of mucus was observed on the gill filaments and body surfaces of the fishes that died as a result of their exposure to lethal concentrations of zinc, cadmium and zinc/cadmium mixture. Similar observation was made by other workers (Daye and Garside, 1976; Alabaster and Lloyd, 1980; Annune *et al.*, 1991) who studied the effects of heavy metals on different fish species. According to Annune *et al* (1991) the accumulation of mucus may result from an increase in the activity of mucous cells subsequent to exposure to heavy metals. This resulted in increase in the production of mucus over the fish body. Solutions of heavy metals suffocate fish by precipitating or coagulating mucoproteins on the gill epithelium. This interfered with the gaseous exchange, secretion of waste products and osmoregulation. The toxic action of metals appeared to be a combined effect of precipitation of mucus on the gills and osmoregulatory stress with death resulting from suffocation. Blood was observed around the gill coverings of the dead fishes. A similar observation was made by Annune *et al.*, (1991). The results suggest that the fishes might have suffered from gill haemorrhage. Zinc, copper and cadmium are known to cause necrotic damage in the gill epithelium (Alabaster and Lloyd, 1980). Therefore, the primary mode of action of toxic metals may be the disruption of gaseous exchange and salt/water balance caused by necrotic damage. Mucus accumulation on gill filaments would be of secondary importance.

The median lethal concentrations of this study suggest that fingerlings of both *C. gariepinus* and *O. niloticus* are tolerant

to zinc and cadmium though the degree of tolerance varies; with *C. gariepinus* being more susceptible to the two metals. The difference in susceptibility may be attributed to the integumental difference in these two species. *Clarias gariepinus* is a naked fish hence the body is in direct contact with the toxicant while *Oreochromis niloticus* has cycloid scales that give some protection from the toxicant. The permeability of various species is of considerable importance in determining the tolerance to metal-poisoning. Bryan (176) found that in *Nereis diversicolor* there is a fairly close relationship between the rates of absorption of metals and their acute toxicity. Corner and Rigler (1958) showed that the much greater resistance to mercury by larvae of the brine shrimp, *Artemia salina* compared with larvae of the barnacle, *Eleminius modestus*, rested on the impermeability of the body surface in *Artemia*. Also, changes in physiological stress, ability of organisms to excrete or detoxicate metals may be attributed to the tolerance of different species to heavy metals. Some species are able to excrete a higher proportion of the heavy metal intake under contamination conditions and thereby regulate the concentration in the body at a fairly normal level. Rainbow trout, *Salmo gairdneri* loses zinc-65 through the gills rather than in urine (Nakatani, 1966).

Schneider (1971) and National Academy of Sciences and National Academy of Engineering (1973) reported lethal levels of zinc to various fishes in different waters as within the range of 0.1-32 mgl^{-1}. The lethal values obtained in this study are within this reported lethal range. Both *C. gariepinus* and *O. niloticus* appeared to be more resistant to zinc than stickleback

which have been killed by 0.5-1.0 $mgdm^{-3}$ zinc within 1-3 days (Matthiessen and Brafield, 1973) and juvenile longfin dace, *Agosia chrysogaster* killed by 0.79 mgl^{-1} zinc within 96 hours (Lewis, 1978). *Clarias gariepinus* appeared to be more susceptible to zinc than juvenile and subadult rainbow trout, *Salmo gairdneri* killed within 96 h by 26 and 24 mgl^{-1} zinc respectively (Meisner and Hum, 1987). This study is limited to the fingerling stage of *C. gariepinus* and *O. niloticus* but the effects of heavy metals on fish are known to vary with the stages of their life cycle and age.

Metal-metal interactions is a process by which metals in their different forms change either the critical concentration or a critical effect of a metal under consideration. In this study, the interaction between cadmium and zinc was examined to determine the effect of biological interaction on the relative toxicities of the two metals. The results showed that zinc/cadmium solutions were more toxic to both *C. gariepinus* and *O. niloticus* than any of the individual metals used for the bioassay tests. Zinc/cadmium mixture exhibited an antagonistic toxicity in *C.gariepinus*. This is not expected due to the chemical similarities of zinc and cadmium (Underwood, 1979). The toxicity of cadmium was increased by intakes of zinc and the toxicity of zinc was inhibited by intakes of cadmium. Nordberg *et al.*, (1972) observed that cadmium treatment increased the levels of zinc in the liver. Therefore, there is a possibility that the synthesis of compounds for detoxicating one metal may increase the affinity of the organism for another. Medici and Taylor (1967) demonstrated the mutual antagonism that exists

between cadmium and zinc and interactions between cadmium, iron and copper in their studies with flour beetle, *Tribolium confusum*. High levels of zinc in that species were shown to reduce the toxicity of cadmium and additional copper slightly reduced the toxic effects of both cadmium and zinc. The antagonistic action of cadmium in *C. gariepinus* may be due to the interchange of cadmium and zinc ions on ligands that mediate the absorption of zinc.

In *Oreochromis niloticus*, zinc/cadmium mixture exhibited synergism. The toxicity of zinc was significantly increased by intakes of cadmium and the toxicity of cadmium was also significantly increased by intakes of zinc. This is expected due to the chemical similarities of the two metals (Underwood, 1979).

The interaction between zinc and cadmium can be explained by competition for protein-binding sites at the mucosal cell and in the tissues. This competition for binding sites includes the zinc metalloenzymes. In interchange of metal-bound proteins, competition for carrier protein may affect the transport of metal ions (Evans and Hahn, 1974; Chmielnicka, 1983). The induction of metal-binding protein, metallothionein is another possible site of metal-metal interaction. The most potent inducers of thionein synthesis are Zn^{2+}, Cd^{2+} and Hg^{2+} (Kagi and Nordberg, 1979). Although these cations do not form complexes with one another, the retention of any of them is increased by the presence of either of the two (Chmielnicka, 1983). Metal-metal interactions may also involve induction of metal binding protein synthesis. Metal binding protein modifies the toxicity of many metals. Cd^{2+} will replace Zn^{2+} and Hg^{2+} will replace Cd^{2+} in the

corresponding metallothionein in the liver (Webb, 1972). Zinc can also, interchange with cadmium in the thionein in the liver (Kagi and Nordberg, 1979). The slope of the dose-effect curves calculated from the toxicity tests differ only slightly. The differences in probit slopes might reflect the differences in the time the metals moved to the site of action in the individual fish species.

The interaction between cadmium and zinc has also being reported in other vertebrates. Reduced absorption of ^{65}Zn has been achieved in the chick by cadmium feeding (Underwood, 1979) and the intracellular distribution of ^{65}Zn in mouse liver cells influenced by injected cadmium or by cadmium feeding (Underwood up cit). The competition between zinc and cadmium for protein-binding sites presumably includes the zinc metalloenzymes. It certainly includes metallothionein which can vary in cadmium and zinc contents while the sums of the two elements remain constant. Although the simultaneous presence of cadmium, zinc and mercury in a low-molecular weight protein isolated from kidney cortex was demonstrated many years ago ((Underwood, 1979), the biochemical function of this compound (Metallotheonein) is still not clear. However, since there was a rapid rise in metallothionein synthesis following the oral or intraperitoneal administration of cadmium to rabbits, rats and Chicks (Underwood, 1979) it seems that one important action is decontamination of the organism from harmful cadmium ions.

The metabolic antagonism between cadmium and zinc at the tissue level, as distinct from the effects of absorption was further apparent from the protection afforded by injected zinc

against the remarkable toxic effects of injected cadmium on the testes and on pregnant rats (Underwood, 1979). Rats protected by zinc salts show no reduction in blood cadmium comparable to that brought about by selenium and the concentration of cadmium in the liver is actually increased (Underwood, up cit). This brings about the possibility that zinc reduces cadmium toxicity by increasing the excretion of cadmium from the organism by kidneys, intestine, or biliary tract. Cadmium feeding does not always reduce the zinc concentrations in the tissues, nor does zinc feeding always reduce cadmium concentrations even though each element increases the toxic effects of the other (Underwood, 1979). Furthermore, cadmium feeding can have a greater effect on the copper status of animals than it has on their zinc status, indicating that cadmium is a more powerful metabolic antagonist of copper than of zinc. This is illustrated by works done by Underwood, (1979) with sheep. High dietary levels of cadmium have been shown to directly depress copper uptake from the duodenum (Underwood, 1979).

5.2 HISTOPATHOLOGY

Observations on the histology of gills and liver of *Clarias gariepinus* and *Oreochromis niloticus* were made to determine the extent of pathological damage after the fishes were exposed to lethal concentrations of zinc and cadmium. Lethal concentrations of zinc (12 and 16 mgl^{-1}) and cadmium (14 $mgCdl^{-1}$) to *C. gariepinus* caused proliferation of epithelial cells leading to complete blanketing of the secondary lamellae (Plates V a,b and VI). Sublethal concentrations of zinc, ammonia and cadmium have

also been known to induce similar effects (Smart, 1976; Tuurola, 1983; Gardner, 1972). Similar caustic effects have also been observed in the interlamellae gill epithelium of mummichogs exposed to mixtures of copper and zinc (12 Zn^{2+}/1 Cu^{2+}) (Eisler and Gardner, 1973). The gills of *O. niloticus* exposed to 26 $mgZnl^{-1}$ showed lamellae disarray and epithelial detachment (Plate VII). Similar observations were made by Drewett and Abel (1983) who worked on pathology of lindane poisoning and of hypoxia in the brown trout, *Salmo trutta*.

The proliferation of epithelial cells which eventually leads to blanketing of the lamellae may be said to be a protective measure by increasing the pollutant blood diffusion distance. Hyperplasia of the lamellae might have caused adjacent lamellae to fuse together and thus decrease the free surface of the lamellae. Extensive fusion of such lamellae may eventually cause gill filaments to grow together into a solid mass and this may impair gaseous exchange. The detachments of the lamellae along the axis of the gill filament might have been a response by the fish to increase the lymphatic space and thus increase the diffusion distance of water to the blood spaces of the secondary lamellae. However, this response can only be substantiated if morphometric measurements were taken. In studies where morphometric measurements were used, it was reported that such detachment increased the diffusion distance of water and the blood spaces of secondary lamellae of the gills of *Salmo gairdneri* exposed to 1 $mgZnl^{-1}$ (Tuurala, 1983).

Abel and Skidmore (1975) suggested that the epithelial detachments which commonly occurs in fish exposed to pollutants

arises out of necessity for the fish to maintain an osmotic barrier in the face of a progressive loss of cells in the epithelium. The resulting redistribution of the diminishing number of viable cells demands that the total exposed gill area be reduced. Such a cellular rearrangement presumably causes an increased oxygen demand by the epithelial cells. In fish exposed to pollutants (other than metabolic inhibitors! the gill epithelial cells will have ready access to oxygen, even though deeper tissues may be hypoxic. Light microscope studies on structural modifications of the gill filament of dogfish, *Scyliorhinus canicula* following treatment with sublethal doses of zinc showed that chloride cells increased significantly in number after exposure to zinc (Crespo *et al.*, 1981). In the present study, chloride cells were not observed in the interlamellar spaces of the primary epithelium of the fish gills as only haernatoxyl in and eosin stains were used. Chloride cells are known to stain paler than neighbouring mucous or pavement cells with toluidine blue. Crespo and Sala (1986) suggested that zinc induces an acceleration of chloride cell turnover both producing hyperactivity in some cells and enhancing their degenerating rates. Cadmium and lead have also been described as accelerating the turnover of a very active cell type, the absorptive cell of the trout intestine (Crespo *et al.*, 1986).

Physiological and biochemical studies of fish exposed to lethal levels of zinc (Skidmore, 1970; Burton *et al.*, 1972) indicated that tissue hypoxia is a result of zinc poisoning and the probable immediate cause of death. Zinc does not appear to be a potent interna] poison (Skidmore, 1970). In the case of

zinc poisoning the tissue hypoxia is the severe gill damage (Skidmore and Tovell, 1972) resulting to inadequate respiratory exchange. Other poison such as detergents (Abel and Skidmore, 1975) which cause severe gill damage at acutely lethal concentrations may act in a similar manner. The gill damage associated with acute toxicity of zinc and cadmium is relatively high compared to that caused by other poisons e.g. lindane (Drewett and Abel, 1983). It is not similar to that which occurs in the early stages of poisoning by zinc (Skidmore and Tovell, 1972) and detergents (Abel and Skidmore, 1975), and which progresses to a more severe condition which is sufficient to cause death. If tissue hypoxia occurs as a result of zinc poisoning, it is likely to be due to loss of co-ordination in the cardiovascular and/or respiratory systems as well as gill damage.

Nevertheless, chronically toxic levels of zinc may affect the gills of fish in some instances. Matthiessen and Brafield (1973) found marked gill changes in *Gasterosteus aculeatus* in sublethal zinc concentrations, such as the appearance of vacuoles and myelin-like bodies in gill epithelium and an increase in gill chloride cells. They concluded that the increase in chloride cells was a direct response to zinc influx, and that such cells are involved in excreting other unwanted ions in addition to chloride ions.

Liver cells of *O. niloticus* and *C. gariepinus* exposed to lethal concentrations of zinc and cadmium showed precipitating cytoplasm indicated by unclear and less homogenous cytoplasmic morphology; hepatic vacuolarity; disintegrating nucleus; disintegrated blood vessels and pyknotic hepatocytes. These

structural impairment of hepatocytes in livers of these freshwater fishes are similar to those reported by Kumar and Pant (1981) in *punctus conchonius* after copper and zinc poisoning. The structural changes in the livers of both cadmium and zinc treated fishes are similar to those reported in fish liver cells following damage or injury (Crespo and Sala, 1986) and are indicative of the occurrence of cell death rather than of any specific cause of cell death. Therefore, the possibility of a direct action of zinc and cadmium on liver cells can not be excluded since it is known that these metals are absorbed by fish (Abdullah *et al.*, 1976; Lovett *et al.*, 1972). In the present study, the occurrence of hepatocyte vacuolarity was not observed in the livers of the control fishes. Alan *et al.*, (1983) observed hepatic vacuolarity in the livers of rainbow trout exposed to lead (Pb) and the control and concluded that the interpretation of liver changes from increased hepatocyte vacuolarity as a result of lead exposure was unreliable. It appears that the major target of zinc and cadmium in the case of fish is the gill epithelium and both the gills and the liver may become damaged by exposure of fish to lethal concentrations of these metals. This has being verified by light microscopic studies of these organs.

5.3 HAEMATOLOGY

The study of haematological parameters of fish is relevant because of their relationship with energetics (metabolite levels), respiration (haemoglobin concentration and related parameters) and defence mechanisms (Leucocytes values or WBCC).

Haematological parameters found in control fishes, *Clarias gariepinus* and *Oreochromis niloticus* in this study are within the normal range of teleost blood parameters. Both fish species presented high RBCC which is typical of teleost. The RBCC in teleost is within the range of 700,000-2,000,000 cells mm^{-3} (Saunders, 1966). Teleost fishes also have bigger corpuscular volumes and very high MCH (near to 300 pg $cell^{-1}$) (Saunders, 1966). However, the MCH values of control fish, *O. niloticus* were found to be very low (77 $\pm$ 1 pg $cell^{-1}$); but that of *C. gariepinus* (284 $\pm$ 11 pg $cell^{-1}$) did not differ significantly ($P > 0.05$) from the value (300 pg $cell^{-1}$) reported by Saunders (1966). The reason for the very low value of MCH in *O. niloticus* is not known.

Metal exposure induces alterations in haematological parameters, generally because of changes in blood water content. Both haemoconcentration and haemodilution have been described in different experiments. The muscle has been known as the water exchange tissue with blood (Waiwood, 1980). For instance, haemoconcentration after copper exposure and haemodilution after zinc exposure have been observed in *Colisa fasciatus* (Mishra and Srivastava, 1979, 1980). In the present study, decrease of haematocrit following the exposure of *C. gariepinus* and *O. niloticus* to zinc and cadmium concentrations may be an indication of haemodilution. Tort and Torres (1988) also reported decrease of haematocrit following 24 h exposure of dogfish, *scyliorhinus canicula* to cadmium contamination and attributed it to haemodilution. However, Waiwood, (1980) and Flos *et al.*, (1987) reported haematocrit increases in different fish species treated

with zinc. Waiwood (1980) attributed increases in haematocrit to the swelling of the red blood cells. Flos *et al.*, (1987) however, attributed increase in haematocrit levels to increase in size of the erythrocytes due to increased protein carbondioxide (PC_{02}) in the blood. These changes could be the result of hypoxia or stress caused by sampling producers (Holeton and Randall, 1967; Soivio *et al.*, 1974 a, b).

The RBCC increased except for *O. niloticus* exposed to zinc. Mishra and Srivastava (1980) reported increase in RBCC in *Colisa fasciatus* exposed to copper at 3ppm for 96 h. They however recorded a decrease in RBCC in the same species exposed to 100 ppm of zinc (Mishra and Srivastava, 1979). EL-Domiaty (1987) also reported decreased values of RBCC in *Clarias lazera* exposed to copper. RBCC elevation observed in the present study could be a consequence of blood cell reserve release combined with cell shrinkage, probably due to osmotic alterations of blood by the action of the metal (Tort and Torres, 1988). Spleen contraction after stress has been detected in fish (Abrahamsson and Wilsson, 1975). Cells released from the spleen which is an erythropoietic organ (Fange and Johansson - Sjobeck, 1975) would then have a lower MCV value. In this study, lower MCV values were observed in *C. gariepinus* exposed to zinc and cadmium salts respectively. A similar pattern has been observed in *Cyprinus carpio* after cadmium exposure (Koyama and Ozaki, 1984) in which haematocrit decreased, RBCC maintained and the circulating reticulocytes increased. These were an indication of the release of pre-mature cells into the blood stream. However, elevated MCV values were recorded in *O. niloticus* exposed to zinc and cadmium salts

respectively.

Measurements of haemoglobin (Hb) in plasma of *C. gariepinus* and *O. niloticus* exposed to sublethal concentrations of zinc and cadmium did not show any increase in values. A similar observation was made by Tort and Torres (1988) who studied the effects of cadmium on haematological parameters of dogfish, *Scyliorhinus canicula*. They recorded a very significant haemoglobin concentration lower than the control after 96 h of exposure. The decline in Hb values could be associated with some action of the metal on haeme metabolism, since interactions between metals and enzymes have been known to be involved in haeme metabolism (Dalwani *et al.*, 1985).

Mishra and Srivastava (1979) reported that 100 ppm of zinc decreased the WBCC in *Colisa fasciatus*. Also, Flos *et al.*, (1987) reported a decrease in WBCC in females of *S. canicula*. Joshi (1980) suggested that developing gonads through a gradually decreasing space in the abdominal cavity depletes feeding in females. This fact could explain the low number of leucocytes, because WBCC in fish blood exhibit a direct correlation with the feeding status (Smirnova, 1965). In this study also, decrease in WBCC was observed except for cadmium-treated *Oreochromis niloticus* in which case there was an increase in WBCC. Matthiessen (1981) reported increase in WBCC in pike (*Hepsetus odoe*) following stress and endosulphan treatment. Leucocytosis has been observed in *S. canicula* after endosulphan aerial spraying (Matthiessen, 1981).

Haematological values related to oxygen transport (RBCC, Hb, haematocrit) and calculated indices (MCV, MCH and MCHC) showed

significant ($P < 0.05$) difference between control and zinc or cadmium treated groups in *C. gariepinus* and *O. niloticus*. Therefore, it appears that sublethal exposure of zinc and cadmium act as a stressor, producing the usual haematological and blood metabolite level responses to an unspecific stressor, except for haemoglobin whose pattern may be attributable to a specific cadmium action on haeme metabolism. The most susceptible haematological parameters of *C. gariepinus* and *O. niloticus* appeared to be haematocrit, Hb and RBCC. Heavy metals cause a stress-inducing effect on fish during the course of long-term exposure as indicated by alterations in blood parameters. In such cases the fish become more susceptible to infectious diseases and may be more vulnerable to predators.

5.4 BIOACCUMULATION OF ZINC

Zinc accumulation in tissues of *Clarias gariepinus* increased with increase in duration of exposure. There was significant difference ($P < 0.05$) in the zinc burden reported for control and exposed fish throughout the assay. Zinc residues in fish tissues was in the order of liver > gut > gills > whole fish > skin > muscles. Zinc enrichment of fish organs has been reported in some laboratory experiments with other fish species (Farmer *et al.*, 1979; Flos *et al.*, 1979; Pierson, 1981). Beveridge *et al.*, (1985) reported highest concentrations of metals in the bone and skin tissues except iron levels which they found to be highest in the haemoglobin-rich livers.

In fish, zinc is generally low in muscles and high in gonads (Brooks and Rumsey, 1974; Badsha and Goldspink, 1982). The

results of this study also show very low accumulation of zinc in muscles. The low accumulation of zinc in muscles may be attributed to a factor such as dilution with growth. Growth may dilute metal concentrations in an organism if growth is faster than rate of metal accumulation (Badsha and Goldspink, 1982; Arnac and Lassus, 1985). Beveridge *et al.*, (1985) and Giesy and Wiener (1977) also reported lowest levels of trace metals in muscle tissues. Jeng and Sun (1981) found that when common carp (*Cyprinus carpio*) were fed high levels of ZnS_{04}, zinc levels increased in skeletal tissues before it did in muscle tissue. In their study, muscle levels did not increase until the skeletal tissues were saturated, which required 8 weeks of feeding a diet containing 1,007 $\mu gZng^{-1}$. Nemcsok *et al.*, (1987) found the order of the concentrations of copper taken up by various organs of carp (*Cyprinus carpio*) as: Skeletal muscle > liver > gills > intestine > kidney > heart > brain. They found the high copper content of the skeletal muscle to inhibit the acetylcholinesterase activity in this organ. They correlated this with the greater activity of the fish at $20^{0}C$. During the course of this movement more copper which has not been transported yet towards other copper storing organs during treatment period, may enter the organ because of the enhanced blood-supply and accelerated metabolic process of the skeletal muscle. The high amount of zinc in fish gonads (Brooks and Rumsey, 1974; Badsha and Goldspink, 1982) is expected as zinc is presumably used for gonad ripening.

The present study recorded the highest accumulation of zinc in the liver. Beveridge *et al.*, (1985) also reported the highest

amounts of iron in haemoglobin rich livers. Nemcsok *et al.*, (1987) found very high amounts of zinc in gills and liver of fish. The high accumulation of zinc in the liver may be related to the fact that the liver plays an important role in its accumulation and detoxication (Reichenback-Klinke, 1972). Since fishes are known to possess the metal-binding protein, metallothionein (Friberg *et al.*, 1971), a sequestering agent, detoxication of these metals in fish liver may be sequestration rather than elimination (Giesy and Wiener, 1977). Increasing metal concentration in liver may represent storage of sequestered products in that organ. McDonald *et al.*, (1976) however, reported accumulation of zinc in skeletal material rather than in the liver.

The gills were observed to have accumulated high amount of zinc in the current study. A similar observation was made by Brooks and Rumsey (1974); Friedman and Shibko (1972); Nemcsok *et al.*, (1987) who studied bioaccumulation of different trace metals to different fish species. Zinc accumulates both in dogfish gills (Crespo *et al.*, 1979) and internal organs (Flos *et al.*, 1979) and induces a depressive effect on tissue respiration consistent with a general situation of oxygen deficiency in the whole fish leading to possible death by hypoxia (Burton *et al.*, 1972). The high accumulation of zinc in gills may be related to the fact that gills play a vital role in zinc uptake; they are in direct contact with the toxicant and the toxicant adheres to the gill surfaces; and also that gills are osmoregulatory organs and may store zinc for excretion. Active transport of ^{65}Zn into gill tissue supports the zinc excretory function of gills (Ronald

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et al., 1987). However, Pentreath (1973) found high concentration of ^{65}Zn in the kidney of plaice and suggested that urine was a major route of zinc excretion. Badsha and Goldspink (1982) however, reported very low accumulation of zinc in fish gills.

Trace metals can be incorporated into fish along two pathways: absorption across the gill surfaces or through the gut tract wall. A relationship between trace metal concentrations in fish and the trophic level of the species studied has been suggested, indicating the importance of the food pathway. Mathis and Cummins (1973) found that significantly higher concentrations of zinc were present in omnivorous fishes than carnivores. Windom *et al.*, (1973) noted that planktivorous marine fishes contained higher concentrations of zinc and cadmium than did carnivorous species from the same location. In the present study very high amount of zinc was found to be accumulated in the gut of *Clarias gariepinus* exposed to sublethal concentration (1.325 $mgZnl^{-1}$) of zinc for 8 weeks. Badsha and Goldspink (1982) also reported high zinc content of roach guts and suggested that food is the main route for intake of metals of this species. Hoss (1964), Pentreath (1976) and Cowx (1982) have shown that food may greatly influence the amount of trace metals in fish. This is further supported by Patrick and Loutit (1978) who reported high zinc uptake when metal-enriched tubificid worms were used as fish food, and by Willis and Sunda (1984) who used ^{65}Zn-enriched *Artemia* species as food of marine fish. Rainbow trout tolerate dietary zinc levels of 1700 $\mu g\ g^{-1}$ without apparent toxicity and without accumulating zinc in the tissues other than liver, blood

and gills (Wekell *et al.*, 1983). In several marine fish species uptake from food even amounts to 60-80% of total zinc uptake (Hoss, 1964; Willis and Sunda, 1984). Also, zinc content of trout in particular depends on uptake from their diet (Dallinger and Kautzky, 1985; O'Grady and Abdullah, 1985).

Mean whole fish concentrations of zinc in *C. gariepinus*(16.17-84 $\mu g\ g^{-1}$Zn dry weight) were within the range of average concentrations for whole fish from relatively uncontaminated aquatic ecosystems (48-173 $\mu g\ g^{-1}$Zn dry weight) (Havre *et al.*, 1973; Kelso and Frank, 1974; Giesy and Wiener, 1977). Lande (1977) found 80 μ g g^{-1}Zn (dry weight) in whole fish from a contaminated fjord in Norway. Zinc accumulation in *C. gariepinus* may probably be via the gill, opercular bone and the skin. It may be explained that the zinc accumulation via the gills is a reflection of direct contact of gills with zinc contaminated water. The direct contact the skin of *C. gariepinus* has with zinc-treated water probably assisted in the accumulation. However, relatively low concentration of zinc was accumulated in the skin probably due to less affinity of inorganic zinc at low concentration with the thiol or suphydryl groups (component of the fish skin) (A De Bruin, 1976). The results from the uptake experiments appeared to suggest that the muscle would be the tissue of choice for routine experiment on zinc content of freshwater fish. This is based on the fact that the concentration of this metal in the muscle has been shown to be consistently less than in any other tissue examined. Sample variation of muscle tissue concentration of zinc with the control was small and the muscle is the tissue most commonly consumed by

the public.

The bioconcentration factors (BCFs) of zinc in fish tissues increased with increase in duration of exposure. The BCF of zinc in tissues of *C. gariepinus* were in the order of liver > gut > gills > whole fish > skin > muscles. The values of BCF in tissues were significantly different ($P < 0.05$) from each other. A comparison of BCFs revealed that of the liver of *C. gariepinus* to be significantly greater ($P < 0.05$) than other organs. Since BCF is the concentration in body tissue per unit concentration in the medium, the liver may therefore be a better indicator organ for measuring zinc concentration in *Clarias* species.

Available evidence supports the concept of trace metal equilibrium being sought by freshwater fish. Abdullah *et al.*, (1976) found that a gradient of zinc and manganese concentrations in freshwater determined the zinc and manganese concentrations in scales of native salmonid fishes sampled along that concentration gradient. Also, Van Collie and Rousseau (1974) found higher concentrations of trace metals in fish from polluted sites and Lovet *et al.*, (1972) related cadmium concentrations in freshwater fish to background levels of cadmium. The study by Abdullah *et al.*, (1976) suggested that the relationship between fish and the environment was a direct one. Nevertheless, the concentrations of trace metals in food has also been shown to play an important role in determining the concentration of trace metals in fish tissues (Hoss, 1964; Pentreath, 1976; Singh and Ferns, 1978). It seems likely however, that trace metal concentrations in the natural food supply are influenced by environmental factors and that the levels of trace metals in

natural food represents the integrated history of the variation in environmental trace metals experienced.

Physical factors may influence trace metal uptake in freshwater organisms for example temperature dependence of zinc uptake as observed in tubificid worms (Dean, 1974). The present study has shown substantial differences in zinc concentrations between tissues of *Clarias gariepinus*. Ronald *et al.*, (1987) found substantial differences in ^{65}Zn concentration between tissues of rainbow trout (Nakatani, 1966), in carp (Saiki and Mori, 1955) and in rats (Heth *et al.*, 1966). These differences may reflect the degree of vascularization and rates of zinc turnover in these tissues. Skeletal abnormalities are frequently observed in fish from metal contaminated environments (Muramoto, 1981; Sloff, 1982) and in mammalian systems a number of heavy metals including zinc have been shown to be potent inhibitors of mineralization process in vitro and in vivo (Bird and Thomas, 1963; Russel *et al.*, 1973). In the present study, skeletal abnormalities were not observed. Although low levels of zinc are thought to be essential for the mineralization process, high concentrations have been shown to inhibit calcium phosphate precipitation both in vitro and in vivo (Bird and Thomas, 1963; Guggenhein and Gaster, 1973; Russel *et al.*, 1973).

Zinc could also affect calcification in a more indirect way. Mugiya (1980) showed that calcium incorporated into regenerating scales is taken up directly from the water by the gills. Gills are susceptible to damage by zinc pollution (Tuurala and Soivio, 1982) which could reduce the amount of calcium taken up and made available for scale calcification. In the clam, *Mulinia*

lateralis, cadmium and mercury have all been shown to significantly reduce calcium uptake (Ho and Zubkoff, 1982).

Decapoda can regulate the zinc content in their body tissues and fluids homeostatically up to high zinc concentrations in water (Bryan, 1967; Giesy *et al.*, 1980; White and Rainbow, 1984; Devineau and Amirard Triquet, 1985). However, Memmert (1987) found zinc content of polluted *Daphnia magna* to increase very fast to a high extent. This shows that the part of zinc bound to particles must have substantially contributed to the zinc content of filter-feeding *Daphnia*. Zinc may also be adsorbed on the body surface as shown for freshwater crayfish (Bryan, 1967). In fish, zinc uptake is through the gills and gastrointestinal walls. Zinc is mainly accumulated in internal organs, the liver, gut, gills, skin but to a small extent in muscle tissues.

5.5 BIOACCUMULATION OF CADMIUM

Cadmium residues in tissues of *Clarias gariepinus* and *Oreochromis niloticus* increased with increase in duration of exposure. Cadmium burden in treated and controlled fishes differed significantly ($P < 0.05$) throughout the assays. Accumulation of cadmium residues in both fishes was in the order of the liver > gut > gills > skin > muscles > whole fish. The distribution of cadmium in the fish species showed that the liver has the highest concentrations of this metal while the muscles and whole fish have the least concentration. A similar observation was made by other authors (Giesy and Wiener, 1977; William *et al.*, 1979; Hodson *et al.*, 1978; Oladimeji, 1980; Beveridge *et al.*, 1985) who studied the accumulation of different

metals in tissues of varied fish species. The high accumulation of cadmium in fish liver may be in connection with the function of the liver as a detoxicating organ. Fish is known to have metal-binding protein, metallothionein (Friberg *et al.*, 1971), a sequestering agent. Detoxication of this metal in fish liver may be sequestration rather than elimination (Giesy and Wiener, 1977). Metal accumulation in liver may represent storage of sequestered products in that organ.

The low accumulation of cadmium in muscle tissue may be related to the diluting effect of muscles as a result of growth. The results of the bioaccumulation tests seems to suggest that the muscles of *C. gariepinus* and *O. niloticus* would be the tissue of choice for routine experiment on cadmium content of these freshwater fishes. This is due to the fact that the concentrations of this metal in the muscles have been shown to be less than in any other tissues examined. The sample variation of muscle tissue concentration of cadmium with the control was relatively small and the muscle is the tissue consumed by the public. The guts of these fishes contained high levels of cadmium accumulation. In fish, the main sites of heavy metal uptake are the gills and the gastrointestinal tract (Pentreath, 1973; Part and Svanberg, 1981; Lovegrove and Eddy, 1982). The importance of uptake from diet may also depend on the concentration in water, as for example, the concentration factor in *Daphnia magna* decreases with increasing cadmium concentration in water (Poldoski, 1979). So at lower heavy metal concentration in water a relatively higher amount may be accumulated in algae or zooplankton and consumed by fish. The high accumulation of

cadmium in the guts of these freshwater fishes may suggest that food path may be the main route for intake of metals in these species.

The results of this study show that not very high amount of cadmium was recorded in the gills and skin of *O. niloticus* and *C. gariepinus*. Other authors (Brooks and Rumsey, 1974; Friedman and Shibko, 1972; Nemcsok *et al.*, 1987) have reported very high accumulation of trace metals in gill tissues. In fish, metal ions are normally excreted via the kidney or chloride cells of the gills (Bryan, 1976; Crespo *et al.*, 1981). Exposure of fish to elevated concentrations of heavy metals induces the synthesis of metallothionein (Noel-Lambot *et al.*, 1978) which bind and detoxify the metal ions (Kojima and Kagi, 1978). However, the animals ability to synthesise these proteins and the proteins' capability for metal binding is finite (Brown and Parsons, 1978). If the limits of the metabolic capabilities for excretion and metal binding are exceeded, toxic effects will result unless the fish has an alternate way of detoxification. As shown for Cd^{2+} in the mussel, *Mytilus edulis* (Carpene and George, 1981) metals transported through the body fluids of fish are probably bound to metallothionein or other organic ligands. Noel-Lambot *et al.*, (1978), have identified Cd, Zn and Cu-binding proteins in the gills and body tissues of the eel, *Anguilla anguilla*. The low accumulation of cadmium in gill tissues obtained from this study is not expected as the gills are in direct contact with the toxicant and the gills are osmoregulatory and excretory organs hence they may store cadmium for excretion.

The skin had relatively lower concentrations of cadmium than

the liver and gut, but invariably higher in exposed fishes than in the controls. This does not agree with Beveridge *et al.*, (1985) who found that most metal occurred in highest concentrations in the bone and skin tissues. The lowest accumulation of cadmium in both species was in whole fish. However, the mean whole fish concentrations of cadmium obtained from each species (0.4-1.32 μg Cd g^{-1} for *C. gariepinus*; 0.04-1.24 μg Cd g^{-1} for *O. niloticus*) were higher than the range of average concentrations (0.08-0.38 μg Cd g^{-1} dry weight) for whole fish from a relatively contaminated aquatic ecosystem reported by Murphy *et al.*, (1978). Lande (1977) reported only 0.30 μg Cd l^{-1} (dry weight) in whole fish from a contaminated fjord in Norway.

Although the accumulation of cadmium residues in *C. gariepinus* and *O. niloticus* was in the same order, (liver > gut > gills > skin > muscles > whole fish), the levels of cadmium varied between these species with the tissues of *C. gariepinus* recording the highest accumulation of cadmium. It appears that the tissues of *C. gariepinus* absorbed cadmium more rapidly than those of *O. niloticus*. The difference in rate of absorption of cadmium in these freshwater fishes may be attributed to the integumental difference in these two species. *C. gariepinus* is a scale less fish and the body is in direct contact with the toxicant whereas *O. niloticus* has scales that provide some protection from the toxicant. Also, the differences in cadmium levels observed in the two species may reflect differences in homeostatic control abilities or a reflection of the degree of vascularization and rates of cadmium turnover in these tissues.

Concentrations of cadmium in fish tissue are very variable and do not seem to be under the strict physiological control evident with essential element, zinc. Cadmium concentrations do not appear to be correlated to length, weight or age of fish (Murphy *et al.*, 1978). In the present study, a fairly uniform size of each fish species was used throughout the study, hence it is not thought that size might have influenced cadmium uptake in any of these freshwater fishes. It was also observed that the *Clarias* species accumulated very high levels of zinc in the body tissues than it did for the cadmium metal. Essential and metabolically useful trace metals such as zinc (Love, 1970; Bowen, 1972) are concentrated in tissue in excess of requirements. Some of this excess is stored in the liver and skeletal material from where it can be drawn in times of shortage or low input of trace metals.

Cadmium is the major cause of Itai-itai disease (Nogawa, 1981) and it is known to cause extreme skeletal abnormalities in fish (Muramoto, 1981 a, b). Cadmium also inhibits calcification in the marine coccolithophorid, *Criscophaera carterae* (Stillwell and Corum, 1982). In the present study skeletal abnormalities were not observed in fish as very low concentrations of cadmium were used.

The BCF of this study increased with increase in time of exposure. The BCF obtained from this study was in the order of liver > gut > gills > skin > muscles > whole fish in both species. Just like in the accumulations of cadmium in fish tissues, the BCF of tissues of *C. gariepinus* were found to be much higher than those of *O. niloticus*. Therefore, *C. gariepinus*

may be a better indicator species for monitoring cadmium concentrations in fish communities. Once metal is taken up from the environment, the fish must either eliminate it through excretion or sequester it to prevent toxic conditions (Bryan, 1979). An easy way to achieve this is by precipitating the metal in an insoluble form since it is the free metal ion which is most toxic to fish (Simkiss, 1977).

CONCLUSION

From acute toxicity tests, alterations of fish's behaviours as a result of their exposure to lethal concentrations of heavy metals was dose-dependent and was reflected in mean score of symptoms of toxicosis. Behavioural changes in fish are sensitive responses to pollutants especially heavy metals. The symptoms of toxicosis particularly the activity of the mucous cells was more on *Clarias gariepinus* which resulted to large deposition of mucous on the gill filaments. Zinc and cadmium ions combine with mucous which covers the gills thereby interfering with respiration by slowing down diffusion in the secondary lamellae, (Davis, 1973). This may lead to death as a result of suffocation. The production of large amount of mucous on the gill filaments confirms the observation of Lauren and McDonald (1985), that the gill is the first target organ affected by heavy metals. Toxic action of metals appeared to be a combined effect of precipitation of mucous on the gills and osmoregulation stress with death resulting from suffocation. Another symptom of toxicosis was swollen abdomen observed in the *Clarias* species exposed to lethal concentrations of cadmium. However, *Clarias* species exposed to lethal concentrations of zinc/cadmium mixture did not show this symptom. Zinc may have caused an inhibition in gastrointestinal absorption of cadmium in these species.

From the LC_{50s} of the study it was shown that fingerlings of *O. niloticus* and *C. gariepinus* were tolerant to zinc and cadmium although the later was more susceptible to both of these metals. Acute toxicity tests also showed that cadmium was more toxic to these freshwater species. The toxic effects of cadmium are

presumably associated with the metals affinity for organic ligands containing sulphur, nitrogen and other electronegative functional groups (Ray and Coffin, 1977). However, zinc/cadmium mixture proved to be the most toxic, producing 100% mortality in less than 96 h of exposure. Acute toxicity tests were designed to determine what concentrations would be lethal to the organisms in an aquatic environment so as to prevent the occurrence of such conditions. Acute toxicity test conducted in the laboratory may only indicate whether survival in nature is likely to be unfavourably affected by a lethal agent and no more. It does not give any idea as to the maximum concentration of the potential lethal agent that will not affect directly or indirectly the success of the species in nature. Knowledge of such concentrations are obtained from sublethal toxicity studies on the effects of the agent on the survival, reproduction, growth and behaviour of the species. These are not areas of emphasis in the current work but may be useful propositions for future studies. Toxic agents may affect an organism of economic importance to man either directly or indirectly by affecting the organisms it feeds on. Food organisms must be able to meet the nutritional budget of the fish if it is to grow and reproduce.

More research should be conducted to determine the concentration of metal pollutants which do not have adverse sublethal or chronic effect on the fish. This is called the safe-level. However, such investigation must indicate what life processes the safe-level refers to (i.e. whether reproduction, respiration, growth etc) because a level safe for one process may not be safe for another. This means there can be no single

formula for permissible concentrations of toxicants in receiving waters; as the various toxic substances have their own peculiarities and physiological, behavioural responses of the organisms may also vary.

Zinc and cadmium caused gill haemorrhage in fish probably due to necrotic damage in gill epithelium. This was confirmed by the histological aspects of this study. The defects caused by lethal concentrations of zinc and cadmium include epithelial proliferation, detachment along the central axis of the gill filament and fusion of the lamellae. Although these changes are clearly visible, the quantitative description of the extent of damage and the recognition of slight damage is more difficult to follow; it would also be important to know the particular way in which these damages influence the secondary lamellae structure. It is also known that the diffusing capacity will be reduced and consequently the fish subjected to some form of hypoxia. Lethal concentrations of zinc and cadmium caused vacuolation, ruptured blood vessels and fibrosis in fish liver cells. These damages rendered the liver ineffective in carrying out its function of detoxication.

Long-term (6 weeks) exposure of *C. gariepinus* and *O. niloticus* to sublethal concentrations of zinc and cadmium showed alterations of blood parameters of these fishes. The most susceptible parameters are haematocrit, Hb and RBCC. The alterations in blood parameters of these fishes is an indication that heavy metals cause stress-inducing effect which may lead to interference and damage to blood cells. These sublethal impairments of the fish's ability to combat infectious diseases

or its capacity to perform and adapt can reduce the chances for survival and the potential for growth, and reproduction. These effect though may be slight for individual members of a population, they may be detrimental to that population's long-term ecological success as measured by its abundance and distribution. The consequences may be as severe as extinction.

Clarias accumulated zinc in conditions of contamination and appeared to concentrate it mainly in liver, gut, gills, skin and muscles. Cadmium residues in tissues of *Clarias gariepinus* and *Oreochromis niloticus* were in the order of liver > gut > gills > skin > muscles > in whole fish. However, the *Clarias* species accumulated and concentrated more cadmium in its body tissues than the *Oreochromis* species. This difference in susceptibility may be an indication in the differences in homeostatic control abilities or a reflection of the degree of vascularization and rates of cadmium turnover in these freshwater fishes. The *Clarias* species accumulated more zinc than it absorbed cadmium. Zinc is an essential element and it is present in uncontaminated environments and may be stored in animal tissues for physiological function. In a polluted environment, both essential and non-essential or toxic metals can accumulate since the rate of intake may be higher than the animals excretory/ detoxication mechanisms. It is noted that the phenomenon of bioaccumulation or bioconcentration of zinc and cadmium is very variable; that the conditions can be defined under which in the present contamination, bioaccumulation will or will not occur; and that even when it does occur, it does not usually appear to be the cause of fish deaths.

In the present study, only the fingerling stage of the freshwater fishes was considered. However, there is a growing realization of the need to examine the effects of heavy metals on all stages of the fish life cycle. Within fish species there can be considerable intraspecific differences in metal tolerance. Within fish communities there can be marked interspecific difference in metal tolerance. There is evidence that indiscriminate discharge of effluent containing zinc and cadmium into our aquatic ecosystem can cause severe harmful effects on the ecology leading to the destruction of fish stock and perhaps other forms of aquatic life. If government's goal of self-reliance in food, particularly protein production is to be achieved, efforts should be made to reduce the discharge of toxic metals into our water bodies.

The effects of heavy metals on freshwater fishes are modified by concentrations of these metals, time of exposure and species. They are further modified by environmental factors such as water hardness, alkalinity, oxygen and carbondioxide concentrations, pH and temperature. These factors and the influence of age, stage in life cycle, behaviour, metabolism, acclimation and selective mortality must be considered when assessing the toxic effects of heavy metals on wild life populations. In laboratory situations these factors can be controlled or accounted for, but in natural situations, they may interact in a more complex fashion. This is especially true of the highly active migratory species where time and pattern of exposure may vary as the fish move around at different times during the life cycle.

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Appendix 1a % Mortality of *Oreochromis niloticus* Exposed to Various Concentrations of Zinc

Time	Triplicate	% Mortality/Zinc Conc. mgl^{-1}					
Hour		20	22	24	26	28	Control
24	A	0	0	20	10	50	0
	B	0	0	20	60	10	0
	C	0	0	0	10	10	0
48	A	0	0	50	50	60	0
	B	0	10	40	70	90	0
	C	0	10	30	20	20	0
72	A	0	0	70	60	70	0
	B	0	20	50	70	90	0
	C	0	10	30	30	50	0
96	A	0	0	70	60	80	0
	B	10	30	50	80	90	0
	C	0	20	40	60	80	0

Appendix 1b % Mortality of *Oreochromis niloticus* Exposed to Various Concentrations of Cadmium

Time	Triplicate	% Mortality/Cd Conc. mgl^{-1}					
Hour		16	18	20	22	24	Control
24	A	0	10	20	70	90	0
	B	20	40	10	40	40	0
	C	0	0	80	90	40	0
48	A	10	20	40	80	90	0
	B	30	60	30	50	50	0
	C	0	0	90	90	60	0
72	A	10	20	50	80	90	0
	B	30	60	30	60	70	0
	C	10	10	90	90	60	0
96	A	30	20	60	80	90	0
	B	10	60	30	60	70	0
	C	0	20	90	90	80	0

Appendix 1c % Mortality of *Oreochromis niloticus* Exposed to Various Concentrations of Zinc/Cadmium Mixture

Time	Triplicate	% Mortality/Zn/Cd Conc. mgl^{-1}					
Hour		20/16	22/18	24/20	26/22	28/24	Control
24	A	0	10	30	30	50	0
	B	10	20	20	30	40	0
	C	0	10	30	40	50	0
48	A	10	30	80	70	100	0
	B	20	20	60	60	80	0
	C	10	40	70	80	100	0
72	A	40	50	80	70	100	0
	B	40	60	70	90	100	0
	C	50	50	90	100	100	0
96	A	60	60	80	90	100	0
	B	50	60	90	100	100	0
	C	50	70	90	90	100	0

Appendix 1c % Mortality of *Oreochromis niloticus* Exposed to Various Concentrations of Zinc/Cadmium Mixture

Time	Triplicate	% Mortality/Zn/Cd Conc. mgl^{-1}					
Hour		20/16	22/18	24/20	26/22	28/24	Control
24	A	0	10	30	30	50	0
	B	10	20	20	30	40	0
	C	0	10	30	40	50	0
48	A	10	30	80	70	100	0
	B	20	20	60	60	80	0
	C	10	40	70	80	100	0
72	A	40	50	80	70	100	0
	B	40	60	70	90	100	0
	C	50	50	90	100	100	0
96	A	60	60	80	90	100	0
	B	50	60	90	100	100	0
	C	50	70	90	90	100	0

Appendix 2a % Mortality of *Clarias gariepinus* Exposed to Various Concentrations of Zinc

Time	Triplicate	% Mortality/Zinc Conc. mgl^{-1}					
		10	12	14	16	18	Control
Hour							
24	A	0	0	0	0	0	0
	B	0	0	0	0	10	0
	C	0	0	10	0	0	0
48	A	0	0	30	50	30	0
	B	0	10	10	20	20	0
	C	0	0	10	10	20	0
72	A	10	10	30	50	60	0
	B	0	30	30	70	50	0
	C	0	10	30	70	70	0
96	A	30	30	50	80	90	0
	B	20	30	50	80	90	0
	C	10	40	40	80	80	0

Appendix 2b % Mortality of *Clarias gariepinus* Exposed to Various Concentrations of Cadmium

Time	Triplicate	% Mortality/Cd Concs. mgl^{-1}					
Hour		8	10	12	14	16	Control
24	A	0	0	0	0	40	0
	B	0	20	10	10	40	0
	C	0	0	0	0	10	0
48	A	0	0	10	50	90	0
	B	0	60	60	60	70	0
	C	0	0	50	0	60	0
72	A	0	0	40	100	100	0
	B	0	60	80	100	100	0
	C	0	20	50	40	60	0
96	A	0	0	60	100	100	0
	B	10	60	90	100	100	0
	C	10	30	70	70	90	0

Appendix 2c % Mortality of *Clarias gariepinus* Exposed to Various Concentrations of Zinc/Cadmium Mixture

Time	Triplicate	% Mortality/Zn/Cd Concs. mgl^{-1}					
Hour		10/8	12/10	14/12	16/14	18/16	Control
24	A	0	20	40	100	100	0
	B	20	30	60	90	100	0
	C	20	20	40	90	100	0
48	A	10	40	60	100	100	0
	B	20	40	70	100	100	0
	C	30	30	50	90	100	0
72	A	20	60	80	100	100	0
	B	30	50	80	100	100	0
	C	30	50	70	90	100	0
96	A	20	60	90	100	100	0
	B	30	50	80	100	100	0
	C	30	50	70	100	100	0

Appendix 3 Acute Toxicity of Zinc to *Clarias gariepinus*. Regression Analysis for 96 Hour LC_{50}

% Mortality (Probit) Y	Log. of Conc. X	XY	X^2	Y	X	$E(X)^2$
4.1584	1.000	4.1584	1	5.11958	1.1368	32.3078
4.5684	1.079	4.9293	1.1642			
4.9172	1.146	5.6351	1.3133			
5.8416	1.204	7.0332	1.4496			
6.1123	1.255	7.6709	1.575			
E=25.5979	5.684	29.4269	6.5021			

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

$$b = \frac{29.4269 - 5.684 \times 5.11958}{6.5021 - 6.46157}$$

$$b = \frac{0.3272}{0.0405}$$

$$b = 8.079$$

$$a = Y - bX$$

$$a = 5.11958 - 8.079 \times 1.1368$$

$$= 5.11958 - 9.1842$$

$$a = -4.0646$$

Choosing Two Values of X

When X = 1.000 and when X = 1.255

$$Y = a + bX$$

$$Y = -4.0646 + 8.079 \times 1.000$$

$$Y = 4.0144$$

When x = 1.255

$$Y = -4.0646 + 8.079 \times 1.255$$

$$Y = 6.0745$$

96 Hour LC_{50} = Value of X when Y = 5

$$Y = a + bX$$

$$X = \frac{Y - a}{b}$$

$$\therefore X = \frac{5 - (-4.0646)}{0.079}$$

$$X = 1.1219$$

Antilog = 13.24

i.e. Actual Conc. = 13.24 $mgZnl^{-1}$

$\therefore$ 96 h LC_{50} = $\underline{13.24\ mgZnl^{-1}}$

96 h Lc_{84} = Value of X when Y = 5.9945

$$X = \frac{5.9945 - (-4.0646)}{8.079}$$

$$\approx 1.245062214$$

Antilog = 17.5829

$\therefore$ LC_{84} = 17.58 $mgZnl^{-1}$

Similarly, LC_{16} = value of X when Y = 4.0055

$$X = \frac{4.0055 - (-4.0646)}{8.079}$$

$$X = 0.998898378$$

Antilog = 9.9746

LC_{16} = 9.97 $mgZnl^{-1}$

Appendix 4 Calculation of Upper and Lower Limits for 96 Hours LC_{50} of *Clarias gariepinus*

$LC_{50} = 13.24$

$LC_{84} = 17.58$

$LC_{16} = 9.97$

$$S = \frac{LC_{84}}{LC_{50}} + \frac{LC_{50}}{LC_{16}/2}$$

$$S = \frac{17.58}{13.24} + \frac{13.24}{9.97/2}$$

$$S = \frac{1.327794562 + 1.327983952}{2}$$

Slope = 1.3278

$$LC_{50} = S\ \frac{2.77}{\sqrt{N^1}}$$

N^1 = Total number of animals used between 16 and 84% expected effects

$N^1 = 120$

$\sqrt{N^1} = \sqrt{120}$

$= 10.954451$

$LC_{50} = 1.3278^{2.77/7.7459}$

$LC_{50} = 1.0743257$

The upper and lower 95% limits of the LC_{50} are given by:-

Upper limit = $LC_{50} \times LC_{50}$

$= 13.24 \times 1.0743257$

Upper limit = 14.65 $mgZnl^{-1}$

$$\text{Lower limit} = \frac{LC_{50}}{LC_{50}}$$

$$= \frac{13.24}{1.0743257}$$

Lower limit = 12.324009 $mgZnl^{-1}$

:. The LC_{50} and 95% confidence limits are 13.24 (12.324-14.65) $mgZnl^{-1}$

Appendix 5 Acute Toxicity of Zinc to *Oreochromis niloticus*

Regression Analysis for 96 Hour LC_{50}

% Mortality (Probit) Y	Log. of Conc. X	XY	X^2	Y	X	$E(X)^2$
3.1616	1.301	4.1132	1.6926	4.73528	1.3771	47.4114
4.0339	1.3424	5.4151	1.802			
5.0828	1.3802	7.0152	1.9049			
5.4316	1.4149	7.6851	2.0019			
5.9661	1.4471	8.6335	2.094			
E=23.676	6.8856	32.8621	9.4954			

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

$$b = \frac{32.8621 - 6.8856 \times 4.7352}{9.4954 - 9.4822}$$

$$b = \frac{0.2574}{0.0132}$$

$b = 19.5$

$a = Y - bX$

$a = 5.7352 - 19.5 \times 1.3771$

$a = -22.1182$

Choosing Two Values of X When

$X = 1.301$ and when $X = 1.4471$

$Y = a + bX$

$Y = -22.1182 + 19.5 \times 1.301$

$Y = 3.2513$

When $x = 1.4471$,

$Y = -22.1182 + 19.5 \times 1.4471$

$Y = 6.10025$

96 Hour LC_{50} = Value of X when

$Y = 5.000$

$$Y = a + bX$$

$$X = \frac{Y - a}{b}$$

$$X = \frac{5 - (-22.1182)}{19.5}$$

$$X = 1.390676923$$

Antilog = 24.5853

i.e. Actual Conc. = 24.59 $mgZnl^{-1}$

:. 96 h LC_{50} = $\underline{\underline{24.59\ mgZnl^{-1}}}$

96 h Lc_{84} = Value of X when

$$Y = 5.9945$$

$$X = \frac{5.9945 - (-22.1182)}{19.5}$$

$$X = 1.441676923$$

Antilog = 27.6488

:. LC_{84} = 27.65 $mgZnl^{-1}$

Similarly, LC_{16} = value of X when

$$Y = 4.0055$$

$$X = \frac{4.0055 - (-22.1182)}{19.5}$$

$$X = 01.339676923$$

Antilog = 21.8613

LC_{16} = 21.86 $mgZnl^{-1}$

Appendix 6 Calculation of Upper and Lower Limits for 96 Hours LC_{50} of *Oreochromis niloticus*

$LC_{50} = 24.59$

$LC_{84} = 27.65$

$LC_{16} = 21.86$

$$S = \frac{LC_{84}}{LC_{50}} + \frac{LC_{50}}{LC_{16}/2}$$

$$S = \frac{27.65}{24.59} + \frac{24.59}{21.86/2}$$

$$\text{Slope} = \frac{1.1244083 + 1.124885636}{2}$$

Slope = 1.1246

$$LC_{50} = S^{\frac{2.77}{\sqrt{N^1}}}$$

$$LC_{50} = 1.1246^{2.77/\sqrt{120}}$$

$LC_{50} = S^{1.1468405}$

$LC_{50} = 1.1246^{1.1468405}$

$LC_{50} = 1.1441597$

Upper limit = $LC_{50} \times LC_{50}$

= 24.59 x 1.1441597

Upper limit = 28.13 $mgZnl^{-1}$

$$\text{Lower limit} = \frac{LC_{50}}{LC_{50}}$$

$$= \frac{24.59}{1.1441597}$$

Lower limit = 12.49 $mgZnl^{-1}$

:. The LC_{50} and 95% confidence limits are 24.59 (12.49-28.13) $mgZnl^{-1}$