TOXICOLOGICAL EFFECTS OF TANNERY EFFLUENT ON *Clarias gariepinus* (Teugels)

BY

GBEM, Terlumun Thaddeus

B.Sc (Hons) Zaria.

A THESIS SUBMITTED TO THE POST GRADUATE SCHOOL, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES
AHMADl BELLO UNIVERSITY
ZARIA, NIGERIA.

JUNE 1998

DECLARATION

I hereby declare that this thesis is a record of my research work and has not been presented by any one in any previous application for a higher degree. Information derived from the published or unpublished works of othersare duly acknowledged by means of reterences.

04-10-98

Thaddeus Terlumun Gbem.
Ahmadu Bello University,
Zaria. Nigeria.
June, 1998.

CERTIFICATION

This thesis entitled "Toxicological Effects of Tannery Effluent on *Clarias gariepinus* (Teugels)" meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Dr.J.K.Balogun
Chairman,Supervisory
Committee/Internal Examiner,
Department of Biological Sciences,
Ahmadu Bello University.

Date 3/2/99

Dr. F. A. Lawal
Member, Supervisory Committee/
Internal Examiner, National
Research Institute for Chemical
Technology, Basawa, Zaria

Date 3/2/99

Dr.S.Awodi
Head, Department of Biological
Sciences, Ahmadu Bello
University,Zaria.

Date 12-3-99

Prof. Olayemi
Dean,Post Graduate School
Ahmadu Bello University, Zaria.

Date 28/09/99

DEDICATION

To the sweet memories of my late grand mother, Aham M. Gbem and my Lucy, for everything.

ACKNOWLEGEMENT

I am greatly indebted to my supervisors, Dr. J. K. Balogun and Dr. F. A. Lawal for their contribution to the success of this work. They were more than academic supervisors. I must praise the foresight of Dr. Balogun for bringing in Dr. Lawal whose rich experience in the field of environmental pollution is really a source of inspiration. Apart from moral, financial and logistic support provided by them, Dr. Lawal provided most of the chemical reagents, without which this work would not have gone any where.

Throughout the days of my life, I would never forget the fatherly advice, encouragement and exposure they offered me. May the Almighty God reward them abundantly. I pray to fulfil their aspirations.

I would also like to express my heart-felt gratitude to Alh.M.L.Balarabe for his keen interest in the work. I must acknowledge his help by allowing me unlimited access to his journals. He also assisted financially in the area of typing. Mr. J. Auta was of much help during the course of my Lab work.

I appreciate the help rendered to me by Mr. Owoeye of NARICT, Kano who, on several occasions personally took me to the effluent collection site. Mal. Bala's role is also greatly appreciated and the managements of GNT and NARICT, for allowing me the use of the tannery effluent and library respectively.

Mr. Clement Osumeje of the Faculty of Medicine, A.B.U., Zaria prepared the tissue slides. It gives me great inspiration to note here that there still exist such a fine gentleman in the present day society. May God bless and reward him.

My big brother, Arc. T. F. Gbem and family's role is hugely appreciated. He and his wife, Aver are a role model for us' to emulate. May God continue to guide and bless them.

My friends and brothers, Y.O. Dan, Japhet (XY), Emma (P. Extra), Nyior, Arc. Doofan (DF), Kingpin, Babs, Tim, Cyril, Stanley constantly helped and encouraged me throughout the course of the work and Constance, for her help and understanding. She is a friend in need.

Finally, to the Almighty father, I give thanks and praise.

ABSTRACT

Clarias gariepinus (Teugels), mean weight 6.0+19 and mean standard length 9.5+ 0.4cm were exposed to characterized Great Northern Tanneries' effluent for acute and chronic toxicity studies.

The physico-chemical parameters of the test effluent were determined using a combination of the American Public Health Association, Environmental Protection Agency and Society for Analytical Chemists' methods.

Cr, Cu, Pb and Zn concentration in the digested liver, gill, gut, skin, bone, musele and wholefish were determined by atomic absorption spectrophotometry. RBC and WBC were counted with hemocytometer using blood obtained by caudal severation. Haemoglobin was estimated using Sahli-Hellige method hematocrit (PCV) by using the micro-hematocrit tube.

The symptoms of toxicosis observed include agitated swimming increased gill ventilation, air gulping, twisting, loss of balance, mucus secretion peeling of skin and death.

With the exception of temperature, hardness, Mg, Ca and Fe, all other pollution parameters studied were acutely higher than the FEPA approved standards.

The 96-h LC_{50} value of 9.5% v/v (95% Confidence limit of 8.7043 - 10.2057) and a slope function of 0.65 were established. The acutely exposed fish were found to have accumulated more metals than the control fish with the liver accumulating significantly higher than other tissues. Total metal burden for the sublethal exposure showed the accumulation in the order of Pb > Cr > Cu > Zn with liver levels significantly higher ($P < 0.05$) than other tissues and metal levels increasing with dose and time.

In the acute studies, except for MCHC, the RBC, WBC, Hb, PCV, MCH and MCV decreased. In the sublethal test, RBC, WBC, Hb, PCV, MCHC and MCV were significantly higher ($P < 0.05$) in the effluent treated fish than in the control, with only the MCH showing no significant variation.

Nature of tissue damage was found to be similar in both acute and sublethal exposures with oedema and hyperplasia in the gills. Oedema of hepatocytes and necrotic damage was found in the livers of the long-term exposed fish. Low ovarian weights and a dose dependent impairment of growth (average changes in fish weight on a biweekly basis) were recorded in the effluent exposed fish.

By implication, tannery effluent would adversely affect fish population due to its effect on reproduction, the accumulation of heavy metals in fish tissues could affect consumers most especially man.

TABLE OF CONTENTS

Title Page i

Declaration ii

Certification iii

Dedication iv

Acknowledgement v

Abstract vii

Table of Contents ix

List of Tables xiii

List of Figures xiv

List of Plates xvi

List of Appendices .....

CHAPTER ONE

1.0 INTRODUCTION 1

CHAPTER TWO

2.0 LITERATURE REVIEW 4

CHAPTER THREE

3.0 MATERIALS & METHODS 12

3.1 Effluents 12

3.1.1 Effluent Parameters Determination 12

3.1.2 Treatment of Effluents for Metals Determination 13

3.2 Experimental Animals 13

3.3 Randomization 14

3.4 Toxicity Test 14

3.4.1 Acute (96-h) Toxicity Test 14

3.4.2 Gill Ventilation Frequency 15

3.4.3 Sublethal Toxicity Test 15

3.5 Ovarian Recrudescence Studies 16

3.6 Growth Studies 16

3.7 Metals Enrichment of Fish tissues 17

3.8 Haematology 17

3.8.1 Haemoglobin Estimation 18

3.8.2 Micro-haematocrit (P.C.V) 18

3.8.3 Total Erythrocytes and Leucocytes 18

3.8.4 Absolute Values 19

3.9 Histopathology 20

3.10 Statistical Analysis 20

CHAPTER FOUR

4.0 Results 21

4.1 Effluent Parameters 21

4.2 Acute Toxicity 22

4.2.1 Fish Behaviour and Symptoms of Toxicosis 22

4.2.2 96-h LC_{50} Value 22

4.2.3 Gill Ventilation Frequency 27

4.2.4 Acute (96-h) Haematology 27

4.2.5 Metals Accumulation in Fish (96-h) 30

4.2.6 96-h Histopathology 31

4.3 Sublethal Toxicity 36

4.3.1 Sublethal Haematology 36

4.3.2 Metals Accumulation 41

4.3.3 Sublethal Histopathology 54

4.3.4 Ovarian Development 58

4.3.5 Growth 62

CHAPTER FIVE

5.0 Discussion 65

5.1 Tannery Effluent Parameters 65

5.2 Fish Behaviour and Symptoms of Toxicosis 65

5.3 Gill Ventilation Frequency 67

5.4 Haematology 69

5.5 Metals Accumulation 71

5.6 Histopathology 78

5.7 Ovarian Development 80

5.8 Growth 80

CONCLUSION AND RECOMMENDATIONS 82

REFERENCES 84

APPENDICES 95

LIST OF TABLES

Table 4.1 Results of Chemical Analysis of effluents 21

Table 4.2 96-h LC_{50} indices for *C. garieprinus* 23

Table 4.3: Haematological Parameters for Acute (10%) Exposure 29

Table 4.4 Mean cell Haemoglobin Concentration Values 30

Table 4.5 Mean Cell Volume (MCV) Values 42

Table 4.6 Mean Cell Haemoglobin 43

Table 4.7 Metals Distribution in Tissues of *C. garieprinus* exposed to ABU dechlorinated tap water 46

Table 4.8: Average weight changes in fish 64

LIST OF FIGURES

Figure 1. Sigmoid Dose Response Curve for Diluted Effluents..... 25

Figure 2.Probit of Mortality Versus Log-dose of Effluents..... 26

Figure 3.Gill ventilation frequency in *C. gariepinus* exposed to different effluent concentration for 96-h..... 28

Figure 4. RBC and WBC values for 96-h..... 31

Figure 5. Haemoglobin, PCV and MCH values for 96-h..... 31

Figure6a-d Metals accumulation in various tissues of *C.gariepinus* exposed to acute effluent concentration for 96-h..... 32

Figures 7a & b: Changes in RBC and WBC values of *C. gariepinus* exposed to sublethal concentration for weeks..... 38

Figures 8a & b. Haematocrit and Hb values of *C. gariepinus* exposed to different effluents concentration for 8 weeks..... 39

Figure 9a-d:Metals accumulation in tissues of fish exposed to 2% effluent concentration for 8 weeks..... 47

Figure 10a-d: Metals accumulation in tissues of fish exposed to 4% effluent concentration for 8 weeks..... 49

Figures 11a-d:Metals accumulation in tissues of fish exposed to 6% effluent concentration for 8 weeks..... 51

Figure 12: Total metals accumulation in fish..... 53

Figure 13: Changes in the ovarian wet weight of control and 6% effluent exposed fish..... 61

Figure 14:Percentage cummulative weight gain/ loss in fish..... 63

Figure 15.Correlation between Pb levels in fish tissues & blood counts (RBC & WBC) in fish 73

LIST OF PLATES

Plate I: Effects of Acute (96-h) Tannery Effluent Conc. on Gills of *C. gariepinus*..... 35

Plate II: Long Term Effects of Tannery Effluent on the Gills of *C. gariepinus*..... 55

Plate III: Long Term Effects of Tannery Effluent on the Liver of *C. gariepinus*..... 57

Plate Iv: Long Term Effect of Tannery Effluent on the Ovaries of *C. gariepinus*..... 60

LIST OF APPENDICES

1. Calculated weights of expected probits 94
2. 95% Confidence Limits 95
3. Regression Equation for Figure 2 96
4. Anova and DMRT for blood parameters in Sublethal exposure 97
5. Anova and DMRT for growth in sublethal test 103
6. Anova and DMRT for metals in 96-h exposure 104
7. Anova and DMRT for metals in 2% test 108
8. Anova and DMRT for metals in 4% test 112
9. Anova and DMRT for metals in 6% test 116
10. Anova and DMRT for Total Metals in 2%, 4% and 6% exposures 120

CHAPTER ONE

1.0 INTRODUCTION

Environmental contamination with chemicals have become a global problem. Data on their accumulation by living organisms are therefore valuable both to assess the safety of these chemicals for man and the extent of contamination of these organisms in the environment. In the tanning of hides and skins, a lot of effluents are generated (soaking, liming, deliming, bating, pickling, tanning and dyeing). This is so most especially because the tanning industry is heavily water dependent.

According to Prasad, *et al* (1981), wastewater from the tanning industry ranks among the most polluting of all industrial wastewaters. This is not surprising when one considers the large and diverse nature of the tannery effluent constituents. Such pollutants reported as contained in tannery effluents include Arsenic (Villa, 1966), Mercury, Lead, Hexavalent Chromium, Sodium Sulphide (Pepper, 1966, IULTCS, 1976). Lawal and Singh (1981) in characterisation of effluents from certain tanneries in Nigeria listed several heavy metals (Arsenic, Cadmium, Chromium, Cobalt, Copper, Iron, Zinc and Lead) as being present above the permissible limits. Other metals such as Sodium, Potassium, Calcium as well as Chlorides were also reported.

Along with other pollutants, heavy metals are known to exert a wide range of effects on fishes ranging from metabolic and physiologic to behavioural and ecological, (Forstner and Wittmann, 1981). The observed effects include disturbances in osmoregulation and respiration, tissue damage especially in the gills, liver and also in the brain, reduced energetic resources and poor growth performance.

Nitrogen and Phosphorus have been reported as being contained in tannery wastewaters and in large amounts (Lawal and Singh, 1981). These two elements form the primary nutrients in freshwater bodies (Vollenweider, 1968) and where discharged directly into freshwater bodies [as it is the case with most Nigerian tanneries (Lawal *et al*, 1996)], there is the danger of eutrophication in such water bodies (Vollenweider, 1968;de Boers, 1994).

The purity and salinity of water bodies are greatly affected by chlorides which are present in large amounts in tannery wastewaters. Biodegradation of organic matter could be drastically reduced or hampered due to the presence of caustic alkalinity which kill useful bacteria.

Schotz and Gillisen (1961) reported the presence of *Bacillus anthracis* in tannery effluents. A few cases are reported where anthrax has been transferred to people through contact with tannery effluents discharged in surface waters (Bhaskaran, 1977).

The high amounts of suspended solids and Biochemical Oxygen Demand (BOD) content of tannery wastewater have been noted (Lawal, *et al*, 1986). Generally, the high alkalinity, suspended matter, sulphides and heavy metals in effluents of tanneries are injurious to fish and other aquatic organisms (Mason, 1991; UNEP, 1991).

Damage to crops caused by tannery wastes when applied on land was reported to be due to the high concentration of salts of calcium, chromium, sodium and ammonium as can be seen in the characterisation of tannery sludge (Skrypski-Mantele and Bridle, 1995). Apart from this, the enrichment of the soil with organic nitrogen waste promotes the development of nitrifiers and denitrifiers and leaves the soil poor in nitrogen and also, denitrification results in a loss of nitrogen directly to the atmosphere (Ramasamy & Krishnamurthi, 1981).

In Nigeria, the tanning of hides and skins is a prominent industry as it serves as a foreign exchange earner. The tanning of hides and skins in the country started centuries ago with the small

scale rural tanneries whereas the large scale mechanised tanneries started about five decades ago (Adewoye, 1985). According to LERIN (1981), majority of the commercial tanneries are concentrated in Kano metropolis which has three major industrial estates - Bompai, Challawa and Sharada. Effluents from Challawa and Sharada estates are discharged untreated into River Challawa and water from this river is used for irrigation, bathing and fishing (Udeh, *et al*, 1997).

Considerable work has been done on the characterisation of tannery effluents in Nigeria (Lawal & Singh, 1981; Lawal, *et al*, 1986; Lawal, *et al*, 1996). Comparing the treatment and disposal methods practiced by major tanneries in the country, Lawal, *et al* (1986), Lawal, *et al*, (1996) concluded that a fair degree of pollution occur from these discharges. Inspite of these findings, studies relating to the toxic effects of tannery waste on the Nigerian biota and its various life - support systems have not been reported.

The study was therefore designed with the following objectives in mind:

1. To characterise the effluents from tanneries with a view of ascertaining possible pollutants present and levels of concentration;
2. To expose a widely and commonly occurring fish, *Clarias gariepinus* (Teugels) to various concentrations of the effluents with a view of determining:

i. The 96-h Lc_{50} of the effluent to the fish (acute toxicity studies);

ii. Long-term (chronic) toxicity studies which include:

a. Effects on behaviour, growth and reproduction (ovarian formation and development),

b. Effects on body physiology,

c. Effects on body tissues (gills and liver) and

d. Amounts of certain toxicants (heavy metals) accumulation in fish.

CHAPTER TWO

2.0 LITERATURE REVIEW

All industrial waste are highly complex and as a result, determining which factor is operating becomes difficult. Measurement of biochemical changes in blood and tissues of fish under exposure to pollutants maybe useful to predict effects upon exposure since biochemical and physiological disruption preceeds manifestation of gross damage.

Several factors are known to affect the toxicity of pollutants. Larger fishes are known to be less susceptible to toxicants than smaller ones (Pickering, *et al*, 1962, Mohammed, 1995). Fish species differ in their responses to pollutants as seen in the case of Amune, *et al* (1994) when they exposed juveniles of ***Oreochromus niloticus*** and ***Clarius gariepinus*** to cadmium. Even within the same fish species, differences could arise from such factors as size, sex and whether the fish were taken in winter or summer as seen by McKnight (1965) when he exposed the mountain white fish, *Prosopium williamsoni* to DDT.

Onwumere (1986) investigated the response of a freshwater fish, *Tilapia nilotica* (L) to treated refinery effluents and observed that though no significant mortality occured at 100% effluent concentration, growth was found to be impaired. Analysis of fish metal burden showed preferential metal accumulation with fish tissues accommulating metals several hundred times above the levels in the refinery effluents. Damage to organs was only found to be confined to the gills. This damage manifested in bulged opercula as a result of swollen gills of fish exposed to effluent concentrations of 30%, 40% and 50%. Oedomatous and clavate globate lamellae were found congested with blood with lamellae fused extensively

Bengtsson: *et al.* (1988) exposed three species of fish. *Myoxocephalus quadricornis*, *Alburnus alburnus* and *Perca fluviatilis* to pulp mill effluents and studied the effects on the vertebrae. They found elevated frequencies of vertebrae deformations and there were alterations in the composition and mechanical strength of the vertebrae.

Saxena and Bhatia (1983) studied the effects of vegetable oil factory effluents on ovarian recrudescence in freshwater fish, *Channa punctatus* and found out that the ovarian weights in effluent-exposed and the control fish did not vary significantly up to 60 days. There was a significant decrease in the ovarian weights of the effluent exposed fish after 90 days and this trend continued till the termination of the experiment. There was retardation in development and also a higher incidence of atresia.

Rajendran, *et. al.*, (1985) exposed a freshwater fish, ***Sarotherodon mosambicus*** to individual and combined tannery effluent and found that for the combined waste, the LC50 was 43% and 13% and 35% for the chrome tanning and soaking wastes respectively.

Ghem *et al:* (1997) exposed juveniles of a freshwater fish, *Oreochromis niloticus* to composite tannery effluents and found the 96-h LC_{50} with their confidence limits to be 15(14.627 - 15.373)% v/v. Symptoms of toxicosis were observed to have included loss of equilibrium, agitated swimming, air gulping and finally death. Mucus was observed on the gills and bodies of dead fish and haemorrage of the gills was also observed.

The synergistic effects of heavy metals in organisms is well known. Lloyd (1961) reported the interactions between zinc in combination with copper and lead, copper and phenols in combination with low oxygen. Heavy metals are prominently present in tannery effluents (Lawai and Singh, 1981).

The use of haematological parameters as indicators of sublethal effects of stress (e.g pollutants) can provide information on the physiological responses fish make to changing external environment. This is because haematological parameters are related to responses of the organism to changing environmental conditions, and therefore can be used to screen the health of fish exposed to toxicants (Gill and Pant, 1981).

Dhillon and Gupta (1983) exposing *Clarias batrachus* to aldrin and Swascofix CD-38 found out that clotting time (CT), Mean Corpuscular Haemoglobin (MCH), Mean Cell Volume (MCV), were decreased with increase in the pollutant concentration and exposure time whereas there was an increase in the values of Haemoglobin (Hb), Red Blood Cells (RBC), White Blood Cells (WBC), Erythrocyte Sedimentation Rate (ESR) and Packed Cell Volume (PCV) with increase in concentration and exposure time. They were of the view that the increase in PCV and Hb in experimental fish might be attributed to the catalysing action of chemicals due to which the body store of iron is incorporated into the Hb. Agrawal *et al:* (1978) also reported polycythemia (abnormal increase of RBC) as evidenced by most significant rise in erythrocyte number ($P < 0.001$) after administration of 0.25% aldrin in normal diet of *Channa punctatus*.

Dhillon and Gupta (1983) explained that the decrease in CT with exposure to pollutants could be as a result of the disturbance in coagulative mechanism. Pollutants such as heavy metals affect hybribolysis adversely by depressing coagulative mechanism. However, studies on the effects of heavy metals on Nigerian freshwater fishes (Oladimeji & Ologunmeta, 1987; Oladimeji & Offem, 1989; Annune, *et al*; 1994) did not specifically look at the effects on CT. These studies looked at effects on certain blood parameters and metal accummulation in tissues and are discussed later in the chapter.

An increase in Prothrombin time (PT) as found by Dhillon and Gupta (1983) might be due to liver diseases or loss of clotting proteins from the blood via the kidneys in renal diseases as in nephrotic syndromes (Dacie & Lewis, 1975).

Panigrahi and Misra (1978) exposed a freshwater fish, *Anabas scandens* to mercury and found a decrease in RBC and Hb. Body weights of treated fish also decreased significantly after 45 days of exposure. Gill and Pant (1981) however found an incipient decrease in RBC and subsequent recovery when they exposed the teleost, *Puntius conchonius* to mercury. They attributed the incipient decrease to haemolysis caused by mercury while the subsequent recovery was ascribed to enhanced erythropoiesis which was triggered as a typical stress response.

Panigrahi and Misra (1978) found blindness and exophthalmic swellings in *Anabas scandens* exposed to Hg over several days. These effects were presumed to have been caused by the effects of mercury on the brain and optic nerves. Neurologigical disorders caused by mercury and secondary poisoning might be important ecological consequences as mercury is known to directly affect several physiological processes (Blair, *et al.*, 1975).

The exposure of the teleost, ***Anabas testudineus*** to cadmium resulted in an increase in the ESR, MCV and MCH (Banerjee and Kumari, 1988). Annune, et al (1994) working on the acute toxicity of cadmium to juveniles of two freshwater fishes, ***Oreochromis niloticus*** and ***Clarias gariepinus*** found the 96-h LC_{50}s to be 19.319 (18.376-20.310) mg/l and 10.850 (10.097-11.659) mg/l respectively. They advanced reasons for the significant difference in susceptibility ($P < 0.05$) between the two species as being integumental.

Davis, et al., (1976), Holcombe, *et al.*, (1976) and Hodson, et al: (1982) reported that rainbow trout, ***Salmo gairdneri***, exposed to sublethal concentrations of lead starting at the egg

stage showed black tails land spinal curvature, elevated concentration of lead in tissues and the inhibition of the erythrocyte enzyme, a-aminolevunic acid dehydratase (ALA-D).

Tilapia nilotica (L.) exposed to water-borne lead suffered a decrease in Hb level and the decrease was dose and time dependent. There was also a decrease in the mean corpuscular haemogloloin concentration (MCHC) and this pointed to the fact that the major effect of lead on blood might be a reduction in Hb (Oladimeji and Ologunmeta, 1987). A reduction in heamoglobin, haematocrit and deformed erythrocytes were reported by Wong, *et al*, (1978) in fish exposed to sublethal concentrations of lead. Hodson, *et al*, (1978) suggested that this decrease could have been as a result of reduction in cellular blood iron which could have led to the reduction in the oxygen carrying capacity and also stimulated erythropioesis. Decrease or depletion of glycogen in the liver of fishes exposed to pollutants have been found to be dose dependent (Gill and Pant. 1981 Oladimeji and Ologunmeta. 1987). The later authors suggested that this decrease might be due to the inefficient adsorption of soluble glucose from the intestine or the breakdown of liver cells which store or synthesise glycogen.

The histopathologic effects of pollutants or toxicants on organs and tissues of fish have also been studied. Kumar and Pant (1981) observed the histopathologic effects of acutely toxic levels of copper and zinc (both heavy metals) on the gills, liver and kidney of ***Puntius conchonius***. The effects of these two metals on the gills were found to be indistinguishable. At 24 hour exposure time, the mucus cells underwent hypertrophy and the cells were completely hollow at the end of two days as the mucus completely dissappeared from them. Along with this change, the gill lamillae were found to have fused along their lenghts or at their tips. There was also detachment and swelling of the epithelial lining of the secondary lamellae from the underlying pillar cells in many places.

Though Wong, *et al;* (1977) found no structural damages in the gills of *Cyprinus carpio* exposed to zinc and copper salts (a typical case of different species response to same toxicant) there was the presence of a veil-like film on the operculum and on the bodies of the dead fish. In addition to this, the dead fish were found to have swollen abdomens as observed by Lewis (1978); Ebele, *et al;* (1990) and Annune, *et al;* (1991, 1994). The formation of this veil-like substance according to Wong, *et al*, (1977) and Annune, *et al*, (1994) was due to the interaction of the heavy metal ions with the mucus leading to the precipitation or coagulation of mucoprotein on the gills hampering gaseous exchange and osmoregulation as water flowing over the branchial chambers could not reach the filament cells. Wong, *et al*, (1977) advanced two other possible death causes as:

i. the disruption of circulation of the blood in the gill capillaries as a result of the impermeability of the gill filament. This was also corroborated by Annune, *et al;* (1991).

ii. a heart block, dropping the heart action to about half.

Many pollutants irrespective of type might firstly cause damage to the gills (since the gills are more or less external) and this might be expected to affect ion and water balance as well as respiration resulting into concomitant tissue hypoxia (Skidmore, 1970). Since even the toxic levels of Zinc and Copper were unable to induce structural alterations in the gills of *C. carpio* (Wong, *et al;* 1977), the gill damage and consequent hypoxia did not appear to be the only causes of fish death. It seems therefore that damage caused to internal organs also played a role in fish death. Wong, *et al;* (1977); Kumar and Pant (1981) found evidences of vacoulation and necrosis in both the livers and kidneys of fishes exposed to metal poisoning. Panigrahi and Misra (1978) found the

liver of *Anabas scandens* exposed to mercury to be paler, tender, more congested and also smaller than those in the control.

Omoregie and Ufodike (1991) studied the toxicological effects of Acutellic 25 EC (an insecticide) on the gills, liver and kidney of the fingerlings of *O. niloticus* under a continuous flow system. The results showed the gills to be mostly damaged with minor necrosis observed in the liver and kidneys at a concentration of 1 ugl^{-1}. There was evidence of fatty degeneration and an initial increase in the opercular ventilation which subsequently decreased to below normal at the end of the 96-h bioassay.

Babatunde (1997) exposed fingerlings of *O. niloticus* to paraquat (gramoxone) and determined the 96-h LC_{50} to be 12.50 mgl^{-1}. Long term exposures were found to have resulted in growth impairment which was dose dependent. There was also decrease in the values of Hb, haematocrit, MCHC and leucocyte count together with a high incidence of haemolysis which the author attributed to haemodilution. Histopathological damages were observed in the gills, liver and brain.

The uptake rates and accummulation of toxicants by various fish organs have been studied. Lovegroove and Eddy (1982) stated that in fish, the main sites of heavy metals uptake are the gills and the gastrointestinal tract. Oladimeji and Ologunmeta (1987) exposed *Tilapia nilotica* (L) in water-borne lead and found the levels of lead accummulated in the whole fish to be directly related to concentration and duration of exposure time. Oladimeji and Offem (1989) exposed fingerlings of *Oreochromis niloticus* and *Clarias lazera* to lead and found tissue accummulation of the metal to be in the order: gill > intestine > liver > skin > muscle > bone and gill > liver > intestine > bone > muscle respectively. Ammune and Iyaniwura (1993) found accummulation of Zinc in the tissues

of two species common in Nigerian waters. *O. niloticus* and *C. gariepinus* to have increased with exposure time. Whereas zinc uptake by the tissues was in the order of liver > gut > gills > whole fish > skin > muscles, cadmium uptake was found to have been of the order: liver > gut > gills > skin > muscles > whole fish. On the whole, the *Clarias* sp was found to have accummulated the two metals more than the *Oreochromis* species ($P < 0.05$) and at a faster rate as shown by differences in their bioconcentration factors (BCFs). The integumental difference in the two species was advanced as a possible reason.

Brooks and Rumsey (1974), Benoit, *et al;* (1976), Holcombe, *et al;* (1976), Farmer, *et al*, (1979) and Badsha & Goldspink (1982) have all reported on the metal enrichment of fish organs. In fish, Zinc has been found to be generally low in muscles and high in gonads (Brooks and Rumsey, 1971; Badsha and Goldspink, 1982).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Effluents

The effluents used for this study were tannery effluent - the composite (combined) type. All effluents used were collected from Great Northern Tanneries factory, Bompai, Kano. Effluents were collected in the mornings (between the hours of 6 and 11 a.m.) so as to ensure a true representation of the composite type. The effluents were then transported in 50l capacity plastic cans to the Department of Biological Sciences, Ahmadu Bello University, Zaria where the effluents were used immediately.

3.1.1 Effluents Parameters Determination

A combination of the standard methods of the American Public Health Association, Environmental Protection Agency and Society for Analytical Chemistry was used for measurement of various parameters. The following effluent parameters were determined; Temperature (°C) by using a mercury bulb thermometer, pH, with a pH meter (portable Beckman) Electrical conductivity (μs/cm) using the portable Griffin type conductivity meter, suspended solids (ppm), Dissolved solids, total solids, total ash, total alkalinity, hardness, sulphide, sulphate, total nitrogen, BOD_5, Dissolved oxygen, Magnesium, Calcium, Zinc, Iron, Chromium, Arsenic, Copper, Sodium and Potassium (all units in ppm).

3.1.2 Treatment of Effluents for Metal: Determination

Due to the complexity and variability of tannery effluents matrix, preliminary treatment is necessary (Lawal, 1988). Suspended solids in the effluents were digested and pre-treated in order to destroy organic matter and bring all the metals into solution. Certain metals such as arsenic are volatile and hence dry ashing is not suitable (Allen, *et al*, 1971). Lawal (1988) investigated several digestion methods on tannery effluents and found the Nitric - sulphuric - perchloric acids methods as described by APHA (1985) to be most suitable.

Procedure:

To 100ml sample of tannery effluent in a 250ml beaker, 25ml of HNO_3, 5ml of H_2SO_4 and 5ml of Perchloric acid (all concentrated) were added. The mixture was carefully heated on a hot plate so that no foaming occurred. Nitric acid was added in small portions till all organic matter destroyed indicated by no further darkening of the solution. The solution was cooled and 40ml of conc. HCL added. The mixture was then filtered and the volume brought up to 100ml in a volumetric flask. Portions of the solution were then taken for heavy metals (As, Cr, Fe, Pb and Zn) for analysis using an Atomic Absorption spectrophotometry (AAS), Buck Scientific, Model 210.

3.2 Experimental animals

All the *Clarias gariepinus* (Teugels) used were obtained form artisanal fishermen at the Ahmadu Bello University Dam and were caught from the creeks. For the toxicity and growth tests, juveniles were used while for the ovarian development (recrudescence) studies, matured specimens (female) were used. Acclimation lasted for a period of three months in the laboratory

which fish were held in 160L capacity holding tanks at a temperature range of 24.5 - fed twice daily at a 2.5% body mass with formulated diet of 35CPL at natural photoperiod

Randomisation:

Finey (1964) suggested a formal randomisation procedure in assigning the specimens to various concentrations so as to avoid the introduction of a systematic error. Randomisation was therefore carried out based on the method of Sprague (1970). This was done by distributing the first set of fish caught into one of each of the test tanks in random order according to the occurrence of 1-10 numerals in a prepared table of random order. This continued until all the test fish were assigned. The problem of position effect was eliminated by randomly assigning the Test tanks.

3.4 Toxicity Tests

Acute (96-h static toxicity test) and chronic or sublethal (8 weeks) toxicity tests were conducted.

3.4.1 Acute (96-h) Toxicity Test:

96 - h static toxicity tests were conducted following the methods of Sprague (1970) and APHA (1985). The static toxicity test is justified because the effluents from the factory enter the receiving ecosystem periodically (personal communication with the AGM, Technical). Fish were exposed to effluent concentrations of 0%, 5%, 10%, 12% and 15% v/v. Eeach concentration was replicated and concentration in each tank renewed after 48 hours.

Fish averaging a weight of 6±0.8g and a standard lenth of 10.0±0.5cm were randomly

l to the various test tanks as described above. Feeding of the fish was suspended 25 hr ommencement of the toxicity tests. The behaviour of the fish was observed before, nd after the toxicity tests. Examination of fish for mortality was done at 15, 30 minutes, 1, 2, 4, 6, 8 and 12 hours and then two times daily until the end of the 96-h exposure period. Fish were considered dead when the opercular movement seized and a gentle prodding with a glass rod elicited no response. Dead fish were removed from the solution. Physico-chemical parameters were measured on a daily basis.

The 96 - h median lethal concentration (LC'_{50}) together with its 95% confidence limits (95%CL) and slope function were determined using probit analysis (Finey 1971, Dick and Dixon, 1983) Blood samples were taken from the moribund and surviving fish for hematological analysis The gills, and liver of the dead fish were also removed for observation and histopathological studies.

3.4.2 Gill Ventilation Frequency, Vf:

During the acute (96-h) toxicity test, the gill ventilation frequency was determined by counting the opercula movement (opening and closing) per minute. More than one count was done at predetermined time intervals (6 hr interval) for different fishes per tank and the average obtained for every day. Results were expressed as averages on a daily basis.

3.4.3 Sublethal Toxicity Test:

The concentration of the effluents here were established based on the results of the 96-h toxicity tests, i.e. the LC_{50} value. Effluent concentration of 0%, 2%, 4% and 6% V/v were used. Fish of average weight 5± 0.2g and standard length 9.5 ± 0.6 cm were used. Each tank (15L Fish

of average weight 5±0.2g and standard lenght 9.5±0.6cm were used. Each tank (45l capacity each) contained 16 fish and each concentration was replicated. Two fish were removed from each treatment at two weeks intervals, examined, blotted dry with soft absorbent paper and weighted, blood samples collected and then dissected for the removal of the fish organs. Concentrations in each tank were renewed after every seven days interval.

3.5 Ovarian Recrudescence Studies:

Four fish autopsised at the beginning of the experiment served as the initial control (baseline). All the treatments received natural photoperiods and feeding was done at 5% body weight twice daily i.e. in the mornings and evenings. Water was changed every seven days and a pre-determined quantity of fresh effluent added immediately (i.e. in the 6% effluent exposed fish).

Five fish were removed from every treatment (0% and 6% v/v) at twenty days interval, blotted dry, weighted and then sacrificed for ovaries. The ovaries were removed, weighted and fixed immediately in aqueous Bouins fluid. For the purpose of comparism, ovarian weights were calculated on the basis of 100g body weight. Cross-sections of the ovaries (7μm thick) were obtained using a rotatory microtome, stained using haemoxyl eosin technique and studied under various magnifications. The occurrence of oocytes in different stages of development in the ovaries were observed.

3.6 Growth Studies:

Fish under growth studies were fed at 5% body weight twice daily (mornings and evenings) natural photoperiod were maintained and effluents changed once every week. Measurement of weight was done on a biweekly basis and percentage cumulative weight determined.

3.7 Metals Enrichment of fish Tissues:

The amount of metal accumulation in fish was investigated in whole fish, gills, liver, gut, skin, muscles and the bones. The metals analysed were Cr,Cu,Pb and Zn. Determination was based on the methods of Hodson, *et al:* (1978) and APHA (1985). Whole fish and each removed fish organ was weighted in an acid - washed , pre-weighed dry crucible and the wet weight taken. These samples were oven dried at 60°C for 48 hrs. Due to the small size of most tissues, grounding was not done. Rather, they were ashed directly using a muffle furnace at 450°C for 12 hours (at the end of which samples were ashed completely).

Digestion procedure:

The sample was dissolved in about 50 - 70 ml of distilled water in a beaker. 5ml conc. HNO_3 was added and the solution brought to a slow boil on a hot plate. This was evaporated to about 20ml, 5ml conc. HNO_3 was again added and the beaker covered with a watch glass and heated till a refluxing action was achieved. Heating was continued with addition of conc. HNO_3 till digestion was completed (indicated by the appearance of a clear bright colored solution). Any remaining residue was dissolved by the addition of 1-2 ml of conc. HNO_3 and slight warming. The walls of the beaker as well as the watch glass were washed down and transferred to a volumetric flask, rinsing the beaker with 2 - 5ml of distilled water. This was then cooled, the solution brought to the 100ml mark with distilled water, mixed thoroughly and portions taken for analysis with AAS, Buck Scientific, model 210.

3.8 Hematology:

Two fish were removed form each treatment tank on a biweekly basis/interval and blood sampled for the estimation of certain hematological parameters. Blood was sampled by severing of the caudal region (Hesser, 1960).

3.8.1 Haemoglobin Estimation (gdl^{-1}):

The Salhli - Hellige method as described by Hesser (1960) was used. The Sahli pipette was filled with blood slightly above the $20mm^3$ mark and the excess blood was removed by blotting with a soft absorbent tissue at the tip. The blood was then expelled into a calibrated transmission test tube containing $10mm^3$ of 0.1N HCl. The pipette was rinsed several times with the acid solution. The solution was allowed to stand for not less than 3 minutes and not more than 5 minutes before reading using a colorimeter at between 530-540 nm. For recording, the calibration charts reading grams percent versus percentage transmission prepared by Miale (1958) was used.

3.8.2 Micro haematocrit (P C V)%:

Blood was properly mixed and drawn into a micro-haematocrit tube by capillary action up to two-third of its length. Centrifugation was done at 10, 550 rev. per minutes for 5 minutes in a micro - haematocrit centrifuge. Readings were made using a micro-haematocrit reader and results expressed as the volumes of erythrocyete per $100cm^3$ or as a percentage.

3.8.3 Total Erythrocytes and Leucocyte (mm)

For the erythrocytes count, Hendricks, diluting solution was used. The standard RBC diluting pipette was used and a 1:200 dilution was made. This was made by drawing blood up to the 0.5 mark and then filling the pipette with the diluting fluid up to the 101 mark. The pipette was shaken for about a minute and a few drops of blood expelled. The haemocytometer (counting chamber) used was the improved Neubauer type. The pipette was touched to the edge between coverslip and the chamber and capillary action drew the diluted suspension of

coverslip and the chamber and capillary action drew the diluted suspension of cells into the chamber. The haemocytometer was then placed under the microscope and the number of cells counted and multiplied by 10^6.

For leucocytes count, Shaw's solution, developed for counting avian leucocytes was found to be satisfactory. This is because in fish blood, both the erythrocytes and leucocytes are nucleated and as a result, solution which would normally lyse human erthrocytes cannot lyse fish erythrocytes (Hesser, 1960). Shaw's solution is made up of two diluting fluids of different compositions. The first diluting solution which was labelled solution A is a composition of Neutral red (25mg), sodium chloride (0.9gm) and a 100 ml of distilled water. The second diluting solution was labelled solution B and is made by combining the following: crystal violet (12 mg), sodium citrate (3.8 gm), formaldehyde (0.4 ml) and a 100 ml of distilled water. When leucocytes counts were made, the red-celled diluting pipette with a 1:200 diluting factor was used. Blood was drawn up to the 0.5 mark, solution A was then added to fill the bulb of the pipette half full and mixed. The pipette was then removed from solution A and filled up to the 101 mark with solution B. The pipette was shaken, a few drops expelled and leucocytes count done by filling the haemocytometer as described for the erythrocytes. The multiplication factor used was 500 (Hesser, 1960).

3.8.4 Absolute Values

The absolute values include:

(i) Mean cell volume, MCV (μ^3)
(ii) Mean corpuscular haemoglobin, MCH ($\mu\mu g$)
(iii) Mean cell haemoglobin concentration, MCHC (%)

All these were obtained from the results of RBC, WBC, haemoglobin and PCV using the formulae given in Dacie & Lewis (1972).

3.9 Histopathology:

The gills and liver of the exposed fish were studied for histopathologveal changes by comparing them with those of the control fish. The organs in question were fixed in 10% neutral buffered formalin for 24 hours. At the end of this period, they were removed, washed and dehydrated using various solutions of ethanol and chloroform: (Alcohol: 70%, absolute; chloroform. 3.1, 1.1, 1.3 and two portions of pure chloroform). Infiltration and blocking was done in paraffin wax (59-60 °C melting point). 7 um were obtained using a rotatory microtome. Staining was with H & E and studied under the microscope. Photomicrograph were taken.

3.10 Statistical Analysis:

Except where otherwise indicated. the analysis of variance (ANOVA) and the DUCAN Multiple Range tests were employed. Analysis was performed using a computer programme, PC - SAS programmes, release 6.04 (SAS Institute Inc. Cary, USA).

CHAPTER FOUR

4.0 RESULTS

4.1 Effluent Parameters

Results of the analysis of the tannery effluents (Lawal *et al* 1997)are presented in Table 4.1 below

Table 4:1 Results of chemical analysis of Tannery effluents (compared with those of the International Standards for discharge into streams) and those of FEPA

Parameter	Amount	FEPA	International Standard
Temperature (°C)	30	< 40	32-35
pH	9.5	6 - 9	5 - 9
Electrical Conductivity (µs/cm)		-	-
Suspended solids (ppm)	14,080	30	40
Dissolved solids (ppm)	4,231	2000	7,500
Total Solids (ppm)	15,040	-	-
Total ash (ppm)	19,271	-	-
Total alkalinity	8,013	-	-
(ppm $CaCO_3$)	1,350	-	-
Hardness (ppm $CaCO_3$)	320	600	-
Chlorides (ppm)	250	0.2	1 (free Cl_2)
Sulphide(ppm)	280	500	-
Sulphate (ppm)	1,036	-	-
Total Nitrogen (ppm)	20.58	50	-
BOD (ppm)	6,150	200	20
Magnesium (ppm)	38.20	200	-
Calcium (ppm)	82.60	< 1	-
Zinc (ppm)	1.04	20	1
Iron (ppm)	3.95	< 1	1
Chromium (ppm)	16.25	0.1	1
Arsenic (ppm)	0.5	< 1	1
Copper	0.15		1
Sodium (ppm)	1782	-	1
Potassium (ppm)	140	-	1

4.2 Acute Toxicity

4.2.1 Fish Behaviour and Symptoms of Toxicosis

At the introduction of the fish into the effluent tanks, fish were noticed laying motionless on bottom of the tanks after an initial fast swimming around the tank. No opercular movement (gill ventilation) was noticed for a brief period that lasted about three (3) minutes. This was then followed by sudden darting to the surface to gulp air.

As time progressed, there was agitated swimming. This commenced earlier in tanks of higher concentrations (10%, 12% and 15%) In mode of swimming, the heads were kept above the surface of the effluent as much as possible. As time progressed, there was air gulping (coughing) twisting, loss of equilibrium or balance as most of time, the fish were noticed swimming in circles with the ventral surfaces turned upwards. Panic - like bursts of swimming were also observed. During the period of quiescence, the fish were vertically still with their mouths barely above the surface and then fish finally sunk to the bottom with no pronounced movements save for weak and irregular opercular movement, and occasionally, shivering fins. In tanks of higher effluent concentrations, peeling of the skin and swollen abdomens were observed before death. In death, the mouths were usually gaped open.

Mucus was observed on the head region of dead fish mostly around the opercular. All these symptoms were absent in the control tanks and no fish mortality was recorded.

4.2.2 96-h LC_{50} Value:

The results of fish death and other parameters set out for the determination o the 96-h LC_{50} value are shown in Table 4.2 below:

Table 4.2: 96-h LC_{50} indices for *Clarias gariepinus* (Juveniles) exposed to Tannery effluent.

Dose (%v/v)	Corresponding Log10 Value	Mor. Ratio	% Mor.	Probits of obser. Mor.	Expected Probits	Weight (w)
0	0.000	0/10	0.00	-	-	-
5	0.699	0/10	0.00	-	1.950	0.01187
8	0.903	4/10	40.0	4.7467	4.500	0.58100
10	1.000	6/10	60.0	5.2533	5.250	0.6865
12	1.097	8/10	80.0	5.8416	5.700	0.5320
15	1.176	10/10	100	-	8.250	0.0145

$\Sigma w = 1.8259$

$\bar{w} = 0.3652$

These results when plotted on a graph showing the proportion of fish kill in percentages against the percentage concentration of challenge solution produced a sigmoid curve as shown in Figure 1. Percentage mortality when transformed into probits and plotted against the log - dose of the effluent gave a linear relationship as shown in Figure 2. From Figure 2, the 96-h LC_{50} determined is 9.5% v/v with a 95% confidence limit range of 8.7943 - 10.2057 % v/v and a slope function of 0.65.

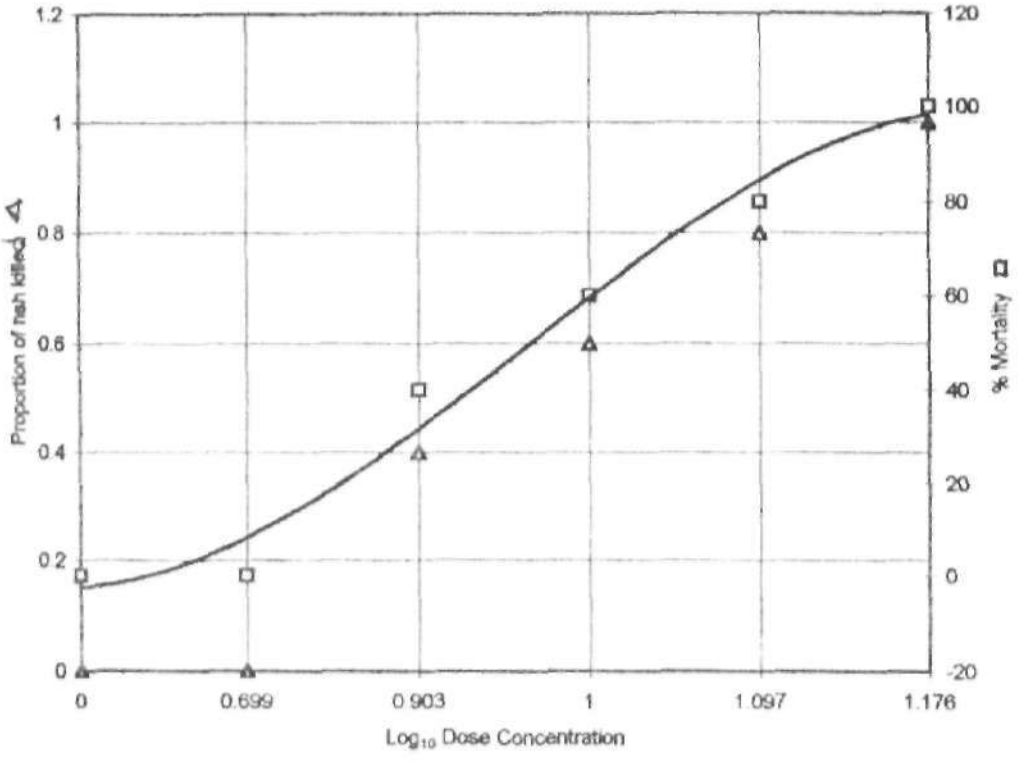


Fig. 1: Sigmoid-dose response curve for diluted effluents

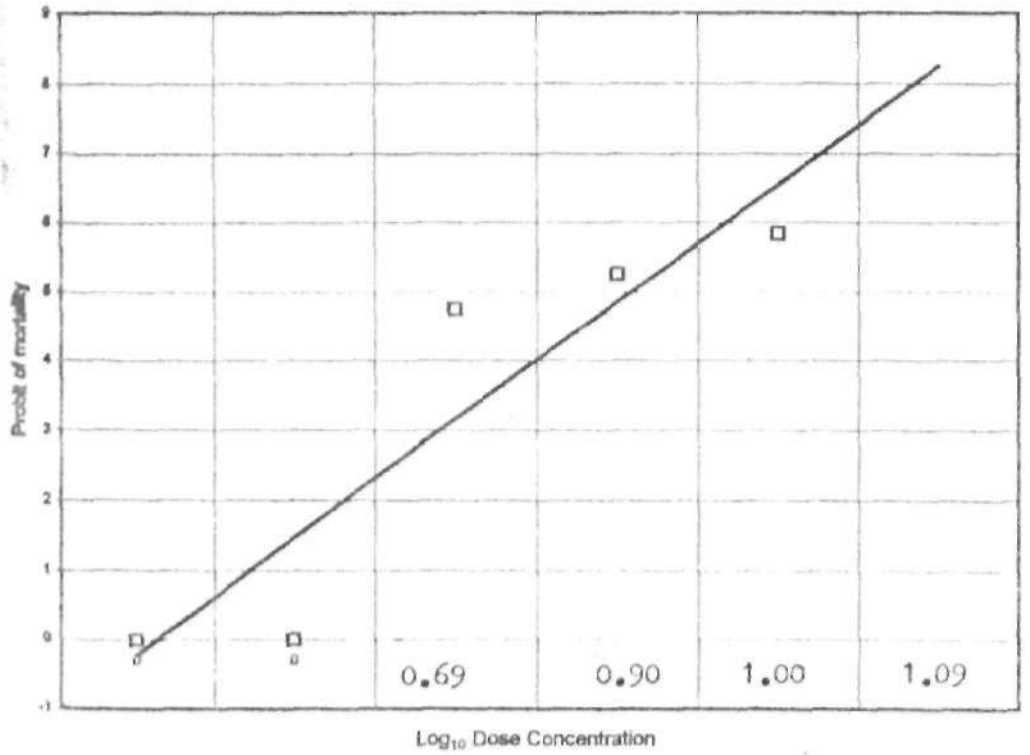


Fig. 2: Probit of mortality versus log-dose of effluents

4.2.3 Gill Ventilation Frequency (Vf.)

The first 24 hr showed lower Vf in the effluent exposed groups than the control group which showed a relatively constant Vf throughout the 96-h test period .Vf increased on the second day (48 hr) above the control values in all the effluent treated fish and the magnitude of increase was in the order of concentration except in the 10% effluent. In the 12% effluent exposed fish, Vf decreased from 120 ventilations/minute in the 76th hr to 116 ventilations/minute in the 96th hour. Though no significant difference ($P > 0.05$) was observed between the different effluent treated fish, there was a significant difference ($P < 0.05$) between the treated fish and the control.

4.2.4 Acute (96 h) Haematology:

Haematological parameters were investigated for fish only in the 10% effluent concentration. This was due to the reason that the number of fish left in 12% effluent concentration was too small to supply enough blood for all the parameters and 8% concentration falls below the 96-h LC_{50} value (which was established to be 9.5% v/v). The 96-h (acute) haematological parameters are shown in Table 4.3 and for the purpose of comparison, those of the control and baseline fishes are also given.

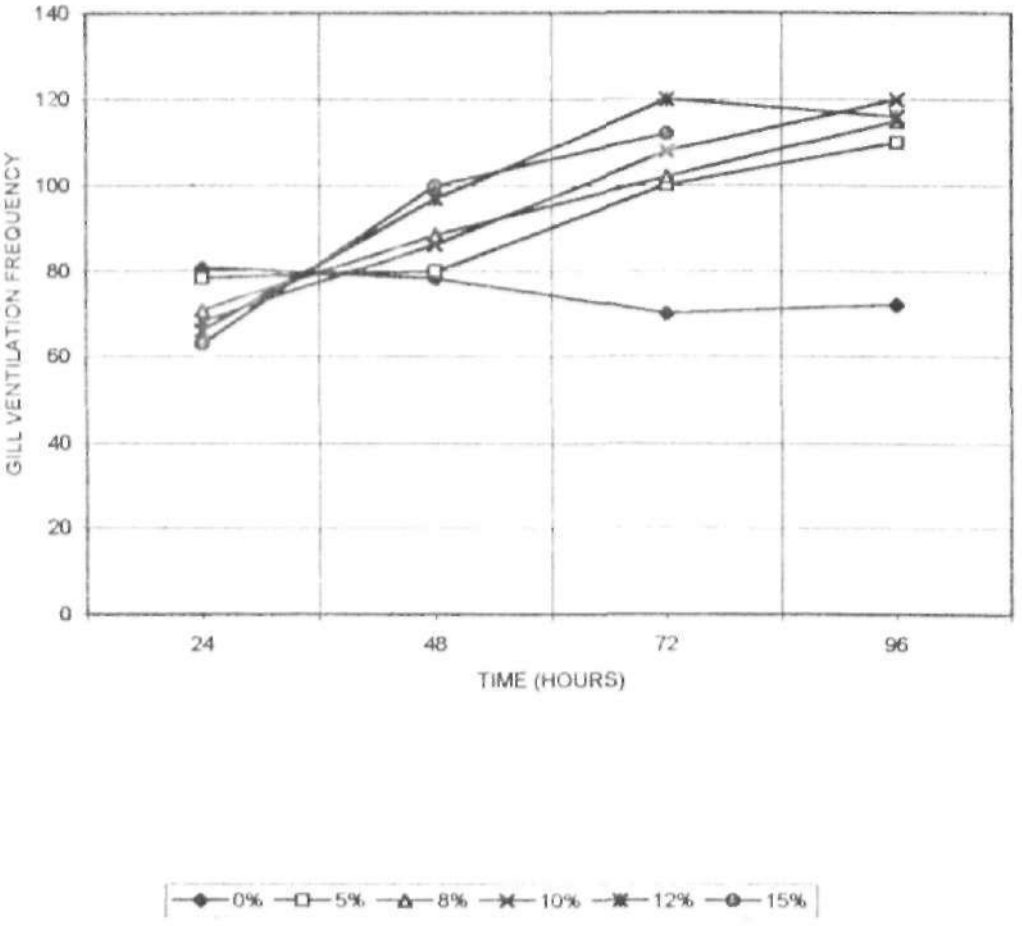


Fig. 3: Gill Ventilation frequency, Vf in C. gariepinus exposed to different conc. of tannery effluent for 96-hr

Table 4.3: Haematological parameters for acute (10‰), control and baseline fish *C. gariepinus:*

Parameter	Acute (10‰)	Control (0‰)	Baseline
Haemoglobin (g/100ml)	5.3±0.65	7.7±0.70	6.7±0.63
RBC (mm^3) x 10^6	56±4.3	250±51.80	260±4.08
WBC (mm^3) x 10^6	2.61±0.17	11.9±0.90	11.48±0.63
PCV or Haematocrit(%)	16±2.49	23±20.05	20± 2.94
MCH (u.ug)	0.947±0.23	0.301±0.057	0.258±0.02
MCHC (%)	33.13±0.13	33.48±0.10	33.58±0.23
MCV (u^3)	2.85±0.14	0.916±0.043	0.76±0.04

Haemoglobin were found to have decreased though the decrease was more pronounced in RBC. WBC and haematocit. as one compares these values with those in the control and baseline fish. MCV and MCH increased in the fish exposed to acute level i.e higher values were obtained in the 10% effluent exposed fish than those in the control and baseline. Changes in RBC and WBC values are presented in figure 4 while those of the Hb, PCV and MCHC are presented in Figure 5.

4.2.5 Metals Accumulation in Fish (96 h):

The 96 h metal (Cr, Cu, Pb, and Zn) uptake by juveniles of *C. gariepinus* is illustrated in figures 6a - d. The 96-h accumulation of metals in the various fish tissues is looked at on three basis: the acute (96-h), the baseline and the control.

Results showed metals concentration to be in the order of acute > baseline > control though statistical analysis showed no significant difference ($p > 0.05$). Results also showed lead to be most rapidly disseminated (among the four metals studied) in the fish tissues followed by chromium.copper and zinc in that order.In terms of 96-h individual metal concentration in the tissues chromium distribution was found to be in the order of;liver>gill>skin>gut>bone> muscles>wholefish: for copper; liver>skin>gill>gut>bone>muscles>wholefish>. For lead: liver skin gills gut bone muscles>wholefish and for zinc, liver>gill>gut>skin>bone>muscles> wholefish. Total metal burden was in the order; liver>skin>gill>gut>bone>musles>wholefish. DMRT showed the levels of the metals to be significantly higher in the liver than in all the other tissues ($P < 0.05$).

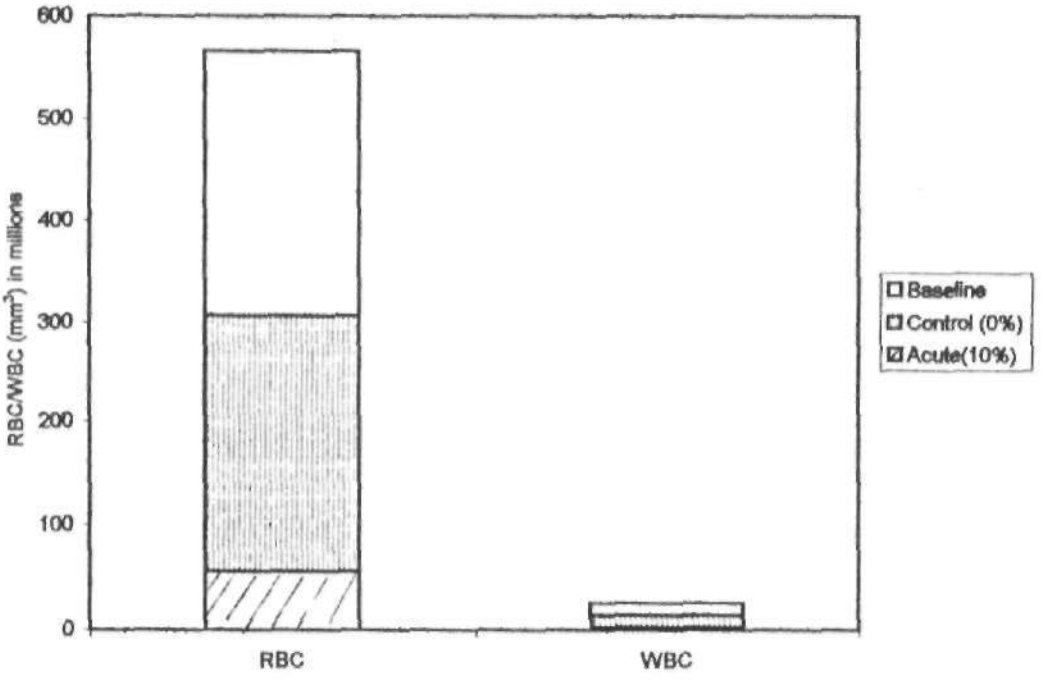


Fig. 4: WBC and RBC of C. gariepinus exposed to acute conc. Of tannery effluent for 96-hr

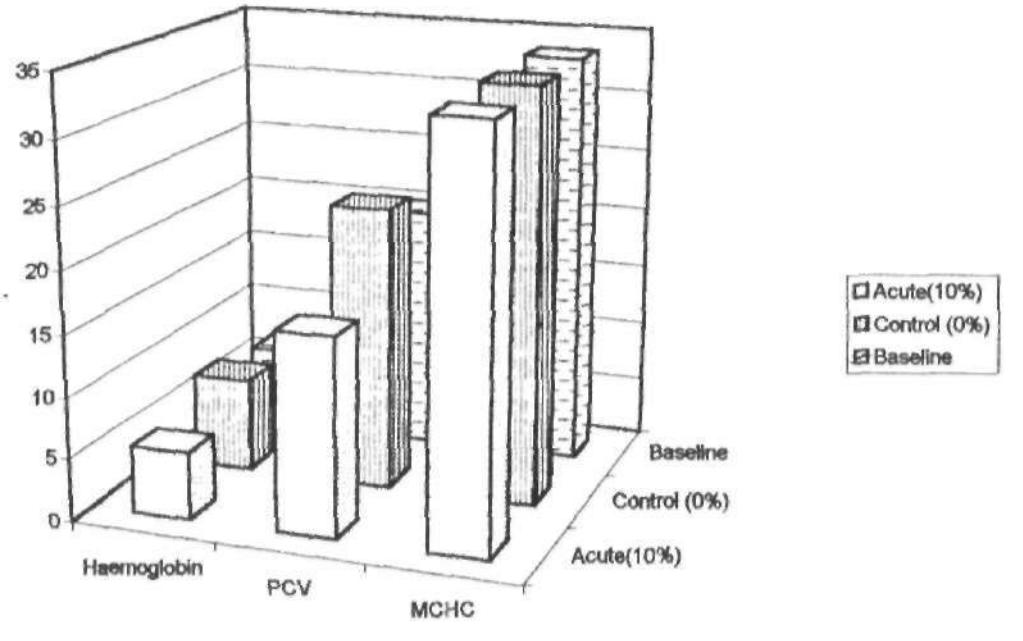
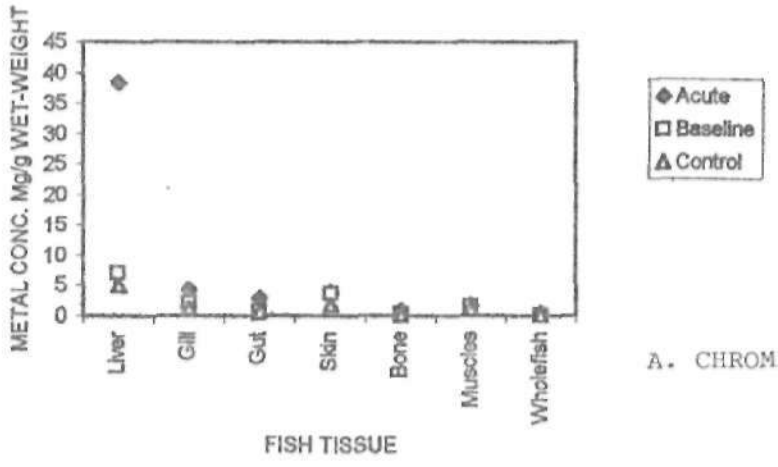
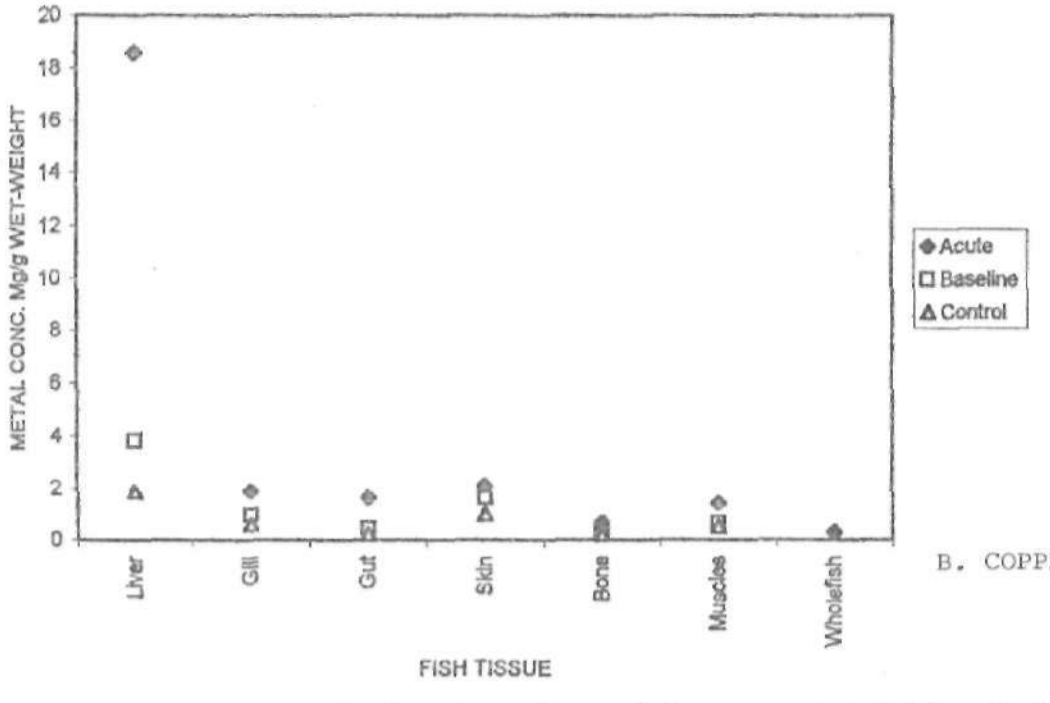


Fig. 5: Hb, PCV and MCHC of C. gariepinus exposed to acute conc. of tannery effluent for 96-h

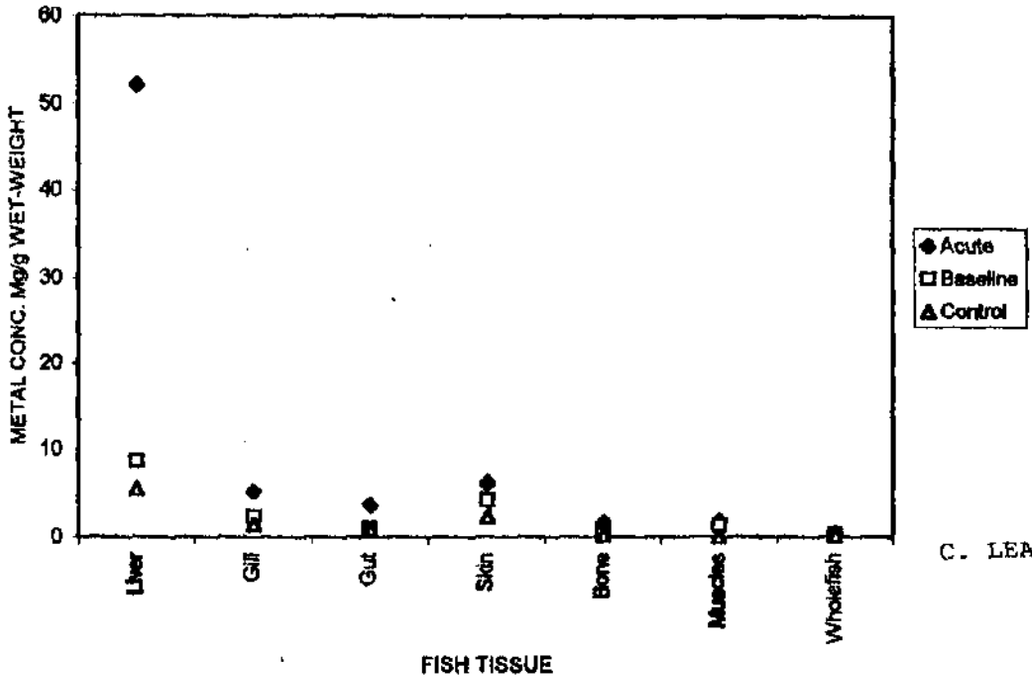


A. CHROMIUM

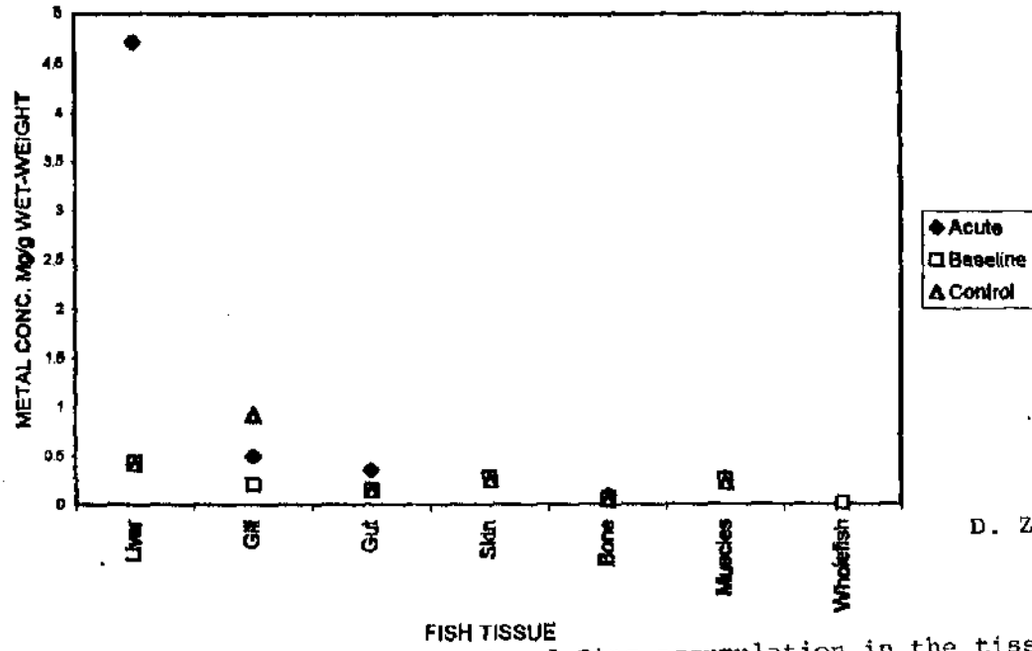


B. COPPER

Fig 6A and B: Showing Chromium and Copper accumulation in the tissues of acute exposed fish, control and baseline.



C. LEAD



D. ZINC

Fig 6C and D: Showing Lead and Zinc accumulation in the tissues of acute exposed fish control and baseline.

4.2.6 96-h Histophathology:

Results for the acute (96-h) toxicity test on the gills are presented on Plate I.

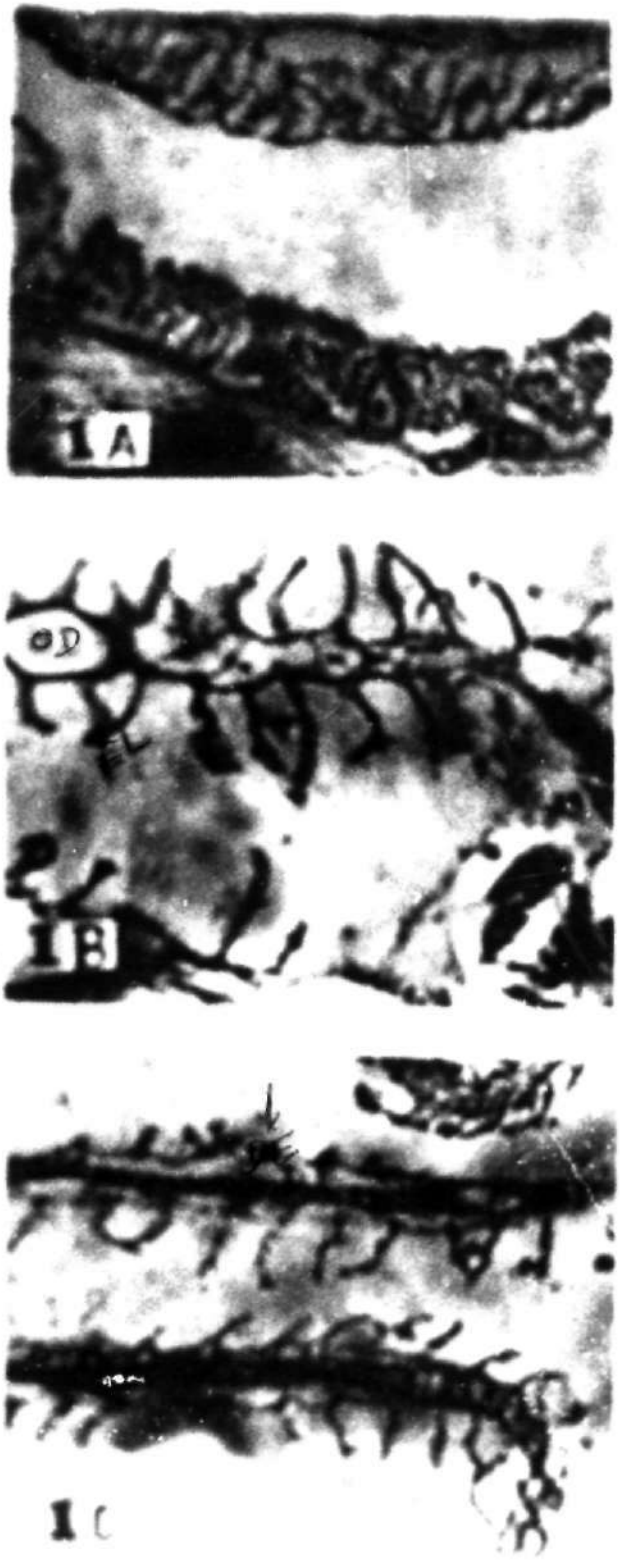
KEY TO PLATE I

PLATE Ia : Gills of the control fish showing well preserved gill architecture with two gill filaments well spaced and lamellae well arranged. Light micrograph (x 400 H & E).

PLATE Ib. Light micrograph of gill from fish killed by 10% effluent concentration. Note the epithelium swollen at a point (OD) and fusion of lamellae (FL) at the tips (x 45, H & E).

PLATE Ic: Light micrograph of gill from fish killed by 15% effluent concentration. Note the appearance of the third type of toxic action, detachment of the epithelial lining (DE) from the underlying core of pillar cells (x 85, H & E stain).

PLATE 1: EFFECTS OF ACUTE (96-h) TANNERY EFFLUENT CONC. ON GILLS OF *C. gariepinus*



4.3 Sublethal Toxicity:

4.3.1 Sublethal Haematology:

Figures 7a and b show the white blood cells (WBC) or leucocytes and the red blood cells (RBC) or erythrocytes count of *C. gariepinus* exposed to various concentrations of tannery effluent.

The WBC in the control fish, 4% and 6% effluent treated fishes increased steadily from week 2 to week 8. There was a decrease of WBC in 2% effluent treated fish from 4.7 x 10^6 mm^3 in week 2 to 0.97 x 10^6 mm^3 in week 8. Statistical analysis (ANOVA) showed significant difference within treatments and between treatments and weeks ($P < 0.05$). The DMRT (Ducan's multiple range Test) showed the means of 6% effluent treated fish to be significantly higher than those of the control and 2% treated fish, 4% and control (0%) higher than 2% while those of 4% and control are not significantly different. The means of week 8, 6 and 4 are not significantly different. However, week 8 mean is significantly higher than week 2.(Appendix 4a).

The RBC in the control fish increased from 251 x 10^6 mm^3 in week 2 to 280 x 10^6 mm^3 in week 8. There was also an increase in the RBC in 4% effluent exposed fish. However, a decrease in RBC was observed in the 2% and 6% treatments. The decrease was more pronounced in the highest concentration (6%) having decreased from 492 x 10^6 mm^3 in week 2 to 161 x 10^6 mm^3 in week 8. Statistical analysis however showed no significant difference between the treatments and between treatments and weeks ($P > 0.05$). DMRT showed no signifi... differences (variations) between the means of the concentrations and between the means of the weeks (Appendix 4b).

The haematocrit (PCV) values increased in the 0%, 2% and 4% exposures or trea... throughout the duration of exposure. In the 6% however, there was an increase from 40%...

value in week 2 to 42% in week 4 and then, a stabilisation back to 40% in week 6 which was maintained up to termination period (week 8). The 6% treatment fish haematocrits were statistically higher ($P < 0.05$) than the other treatment, indicating that the changes were dose dependent. DMRT showed the means of all the effluent concentrations to be statistically insignificant but all being significantly higher than that of the control. Likewise, the means of weeks 8, 6 and 4 were not significantly different from each other but all are significantly higher than that of week 2. Figure 8a shows the changes in haematocrit values.

Haemoglobin (Hb) on the whole increased in all the exposures. There was however, a slight decrease in the 6% effluent exposed fish as Hb values, from 14g/100ml in week 4 deceased to 13.9g/100ml in week 6. Statistically, there was a significant difference between the Hb of the control fish and the exposed fish ($P < 0.05$). Changes in Hb values are shown in figure 8b.

In the 'absolute values', mean cell haemoglobin concentration (MCHC) was more or less maintained throughout the period of exposure as shown in Table 4.4. Statistical analysis (ANOVA, DMRT) showed no significant difference both within and between treatments (concentrations) and weeks ($P > 0.05$).

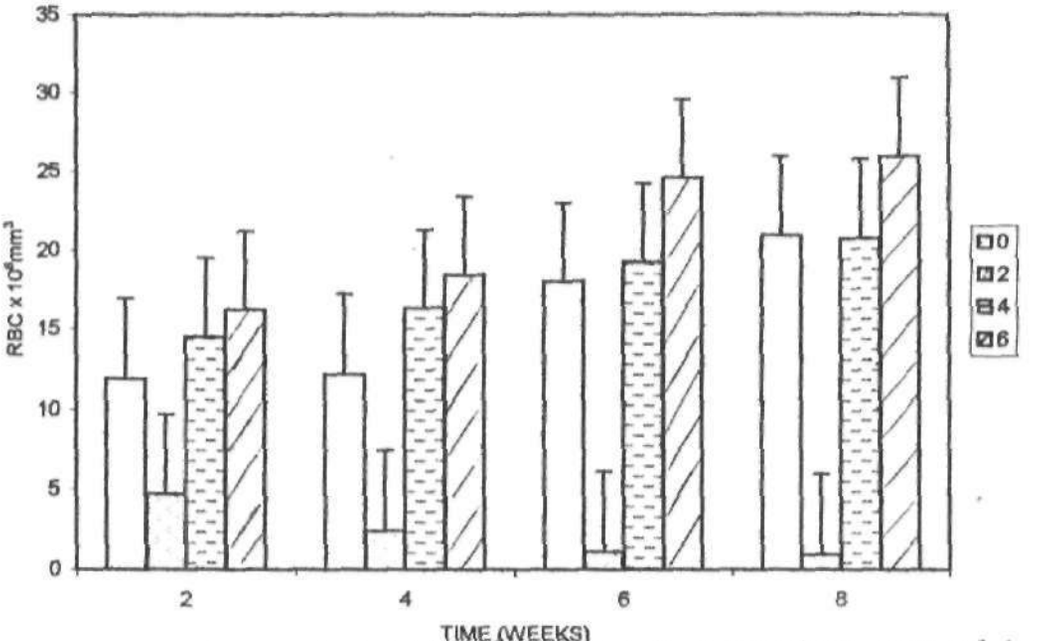


Fig.7a: Changes in RBC ($x10^6 mm^{-3}$) of C. gariepinus exposed to various tannery effluent conc. for 8 weeks (Bars represent SE of mean)

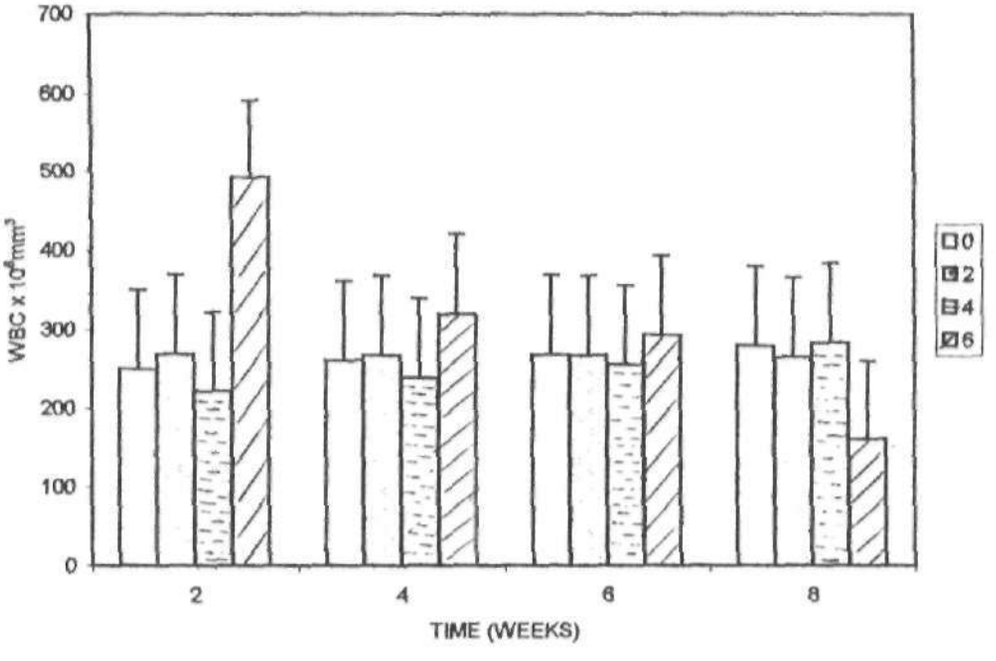


Fig. 7b: Changes in WBC (x $10^6 mm^{-3}$) values of C. gariepinus exposed to various tannery conc. for 8 weeks (bars represent SE of mean)

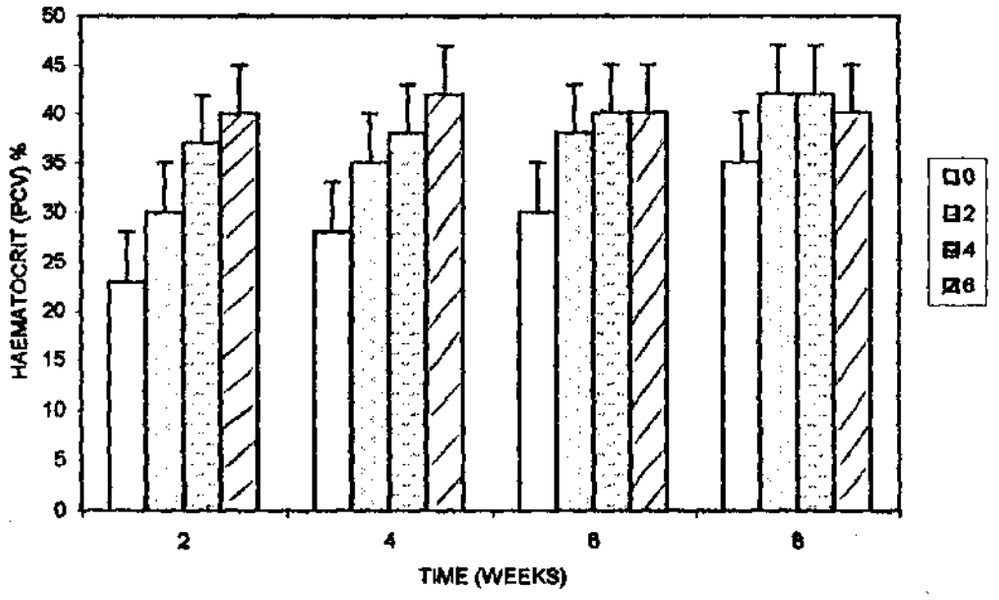


Fig. 8a: Haematocrit (%) values of C. gariepinus exposed to different tannery effluent conc. for 8 weeks (Bars represent S.E. of Mean)

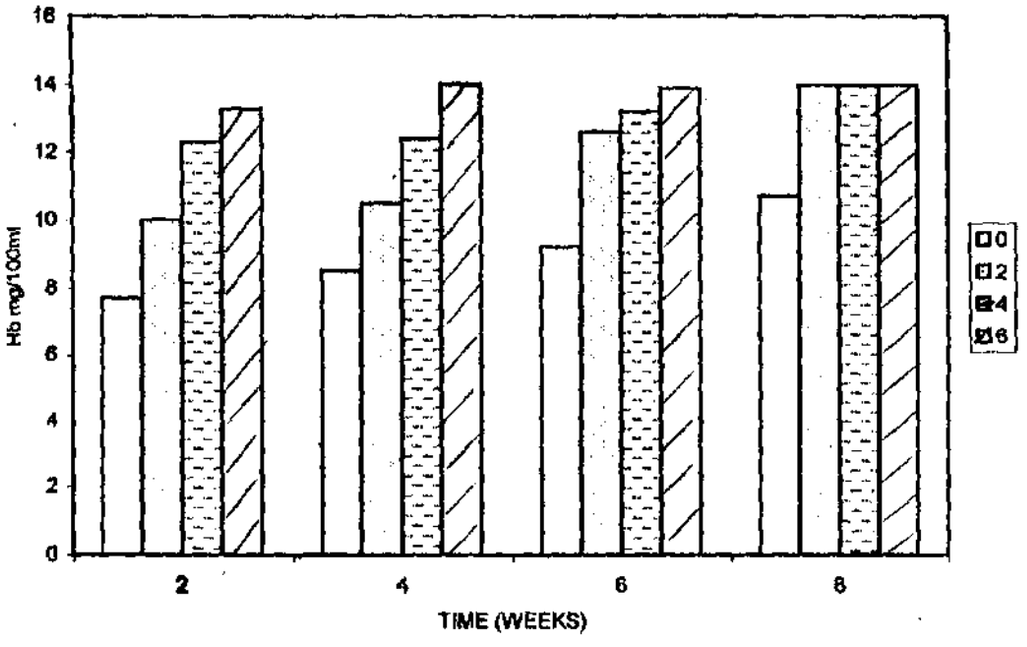


Fig. 8b: Hb (%) values of C. gariepinus exposed to tannery effluent for 8 weeks.

Table 4.4. MCHC values (‰) of juveniles of *C. gariepinus* exposed to various sublethal concentrations of tannery effluents for 8 weeks (mean ± S.E.M.)

Effluent Conc. ‰ v/v	Duration of Exposure (Weeks)			
	2	4	6	8
0	33.38±0.40	30.36±0.90	33.66±0.45	33.44±0.84
2	33.33±0.22	30.00±0.36	33.16±0.76	33.33±0.66
4	33.34±0.56	32.33±0.72	233.00±1.02	33.50±0.36
6	33.25±0.27	33.33±0.34	32.20±0.63	32.20±0.84

Analysis of variance showed that there exist a significant difference ($P < 0.05$) between the MCHC of 6% fish and those of the rest exposures. DMRT for concentrations showed the means of 4%, 2% and 0.% fish to be significantly higher than those of 6% treated fish. In terms of weeks, there was no significant difference.

The M.C.H. results are presented in Table 4.6 and showed a decrease though not a significant one ($P > 0.05$) in only one of the treatments, 4% and an increase in treatment 0%, 2% and 6% (not significant) DMRT produced on significant difference between the means.

4.3.2 Metal Accumulation:

Fish were exposed to three levels of effluent concentrations those were 2%, 4% and 6% in addition to the control.

At 2% Effluent Concentration:

Results show Pb to be more accumulated in fish tissues, followed by chromium, copper and then zinc in that order. In terms of tissue enrichment of individual metals, Cr is seen to be distributed in the order; liver > gut > skin > bone > muscle > wholefish. Statistical analysis showed the accumulation in the liver to be highly significant ($P < 0.05$) compared with that of other tissues and that there was a significant difference($P < 0.05$) between weekly accumulation with the distribution occurring in the order of week 8 > 6> 4> 2. Incidently, a similar pattern was observed for all the other metals.

Table 4 .5 Mean cell volume (μ^3) of juveniles of *C. gariepinus* exposed to tannery effluents for 8 weeks (Mean ± S.E.M).

Effluent Conc. % v/v	Duration of Exposure (Weeks)			
	2	4	6	8
0*	0.916±0.14	1.069±0.20	1.115±0.42	1.143±0.36
2	1.110±0.11	1.306±0.21	1.418±0.33	1.579±0.45
4	1.667±0.37	1.583±0.46	1.563±0.43	1.497±0.25
6	0.813±0.21	0.131±0.06	1.136±0.23	0.248±0.02

*Statistically lower ($P < 0.05$) than other treatments.

Table 4.6 Mean cell haemoglobin (μ. μg) values for juveniles of *C. gariepinus* exposed to tannery effluent for 8 weeks (Mean ± S.E.M).

Effluent Conc % v/v	Duration of Exposure (Weeks)			
	2	4	6	8
0	0.307±0.14	0.324±0.06	0.346±0.02	0.382±0.03
2	0.370±0.12	0.392±0.08	0.470±0.05	0.526±0.10
4	0.554±0.03	0.517±0.06	0.517±0.13	0.493±0.16
6	0.270±0.05	0.412±0.11	0.473±0.06	0.870±0.18

Individual tissues enrichment by various metals is shown for copper to be in the order of liver > gut > skin > gill > muscle > bone > wholefish. For lead, it is liver > gut > gill > skin > bone > muscle > wholefish and for zinc, it is liver > skin > gut > gill > bone > muscle wholefish. Results for these are illustrated on figures 9a - d.

At 4% Effluent concentration:

The concentration pattern exhibited at this level of exposure is similar to that obtained in the 2% effluent concentration with Pb accumulation > cr > cu > zn. For metals enrichment of fish tissues, statistical analysis showed the liver to contain higher amounts than all the other tissues ($P < 0.05$) and this was found to be true for all the metals studied here. Chromium uptake by tissues was in the order: Liver > Gill > Gut > Skin > Bone > Muscles > wholefish. Copper was in the order; Liver > Gill > Skin > Gut > Bone > wholefish > muscles. For lead: liver > Gill > Skin > Gut > Bone > wholefish > Muscles and for Zinc, the order liver > Gill > Bone > Skin > Gut > Muscles > wholefish was found. DMRT showed a significant difference between weekly accumulations with week 8 having the highest enrichment levels and distributed in the order of 8 > 6 > 4 > 2. These results are presented in figures 10a - d.

At 6% effluent concentration:

At higher effluent concentration of 6%, total tissues metal burden increased. Total burden increased in the pattern of Pb > Cr > Cu > Zn with regards to metal element. Highest levels of distribution are attained here indicating increased uptake by tissues at elevated levels of environmental concentration. Individual metal enrichment of the tissues showed the liver having significantly ($P < 0.05$) higher levels of accumulation than other tissues for all the metals.

Significant differences are also observed in terms of weeks with the highest levels attained in week 8 (8 > 6 > 4 > 2). Cr enrichment of the tissues followed the order; liver > Gill > Gut > Skin > Bone > Muscle > Whitefish. Cu was of the order; Liver > Gill > Gut > Skin > Bone > Muscle > wholefish. For lead, Liver > Gill > Gut > Skin > Bone > Muscle >wholefish and for Zn ; Liver > Gill > Gut > Bone> Skin > Muscles > wholefish. (See figures 11a - d).

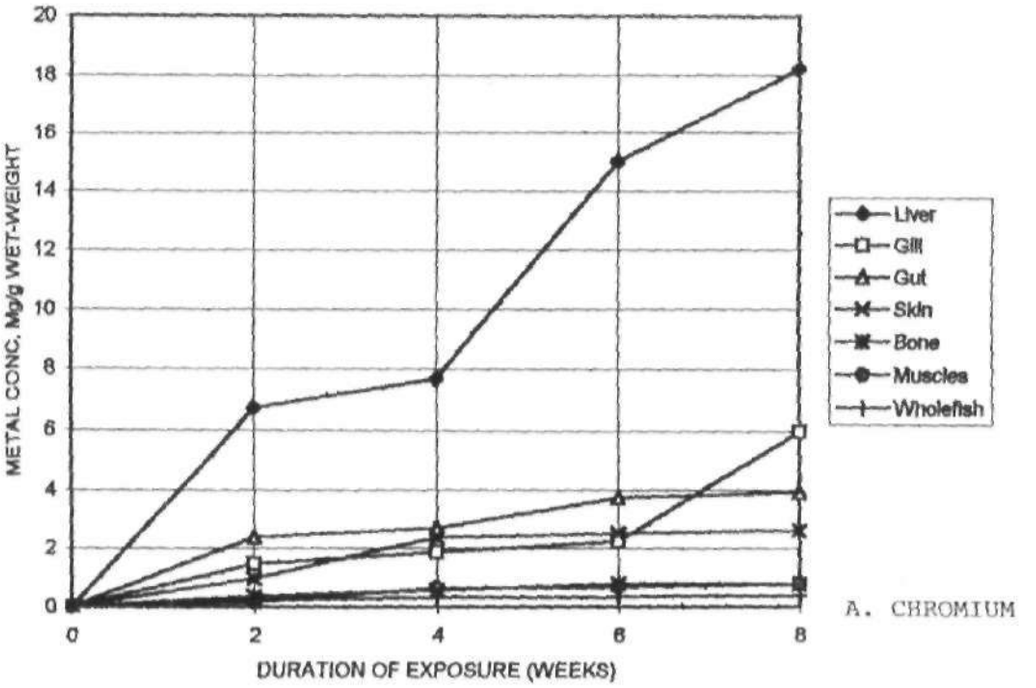
Total metal (Cr, Cu, Pb and Zn) Burden:

Figure 12 shows the total amount of each metal (Cr, Cu, Pb and Zn) accumulated by various fish tissues and the information is also illustrated on figure 12. The metals are accumulated in the order of Pb > Cr > Cu > Zn with Pb and Cr levels of accumulation significantly higher ($P < 0.05$) than Cu and Zn. Metals enrichment of fish tissues also increased with time with the amount accumulated in weeks $2 < 4 < 6 < 8$. Statistical analysis showed significant difference ($P < 0.05$) between weeks accumulation. Metal burden of all levels of tannery effluent exposure are significantly higher than those of the control ($P < 0.05$).

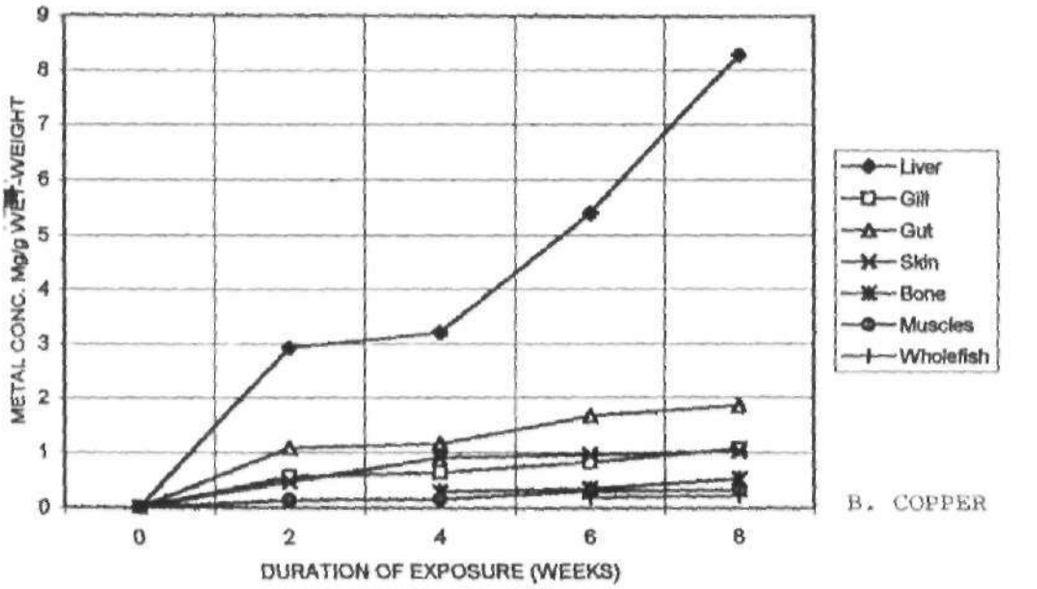
The control data is presented in Table 4.10 and shows accumulation in all the tissues and wholefish to decrease with time. That is, accumulation levels in week 2 > 4 > 6 > 8.

Table 4:7 Showing metals (Cr, Cu, Pb & Zn): Distribution in tissues of juveniles *Clarias gariepinus* exposed to ABU. Dechlorinated water (control) Mean conc. In mg/g wet weight ± S.E.M.

METAL	BODY TISSUE	DURATION OF EXPOSURE (WEEKS) 2	4	6	8
CHRO-MIUM	LIVER	5.488±1.2	3.610±1.1	2.222±0.6	1.102±0.23
	GILL	2.240±0.84	1.141±0.52	1.769±0.34	1.004±0.12
	GUT	0.406±0.14	0.316±0.21	0.116±0.03	0.095±0.02
	SKIN	0.594±0.1	0.451±0.23	0,.326±0.14	0.103±0.03
	BONE	0..402±0.2	0.459±0.02	0.432±0.025	0.420±0.06
	MUSCLE	0.125±0.03	0.067±0.01	0.060±0.004	0.050±0.003
	WHOLEFISH	0.300±0.1	0.091±0.003	0.064±0.002	0.032±0.012
COPP-ER	LIVER	2.927±0.7	1.536±0.5	0.038±0.01	0.030±0.00-
	GILL	0.0558±0.25	0.487±0.06	-	-
	GUT	0.362±0.03	0.177±0.02	0.063±0.002	-
	SKIN	0.331±0.01	0.211±0.04	-	-
	BONE	0.225±0.01	0.183±0.05	-	-
	MUSCLE	-	-	-	-
	WHOLEFISH	0.130±	-	-	-
LEAD	LIVER	6.141±1.66	3.945±1.52	31.028±0.23	2.002±1.01
	GILL	2.510±1.02	1.553±0.13	1.002±0.03	0.865±0.20
	GUT	3.684±0.45	0.937±0.24	0.840±0.26	0.641±0.30
	SKIN	0.875±0.56	0.564±0.04	0.489±0.12	0.40±0.02
	BONE	0.972±0.82	0.510±0.12	0.474±0.17	0.382±0.14
	MUSCLE	0.132±0.02	0.095±0.02	0.008±0.004	-
	WHOLEFISH	0.31±0.05	0.109±0.006	0.092±0.002	0.084±0.006
ZINC	LIVER	0.404±0.07	0.340±0.10	0.290±0.04	0.250±0.06
	GILL	0.306±0.14	0.258±0.07	0.251±0.013	0.230±0.02
	GUT	0.140±0.03	0.139±0.08	0.090±0.002	0.075±0.01
	SKIN	0.098±0.03	0.120±0.03	0.107±0.0.04	0.066±0.03
	BONE	0.193±0.015	0.113±0.02	0.109±0.05	0.105±0.03
	MUSCLE	0.098±0.004	0.016±0.002	0.023±0.003	0.020±0.004
	WHOLEFISH	0.036±0.007	0.028±0.006	0.027±0.002	0.009±0.001

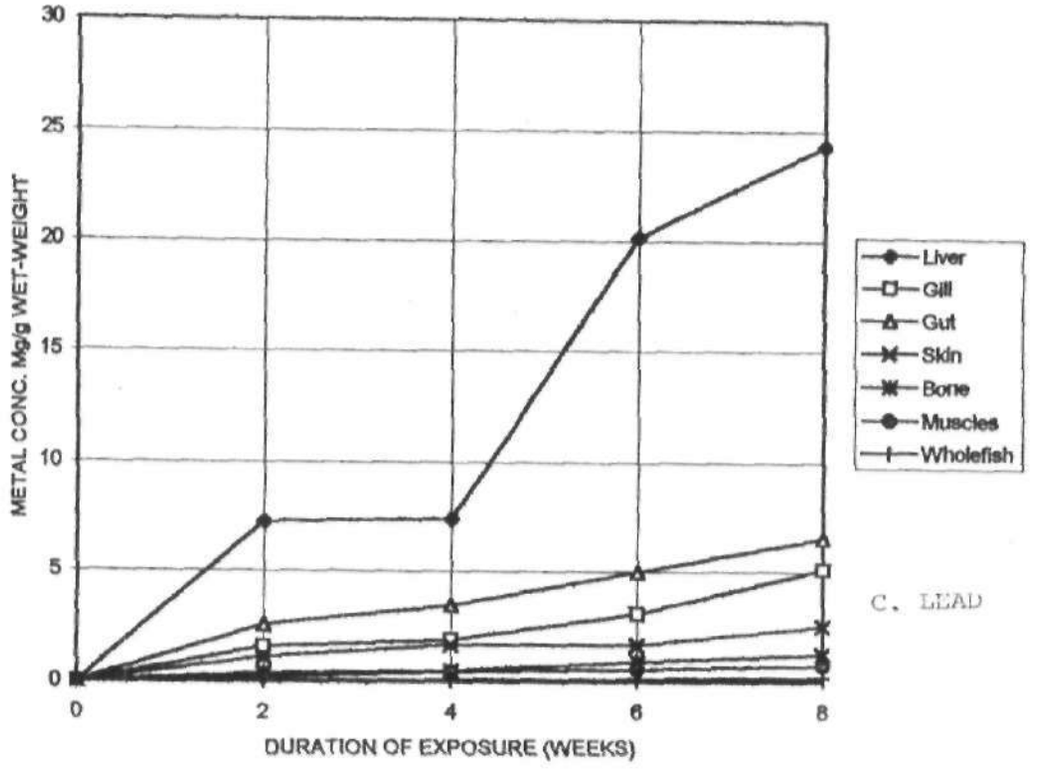


A. CHROMIUM

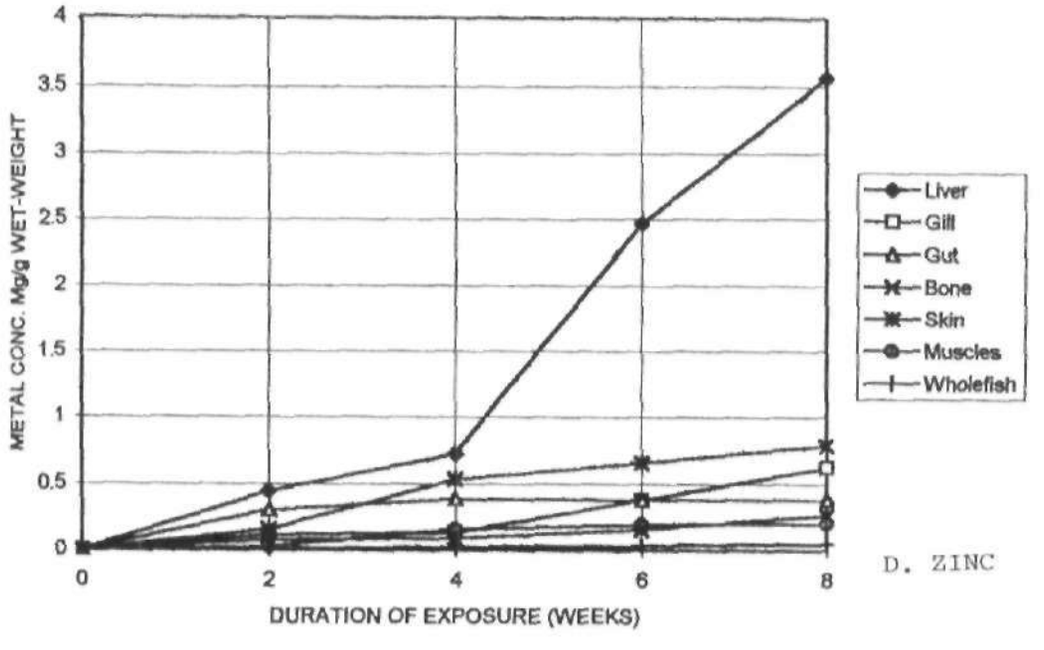


B. COPPER

Fig 9 A and B: Chronium and Copper distribution in tissues of C. gariepinus exposed to 2% tannery effluent.

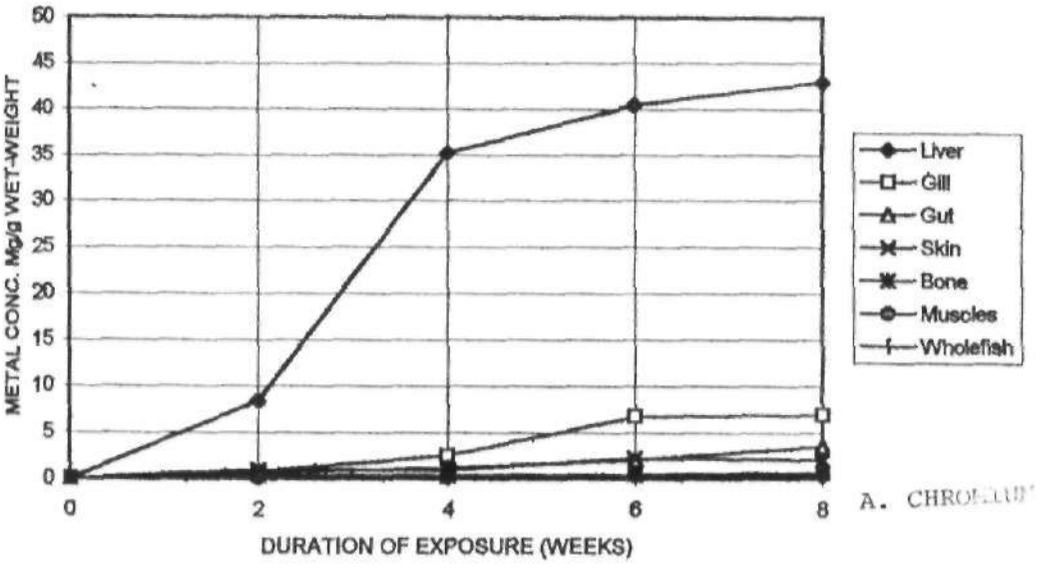


C. LEAD

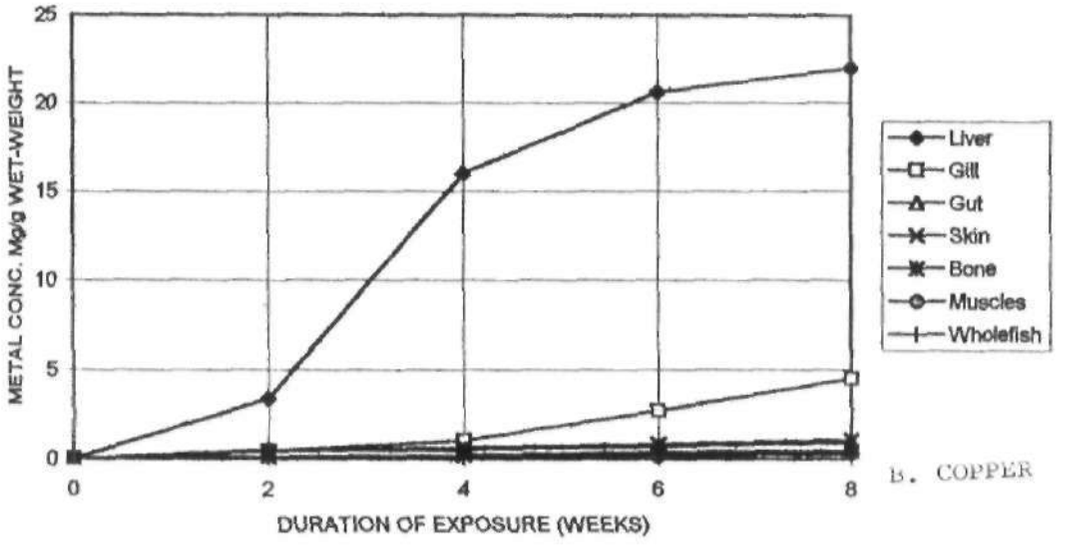


D. ZINC

Fig. 9(C)-(D): Pb and Zn distribution in tissues of C. gariepinus exposed to 2% tannery effluent concentration

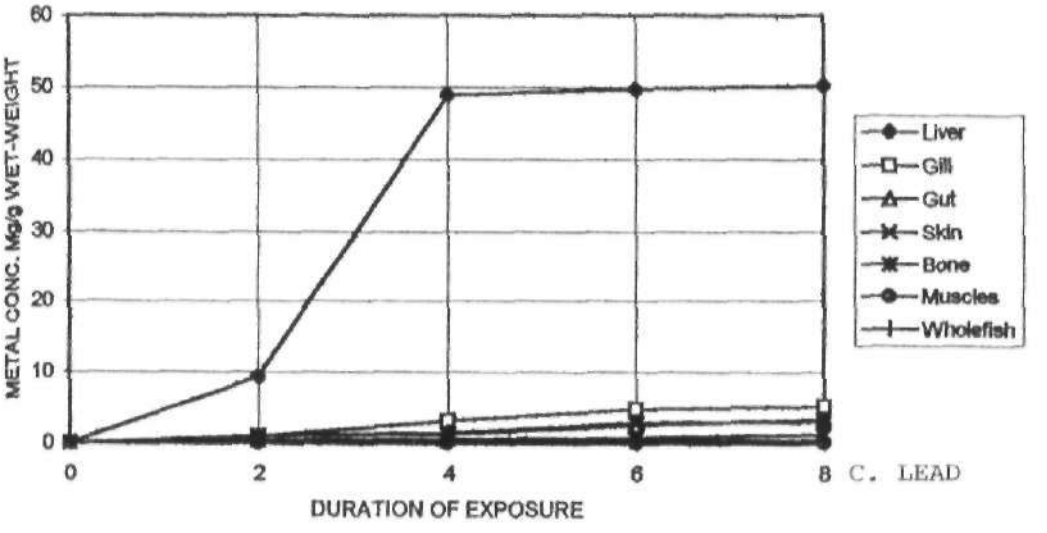


A. CHROMIUM

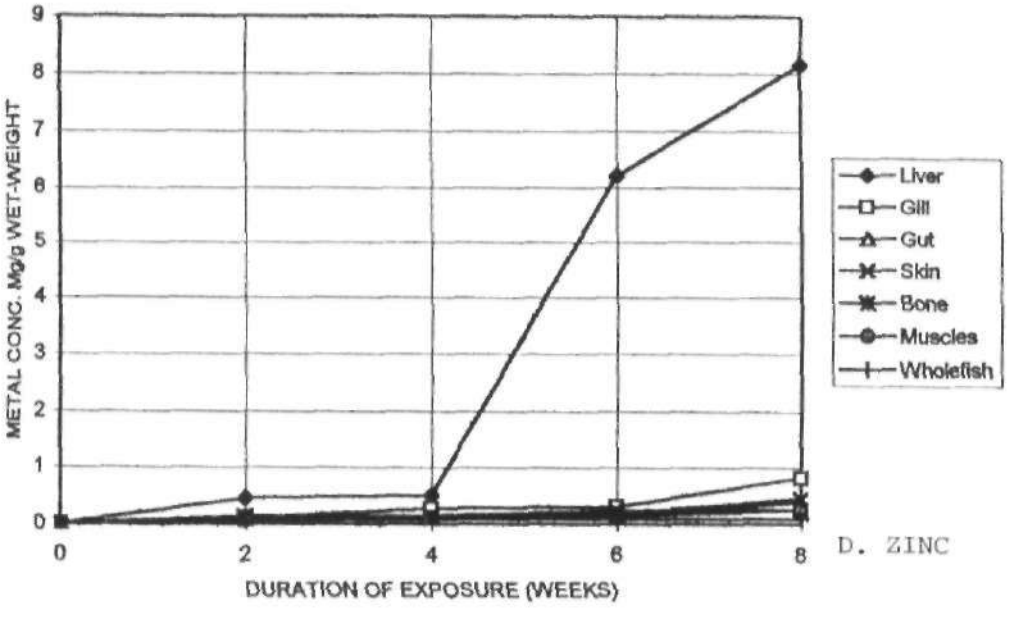


B. COPPER

Fig 10 A and B: Chromium and Copper in tissues of C. gariepinus exposed to 4% tannery effluent.

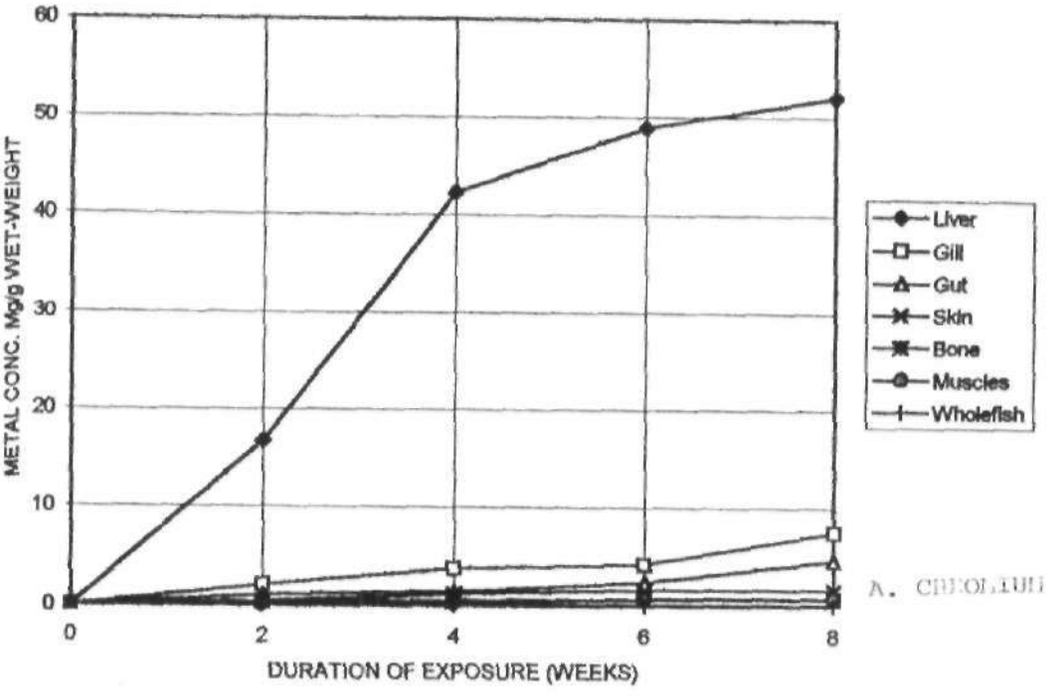


C. LEAD

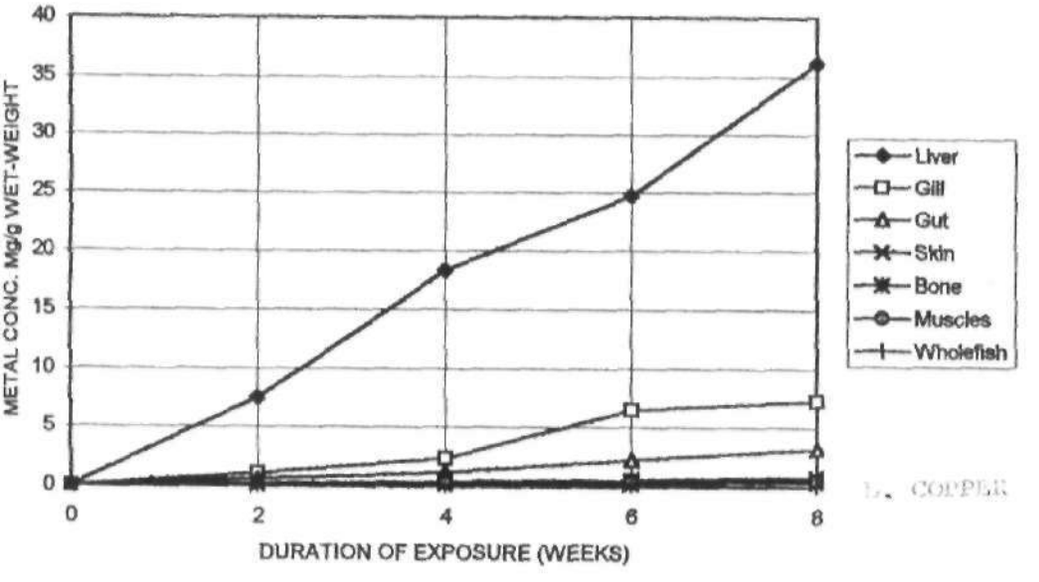


D. ZINC

Fig.10(c)-(d): Pb and Zn in tissues of C. gariepinus exposed to 4% tannery effluent concentration

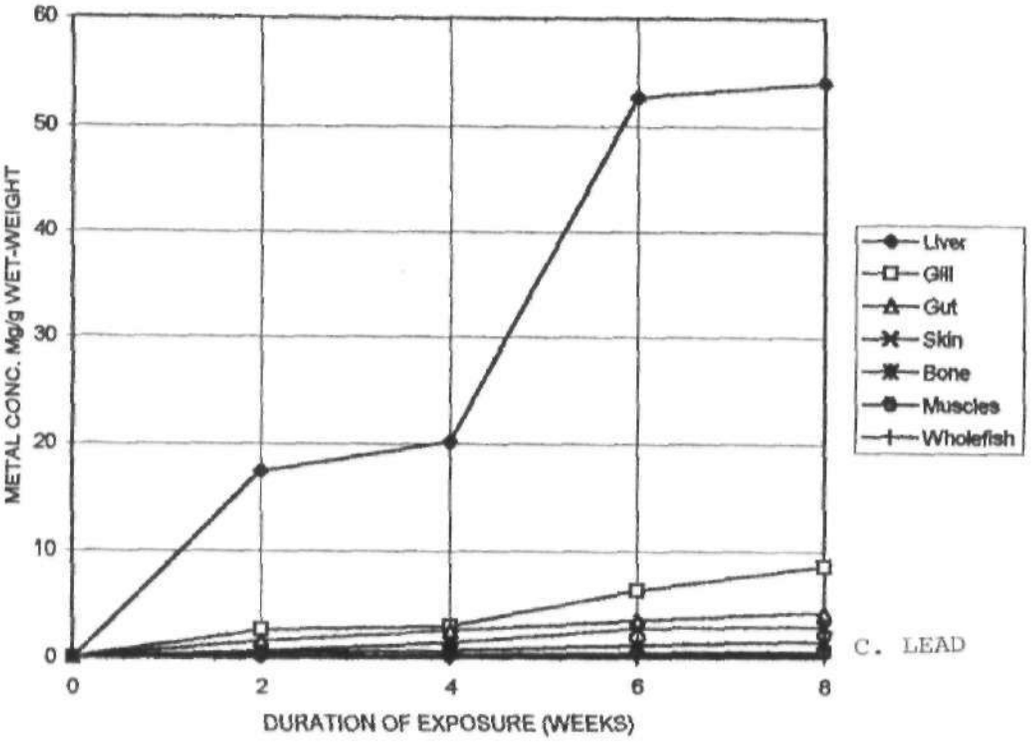


A. CHROMIUM

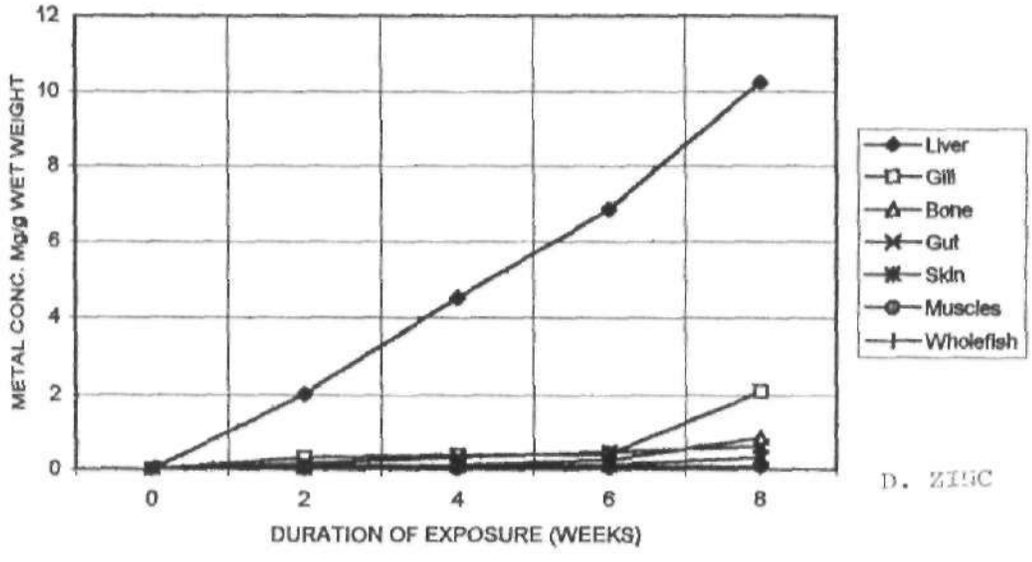


B. COPPER

Fig 11 A and B: Chromium and Copper distribution in tissues of C. gariepinus exposed to 6% tannery effluent.



C. LEAD



D. ZINC

Fig.11(c)-(d): Pb and Zn distribution tissues of C. gariepinus exposed 60 6% tannery effluent concentration

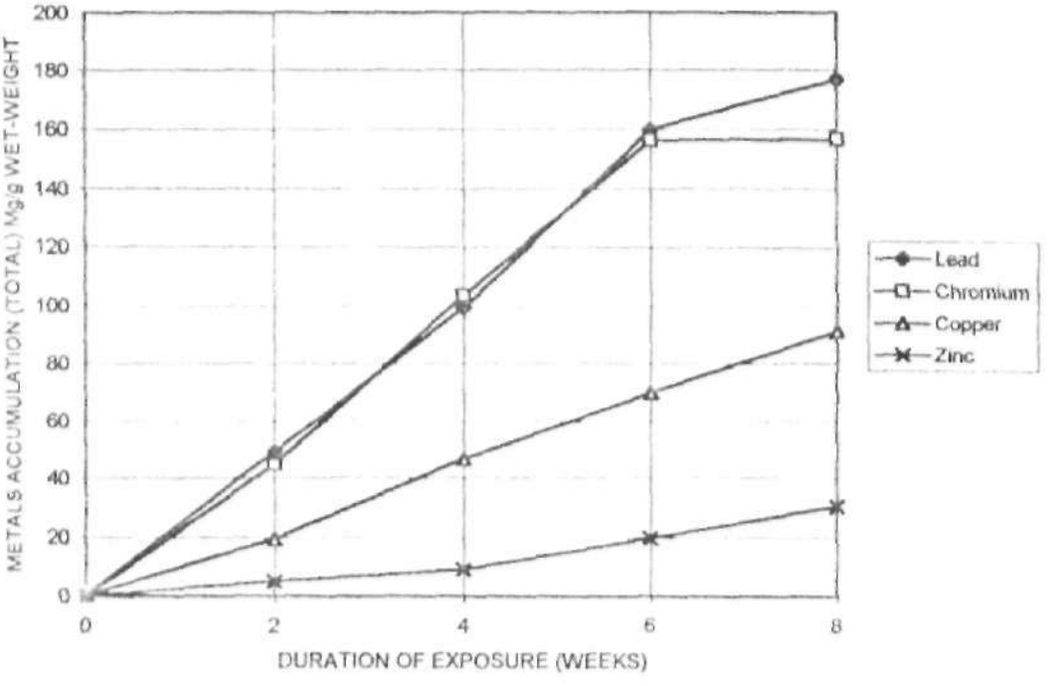


Fig. 12: TOTAL METALS (Cr, Cu, Pb & Zn) ACCUMULATION IN TISSUES OF JUVENILES OF C. gariepinus EXPOSED TO TANNERY EFFLUENT

4.3.3 Sublethal Histopathology:

The sublethal histopathological results are presented on Plates II and III.

KEY TO PLATE II

PLATE IIa:

Light micrograph of control fish gill. x 350, H & E stain. Note the regulary spaced gill limillae and well arrgned secondry lamillae.

PLATE IIb:

Light micrograph of gill of fish exposed to 4% tannery effluent for four (4) weeks. Note the slight epithelial swellings (FS) blocking the epithelial space at a point. x450 H & E stain.

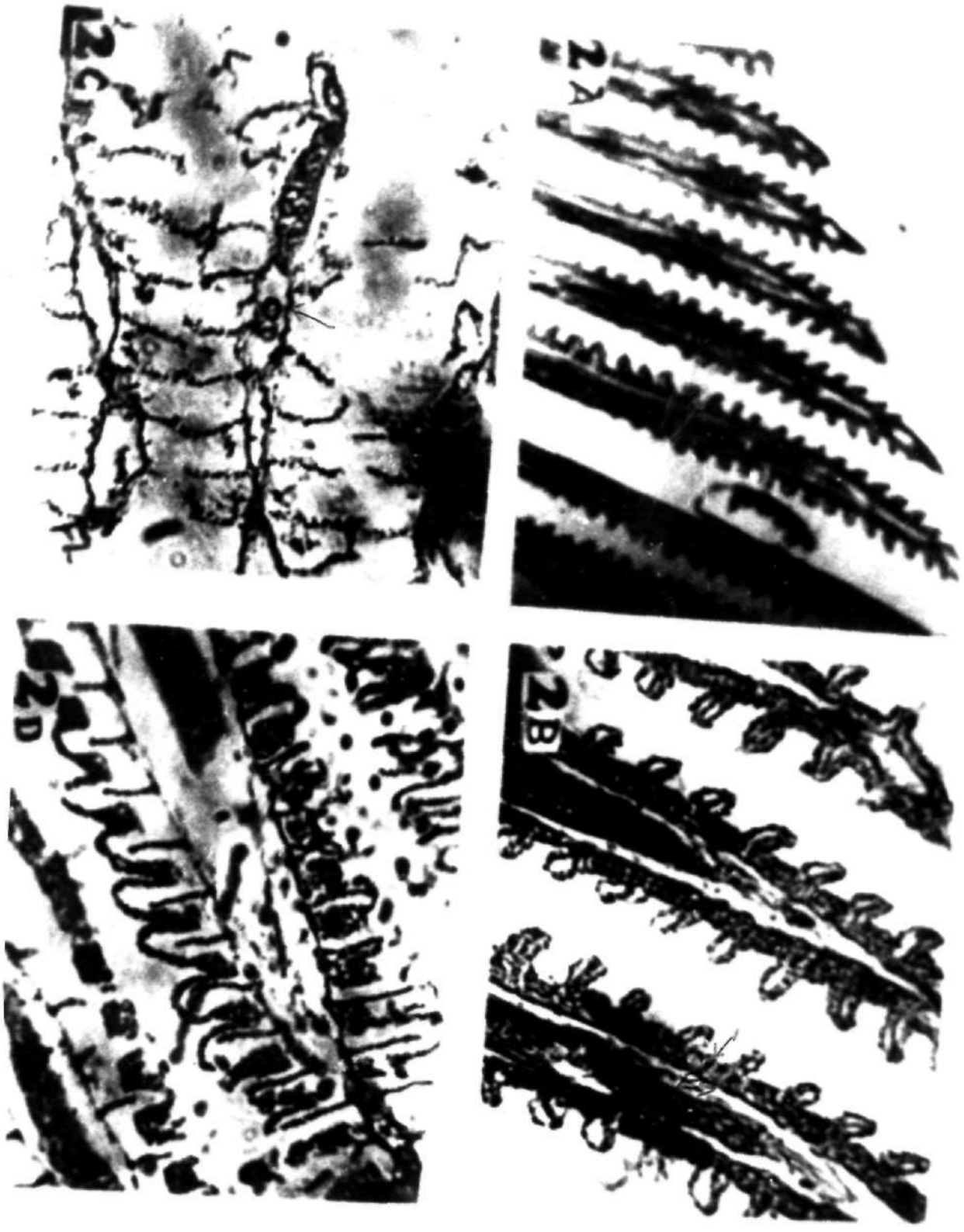
PLATE IIc:

Light micrograph of gill of fish exposed to effluent of 6% concentration level. (x 100, H & E stain). Note the conspicious oedema (OD) and hyperplasia (FL) of the gill lamellae.

PLATE IId:

Same as plate IIc above but at a higher magnification (x 450). Note the destruction of the secondary lamillae at the tips (DT) and also sloughed off celular debris in the underlying pillar cells (PC).

PLATE II: LONG TERM EFFECTS OF TANNERY EFFLUENT ON THE GILLS OF *C. gariepinus*



KEY TO PLATE III

PLATE III

Light micrograph of lvier of fish exposed to 0% effluent (dechlorinated ABU Community water).

(a) x 45, H & E stain. Observe the liver plates that anastomose freely and note also the limited space occupied by the sinusoids .

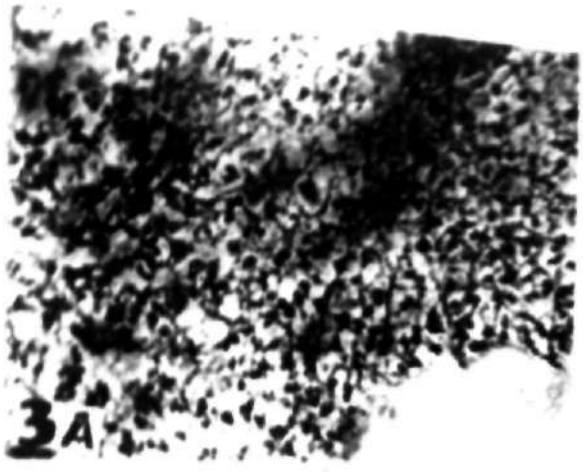
(b) same as (a) above but at x300, H & E. Note that the postal area is not shown.

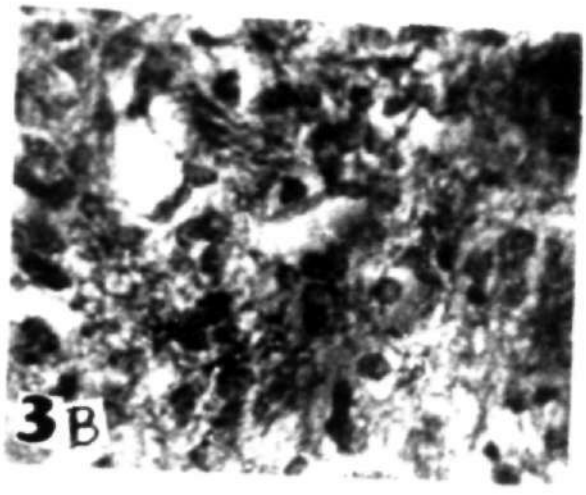
(c) Liver of fish exposed to 2% tannery effluent for 2 weeks - (x 45, H & E stain). Note the appearance of dark particles indicating necrotic(NE) damage in the liver.

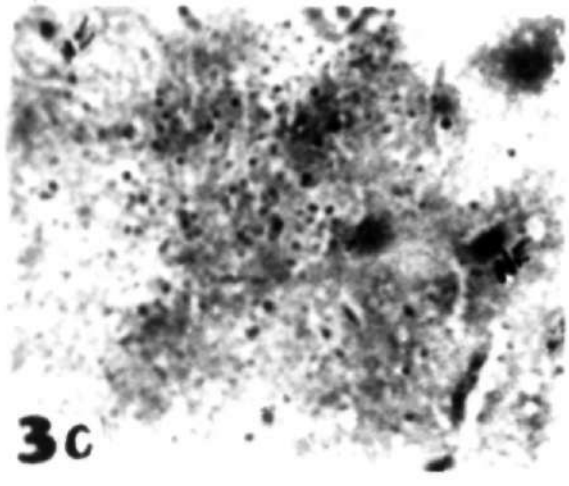
(d) Liver of fish exposed to 6% effluent concentration for 4 weeks showing typical globular bodies surrounded by open spaces (x 250, H & E stain).

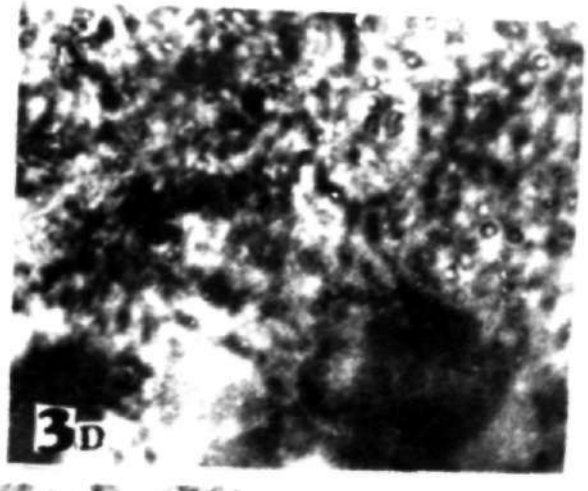
(e) Liver of 6% effluent exposed for 8 weeks x 300, H & E stain.Note necrotic damage with oedematous hepatocytes.

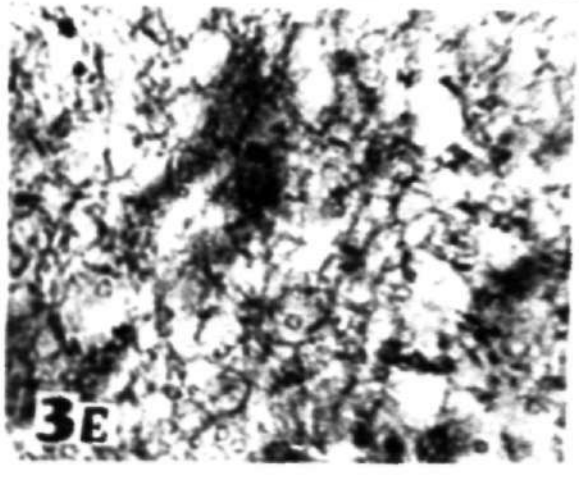
PLATE III: LONG TERM EFFECT OF TANNERY EFFLUENT ON THE LIVER OF *C. garieipinus*











4.3.4 Ovarian Development:

Figure 13 shows chagne in weight (wet -weight) of the ovaries of fish exposed to tennary effluent at 6% concentration and those of the control fish. There was retardation in the ovarian weighis of effluent exposed fish as compared with those of the control fish, though no significant differenct (< 0.05) was recorded. Histological studies showed more maturing and mature ovaries in the control than in the effluent fish as shown in Plate IV.

OVARIAN PLATES

Plate IV a - e show the various stages of ovarian development in fishes exposed to 6% tannery effluent and those exposed to ABU dechlorinated community tap water. Stages of oogenesis are identified on the basis of nuclear and cytoplasmic characteristics. The various stages are:

STAGE I:

Immature (pre vitellogenic) Primary oocytes. Note that oocytes in this stage are non-yolky.

STAGE II:

Maturing (Vitellogenic) oocytes.

STAGE III:

Mature oocytes. The yolk here is fully formed.

KEY TO PLATE IV

PART IV a:

Light micrograph of T.S of ovaries from a baseline fish (x40, H & E stain after fixing in Bouin's fluid). Note the even distribution of oocytes.

PLATE IV b.

Light micrograph of T.S of fish oocytes exposed to 0% effluent concetration,i.e ABU dechlorinated water at 6 weeks (x 150 H & E stain). Note the large number of mature and maturing oocytes.

PLATE IV c

Light micrograph of the oocytes (T.S) of 6% effluent exposed fish at 4 weeks (x350, H & E)Note that most oocytes are in the stage II with small yolk..

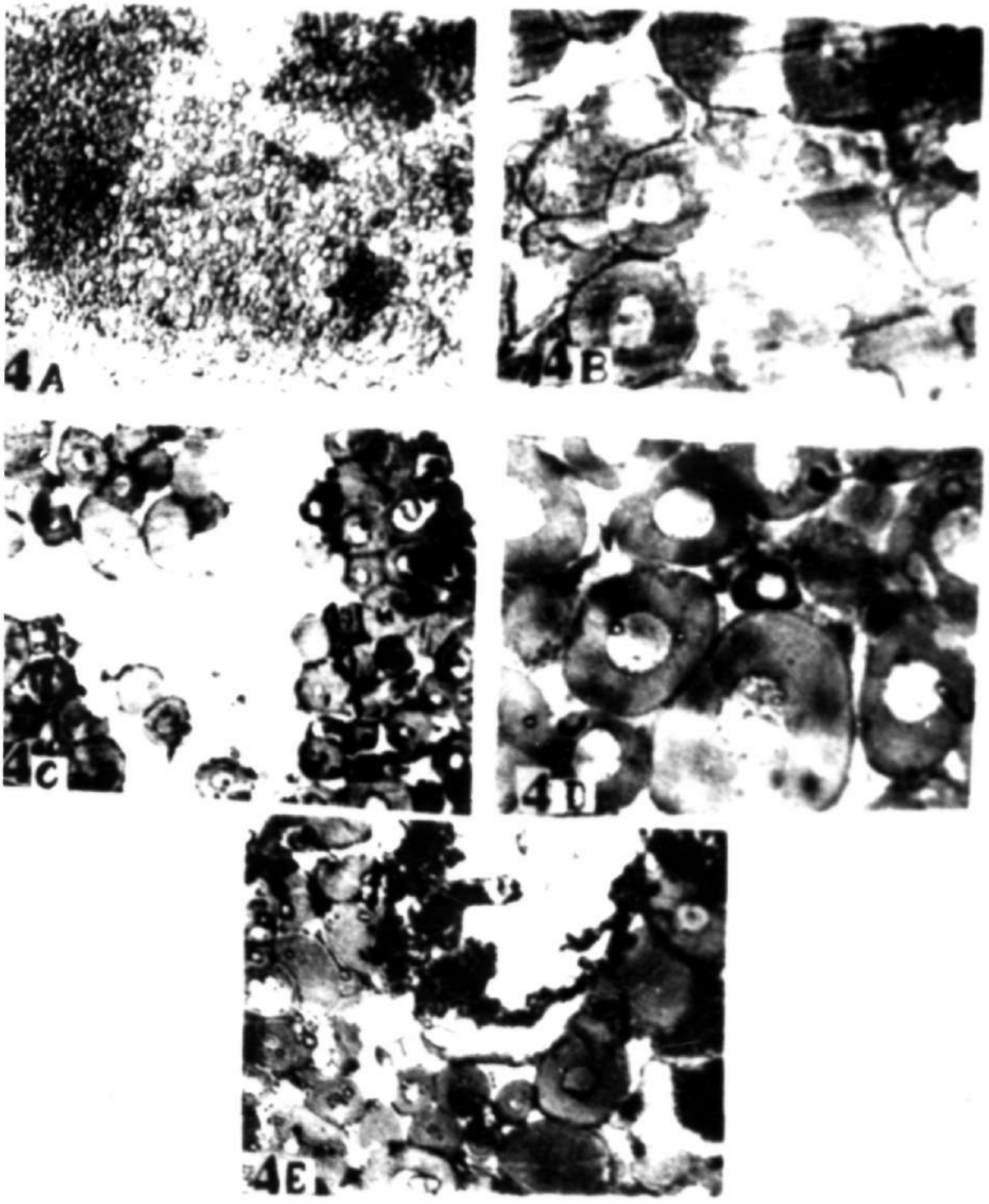
PLATE IV d:

Light meirograph showing the T.S of 6% effluent treated fishat 6 weeks (x 450, H & E stain). Note the few mature oocytres, dominated by maturing or stage II oocytes.

PLATE IV e.

6% effluent treated fish oocytes (T.S) at 8 weeks (x350, H & E stain) There are more stage II oocytes at this duration of exposure. There is an incidence of atresia (AT).

PLATE IV: LONG TERM EFFECT OF TANNERY EFFLUENT ON THE OVARIES OF *C. garnepinus*



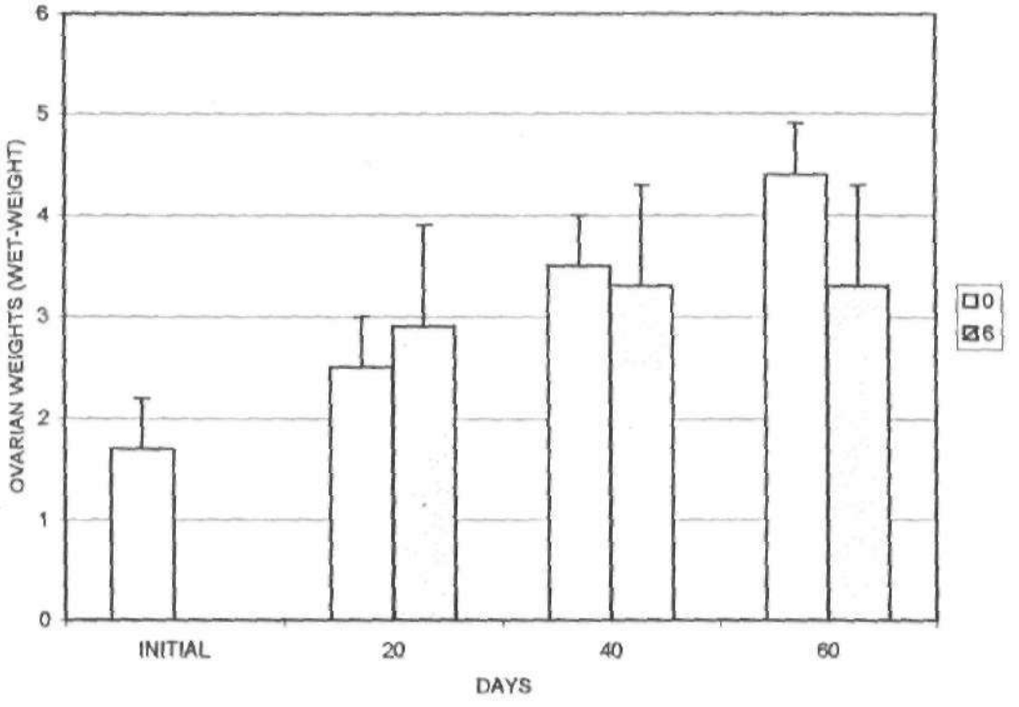


Fig. 13: CHANGES IN THE OVARIAN WET-WEIGHT OF CONTROL AND 6% EFFLUENT EXPOSED FISH (BARS INDICATE S.E. OF MEAN)

3.4.5 Growth:

Growth results are presented in Figure 14 and Table 4.8. Weight increase was observed in the control fish and in 1% and 2% effluent treated fish, though the increase was statistically higher (P 0.05). In 0% and 1% than in 2%. In the 4% and 6% exposed fish, there was a slight weight gain in week 2 and then, a trend of continous decrease throughout the remainging exposure period in 6% effluent fish, and a slight recovery in week 8 (from - 5.30% to -3.51%) in 4% effluent exposed fish. Subjecting the results to DMRT showed the means of concentrations 0% and 1% to be significantly higher than tha other exposures (2%, 4% and 6%).

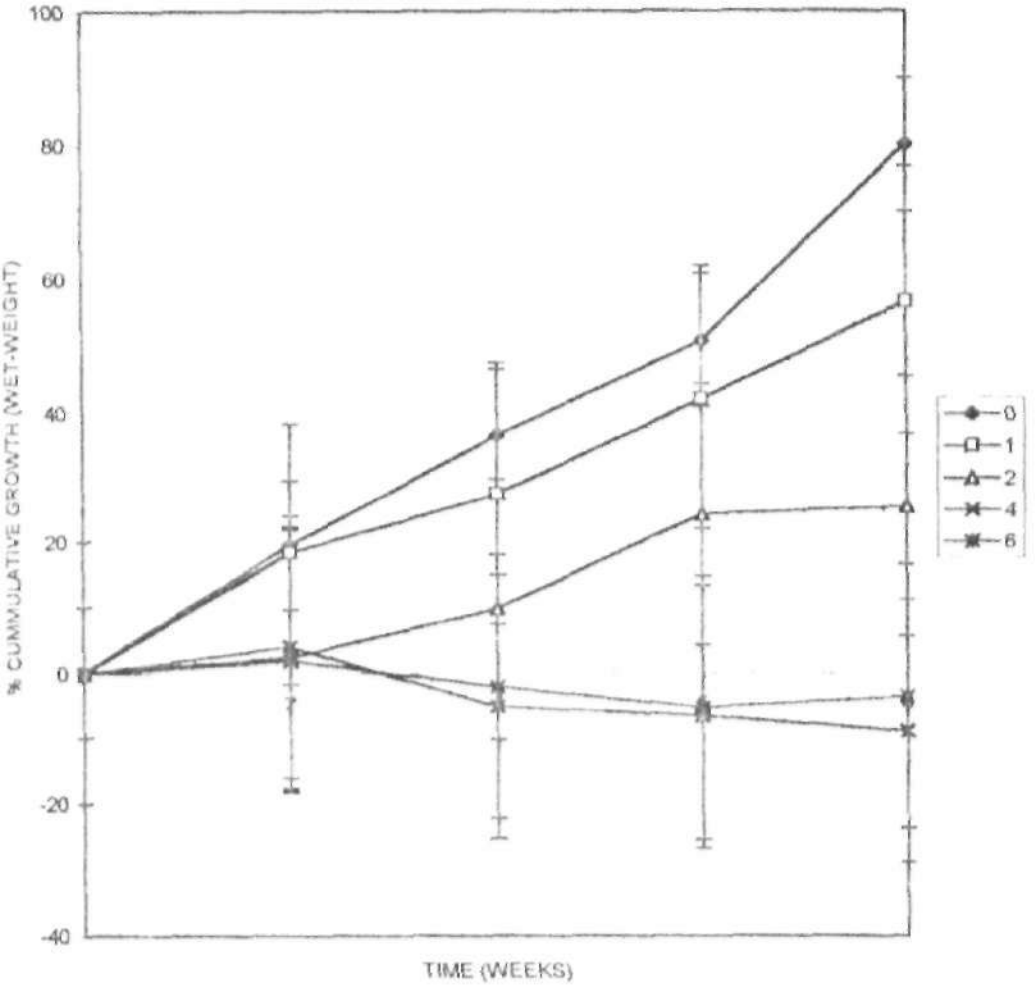


Fig. 14: Showing % cumulative wet-weight gain or loss in C. gariepinus exposed to different tannery effluent concentrations (bars represent SE of mean)

Table 4.8 Average wet-weight changes in control and exposed fish (with S.E.FM)
Note. Percentage commulative figures are in parenthesis.

EFFLUENT CONC. % V/V	WET - WEIGHT CHANGES PER WEEK				
	INITIAL	2	4	6	8
0*	4.202±0.19 (0)	5.024±0.72 (19.56)	5.822±0.89 (36.55)	6.340±0.42 (50.88)	7.564±1.12 (80.00)
1*	2.201±0.78 (0)	2.601±0.41 (18.31)	2.805±0.27 (27.44)	3.126±0.34 (42.03)	3.451±0.64 (56.79)
2	2.424±0.32 (0)	2.482±0.46 (02.39)	2.660±0.36 (09.74)	3.013±0.44 (21.30)	3.044±0.61 (25.58)
4	5.702±0.49 (0)	5.810±0.62 (01.89)	5.585±0.80 (-02.07)	5.400±0.42 (-5.30)	5.502±0.57 (-3.51)
6	6.702±0.27 (0)	6.971±0.71 (4.01)	6.360±0.51 (-5.10)	6.254±0.41 (-6.68)	6.105±0.32 (-8.91)

*Significantly higher ($P < 0.05$) than other treatments.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Tannery Effluent Parameters

Except for temperature which was below the acceptable standard levels, the rest of the other parameters were found to be higher than the standard acceptable levels. It is therefore evident from the results that the discharge of these effluent is causing a fair degree of environmental pollution. These findings agree with those of Lawal and Singh, (1981) and Lawal *et al* (1996).

5.2 Fish Behaviour and Symptoms of Toxicosis.

The behaviour described for acutely tannery effluent intoxicated fish indicate toxic effect of the effluents on the central nervous system (CNS). The primary acute actions of the effluent could be that one, few or several toxicants from the effluent accumulated internally and impaired cerebral oxidative energy metabolism and resulted in depletion of high energy phosphate compounds in the brain, as was proposed by Smart (1978) for ammonia intoxication to *Salmo gairdneri*. One could therefore understand the increase in gill ventilation frequently, Vf (explained later).

The accumulation of mucus on the bodies and the gills of dead and moribund fish was also found by Annune, *et al*; (1994) when the authors exposed *C. gariepinus* to cadmium. Ghem, *et al*; (1997) found mucus on the gills and bodies of *O. niloticus* exposed to acute levels of tannery effluent. Similar observations have been made when fishes were exposed to heavy metals (Lewis

1978, Ebele, *et al*, 1990, Annune, *et al*: 1991). Tannery effluent contain both ammonia and heavy metals (Lawal and Singh, 1981, Lawal, *et al*: 1996).

The most likely explanation for the accumulation of mucus on the gills and bodies of the acutely exposed fish could be due to increased activity of mucus cells subsequent to exposure which could have resulted in the production of mucus over the body. The precipitation of mucoprotein on the gill filaments could have impaired respiration and osmoregulation and hence resultant death. Similar findings were made by Westfall, (1945), Skidmore (1970), Wong, *et al*, (1977), Annune, *et al* (1991, 1994) and Olbem, *et al*, (1997). The disruption of osmoregulation could have resulted also from the disruption of the gill architecture (as shown in plates I and II) and hence destruction of the osmoregulatory function of the gills.

The swollen abdomens in dead fish suggests that the toxicity of tannery effluent is not restricted only to the external organs (gills and skin). Wong, *et al*; (1977) could not find structural damages in the gills of *Cyprinus carpio* exposed to Zinc sulphate despite death of the exposed fish. Further investigation revealed structural damages to the liver (an internal organ). The swollen abdomen could be as a result of necrotic damage to the gut.

Oladimeji and Offem (1989) found peeling of skin in *C. lazera* exposed to lead. The present study also found peeling of skin. Progressive acidification of the medium (as indicated by a fall in pH) could have resulted into more toxicity as the metal free ions are released from their binding sites into the medium and hence more toxic effects on the skin which is indirect contact with the water.

5.3 Gill Ventilation Frequency, VF:

Observation on gill ventilation frequency, VF, also referred to as opercular ventilation are reported to be a strong indicator of stress when fishes ar in an unfavorable environment . Smart (1978) and Knoph (1996) noted that in the Atlantic Salmon exposed to ammonia, VF increased with time up to 70% of survival time, and decreased again as time for death was approached. A similar decrease in VF was observed in the 12% effluent exposed fish from 120 ventilation per minute at 76-h to 116 ventilation per minute at 96-h.

Plasma catecholamaines were found to stimulate gill ventilation in teleosts (Aota, *et al.* 1990, McKenzie, *et al.* 1991). And their levels were increased in teleosts during short term ammonia exposure (Jeney, *et al*; 1992). Thus, increased VF in exposed fish could be due to increased plasma catecholamine levels but it could equally be a more direct effect of tannery effluent. Plasma ammonia level was described to increase with the water ammonia level for several teleosts (Wilson and Taylor, 1992; Knoph and Olsen, 1994) and Wichser and Kazami (1974) had proposed ammonia to be a central respiratory stimulant in mammals. Smart (1978) suggested that ventilation stimulations in fish (trout) exposed to ammonia was as a result of disturbances in cellular metabolism resulting in an increased oxygen demand by tissues. Begum (1987) later found a depressed oxidative metabolism at the level of the mitochondria. Tannery effluents contain high levels of ammonia and ammonia could actually increase in concentration in the experimental tanks due to rapid excretion as a result of toxicant effects.

The toxicity of ammonia solutions is due to the un-ionized ammonia molecules and that this unionized proportion of ammonia increased with a rise in pH value. Lloyd and Herbert (1960) however, showed that the toxicity of ammonium salts is dependent, not on the pH value of the bulk solution, but on that of the water around the gill surface. As the oxygen concentration of the

test water is reduced, carbondioxide excreted at the gill surface is also reduced and this leads to pH increase around the gill surfaces resulting into an apparent increase in the toxicity of ammonia. This increase in toxicity will become greater as CO_2 in the bulk solution is reduced. The toxicity is further increased in waters of reduced O_2 concentration due to the reduction in the concentration of excreted CO_2 at the gill surface.

At pH below 8.5, a significant proportion of sulphide compound is converted to H_2S . Apart from its known effects on aquatic life and humans (McKee and Wolf, 1963, SLTC, 1965, Pepper, 1966 and Sharphouse, 1984), H_2S a foul smelling gas and highly reducing in nature is known to deplete oxygen content in waters where it is found. The observed effects therefore could have resulted from the direct actions of ammonia and/or in combination with other toxicants present such as sulphide, phenols, heavy metals etc. Heavy metals have been implicated in respiratory disturbances due to their attack on the cytochrome system (Blair, *et al:* 1975).

Although the toxic actions of ammonia, heavy metals and other toxicant are probably not the same, a common effect on their toxicity exist and this results from the reduction in the concentration of dissolved oxygen. According to Lloyd (1961), this suggests a physiological reaction by the fish to such a change of the environment and it is independent of the nature of the poison or toxicant. The most obvious reaction of fishes exposed to environments of low dissolved oxygen is the increase in the volume of water passed over the gills. This therefore increases the amount of the toxicant reaching the gill epithelium, the site at which most toxicant are absorbed.

Increased Vf could also be connected or linked to the disturbances in the gill function as the fish struggles to offset the damage. Generally, Vf were lower in the effluent exposed fish than the control fish at 24 hr. It was observed that when fish were firstly introduced in the effluent tanks, the fish closed their mouths and occasionally darted up to take oxygen at the surface. This

been an avoidance behaviour. The decreased VI with concentration in the first 24 hr and its subsequent increase in the next 24 hr (at 48 hr) agrees with the findings of Babatunde (1997) when she exposed *O. niloticus* to paraquoat.

5.4 Haematology:

In the acute test (96-h), the Hb, RBC, WBC, PCV (haematocrit) were decreased significantly ($P < 0.05$) compared with the control and baseline. MCV and MCH were increased ($P < 0.05$) whereas MCHC showed no significant changes ($P > 0.05$). In the sublethal (8 weeks test) WBC, haematocrit and haemoglobin increased above the control values while RBC and MCH were decreased. MCHC and MCH showed no significant changes (DMRT). The changes in the sublethal test were both dose (concentration) and time dependent.

McLeay (1973) exposed fish to pulp mill effluent and observed a significant decrease in RBC and haematocrit. These effects were attributed to the possibility of erythrocyte production inhibition and also an increase in the rate of erythrocyte destruction or haemodilution. Pulp mill effluent contain several toxicants including heavy metals (Martel *et al* 1994). Heavy metals like arsenic, have been implicated in blood haemolysis and disturbed erythrocyte production in humans due to its effects on the bone marrow (Kyle and Pease, 1965). Arsenic is a major pollutant in tannery effluent (Lawal, *et al* 1986). Wedemeyer and Yasutake (1977) suggested that haemodilution could be responsible for the decrease in these parameters because of disturbances in osmoregulation.

Considerable decrease in Hb , RBC could have directly affected the respiration (both rate and volume) causing death in the 96-h tests. Heavy metals are known to affect the cytochrome

system (Blair *et al.* 1975). When they attack directly, respiration is completely inhibited, and when the attack is on the side chains, the respiration is greatly reduced finally leading to death (Blair *et al;* 1975).

Panigrahi and Misra (1978) found RBC, Hb and PCV of fish exposed to mercury to be depressed and this depression was dose and time dependent. Banerjee and Kumari (1988) found the total erythrocyte count (TEC), PCV and haemoglobin of *Anabas scandens* exposed to $ZnCl_2$, $CdCl_2$, and $HgCl_2$ to be decreased significantly whereas ESR, MCV and MCH increased in $HgCl_2$ and $CdCl_2$.

Musa(1993) exposing *C. gariepinus* to oxalate (malachite green) observed reduced RBC, PCV and Hb. He also observed destruction of the gill architecture by the chemical and hence disruption of osmoregulation. The decrease in these parameters were suggested to have been caused by the effect of the chemical on membrane ATPase, the glycolytic enzyme present in erythrocyte, hence the resultant heamodilution.

Zambariborsch and Bin (1977) found marked decrease in RBC and WBC in *Heteropneustes fossilos* exposed to heptachlor and they rejected the heamodilution factor as a possible cause due to the occurrence of hyperchor anemia at the 48-hr and 96-hr periods of the test. According to them, blood dyserasia was more likely the explanation for these changes and could have been induced by the chemical interfering with haemopoiesis and or alteration of cell membrane by hydrolysis of the acetylcholine in the body fluids.

In the long term study however, there was an increase in WBC and a decrease in RBC, especially in the highest level of exposure (6%) and hemodilution could not be implicated as a factor. Hb and haematocrits (PCV) were also significantly increased over the control values in the

(1969) determined that changes in haematocrits of brook trout, *Salvalinus fountinalis* in relation to acclimation temperature could be explained by changes in blood cell volume, MCV, but this was over an extended period of time. In the present study, there were significant changes ($P < 0.05$) in MCV values. *In vitro* changes could occur if the blood was placed initially in aerobic or anaerobic condition. An aerobic environment could cause a decline of greater than 10% of the original haematocrit where an hypoxic condition could cause an increase of 10-30%.

In this study, the haematocrit decreased by 30% as compared to the control and by 20% as compared to the baseline in the 96-h study. Since oxygen conditions in the control and baseline were relatively better (higher D.O) than those of the test and no gill damages in both control and baseline (to have impaired oxygen uptake thereby causing an hypoxic condition), the haemodilution factor and changes in MCV could be advanced as the two major factors in influence.

In the sublethal experiments, the average haematocit values increased over the control (original) values to the tune of between 15% and 35%. Casillas and Smith (1977) found a range of between 10% and 30% in *Onchorhychus mykiss* (formerly *Salmo gairdneri*). It would therefore appear plausible that in addition to haemoconcentration, a slightly hypoxic environment was created *in vitro* by muscular excertion during stress and hence increased demand for oxygen.

sublethal test. Dhillon and Gupta (1983) exposed the freshwater teleost, *Clarias batrachus* to aldrin (an organochlorine pesticide) and swascofix CD-38 (an ionic detergent) and found WBC, Hb, PCV and erythrocyte sedimentation rate (ESR) values to have increased with increase in dose and time of exposure. They attributed the increase in Hb and the haematocirt in experimental fish to the catalyzing action of the chemicals which resulted in the incorporation of the body iron store in the Hb. However, Panigrahi and Misra (1978) found the RBC and Hb of *Anabas scandens* exposed to Hg to be depressed. Al-Akel *et al.* (1988) exposed *O. niloticus* to Cd and found a depletion in Hb with increasing concentration and time.

Gill and Pant (1981) exposed the teleost, *Puntius conchonius* to mercury for a period of 8 weeks and found an initial incipient decrease in RBC with subsequent recovery. A similar situation was observed in one of the treatments (i.e 8%). The incipient decrease could be ascribed to haemolysis and the subsequent recovery to erythropoiesis. Erythropoiesis is triggered as a typical stress response. Most pollutants damage the gills and hence a fall in oxygen capacity of the blood which is partly offset by liberation of erythrocytes in the general circulation (Gill and Pant, 1981)

For WBC or leucocytes, except in 2% effluent exposed fish (where there was a continuous decrease with time) showed a continuous increase in all other treatments. It was interesting to note here that, in the 2% effluent exposed fish, both the WBC and RBC showed a continuous decrease. The haemodilution factor could be advanced for this changes in this exposure level, though dyscrasia, as reported by Zambariborsch and Biut (1977) could equally have been responsible.

Results have shown that tannery effluent may not have a significant effect on MCHC as shown by no significant ($P > 0.05$) changes in the values, both in 96-h and sublethal studies. An increase in the haematocirt (PCV) values in the sublethal could be explained as haemoconcentration since significant changes occurred in erythrocyte count. Huston and DeWilde

5.5 **Metal Accumulation:**

Metal accumulation was found to be higher in the exposed fish than both control and baseline fish in the 96-h study. Chromium, copper and lead were found to be significantly higher ($P < 0.05$) than zinc with the accumulation in the order of Pb > Cr > Cu > Zn. For all these metals, the liver, gill, gut and the skin were seen to have concentration values higher than the remaining tissues, though the highest values occurred in the liver.

Similar patterns were obtained in the sublethal exposures. Here, the levels of metal accumulated in the tissues and wholefish were directly related to concentration (dose) and duration (time) of exposure and for all the exposures, lead was more accumulated than the other metals. Metals uptake seems directly related to concentration in effluent. This agrees with the findings of Hodson, *et al* (1978), Offem (1983) Oladimeji and Ologunmeta (1987), Oladimeji and Offem (1989). These authours exposed various fishes to Pb and found concentration in tissues to be directly related to concentrations in water. Radhakrishnainah (1988) found the accumulation of copper in the organs of *O. niloticus* to be dose and time dependent. Annune and Iyaniwura (1993) exposed *O. niloticus* and *C. gariepinus* to zinc and cadmium and observed tissue accumulation in both fishes to be dose and time dependent. Berveridge, *et al*; (1985) reported highest amounts of Fe in Hb - rich livers. Nemesok, *et al*; (1987) also reported high amounts of Zn in fish liver. Annune and Iyaniwura (1993) also reported the livers of *O.niloticus* and *C. gariepinus* to have accumulated Zn and Cd more than the other tissues. Once a metal is taken up from the environment, the organism must either eliminate it through excretion or sequester it to prevent toxic condition (Bryan, 1979). An easy away to achieve this is by precipitating the metal ion in an insoluble form since it is the free metal ion which is most toxic (Simkiss, 1977). Fishes are known

to possess the metal binding protein, metallothionein (Friberg, *et al*; 1971) which is a sequesting agent. The detoxication of these metals in fish liver may be by sequestration rather than elimination (Giesy and Wiener, 1977, Phillips and Rainbow, 1989). The high levels of metals in the liver of *C. gariepinus* may actually be an indication of the storage of sequestered products in it.

C. gariepinus also accumulated high amounts of metals in the gills, agreeing with the studies of Brooks and Rumsey (1974), Nemcsok, *et al* (1987) and Amune and Iyaniwura (1993) who studied the bioaccumulation of trace metals in several fish species including *C. gariepinus*. Zinc accumulated in dog fish gills (Crepso, *et al*; 1979, 1981) and internal organs (Flos, *et al.* 1979) inducing a depressive effect on tissue respiration consistent with a general oxygen deficiency in the whole fish leading to possible death by hypoxia (Burton, *et al*; 1972). The elevated levels of Zn in the gills could be related to the active uptake of metals by gills, being in direct contact with the toxicant which adheres to the gill surfaces, and also,the gill being an osmoregulatory organ, having chloride cells, may store zinc for excretion (Bryan, 1976; Crepso, *et al;* 1981). The active transport of ^{65}Zn into the gills supports the excretory role of the gills (Ronald, *et al;* 1987). The accumulation of these metals in the gills as shown in the present study could therefore be attributed to the above factors.

Exposure of fish to elevated concentrations of heavy metals induces the synthesis of metallothioneins (Noel - Lambot, *et al*; 1978). These bind and detoxify the metal ion (Kojima and Kagi, 1978). The fishes ability however, to synthesis these proteins are finite (Borwn and Parsons, 1978). If the limits of the metabolic capabilities for excretion and toxicant binding are exceeded, toxic effects will result, unless the fish has an alternate way of detoxification. In fishes with scales, such alternate detoxification process may be calcification as suggested by Simkiss (1977). Sauer

and Watabe (1984). *C. gariepinus* however, is a scaleless fish. The absence of scales could be a major reason for lower susceptibility to toxicant between it (*C. gariepinus*) and other scale-possessing fishes like *O. niloticus*.

This is supported by the findings of Oladimeji and Offem (1989) and Annune, et al (1994). The former authors exposing *C. lazera* and *O. niloticus* to Pb found both the 48 - hr and 96-h LC_{50} values for *O. niloticus* to be higher than those for *C. lazera*. Annune, *et. al.* (1994) compared the susceptibilities of juveniles of *C. gariepinus* and *O. niloticus* to cadmium and found *C. gariepinus* to be more susceptible with lower LC_{50} values. They attributed the differences in susceptibilities to integumental variation in these two species. *C. gariepinus* lacks scales hence the body is in direct contact with the toxicant whereas *O. niloticus* have cycloid scales. Offem, et al. (1997) found the 96-h LC_{50} for *O. niloticus* to be 15% as compared to the 9.5% v/v obtained for *C. gariepinus* in this present study. The scales therefore help in detoxification by acting as toxicant sinks. The uptake of metals by the scales has been shown by Sauer and Watabe (1984) to be an active process.

The permeability of various species of fish to toxicant such as heavy metals is of considerable importance in determining their tolerance to metals (Cornes and Rigler, 1958, Bryan 1976). There is also a possibility that the synthesis of compounds for detoxicating one metal may increase the affinity of the organism for another. It was observed by Nordeberg, *et. al.* (1972) that Cd treatment increased the level of Zn in the liver of the exposed fish. Looking at the levels of all metals considered in this study, Zinc levels are the lowest. It is possible that such an interaction might have occurred leading to variations in the levels of different metals accumulated.

In biological systems, metals tend to bind to organic ligand in competition with hydrogen ion such that a decrease in pH would tend to dissociate such metal ions from the ligand (Valee and Wacker, 1970). The progressive acidification of the medium may be sufficient to release the metals for interaction with the skin. This may actually explain the peeling of the skin in the acutely toxic experiments and elevated metal ion concentration in the skin of sublethal animals, counter balancing the low affinity for certain inorganic metals associated with the low thiol or sulfyryl groups of the fish skin (A DeBruin, 1976).

The main sites of heavy metal uptake in fish are the gills and gastro intestinal tract (Pentreath 1973; Part and Svanberg, 1981; Lowegrove and Eddy, 1982; Annune and Iyaniwura, 1993). There is high concentration of metals in the gut tissues. A relationship between trace metal concentration in fish and the amount in water, sediments, food and feeding habit has been suggested (Douben, 1990, Smith, et al; 1996). Badsha and Goldspink (1982) reported high zinc content in roach gut.

In fish, Brooks and Rumsey (1974), Badsha and Goldspink (1982) found zinc to be generally low in muscles. Oladimeji and Offem (1989) found lead accumulation in *O. niloticus* and *C. lazera* to be least in the muscle tissue. Annune and Iyaniwura (1993) foud low levels of zinc and cadmium in the muscle tissue of *O. niloticus* and *C. garriepinus*. The present study also found metal accumulation to be low in muscle tissue. This may be attributed to growth factor as growth may dilute toxicant concentration in an organism if growth is faster than accumulation (Badsha and Goldspink, 1982; Arnac and Lassus, 1985). Beveridge, et al; (1985) found lowest levels of trace metals in the muscles. Jeng and Sun (1981) fed high levels of $ZnSO_4$ to *Cyprinus carpio* and found zinc levels to be higher in the skeletal tissues, and that muscle levels were not increased till skeletal tissues were saturated. The present study found higher levels in the bone

the muscle. Muscle would therefore appear to be the tissue of choice for routine environmental investigation in metal contamination of fish, but for accidental metal contamination, liver, gill and gut analysis in addition to muscle would be suggested.

Two phases of heavy metals uptake were observed. These were, a fast phase of uptake followed by a slow phase. Similar observation were also made by Oladimeji and Offem (1989) in the case of Pb uptake by various invertebrates and fish. Joyner (1961) had earlier explained this phenomenon as resulting from the fact that a portion of the toxicant taken up by fish is initially adsorbed unto mucus substances secreted at the surfaces in contact with the internal environment like gills, skin and intestine. This is then followed by absorption by diffusion of ions across the semi-permeable membranes onto the body fluids, onto the binding sites on or within specific cells in the body fluids and in internal organs. The rapid initial uptake seen in most cases in this study therefore represents a phase in which the first sorption mechanism is predominant and the period of declining accumulation could be caused by increasing acidity, providing more H for free metal ions attachment, and release from their binding sites into the water.

5.6 Histopathology:

The main point of action or target of pollutants in fishes is considered to be the gills. The present study found that the gills were greatly affected. An early hypertrophic response of the gills was observed in the effect of tannery effluent. There was a conspicuous secretion of mucus. This response could have been due to the presence of heavy metals in the effluent as was established by Westfall. (1917); Wong, *et al;* (1977); Kumar and Pant (1981); Ebele, *et al;* (1990) and Amune, *et al;* (1991, 1994) or any of the other several toxicant contained in the effluent. The above authors explained this phenomenon as a result of the interaction of the heavy metals with the

mucus glands. The fusion of the gill lamellae at the tips agrees with the findings of Kumar and Pant (1981) and Babatunde (1997). The former authours explained this fusion to be due to the sticky action of the mucus. Skidmore and Tovell (1972) found detachment of the lamellar epithelium in the gills of rainbow trout exposed to Zinc sulphate. The present study also found detachment and oedema of the epithelium. This could be as a result of hypertrophic reaction of the toxicant with the epithelial cells. Ufodike and Onusiriuka (1990) exposed *C.garieptinus* to inorganic fertilizer and observed similar damages.

Death in fishes exposed to toxicant is mostly caused by disruption in the respiratory process due to gill damage (Skidmore, 1970, Skidmore and Tovell, 1972, Annune, *et al*; 1991, 1994). But Wong, *et al*; (1977) could not find structural damages in the gills of *C.carpio* exposed to zinc and copper salts. It therefore means that, other causes of death apart from gill damage and consequent hypoxia are involved. The present study found the liver, an internal organ to be damaged too. The damage was mainly necrotic and oedematous in nature. Omoriegie and Ufodike (1991) exposed *O. niloticus niloticus* to Actellic 25 EC and found necrotic damage in the hepathocytes of the liver. Kumar and Pant (1981) found structural impairment leading to destruction of hepathocytes in *P.conchonius* exposed to copper and zinc. Wong, *et al*; (1977) found necrotic damage (coagulative type) of the hepatic cells and also oedema. The present study found all the above mentioned damages in the liver cells. There was also the appearance of globular structures and this observation was also made by Kumar and Pant (1981).

According to Omoriegie and Ufodike (1991) the necrotic damage of the liver tissue could be due to the inability of the fish to regenerate new liver cells due to their constant flooding with the toxicant and that the necrotic damage could also have been due to the excessive work required by the liver to get rid of the toxicant during the process of detoxification. The appearance of the

globular structures in week 4 and their disappearance in week 8 suggest that their presence might be an intermediate step in the formation of necrosis (Kumar and Pant, 1981).

5.7 Ovarian Development:

The retardation in ovarian weight (low ovarian weights) in the effluent exposed fish could be associated with the low occurrence of stages II and III ovaries in those group of fish. Saxena and Bhatia (1983), exposing a freshwater teleost, *Channa punctatus* to pulp mill effluent found similar results and that weight differences were significant after 60 days of exposure. The authors attributed the slow development of ovaries in the exposed fish to low levels of gonadotropin. Though no determination of gonadotropin levels was done in this study, it may not be unlikely that tannery effluent could be implicated in reducing the production of the gonadotropin.

5.8 Growth:

A does-dependent impairment of growth was observed in *C. gariepinus* exposed to tannery effluent with the highest concentration (i.e. 6%) exhibiting the most marked suppression. The control fish exhibited the highest growth followed by the 1% and then 2% 4% 6%. Similar findings have been made when other fishes were exposed to toxic effluents. Onwumere (1986) exposed *O. niloticus* to treated NNPC Refinery effluent and found that growth was impaired and the impairment was dose dependent. Several effluent constituents have been studied and found to impair growth. Oladimeji and Ologunmeta (1987) exposed *O. niloticus* to certain concentrations of lead and found growth impairment to be dose - dependent.

Growth can be affected by toxic chemicals directly or, indirectly due to effects on feeding or maintenance because these processes are intimately linked to each other. The Dynamic Energy Budget (DEB) theory provides a mechanistic basis for the link that has been tested against experimental data for many species (Kooijman, 1993, Kooijman and Bedaux, 1996). Reduced physical activities especially feeding was noticed in the 4% and 6% effluent exposed fish. This could have contributed to weight loss.

Changes in carbohydrates metabolism occurs in fish exposed to various stressful conditions. The secretion of catecholamine and adrenocorticoid in stressful conditions by fish has been reported (Fager, 1967, Smart, 1978). This leads to marked changes in carbohydrates reserves which according to Wedemeyer, *et al*; (1984) causes hyperglycemia.Fishes are noted to increase their metabolic activities and excretion of toxicant, hence making more energy available for homeostatic maintenance than storage. For example, Moles and Rice (1983) noted a depletion of liver and muscle glycogen in fish exposed to hydrocarbons.

Gills of exposed fish were also damaged . Therefore, the growth reduction observed in this study could have been due to reduced carrying capacity of the blood resulting in the inefficient utilization of assimilated food or to inhibition of certain enzymes of the metabolic pathways.

Changes in carbohydrates metabolites under toxic conditions have been reported in vertebrates. Mahaffey (1980) and Gill and Pant (1981) observed decreased glucose absorption in lead treated rats and *Pontius conchonius* respectively. Oladimeji and Ologunmeta (1987) observed a dose dependent depletion of liver glycogen in *O. niloticus* exposed to lead and attributed it as probably due to the inefficient absorption of soluble glucose from the intestine or breakdown of liver cells which store or synthesis glycogen. The changes in the liver cells could as well have led

to a similar phenomenon described above. Omoregie, *et al*; (1985) observed hypoglycemic conditions in *O.niloticus* exposed to petroleum effluent.

CONCLUSION AND RECOMMENDATIONS

The effluent produced by the Great Northern Tanneries (GNT LTD) Kano, contributes to the contamination of the environment to a fair degree; fish exposed to acutely toxic levels of tannery effluent showed symptoms of toxicosis that included agitated swimming, loss of balance, increased gill ventilation, air gulping, coughing, twisting, swollen abdomens, increased mucus secretion over the body surface, precipitation of the mucus and finally death after a period of quiescence: That the 96-h LC_{50} value established for the composite effluent was 9.5% v/v and a 95% confidence limit range of 8.7943 - 10.2057. A slope function of 0.65 was found:

The hematological parameters of RBC, WBC, Hb, Haematocrit (PVC), MCH and MCV were in both the acute and chronic exposures, adversely affected by either increasing above or decreasing below the control values. In most of the treatments, the variations were statistically significant at ($P < 0.05$). Only the MCHC was not affected: fishes both in the acute and chronic exposures accumulated the four metals (chromium, copper, lead and zinc) studied far above the control fishes ($P < 0.05$) and the individual metal distribution was in the order of lead > chromium > copper > zinc. In terms of tissue accumulation, the liver accumulated more than any other organ studied; Histopathological studies showed that the gill and liver of fish exposed to tannery effluent were damaged to various degrees, and Growth of fish and ovarian development were retarded in the effluent exposed fish. The following recommendations are made:

1. Rather than employ 'end of pipe' strategies in solving pollution problems associated with tannery wastes, cleaner production which aim at 'prevention of production of pollutants'

1. Rather than employ 'end of pipe' strategies in solving pollution problems associated with tannery wastes, cleaner production which aim at 'prevention of production of pollutants' using cleaner technologies be encouraged. This however involves a total societal commitment.

2. In a society like ours, where awareness (knowledge of pollutants and pollutants effects) of the general public is lacking, societal commitment may be difficult. Awareness should therefore be created in the general public.

3. Where wastes are produced, level of toxic pollutants be reduced before discharge into streams. This could be achieved by physical, chemical and biological treatments of the waste. Recycling of wastes should also be practiced.

4. The Federal Environmental Protection Agency (FEPA) should strive to monitor and ensure that the fixed standards for waste discharge are strictly adhered to by the industries.

5. Knowing the median lethal concentration, that is the concentration that kills 50% of the fish is not an acceptable goal to control toxicity in a receiving water. An application factor (AF) is used to reduce the lethal concentration that would allow for complete survival of all stages of the animals life history. This concentration is referred to as the 'safe concentration' and is a product of 96-h LC_{50} and AF. AFs are usually developed taking into consideration long- term or chronic effects of the toxicant on life history factions as growth, fecundity hatching success, food conversion and behaviour. It is therefore recommended that further research be conducted on the long term effects of tannery effluent on fecundity, hatching success and food conversion so that an AF factor could be established.

REFERENCES

A deBruin A. (1976) *Biochemical toxicology of environmental agents.* Biomedical Press Elsevier. North Holland. 210 pp.

Adewoye. R.O. (1985) The Development of Nigerian Leather and Leather Products Industries and the place of Kwara State. *J. Leath. Res.* 3 (3): 46- 61.

Al-Akel. A.S.; M.J.K. Shamsi. H.F. Al-Kahem. M.A. Chaudhary and Z. Ahmad (1988).Effect of cadmium on cichlid fish, *Oreochromis niloticus:* Behavioural and Physiological responses. *J. Univ. Kuwait* (Sci). 15(2): 341-346.

Allen. S.E.; H.M. Grimshaw, J.A. Parkinson and C. Quarmby (1974): *Chemical Analysis of Ecological Materials.* Blaxwell Scientific Publications, Oxford.

American Public Health Association: American water works Association and Water pollution Control Federation (1985). *Standard Methods for Examination of Water and Waste water.* 16th Edition. APHA. New York.

Annune P.A ; A.A Oladijemi and S. O Ebele (1991):Acute Toxicity of zinc to the fingerlings of *Clarias lazera* (Cuv. and Val) and *Oreochromis niloticus* (Trewavas) J. *Aquatic Sci* 6:19-22

Annune. P. A. and T. T. Ivaniwura (1993):Accumulation of Two Trace Metals in Tissues of fresh water fishes, *Oreochromis niloticus and Clarias' gariepinus. J. Aquatic food Product Technology* 2(3):5-18.

Annune. P.A; S.O. Ebele & A.A. Oladimeji (1994) Acute Toxicity of Cadmium to juvenile of *Clarias gariepinus* (Teugels) and *Oreochromis niloticus* (Trewavas). J. *Environ. Sci Health.* A29 (7) 1357- 1365.

Aota. S; K.D. Holmgren. P. Gallangher and D.J. Randal (1990): A possible role for catecholamines in the ventilatory responses associated with internal acidosis or external hypoxia in rainbow trout. *Onchorhynchus mykiss. J. Exp. Biol.* 151, 57-70.

Arnac. M. And C. Lassus (1985):Heavy metal accumulation (Cd, Cu, Pb and Zn) by Smelt *(Osmerus mordax)* from the North Shore of St. Lawrence Estuary. *Wat. Res.* 19:725-734.

Babanunde . M (1997). :Toxicity of paraquat (Gramoxone) to *Oreochromis niloticus* (Trewavas). M.Sc Thesis Department of Biological Sciences, Ahmadu Bello University, Zaria. pp123

Badsha, K.S. and C.R. Goldspink (1982):Preliminary observations on the heavy metal content of four species of freshwater fishes in N.W. England. *J. Fish Biol.* 21. 21: 251-267.

Banerjee. V. And K. Kumari (1988):Effect of Zinc, Mercury and Cadmium on erythrocyte and related parameters in the fish *Anabas testudineus*. *Environ. Ecol.* 6(3) 737-739.

Begum. S.J. (1987)Biochemical adaptive responses in glucose metabolism of fish (*Tilapia mossambica*) during ammonia toxicity. *Curr. Sci.* 56, 705-708.

Bengtsson, B.E. A. Bengtsson and U. Taernlund (1988):Effects of Pulp mill effluents on vertebrae of fourhorn sculpin. *Myoxocephalus quadricornis*. Bleak. *Alburnus alburnus* and perch, *Perca fluviatilis*. *Arch. Environ. Contam. Toxicol.* 17(6): 789-797.

Benoit. D.A; E.N. Leonards, G.M. Christensen and J.T. Fiandt (1976):
Toxic effects of cadmium on three generations of brooktrout (*Salvelinus fontinalis*). *Trans. Am. Fish Soc.* 105: 550-560.

Beveridge, M.C. M. E. Stafford and R. Coutts (1985):Metal concentrations in the commercially exploited fishes of an endoheic saline lake in the tin-silver province of Bolivia. *Agriculture and Fish Management.* 1: 41-53.

Blair. A.M. J.N; B. Clark; A.J. Clarke & P. Wood (1975):Tissue concentrations of mercury after chronic dosing with theomersal.*Toxicology*. 3, 171-176.

Boers, P.C.M.(1991).The Release of Dissolved Phosphorus from Lake Sediments. Ph.D Thesis.Limnological Institute, the Netherlands. pp139

Brooks. R.R. and D. Rumsey (1974):Heavy metals in some New Zealand Commercial sea fishes. *N.Z.J. Mar. Freshwat. Res.* 8(1): 155-166.

Brown. D.A.& T.R. Parsons (1978):Relationship between cytoplasmics distribution of mercury and toxic effects to Zooplankton and chum salmon (*Onchorhynchus keta*) exposed to mercury in a controlled ecosystem. *J. Fish Res. Board Can.* 35: 880-884.

Bryan, G.W. (1976):Heavy metal contamination in the sea. In: *Marine Pollution,* edited by R.Johnston. Academic Press, New York, Pp. 185-302.

Bryan. G.W. (1979):Bioaccumulation of marine pollutants. *Phil. Trns. R. Soc. London* B. 286, 483-505.

Burton. D.T; A.H. Jones, and J. Cairns (1972):Acute Zinc toxicity of rainbow trout (*Salmo gardneri*) Confirmation of the hypothesis that death is related to tissue hypoxia. *J. Fish Res. Bd. Can.* 29: 1463-1466.

Casillas E. & I. S. Smith (1977)Effect of stress on Blood coagulation and Haematology in Rainbow trout (*Salmo gairdneri*). *J. Fish Biol.* 10, 481-491.

Cornet, E.D.S. & F.H. Rigler (1958) The modes of action of toxic agents.III. Mercuric chloride and N.-anylmecuric chlorides on crustaceans. *J. Mar. Biol. Assoc. United Kingndom* 37: 85-96.

Crepso, S., R. Flos, . Balasch and G. Alonso (1979): Zinc in the gills of the dog fish (*Scyliorhinus canicula L.*) related to experimental aquatic zinc pollution. *Comp. Biochem. Physiol.*630: 261-266.

Crepso, T.E. Soriano, C. Sampera and J. Blasch (1981):Zinc and Copper excretory organs of the dog fish *Scyliorhinus canicula* and chloride cell response following treatment with Zinc sulphate. *Mar. Biol.* 65: 117-123.

Dacie, J.V. and S.M. Lewis (1975): *Practical Haematology*(5th Ed) Livingstone, Edinburgh.629pp

Davis, P.H; J.B. Groetd, Jr. J.R. Sidey and N.F. Smith (1976): Acute and chronic Toxicity of lead to rainbow trout, *Salmo gairdneri (R.)* in hard and soft water. *Wat Res.* 10: 199-206.

Dhillon, S.S. & A.K. Gupta (1983):A clinical approach to study the pollutants intoxication in freshwater teleost (*Clarias batrachus*). *Water, Air, and Soil Pollut.* 20(1): 63-68.

Dick, P.T. & G. Dixon (1985): Changes in circulating blood cell levels ofrainbow trout(*Salmo gairdneri R.*) following acute and chronic exposures to copper*J. Fish Biol* 26:475-481.

Douben, P.E.T. (1990):A mathematical model for cadmium in the stone loach (*Noemacheilus barbatulus L.*) from the river Ecclesbourne, Derbryshire. *Ecotox. & Envir. Saf.* 19: 160-183.

Ebele, S. O; A.A. Oladimeji and J.A. Daramola (1990).Molluscicidal and Pisscicidal properties of copper (II) tetraoxosulfate(VI) on *Bulinus globosus* (Morelet) and *Clarias anguillaris* (L) *Aquatic Toxicol* 17: 231-238.

Fager, U.H.M.(1967):Plasma cortisol concentrations in relation to stress in adult sokeye salmon during freshwater stage of life cycle. *General Comparative Endocrinology* 8: 197-207.

Farmer, G.J. D. Ashfield and H.S. Samant (1979):Effects of zinc on juvenile Atlantic salmon, *Salmo salar*: acute toxicity, food in take, growth and bioaccumulation. *Environ. Pollut* 19: 103-106.

Federal Environmental Protection Agency (1991):*Guidelines and standards for Environmental pollution control in Nigeria.* FEPA Document. Pp. 230.

Finney, D.J.(1964) *Probit Analysis:* A statistical treatment of the sigmoid response curve. 2nd ed. London, C U P. 318p

Flos, R; A Caritat and J Balasch (1979):Zinc content in organs of dogfish (*Scyliorhinus canicula*) subjected to sublethal experimental aquatic zinc pollution. *Comp. Biochem. Physiol.* 64C: 77-81.

Forstner U and G.T.W. Wittmann (1981): *Metal pollution in Aquatic Environment.* Springer,Berlin.

Fraser, J. (1980): Acclimation to lead in the freshwater isopod *Asellus aquaticus*. *Oecologia* 45:419 - 420.

Fribern, L.; M. Piscater and G. Nordberg (1971):*Cadmium in the Environment.* Chemical Rubber C., Cleveland.

Gbem, T T ; F A Lawal, I K Balogun and J. Auta (1997):
Acute Toxicity of Tannery Effluents to a freshwater fish, *Orechromis niloticus* (Trewavas). *Conf. Proc. Chemical Engineers Society of Nigeria.* Hamdala Hotel, Kaduna, Nov. 13-15, 1997, Pp. 125-129.

Giesy, J.P. and J.G. Wiener (1997).Frequency distribution of trace metal concentrations in five freshwater fishes. *Trans. Am. Fish Soc.* 106: 393-403.

Gill, T S & J C. Pant (1981): Effects of sublethal concentrations of Mercury in a Teleost,*Puntius conchonius*: Biochemical and Haematological Responses. *Indian J. exp. Biology* 19: 571-573.

Hesser, E F. (1960):Methods for routine fish haematology. *Prog. Fish Cult.* Vol. 22: 164-171

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

Hodson, P.V; D.C. Dixon, D.J. SPRY, D.N. White and J.B. Sprague (1982)
Effects of growth rate and size of fish on rate of intoxication by water-borne lead. *Can.Journal of fisheries and Aquatic science.* 39: 1225-1251.

Holcombe, G.W.; D.A. Benni, E.N. Leonard and J.W. Mckim (1976):
Long term effects of lead exposure on the generation of book trout, *Salvelinus fontinalis*. *J. Fish Res. Bd. Can.* 33: 1731-1741.

Houston & DeWilde (1969):Comp. Biochem. Physiol. 28; 877-885.

International Union of Leather Technical and Chemical Society (1976): Meeting of Tannery Wastes Commission, Svendborg, Denmark, May 1975. Via *J. Soc. Leath Tech Chem;* 60.95.

Jeney Z. J. Nemesok, G. Jeney and J Olah (1992):Acute effect of sublethal ammonia concentrations on common carp (*Cyprinus carpio* L). I. Effect of ammonia on adrenaline and noradrenaline levels in different organs *Aquaculture* 1045: 139-149.

Jeng. S.S. & J.C. Sun (1981):Effects of dietary levels of zinc concentrations in tissues of common carp. *J. Nutr.* III: 134-140.

Joyner. A.H. (1961):*'Mercury Accumulation in food chains'* Thomas Publishers, Springfield,Ill.

Knoph, M.B.& Y.A. Olsen (1994): Subacute Toxicity of Ammonia to Atlantic Salmon (*Salmo salar* L). In Seawater; Effects on Water and Salt Balance,Plasma Cortisol and Plasma Ammonia Levels. *Aquatic Toxicol.* 30; 295-310

Knoph, M.B. (1996):Gill ventilation frequency and mortality of atlantic salmon (*Salmo salar* L) exposed to high ammonia levels in seawater. *Wat. Res.* Vol. 30 No.4: 837-842.

Kojima, Y. & J.H.R. Kagi (1978) Metallothionein. *Trends Biochem Sci.* 3, 90-93.

Kooijman. S.A.L.M.(1993):*Dynamic Energy Budget in Biological Systems. Theory and applications ecotoxicology.* Cambridge University Press P. 350.

Kooijman. S.A.L.M. & J.J.M. Bedaux (1996):Analysis of toxicity tests on fish growth. *Wat. Res.* 30(7): 1o33-1644.

Kumar, S. & S.C. Pant (1981): Histopathologic Effects of Acutely Toxic Levels of Copper & Zinc on Gills, Liver and Kidney of *Puntius conchonius* (Ham). *Indian J exp. Biology* 19: 191-194.

Kyle, R.A. and G.L. Pease (1965) Haematologic Aspects of Arsenic Intoxication. *The New Engtand J. Medicine* 237 (1): 18-23.

Lawal, F.A. (1988). Studies on Arsenic and other toxic pollutants in vegetable Tannery wastewaters. Ph.D thesis, Department of Chemistry,ABU, Zaria. pp 158

Lawal, F.A. and K. Singh (1981) Analytical Studies of Wastewater Effluents of some tanneries of Kano and Sokoto states for possible environmental pollution. *Bull. Chem Soc. Nigeria.*6: 68-75

Lawal F.A. R.O. Adewoye and K. Singh (1986):Studies on Major pollutants in vegetable tannery wastewaters. *J. Leather Res.* 4(4): 63-74.

Lawal, F.A., I.D. Owoeye, M. U. Udeh and O.D. Ojo (1996): Effects of Discharged Industrial Pollutants on the Quality of Challawa River, Soil and rural Dwellers. *(NEST Commissioned Report).*

Lawal, F.A.; I.D. Owoeye & M.U. Udeh (1997): Analysis of effluent from Great Northern Tanneries, Co. (Unpublished).

Leather Research Institute of Nigeria, Zaria (1981). *Annual Reports.*

Lewis, M.(1978) Acute toxicity of copper, Zinc and Manganese in single and mixed salt solutions to juvenile longfin dace, *Agosia, chrysogaster. J. Fish Biol.* 13: 695-700.

Lloyd, R. & D.W.M. Herbert. (1960) The influence of carbondioxide on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdneri* Richardson). *Ann appl. Biol.* 48: 399-404.

Lloyd, R.(1961).Effect of Dissolved Oxygen concentrations on the toxicity of several poisons to Rainbow Trout (*Salmo gairdneri* Richardson). *J. exp. Biol* 38, 47 - 455.

Lovegrove, S.M. and B. Eddy (1982): Uptake and accumulation of zinc in juvenile rainbow trout, *Salmo gairdneri. Environ. Biol. Fish.* 7, 285 - 289.

Mahaffey, L.R. (1980): Nutrient Lead interaction. In: Singhal, R.L. & J.A. Thomas (eds). *Lead Toxicity.* Urban and Schwarzenberg Baltimore - Munich

Martel, P.H., E.G. Kovac, B.I. O'Connor & R.H. Voss (1994):A survey of pulp and Paper Mill Effluents for their potential to induce mixed function oxidase enzyme activity in fish *Wat. Res.* Vol. 28(8) 1835-1844.

Mason, C.F. (1991):*Biology of freshwater pollution.* 2nd edition. John Wiley and Sons Inc. New York. pp. 73-75.

Mehee, J. E. and H. Wolf (1963):*Water Quality Criteria.* 2nd ed. The Resources agency of California State Water Quality Board Publication No.3A; 294-297.

McKenzie, D.J; S. Aota and D.J. Randall (1991): Ventilatory and cardiovascular responses to Blood pH, Plasma P_{CO_2}, blood O_2 content, and catecholamines in an air breathing fish, the bowfin (*Amia calva*). *Physiol. Zool.* 64; 432-450.

McKnight, I.M. (1965) A haematological study on the mountain white fish. *Prosopium williamson. J. Fish Res. Bd Canada.* 23(1):

McLeay, D.J. (1973):Effects of a 12-h and 25 day Exposure to Kraft Pulp mill Effluent on the Blood and Tissues of Juvenile Coho Salmon (*Oncorhynchus kisutch*). *J. Fish Res. Bd Canada* 30: 395-400

Miale, J.B. (1958): *Laboratory Medicine - haematology*. 1st ed. 735pp. Illus.C.V. Mosby Co. St.Louis.

Mohammed, B. (1995) Haematological effect of lindane on *Oreochromis niloticus* M.Sc. Thesis. Federal University of Technology Minna, Nigeria

Moles, A. And S.D. Rice (1983): Effects of crude oil and naphthalene on growth, caloric content and fat content of pink salmon juveniles in seawater. *Trans. Amer. Fish Soc.* 112:205-211.

Murad, A. A.H. Houston and L. Samson (1986): Haematological Response to Reduced Oxygen carrying capacity, increased temperature and hypoxia in Goldfish (*Carassius auratus*). *J. Fish Biol.* 36: 289-305.

Musa, S.O. (1993) Investigation into haematological changes in *Clarias gariepinus* exposed to acute and sublethal levels of malachite green M.Sc. Thesis. University of Jos, Jos, Nigeria.

Naylor, C., Pindar, L. And P. Calow (1990): Inter-and Intraspecific variation in sensitivity to toxins: the effects of acidity and zinc on the freshwater crustaceans *Asellus aquatius* (L.) and *Gammarus pulex* (L.) *Wat. Res.* 24: 757-762

Nemcsok, J., L. Orban, B. Asztalos and E. Vig (1987): Accumulation of pesticides in the organs of carp (*Cyprinus carpio* L.) at 4°C and 29 °C *Bull. Environ. Contam. Toxicol* 39(3). 370-378.

Noel-Lambot, F., C. Gerday and A. Disteche (1978) Distribution of Cd, Zn and Cu in liver and gills of the eel *Anguilla anguilla* with special reference to metallothioneins. Comp. *Biochem. Physiol.* 61C. 177-187.

Nordberg, M., M. Piscator and O. Vesterberg (1972): Separation of two forms of rabbit metallothionein by isoelectric focussing. *Biochem J.* 126: 191-198.

Offem, B.O. (1983) Toxicity and residue accumulation of Lead in some fish and invertebrates. M.Sc. Thesis. Department of Biological Sciences, Ahamdu Bello University., Zaria.

Oladimeji, A.A. and B.O. Offem (1989) Toxicity of lead to *Clarias lazera*, *Oreochromis niloticus*, *Chironomus tetans* and *Benacus sp. Water, Air* and *Soil pollution* 44: 191-203

Oladimeji, A.A. and R.T. Mohammed (1987): Toxicity of water borne lead to *Tilapia niloticus* (L.). *Nigerian J. Appl. Fish and Hydrobiol.* 2: 19-24.

Omoregie, E. And E.B.C. Ufodike (1991) Histopathology of *Oreochromis niloticus niloticus* exposed to Actellic 25. EC. *J. Aquatic Science* 6: 13-17.

Omoregie, E. E. B.C. Ufodike and C.O.E. Onwuliri (1995) Effects of petroleum Effluent on Carbohydrate Reserves of the Nile *Tilapia, Oreochromis niloticus* (L.) *West Afr. J. Biol. Sci.* 3(182): 70-76.

Onwumere, B.G. (1986) Toxicity of treated effluents from NNPC Refinery, Kaduna to *Tilapia. nilotica* L. M.Sc Thesis. Department of Biological Sciences, Ahamdu Bello Univeristy,Zaria.

Panigrahi, A.K. and B.N. Misra (1978):Toxicological effects of mercury on a freshwater fish, *Anabas scandens*. Cuv. & Val. and their Ecological Implications. *Environ. Pollut.* 16: 31-39.

Part, P. and O.S. Vanberg (1981):Uptake of cadmium in perfused rainbow trout (*Salvelinus gairdneri*) gills. *Can. J. Fish Aquat Sci.* 38: 917-924.

Pentreath, R.J. (1973):The accumulation and retention of 65 Zn and ^{54}Mn by the Plaice, *Pleuronectis platessa* L. *J. Exp. Mar. Biol. Col.* 12: 1-18.

Pepper, K.W. (1966) *J. Amer. Leath.Chem. Assoc.* 61, 570.

Phillips, D.J.H. & P.S. Rainbow (1989) Strategies of trace metal sequestration in aquatic organisms. *Mar Envir. Res.*28, 207-210

Pickering, Q.H. C. Henderson and A.E. Lenke 91962). The toxicity of organo phosphorus insecticides to different species of warm water fish. *Trans Am. Fish Soc.* 91(2): 175-184

Pontasch, K.W. and J. Cairns, Jr. (1989) Establishing and maintaining laboratory - based microcosms of riffle insects communication their potential for multispecies toxicity tests. *Hydrobiologia* 175: 49-60.

Prasad, B.G.S.; B.V.S.G. Rao, W Madharakrishna and C.A. Sastry (1981) *Proceedings of 16th I.C.T. Madras*, India.

Radhakrishnaiah, K. (1988) Accumulation of copper in the organs of freshwater fish. *Labeoo rohita* (Hamilton) on exposure to lethal, and sublethal concentration of copper. *J. Environ. Biol.* 9(3) Suppl. 319-326.

Rajendran, S; A. Kolanchiappan and P. Baskar (1925):A study on the toxicological (acute) aspects of tannery waste water in fish. *Sarotherodon mosambicus*. *Leather Science* 32(12) 305-310.

Ramasamy P. And V.S. Krishnamurthi (1981)Zinc uptake and its contaminants of soil micro organisms. *Leather Science* 28(1): 403-410.

Ronald, W.H. V.S. Craig and M.N. Koziol (1987). Absorption, body distribution and excretion of dietary zinc in rainbow trout (*Salmo gairdneri*). *Fish Physiol. Biochem.* 3(3). 133-143.

Sauer, G. R. and N. Watabe (1984) Zinc uptake and its effect on calcification in the scales of mummicoog. *Fundulus hetrochitus*. *Aquatic Toxicol* 5: 51-66.

Saxena, P.K. and R. Bhana (1983):Effects of vegetable oil factory effluents on ovarian recrudescence in the freshwater teleost, *Chana punctatus* (BL). *Water, Air and Soil pollution*. 20 No.1 55-61.

Scholtz, T. H. And O. Gillisen (1961): Anthrax in Leather factory waste *JALCA*, 56 511(Abst).

Sharpbaise, J. H. (1983):*Leather Technicians's hand book*. Leather producers' Association.Northampton, NP. 455.

Simkiss, K., (1977). Biomineralisation and detoxification. *Calcif. Tissue Res.* 24; 199-200.

Skidmore, J.F.(1970):Respiration and Osmoregulation in rainbow trout with gills damaged by zinc sulphate. *J. Exp. Biol.* 52: 481-494.

Skidmore, J.F. and P.W. A. Tovell (1972): Toxic effects of Zinc sulpahte on the gills of rainbow trout. *Wat. Res.* 6: 217-230.

Skrypski-Mantele, S; T.r. Bridle (1995): Environmentally sound Disposal of Tannery Sludge. *Wat. Res.* 29(1): 1033-1039.

Smart, G. (1978):Investigations of the Toxic Mechanisms of Ammonia to Fish Gas Exchange in Rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations *J. Fish Biol.*12, 93-104.

Smith, S.; M.H. Chen ,R.G. Bailey & W.P. Williams (1996):Concentration and distribution of copper and cadmium in water, sediments, detritus, plants and animals in a hardwater lowland river. *Hydrobiologia* 341; 71-80.

SLTC (1981)-*Official Methods of Analysis*. SLT, Moulton, Northampton, SLT, 2.2-SLT. 2/3h.

Sprague, J.B. (1970) Measurement of Pollutant toxicity to fish II: Utilising and applying bioassay results. *Wat Res.* 4. 3-32.

Ufodike, C.B.C. and Onusiruka, B.C. (1990): Acute toxicity of inorganic fertilizer to African Catfish, *Clarias gariepinus* (Teugels). *Aquaculture and Fisheries Management*. 21: 181-185

Udeh, M.U; F.A. Lawal; I.D. Owoeye, I.D. Ojo, E.M. Okonkwo & I. A. Jaieyola (1997):The effects of discharged industrial pollutants on quality of Challawa River in Kano. *National Research Institute for Chemical Technology Seminar Series* held at NARICT Conf. Hall. 30th July 1997. 10pp.

UNEP (1991). *Tanneries and the Environment*. A Technical Guide. Technical Report Series.No.4.

Vallee, B.L., and W.E. C. Wacker (1970) *Metalloproteins*. In: The proteins:composition,structure and function. 2nd ed; vol.5, edited by H. Neurath, Academic Press,New York, 192 pp.

Villa, L. (1966) *J. Amer. Leath. Chem. Assn.* 61: 414.

Villa, L. (1966): Wastewater in the tanning industry and its purification, *JALCA*, 61 414-422.

Vollenweider, R.A. (1968) Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to Nitrogen and phosphorus as factors in eutrophication.*OECD Report*. Paris.

Wedemeyer, G.A. and W. T. Yasutake (1977): Clinical Methods for the assessment of the effects of environmental stress on fish health. *Tech. Paper* No. 89 *U.S. Fish and Wildlife service*. Washington. DC. USA 19pp.

Wedemeyer, G.A.; D.J. McLeay and C.P. Goodyear (1984): Assessing the tolerance of fish and fish populations to environmental stress. In: *Contaminant effects on fisheries* (Editor: Cairns, V.W.; P.V. Hodson and J.O. Nriagu).John Wiley and Sons, New York, USA 161-193.

Westfall, B.A. (1945) Coagulation film anoxia in fishes. *Ecology* 26: 283-287.

Wichser, J and H. Kazemi (1974): Ammonia and Ventilation: Site and Mechanism of action.*Resp. Physiol.* 20; 393-406.

Wilson, R. W., Aand L.W. Taylor (1992). Transbranchial ammonia gradients and acid -base responses to high external ammonia concentration in rainbow trout (*Onchorhynchus mykiss*) acclimated to different salinities. *J. Exp.Biol.* 166, 95-112.

Wintrobe, M.M. (1934). Variations in the size and hemoglobin content of erythrocytes in the blood of various invertebrates. *Folia Haematol.* 51: 32-49.

Wong, M.H; K.C. Luk & K.Y. Choi (1977): The Effects of Zinc and copper on *Cyprinus carpio* and *Ctenopharyngodon idellus*. *Acta anat.* 99: 450-454.

Wong, P.T.S, B.A. Silverberg, Y.K. Chan and P.V. Hodson (1978): Lead and the aquatic biota. *J. Fish Biol* 21: 411-415.

Zamparborsch, F. S. and L. Biu (1977): The effects of hexachloran (Lindane) on some haematological and biochemical indices of blood in the round oby (*Gobulus melanostomus*) *Pallas. Vapr. Chtd.* 17: 569-572.

APPENDICES

APPENDIX I:

CALCULATED WEIGHTS OF EXPECTED PROBITS

For 5.25:

Expected	Weight
5.0	0.637
1.0	0.439
1.0	0.198

If 1.0 0.198

then 0.25 = ?

$$0.25 = \frac{0.198 \times 0.25}{1.0}$$

$$= 0.0495$$

therefore 5.25 = 0.637 + 0.0495 = 0.6865

For 1.95:

Expected	Weight
2.4	0.040
2.0	0.015
0.4	0.025

If 0.01 0.025

then 0.04 = ?

$$0.05 = \frac{0.025 \times 0.05}{0.4}$$

$$= 0.00313$$

therefore 1.95 = 0.015 + 0.00313 = 0.01187

APPENDIX 2:

95% CONFIDENCE LIMITS

$$Slog_{10} = \frac{1}{b\sqrt{pnw}}$$

Where:

b	=	slope of the log dose probit line	=	0.65
p	=	number of dilutions	=	5
n	=	number of fish in each group	=	10
w	=	average weight of the obserbvations	=	0.3652

therefore $Slog_{10} = \frac{1}{\sqrt{0.655x10x0.3652}}$

$$= \frac{1}{2.77756}$$

$$= 0.3600$$

Log_{10} (95% C.I.) $Log_{10}\ LC_{50} \pm 1.96(0.3600)$

$= Log_{10}\ LC_{50} \pm 0.7057$

$= 8.7913 - 10.2057$

APPENDIX 3
REGRESSION EQUATION FOR FIGURE 2.

	X	Y	X^2	XY			
	0.903	4.75	0.815	4.289	X	=	0.994
	1.000	5.52	1.000	5.250	Y	=	5.280
	1.079	5.58	1.164	6.301			
	----	---	----	----			
E =	2.982	15.84	2.979	15.840			

$$b = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/n}{\Sigma X^2 - (\Sigma X)^2/n}$$

b = 0.095/0.015

= 6.333

a = Y - bx

= 5.280 - 6.333 x 0.994

= -1.012

Y = a + bx

Y = -1.012 + 6.333x

APPENDIX 4a:
Anove table for WBC of *C gariepinus* exposed to various sublethal concentrations of tannery effluent for Eight weeks.

Source	DF	SS	MD	F	Pr > F
Model	6	909.1268	151.5211	17.27	0.002
Error	9	78.9673	8.7741		
Corrected Total	15	988.09			

R-Square	CV	Root MSE	WBCM
0.920081	20.77130	2.962117	14.2

Source	DF	Anova SS	MS	F Values
Conc.	3	826.9604	275.6535	31.42
WEEKS	3	82.1664	27.3888	3.12

Duncan's Multiple Ragne Test for WBC.
Note: Means with the same letters are not signficant different.

DUNCAN GROUPING	MEANN		CONC
A	21.300	4	5
A	17.675	4	4
B	15.775	4	0
C	2.293	4	2

DUNCAN GROUPING	MEANN		WEEK
A	17.192	4	8
B A	15.725	4	6
B A	12.325	4	4
B	11.800	4	2

Alpha = 0.05 dif = 9 mse = 8.77414

APPENDIX 4b:
Anova table for RBC of C. GARIEPINUS exposed to various sublethal concentrations for eight weeks.

SOURCE	DF	SUM OF SQUARTES	MEAN SQUARE	F.VALUE	Pr>F
Model	6	17522.87500	2920.479	0.52	0.7796
Error	9	50133.56250	5603.729		
Corrected Total	15	67956.43750			

R - Square	C.V	Root MSE	RBC mean
0.257855	27.20257	74.85806	275.188

SOURCE	DF	ANOVA SS	MS	F.VALUE	Pr > F
Conc.	3	9929.688	3309.896	0.59	0.6365
Weeks	3	7593.188	2531.063	0.45	0.7224

DUNCAN'S MULTIPLE RANGE TEST FOR RBC
Note: Mens with the same letter are not signfiicantly different.

DUNCAN GROUPING	MEANN	CONC.
A	316.75	4 6
A	268.00	4 2
A	265.50	4 0
A	250.50	4 4

DUNCAN GROUPING	MEANN	WEEKS
A	308.75	4 2
A	272.50	4 4
A	271.75	4 6
A	247.75	4 8

Alpha = 0.05 df = a MSE = 5603.729

APPENDIX 4c:

Anova table for haematocrit (pcv) of *C. gariepinus* exposed to various sublethal concentrations of tannery effluent for eigh weeks.

SOURCE	DF	SS	MS	F.VALUE	Pr > F
Model	6	427.0000	71.1667	10.68	0.0012
Error	9	60.0000	6.667		
Corrected Total	15	187.0000			

R -Square	C.V	Root MSE	PCV MEAN
0.876797	7.122728	2.581989	36.2500

SOURCES	DF	ANOVA-SS	MS	F-VALUE	Pr > f
CONC.	3	318.5000	106.1667	15.93	0.0006
WEEKS	3	108.5000	36.1667	5.42	0.0209

DUNCAN'S MULTIPLE RANGE TEST FOR PCV

NB. Means with the same letter are not significantly different.

DUNCAN GROUPING	MEANN	CONC.
A	40.500	4 6
A	39.250	1 3
A	36.250	4 2
B	29.000	4 1

DUNCAN GROUPING	MEANN	WEEKS
A	39.750	4 8
A	37.000	4 6
B A	35.750	4 4
B	32.500	4 2

Alpha = 0.05 dif = 9 MSE = 6.666667

APPENDIX 4d

Anova table for haemalobin (h6) of *C. gariepinus* exposed to various sublethal concentrations of tannery effluent for eight weeks.

SOURCE	DF	SS	MS	F-VALUE	Pr > F
Model	6	64.9638	10.8240	20.3	0.0001
Error	9	4.7456	0.5273		
Corrected Total	15	69.6894			

R - Square	CV	Root MSE	H6 Mean
0.931905	6.1045295	0.726149	11.8937500

SOURCE	DF	ANOVA SS	MEAN SQUARE	F-VALUE	Pr > F
CONC.	3	52.1869	17.3956	32.99	0.0001
WEEKS	3	12.7569	4.2523	8.06	0.0064

DUNCAN'S MULTIPLE RANGE TEST FOR HAEMOGLOBIN.
Note: Mean swith the same letter are not significantly different

DUNCAN GORUP	MEANN		CONC.
A	13.500	4	6
A	12.975	4	4
B	11.775	4	2
C	9.025	4	0
DUNCAN GROUPING	MEANN		WEEKS
A	13.175	4	8
A	12.225	4	6
B	11.350	4	4
C	10.825	4	2

Alpha = 0.05 dif = 9 MSE = 0.527292.

APPENDIX 4e:

Anoval table for mchc of *C. gariepinus* exposed to varions sublethal concentrations of tannery effluent for eight weeks.

SOURCE	DF	SS	MS	F-VALVE	Pr > F
Model	6	11.8532	1.9755	1.56	0.2645
Error	9	11.4323	1.2703		
Corrected Total:	15	23.2855			
R-Square		C.V	Root MSE	MCHC Mean	
0.509010		3.119879	1.127054	32.6693750	

SOURCE	DF	ANOVA SS	MS	F-VALVE	Pr > F
CONC.	3	2.2024	0.7341	9.89	0.0033
WEEKS	3	0.1721	0.05474	0.77	0.5380

DUNCANS MUI TIPLE RANGE TEST FOR MCV.
NB. Means with the same letter are not significantly different.

DUNCANS GROUPING	MEANN	N	CONC.
A	1.573	4	4
B A	1.353	1	2
B	1.061	4	0
C	0.582	4	6

DUNCAN GROUPING	MEAN	N	WEEKS
A	1.308	4	6
A	1.127	4	2
A	1.112	4	8
A	1.022	4	4

Alpha = 0.05 df. = 9 MSE = 0.074257.

APPENDIX 4g:

Anova table for mch of *C. garieipinus* exposed to various sublethal concentration for eight weeks

SOURCE	DF	SS	MS	F-VALUE	Pr > F
Model	6	0.1661	0.02768	1.85	0.1956
Error	9	0.1348	0.01498		
Corrected Total	15	0.3009			

R-Square	C.V	Root MSE	MCH Mean
0.551980	27.12783	0.122397	0.451178750

SOURCE	DF	ANOVA SS	MS	F-VALUE	Pr > F
CONC.	3	0.08232119	0.02744040	1.83	0.2116
WEEKS	3	0.08379519	0.02793173	1.86	0.2060

DUNCAN'S MULTIPLE RANGE TEST FOR MCH

Nb: Note that Means with the same letter are not significantly different

DUNCAN GROUPING	MEANN	CONC.
A	0.5202	4
A	0.5062	6
A	0.4395	2
A	0.3387	0

DUNCAN GROUPING	MEANN	WEEKS
A	0.5677	8
A	0.4505	6
A	0.4112	4
A	0.3753	2

APPENDIX 5

Anova and duncan tables for growth in *C. gariepinus* exposed to various sublethal concentrtions of tannery effluent for eight weeks.

ANOVA

SORUCE	DF	SS	MS	F-VALUE	Pr > F
Model	7	9506.332820	1358.047546	7.86	0.0011
Error	12	2073.025680	172.752140		
Corrected Total	19	11579.358500			

R-Square	CV	Root MSE	Growth mean
0.820972	71.45159	13.15332	18.39500000

SOURCE	DF	ANOVA SS	MEAN SQUARE	F-VALUE	Pr > F
CONC	4	8249.986400	2062.496600	11.94	0.004
WEEKS	3	1256.346420	418.782140	2.42	0.1163

DUNCAN MULTIPLE RANGE TEST FOR GROWTH

Nb: Means with same letters are not significantly different.

DUNCAN GROUPING	MEANN	CONC
A	16.748	1 0
A	36.143	3 1
B	15.503	4 2
B	-2.247	4 4
B	-4.170	4 6

DUNCAN GROUPING		MEANN	WEEKS
	A	29.990	5 8
B	A	21.046	5 6
B	A	13.312	5 4
B	A	9.234	5 2

B Alpha = 0.05 dif = 12 MSE = 172.7521

APPENDIX 6a:
Anova and Table for 96-h accumulation to chromium in the various tissues of *C. griepinus* exposed to tannery effluent

ANOVA

SOURCE	DF	Sum of Square	Mean Square	F-value	Pr > F
Model	8	772.40	96.55	2.09	0.1213
Error	12	555.65	46.30		
Corrected Total	20	1328.06			

R-Square	C.V	Root MSE	Chromium Mean
0.5816	182.93	6.8047	3.7198

SOURCE	DF	ANOVASS	Mean Square	F.value	Pr > F
Body tissues	6	618.87	103.15	2.23	0.1120
Vars	2	153.53	76.77	1.66	0.2314

Duncans Multiple Range Test for Chromium accumulation.
Note: Means with same letters are not significantly different.

Duncan grouping	Mean	N	Body tissues	Duncan grouping	Mean	N	Vars
A	16.802	3	1	A	7.516	7	1
B	3.162	3	4	A	2.216	7	2
B	2.557	3	2	A	1.427	7	3
B	1.468	3	6				
B	0.465	5		B	0.209	3	7

Alpha 0.05 df 12 MSE 46.30444

APPENDIX 6b:
Anova and DAKT Tables for the accumulation of copper in the tissues of *C. gariepinus* exposed to tennary effluent for 96.hr.

Anova

SOURCE	DF	Sum of Squares	Mean Square	F.Value	Pr > F
Model	8	181.61	22.7008	2.10	0.1188
Error	12	129.61	10.8007		
Corrected Total	20	311.21			

R-Square	C.V.	RootMSE	Copper Mean
0.5835	178.41	3.2864	1.8421

SOURCE	DF	ANOVASS	Mean Square	F.Value	Pr > F
Body tissues	6	140.8465	23.4744	2.17	0.1189
Vars	2	40.7603	20.3801	1.89	0.1938

Duncans Multiple Ragne Test for Copper accumualtion.
Note: Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Body Tissue
A	8.091	3	1
B	1.561	3	4
B	1.154	3	2
B	0.866	3	6
B	0.756	3	3
B	0.363	3	5
B	0.160	3	7
A	3.793	7	1
A	1.104	7	2
A	0.629	7	3

Alpha = 0.05 Df. = 12 MSE = 10.80066

APPENDIX 6c:
A nova and DMRT Tables for the accumualtion of lead in the tissues of *C. gariepinus* exposed to tannery effluent for 96.hr.

Anova

SOURCE	DF	Sum of Squares	Mean Square	F.value	Pr > F
Model	8	1379.32	172.42	1.92	0.1480
Error	12	1075.45	89.62		
Corrected Total	20	2154.77			
	R-Square	C.V	Root MSE	Lead mean	
	0.5639	9197.56	9.4668	4.7919	

SOURCE	DF	Anova SS	Mean Square	F.value	Pr > F
Body tissues	6	1075.75	179.29	2.00	0.1444
Vars	2	303.57	151.79	1.69	0.2250

Duncan's Multiple Range Test for Lead accumualtion.
Note: Means with same letters are not significantly different.

Duncan Grouping	mean	N	Body Tissues
A	22.056	3	1
B	4.248	3	4
B	2.957	3	2
B	1.844	3	3
B	1.422	3	6
B	0.772	3	5
B	0.245	3	7
A	10.141	7	1
A	2.593	7	2
A	1.642	7	3

Alpha = 0.05 df = 12 MSE = 89.6209

APPENDIX 6d:

Anova and DMRT Tables for the accumulation of Zinc in the tissues of *Clarieginus* exposed to tannery effluent for 96-hr.

Anova

SOURCE	DF	Sum of Square	Mean Square	F-value	Pr > F
Model	8	9.6801	1.2100	1.43	0.02770
Error	12	10.1341	0.8445		
Corrected Total	20	19.8142			

R-Square	C.V	RootMSE	Zinc Mean
0.1885	217.58	0.9190	0.4224

SOURCE	DF	Anova SS	Mean Swuare	F.Value	Pr > F
Body tissues	6	7.3714	1.2286	1.45	0.2729
Vars	2	2.3087	1.1544	1.37	0.2919

Duenan's Multiple Ragne Test for Zinc accumualtion
Note: Means withi he same letter are not significantly different.

Duncan Grouping	Mean	N	Body Tissues
A	1.852	3	1
B	0.299	3	2
B	0.271	3	4
B	0.248	3	6
B	0.214	3	3
B	0.068	3	5
B	0.005	3	7
A	0.891	7	1
A	0.197	7	2
A	0.179	7	3

Alpha 0.05 dl = 12 MSE = 0.844507.

APPENDIX 7a:
Anova and DNRT Tables for the accumulation of chromium in the tissues of *C. gariepinus* exposed to 2% tannery effluent for 8 weeks.

ANOVA

SORUCE	DF	Sum of Squares	Mean Square	F-value	Pr > F
Model	9	430.6456	47.8495	11.49	0.0001
Error	18	74.9406	4.1634		
Corrected Total	27	505.5863			

R-Square	C.V.	Root MSE	Chromium Mean
0.8518	66.1734	2.0404	3.0835

Source	DF	Anova SS	Mean Square	F-value	Pr > F
Body tissues	6	398.6686	65.7781	15.80	0.0001
Weeks	3	35.9769	11.9923	2.88	0.0645

Duncans Multiple Range Test for Chromium accumulation.
Note: Mean with he same letter are not significantly different.

Duncant Grouping	Mean	N	Body Tissues
A	11.897	4	1
B	3.177	4	3
B	2.882	4	2
B	2.112	4	4
B	0.623	4	5
B	0.570	4	6
B	0.323	4	7
A	4.662	7	8
B A	3.611	7	6
B A	2.308	7	4
B	1.754	7	2

Alpha = 0.05 df = 18 MSE = 4.1634

APPENDIX 7b:
Anova and DMRT Tables for the accumuallion of Copper in the tissues of *C. gariepinus* exposed to 2% tannery effluent for 8 weeks.

ANOVA

an Square	F-valuePr > F				
Model	9	75.3076	8.3675	11.03	0.0001
Error	18	13.6561	0.7587		
Corrected Total	27	88.9636			

R-Square	C.V.	Root MSE	Copper Mean
0.8465	70.7569	0.8710	1.2310

Source	DF	Anova SS	Mean Square	F-Value	Pr > F
Body tissues	6	69.5951	11.5992	15.29	0.0001
Weeks	3	5.7125	1.9042	2.51	0.0914

Duncans Multiple Range Test for Copper.
Note: Mean with the same letter are not significantly different.

Duenan Grouping	Mean	N	Body Tissues
A	1.950	4	1
B	1.435	4	3
B	1.834	4	4
B	0.772	4	2
B	0.092	4	5
B	0.230	4	6
B	0.103	4	7
A	1.897	7	8
B A	1.384	7	6
B A	1.905	7	4
B	0.738	72	

Alpha = 0.05 df = 18 MSE = 0.7587.

APPENDIX 7c:
Anova and DMRT Tables for the accumulation of lead in the tissues of *C griepinus* exposed to 2% tannery effluent for 8 weeks.

Source	DF	Sum of squares	Mean Square	F-value	Pr > F
Model	9	710.5101	78.9456	8.06	0.0001
Error	18	176.3734	9.7985		
Corrected Total	27	886.8835			

R-Square	C.V	Root MSE	Lead Mean
0.8011	87.2001	3.1303	3.5897

Source	DF	Anova SS	Mean Square	F-value	Pr > F
Body tissues	6	636.1049	106.0175	10.82	0.0001
Weeks	3	74.3452	24.7817	2.53	0.0907

Duncans Multiple Range Test for Lead
Note: Mean with same letter are not significantly different.

Duncan Grouping	Mean	N	Body tissues
A	14.767	4	1
B	12.257	4	3
B	9.817	4	2
B	7.775	4	1
B	6.734	4	5
B	6.500	4	6
B	6.120	4	7
B	5.802	4	8
B A	4.493	4	6
B A	2.194	4	4
B	1.869	4	2

Alpha = 0.05 DF = 18 MSE = 9.7985

APPENDIX 7d:
Anova and DMRETYAbles for the accmulation of Zinc in the tissues of *C. goriepinus* exposed to 2% tannery effluent for 8 weeks.

ANOVA

Source	DF	Sum of Squares	Mean Squares	F. Value	Pr > F
Model	9	10.8498	1.2055	4.31	0.0041
Error	18	5.0379	0.1299		
Corrected Total	27	15.8877			

R-Square	C.V.	Root MSE	Zinc Mean
0.6829	111.48	0.5290	0.4746

Source	DF	Anova SS	Mean Square	F-value	Pr > F
Body tissues	6	8.8687	1.4781	5.28	0.0027
Weeks	3	1.9811	0.6604	2.36	0.1056

Duncals Multiple Range Tst for Zinc.
Note that means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Body Tissues
A	1.800	4	1
B	0.534	4	5
B	0.362	4	3
B	0.311	4	2
B	0.146	4	4
B	0.143	4	6
B	0.027	4	7

Alpha = 0.05 df = 18MSE = 0.2799

APPENDIX 8a
Anova axnd DMRT Tables for the accumulation of chromium in th tissues of *C.gariepinus* exposed to 4%o tannery effluent for 8 weeks.

ANOVA

Soruce	DF	Sum of squares	Mean squares	F-value	Pr > F
Model	9	3364.86	373.87	10.99	0.0001
Error	18	612.47	34.03		
Corrected Total	27	3977.33			

R-Square	C.V.	ROOT MSE	Chromium Mean
0.8460	100.87	5.8332	5.7828

Source	DF	Anova SS	Mean Square	F-value	Pr > F
Body Tissues	6	3182.592	530.43	15.59	0.0001
Weeks	3	182.261	60.75	1.79	0.1860

Ducnans multiple Raene Test for chromium
Note: Means witht he same letter are not significantly different.

Duncan Grouping	Mean	N	Body Tissues
A	31.707	4	1
B	4.219	4	2
B	1.847	4	3
B	1.689	4	4
B	0.461	4	5
B	0.282	4	7
B	0.275	4	6
A	8.191	7	8
A	7.504	7	6
A	5.180	7	4
A	1.626	7	2

Alpha = 0.05 df = 18MSE = 34.02589.

APPENDIX 8b
Anova and DMRT Tables for Copper accumulation in the tissues of *C. gariepinus* exposed to 4% tannery effluent for 8 weeks.

ANOVA

Source	DF	Sum of squares	Mean squares	F-value	Pr > F
Model	9	814.7897	90.5322	9.23	0.0001
Error	18	176.5553	9.8086		
Corrected Total	27	991.3450			

R-Square	C.V.	Root MSE	Copper Mean
0.8219	111.55	3.1919	2.8076

Source	Df	Anvoa SS	Mean Square	F-Value	Pr > F
Body Tissues	6	764.1334	127.3556	12.98	0.0001
Weeks	3	50.6563	16.8854	1.72	0.1984

Duncans Multiple Ragne Test for Copper.
Note: Means with same letter are not significantly different.

Duncan Grouping	Mean	N	Body Tissue
A	15.506	4	1
B	2.161	4	2
B	0.738	4	4
B	0.660	4	3
B	0.300	4	6
B	0.169	4	7
B	0.119	4	5
A	4.223	7	8
A	3.662	7	6
A	2.654	7	4
A	0.691	7	2

Alpha = 0.05 df = 18 MSE = 9.8086

APPENDIX 8c:
Anova and DMRT Tables for Lead accumulation in the tissues of *C. gariepinus* exposed to 4% tannery effluent for 8 weeks.

Anova.

Source	DF	Sum of Squares	Mean Squares	F-Value	Pr > F
Model	9	5225.19	580.577	10.50	0.0001
Error	18	995.30	55.291		
Corrected Total	27	6220.42			

R-Square	C.V.	Root MSE	Lead Mean
0.840006	016.9811	7.458	6.9505

Source	DF	Anova SS	Mean Square	F-Value	Pr > F
Body Tissues	6	4980.8171	830.1362	15.01	0.0001
Weeks	3	244.3767	81.4589	1.47	0.2553

Duncans Multiple Raene Test for Lead.
Note: Mean with the same letter are not significantly different.

Duncan Grouping	Mean	N	Body Tissues
A	39.510	4	1
B	3.536	4	2
B	2.150	4	4
B	2.098	4	3
B	0.790	4	5
B	0.383	4	7
B	0.186	4	6
A	9.156	7	8
A	8.771	7	6
A	7.990	7	4
A	1.886	7	2

Alpha = 0.05 df = 18 mse = 55.29054

APPENDIX 8d:
Anova and DMRT Tables for Zinc accumulation inthe tissues of *C. gariepinus* exposed to 4% tannery effluent for 8 weeks.

ANOVA:

Source	DF	Sum of square	Mean square	F-Value	Pr > F
Model	9	54.7319	6.0813	2.88	0.0270
Error	18	38.0613	2.1145		
Corrected Total	27	92.7932			

R-Square	C.V.	Root MSE	Zinc Mean
0.5898	202.07	1.4541	0.7196

Source	DF	Anova SS	Mean square	F-Value	Pr > F
Body tissues	6	45.5973	7.5990	3.59	0.0161
Weeks	3	9.1381	3.0460	1.44	0.2640

Dunans multiple Raene Test for Zinc.
Note: Means witht eh same letter are not significantly different.

Duncan Grouping	Mean	N	Body tissues
A	3.839	4	1
B	0.038	4	2
B	0.203	4	5
B	0.194	4	4
B	0.182	4	3
B	0.161	4	6
B	0.081	4	7
A	1.494	7	8
A	1.042	7	6
A	0.196	7	4
A	0.146	7	2

Alpha = 0.05 df = 18 MSE = 2.11458

APPENDIX 9a:
Anova and DMRT Tbles for chromium accumulation in the tissue of *C. gariepinus* exposed to 6% tannery effluent for 8 weeks.

ANOVA

Source	DF	Sum of squares	Mean squares	F-Value	Pr > F
Model	9	5276.33	586.26	17.01	0.0001
Error	18	620.51	34.47		
Corrected Total	27	5896.84			

R-Square	C.V	Root MSE	Chromium Mean
0.8948	83.6075	5.8713	7.0225

Source	DF	AnnovaSS	Mean square	F-value	Pr > F
Body tissues	6	5101.1457	850.191	24.66	0.0001
Weeks	3	171.1802	58.393	1.69	0.2040

Duncans Multiple Ragne Test for Chromium.
Note: Means with the same letter are not significantly different.

Duncan Grouping	mean	N	Body Tisues
A	39.913	4	1
B	4.408	4	2
B	2.428	4	3
B	1.237	4	4
B	0.607	4	5
B	0.484	4	6
B	0.081	4	7
A	9.661	7	8
A	8.372	7	6
A	7.065	7	4
A	2.992	7	2

Alpha 0.05 df 18 MSE =

APPENDIX 9b:
Anova and DMRT Tables for Copper accumulation int he tissues of *C. griepinus* exposed to 6% tannery effluent for 8 weeks.

ANOVA:

Source	DF	Sum of Squares	Mean squares	F-value	Pr > F
Model	9	1603.36	178.15	9.30	0.0001
Error	18	344.75	19.15		
Corrected Total	27	1948.11			

R-Square	C.V.	Root MSE	Copper Mean
0.823015	106.2283	4.376379	4.119778571

Source	DF	Anova SS	Mean Square	F-Value	Pr > F
Body tissues	6	1484.28	247.38	12.92	0.0001
Weeks	3	119.08	39.69	2.07	0.1397

DMRT:
Note: Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Body Tissues
A	21.633	4	1
B	4.273	4	2
B	1.788	4	3
B	0.486	4	4
B	0.374	4	6
B	0.258	4	5
B	0.025	4	7
A	6.931	7	8
B A	4.952	7	6
B A	3.242	7	4
B	1.355	7	2

Alpha= 0.05 df = 18 MSE = 19.15269

APPENDIX 9c:

Anova and DMRT Tables for Lead accumulation in the tissues of *C. gariepinus* exposed to 6% tannery effluent for 8 weeks.

ANOVA.

Source	DF	Sum of squares	Mean square	F-value	Pr > F
Model	9	4358.03	484.23	9.07	0.0001
Error	18	960.71	53.37		
Corrected Total	27	5318.71			

R-Squares	C.V	Root MSE	LEAD MEAN
0.819373	107.4842	7.305665	6.79696

Source	Df	Anova SS	Mean square	F-Value	Pr > F
Body Tissues	6	4074.86	679.14	12.72	0.0001
Weeks	3	283.18	94.39	1.77	0.1892

DMRT:

Note: That means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Body Tissues
A	36.078	4	1
B	5.162	4	2
B	3.009	4	3
B	1.940	4	4
B	0.949	4	5
B	0.317	4	6
B	0.124	4	7
A	10.324	7	8
A	9.586	7	6
A	4.017	7	4
A	3.260	7	2

Alpha = 0.05 dif = 18 MSE = 53.37271.

APPENDIX 9d:
Anova and DMRT Tables for Zinc accumulation in tissues of *C. gariepinus* exposed to 6% tannery effluent for 8 weeks.

ANOVA.

Source	Df	Sum of Squares	mean square	F-value	Pr > F
Model	9	120.4389	13.3821	8.27	0.0001
Error	18	29.1351	1.6186		
Corrected Total	27	149.5739			

R-Square	C.V.	Root MSE	Zinc Mean
0.805213	116.2705	1.272248	1.09421

Source	DF	Anova SS	Mean Square	F-value	Pr > F
Body tissues	6	110.0216	18.3369	11.33	0.0001
Weeks	3	10.4173	3.4724	2.15	0.1301

DMRT
Note: Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	Body Tissues
A		3.913	4	1
B		0.799	4	2
B		0.386	4	4
B		0.327	4	3
B		0.148	4	5
B		0.047	4	6
B		0.039	4	7
A		2.037	7	8
B	A	1.166	7	6
B	A	0.785	7	4
B		0.389	7	2

Alpha = 0.05 df - 18 MSE - 1.6186

APPENDIX 10
Anova and DMRT Tables for Total metal (Cr, Cu, Pb and Zn) burden in *C. gariepinus* exposed to 2%, 4% and 6% tannry effluent for 8 weeks.

ANOVA

Source	DF	Sum of Squares	Mean squares	F-value	Pr > F
Model	6	45419.99	7569.10	16.39	0.0002
Error	9	4157.10	461.90		
Corrected Total	15	49577.10			

R-Square	C.V.	Root MSE	Metal Mean
0.916149	28.22395	21.49185	76.1416

Source	DF	Anova SS	Mean Square	F-value	Pr > F
Metal	3	28822.54	9607.51	20.80	0.0002
Weeks	3	16597.46	5532.49	11.98	0.0017

DMRT:
Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Metal
A	121.36	4	Lead
A	110.39	4	Chromium
B	56.82	4	Copper
C	16.02	4	Zinc
A	113.93	4	8
B A	96.51	4	6
B	64.62	4	4
C	29.52	4	2

Alpha = 0.05 df = 9 MSE = 461.8998.

APPENDIX 10
Anova and DMRT Tables for Total metal (Cr, Cu, Pb and Zn) burden in *C. gariepinus* exposed to 2%, 4% and 6% tannry effluent for 8 weeks.

ANOVA

Source	DF	Sum of Squares	Mean squares	F-value	Pr > F
Model	6	45419.99	7569.10	16.39	0.0002
Error	9	4157.10	461.90		
Corrected Total	15	49577.10			

R-Square	C.V.	Root MSE	Metal Mean
0.916149	28.22395	21.49185	76.1416

Source	DF	Anova SS	Mean Square	F-value	Pr > F
Metal	3	28822.54	9607.51	20.80	0.0002
Weeks	3	16597.46	5532.49	11.98	0.0017

DMRT:
Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Metal
A	121.36	4	Lead
A	110.39	4	Chromium
B	56.82	4	Copper
C	16.02	4	Zinc
A	113.93	4	8
B A	96.51	4	6
B	64.62	4	4
C	29.52	4	2

Alpha = 0.05 dI = 9 MSE = 461.8998.