THERAPEUTIC EFFECT OF L-ASCORBIC ACID ON LEAD NITRATE INDUCED PATHOLOGY IN SOME ORGANS OF THE AFRICAN CATFISH *Clarias gariepinus*

IKEOGU, CHIKA FLORENCE
PG/PhD/2013597002P

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF ZOOLOGY, FACULTY OF BIOSCIENCES, NNAMDI AZIKIWE UNIVERSITY AWKA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD) IN FISHERIES AND AQUACULTURE.

MARCH 2020

CERTIFICATION

I, CHIKA FLORENCE IKEOGU, with registration number PG/PhD/2013597002P, hereby certify that I am responsible for the work submitted in this dissertation and that this is an original work which has not been submitted to this university or any other institution for the award of a degree or a diploma.

_____ _____

Signature of Candidate Date

APPROVAL PAGE

This dissertation titled "Therapeutic effect of L-ascorbic acid on lead nitrate induced pathology in some organs of the African catfish *Clarias gariepinus"*which was carried out by Ikeogu,Chika Florence;Registration number PG/PhD/2013597002P,has been approved for the award of Doctor of Philosophy(PhD) degree in the Department of Zoology, Faculty of Biosciences, Nnamdi Azikiwe University Awka.

By:

Prof.C.I.Nsofor **SUPERVISOR**	**Date**
Prof. C.E. Akunne **HEAD OF DEPARTMENT**	**Date**
Dr. S. C. Onuorah **SUB DEAN FACULTY OF BIOSCIENCES**	**Date**
Prof. J. E Eyo **EXTERNAL EXAMINER**	**Date**
Prof. M.U. Orji **DEAN, FACULTY OF BIOSCIENCES**	**Date**
Prof. P. K. Igbokwe **DEAN, SCHOOL OF POSTGRADUATE STUDIES**	**Date**

DEDICATION

I Ikeogu, Chika Florence dedicate this work to my loving husband, Dr. Ikeogu Emeka Ubah.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Prof. Mrs. Charity IfeyinwaNsofor,my supervisor.She has been a source of inspiration and encouragement to me.She guided me morally and academically throughout this PhDprogramme. I thank her greatly for counting me worthy tobenefit from her wealth of experience.

My appreciation also goes to Prof. Innocent Nwaogu of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, for permitting me to use their facilities for the bench work.

I appreciate the inputs from Prof. AbuchiIgwebuike, late Prof. D.N. Ezeasor,late Prof. E.L.C. Nnabuife,Prof. Baumgartner Wes, Prof. Chidi Akunne, Dr. Austin Ngene, Prof. Cordelia Ebenebe, Prof. Innocent Igwilo, Prof Angela Ufele, Dr. Obiora Ikpeze, Dr. Chika Ikele, Dr. Ndukwe Okorie, Dr. Uko Ibeabuchi and Mr. Moses Okoli.

I also acknowledge the encouragement and support from my family; Dr Emeka Ikeogu (husband), Dr Chidinma Ikeogu (daughter), Dr. Nnamdi Ikeogu (son) and Dr.Uche Ikeogu (daughter).To my late mother Mrs. Dorothy Uzoh,who wished to see the end of this academic journey,but was called home on 2nd December 2017,I say' continue to rest in the bosom of the Lord'. My siblings; Mrs. Chinwe Ike-Belonwu, Mrs. Uche Onwugbufor, Mr. Tagbo Uzoh and Pastor Izuchukwu Uzoh were very supportive.

I am grateful to Mr. Emmanuel Asogwa, Mr.Somadina Obodoefuna and Miss Christy Ikegbo for taking care of the experimental animals. I cannot forget the support from my colleagues in the Department of Fisheries and Aquaculture, Nnamdi Azikiwe University Awka; Kenechi Okpala-Ezennia, Nkechi Onyeyili, Gloria Anachuna, Oluchi Ezeike and Ogbonnaya Hannah.The home front was well covered by Simon, Oluebube, Abel, Chidera and Oluchukwu.

Finally and most importantly, I appreciate divine help, guidance and protection given to me by the Almighty God, who makes all things beautiful in His time. To Him be all the glory. Amen.

ABSTRACT

The objective of the study was to determine the toxic effects of sub lethal concentrations of lead nitrate, its tissue residues, and the possible therapeutic effects of L-ascorbic acid on lead nitrate toxicity in *Clarias gariepinus*. This study was based on 48 hours acute toxicity tests carried out on four *Clarias gariepinus* juveniles of mean weight 39.80±1.54g and mean length 21.05± 1.23cm. The LC_{50} of lead nitrate was 60mg/l.The LC_{50} of vitamin C (Kepro®) was 175mg/l. Sublethal concentrations of 0,5,10, and 15mg/l lead nitrate were employed in experiment 1 investigating the chronic effects of lead nitrate on *Clarias gariepinus* juveniles for 91 days (13 weeks). 50 and 100mg/l of kepro® vitamin C powder were employed as bath treatment to the 91 day lead exposed experimental fish for 7 days. The effects of lead nitrate on haematological parameters; PCV, RBC count, Hb concentration, TWBC count, and DWBC were assessed using standard methods before lead exposure to obtain day zero data, after 91 day lead exposure, and after 7 day treatment with 50 and 100mg/l kepro® vitamin C. Biochemical parameters investigated were serum enzymes: AST, ALT, ALP and urea. The histology of the gills, liver, and stomach were also carried out to assess the histopathological effects of 91 day exposure to lead nitrate and 7 days treatment with vitamin C on these organs. Haematology, enzymology and histology of the gills, liver and stomach of *Clarias gariepinus* were also carried out with concurrent exposure to lead nitrate and vitamin C in experiment 2 for 91 days to assess the effects of vitamin C on the chronic effects of lead nitrate on the blood, serum enzymes, gills, liver, and stomach of *Clarias gariepinus* in the same aquaria. Lead accumulation in the muscles of *Clarias gariepinus* was determined in both experiments through A.A S. Results of experiment 1 showed significant decrease ($P<0.05$) in PCV, Hb, and RBC of 91 day lead nitrate exposed groups when compared to day zero and control groups. This signifies anaemia. A significant increase ($P<0.05$) in TWBC in the fish exposed to varying concentrations of lead when compared to the control and day zero group is termed leucocytosis. Enzymes assay showed significant decrease ($P<0.05$) in AST, ALT and ALP levels in the lead nitrate exposed groups when compared to the control. A significant increase ($P<0.05$) in the urea of the lead nitrate exposed group when compared to the control group is termed azotemia and is an indication of poor renal function. There was a significant increase in the concentration of lead in the muscle of the lead exposed groups when compared to the control group. The histology of gills showed distorted lamellae, necrotic primary and secondary lamellae, severely damaged lamellae in the lead exposed groups when compared to the control a group. The liver histology of *Clarias gariepinus* exposed to varying concentrations of lead nitrate showed cytoplasmic vacuolations,pyknotic nuclei and degenerated hepatocytes. The liver of the control showed normal liver parenchymal cells. The histology of the stomach of *Clarias gariepinus* exposed to varying concentrations of lead nitrate showed mucosal haemorrhages, broken crypts and degeneration of the columnar epithelial cells of the mucosa, when compared to the normal cells of the control. The results of the 7 day treatment of the lead exposed groups with 50mg/l and 100mg/l vitamin C showed a significant increase ($P<0.05$) in PCV,Hb and RBC between the exposed and treated groups. There was no significant difference ($P>0.05$) between the 50mg/l vitamin C and 100mg/l vitamin C groups. Treatment of the lead nitrate exposed group with vitamin C showed that there was no significant difference ($P>0.05$) between the TWBC of exposed and treated groups and between the 50mg/l vitamin C and 100mg/l vitamin C. Treatment of the lead nitrate exposed group with vitamin C showed a significant decrease ($P<0.05$) in AST, ALT and ALP between the lead exposed groups and the vitamin C treated groups. Treatment of lead

exposed group showed a significant decrease ($P<0.05$) in urea level between the lead exposed group and vitaminC treated groups. The 100mg/l vitamin C showed more significant decrease ($P<0.05$) in urea level than the 50mg/l vitamin C. The histopathology of the gills, liver and stomach caused by lead nitrate toxicity was significantly resolved ($P<0.05$) when treated with 50mg/l vitamin C and 100mg/l vitamin C with 50mg/l vitamin C having better therapeutic effect than 100mg/L vitamin C. There was a significant decrease ($P<0.05$) in lead accumulated in fish muscle after treatment of the lead nitrate exposed group with vitamin C. The 50mg/l vitamin C was more effective in reducing lead level in the muscle of the exposed fish than the 100mg/l.Results of experiment 2 showed that there was no significant difference ($P>0.05$) between the pathology of *Clarias gariepinus* exposed to lead nitrate and the group exposed concurrently to lead and vitamin C. The toxicity of lead nitrate could not be reversed by Vitamin C during concurrent exposure. The study concluded that lead is toxic to *Clarias gariepinus* at sublethal concentrations and that vitamin C at the water bath dose of 50mg/l administered for 7 days can reverse the damaging effects of lead on fish. It is therefore recommended that fish from lead polluted waters can be made safe by treatment with 50mg/l vitamin C for 7 days. Also bioaccumulated lead residues in the muscles of lead contaminated fish can significantly be reduced by 50mg/l Vitamin C for 7 days as a food safety measure.

TABLE OF CONTENTS

	Title page	i
	Certification	ii
	Approval	iii
	Dedication	iv
	Acknowledgement	v
	Abstract	vi
	Table of Contents	vii
	List of Tables	viii
	List of Figures	ix
	List of Plates	**x**
	List of Abbreviations	xi
	CHAPTER ONE	**1**
	INTRODUCTION	1
1.1	General Background	1
1.2	Statement of Problem	5
1.3	Justification for the study	6
1.4	Aim for the study	7
1.5	Specific Objectives	**7**
	CHAPTER TWO	**8**
	LITERATURE REVIEW	8
2.1	Acute Toxicity Tests	8
2.2	Heavy Metals and the Aquatic Ecosystem	8
2.3	Mechanism of Metal Bio-accumulation in Fishes	9
2.4	Mechanisms of Histopathological Damage	10
2.5	Mechanisms of Metal Induced Oxidative Damage	13
2.6	Lead and Oxidative Stress	14
2.6.1	Chemical forms and biological properties of lead	15
2.6.2	Ionic mechanism of lead toxicity	18
2.6.3	Toxicity of lead on cell membranes	19
2.6.4	Lead and the environment	21
2.6.4.1	Birds	24
2.6.4.2	Mammals	25
2.6.4.3	Micro-organisms	25
2.6.4.4	Aquatic organisms	26
2.6.5	Effect of lead toxicity on organs of living organisms	28
2.6.5.1	Effect on the nervous system	28
2.6.5.2	Effect on the hematopoietic system of d-amino levulinic acid (ALA)	30

	induced generation of reactive oxygen species	
2.6.5.3	Renal effects of lead	32
2.6.5.4	Physical impairment	33
2.6.5.5	Reproductive health effects of lead	34
2.6.5.6	Effect of lead on bone	35
2.6.5.7	Effect of lead on liver	36
2.7	Oxidative Stress	36
2.7.1	Oxidative stress and antioxidant defenses	38
2.7.2	Role of antioxidants in protecting lead induced oxidative stress	39
2.8	Vitamin C: Function and Essentiality	41
2.8.1	Antioxidant effect of vitamin c in metal induced organisms	43
	CHAPTER THREE	44
	MATERIALS AND METHODS	44
3.1	Experimental Animals	44
3.2	Experimental Design	46
3.2.1	Experiment A: Determination of LC_{50} of lead nitrate in C. *gariepinus*	46
3.2.2	Experiment B: Determination of LC_{50} of ascorbic acid (kepro vitamin C)	46
3.2.3	Experiment I: Chronic toxicity of lead nitrate in C. *gariepinus* juvenile and ameliorating effect of ascorbic acid	47
3.2.4	Treatment with ascorbic acid for 7 days	49
3.2.5	Experiment 2a: Effects of concurrent exposure of *Clarias gariepinus* juveniles to lead nitrate and 50 mg/l ascorbic acid for 91 days	52
3.2.6	Experiment 2b: Effects of concurrent exposure of *Clarias gariepinus* juveniles to lead nitrate and 100 mg/l ascorbic acid for 91 days	54
3.3	Studies on Haematological Parameters	56
3.3.1	Determination of packed cell volume (PCV)	56
3.3.2	Determination of haemoglobin concentration (Hb)	56
3.3.3	Red blood cell (RBC) count	57
3.3.4	Total white blood cell (TWBC) count	57
3.3.5	Differential white blood cell (DWBC) count	57
3.4	Biochemical Assay	58
3.5	Serum Biochemistry Determinations	58
3.5.1	Alanine amino transferase (ALT)	58
3.5.2	Aspartate amino transferase (AST)	59
3.5.3	Alkaline phosphatase (ALP)	60
3.5.4	Determination of blood urea	61
3.5.5	Determination of lead concentration in *C. gariepinus* muscle	62
3.6	Histopathological Examinations	62

3.7	Data Analysis	63
		64
	CHAPTER FOUR	
	RESULTS	64
4.1	Acute Toxicity of Lead Nitrate in *Clarias gariepinus* Juveniles	64
4.2	Acute Toxicity of Vitamin C (Kepro®) in *Clarias gariepinus* Juveniles	66
4.3	Chronic Toxicity of Lead Nitrate in *Clarias gariepinus* Exposed for 91 days	68
4.3.1	The Effect of lead nitrate on haematological parameters of *Clarias gariepinus* after 91 days exposure to varying concentrations	68
4.3.2	The Effect of 7 day therapy with vitamin Con the haematological parameters of *Clarias gariepinus*	71
4.3.3	The effects of lead nitrate on the serum biochemistry of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91days	75
4.3.4	The effect of 7 day therapy with vitamin C on the serum chemistry of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days	75
4.3.5	The levels of lead accumulation in the muscle of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days	78
4.3.6	The effect of vitamin C on the levels of lead in the muscles of *Clarias gariepinus* exposed to lead nitrate	78
4.4	Histology of the Gills, Liver, and Stomach of *Clarias gariepinus* exposed to varying concentrations of Lead Nitrate for 91 days and treated with vitamin C	81
4.4.1	Histopathological changes in the gills of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days	81
4.4.2	The effect of vitamin C on the histopathological changes in the gills of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days	86
4.4.3	Histopathological changes in the liver of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days	89
4.4.4	The effect of vitamin C on the histopathological changes in the liver of *Clarias gariepinus* exposed to varying concentrations of lead nitrate	94
4.4.5	Histopathological changes in the stomach of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days	97
4.4.6	The effect of vitamin C on the histopathological changes in the stomach of *Clarias gariepinus* exposed to varying concentrations of lead nitrate	102

4.5	*Clarias gariepinus* juveniles concurrently exposed to lead nitrate and vitamin C in varying concentrations.(Experiment 2)	107
4.5.1	The effects of concurrent exposure to lead nitrate and vitamin C on the haematological parameters of *Clarias gariepinus* juveniles	107
4.5.2	Serum biochemistry (AST, ALT, ALP and Urea) of *Clarias gariepinus* concurrently exposed to lead nitrate and vitamin C for 91 days	111
4.5.3	The effect of Vitamin C on the histopathological changes in the gills, liver and stomach of *Clarias gariepinus* concurrently exposed to lead and vitamin C	113
	CHAPTER FIVE	**127**
	DISCUSSION	127
5.1	Discussion	127
5.2	Contribution to Knowledge	138
5.3	Conclusion	138
5.4	Recommendation	138
	References	139
	Appendices	177

LIST OF TABLES

Table		page
1	Toxicity of lead in aquatic environments and bio concentration factors	27
2	Proximate analysis of 3mm skretting fish pellets used in the study	45
3	Experimental design of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days.	48
4	7 day treatment with 50mg/l vitamin C after 91 days exposure of *Clarias gariepinus* to varying concentration of lead nitrate.	50
5	7 day treatment with 100 mg/l vitamin C after 91 days exposure of *Clarias gariepinus* to varying concentration of lead nitrate.	51
6	Exposure of *Clarias gariepinus* juvenile to varying concentrations of lead nitrate and 50 mg/l vitamin C for 91 days.	53
7	Exposure of *Clarias gariepinus* juvenile to varying concentrations of lead nitrate and 100 mg/l vitamin C for 91 days.	55
8	Determination of LC_{50} of lead nitrate in *Clarias gariepinus* juveniles	65
9	Determination of LC_{50} of Vitamin C. (Kepro®) in *Clarias gariepinus* juveniles	67
10	Haematological values of *Clarias gariepinus*exposed to varying concentrations of lead nitrate for 91 days and treated with 50 and 100 mg/l vitamin C for 7 days	179
11	Mean value of liver enzymes and urea of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days and treated with either 50 mg/l or 100 mg/l vitamin C for 7 days	180
12	Mean value of lead concentration (ppm) in the muscle of *Clarias gariepinus* exposed to lead nitrate for 91 days and treated with 50 and 100 mg/l vitamin C	181
13	The mean haematological values of *Clarias gariepinus*concurrently exposed to lead nitrateand Vitamin C for 91 days.	182
14	Mean value of liver enzymes and urea of *Clarias gariepinus* concurrently exposed to lead nitrate and vitamin C for 91 days.	183

LIST OF FIGURES

Figure		Page
1	Means of haematological parameters [PCV, RBC, Hb, and TWBC] of *Clarias gariepinus* exposed for 91 days to lead-nitrate, and for 7dayspost-exposures to 50mg/l and 100mg/l of Vitamin C, respectively.	70
2	Differential White Blood Counts of *Clarias gariepinus*at 91 days exposure to lead nitrate, and at 7days post-exposures to 50mg/l and to 100mg/l of Vitamin C.	74
3	Mean values of serum enzymes [AST, ALT, ALP and Urea] of *Clarias gariepinus* at 91 days exposure to lead nitrate, and at 7days post-exposures to 50mg/l and to 100mg/l of Vitamin C.	77
4	Mean values of lead nitrate concentration (ppm) in muscles of *Clarias gariepinus*at 91 days exposure to Lead nitrate, 7days post-exposures to 50mg/l of Vitamin C, 7days post-exposures to 100mg/l of Vitamin C, and 91 days exposure to a combination of Lead nitrate and 100mg/l of Vitamin C.	80
5	Mean values of PCV, Hb, TWBC of *Clarias gariepinus*at 91 days concurrent exposuresto lead nitrate concentrations, 50mg/l of Vitamin C, 100mg/l of Vitamin C	108
6	Differential White Blood Counts of *Clarias gariepinus* at 91 days concurrent Exposures to lead nitrate concentrations, 50mg/l of Vitamin C, 100mg/l of Vitamin C.	110
7	Mean values of serum enzymes [AST, ALT, ALP and Urea] of *Clarias gariepinus* at 91 days concurrent exposures to lead nitrate concentrations, 50mg/l of Vitamin C, and 100mg/l of Vitamin C.	112

LIST OF PLATES

Plate		Page
1	Photomicrograph of control gill exposed to 0mg/l lead, showing normal gill structure, striated abductor muscle acellular bone and intact gill lamellaeH&EX100	82
2	Photomicrograph of gills exposed to 5mg/l Lead showing minor distorted lamellae. H&EX100	83
3	Gills exposed to 10mg/l lead showing distorted and curled gill lamellae H&E X 100.	84
4	Gills exposed to 15mg/l lead showing severely destroyed, irregularly shaped lamellae H&E X 100.	85
5	Gill exposed to 10mg/l lead and treated with 50 mg/l vitamin C showing intact gill lamellae H&E X 100.	87
6	Gill exposed to 15mg/l lead and treated with 100mg/l vitamin C showing enlargement of the central venous sinus with clear/intact afferent arteries. H&E X 100.	88
7	Photomicrograph of normal liver of *C. gariepinus* exposed to 0mg/l lead showing normal liver architecture with intact hepatocytes and central vein. H&E X 100.	90
8	Photomicrograph of liver of *C. gariepinus* exposed to 5mg/l lead showing cytoplasmic vacuolations. H&E X 100.	91
9	Photomicrograph of liver of *C. gariepinus* exposed to 10mg/l lead showing hepatocytes lacking nucleus and undergoing cell death H&E X 100.	92
10	Photomicrograph of liver of *C. gariepinus* exposed to 15mg/l lead showing cytoplasmic vacuolations and necrosis of hepatocytes. H&E X 100.	93
11	Liver exposed to 10mg/l lead and treated with 100mg/l vitamin C showing prominent central vein and sinusoid.	95
12	Liver exposed to 15mg/l lead and 100mg/l vitamin C showing minor congestion of the central vein and necrosis.	96
13	Photomicrograph of normal stomach of *Clarias gariepinus* showing intact mucosa, muscularis mucosa and gastric pit. H&E X 100.	98
14	Stomach exposed to 5mg/l lead showing minor submucosal haemorrhages in the stomach tissue. H&E X 100	99
15	Stomach exposed to 10mg/l lead showing minor submucosal haemorrhages in the stomach tissue. H&E X 100.	100
16	Stomach exposed to 15mg/l lead showing minor submucosal haemorrhages, erosion of the mucosal lining and degenerative changes in the muscularis mucosa.	101

17	Stomach exposed to 5mg/l lead and treated 50mg/l vitamin C showing intact mucosaand muscularis mucosa. H&E X 100.	103
18	Stomach exposed to 5mg/l lead and treated 100mg/l vitamin C showing intact stomach mucosa and gastric pits. H&E X 100.	104
19	Stomach exposed to 15mg/l lead and treated 50mg/l vitamin C showing multiple lesions in the mucosa. H&E X100.	105
20	Stomach exposed to 15mg/l lead and treated 100mg/l vitamin C showing intact mucosa muscularis and serosa	106
21	Gill exposed to concurrent 10mg/l lead and 50mg/l vitamin C showing minor distorted lamellae H&E X 100.	115
22	Gill exposed to concurrent 10mg/l lead and 100mg/l vitamin C showing degenerated gill lamellae. H&E X 100.	116
23	Gill exposed to concurrent 15mg/l lead and 50mg/l vitamin C showing multiple curling of the lamellae. H&E X 100.	117
24	Gill exposed to concurrent 15mg/l lead and 100mg/l vitamin C showing severe destruction of the gill lamellae. H&E X 100.	118
25	Liver exposed to concurrent 5mg/l lead and 50mg/l vitamin C showing intact central vein and congested sinusoid.	119
26	Liver exposed to concurrent 5mg/l lead and 100mg/l vitamin C showing intact central vein, congested interlobular ducts and fibrosis of the hepatocytes.	120
27	Liver exposed to concurrent 15mg/l lead and 50mg/l vitamin C showing intact central vein and congested interlobular ducts with massive degeneration of the hepatic tissues.	121
28	Liver exposed to concurrent 15mg/l lead and 100mg/l vitamin C showing extensive fatty degeneration of the hepatic tissues, congestion and erosion of the interlobular ducts	122
29	Stomach exposed to concurrent 5mg/l lead and 50mg/l vitamin C showing loosened circular muscle and intact mucosa.	123
30	Stomach exposed to concurrent 5mg/l lead and 100mg/l vitamin C showing submucosal haemorrhages, intact muscularis and intact mucosa.	124
31	Stomach exposed to concurrent 15mg/l lead and 50mg/l vitamin C showing submucosal haemorrhages and cellular infiltration.	125
32	Stomach exposed to concurrent 15mg/l lead and 100mg/l vitamin C showing distorted mucosa with lumen filled with debris And fibrosis of the muscularis mucosa.	126

LIST OF ABBREVIATIONS

µg/dl	microgram per deciliter
µg/l	microgram per litre
A.A.S.	Atomic absorption spectrophotometry
A.L.A.-D.	Delta amino levulinic acid
A.L.P.	Alkaline phosphatase
A.L.T.	Alanine amino transferase
A.S.T.	Aspartate amino transferase
B.B.B.	Blood brain barrier
B.L.M.	Biotic ligand model
C.N.S.	Central nervous system
Ca^{2+}	Calcium ion
CAT	Catalase
Cd	Cadmium
CO_2	Carbondioxide
Cr	Chromium
Cu	Copper
D.N.A.	De- oxyribonucleic acid
D.W.B.C.	Differential white blood cell
F.I.A.M.	Free ion activity model
Fe	Iron
Fe^{2+}	Iron II ion
G.A.B.A.	Gamma amino butyric acid
H. & E.	Haematoxylin and eosin
H.P.I. axis	Hypothalamo - pituitary - interrenal axis.
H_2O_2	Hydrogen peroxide
Hb.	Haemoglobin
Hg	Mercury
I.P.C.S.	International Programme on Chemical Safety
I.Q.	Intelligence quotient
LC_{50}	Lethal concentration fifty
M cell	Mauthner cell
M.D.A.	Malondialdehyde
M.T.	Metallothioneins
mg/kg	milligram per kilogram
mg/l	milligram per litre
Mg^{2+}	Magnesium ion
Na^{+}	Sodium ion
O.E.C.D.	Organization for Economic Co-operation and Development
O^{2-}	Superoxide ion
$OH^{\cdot}$	Hydroxyl radical
P.C.V.	Packed cell volume
P.N.S.	Peripheral nervous system
Pb	Lead
$Pb_3(PO_4)_2$	Lead phosphate
pH	Hydrogen ion concentration
R.B.C.	Red blood cell
R.N.A.	Ribonucleic acid
R.O.S.	Reactive oxygen species
S.O.D.	Superoxide dismutase
S.P.M.	Subcellular partitioning model

T.W.B.C.	Total white blood cell
W.H.O.	World Health Organisation
Z.P.P.	Zinc protoporphyrin
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 General Background

Freshwater contamination with heavy metals such as lead is increasingly becoming a subject of great concern over the past decade (Al-Awady, 2011) not only because of their threat to potable water supplies but also because of the damage caused to aquatic life especially fishes, (Tawari-fufeyin *et al.*,2008). Fishes are widely used to evaluate the health of aquatic ecosystem because pollutants build up in the food chain and are responsible for the adverse effects and death of aquatic organisms(Vinodini and Narayanan, 2008).

Fishes therefore are exposed to unnaturally high levels of lead and its products in the environment as introduced by manufacturing and mining activities (Moulis,2010). Several researchers have reported that toxic and non-biodegradable heavy metals such as lead accumulates in many fish species causing toxicological effects (Gordon *et al.*,2002; Khoshnood *et al.*,2011). Lead has been recognized as a strong biological poison because of its persistent nature, toxicity, tendency to accumulate in organisms and under food chain amplification (Olowu *et al.,* 2010; Al-Awady, 2011). Lead is an extremely common heavy metal that can be found almost anywhere. Common sources of lead include water, paint, electric storage batteries, insecticides, autobody shops, gasoline etc. Lead is therefore a public health hazard. Low levels of lead in children can cause the following side effects; nervous system and kidney damage, learning disabilities, attention deficit disorder, decreased intelligence, speech and language disorder, behavioural problems, poor muscle co-ordination, decreased muscle and bone growth, hearing damage, seizures, unconsciousness and death (Newman *et al.*, 2013). In adults, high levels of lead in their systems can cause the following;

increased chances of illness during pregnancy, harm to fetus, including brain damage or death, fertility problems in both men and women, high blood pressure, digestive problems, nervous

disorders, memory and concentration problems, muscle and joint pains (Rubin and Strayer,2008). Lead is an environmental toxin that interferes with a wide range of body processes and also induces pathological responses in many tissues and organs including the kidneys, liver, heart, reproductive, nervous and endocrine organs (Flora *et al.,* 2008). Most of the dysfunctions produced by the absorption of lead are due to the lead's ability to mimic and inhibit the actions of calcium (Anjali, 2000).In fish lead *in vivo* interferes with the normal cell function and physiological processes. Some of the effects of lead include peripheral and central nervous systems damage, blood disorders, reproductive toxicity and interference with Vitamin D and calcium metabolism. In the central nervous system, lead increases the permeability of the blood brain barrier (BBB) which results in brain oedema (Jeong-Hyeon *et al.*,2013). In addition, lead has an extremely high affinity for erythrocytes (Salman,2014) and is a known inhibitor of dehydrogenase of delta amino- levulinic acid (ALA-D) an enzyme participating in heme synthesis which may cause deformities of fish erythrocytes,membrane disruption and often induces anaemia in fish (Olanike *et al.*,2008; Horiguchi *et al.,* 2011).

Toxicological studies of chemicals on animals have been used to detect the potential hazards posed by chemicals to man. In several decades aquatic toxicology has moved from a descriptive approach which was necessary to explore those concentrations of single toxicants within the water that were not compatible with the life of individual fishes, to concentrations that do not cause death over the short term but do harm to the individual thus making it expend resources to survive in the midst of altered equilibrium (Guillio and Hinton, 2008).

The exposure and effects of chemicals can be studied by biomarkers; specific changes (response) in biological parameters reflecting specific toxicity mechanism such as over production of reactive oxygen species (ROS) and oxidative stress (Boelesterli, 2003). Sublethal concentrations of lead cause toxicity which results into oxidative damage in fish tissues. It produces oxidative stress through the generation of free radicals in which reactive oxygen species are important in causing damage to cells and tissues (Verma and Belsare,2005).Lead depletes major antioxidants in the cell especially thiol-containing antioxidants and enzymes, and can cause significant increases in reactive oxygen species (ROS) production resulting in oxidative stress, leading to various dysfunctions in lipids, proteins and de-oxy ribonucleic acid (DNA) (El-Badawi 2005).

Biomarkers illustrate the multiple organ, tissue and cellular sites of action and the spectrum of responses that are possible. Biomarkers and validated methods have been designed and are now in use to assess chemical exposures and responses from various forms of toxicity (Guillio and Hinton, 2008).

Haematological parameters have been used as tools in order to determine the specific and non-specific effects of environmental and physical stress. Fish blood indices have been increasingly examined as valuable parameters for the presence of toxicants.Changes in the haematological profiles is used as an important tool for the evaluation of pathological conditions of fish (Al-Rudainy *et al.,* 2015).

Recently attention has been devoted to assess the toxic effects of xenobiotics on endocrine system of stressed animals. In natural and in culture conditions, stress is a common phenomenon which elicits compensations or adaptive responses. These responses occur in many target organs especially those under multiple endocrine control. The hypothalamo-pituitary-interrenal (HPI) axis

of fish is activated during acute exposure to stressors (Hontela, 2005). Endocrine responses through their integration and early warning capacity may serve as potential indicators, which may be useful in the detection and assessment of sublethal toxic stress in fish exposed to polluted environments (Hontela *et al.*,2003) and these responses are centered on the activation of the HPI axis.

Histopathological changes in cells of tissues and organs are useful biomarkers in studying the effects of lead on biological tissues. Authman *et al.* (2015)observed a very high number of rodlet cells (RCs) in the epidermis of common carp and rainbow trout kept in lead polluted water. Hepatocyte vacuolization, hepatic cirrhosis, necrosis, shrinkage, parenchymal degeneration, nuclear pkynosis and increase of sinusoidal spaces were distinct changes observed in the liver of lead exposed *Clarias gariepinus* (Olojo *et al.*,2005). Acute lead toxicity is initially characterized by damaging gill epithelium and ultimately suffocation. Two types of structural alterations of gill,defence/compensatory responses and direct deleterious effects were observed in chronic lead exposed *Clarias gariepinus*. The necrosis and desquamation of gill epithelium as well as lamellar curling and aneurysms were direct deleterious effects reported in chronic lead exposed *Clarias gariepinus* (Olojo *et al.*, 2005). Low levels of lead pollution could cause some adverse effects on fish health and reproduction (Ercal *et al.*,2001).

The currently approved approach against lead toxicity is by using chelating agents which will bind with and withdraw lead from lead-burdened tissues. But some toxic effects of such agents necessitate research on alternative therapeutic approaches particularly the use of natural compounds (Najarnezhad *et al.*,2010). L-ascorbic acid (Vitamin C) as one of the most important antioxidants spares the other oxidants by forming the first line of defence against free radicals and peroxides which are generated during cellular metabolism. L-ascorbic acid reduces lead level in the body by

decreasing the rate of intestinal absorption (Gurer *et al.*,2001). L-ascorbic acid complexes with lead *in vitro* thus acting as a potential chelating agent and seem to alleviate lead-induced vitamin deficiency and metabolic disturbances.The effect of toxic metal can be protected by L-ascorbic acid. L-ascorbic acid is a powerful reducing agent and has been shown with numerous *in vitro* studies to scavenge a number of ROS (Chatterjee *et al.*,1995). L-ascorbic acid not only confers protection against lead toxicity but can also perform therapeutic role (Bhattacharjee *et al.*, 2003)

1.2 Statement of Problem

Clarias gariepinus, the African mudfish is commonly found in the Nigerian inland waters which can be contaminated with heavy metals. Contamination of heavy metals can lead to fish mortality, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans eating these fishes. Due to its physical and chemical properties, lead is being utilized in many industries, as a result, through industrial effluents, sewages, electricity storage batteries, leaded paints and gasoline etc, lead has found its way into fresh waters . Having an accumulative behaviour, exposure to prolonged low levels of lead can induce high accumulation in tissues without causing mortality of fish.The Fish may be alive but the organs and tissues are diseased. To date a vast report on the remediation effects of L-ascorbic acid (Vitamin C) on heavy metal contaminated African mudfish exists. There is therefore a need to undertake studies that can help to interpret the role of L-ascorbic acid on remediation of the damaging effects of lead nitrate to the tissues of the African mudfish.

1.3 Justification for this Study

The toxicity of any chemical alters the physiological state of the animals, thereby impairing various metabolic activities. Therefore, to have a clear understanding of how these chemicals cause injury to the tissues, it is essential to:

a. Understand the therapeutic role of L-ascorbic acid on lead nitrate affected tissues.

b. Investigate the ability of L-ascorbic acid to reverse the damaging effects of lead nitrate on fish tissues, hence the need for this study.

c. Investigate the ability of L-ascorbic acid to reverse the damaging effects of lead nitrate on the blood of fish.

d.Investigate the ability of L-ascorbic to reverse the damaging effects of lead nitrate on the serum enzymes and urea of fish.

e. Study the histopathological changes that might take place in tissues in response to these chemicals.

f.*Clarias gariepinus*will be used for this study because of its potential tolerance to high concentrations of heavy metals and high consumption demand.

The target organs for this study were chosen for the following reasons;

i. Bloodis the medium for the circulation of nutrients, gases, and other metabolites.

ii. Gills are the organs for respiration and osmoregulation.

iii.Liver is the organ for bile and protein synthesis as well as detoxification of metabolites.

iv.Stomach is a digestive organ,producing gastric acid.

1.4 Aim:

The aim of this study is to investigate the therapeutic effects of L-ascorbic acid (vitamin C) on lead nitrate induced pathology in some organs of the African catfish *Clariasgariepinus.*

1.5 Specific objectives were to determine the:

i.acute toxicity (LC_{50}) of lead nitrate in Clarias gariepinus juveniles.

ii.acute toxicity (LC_{50}) of Kepro Vitamin C in Clarias gariepinus juveniles.

iii.chronic effects of sublethal concentrations of lead on the blood and serum enzymes of *Clarias gariepinus*.

iv.therapeutic effects of L-ascorbic acid (Vitamin C) on the lead induced pathology of the blood and serum enzymes of *Clarias gariepinus.*

v. chronic effects of sublethal concentrations of lead on the histology of the gills, liver and stomach of *Clarias gariepinus*.

vi therapeutic effects of L-ascorbic acid on the lead induced pathology of the gills, liver, and stomach of *Clarias gariepinus.*

vii.level of lead accumulation in the muscle of *Clarias gariepinus* exposed to chronic lead toxicity.

viii.effect of L–ascorbic acid on the accumulated lead in the muscles, of *Clarias gariepinus* exposed to chronic lead toxicity.

ix.effect of L-ascorbic acid on the lead induced pathology in the blood, serum enzymes,gills, liver and stomach of *Clarias gariepinus* during concurrent exposure.

Formatted: Font: 11 pt, Font color: Auto

Formatted: Font color: Auto

CHAPTER TWO

LITERATURE REVIEW

2.1 Acute Toxicity Test

Toxicity bioassays are the tools that allow the determination under specific and controlled conditions, the physical and or the chemical effects of a substance on a test organism. A classic study to characterize the potential toxicity of such substance is the acute lethal toxicity test in which the lethal concentration (LC_{50}) value is determined. This is the concentration of a substance that causes the death of 50% population of the test organisms in stipulated time in hours which could be 24, 48 or 96 hours (USEPA, 2002).

According to Adedeji *et al.*(2008), acute toxicity is usually caused by exposure of an organism to a large dose of a toxic compound for a short period of time with a rapid effect being produced usually causing mortality. This test may equally be used to determine the median lethal concentration of a compound over a given period of time.

2.2Heavy Metals and the Aquatic Ecosystem

Metals, especially heavy metals, are important contaminants of aquatic environments worldwide. Metal pollution has increased with the technological progress of human society. Industry, mining, advanced agriculture, household waste, and motor traffic are all among the activities considered to be major sources of metal pollution. Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments (Luoma and Rainbow, 2008).

Fish are important component of human nutrition, and those from contaminated sites present potential risk to human health. Since fish occupy the top of the aquatic food chain, they are suitable

Formatted: Font:

Formatted: Font color: Auto

Formatted: Font:

Formatted: Font: Font color: Auto

Formatted: Font: Font color: Auto

bio-indicators of metal contamination. Metals are well-known inducers of oxidative stress, and assessment of oxidative damage and antioxidant defenses in fish can reflect metal contamination of the aquatic environment (Livingstone, 2003).Speciation of metals, their solubility and complexity, are important factors that influence the toxicity of metals in the aquatic environment. The amount of dissolved metal strongly depends on water pH. The interaction of metals can alter their toxic effects on aquatic organisms both positively and negatively (Jezierska and Witeska, 2001). Different modes of exposure to metals also play a role in metal toxicity. Fish take up metals through the gills, digestive tract and body surface (Tao *et al.*,2001; Kamunde *et al.*, 2002).

2.3 Mechanism of Metal Bio-Accumulation in Fishes

Formatted: Font: Font color: Auto

Formatted: Font: Font color: Auto

Fishes are continuously exposed to waterborne and particulate heavy metals due to continuous flow of water through gills and through food sources. Metals that bio-accumulate in different tissues follow different patterns of bio-accumulation factors (Fatima and Usmani, 2013). The mechanism of bio-accumulation of heavy metal in fish includes different processes in dynamic manner. Both physiological/biochemical responses and metal geochemistry are responsible for the differences in metal concentrations observed in different populations of aquatic species. It was confirmed that the internalization of metals into the cells of gills and internal epithelia follows similar mechanisms from different bioaccumulation studies (Noegrohati, 2006).

Formatted: Font color: Auto

Since decades, study of metal bioaccumulation has led to the formulation of many models. The Free Ion Activity Model (FIAM), proposed in the 1980s, gives an insight in the study of metal uptake in different species of aquatic organisms (Campbell, 1995). Few years later, the Biotic Ligand Model (BLM), was introduced which is based on the interaction of the free metal ion with the proposed biological site of action, fish gill being the initial site of action (Paquin *et al.*,2002). After

these two, the Sub-cellular Partitioning Model (SPM) came. This directly addresses toxicity within organisms in terms of subcellular components of accumulated metal, a variation of a tissue residue approach (Wang and Rainbow, 2007). A simple Biokinetic Model including all the processes that result to metal bio-accumulation was quantitatively given by Wang and Rainbow (2008). Thus, metal bio-accumulation in an organism is controlled by the balance between uptake and elimination.

A number of factors such as sex, age, season, spawning period, variability of food habitats and pollutant exposure and phylogenetic differences in regulatory mechanisms, may influence the uptake, retention and bio-accumulation of trace contaminants in fish tissues (Nesto *et al.*,2007). Zhao *et al.*(2012) showed correlation of heavy metals in the tissue of fish to their living environments both qualitatively and quantitatively and there was diverse metal bio-accumulation characteristics which was significantly affected by environmental factors and living habits. The bio-accumulation model showed that the uptake efficiency factor of essential heavy metals such as copper (Cu) and zinc(Zn) decreases as exposure concentration increases, due to homeostasis regulation, while for non-essential heavy metals like mercury(Hg),it increases as the exposure concentration increases and excretion was observed as manifestation of homeostasis regulation (Noegrohati, 2006).

2.4 Mechanism of Histopathological Damage

Histopathological damage in tissues is an outcome of various biochemical and physiological interactions within the cell owing to exposure to various aquatic toxicants. Heavy metals generate reactive oxygen species (ROS) which damage proteins, lipids and DNA content of exposed animal cells, which on gross level can be visualized through histopathology. Heavy metals grouped as Redox-active (Fe, Cu, Cr, etc) undergo redox cycling whereas redox-inactive metals (such as Pb, Cd and Hg) undergo covalent electron sharing with cells major antioxidant enzymes (Thiols). Both types

lead to the production of ROS as hydroxyl radical (OH), Superoxide radical (O^{2-}) or hydrogen peroxide (H_2O_2) which deplete cells intrinsic antioxidant defense. ROS lead to lesions to lipids, proteins and DNA which can be visualized through cross index i.e., histopathology of tissues (Ercal *et al.,* 2001). Histopathology is a broader term and mirror of effects of exposure to a variety of anthropogenic pollutants (Hinton *et al.,*1992). Histopathology thus is a long term and reliable biomarker of toxicant exposure. These metals undergo metabolic activation that induces a cellular change in affected fish. The tissue lesions and apoptosis which arise from bio-accumulation stimulate necrotic alterations in the fish cells with an inflammatory defensive reaction (Roganovic-Zafirova *et al.,* 2003). Below are few mechanistic insights of metal toxicity leading to microscopically visible alterations;

a.Heavy metal ions can enter blood vessels, some of them are carried by proteins like albumin and can be taken up by endothelial cells lining the vessels (Wagner *et al.,*1998).

b.Heavy metal ions induce mechanisms of gene activation in endothelial cells like pro-inflammatory mediators, indicating that corroding metal ion containing biomaterials can provoke inflammatory reactions by known and unknown, intracellular signalling pathways (Wagner *et al.,* 1998). Thus blood profile changes with respect to heavy metal exposure has become sensitive bioindicators of heavy metal pollution as also shown by some authors (Baltova and Velcheva, 2005; Kori-Siakpere and Ubogu, 2008; Maheswaran *et al.,* 2008).

Teleost liver is a major organ for heavy metal metabolism thus frequently studied by many authors (Canli *et al.,*1998; Javed, 2005; Vinodhini and Narayanan, 2008) to observe different deformities. Fish hepatocytes have relatively more glycogen/lipid content which lead to hepatocytes being more vacuolated (Weber and Gingerich, 1982). Macrophage aggregates act as repositories for

products of cell membrane and erythrocyte breakdown which include lipofuscin, ceroid, hemosiderin and melanin (Wolke, 1992). Reason behind hepatocellular enlargement is organelle proliferation (hypertrophy), failed mitotic division of hepatocytes (megalocytosis) and vacuolar swelling of endoplasmic reticulum cisternae (hydropic degeneration) (Hinton *et al.*,1992). Toxic chemicals lead to increased number of organelles as myelinated bodies, mitochondria, glycogenosomes, peroxisomes and lysosomes and changes in rough endoplasmic reticulum. Due to toxicology of chemicals, hepatocytes hypertrophy is accompanied by basophilia as a result of loss in glycogenic vacuolization and increased mRNA content (Wester *et al.*,2003).

Kidney is another target organ for metabolism and removal of waste from blood and studied for metal bioaccumulation (Ambedkar and Muniyan, 2011; Fatima and Usmani, 2013), following heavy metal exposure. Kidneys tend to follow specific metabolic processes. Macrophages are the key defensive cells dealing with foreign materials and debris (Blazer *et al.*,1994). The macrophage comprises lipofuscin, melanin and haemosiderin pigment in heavy metal intoxicated kidney tissues i.e., contaminants influence macrophage pigment composition (Kruger *et al.*,1996).

Gills are another organ of concern for heavy metal toxicology as it shows significant high bioaccumulation factor owing to the fact that gills have larger surface area and come in direct contact with heavy metal laden water. Different scholars have given insight into the various deformities such as epithelial lifting, interstitial oedema, leucocytic infiltration, hyperplasia of the epithelial cells, lamellar fusion, vasodilation and necrosis that arise due to heavy metals (Martinez *et al.*,2004; Al-Attar, 2007; Taweel *et al.*,2011; Fatima and Usmani, 2013).

Muscle and integument are the least affected tissues in terms of bio-accumulation of heavy metals,(Javed, 2005; Al-Weher, 2008; Nicula *et al.*,2009; Rauf *et al.*,2009; Javed and Usmani, 2011)

as these are major edible parts and relished protein diet. Other scholars (Ajmal *et al.*,1985; Obasohan, 2008; Pourmoghaddas and Shahryari, 2010; Sen *et al.*,2011) estimated heavy metal in fish taken as whole. The muscle and integument are of prime concern as the fish may not only be consumed by local population but may be transported to other regions for economy.

2.5 Mechanisms of Metal-Induced Oxidative Damage.

The involvement of metals in oxidative damage is multi-faceted. In general, metals produce free radicals in two ways: Redox active metals such as iron, copper, chromium, and vanadium generate ROS through redox cycling. Metals without redox potential, such as mercury, nickel, lead and cadmium impair antioxidant defenses, especially those involving thiol-containing antioxidants and enzymes. A third important mechanism of free radical production is the Fenton reaction, by which ferrous iron (II) is oxidized by hydrogen peroxide to ferric iron (III), a hydroxyl radical, and a hydroxyl anion (Valko *et al.*,2005). The superoxide radical can reduce iron to its ferrous form. Copper, chromium, vanadium, titanium, cobalt, and their complexes can also be involved in the Fenton reaction (Lushchak, 2011).Activation of redox-sensitive transcription factors such as activator protein 1(AP-1), p53, and NF-κB is another mechanism by which metals can participate in producing oxidative stress. These transcription factors control the expression of protective genes which repair DNA and influence apoptosis, cell differentiation and cell growth (Valko *et al.,* 2005).

2.6 Lead and Oxidative Stress.

Formatted: Font: Font color: Auto

Formatted: Font: Font color: Auto

Lead is an element and a metal (atomic number 82). It is soft, has a low melting point (327.5 °C), a high density (11.34 g/cm^3) and is found naturally in a variety of minerals including galena, cerussite and anglesite. Lead has been confirmed as a principal lethal metal pollutant to both humans and other living organisms. Occupational and environmental exposures to this toxic metal remain a global health problem (Abdel Moneim, 2016). This ubiquitous pollutant has been detected in biological systems predominantly in environmental and industrial areas. It persists in nature as oxides or salts and lingers in soil, water, dust and in manufactured products (Shatha *et al.*, 2016). Since ancient times, anthropogenic use of lead has been on the increase with each passing century with that of the 21st century surpassing the sum of preceding eras (El-Nekeet *et al.*, 2009). Plants are usually exposed to lead through water uptake, while man and animals through their consumption of such plants (Hedayati and Darabitabar, 2017). Environmental lead enters the body via inhalation of airborne contaminated dust or ingestion of contaminated food and water into the digestive tract. Once absorbed, lead diffuses swiftly via the bloodstream to diverse systems and organs including the liver, kidneys, brain and to well calcified tissues including bones and teeth (Alya *et al.*,2015). Complete regulation and aversion of exposure to lead is yet to be accomplished. So far, no such level of lead has been ascribed beneficial to the body likewise, no "safe" exposure level has been specified (Flora *et al.*, 2012). Nevertheless, lead levels of 10 µg/dl (equivalent to 0.48 µmol/l) in the blood or higher has been confirmed to be toxic and induces cognitive impairments, hypertension, neurological

disorders and other lead-induced perturbations (Patrick, 2006a). Lead intoxication is an insidious risk, capable of triggering irreversible health consequences. It interferes with physiological processes and alters systemic functions; resulting in grave disorders (Kalia and Flora, 2005). Blood lead levels of about 40 - 60 µg/dl following occupational exposures result in acute and more commonly chronic toxicity. If untreated it becomes severe; characterized by persistent nausea, encephalopathy, fatigue, frenzy, spasms and coma. Lead-induced oxidative damage has been proposed as one of the important mechanisms of lead-related pathologies (Patrick, 2006b).Lead is a metal without redox potential, therefore it causes oxidative stress in fishes by impairing antioxidant defenses especially those involving thiol-containing antioxidants and enzymes (Stohs and Bagchi 1995). Lead promotes formation of reactive oxygen species in fish. Elevated levels of ROS lead to oxidative damage in form of lipid peroxidation, protein and DNA oxidation and enzyme inactivation Sevcikova *et al.*, 2011). In addition, chelating agents exert detrimental effects and are incapable of alleviating some toxic effects of lead (Ajayi *et al.*, 2009).

In recent times, several studies have been focused on sourcing for alternatives and potentially safe treatments for lead toxicity. Consequently, to mitigate adverse effects of lead toxicity, natural compounds with both chelating and antioxidant activities are considered as good candidates (Bokara *et al.*, 2008).

Formatted: Font color: Auto

2.6.1 Chemical forms and biological properties of lead

Lead (Pb) is a bluish gray heavy metal that occurs naturally in various mineral forms in the earth's crust. Metallic lead is resistant to corrosion, because, when it is exposed to air or water, thin films of lead compounds (oxides, carbonates, chlorides,nitrates, acetates,phosphates etc.) are formed and protect this metal from further attacks (Encyclopaedia Britannica). It has been widely used for

centuries because it is readily shaped, molded and resistant to corrosion. Lead can exist in three forms: metallic, inorganic, and organic. Lead in the environment rarely occurs in its elemental state, but rather in its oxidation state (Pb^{2+}) in various ores throughout the earth such that lead ores are widely distributed around the world (Smith 2001). The phasing out of leaded gasoline for transportation vehicles between 1973 and 1995 and the removal of lead from paint by 1978 have resulted in substantial lowering of mean blood lead levels. However, because lead is a persistent metal, it is still present in the environment, water, soil, and dust (Patrick 2006).

In this regard, the work by Patterson (1956), who determined the age of the earth by a uranium-lead isotopic method, needs to be mentioned. Using a Canyon Diablo meteorite, Patterson was able to make an accurate measurement, calculating that the earth was 4.55 billion years old. But in his study he discovered a disturbing and constant presence of lead in the atmosphere mainly due to tetraethyl lead used as an anti-knock gasoline. The presence of lead in the blood of human beings has considerably diminished; but in any case, the human beings today have about 625 times more lead in his body than people did 100 - 120 years ago.In the majority of adults, chronic lead poisoning comes from exposures to work places and can occur in numerous work settings, such as manufacturing, lead smelting and refinement, or it may be caused by use of batteries, pigments, solder, ammunitions, paint, car radiators, cable and wires, and certain cosmetics (Brodkin *et al.*, 2007). Diagnosis of lead toxicity was traditionally based on significantly elevated blood lead levels. These are an indicator of circulating lead that discloses variation in recent external lead exposure as well as of lead that has been mobilized by tissue stores (mostly bones). Lead levels in tibia and patella provide an indication of cumulative dose over decades (particularly cortical tissue in tibia) as well as the largest pool of lead in the body that is available for mobilization into blood. The latter phenomenon is heightened at

times of high bone resorption (eg during pregnancy, aging, post menopause) (Hu *et al.,* 2007) Inorganic lead is absorbed from the respiratory or gastrointestinal tract but not through the skin. Approximately 90% of the total body burden of lead is stored in bone and the remainder is in blood stream and soft tissue (Philip and Gerson, 1994). Gastrointestinal absorption varies depending on nutritional status and age. Iron is believed to impair lead uptake in the gut, while iron deficiency is associated with increased blood lead concentrations in children (Bradman *et al.,* 2001). Lead exposure in pregnant animals usually occurs through the oral route. It is known that absorption of this metal increases during pregnancy. Lead crosses the placenta and it accumulates in the fetus. Accumulation of lead occurs in the fetal brain owing to lack of blood-brain barrier (BBB). Lead also accumulates in the placenta in times of fetal stress (Gupta, 2012). Once absorbed, the circulating lead is bound to erythrocytes for approximately 30-35 %, while only 1 % of absorbed lead is found in plasma and serum and it is dispersed into the soft tissues of liver, renal cortex, aorta, brain, lungs, spleen, where it accumulates as lead phosphate ($Pb_3(PO_4)_2$ in the following 4-6 weeks (Begovic *et al.,* 2008). Lead is primarily excreted via the kidneys, while a small amount is excreted in faeces and with sweat (Sinicropi *et al.,* 2010). The most common symptom of acute inorganic lead poisoning is gastrointestinal colic. Chronic exposure to Pb^{2+} produce damage to hematopoietic, nervous, gastrointestinal and renal systems.

The effects of lead exposure are a health concern for all humans, but especially during early childhood because children are most at risk. Exposure to excessive amounts of inorganic lead during the toddler years may produce lasting adverse effects on brain function. Maximal ingestion of lead occurs at an age when major changes are occurring in the brain synaptic connections (Goldstein 1990). Organolead compounds, as tetramethyllead and tetraethyllead are readily absorbed by

inhalation and through the skin as well as by gastrointestinal tract. Tetraethyllead is metabolized to triethyllead, and this demethylated compound is excreted with the urine (Pim de Voogt 2016). Tetraethyllead and its metabolites are toxic especially for the brain. Toxicity appears with headache, restlessness, nervousness and anxiety (Beattie *et al.,* 1972): Severe symptoms including convulsion, delirium, coma, abdominal pain and peripheral neuropathy. The neurotoxic effects of organolead compounds are associated with urinary lead concentrations higher than 30 mg/l (Macintyre, 1994). In 2004, the International Agency for Research on Cancer (IARC) classified metallic lead and inorganic lead as probable human carcinogens (IARC group 2A), while organic lead remained unclassifiable Pim de Voogt (2016).

2.6.2 Ionic mechanism of lead toxicity.

Ionic mechanism of action for lead mainly arises due to its ability to substitute other bivalent cations like Ca^{2+}, Mg^{2+}, Fe^{2+} and monovalent cations like Na^{+} (though bivalent cations are more readily substituted), affecting various fundamental biological processes of the body (Lidsky & Schneider, 2003). Significant effects have been found on various fundamental cellular processes like intra and intercellular signaling, cell adhesion, protein folding and maturation, apoptosis, ionic transportation, enzyme regulation, release of neurotransmitters, etc (Garza *et al.,* 2006). The ionic mechanism contributes principally to neurological deficits, as lead, after replacing calcium ions, becomes competent to cross the blood-brain barrier (BBB) at an appreciable rate. After crossing the BBB, lead accumulates in astroglial cells (containing lead binding proteins). Toxic effects of lead are more pronounced in the developing nervous system comprising immature astroglial cells that lack

lead binding proteins. Lead easily damages the immature astroglial cells and obstructs the formation of myelin sheath, both factors involved in the development of BBB (Mohammed *et al.*, 2016).

Lead, even in picomolar concentrations, can replace calcium, thereby affecting key neurotransmitters like protein kinase C, which regulates long term neural excitation and memory storage. It also affects the sodium ion concentration, which is responsible for numerous vital biological activities like generation of action potentials in the excitatory tissues for the purpose of cell to cell communication, uptake of neurotransmitters (choline, dopamine and gamma amino butyric acid (GABA) and regulation of uptake and retention of calcium by synaptosomes. This interaction between lead and sodium seriously impairs the normal functioning of the aforementioned sodium dependent processes (Bressler *et al.*, 1999).

2.6.3 Toxicity of lead on cell membranes

Lead is known to have some toxic effects on membrane structure and functions (Donaldson and Knowles, 1993). The effects on red blood cell (RBC) membranes in particular, are intensely analyzed because RBCs have a high affinity for lead, contain a majority of the lead found in the blood stream, and are more vulnerable to oxidative damage than many other cells (Leggett, 1993). Osmotic and mechanic susceptibilities of RBC were reported to increase during lead toxicity (Waldron, 2006) accompanied by decreased deformity and a shortened life span. The biochemical basis for those toxic effects still needs to be answered. However, activities of some membrane-bound enzymes (Raghavan, 1981) and composition of membrane proteins in RBC were also found to be altered by lead exposure (Fukumoto *et al.*, 1983). It is not clear whether oxidative stress is the cause or the consequence of these reported toxic effects of lead, but lead exposure may probably further

increase the susceptibility of membranes by altering their integrity via deteriorating their components. Besides directly inducing the generation of ROS, a molecule can indirectly induce oxidative stress by increasing the vulnerability of membranes to the attack of ROS (Gurer-Orhan & Ercal 2000).The major constituents of biological membranes are lipids and proteins. The lipid molecules found in membranes contain hydrophobic, fatty acid side-chains. The "first chain initiation" is the initial step of a peroxidation sequence in a membrane or polyunsaturated fatty acids. This refers to the attack of any species with sufficient reactivity to abstract a hydrogen atom from a methylene group of the fatty acids. The presence of a double bond in the fatty acid weakens the carbon-hydrogen (C-H) bonds on the carbon atom adjacent to the double bond and therefore makes H removal easier. Therefore, fatty acids with zero, one, or two double bonds are more resistant to oxidative attack than are the polyunsaturated fatty acids that have more than two double bonds (Halliwell and Gutteridge, 2009). Several studies have focused on the possible toxic effects of lead on membrane components and identified a correlation between these effects and lead-induced oxidative damage. Yiin and Lin (1998) demonstrated a marked enhancement in malondialdehyde (MDA) concentrations following incubation of linoic, linolenic, and arachidonic acid with lead. The concentrations of generated MDA were increased with regard to the number of double bonds of fatty acids (Yiin and Lin, 1998), suggesting possible association of a peroxidation process. Several studies pointed to increased arachidonic acid and the arachidonate/linoleate ratio in liver, serum, and RBC membranes of lead-exposed chicks (Lawton and Donaldson, 1991; Halliwell and Gutteridge, 2009). A mechanism for those changes in the fatty acid composition of membranes was suggested by Lawton and Donaldson (1991). They observed a decrease in the *in vitro* capacity of the microsomal enzyme system of fatty acid elongation that lengthens linoleic acid (18:2) to the 20-carbon precursor

of arachidonic acid (20:4). How such an effect can paradoxically lead to increases in tissue arachidonic acid was suggested to be partially explained by the relative rates of the fatty acid elongation and desaturation steps.Since fatty acid chain length and unsaturation are important determinants of membrane susceptibility to peroxidation, as mentioned above, the authors suggested that lead-induced arachidonic acid augmentation might be responsible for the enhanced lipid peroxidation in those membranes (Lawton and Donaldson, 1991).

On the other hand, lead is shown to bind strongly to phosphotidylcholine membranes *in vitro* (Hoogeveen, 2000). Shafig-ur-Rehman and Abdullah (2003) reported an alteration of the composition of RBC membrane phospholipids, indicating a decrease in the levels of phosphotidylcholine. Furthermore, in a detailed study, lead, phospholipid, and lipid peroxidation levels were determined in various regions of the brains of lead-exposed rats. The percentages of increase in the rate of lipid peroxidation and decrease in the phospholipid level were shown to follow a pattern similar to that of lead concentrations in the brain areas. Taken together, these data suggest that altered lipid composition of membranes may result in altered membrane integrity, permeability and function. These would increase the susceptibility to lipid peroxidation (Gurer & Ercal 2000).

2.6.4 Lead and the environment

Lead occurs naturally in the environment on account from human activities and it continues to be a significant public health problem in developing countries (Tong and McMichael, 1999; Grant and Davis 1989), where there are considerable variations in the sources and pathways of exposure (USEPA, 1986).

Exposure attributable to miscellaneous sources may be even more significant than universal exposure associated with leaded petrol, especially for people living in poverty (IPCS, 1995).

Exposure to lead from lead mining (Ajumobi *et al.,* 2014), smelting of leads ores, as well as other ores (zinc, copper, iron, gold (Dooyema *et al.,* 2012) and silver) in which lead is by-product or contaminant, battery factories and cottage industries is a significant environmental hazard in developing countries. Electrical utilities released into the atmosphere lead in flue gas from the burning fuels, such as coal, in which this element is a contaminant. As a result of human activity, environmental levels of lead increased more than a hundredfold over the past three centuries. The greatest increase occurred in the past century between the years 1950 - 2000 and reflected increasing worldwide use of tetraethyl lead and tetramethyl lead as gasoline additives to increase octane rating (Pim de Voogt 2016). Since gasoline additives have been banned, the level of lead in the atmosphere has dropped dramatically. Tetraethyl and tetramethyl lead, once added to gasoline, are no longer present in significant quantities in air. In fact, when exposed to sunlight, they decompose rapidly to trialkyl and dialkyl lead compounds and to lead oxides by direct photolysis, and reacting with hydroxyl radicals and ozone. But it is necessary to emphasize that in the winter tetraethyl and tetramethyl lead have half-lives of up to several days since the atmospheric hydroxyl radical's concentration is lower than in summer (DeJonghe and Adams, 2006).

In parallel with enforcement of the reduction of lead gasoline in Italy, Annibald*et al.* (2009) have studied the lead content of Adriatic seawater. In the years from 2000 to 2004, seawater was collected systematically at three sites along the coast line close to the city of Ancona. The results showed that the lead content of the seawater diminished from a median value of 0.25 nmol/L in 2000-2001 to 0.12 nmol/L in 2003-2004. This decrease has been correlated to the concurrent decrease of lead in gasoline in Italy with a reduction of lead emission to the atmosphere.

Lead is also released into the air during burning of coal and oil. In fact, in the last 15-20 years the total lead emission from electric steam increased due to the increased demand for electric power and an increased use of coal and natural gas as fuel sources to generate electricity. Burning these sources of energy recklessly, have resulted in increased levels of CO_2 and thus, global warming due to greenhouse effect (Pim de Voogt 2016).

Once small lead particles get into the atmosphere, they can travel long distances about 10 km from emission sources (Berndtsson, 1993), before they fall by rain to land or into surface of rivers, lakes and sea. Sources of lead in dust and in soil include not only lead that falls to the ground from the air, but also weathering and chipping of lead-based paint from buildings and bridges. Higher levels of lead in soil are found near roadways (Nielsen, 1984). Once lead falls onto soil, it sticks strongly to soil particles for many years in the upper layer of soil. Small amounts of lead may enter in rivers, lakes and sea when the soil particles are moved by rain water, or when lead is released by acid rain (Pim de Voogt 2016).

The fate of lead in soil is affected by the absorption at mineral interfaces and the formation of relatively stable organic-metal complexes or chelates with soil organic matter. This process is dependent on factors such as soil pH, soil type, organic matter content of soil, and cation exchange capacity (Reddy *et al.,* 1995). Most lead is strongly retained in soil and very little is transported through runoff to surface of water. Clays, silts, iron and manganese oxides, and soil organic matter may bind lead electrostatically as cation exchange resin, as well as chemically for specific adsorption (Reed *et al.,* 1995).

The amount of soluble lead in surface water depends upon the pH of the water and the concentration of dissolved salts. Equilibrium calculations show that at pH 5.4 the solubility of lead is

about 30µg/l in hard water and approximately 500 µg/l in soft water. Sulfate ions, if present in soft water,decrease the lead concentration through the formation of insoluble lead sulfate. The lead carbonate limits the amount of soluble lead considering also the partial pressure of CO_2, pH and temperature (USEPA, 1986).

Plants and animals may bio-concentrate lead and the high lead concentrations are found in aquatic and terrestrial organisms. This occurs when these living beings have habitats near lead mining and smelting, areas affected by high automobile and truck traffic, sewage sludge and spoil disposable areas, sites where dredging have occurred, and in urban and industrialized areas (McGrath *et al.,* 1994). Lead may be present on plant surfaces on account of atmospheric deposition; but its presence in internal plant tissues indicates biological uptake from the soil and leaf surface. Lead may be taken up in edible vegetables and fruits from the soil via the root system, by direct foliar uptake and translocation within the plant. As already mentioned, the amount of lead in soil that is bio-available to a vegetable plant,depends on factors such as cation exchange capacity, pH of soil, amount of organic matter present and type of fertilizer added to the soil (Holmgren *et al.,* 1993).

Uptake of lead in animals may occur on account of inhalation of contaminated ambient air or ingestion of contaminated foods. However, lead is not biomagnified in aquatic or terrestrial food chains, as for other metals, for example mercury. In aquatic organisms, lead levels are usually highest in benthic organisms and algae, and lowest in upper trophic level predators such as carnivorous fishes (Tulasi *et al.,* 1992).

2.6.4.1 Birds

The well-documented environmental effect of lead contamination is the effect of lead on waterfowls. Lead shot taken by birds into their gizzards is a source of severe lead contamination.

Also lead sinkers used for angling have been demonstrated to be taken by birds. In the gizzard, the lead is slowly ground down resulting in the release of lead. It results in high organ levels of lead in blood, kidney, liver, and bone. Metallic lead is highly toxic to birds when ingested as lead shots; ingestion of a single pellet of lead shot is fatal in some bird species. The sensitivity varies between species and is dependent on diet. Since birds have been found in the wild with large numbers of lead shot in the gizzard, this poses a major hazard to those species feeding on river margins and in fields where many shots have accumulated (Tulasi *et al.,* 1992).

2.6.4.2 Mammals

There are many reports of lead levels in wild mammals, but few report of toxic effects of the metal in the wild or in non-laboratory species (WHO, 1989). In all species of experimental animalsstudied, lead has been shown to cause adverse effects to several organs and systems, including the blood system, central nervous system, the kidneys, and the reproductive and immune systems (Mohammed *et al.*, 2016).

2.6.4.3 Microorganisms

Studies have shown that lead can hamper mineralization of nitrogen in soil in acidified areas (Alloway, 2013). However, lead compounds are in general not very toxic to microorganisms and lead compounds contrary to mercury and chromium compounds have not been used as biocides (European Commission 2002). In general, inorganic lead compounds are of lower toxicity to microorganisms than are tri alkyl and tetra alkyl lead compounds. There is evidence that lead tolerant microbial strains exist and that tolerance may develop in other microbes (Rodriguez-Sanchez *et al*., 2017).

2.6.4.4 Aquatic organisms

Lead is toxic to all aquatic biotic components, and organisms higher up in the food chain may experience lead poisoning by ingestion of food contaminated with lead. Depuration is relatively rapid; in the case of rainbow trout exposed to tetra methyl lead the half-life values of depuration are about 35-45 h (Eisler, 1988).

The toxicity of inorganic lead salts is strongly dependent on environmental conditions such as water hardness, pH, and salinity, a fact which has not been adequately considered in most toxicity studies. Lead is unlikely to affect aquatic plants at levels that might be found in the general environment.In communities of aquatic invertebrates, some populations are more sensitive than others and community structure may be adversely affected by lead contamination (WHO 1989).Early developmental stages are more vulnerable than adult stages. However, populations of invertebrates from polluted areas can show more tolerance to lead than those from non-polluted areas. Typical symptoms of lead toxicity include spinal deformity and blackening of the tail region.The typical lead levels in aquatic environments are low (seawater: below 1µg/l; freshwater: below 5µg/L (OECD, 1993) compared to the lead levels causing effects. However, it cannot be ruled out that lead may affect fish and other organisms in areas where the lead burden is highest. The toxicity of lead to aquatic organisms and their various bio-concentration factors indicated that all aquatic organisms uptake lead, with bio-concentration factors ranging from 0.4 -540,000 µg/l (mg/kg) in fish as shown in table 1.

Table 1: **Toxicity of lead in Aquatic Environments and Bio-concentration Factors**

Species, effect	**concentrations**
Toxicity in freshwater	
Plankton algae (EC_{50}- growth rate, etc.)	**140-11,000**
Crustaceans (chronic)	**10- 200**
Crustaceans (acute)	**100-224,000**
Fish (chronic)	**0.4- 220**
Fish (acute)	**1,000- 540,000**
Toxicity in the marine environment	
Plankton algae (EC_{50}- growth rate, etc.)	**20 – 950**
Crustaceans (chronic)	**20 - 40**
Crustaceans (acute)	**50 - 27,000**
Fish (acute)	**300 - > 10,000**
Bioaccumulation	
Algae (bio- concentrations factor)	**2,000 - 4,000**
Invertebrates (bio- concentrations)	**400 – 12,400**

(source: Bronnum and Hansen, 1998)

2.6.5 Effects of lead toxicity on organs of living organisms

2.6.5.1 Effect on the nervous system

The nervous system appears to be the most sensitive and chief target for lead induced toxicity when compared to other systems.Both the central nervous system(C.N.S) and the peripheral nervous system(P.N.S.) become affected on lead exposure. The effects on the peripheral nervous system are more pronounced in adults while the central nervous system is more prominently affected in children (Bellinger, 2004). Encephalopathy (a progressive degeneration of certain parts of the brain) is a direct consequence of lead exposure and the major symptoms include dullness, irritability, poor attention span, headache, muscular tremor, loss of memory and hallucinations (Flora *et al.*,2012). More severe manifestations occur at very high exposures and include delirium, lack of coordination, convulsions, paralysis, coma and ataxia (Flora *et al.*, 2006). Fetuses and young children are especially vulnerable to the neurological effects of lead as the developing nervous system absorbs a higher fraction of lead.

The proportion of systemically circulating lead gaining access to the brain of children is significantly higher when compared to adults. Children may appear inattentive, hyperactive and irritable even at low lead exposure. Children with greater lead levels may be affected with delayed growth, decreased intelligence, short-term memory and hearing loss. At higher levels, lead can cause

Formatted: Font: Not Italic, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: Not Italic, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: Not Italic, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: Not Italic, Font color: Auto

Formatted: Font color: Auto

Formatted: Font color: Auto

Formatted: Font: Not Italic, Font color: Auto

permanent brain damage and even death (Cleveland *et al.,* 2008). There is evidence suggesting that low level lead exposure significantly affects intelligent quotients (IQs) along with behavior, concentration ability and attentiveness of the child. Repercussions of lead exposure on the peripheral nervous system have also been observed in the form of peripheral neuropathy, involving reduced motor activity due to loss of myelin sheath which insulates the nerves, thus seriously impairing the transduction of nerve impulses, causing muscular weakness, especially of the exterior muscles, fatigue and lack of muscular coordination (Sanders *et al.,* 2009).

Lead decreases heme synthesis and erythrocyte concentrations (Bolognani Fantin *et al.,* 1989; Tabche *et al.,* 1990) in fishes within a few days.

Cumulatively, lowered oxygen uptake and transport abilities, and ion exchange capacities at gill surfaces (Tabche *et al.,* 1990) could result in decreased swimming capacity. Yet,another explanation for decreased swimming capacity is lead induced C.N.S. and P.N.S. dysfunction. The medulla, a critical site for respiratory control, contains both cholinergic and catecholaminergic nuclei that control such functions as mucus secretions on the gill surface, opercular stroke frequency, gill and systemic blood vessel vasoconstriction, and heart stroke (Randall, 1970; Butler and Metcalfe, 1983; Smith, 1994). Lead interferes with selected muscarinic receptors in the brain and may be an important mechanism for some lead induced behavior ral alterations (Costa and Fox, 1983; Schulte *et al.,* 1994; Cory-Slechta and Pokora, 1995). Group responses to directional stimuli are directly controlled by two interconnected, neural pathways, the Mauthner cells (M-cell) and lateral line system, that induce highly coordinated and uniform group movements away from the perturbation (Partridge, 1982; Eaton *et al.,*1991). This highly polarized, stereotypical startle reflex is controlled by the M-cell located on the medulla surface. Sensitive to transitory displacements of water, the lateral

line gives information to individuals regarding position and velocity within a group (Partridge, 1982).

2.6.5.2 Effect on the haematopoietic system and d-amino levulinic acid (ALA) - induced generation of reactive oxygen species

Lead directly affects the haematopoietic system through restraining the synthesis of haemoglobin by inhibiting various key enzymes involved in the haeme synthesis pathway. It also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes. The combined aftermath of these two processes leads to anaemia (Cornelis, 2005). Anaemia caused on account of lead poisoning can be of two types: haemolytic anaemia, which is associated with acute high level lead exposure, and frank anaemia, which is caused only when the blood lead level is significantly elevated for prolonged periods (Vij, 2009). Lead significantly affects the haeme synthesis pathway in a dose dependent manner by down-regulating three key enzymes involved in the synthesis of haeme. δ-amino levulinic acid dehydratase (ALAD), a cytosolic enzyme that catalyzes the formation of porphobilinogen from δ-aminolevulinic acid (ALA), aminolevulinic acid synthetase (ALAS), a mitochondrial enzyme that catalyzes the formation of aminolevulinic acid (ALA) and finally, the mitochondrial enzyme ferrochelatase that catalyzes the insertion of iron into protoporphyrin to form haeme (Piomelli, 2002). The initial and final steps of haeme synthesis take place in the mitochondria, whereas the intermediate steps take place in the cytoplasm. Lead inhibits the three aforementioned vital enzymes of this pathway but its effect on ALAD is more profound and its inhibition has been used clinically to gauge the degree of lead poisoning. Inhibition of ALAD results in the accumulation

Formatted: Font color: Auto

Formatted: Font: Not Italic, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: Not Italic, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: Not Italic, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: Not Italic, Font color: Auto

of aminolevulinic acid, detectable in the plasma and urine even at blood lead levels of less than 10 µg/dl. Although ALAD inhibition is first noted at blood lead levels of 10-20 µg/dl, haeme biosynthesis does not decrease until the activity of ALAD is inhibited by 80-90%, which occurs at a much higher blood lead concentration of about 55 µg/dl (Ahamed *et al.*, 2005).

Inhibition of ferrochelatase results in increased excretion of coproporphyrin in urine and accumulation of protoporphyrin in erythrocytes. Moreover, inhibition of this enzyme results in the substitution of iron by zinc in the porphyrin ring forming zinc protoporphyrin (ZPP). The concentration of ZPP thus gets increased, which can also be used as an indicator to monitor the level of lead exposure (Jangid *et al.*, 2012). Thus, the collective inhibition of these three key enzymes blocks the haeme production via the haeme synthesis pathway. The mechanism responsible for shortening the life cycle of erythrocytes is not well understood. One of the earliest observed hematological effects of lead revealed basophilic stipplings of red blood cells (presence of dense material in red blood cells), which is also a potential biomarker for the detection of lead poisoning. These aggregates are degradation products of ribonucleic acid (Patrick, 2006b).

Lead molecules that enter into the aquatic system exert a specific toxic effect on fish blood and tissues (Mousa and Khattab, 2003; Vosyliene and Kazlauskiene, 2004).

Metabolic effects include hyperglycemia, depletion of tissue glycogen reserves, catabolism of muscle protein, and altered blood levels of protein, cholesterol, and free fatty acids (Thomas, 1990; Jobling, 1994; Wendelaar,1997).

In a study by Vinodhini and Narayanan (2008), they found out that the blood of common carp showed significant increase in glucose during 32 days of lead intoxication. This might be due to the vulnerable stress induced by the heavy metals which resulted in hyperglycemia. Lead increases the

Formatted: Font color: Auto
Formatted: Font: Not Italic, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: Not Italic, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: Not Italic, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: Not Italic, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: Not Italic
Formatted: Font: Not Italic, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt
Formatted: Font: Font color: Auto
Formatted: Font: 12 pt

glucose content in the blood, because of intensive glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and aminoacids (Almeida *et al.*,2001). Accordingly, the blood cholesterol level was significantly ($p<0.01$) increased in heavy metal exposed experimental groups including lead. The increased levels of cholesterol make the fish develop weakness in its body and its swimming ability. Cholesterol is the most important sterol occurring in animal fats. It is equally distributed between plasma and red blood cells, but in adrenal cortex, it occurs in the esterified form. The cholesterol occurs as white (or) faintly yellow almost odorless granules (Mousa and Khattab, 2003).

2.6.5.3 Renal effects of lead

Several heavy metals have been reported to stimulate inter-renal activity and plasma corticosteroid and glucose levels in fish (Pratap and Wendelaar, 1990). Hypersecretion of adrenalin and cortisol are considered primary stress responses. These effects trigger a broad suite of biochemical and physiological alterations called secondary stress responses. Renal dysfunction occurs mostly at high levels of lead exposure (>60 µg/dl) but damage at lower levels has also been reported (~10 µg/dl) (Grant, 2008). Renal functional abnormality can be of two types: acute nephropathy and chronic nephropathy. Acute nephropathy is characterized functionally by an impaired tubular transport mechanism and morphologically by the appearance of degenerative changes in the tubular epithelium along with the occurrence of nuclear inclusion bodies containing lead protein complexes. It does not cause protein to appear in the urine but can give rise to abnormal excretion of glucose, phosphates and amino acids, a combination referred to as Fanconi's syndrome. Chronic nephropathy on the other hand, is much more severe and can lead to irreversible functional and morphological changes. It is characterized by glomerular and tubulointerstitial changes, resulting

Formatted: Font:

Formatted: Font: 12 pt

Formatted: Font:

Formatted: Font: 12 pt

Formatted: Font:

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

in renal breakdown, hypertension and hyperuricemia (Rastogi, 2008). On exposure of *P. lineatus* to lead, after 6 hours, the organ that accumulated the highest amount was the kidney, which plays an important role in the excretion of toxic substances (Streit, 1998), and much of the Pb that enters the body is eliminated through the urine, after passing through glomerular filtration (Alves and Wood, 2006). The kidney also plays an essential role in the water and electrolyte balance and in the maintenance of a stable internal environment (Palaniappan *et al.,* 2009) and numerous channels, transport mechanisms and enzymes can be found in the renal cells, many of which have a high affinity for metals such as lead (Patel *et al.*,2006). Analyses of accumulated lead in distinct portions of the kidney have demonstrated that the posterior segment of the kidney retains more metal than the anterior portion, due to its greater involvement in ion reabsorption (Alves and Wood, 2006). Due to its functions, the kidney is an organ of high metabolic activity that is capable of producing metallothioneins (MT), which act as protectors against the action of metals (Cicik *et al.,* 2004). Histopathological studies have shown that the presence of lead may also induce the formation of inclusion bodies in the cells of the renal tubules of rats (Moore and Goyer, 1974) and birds (Locke *et al.*, 1966). These bodies which are precipitates of lead-binding proteins, help to prevent lead from continuing in circulation, and may be one of the reasons for the occurrence of a large quantity of lead in the kidney of rainbow trout (*O. mykiss*) after acute exposure (Patel *et al.*, 2006). As a result of these two processes, much of the circulating lead may be retained in the kidney to prevent damage to other organs.

2.6.5.4 Physical impairment

Lead exposure can inhibit essential physiological functions in salmonids. The exposure of juvenile rainbow trout to 13 µg/L of Pb for 2 weeks caused a reduction in red blood cell enzyme

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

(delta-aminolevulinic acid dehydratase; ALA-D) activity, and the activity was significantly reduced after 4 weeks compared to control fish; at 4 months, enzyme activity of exposed fish was reduced to 60% of control fish levels (Hodson *et al*., 1976). The effect of Pb on ALA-D increases both with concentration and exposure time (Hodson *et al.,* 1976). Delta-aminolevulinic acid dehydratase is responsible for the production of haemoglobin, an essential oxygen-transport protein in red blood cells. In a follow-up study, Hodson *et al.* (1977) reported a 20% reduction in ALA-D activity in juvenile rainbow trout exposed to 10 µg/L Pb after only 2 weeks compared to control fish. Red blood cell enzyme activity of juvenile brook trout was inhibited by 20 - 45 % only during exposure to 90 - 100 µg/L Pb for 2 weeks; 50 - 60 µg/L Pb over the same time period had little effect (Hodson *et al.*, 1977). The maximum acceptable toxicant concentration for juvenile rainbow trout exposed to Pb has been estimated at between 3.0 and 13 µg/l in waters of alkalinity between 26 and 90 mg/l (Hodson *et al*., 1976).

2.6.5.5 Reproductive health effects of lead

Lead causes a number of adverse effects on the reproductive system of both men and women. Common effects seen in men include: reduced libido, abnormal spermatogenesis (reduced motility and number), chromosomal damage, infertility, abnormal prostatic function and changes in serum testosterone. Women on the other hand, are more susceptible to infertility, miscarriage, premature membrane rupture, pre-ecclampsia, pregnancy hypertension and premature delivery (Flora 2011). Moreover, during the gestation period, direct influence of lead on the developmental stages of the fetus has also been reported (Saleh *et al.,* 2009). In fish, sub-lethal lead exposure can cause endocrine dysfunction. For example, Ruby *et al.* (2000) reported decreased transformation of spermatogonia to spermatocytes in sexually maturing male rainbow trout exposed to 10 µg/l lead for 12 days.

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Additionally, two-year old female rainbow trout exposed to 10 µg/l lead for 12 days showed significantly reduced oocyte (cells from which eggs develop) growth compared to control fish (Ruby *et al.*, 2000). Weber (1993) observed that multiple effects on reproductive behavior and overall reproductive success in adult fathead minnows, where lead suppressed spermatocyte production and retarded ovarian development, decreased the number of eggs oviposited, increased interspawn periods, and suppressed embryo development. Thomas (1990) reported the decreases in estradiol levels in female Atlantic croaker following exposure to dietary lead. Khan (2000) demonstrated that lead and Aroclor 1254 significantly decreased leuteinizing hormone (LH) levels in Atlantic croaker in response to stimulation by an leuteinizing hormone releasing factor (LHRF) analog *in vivo* and reduced gonadal growth when administered at a dose of 15 mg/kg body weight for 30 days. Kidd *et al.* (2007) observed that a chronic exposure of fathead minnow (*Pimephales promelas*) to low concentrations of thepotent 17α-ethynylestradiol led to feminization of males and ultimately caused a near extinction of this species from the lake.

2.6.5.6 Effect of lead on bone

The primary sites of lead storage in the human body are the bones (Renner, 2010). There are two compartments in bones where lead is believed to be stored. The exchangeable pool present at the surface of bone and the non-exchangeable pool located deeper in the cortical bone. Lead can enter into plasma at ease from the exchangeable pool but can leave the non-exchangeable pool and move to the surface only when bone is actively being re-absorbed (Patrick, 2006). Stable lead isotope methodology showed that bones contribute around 40 -70% of lead released intoblood in adults. In adults, 85 -95% of the lead is stored in bones, in contrast to 70% in children, resulting in higher concentration of lead in soft tissues in children. The storage and the mobilization of lead in bones

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

depend on several factors, like dose/rate of lead exposure, age, pregnancy, gestation and race. In fish, Weber(1993) indicated that lead reaches very high bio-concentration factors (internal: ambient concentrations), remains in various tissues, especially bone, even after lead is no longer present in the water, and may decrease from specific tissues at a very slow rate. A similar pattern of bone lead mobilization has been observed in humans (Todd *et al.*, 1996).

2.6.5.7 Effects of lead on liver

The liver of fish can be considered a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health (Myers *et al.*, 1998), and exhibit the effects of a variety of environmental pollutants (Hinton *et al.*, 1992). Moreover, the liver play a major role in complex enzymatic processes of tetraiodothyronine (thyroxine)-tri-iodothyronine (T4-T3) conversion.

The fibrosis, steatosis, hyperemia and necrosis; which forms a rectangle of hepatic tissue changes, are similar to those reported for fish caught in contaminated water or exposed ones to various chemicals in laboratory conditions (Olojo *et al.*, 2005; Camargo and Martinez, 2007; Wahbi and El-Greisy, 2007; Aniladevi *et al.*, 2008). Fibrosis and local blood congestions in the liver sinusoids of the flounder, *Platichthys flesus*, the ruffe, *Gymnocephalus cernua*, and the smelt, *Osmerus eperlanus*, were reported as a consequence of pollution by Peters *et al.* (1987). Radhaiah and Jayantha (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, ruptured blood vessels and pyknotic nuclei in the liver of *Tilapia mossambica* exposed to fenvalerate. Tilak *et al.*, (2005) observed the same changes in liver of *Catla catla* exposed to chlorpyrifos.

2.7 Oxidative stress

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Oxidative stress represents an imbalance between the production of free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Flora,2011). This has been reported as a major mechanism of lead induced toxicity. Under the influence of lead, onset of oxidative stress occurs on account of two different pathways operative simultaneously; first comes the generation of ROS**,** like hydroperoxides, singlet oxygen and hydrogen peroxide (H_2O_2), and second, the antioxidant reserves become depleted(Flora2002).

The antioxidant defenses of the body come into play to nullify the generated ROS. The most important antioxidant found in cells is glutathione (GSH). It is a tripeptide having sulfhydryl groups and is found in mammalian tissues in millimolar concentrations. It is an important antioxidant for quenching free radicals (Mates, 2000). Glutathione exists in both reduced (GSH) and oxidized form (GSSG). The reduced state of glutathione donates reducing equivalents ($H^+ + e^-$) from its thiol groups present in cysteine residues to ROS and makes them stable. After donating the electron, it readily combines with another molecule of glutathione and forms glutathione disulfide (GSSG) in the presence of the enzyme glutathione peroxidase (GPX). GSH can be regenerated from GSSG by the enzyme glutathione reductase (GR) (Deponte 2013). Under normal conditions, 90% of the total glutathione content exists in reduced form (GSH) and around 10% is in the oxidized form (GSSG). Under conditions of oxidative stress, the concentration of GSSG is much higher than that of GSH.

Lead has electron sharing capability that results in the formation of covalent attachments. These attachments are formed between the lead moiety and the sulfhydryl groups present in antioxidant enzymes, which are the most susceptible targets for lead and which eventually get inactivated (Flora *et al.*,2012). Lead inactivates glutathione by binding to sulfhydryl groups present in it. This results in

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

synthesis of GSH from cysteine via the γ-glutamyl cycle, which is usually not effective in replenishing the supply of GSH (Hultberg *et al.*, 2001). Similarly, lead inactivates enzymes like δ-amino levulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase, which further depresses the glutathione levels (Ahamed and Siddiqui, 2007).

A few other notable antioxidant enzymes that are rendered inactive by lead include super oxide dismutase (SOD) and catalase (CAT). Decrease in SOD concentration reduces the disposal of superoxide radical, whereas reduction in CAT impairs scavenging of superoxide radical ($O_2^{\bullet -2}$). Apart from targeting the sulfhydryl groups, lead can also replace the zinc ions that serve as important co-factors for these antioxidant enzymes and inactivates them (Flora and Gupta, 2007).

2.7.1 Oxidative stress and antioxidant defenses

Oxidative stress is an unavoidable aspect of aerobic life. It is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses in living organisms (Nishida, 2011). Reactive oxygen species are induced by substances such as transitional metal ions, pesticides, and petroleum pollutants (Slaninova –*et al.*, 2009; Lushchak, 2011). Free radicals are also produced by endogenous cellular sources during normal cell metabolism. Mitochondrial respiration is the main endogenous source of ROS. Elevated production of ROS can cause oxidation of proteins and lipids, alterations in gene expression, and changes in cell redox status (Livingstone, 2003).

Mechanisms of antioxidant defenses in fish include the enzyme system and low molecular weight antioxidants, similar to those in mammals, although the specific isoforms of enzymes in various fish species have not been well identified (Giullio and Hinton, 2008). Superoxide dismutase

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

(SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-s-transferase (GST) are the main antioxidant enzymes and important indicators of oxidative stress (Wu *et al.*, 2011). Reduced glutathione (GSH) and oxidized glutathione disulphide (GSSG) play a key role in non-enzymatic antioxidant defence. Metal-binding proteins such as ferritin, ceruloplasmin, and metallothioneins (MTs) have special functions in the detoxification of toxic metals, and also play a role in the metabolism and homeostasis of essential metals (Kelly *et al.*, 1998).

Metallothioneins are low molecular weight proteins rich in cysteine residues that can bind various metals, including mercury, silver, copper, cadmium, lead, zinc, and cobalt, with varying affinities (Hamer, 1986). It has been reported that different fish species possess different isoforms of MTs (Smirnov *et al.*,2005). Metallothioneins are involved in the regulation of the essential metals copper and zinc and in the detoxification of non-essential metals (Amiard *et al.*, 2006). Zinc has an essential function in the activation of metal-regulated transcription factors which initiate expression of the MT genes (Roesijadi, 1996).

2.7.2 Role of antioxidants in protecting organs in lead induced oxidative stress

Lead stimulated oxidative stress is a state that involves the generation of free radicals beyond the permissible limits, depleting at the same time the antioxidant reserves and thus hampering the ability of the biological system to reverse the resulting effects. Free radicals generation starts a chain reaction that results in lipid peroxidation, disruption of cell membrane, protein oxidation and oxidation of nucleic acids like DNA and RNA leading to cancer (Gurer and Ercal, 2000). Research findings have suggested that administration of various antioxidants can prevent or subdue various toxic effects of lead and generation of oxidative stress in particular. An *antioxidant* is a substance

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

which, when present at a low concentration as compared to that of the oxidizable substrate, can prevent the oxidation of that substrate. Generally, an antioxidant can prevent lead toxicity in three ways;

i. Inactivating the generated ROS at molecular level, thereby terminating the radical chain reaction (chain breaking).

ii. Chelating the lead ion and preventing further formation of ROS.

iii. Chelating lead and maintaining it in a redox state, which leads to its incompetency to reduce molecular oxygen. (Garcia-Medina *et al.*, 2010)

Antioxidants may be broadly grouped according to their mechanism of action: primary or chain breaking antioxidants and secondary or preventive antioxidants. Primary antioxidantsare compounds capable of scavenging free radicals that are responsible for initiation or propagation of the chain reaction through chain breaking mechanism. This is done by donating free electrons to ROS and lipid radicals present in the biological system and converting them into stable molecules. This prevents or delays the oxidation process and prevents lipid peroxidation, which can cause membrane damage. Common primary antioxidants include flavonoids, tocopherol and ascorbic acid (Vaya and Aviram, 2000). On the other hand, secondary antioxidants(like low molecular weight polyphenols) are those which mainly act by slowing down the rate of the oxidation reaction (Flora *et al.*, 2012).

The major difference between primary and secondary antioxidants is that the latter do not convert free radicals into stable molecules. They are capable of chelating heavy metals like lead (Wanasundara and Shahidi, 2005).

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

2.8 Vitamin C: Function and essentiality

Vitamin C is known to perform numerous biochemical and physiological functions in both plant and animal metabolism (Tolbert, 1979). Most animals can synthesize this vitamin in the form of ascorbic acid in amounts sufficient to prevent the clinical symptoms of deficiency collectively known as scurvy. However, primates, guinea pigs, fish, shrimp, and some insects, bats and birds require a dietary source of vitamin C to prevent or reverse scorbutic symptoms. Among these species, dietary essentiality of vitamin C in fish and shrimp probably results from an absence or insufficiency of L-gulonolactone oxidase (Wilson, 1973; Yamomoto *et al.*, 1978).

Ascorbic acid is a strong reducing agent that provides electrons to functional groups of other biochemicals and free radicals found in the aqueous phase of biologic fluids. Two biochemical reactions commonly associated with the function of ascorbic acid in animals are hydroxylation and reduction. There may also be other as yet undetermined functions of ascorbic acid as suggested by Tolbert (1979).

Over the past 30 years a great deal of research has been conducted to study the function of ascorbic acid in aquatic species. Effects of dietary ascorbic acid on growth, morphogenesis, reproduction, and adaptation have been studied extensively in carp, catfish (Lovell, 1973; Wilson and Poe, 1973; Launer *et al*.,1978,Lim and Lovell,1978;Lovell and Lim,1978;Mayer *et al.*, 1978; Lovell, 1982; Li and Lovell 1985), trout and salmon (Sandnes *et al.*,1984; Grant *et al.*,1989), shrimp (Lightner *et al.*, 1977), tilapia (Jauncey *et al*.,1985; Soliman *et al.*, 1986) and snake heads (Mahajan

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

and Agrawal, 1980). In all of these species the most studied, and perhaps best understood function of ascorbic acid is its role as a co-factor in hydroxylating lysine and proline of collagen. Collagen is the major component of connective tissue, including bone and cartilage. Impaired collagen formation results in the classical vitamin C deficiency known as scurvy, whose symptoms include lordosis and scoliosis, as well as poor growth, anorexia, reduced wound healing efficiency, and haemorrhage(Tucker and Halver 1984).They cited other evidence supporting a similar role of ascorbic acid in hydroxylation reactions in carnitine synthesis. They suggested that early vitamin C deficiency symptoms of lethargy and fatigue may be due to depleted muscle carnitine. These symptoms have been reported in trout (Grant *et al.*, 1989) and described similarly as prolonged periods of torpor.

Ascorbic acid also serves as a cofactor in hydroxylation reactions involved in excretion of drugs and toxicants. Its role in detoxification of organochloride pesticides was investigated by Wagstaff and Street (1971). It was shown that ascorbic acid was required to perform specific detoxification reactions in the liver. Mayer *et al.* (1978) found that exposure to the pesticide toxaphene resulted in reduced levels of whole body vitamin C activity and decreased backbone collagen in fathead minnows and channel catfish. This led to the hypothesis that hydroxylation reactions may compete with one another for available vitamin C activity, thereby increasing the requirement for ascorbic acid. Studies with trout showed that ascorbic acid also plays a role in iron metabolism. Hilton *et al.* (1978) reported increased iron levels in the spleens of scorbutic fish, along with liver iron levels and haematocrit readings that were positively correlated to dietary levels of supplemental ascorbic acid. Thesedata lead to the conclusion that ascorbic acid may control the release of iron within spleen tissue, thereby affecting the redistribution of iron stores.

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Apart from these biochemical processes affecting growth and morphogenesis of various species of fish, vitamin C activity also has been linked conclusively to reproduction as well as adaptive responses such as disease resistance. Sandnes *et al.* (1984) and Soliman *et al.* (1986) showed that fish transfer ascorbic acid to eggs just before spawning, where it is used in larval development. Other researchers have observed increased resistance to bacterial infections by channel catfish (Lovell, 1982, Li and Lovell, 1985) and reduced mortality in trout caused by the protozoan *Ichthyophthirius multifiliis* (Wahli *et al.*, 1985; 1986; 1995).

2.8.1 Anti-oxidant effect of vitamin C in metal induced organisms

Vitamin C also known as ascorbic acid is a water-soluble vitamin required for physiological growth and development. It is needed for collagen synthesis which is necessary for the formation of tendons, ligaments, skin, and blood vessels. Vitamin C aids in wound healing and scar tissue formation. It restores and sustains bones, teeth and cartilage acting as a major antioxidant (Ganesh *et al.*,2016). Vitamin C is a potent antioxidant which decreases oxygen, nitrogen, and sulfur centered free radicals (Niki, 1991). Lead (200 mg/kg diet) had an inhibitory effect on the growth of broilers and appeared to be inducing lipid peroxidation. The addition of ascorbic acid to the diet reduced the plasma malondialdehyde levels induced by lead and tended to reduce the inhibitory effect of lead on growth. It was concluded that the addition of higher doses of ascorbic acid to the diet may be more efficacious in fully reversing the negative effect of lead on growth (Zeynep, 2005).

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

CHAPTER THREE

MATERIALS AND METHOD

3.1 Experimental animals

Three hundred and forty (340) juveniles of *Clarias gariepinus* weighing 40.60±1.48g and 20.45±1.51cm length were collected from Chi Farms Ltd., Ibadan, and transported in one fifty litre capacity plastic tank containing 30 (thirty) liters of water to Onitsha where they were acclimatized in twenty four (24) aquaria tanks for 14 days. The juveniles were fed 3mm local fish pellets at 3% biomass at 9.00am and 5.00 pm respectively. The fish pellets brandnamed Skretting is manufactured by Skretting Nigeria Ltd. Ibadan Nigeria. The 3mm pellets contain the following feed ingredients as shown in table 2.

Formatted: Font color: Auto
Formatted: Left
Formatted: Centered
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

Table 2: **Proximate Analysis of 3mm Skretting Fish Pellets Used In The Study.**

Feed ingredients	Quantity
Crude protein	45%
Crude fat	14%
Crude fibre	2.3%
Ash	8%
Calcium	1.6%
Phosphorous	1%
Lysine	2.5%
Methionine	0.9%
Copper Sulphate	5mg/kg
Vitamin A	5000iu/kg
Vitamin D_3	750iu/kg
Vitamin E	150mg/kg
Vitamin C	100mg/kg

(Feed Analysis:Source Skretting Nigeria Limited, Ibadan)

3.2 Experimental design: The experimental design used in this study was latin square

3.2.1 Experiment A: Determination of LC_{50} of lead nitrate on *Clarias gariepinus*

The stage 1 of the acute toxicity test for lead nitrate was carried out using 4 juveniles of mean weight 39.80 ± 1.54g. and mean length 21.05 ± 1.23cm. They were divided into one juvenile each in 4 aquaria tanks. They were treated with 20, 100, 300 and 600mg/l of lead nitratere spectively.

Acute toxicity (LC_{50}) was calculated using the formula of (Enegide *et al.*, 2013) thus

$$LC_{50} = \frac{[M_0+M_1]}{2}$$

Where: Mo = Highest dose of test substance that recorded no mortality.

M_1 = lowest dose of test substance that gave mortality

$$LC_{50} = \frac{[M_0+M_1]}{2} = \frac{20mg/l + 100mg/l}{2} = \frac{120}{2}$$

LC_{50} of lead nitrate for *C. gariepinus* juveniles was 60mg/l

The LC_{50} of 60mg/l -was divided by 12 to obtain 5mg/l as the least sub lethal dose which was subsequently increased to obtain the other doses of lead nitrate employed in the study. The sub lethal doses of lead nitrate for experiments 1 and 11 were 0mg/l, 5mg/l, 10mg/l and 15 mg/l.

3.2.2 Experiment B: Determination of LC_{50} of ascorbic acid on *Clarias gariepinus*

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

The LC_{50} of ascorbic acid (vitamin C) used in experiment I and 1I, was determined according to Enegide *et al.* 2013. The doses of ascorbic acid given to the stage 1 juveniles were 50, 100, 150, 200 and 400 mg/l of ascorbic acid respectively.

$$LC_{50}\text{ (Ascorbic acid; kepro vitamin C)} = \frac{[M_0+M_1]}{2}$$

$$= \frac{150mg/l + 200mg/l}{2} = \frac{350mg/l}{2}$$

LC_{50} for Ascorbic acid for *C. gariepinus* juveniles was = 175mg/l

The lethal concentration for vitamin C was divided by 3.5 to obtain the first therapeutic dose of 50mg/l which was subsequently doubled to 100mg/l .Both doses of Vitamin C were employed as therapeutic doses for experiments 1 and 11.

3.2.3 Experiment I: Chronic toxicity of lead nitrate in *Clarias gariepinus* juveniles and ameliorating effect of ascorbic acid

One hundred and forty four (144) juveniles of *Clarias gariepinus* (mean weight43.20±1.41g and mean length 20.75±1.96cm), divided into four groups using simple purposive sampling technique based on weight, were used to perform this study. Juvenile catfishes in groups IA, IB, IC and ID were exposed to lead nitrate at 0, 5, 10 and 15mg/l respectively for 91 days, in three replicates each containing twelve catfish juveniles (Table 3). Water change and lead nitrate renewal was done every three days to maintain toxicant strength and level of dissolved oxygen (DO). Water quality parameters; DO, pH and temperature were monitored during the experiment. The juveniles were fed 3mm Skretting® fish pellets at 3% body weight twice daily. Clinical observations were made during the experiment. Blood and serum samples were collected and analysed for haematological parameters on day zero to obtain baseline data and day 91 of the study to determine the chronic effects of lead nitrate on the haematological parameters. Samples of the gills, liver and stomach were

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto

collected after 91 day exposure to lead nitrate, for histological assessment after anesthesia with Tricaine S.

Table 3:**Experimental Design for the Exposure of *Clarias Gariepinus* to Varying Concentrations of Lead Nitrate in Three Replicates for 91 Days.**

R / T	**T_1 (A1)**	**T_2 (1B)**	**T_3 (1C)**	**T_4 (1D)**
R_1	0 mg/l lead	5mg/l lead	10mg/l lead	15mg/l lead
R_2	0 mg/l lead	5mg/l lead	10mg/l lead	15mg/l lead
R_3	0 mg/l lead	5mg/l lead	10mg/l lead	15mg/l lead

Experimental design 1a

3.2.4 Treatment with ascorbic acid for 7 days:

The fish in the experimental aquaria exposed for 91 days to varying sublethal concentrations of lead nitrate were divided into two groups for 7 days treatment with vitamin C at 50 and 100mg/l (Borane and Zambare, 2006) respectively as shown in Tables 4 and 5.At the end of 7 day treatment, blood and tissue samples were assessed to determine the effects of Ascorbic acid on the lead nitrate induced pathology of *Clarias gariepinus*.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Table 4: **7 Day Treatment With 50 Mg/L Vitamin C After 91 Day Exposure Of *C. Gariepinus*to Varying Concentrations of Lead Nitrate.**

R \ T	1A	1B	1C	1D
R1	50mg vit C	5mg/l lead 50mg/l vit C	10mg/l lead 50mg/lvit C	15mg/l lead 50mg/lvitC
R2	50mg vit C	5mg/l lead 50mg/l vit C	10mg/l lead 50mg/lvit C	15mg/l lead 50mg/lvitC
R3	50mg vit C	5mg/l lead 50mg/l vit C	10mg/l lead 50mg/lvit C	15mg/l lead 50mg/lvitC

Experimental design 1b

Table 5: **7 Day Treatment With 100 Mg/l Vitamin CAfter 91 Day Exposure of *Clarias Gariepinus* to Varying Concentrations of Lead Nitrate.**

R / T	$1A_2$	$1B_2$	$1C_2$	$1D_2$
R_1	100mg/l Vit. C	5mg/l lead 100mg/l vit. C	10mg/l lead 100mg/l vit. C	15mg/l lead 100mg/l vit. C
R_2	100mg/l Vit. C	5mg/l lead 100mg/l vit. C	10mg/l lead 100mg/l vit. C	15mg/l lead 100mg/l vit. C
R_3	100mg/l Vit. C	5mg/l lead 100mg/l vit. C	10mg/l lead 100mg/l vit. C	15mg/l lead 100mg/l vit. C

Experimental design 1c

3.2.5 Experiment 2a: Effects of concurrent exposure of *Clarias gariepinus*juveniles to leadnitrate and 50 mg/l ascorbic acid for 91 days

2a. Seventy two (72) juveniles of *Clarias gariepinus* of weight (21.50 ±1.53g) and length 10.60±1.84cm) were assigned into four groups namely $2A_1$, $2B_1$, $2C_1$ and $2D_1$ and treated with 0, 5, 10 and 15mg/l of lead nitrate in three replicates. The control group (0mg/l lead) was treated with 50 mg/l vitamin C, the other groups were also treated with 50mg/l of ascorbic acid (Table 6). At the end of 91 days blood, serum and tissue samples were collected for various analyses.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Table 6: **Exposure of *Clarias Gariepinus* Juveniles to Varying Concentrations of Lead Nitrate and 50mg/l Vitamin C For 91 Days**

R / T	$2A_1$	$2B_1$	$2C_1$	$2D_1$
R_1	50mg/lvit C	5mg/l lead 50mg/l vit. C	10mg/l lead 50mg/l vit. C	15mg/l lead 50mg/l vit. C
R_2	50mg/lvit C	5mg/l lead 50mg/l vit. C	10mg/l lead 50mg/l vit. C	15mg/l lead 50mg/l vit. C
R_3	50mg/lvit C	5mg/l lead 50mg/l vit. C	10mg/l lead 50mg/l vit. C	15mg/l lead 50mg/l vit. C

Experimental design 2a

Formatted ...
Formatted ...
Formatted ...
Formatted: Font: Not Bold, Font color: Auto
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...

3.2.6 Experiment 2b: Effects of concurrent exposure of *Clarias gariepinus* juveniles to lead nitrate and 100 mg/l vitamin C for 91 days.

2b. This experiment was carried out with seventy-two juveniles of *Clarias gariepinus* of weight (20.80 ± 1.41g and length 11.50 ± 1.32cm),divided into four groups and exposed to 0, 5, 10 and 15mg/l of lead nitrate in three replicates. The control group was treated with 100mg/l of vitamin C and 0 mg/llead nitrate while the other exposed groups were treated with 100mg/l vitamin C (Table 7).This experiment also lasted for 91 days after which blood, serum and tissue samples were collected for various analyses.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Table 7: **Exposure of *Clarias Gariepinus* Juvenile to Varying Concentrations of Lead Nitrate and 100 Mg/l Vitamin C for 91 Days.**

R T	$2A_2$	$2B_2$	$2C_2$	$2D_2$
R_1	100mg/l vit C	5mg/l lead 100mg/l vit. C	10mg/l lead 100mg/l vit. C	15mg/l lead 100mg/l vit. C
R_2	100mg/l vit C	5mg/l lead 100mg/l vit. C	10mg/l lead 100mg/l vit. C	15mg/l lead 100mg/l vit. C
R_3	100mg/l vit C	5mg/l lead 100mg/l vit. C	10mg/l lead 100mg/l vit. C	15mg/l lead 100mg/l vit. C

Experimental design 2b

3.3 Studies on haematological parameters

Blood samples were collected by cardiac puncture using sterile syringe and needle from the juveniles of *Clarias gariepinus*. The collected blood was put into EDTA sample bottles to prevent the blood from clotting and was properly labeled.

3.3.1 Determination of packed cell volume (PCV)

The packed cell volume (PCV) was determined by the micro haematocrit method (Thrall and Weiser, 2002). Non heparinized micro-capillary tubes were almost filled with the anti-coagulated blood sample and one end sealed with plasticine. The filled tubes were centrifuged at 10,000 revolutions per minute for 5 minutes using a micro haematocrit centrifuge (Hawksley, England). The PCV was read as a percentage on the micro haematocrit reader (Thrall and Weiser, 2002).

3.3.2 Determination of haemoglobin concentration (Hb)

The haemoglobin (Hb) concentration was determined by the cyanomethaemoglobin method (Higgins *et al.*, 2008). The blood sample (0.02ml) was added to 5ml of Drabkins reagent in a clean test tube. This was mixed gently and kept at room temperature for 20 minutes to react. The absorbance of both sample and standard were read, against a working reagent blank at a wavelength of 540 nm using spectrophotometer (Lab-Tech, India). The haemoglobin concentration of the blood sample was obtained by multiplying the absorbance of the sample with the factor derived from the

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

absorbance and concentration of the standard. The haemoglobin concentration was expressed in mg/dl.

3.3.3 Red blood cell (RBC) count

The RBC was determined by the haemocytometer method (Thrall and Weiser, 2002), blood sample (0.02ml) was added to 4ml of red blood cell diluting fluid (sodium citrate, formaldehyde solution and distilled water) in a clean test tube, to make a 1:200 dilution. A drop of the diluted blood was charged onto the Neubauer counting chamber and allowed to settle for 2-3 minutes. The high dry objective (X40) of the light microscope was used in carrying out the erythrocyte count, in the five groups of 16 small squares. The number of erythrocytes enumerated for each sample was multiplied by 10,000 to obtain the erythrocyte count per micro litre of blood. (Thrall and Weiser, 2002).

3.3.4 Total white blood cell (TWBC) count

The total white blood cell count was determined by the haemocytometer method (Thrall and Weiser, 2002). Blood sample (0.02ml) was added to 0.38ml of white blood cell diluting fluid (glacial acetic acid tinged with gentian violet) in a clean test tube, to make a 1:20 dilution. A drop of diluted blood was charged onto the Neubauer chambers and allowed to settle for 2 minutes. The X10 objective lens of the light microscope was used in making a total count of white blood cells on the four corner squares. The number of cells counted for each blood sample was multiplied by 50 to obtain the total leukocyte count per micro litre of blood (Thrall and Weiser, 2002).

3.3.5 Differential white blood cell (DWBC) count

Smears for differential leukocyte counts were prepared on clean slides and stained by the Leishman stain (Thrall and Weiser, 2002). The differential leukocyte count was enumerated by the battlement counting method (Coles, 1986). The X100 (oil immersion) objective lens of the light microscope was used for the differential leukocyte count and the different cells of the leukocytic series were identified and scored using the differential cell counter (Thrall and Weiser, 2002).

3.4 Biochemical Assay

Assay kits for the estimation of ALT, ASP, ALP and blood urea were the products of Randox® laboratory United Kingdom.

3.5 Serum biochemistry determinations

Blood samples were collected by cardiac puncture using sterile syringe and needle. The collected blood was put into plain sample bottles (with anti-coagulant) and allowed to clot. The clotted blood meant for serum biochemistry were separated from clear serum by centrifugation at 2000 rpm for 15 minutes and properly labeled. All serum biochemistry determinations were carried out following standard procedure using Randox test kits (Randox United Kingdom). Blood and serum samples were packaged in ice and transported to the Department of Veterinary Medicine laboratory, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

3.5.1 Alanine amino transferase (ALT)

ALT assay was carried out using the ALT kit from Randox Laboratories, United Kingdom. The kit is designed for the quantitative *in vitro* determination of ALT in serum. The kit contains two reagents R_1 and R_2, R_1 contains buffer made up of phosphate buffer 100mmol/L,pH 7.4, L-Alanine 200mmol/l and α −oxoglutarate 2.0mml/l. R_2 contains 2,4- dinitrophenylhydrazine 2.0mmol/l.

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

The principle is based on the following biochemical reaction;

$$\alpha - oxoglutarate + L - alanine \xrightarrow{apt} L - glutamate + pyruvate$$

Alanine amino transferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4 -dinitrophenylhydrazine (Oseni *et al.*, 2018).Serum sample (0.7ml) was mixed with 0.5ml of R_1 solution and incubated for 30minutes at 37^{o}C, 0.5ml of R_2 solution was added, the mixture was allowed to stand for 20 minutes at 20 to 25^{o}C (room temperature). Sodium hydroxide (5ml) was added and mixed. The absorbance of the serum sample was read against the reagent blank, after 5 minutes at 540nm using a UV spectrophotometer. The reagent blank contains 0.5ml R_1 solution, 0.1ml distilled water, 0.5ml R_2 solution and 5ml sodium hydroxide. The ALT activity in the serum was obtained from the matching table and expressed in international unit per litre (iu/l).

3.5.2 Aspartate amino transferase (AST)

AST assay was carried out using the AST kit from Randox Laboratories, United Kingdom. The kit being designed for the quantitative *in vitro* determination of AST in serum contains two reagents R_1 and R_2.R_1 contains buffer made up of phosphate buffer 100mmol/l, pH 7.4, L-Aspartate 100mmol/l.R_2 contains 2,4- dinitrophenylhydrazine 2.0mmol/l.

The principle is based on the following reaction;

$$\alpha - oxoglutarate + L - aspartate \xrightarrow{GOT} L - glutamate + oxaloacetate$$

Aspartate amino transferase (AST) is measured by monitoring the concentration of oxaloacetate by hydrazone formed with 2,4- dinitrophenylhydrazine (Oseni *et al.*, 2018). Serum sample (0.1ml) was mixed with 0.5ml of R_1 solution and incubated for 30minutes at 37^{o}C. R_2(0.5ml) was added, the

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted

mixture was allowed to stand for 20mins at 20-25^{0}C (room temperature). Sodium hydroxide (5.0ml) was added and the absorbance of the serum sample was read against the reagent blank after 5mins at 540nm using UV spectrophotometer. The blank reagent contains 0.5ml R_1, 0.1ml distilled water, 0.5ml R_2 and 5.0ml sodium hydroxide. The activity of the AST in the serum was obtained from the matching table and expressed in international unit per litre (iu/l).

3.5.3 Alkaline phosphatase (ALP)

ALP assay was carried out using the ALP kit from Randox Laboratories, United Kingdom.The ALP kit was designed for the quantitative *in vitro* determination of alkaline phosphatase (ALP) in serum and plasma. The kit employs a colorimetric method which is an optimized standard method according to Deutsche Geseilschaftfur Klinische chemie (DGKC) (1972) which is a kinetic method.The kit contains 2 reagents R_1,a buffer which contains diethanolamine buffer 1mmol/L, pH 9.8 and magnesium chloride 0.5mmol/l and R_2 which is p-nitrophenylphosphate 10 mmol/l. The principle is based on the following biochemical reaction;

$$P-nitrophenylphosphate + H_20 \xrightarrow{ALP} Phosphate + P-nitrophenol$$

The alkaline phosphatase present in the serum sample catalyses the hydrolysis P-Nitrophenylphosphate (PNPP) during which P- Nitrophenol and Phosphate are released.Mg^{2+} ions enhance the activity.The increasein absorbance at 405nm correlates with the activity of serum alkaline phosphatase according to assay kit manufacturer's instructions. Serum sample (0.02ml) was mixed with 1.00ml of R_1 at 30^{o}C. The initial absorbance of the mixture was read at 405nm, after which other absorbances were read after 1, 2 and 3 minutes using a UV spectrophotometer to

Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto

determine the change of optical density (ΔA). The ALP activity was calculated using the formula below,to express the value of ALP in *iu*/L.

$$\frac{iu}{L} = 2760 \times \Delta A405nm/min.$$

3.5.4 Determination of serum urea

Serum urea was determined using the urea Randox Kit from Randox Laboratories United Kingdom. The method is called Urease-Berthelot Colorimetric method for the quantitative *invitro* determination of urea in serum, plasma and urine.The kit contains four reagents R_1a 1.0ml Urease R_1b 37ml sodium nitroprusside ,R_2 110ml phenol concentrate,R_3 22ml hypochlorite concentrate, and R_4 5.5ml standard calibrator. It is based on the principle of hydrolysis. Serum urea is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction;

$$urea + H_20 \xrightarrow{urease} 2NH_3 + CO_2$$

$$NH_3 + hypochlorite + phenol \rightarrow indophenol(blue\ compound)$$

10µl of the serum sample was mixed with reagent 1 and incubated at 37°C for 10 minutes. Reagent 2(2.50ml) and reagent 3(2.50ml) were added immediately and incubated at 37°C for 15 minutes. The standard calibrator(10µl) was mixed with 100µl of reagent 1 and incubated at 37°C for 10 minutes. The absorbance of sample (A sample) and standard (A standard) was read against the blank using a UV spectrophotometer at 540nm.Serum urea concentration was obtained by applying the formula below and the serum urea was measured in mmol/l or mg/dl.

Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

$$serum\ urea\ concentration = \frac{A\ sample}{A\ standard} \times standard\ conc.$$

= mg/dl or mmol/L

3.5.5 Determination of lead concentration in *Clarias gariepinus*muscle

Lead level analysis in the fish muscle was conducted using Agilent FS 240 Atomic Absorption Spectrophotometer (AAS) according to the method of American Public Health Association APHA (2012).Fish sample (5g) was weighed into a digestion flask and 20ml of acid mixture (650ml conc. HNO_3, 80ml Perchloric acid and 20ml conc. H_2SO_4) was added to the flask. The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to the 100ml mark. 100ml of the diluted digested sample was transferred into a glass beaker of 250ml volume, to which 5ml of concentrated nitric acid was added and heated to boil till the volume reduced to about 20ml. Adding concentrated Nitric acid in increments of 5ml resulted to complete dissolutions of all residues. The mixture was cooled, transferred and made up to 100ml using metal free distilled water. The sample was aspirated into the oxidizing air-acetylene flame, to observe the sensitivity for absorption. Standard lead solutions in the optimum concentration range were prepared. The quantity of lead in the digested fish sample was read from the AAS in µg/l (ppm)

3.6 Histopathological examinations

The gills, liver and stomach of the experimental fish were collected and fixed in 10% formal saline. They were dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax. Cut sections (5µm thick) were stained with haematoxylin and eosin (H&E) stain for histological

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto

examination using light microscope (Bancroft and Stevens, 1977). Photomicrographs of histological sections were taken with a Moticam image plus 2.0 digital ~~camera~~cameras.

Formatted: Font color: Auto

3.7 Data analysis

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Data collected were analyzed using one-way analysis of variance (ANOVA). The difference between the means was separated by least significant differences using GenStat Release statistical package 7.2 DE (PC windows). A difference in values less than a probability of 0.05% was considered significant.The results were presented as mean and standard error of mean using microsoft excel version 2010 to present the data in form of bar charts. Error bars indicated significant differences at $P<0.05$ among the variables.

Formatted: Font color: Auto

CHAPTER FOUR

RESULTS

4.1 Acute Toxicity of lead nitrate in *Clarias gariepinus* Juveniles

The results of the 48 hour acute toxicity study of lead nitrate in *Clarias gariepinus* juveniles carried out with four juveniles mean weight 39.80 ± 1.54g, mean length 21.05±1.23cm, one in each aquarium tank are shown in Table 8.

There was no mortality at 20mg/l concentration of lead nitrate. Mortality occurred in 100, 300 and 600mg/l of lead nitrate after 48 hour exposure.

The LC_{50} of lead nitrate was 60 mg/l

Formatted: Left

Formatted: Centered

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Table 8: Determination of LC_{50} of Lead nitrate in *C. gariepinus* juveniles

Tank	Dose	No of Fish	Observation after 48 Hours
1.	20 mg/l	1	Alive
2.	100 mg/l	1	Dead
3.	300 mg/l	1	Dead
4.	600 mg/l	1	Dead

According to Enegide *et al* (2013)

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

4.2 Acute toxicity of Vitamin C in *Clarias gariepinus* juveniles.

The results of the 48-hour acute toxicity study of vitamin C in *Clarias gariepinus* carried out with four juveniles mean weight 43.0±0.18g, mean length 21.6 ± 0.63cm placed one in each a

There was no mortality at 50mg/l,100mg/l and 150mg/l of Vitamin C. However mortality occurred at 200mg/l and 400mg/l of vitamin as shown in Table 9.

The LC_{50} of Vitamin C (Kepro®) was 175mg/l.

The doses of 50mg/l and 100mg/l were used for the assessment of their therapeutic effects on the pathology induced by lead nitrate on *Clarias gariepinus*, after chronic exposure for 91 days.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Table 9: Determination of LC_{50} of Vitamin C in *C. gariepinus* juveniles

Tank	Dose	No of Fish	Observation after 48 hours
1.	50 mg/l	1	Alive
2.	100 mg/l	1	Alive
3.	150 mg/l	1	Alive
4.	200 mg/l	1	Dead
5.	400 mg/l	1	Dead

According to Enegide *et al* (2013)

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font color: Auto
Formatted: Font color: Auto
Formatted: Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

4.3 Chronic toxicity of lead nitrate in *Clarias gariepinus* exposed for 91 days

Clarias gariepinus juveniles in group 1A had good feeding response and were swimming swiftly while those in 1A,1B and 1C had reduced feed intake, were lethargic and swimming sluggishly as the concentration of the lead nitrate increased from 5 to 15 mg/l. Coagulation of the skin mucus was observed in 1B,1C and 1D in increasing measure as the concentration of lead nitrate increased

4.3.1 The effect of lead nitrate on haematological parameters of *Clarias gariepinus* after 91 day exposure to varying concentrations.

The results obtained from the study revealed a significant decrease ($P<0.05$) in the mean PCV values of *Clarias gariepinus* juveniles exposed to lead nitrate for 91 days, with group 1D showing the highest decrease (Figure 1/Table 10) The least significant differences of means ($P< 0.05$) showed that lead nitrate exposure on the fish had a significant effect on catfish PCV between group 1A (control group) and groups 1B, 1C and 1D.

Figure1 showed significant decrease ($P< 0.05$) in the mean haemoglobin content of the blood of fish in groups 1C and 1D when compared to group 1A. There was no significant difference ($P>0.05$) in the mean haemoglobin content of the group 1B when compared to group 1A. The least

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto

significant difference of means (P<0.05) showed that lead nitrate exposure had a significant effect on the mean haemoglobin of the fish in groups 1C and 1D when compared to 1A and not on the mean haemoglobin of the fish in group 1B when compared to 1A.

Formatted: Font color: Auto

Figure 1 showed a significant decrease (P<0.05) in the mean RBC of fish in groups 1C and 1D when compared to 1A. There was no significant difference (P>0.05) in the mean RBC of group 1B when compared to group 1A. The least significant differences of means (P<0.05) showed that lead nitrate exposure had a significant effect on the mean RBC of fish in groups 1C and 1D when compared to group 1A.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

There was no significant difference (P>0.05) in the mean TWBC values of fish in groups 1B, 1C and 1D when compared to group 1A. The least significant differences of means (P<0.05) showed that lead nitrate had no significant effect on the mean TWBC in groups 1B,1C and 1D when compared to group 1A.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

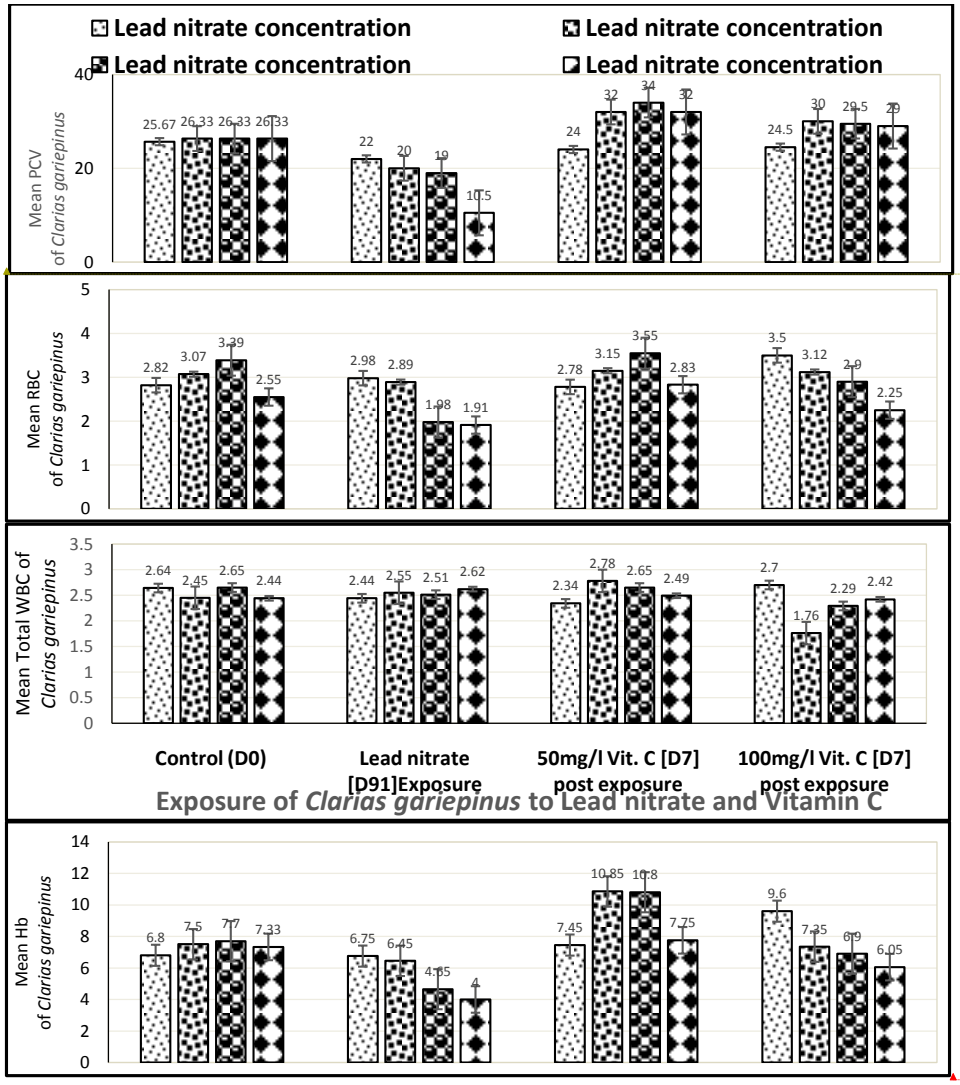

Figure 1: Means of haematological parameters [PCV, RBC, Hb, and TWBC] of *Clarias gariepinus* exposed for 91 days to Lead-nitrate, and for 7days post-exposures to 50mg/l and 100mg/l of Vitamin C, respectively. Error bars (Excel version 2010) indicate significant differences ($p<0.05$) between the variables.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.3.2 The effect of 7 day therapy with Vitamin C on haematological parameters of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

The mean haematological values of *Clarias gariepinus* treated with 50mg/l and 100mg/l of vitamin C for 7 days after 91 day exposure to varying concentrations of lead nitrate are shown in Figures 1 and 2.Figure 1 revealed a significant increase ($P<0.05$) in the mean PCV values of groups 1B,1C and 1D when compared to 1A. The least significant difference between means showed that there was a significant difference ($P<0.05$) between the mean PCV of 50mg/l vitamin C group and 100mg/l vitamin C group with 50mg/l vitamin C group showing more protective effect than the 100mg/l vitamin C group.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Figure 1 showed a significant increase ($P<0.05$) in the mean haemoglobin values of fish in groups 1B,1C and 1D when compared to 1A. Least significant differences between means showed that there was a significant difference ($P<0.05$) between the mean Hb values of 50mg/l Vitamin C and 100mg/l Vitamin C treated groups with 50mg/l vitamin C group showing more protective effect than the 100mg/l Vitamin C groups. Results obtained showed significant increase ($P<0.05$) in mean RBC values of fish in group IB, IC, and 1D when compared to 1A. The least significant difference between means showed that there was a significant difference ($P<0.05$) between the mean RBC values of 50mg/l Vitamin C and 100mg/l Vitamin C treated groups, with the 50mg/l vitamin C group showing more protective effect than 100mg/l vitamin C group. There was a significant increase ($P<0.05$) in the mean TWBC values of fish in groups 1B,1C and 1D when compared to group 1A. The least significant differences between means showed that Vitamin C had a significant effect ($P<0.05$) on the mean TWBC values of groups 1B,1C, and 1D when compared to 1A. The 100mg/l

Formatted: Font: 12 pt, Font color: Auto

vitamin C treated group had a more protective effect (P<0.05) than the 50mg/l Vitamin C treated groups.

Figure 2 (Table 10) showed a significant decrease (P<0.05) in the mean lymphocyte count of fish in group 1A, 1B and 1D when compared to day zero data. There was a significant increase (P<0.05) in the mean lymphocyte count of fish in groups 1C when compared to 1A and day zero data.There was a significant increase (P<0.05) in the mean monocyte count of fish in groups 1A,1B,1C, and 1D when compared to the day zero data. The least significant differences of means (P<0.05) showed that lead nitrate had a significant effect on the mean monocyte count of fish in groups 1A,1B,1C and 1D when compared to the day zero data with the highest significant increase (P<0.05) on the mean monocyte count of fish in group 1B.

Figure 2 showed a significant increase (P<0.05) in the mean neutrophil count of fish in groups 1A,1B,1C and 1D when compared with day zero mean neutrophil count. Figure 2 also showed a significant increase (P<0.05) in the mean lymphocyte count of fish in group ID treated with 100mg/l Vitamin C when compared to the group treated with 50mg/l Vitamin C. The least significant differences between means showed a significant increase (P<0.05) in the mean lymphocyte count between the control 1A and treated groups 1B, 1C and 1D, with the highest increase in the group 1D treated with 50mg/l Vitamin C. The figure showed a significant increase (p<0.05) in the mean monocyte count of fish occurring in group 1A, 1B and 1D treated with 50mg/l Vitamin C when compared with the group treated with 100mg/l Vitamin C with the highest mean monocyte count occurring in group 1C exposed to 10mg/l lead nitrate and treated with 100mg/l Vitamin C. The results showed a significant increase (P<0.05) in the mean neutrophil count of fish exposed to lead and those treated with 50mg/l Vit.C and 100mg/l Vit.C. The least significant

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

differences between means showed a significant increase ($P<0.05$) between the mean neutrophil count of the 50 mg/l vitamin C treated group than the 100 mg/l vitamin C treated group with the highest increase occurring in 1B (group exposed to 5mg/l lead nitrate) and treated with 50 mg/l vitamin C.

Formatted: Font color: Auto

Formatted: Font color: Auto

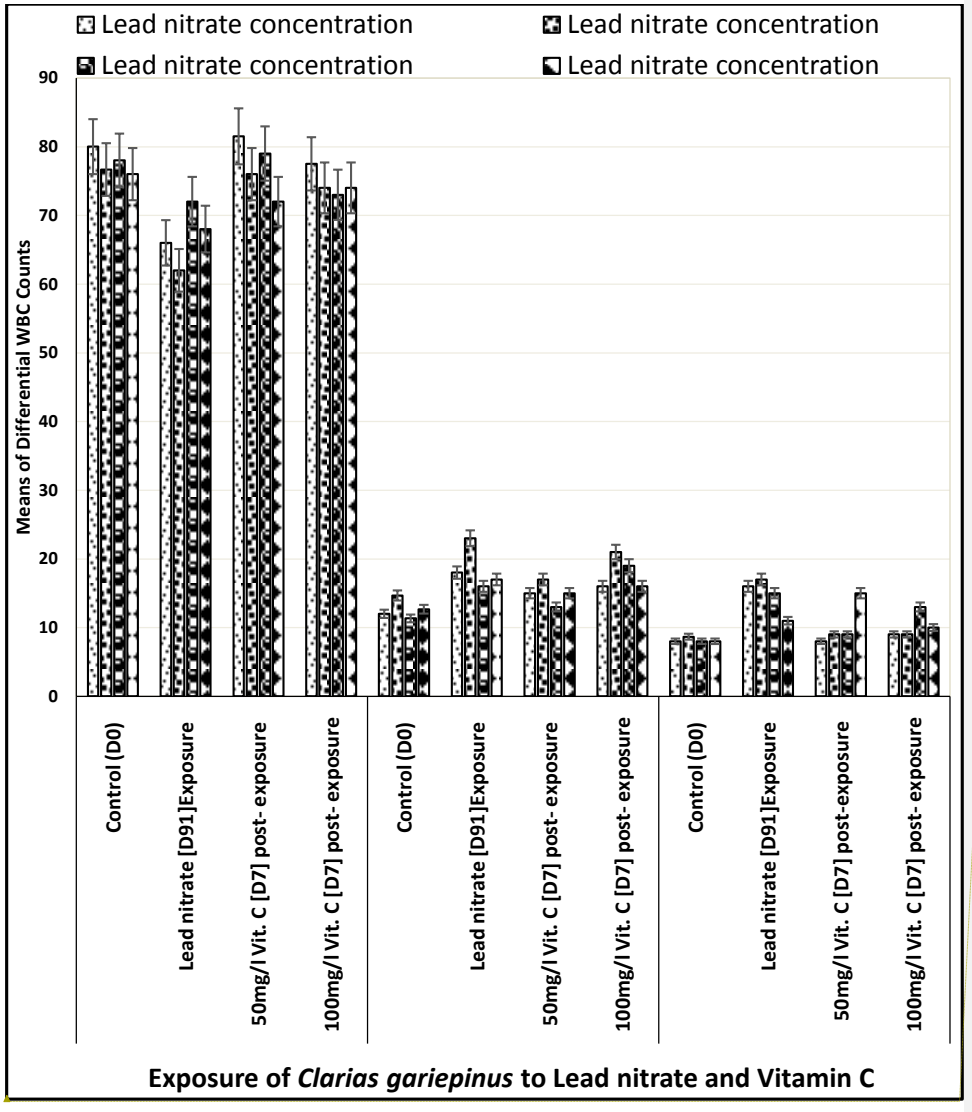

Figure 2:Differential white blood cell counts of *Clarias gariepinus* at 91 days exposure to lead nitrate and at 7 days post- exposures to 50 mg/l and 100 mg/l of vitamin C. Error bars indicate significant differences (P<0.05) between the variables (MS Excel version 2010).

Formatted: Font color: Auto

Formatted: Font color: Auto

Formatted: Font color: Auto

4.3.3 The effect of lead nitrate on the serum biochemistry of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91days.

The results of the serum biochemical values are as shown in figure 3 (Table 11). The results showed significant decrease ($P<0.05$) in the mean values of AST of fish in groups 1B, 1C and 1D when compared to 1A.The least significant differences between means showed lead nitrate had a significant effect ($P<0.05$) on the mean values of AST values of fish in groups 1B 1C and 1D when compared to 1A.

There was a significant decrease ($P<0.05$) in the mean values of ALT of fish in 1B, 1C and 1D when compared to 1A. The least significant difference between means showed that lead nitrate had a significant effect ($P<0.05$) on the mean ALT values of fish in groups 1B 1C and 1D when compared to 1A, with the least difference occurring in group 1C.

Figure 3 showed a significant increase ($P<0.05$) in the mean values of ALP of fish in groups 1B and 1C, and a significant decrease ($P<0.05$) in group 1D when compared to 1A.

There was a significant increase ($P<0.05$) in the mean values of urea of fish in groups 1B, 1C and 1D when compared to 1A, with highest increase occurring in group 1D. The least significance differences between means showed that lead nitrate had a significant effect ($P<0.05$) on the mean urea values of fish in groups 1B, 1C and 1D when compared to 1A.

4.3.4. The effect of 7 days therapy with vitamin C on the serum chemistry of *Clarias Gariepinus* exposed to varying concentrations of lead nitrate for 91 days.

The results of the serum biochemical values of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days and treated with 50mg/l and 100mg/l vit. C for 7 days are shown in Figure 3 and Table 11.

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

The results showed significant decrease (P<0.05) in the mean AST values of the fish exposed to varying concentrations of lead nitrate when compared with the day zero data and control treatment with 50 mg/l vit. C (P<0.05) resulted in a significant decrease of the mean AST values when compared to control, while treatment with 100 mg/l vit. C resulted in a significant increase (P<0.05) in the mean AST values of all experimental groups including the control when compared to the day zero data and the 50 mg/l vit. C treated group.

The results showed a significant decrease (P<0.05) in the mean ALT values of fish in the 50 mg/l vit C and 100 mg/l vit. C treated groups, when compared to day zero data and control except in the 5mg/l lead nitrate group treated with 100 mg Vitamin C and that showed mean ALT value of 46.00 ± 1.00 iu/l

The results showed a significant increase (P<0.05) in the mean ALP values of the fish exposed to varying concentrations of lead nitrate when compared with the control and day zero data groups. Treatment with 50mg/l Vitamin C resulted in a significant decrease (P<0.05) in the mean ALP values when compared to the day zero data and control group, while treatment with 100 mg/l resulted in a significant increase (P<0.05) in the mean ALP values when compared to the day zero data and control groups.

The results showed significant decrease (P<0.05) in the mean urea values of 50 mg/l vit C and 100mg/l vit C treated groups. The least significant difference (P<0.05) between means showed a significant difference (P< 0.05) between the lead exposed group and the Vit. C. treated groups, with the least significant mean urea value occurred in the 5 mg/l lead exposed group treated with 100 mg/l Vitamin C.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

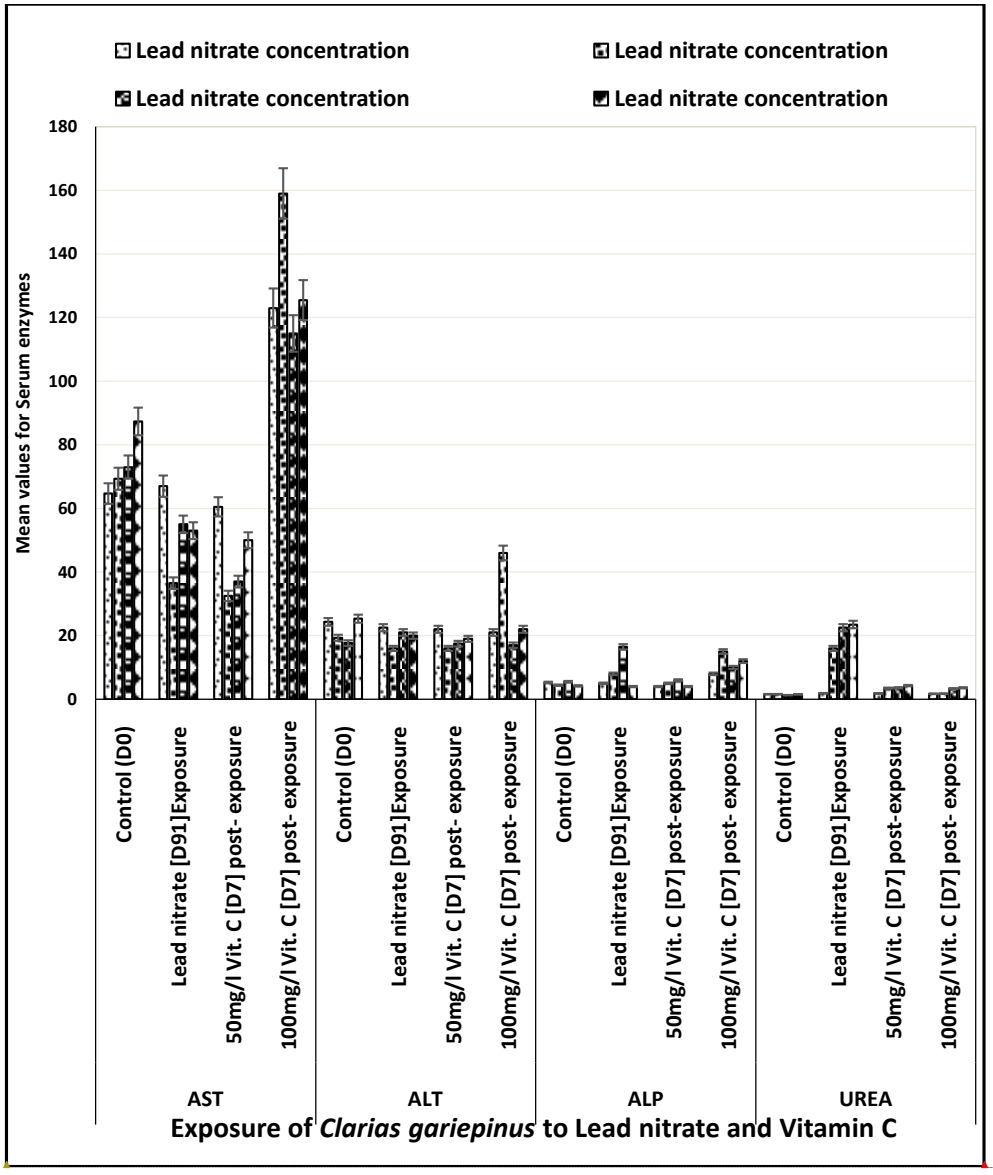

Figure 3 : Mean values of serum enzymes [AST, ALT, ALP and Urea] of *Clarias gariepinus* at 91 days exposure to Lead nitrate, and at 7days post-exposures to 50mg/l and to 100mg/l of Vitamin C. Error bars indicate significant differences ($p<0.05$) between the variables (MS Excel version 2010).

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.3.5 The levels of lead nitrate in the muscle of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days

The results of the mean values of lead nitrate retained in the muscle of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days are shown in figure 4 (Table 12). The results showed significant increase ($P<0.05$) in the mean values of lead nitrate retained in the muscles of *Clarias gariepinus* after 91 days exposure to varying concentrations of lead nitrate in groups 1B,1C and 1D when compared with 1A.

The least significant differences of means showed that lead nitrate significantly deposited ($P<0.05$) in the muscles of the fish in groups 1B,1C, and 1D when compared to 1A and day zero data.

4.3.6 The effect of Vitamin C on the levels of lead in the muscle of *Clariasgariepinus* exposed to lead nitrate.

The results of the concentration of lead retained in the muscles of *Clarias gariepinus* treated with Vitamin C for 7 days after 91 days exposure to lead are presented in figure 4 (Table 11). The results showed a significant increase ($P<0.05$) in the mean muscle lead concentration in the fish in the experimental groups when compared to the day zero data and control groups. A significant increase ($P<0.05$) also occurred in the mean muscle lead concentration in 1B, 1C, and 1D when compared to day zero data and control group. The highest lead concentration was found in group 1C (10mg/l lead nitrate group) while the least was in group 1B (5mg/l ead nitrate group).

The results showed that in the Vitamin treated groups, there was a significant difference ($P<0.05$) between the 50mg/l Vitamin C treated group and the 100mg/l Vitamin C treated group. The 50mg/l Vitamin C treated group showed increased levels of lead concentration in the muscles of the

treated fish when compared to the day zero data, control and 100mg/l Vitamin C treated group. In the 100mg/l Vitamin C treated group, there were little or no traces of lead in the muscles of the fish in groups $2A_2$, $2B_2$, $2C_2$ and $2D_2$ when compared to the 50mg Vitamin C treated group. The results of the lead concentration in the muscles of *Clarias gariepinus* treated concurrently with lead and 100mg/l Vitamin C for 91 days are also shown in figure 4(Table 12). The results showed a significant increase ($P<0.05$) in the mean muscle lead concentration of fish in groups $2B_2$, $2C_2$ and $2D_2$ when compared to $2A_2$ (control) and the day zero data.

Formatted: Font color: Auto

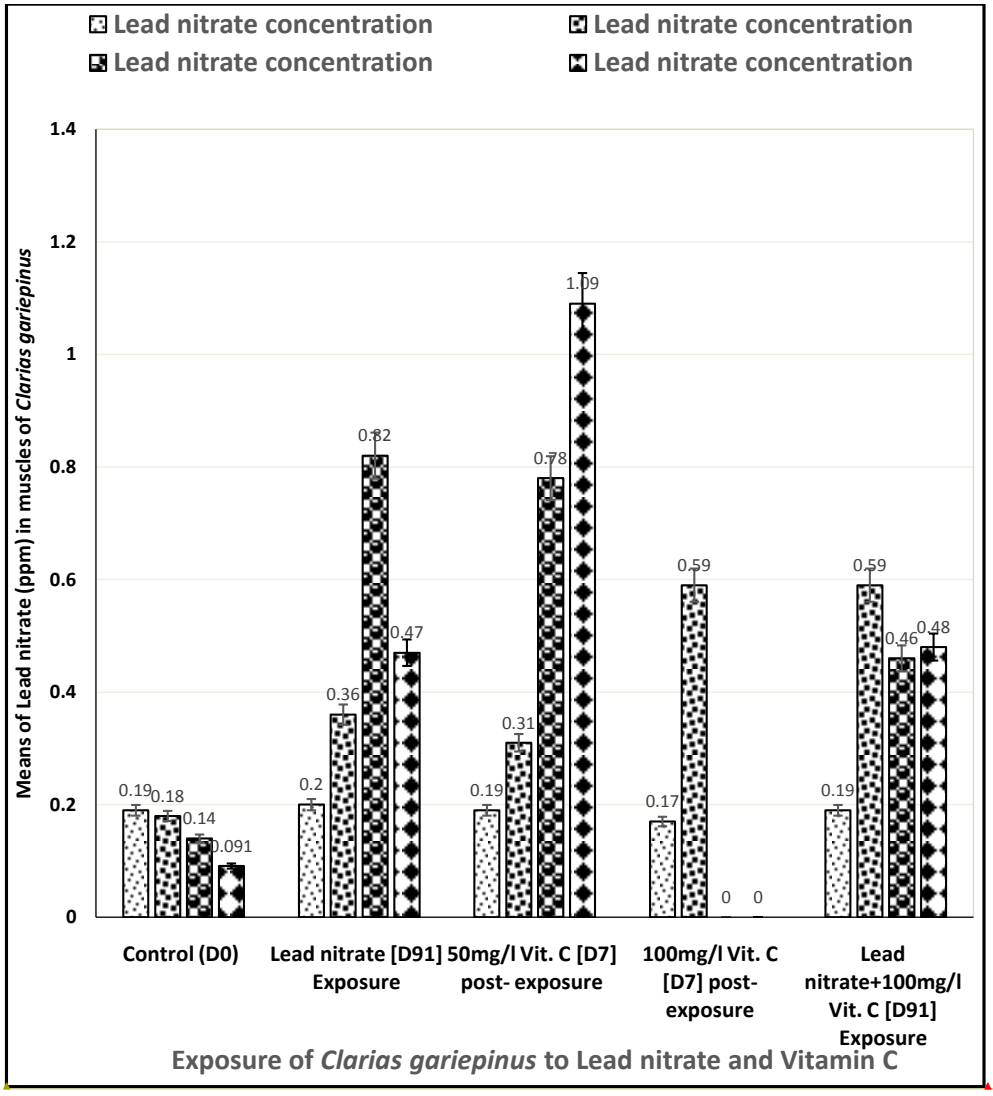

Figure 4: Mean values of Lead nitrate concentration (ppm) in muscles of *Clarias gariepinus* at 91 days exposure to Lead nitrate, 7days post-exposures to 50mg/l of Vitamin C, 7days post-exposures to 100mg/l of Vitamin C, and 91 days exposure to a combination of Lead nitrate and 100mg/l of Vitamin C. Error bars indicate significant differences ($p<0.05$) between the variables (MS Excel version 2010).

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.4 Histology of the gills, liver and stomach of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days.

Histopathological -changes in the gills of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days.

4.4.1

The observed changes in the gills of *Clarias gariepinus* exposed to varying concentrations of lead nitrate showed a typical structural organization of the gill in the control group 1A (0mg/l lead nitrate) characterized by accelular bones, adductor muscle, intact structural arrangement of the primary and secondary lamellae **(Plate 1)**.

The gills of *C.gariepinus* exposed to 5 mg/l lead nitrate (group 1B) showed minor distorted lamellae (**Plate 2).** In group 1C (10mg/l lead nitrate), the photomicrograph of the gill showed distorted and necrosed primary and secondary lamellae. **(Plate 3)**.

The gills of the fish in group 1D (15mg/l lead nitrate) were severely destroyed and irregular in shape (**Plate 4).**

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Normal, Justified, Line spacing: single
Formatted: Font: 12 pt, Font color: Auto
Formatted: List Paragraph, Left, Outline numbered + Level: 3 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0 cm + Indent at: 1.27 cm
Formatted: Font: Bold
Formatted: Font: (Default) Times New Roman, Font color: Auto
Formatted: List Paragraph, Left, Indent: First line: 0 cm
Formatted: Font color: Auto
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman, Font color: Auto
Formatted: List Paragraph, Left
Formatted: Font: (Default) Times New Roman
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

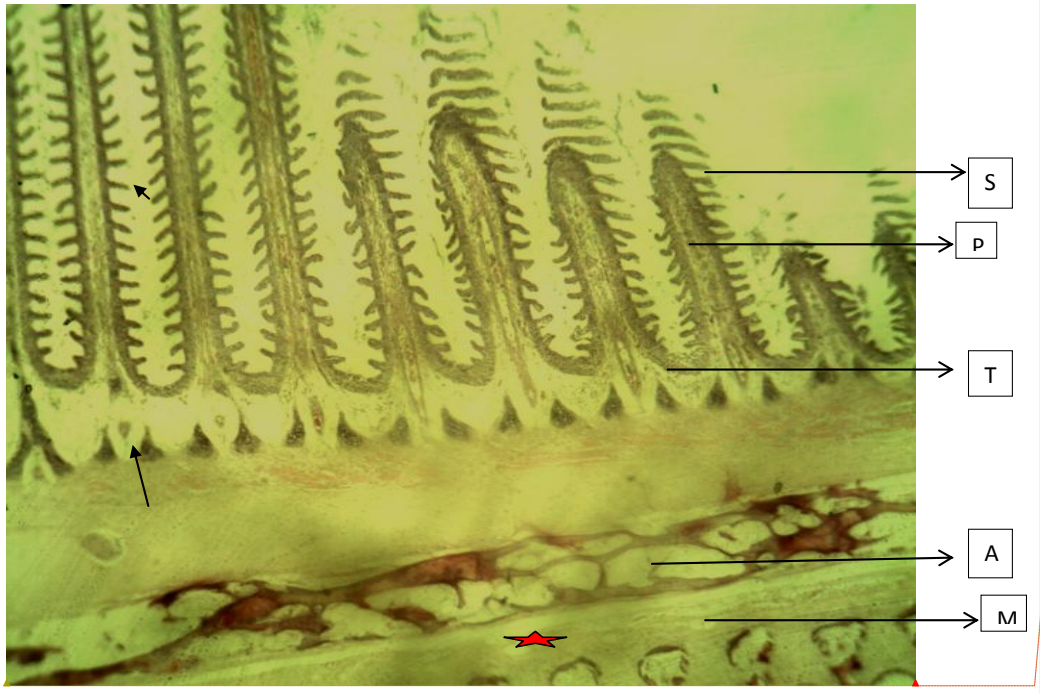

PLATE 1: Photomicrograph of control gill exposed to 0mg/l lead. Showing normal gill structure, striated abductor muscle (M), acellular bone (A), intact primary (P) and secondary (S) gill lamellae, afferent arteries (T). H& E X100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

PLATE 2: Photomicrograph of gills exposed to 5mg/l Lead showing minor distorted lamellae (D) and hyperemic afferent arteries (H).
H&E X 100.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

PLATE 3: Gills exposed to 10mg/l lead showing curled gill lamellae and hyperemic afferent arteries (H). H&E X 100.

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

PLATE 4: Gills exposed to 15mg/l lead showing severely destroyed, irregularly shaped Lamellae (I) H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.4.2 The effect of vitamin C. on the histopathological changes in the gills of *Clarias gariepinus* exposed to varying concentrations of lead nitrate.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

The observed histological changes in the gills of *Clarias gariepinus* exposed to 10 mg/l of lead nitrate showed distorted and necrotic primary and secondary lamellae (Plate 3). Treatment with 50mg/l Vitamin C resulted in the restoration of the primary and secondary lamellae as in (Plate 5). The gills of *C.gariepinus* exposed to 15mg/l Lead nitrate showed severely damaged and irregularly shaped lamellae (Plate 4). However, treatment with 100mg/l vitamin C showed restoration of the lamellae, enlargement of the central venous sinus with clear afferent intact arteries as shown in (Plate 6).

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Plate 5: Gill exposed to 10mg/l lead and treated with 50 mg/l vitamin C showing intact gill lamellae (black arrow) with clear/intact afferent arteries (A) H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

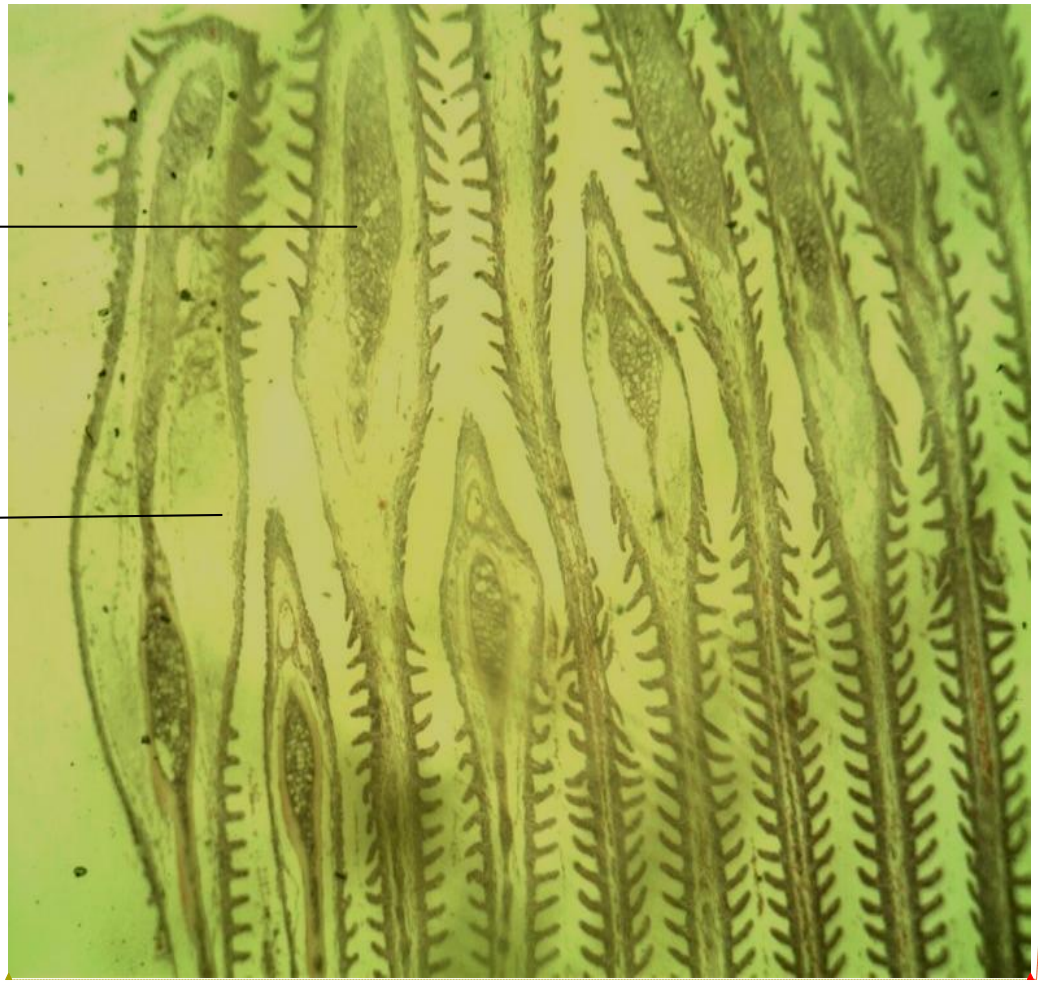

Plate 6: Gill exposed to 15mg/l lead and treated with 100mg/l vitamin C showing enlargement of the central venous sinus arteries (V) with clear intact afferent arteries (A) H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.4.3 Histopathological changes in the liver of Clarias gariepinus **exposed to varying concentrations of lead nitrate for 91days**

The photomicrograph of the liver tissues of *C.gariepinus* exposed to 0 mg/l lead nitrate (1A, control group) showed prominent central vein and intact hepatocytes **(plate 7).**The photomicrograph of liver of group 1B (5mg/l lead nitrate) showed cytoplasmic vacuolations (**plate 8)** liver of group 1C (10mg/l lead nitrate) showed pyknotic nuclei in the hepatocytes **(Plate 9)**. The liver of group 1D (15mg/l lead nitrate) showed degenerated hepatocytes (**Plate 10**)

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

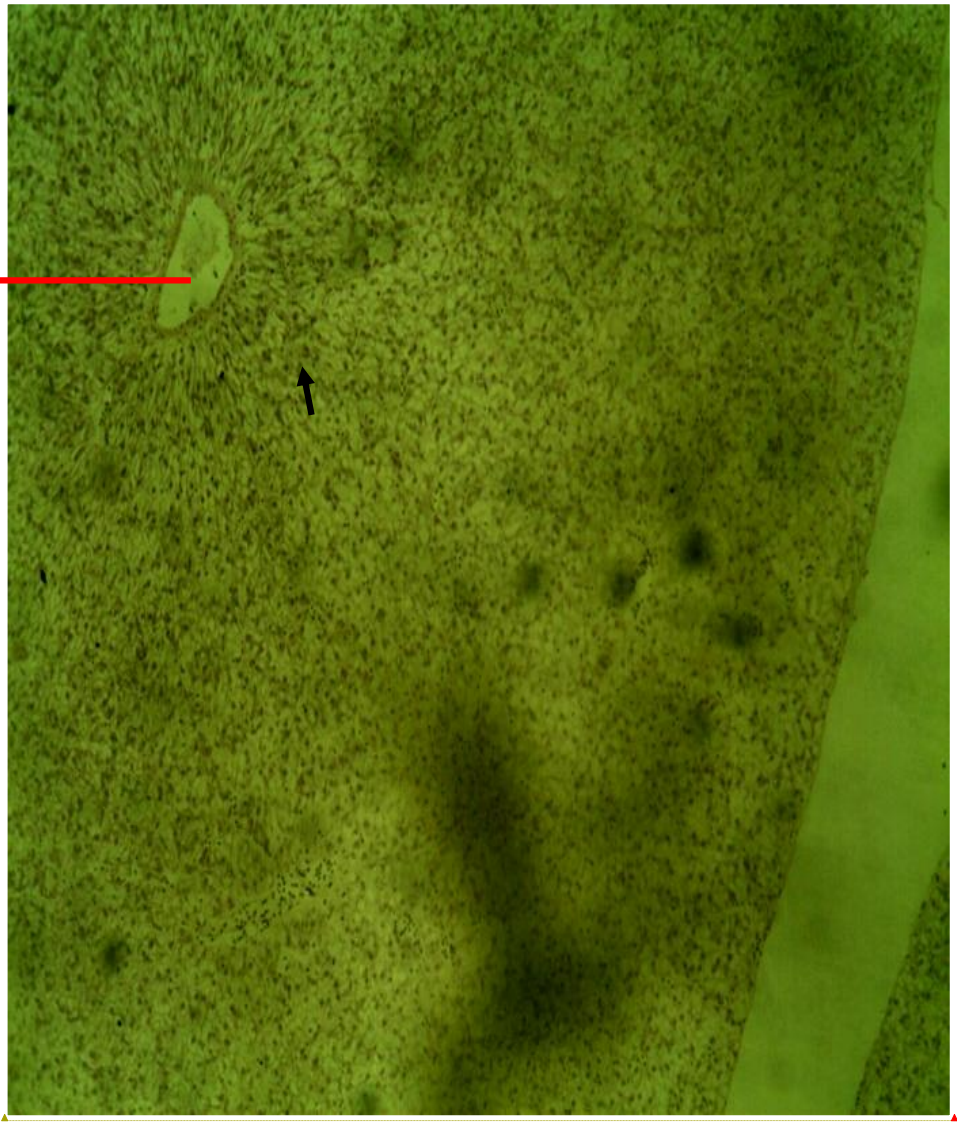

PLATE 7. Photomicrograph of normal liver of *C. gariepinus* exposed to 0mg/l lead showing Normal liver architecture with intact hepatocytes (black arrow) and central vein (CV). H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

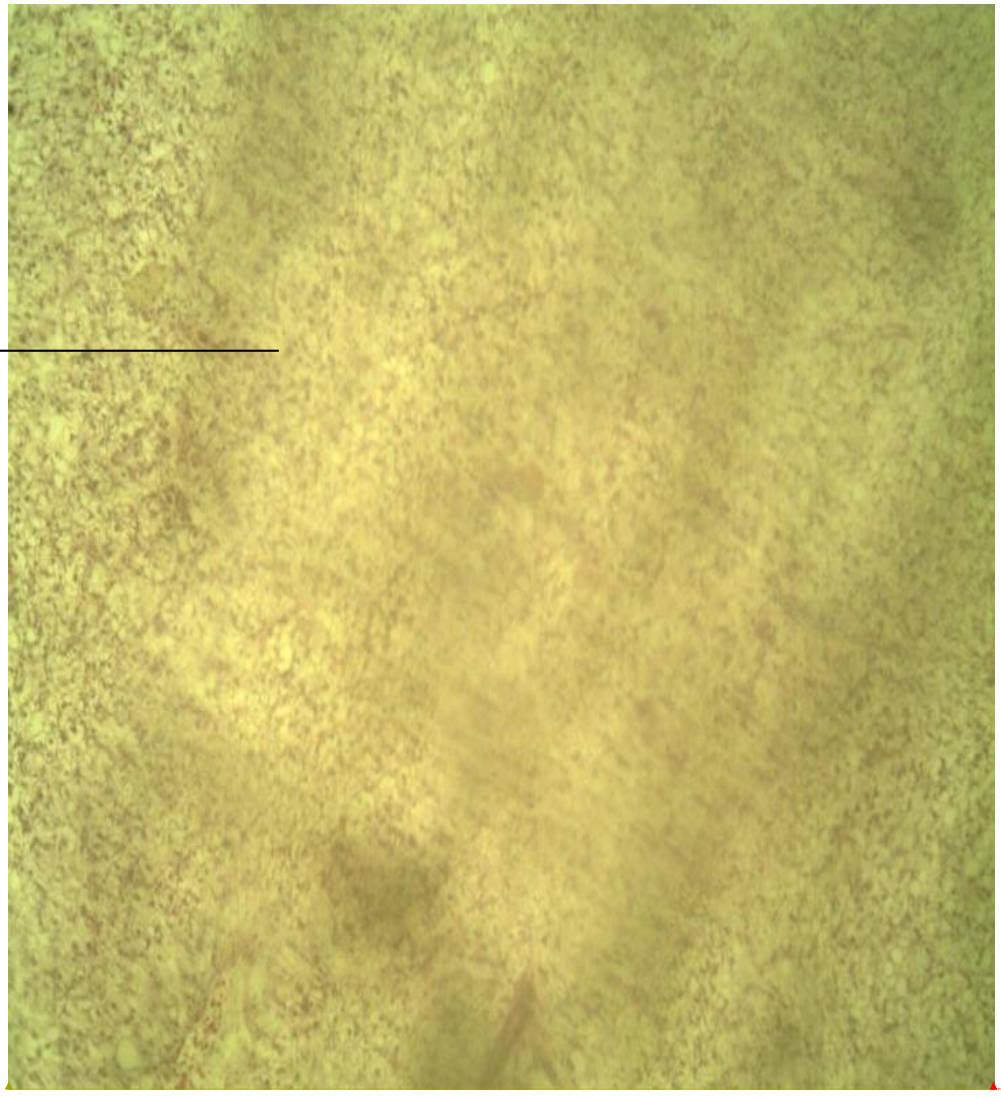

PLATE 8: Photomicrograph of liver of *C. gariepinus* exposed to 5mg/l lead showing cytoplasmic vacuolations (C) H&E X 100.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

PLATE 9: Liver of *C. gariepinus* exposed to 10mg/l lead showing hepatocytes lacking nucleus and undergoing cell death (AP) H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

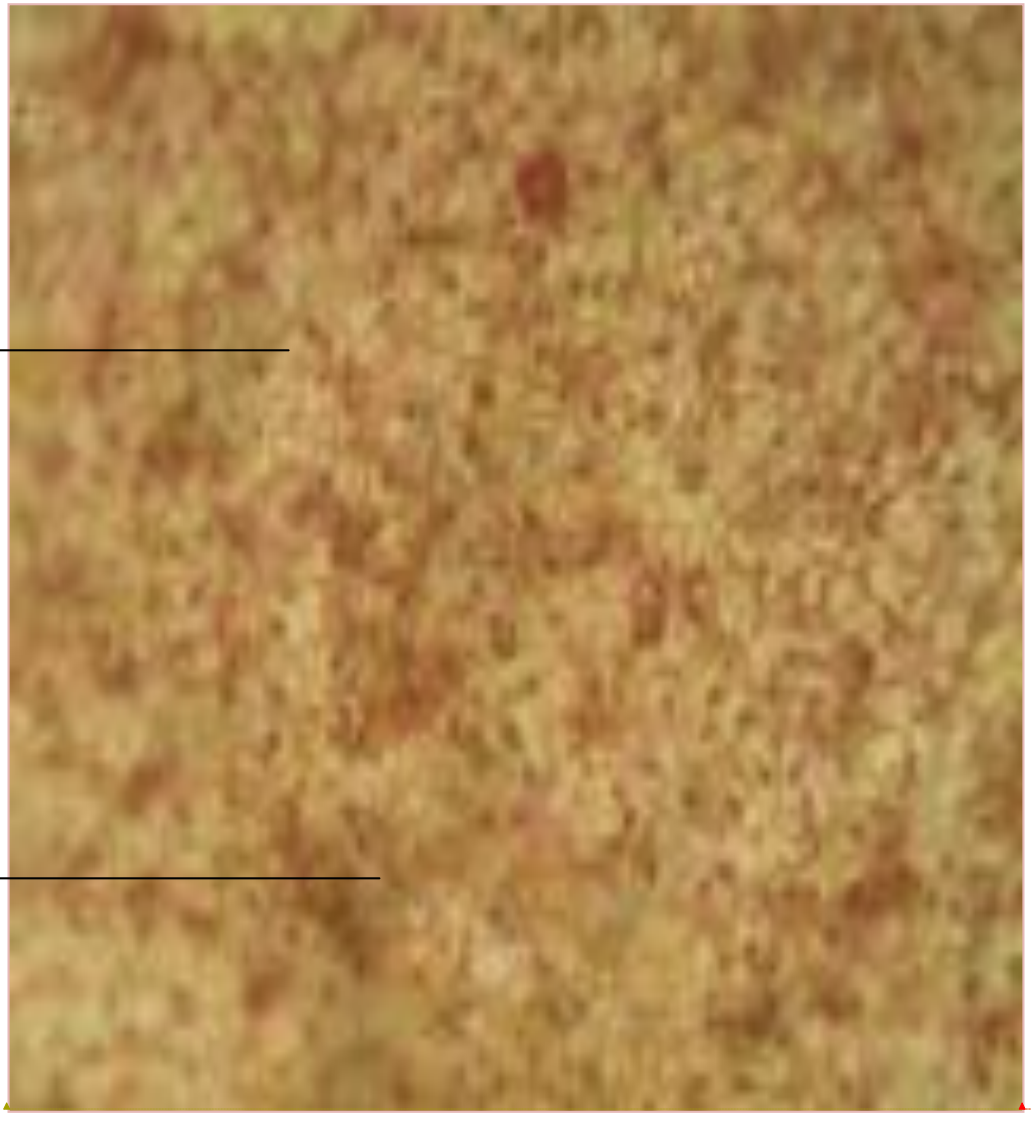

PLATE 10: Liver of *C. gariepinus* exposed to 15mg/l lead showing cytoplasmic vacuolations (C) and necrosis of hepatocytes (N) H&E X 100.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.4.4 The effect of vitamin C. on the histopathological changes in the Liver of *Clarias gariepinus* exposed to varying concentrations of lead nitrate.

Observed histological changes in the liver of *C. gariepinus* exposed to 5 mg/l lead nitrate showed cytoplasmic vacuolations (Plate 8). Treatment with 50mg Vitamin C for 7 days after 91 days exposure showed liver with prominent central vein and sinusoids (Plate 11). The liver of *C. gariepinus* exposed to 15 mg/l lead nitrate showed degenerated hepatocytes (Plate 10). Treatment with 100mg/l Vitamin C for 7 days post 91 days exposure resulted in regenerated hepatocytes, with minor congestion of the central vein and necrosis as shown in (Plate 12).

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

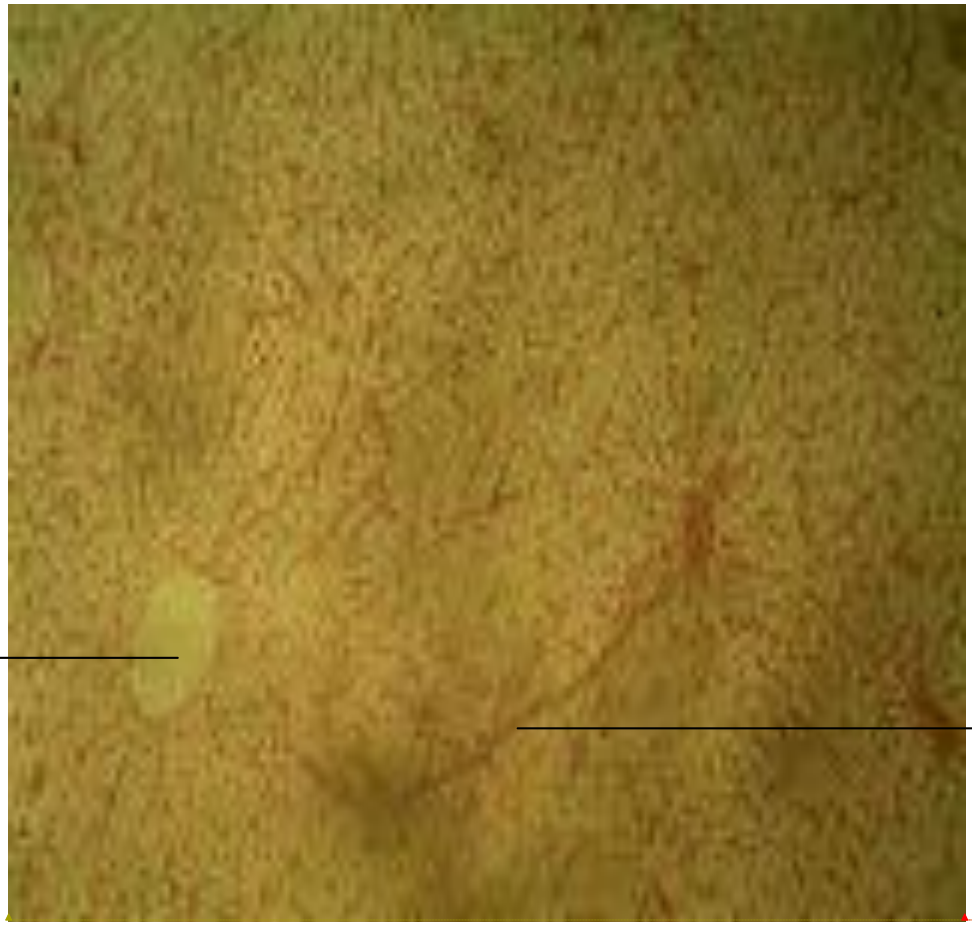

Plate 11: Liver exposed to 5mg/l lead and treated with 50mg/l vitamin C showing prominent central vein (CV) and sinusoid (S). H& E X100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font: 11 pt, Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font: 11 pt, Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font: 11 pt, Font color: Auto

Formatted: Font: 11 pt, Not Bold, Font color: Auto

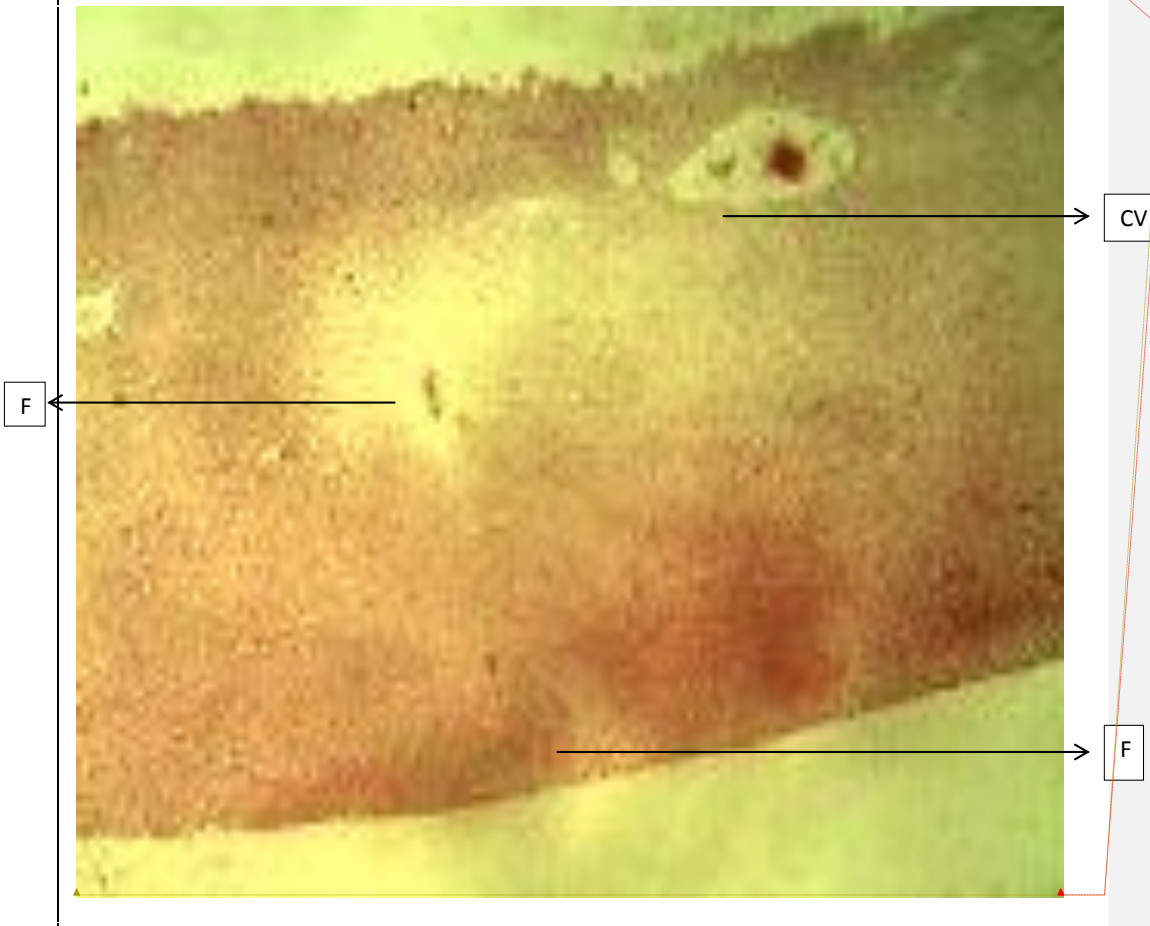

Plate 12: Liver exposed to 15mg/l lead and 100mg/l vitamin C showing minor congestion of the central vein (CV) and necrotic foci (F), H & E X100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font: 11 pt, Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font: 11 pt, Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.4.5 Histopathological changes in the stomach of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days.

The photomicrograph of the stomach tissue of *Clarias gariepinus* exposed to 0 mg/l lead nitrate (1A, control group) showed normal stomach histoarchitecture characterized by intact mucosa, muscularis mucosa (white star) and gastric pits (black arrow). **(Plate 13).**The group exposed to 5 mg/l lead nitrate (1B) showed haemorrhages in the mucosa **(Plate 14).**

The group exposed to 10mg/l lead nitrate (1C) showed petechial haemorrhages in the muscularis mucosa, broken crypts and necrosis and degeneration of the columnar epithelial cells. **(Plate 15).**The group exposed to 15 mg/l lead nitrate (1D) showed haemorrhages in the muscularis mucosa and degeneration of the epithelial mucosal cells. (**Plate 16)**

Formatted: Font: 12 pt, Font color: Auto
Formatted: Line spacing: single
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Line spacing: single
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

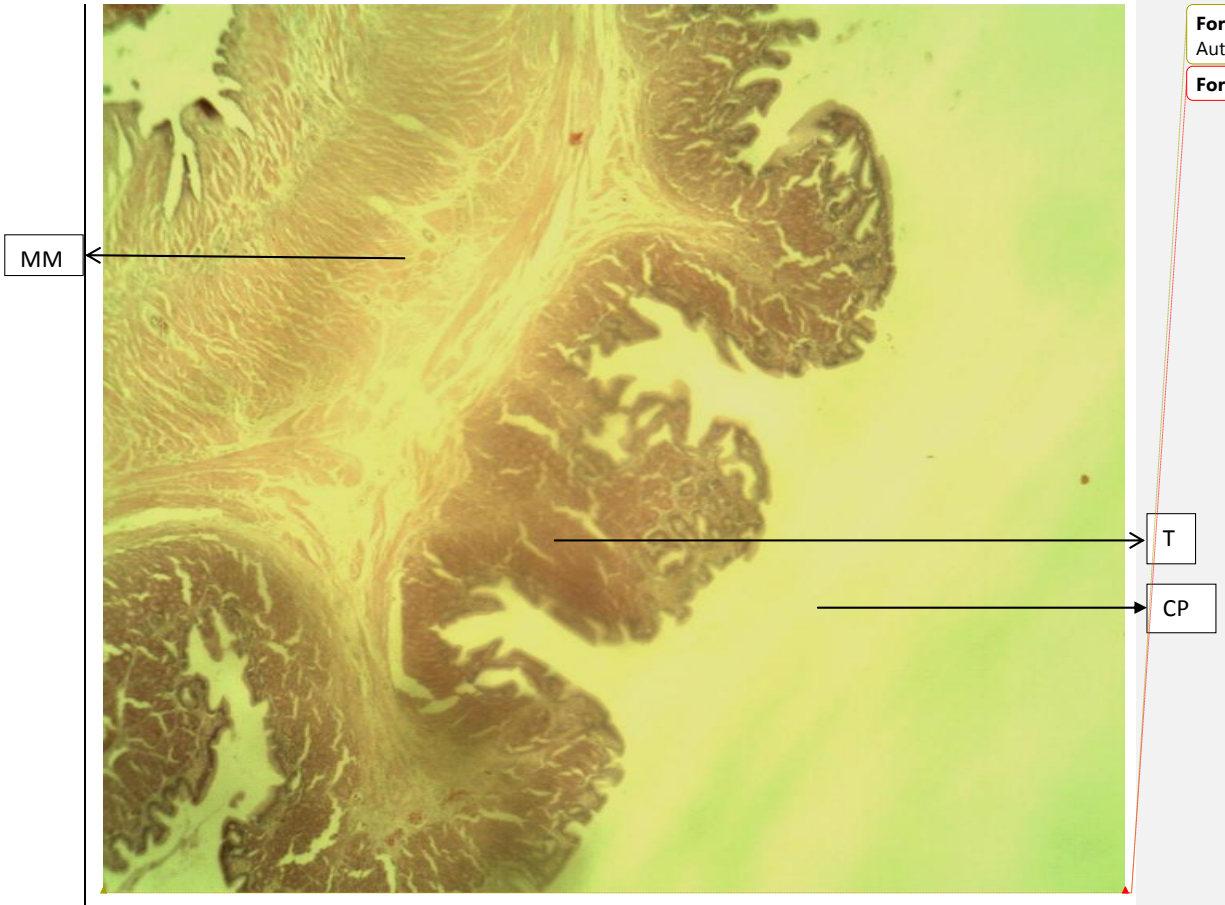

PLATE 13: Photomicrograph of normal stomach of *Clarias gariepinus* intact mucosa (T), muscularis mucosa (MM) and gastric pits (CP). H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

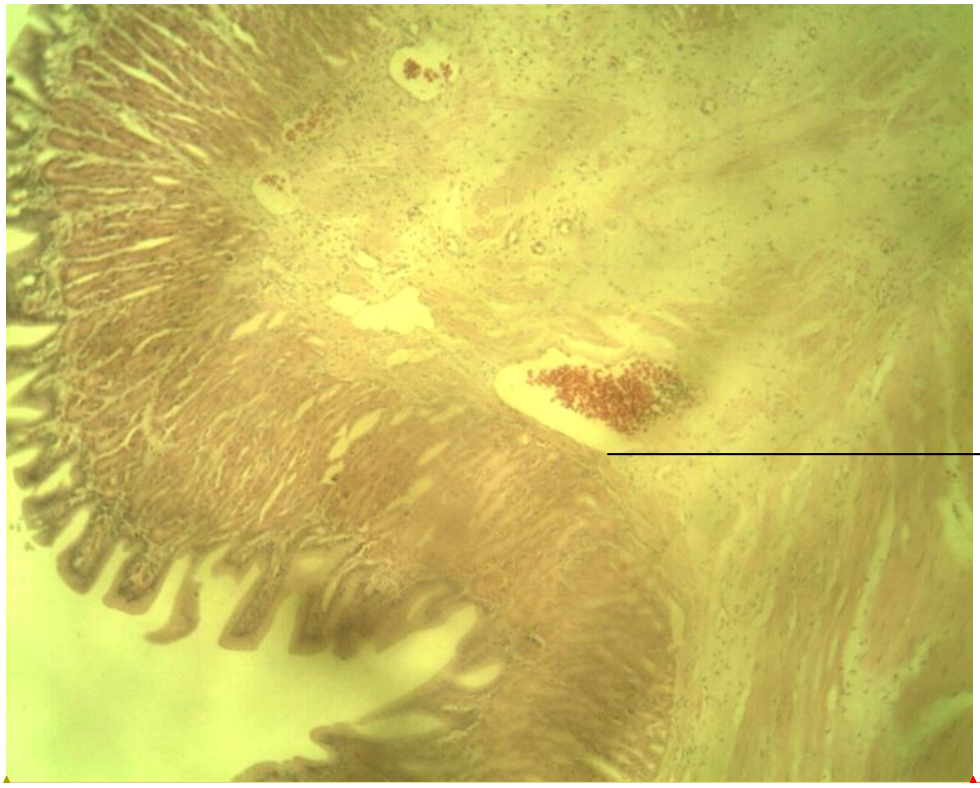

PLATE 14: Stomach exposed to 5mg/l lead showing minor submucosal haemorrhages in the stomach tissue (SH) H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

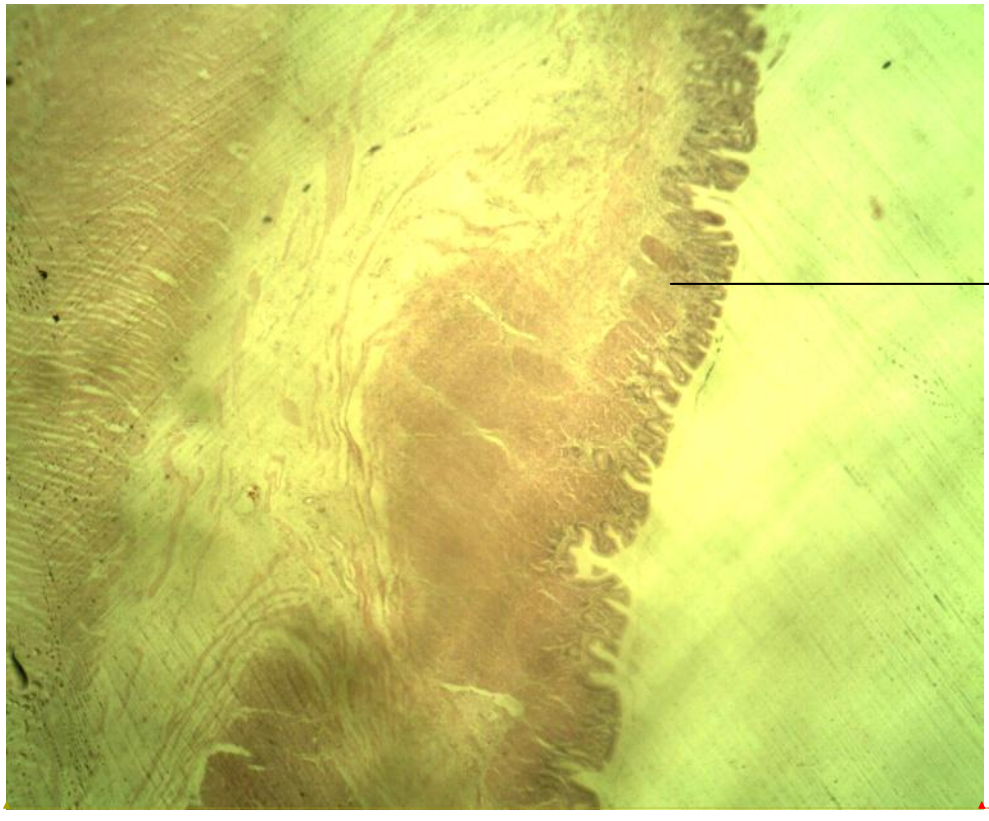

PLATE 15: Stomach exposed to 10mg/l lead showing minor submucosal haemorrhages in the stomach tissue (SH) H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

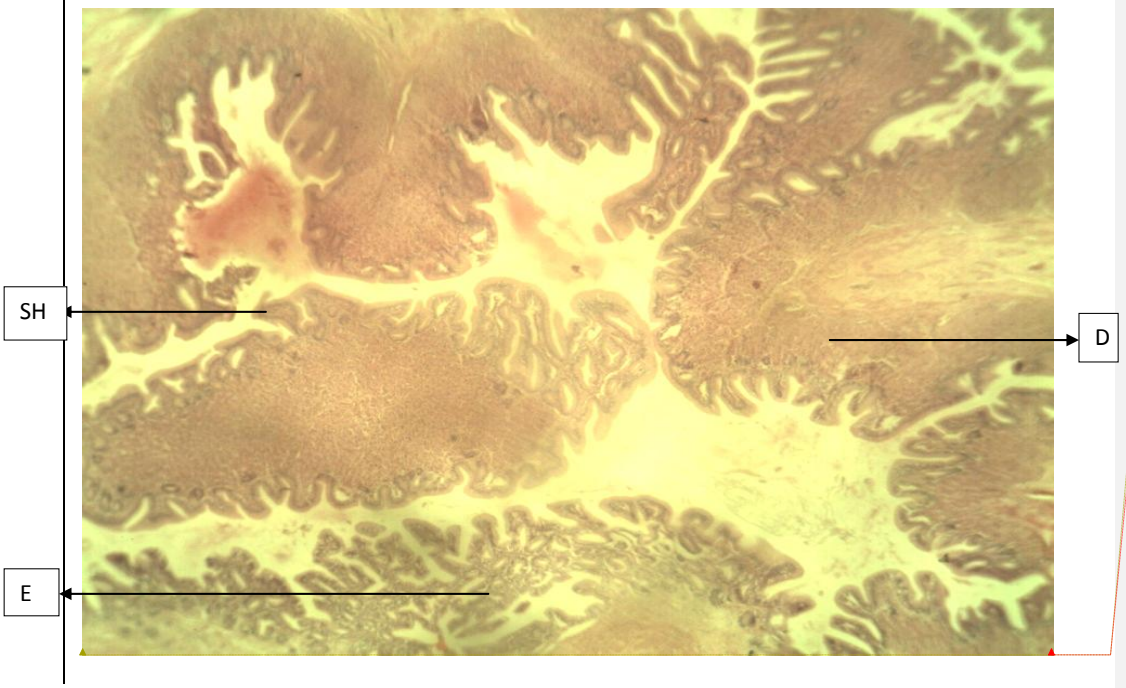

PLATE 16: Stomach exposed to 15mg/l lead showing minor submucosal haemorrhages (SH) erosion ofthe mucosal lining (E) and degenerative changes in the muscularis mucosa (D), H&E X100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.4.6 The effect of vitamin C on the histopathological changes in the stomach of *Clarias gariepinus* exposed to varying concentrations of lead nitrate

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

The observed histological changes in the stomach of *Clarias gariepinus* exposed to 5mg/l of lead nitrate showed haemorrhages in the mucosa (Plate 14). However, treatment with 50mg/l Vitamin C shows intact mucosa and muscularis mucosa (Plate 17) while treatment with 100mg/l vitamin also resulted in intact stomach mucosa and gastric pits (Plate18). The stomach of *C. gariepinus* exposed to 15mg/l lead nitrate showed haemorrhages in the mucosa, degeneration of the mucosal epithelial cells (Plate 16). Treatment with 50mg/l Vitamin C resulted in minor lesions in the stomach mucosa (plate 19) while 100mg/l Vitamin resulted in intact muscularis mucosa (Plate 20).

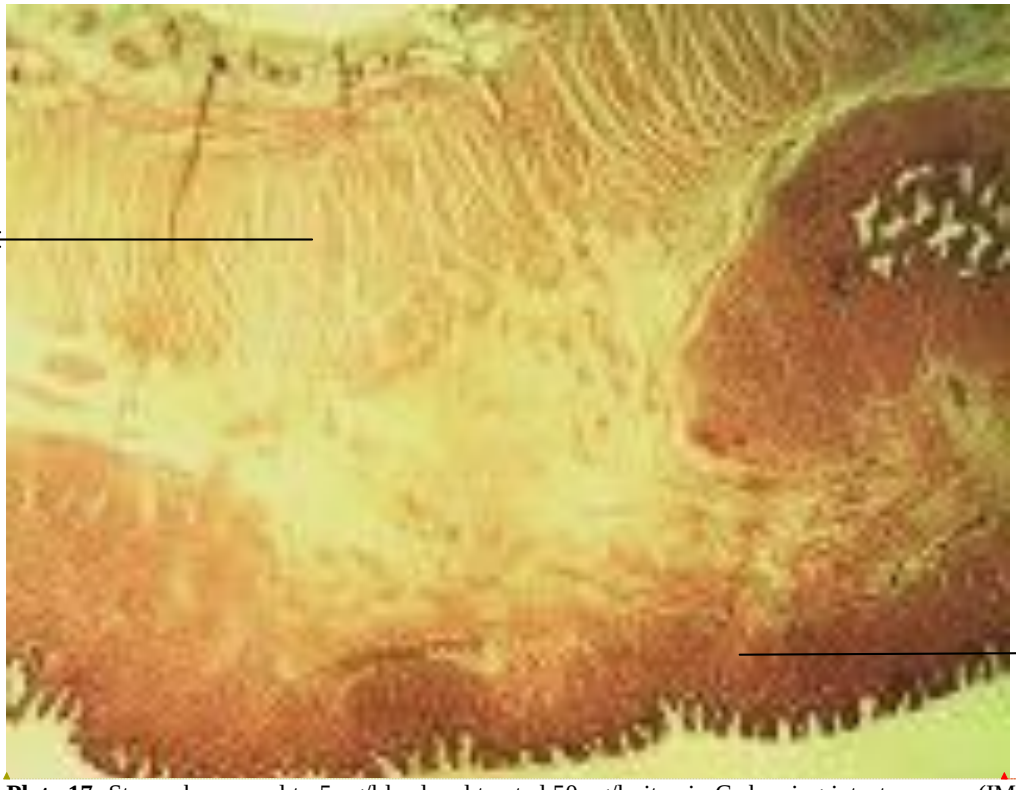

Plate 17: Stomach exposed to 5mg/l lead and treated 50mg/l vitamin C showing intact mucosa (IM) and muscularis mucosa (MM) H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

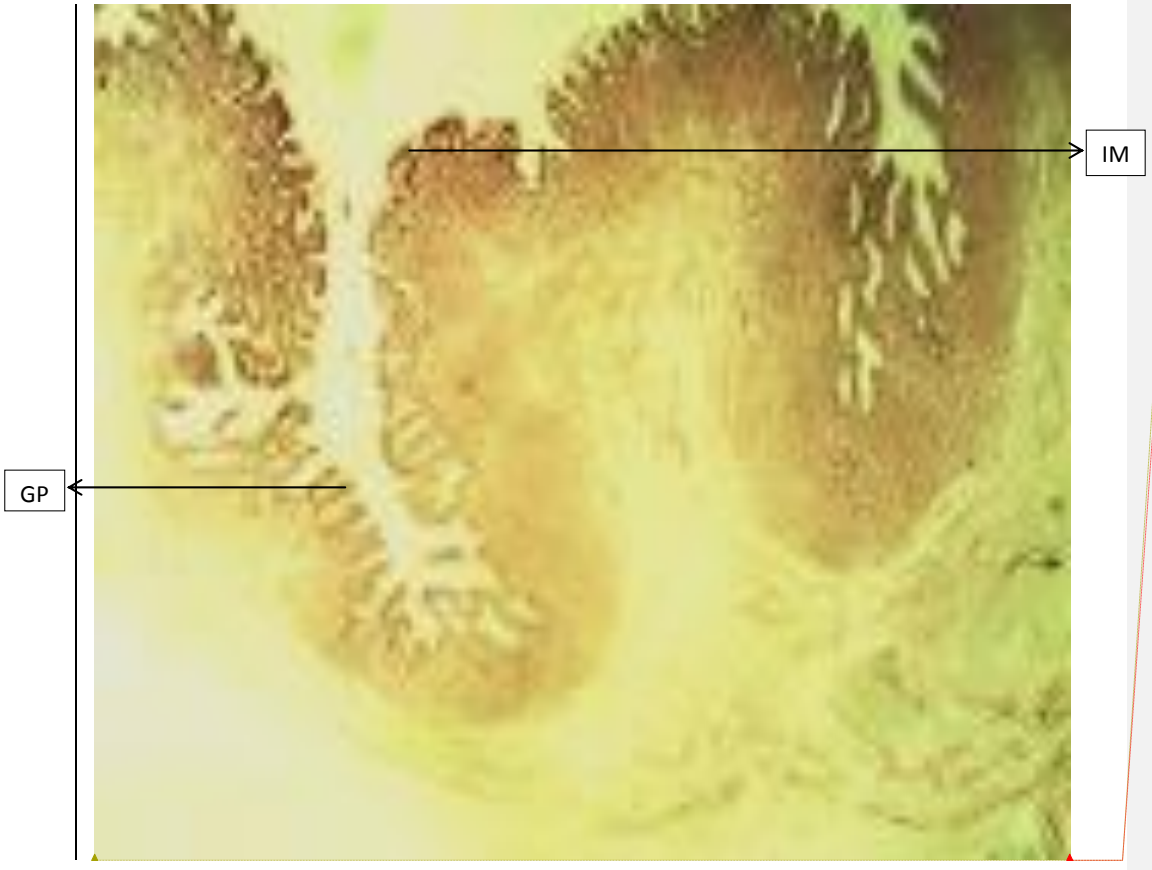

Plate 18: Stomach exposed to 5mg/l lead and treated 100mg/l vitamin C showing intact stomach mucosa (IM) and gastric pits (GP). H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

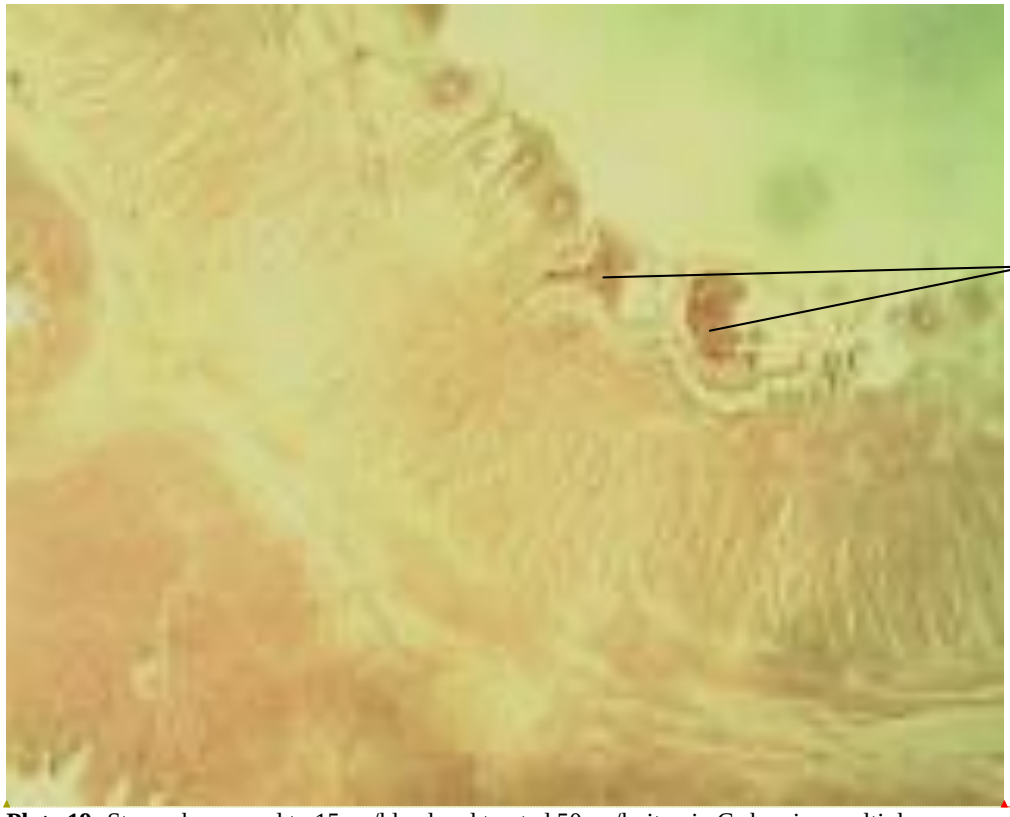

Plate 19: Stomach exposed to 15mg/l lead and treated 50mg/l vitamin C showing multiple lesions in the mucosa (LM). H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

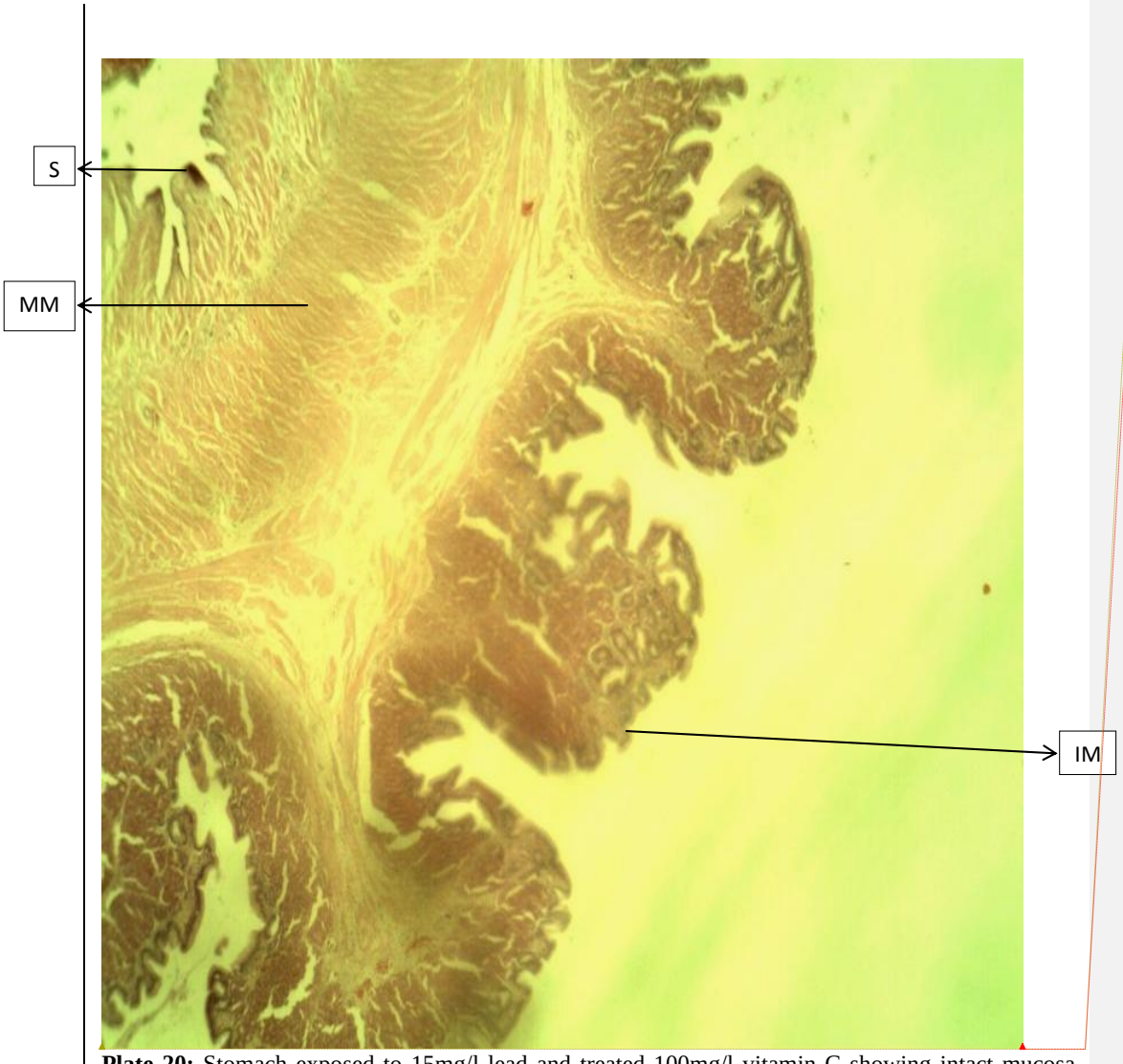

Plate 20: Stomach exposed to 15mg/l lead and treated 100mg/l vitamin C showing intact mucosa muscularis (MM), intact mucosa (IM) and serosa (S).H & E X100

☆

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.5.*Clarias gariepinus* concurrently exposed to lead nitrate and Vitamin C. in varying concentrations (Experiment 2)

4.5.1: The effects of concurrent exposure to lead nitrate and Vitamin C on the haematological parameters of *Clarias gariepinus*

The results of the mean haematological values of *Clarias gariepinus*concurrently exposed to lead nitrate and vitamin C in varying concentrations are shown in Figure 5 (Table 13).

The results showed a significant decrease ($P<0.05$) in the mean PCV of fish in the experimental groups when compared to the day zero data and control groups with the least mean PCV of 13.00 ± 1.00 % in group $2C_2$. However, there was a significant increase ($P<0.05$) in the mean PCV of fish treated concurrently with 10 mg/l lead nitrate and 50mg/l Vit.1C (group $2C_1$).

There was a significant difference ($P<0.05$) between the mean Hb values of fish in groups, $2B_2$, $2C_2$ and $2D_2$ when compared to day zero data, control and $2B_1$, $2C_1$ and $2D_1$, with the least mean Hb value of 3.70 ± 0.30 mg/dl in group $2B_2$ (5mg lead and 100mg/l vitamin C).

The results showed a significant decrease ($P<0.05$) in the mean RBC values of fish in groups $2B_2$, $2C_2$ and $2D_2$, when compared to baseline, control and $2B_1$, $2C_1$, and $2D_1$, with the least mean RBC value in group $2B_2$ (5mg lead and 100mg/l vitamin C).

The results showed that there was no significant difference ($P>0.05$) between the mean TWBC of day zero data and the lead /100mg/l vitamin C group. However, there was a significant difference ($P<0.05$) between the TWBC of the 10mg/l lead nitrate/50 mg/l vitamin C ($2D_1$)and the 10mg/l lead nitrate /100mg/l vitamin C of *Clarias gariepinus* ($2D_2$) concurrently exposed to lead nitrate and vitamin C.

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font color: Auto

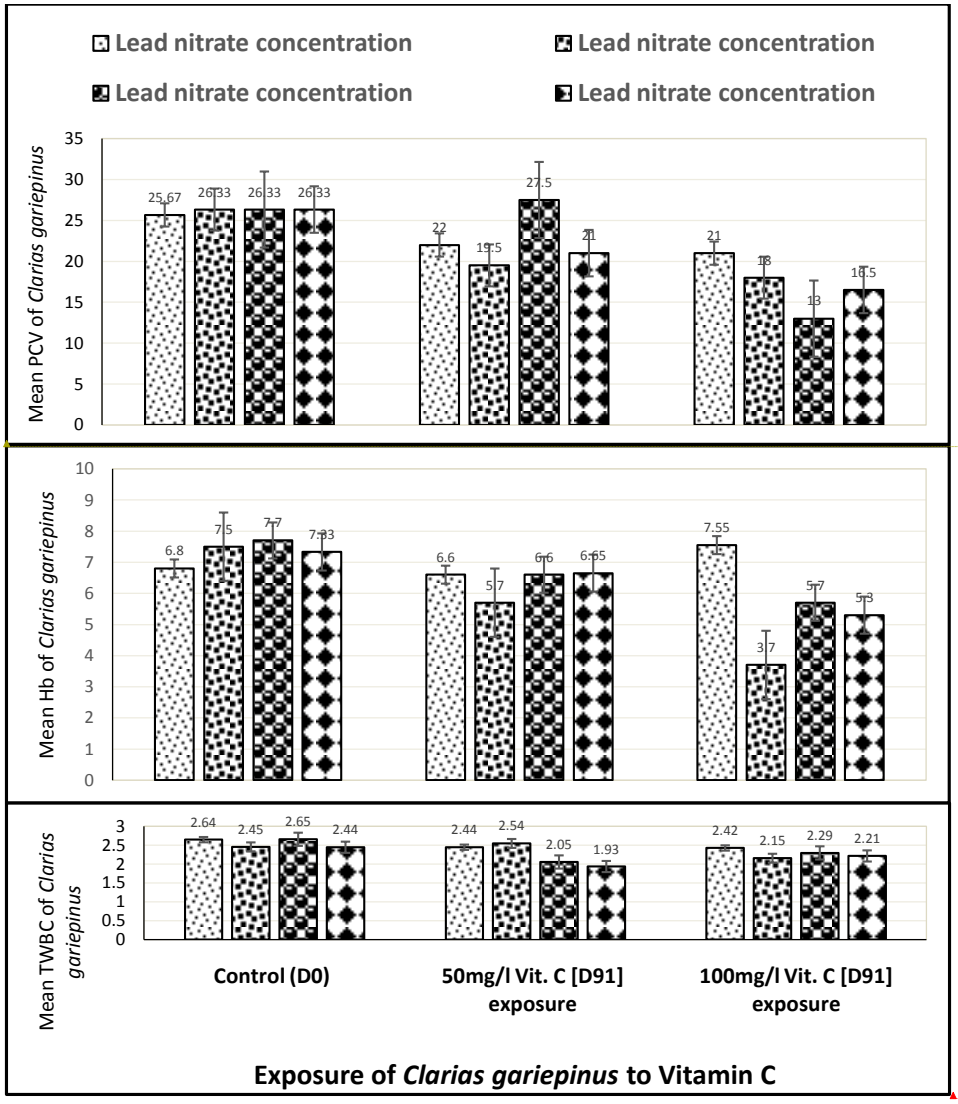

Figure 5: Mean values of PVC, Hb, TWBC of *Clarias gariepinus* at 91 days concurrent exposures to Lead nitrate concentrations, 50mg/l of Vitamin C, 100mg/l of Vitamin C. Error bars indicate significant differences ($p<0.05$) between the variables (MS Excel version 2010)

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

The results of the mean DWBC count of *Clarias gariepinus* concurrently exposed to lead nitrate and vitamin C in varying concentrations are shown in figure 6 (Table 13).There was a significant increase ($P< 0.05$) in the mean lymphocyte count of lead/50mg/l vitamin C group when compared to the lead/ 100mg/l vitaminC group, except in group 2D where $2D_1$ was significantly higher than $2D_2$. There was a significant decrease ($P<0.05$) between the mean monocyte values of fish in the control group and the experimental groupswhen compared to the day zero data. Between the two experimental groups, there was no significant difference ($P>0.05$) between the mean monocyte counts. The results showed a significant increase ($P<0.05$) in the mean neutrophil count of the fish between the day zero data and the experimental groups with the highest mean neutrophil count in group $2B_1$ (5 mg lead and 50mg/l Vitamin C). There was no significantdifference ($P>0.05$) in the mean neutrophil count of the fish in the 15mg/l lead nitrate/ 50mg/l Vitamin C group when compared to the control group, butthere was a significant increase $P<0.05$) in the neutrophil count of 15mg/l lead nitrate/100mg/l vitamin C group when compared to the 15 mg/l lead/50mg/l vitamin C.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

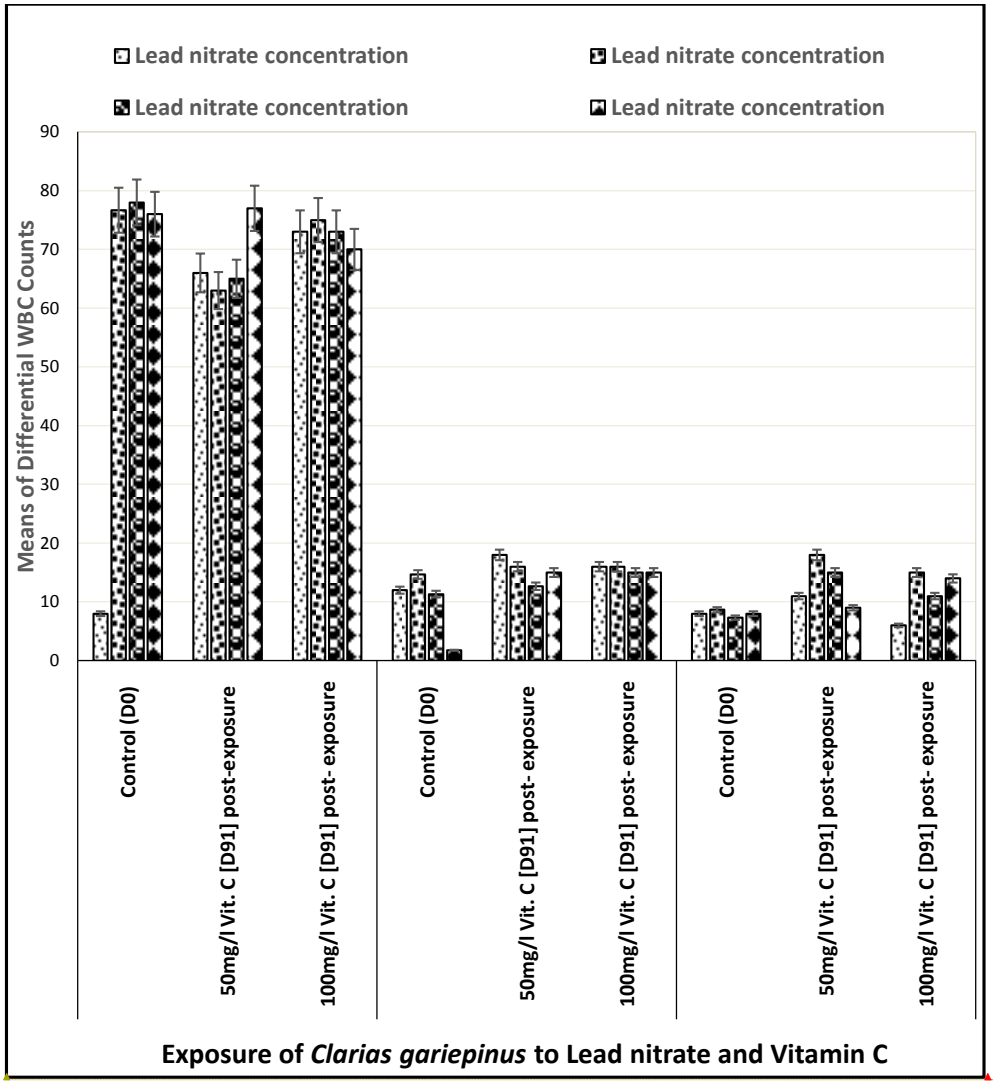

Figure 6: Differential White Blood Counts of *Clarias gariepinus* at 91 days concurrent exposures to Lead nitrate concentrations, 50mg/l of Vitamin C, 100mg/l of Vitamin C. Error bars indicate significant differences ($p<0.05$) between the variables (MS Excel version 2010

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.5.2 Serum biochemistry (AST, ALT,ALP) and Urea of *C. gariepinus* concurrently exposed to lead nitrate and Vitamin C for 91 days.

The results of the serum biochemical values of *Clarias gariepinus* given concurrent treatment of lead nitrate and Vitamin C for 91 days are presented in Figure 7 and Table 14.

The results showed a significant increase ($P<0.05$) in the mean AST values of groups $2B_1$, $2B_2$ and $2D_1$, when compared with the control group.The highest mean AST value occurred in group $2D_1$, (15mg lead and 50mg/l Vitamin C).

Similar result occurred in the mean ALT values of groups$2B_1$, $2B_2$ and $2D_1$ and $2D_2$ when compared with control group. Highest increase in mean ALT values occurred in groups $2D_1$ (15mg lead and 50mg/l Vitamin C).

The results showed no significant difference ($P>0.05$) in the mean ALP values of the experimental groups when compared with the day zero group. There was no significant difference ($P>0.05$)between the mean values of $2A_1$, $2B_1$, $2C_1$, and $2D_1$ (50mg/l Vitamin C) and $2A_2$, $2B_2$, $2C_2$ and $2D_2$ (100mg/l Vitamin C) when *Clarias gariepinus* juveniles were concurrently exposed to lead nitrate and vitamin C. There was no significant increase ($P>0.05$) in the mean urea values of the experimental groups when compared to day zero control group. There was no significant difference ($P>0.05$) between the mean urea values of the 50mg/l lead group and 100mg/l lead group.

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

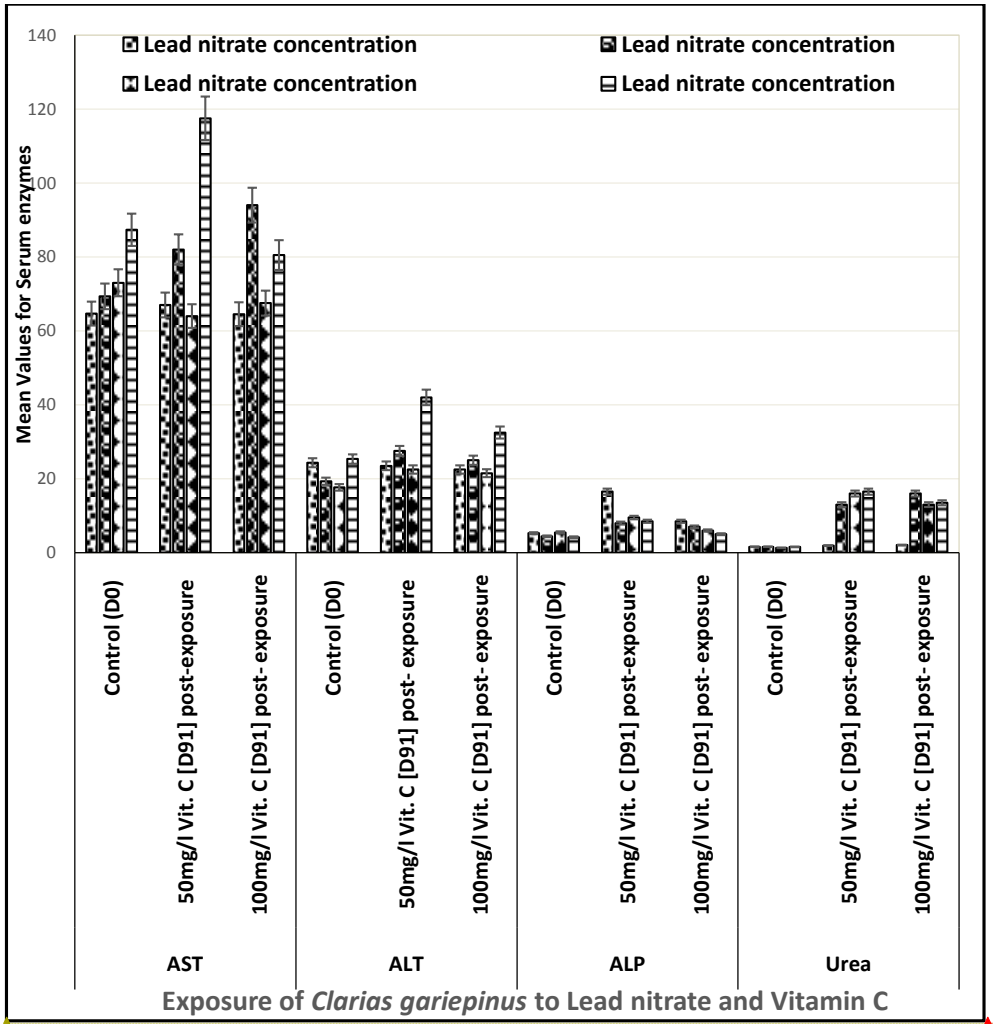

Figure 7: Mean values of serum enzymes [AST, ALT, ALP and Urea] of *Clarias gariepinus* at 91 days concurrent exposures to Lead nitrate concentrations, 50mg/l of Vitamin C, and 100mg/l of Vitamin C. Error bars indicate significant differences ($p<0.05$) between the variables (MS Excel version 2010).

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

4.5.3 The effect of Vitamin C on the histopathological changes in the gills, liver and stomach of *Clarias gariepinus* concurrently exposed to lead and Vitamin C

The observed histological changes in the gills of *Clarias gariepinus* exposed to 10mg/l of lead nitrate and 50mg/l vitamin C showed distorted and necrotic primary and secondary lamellae (Plate 21),while gills exposed to 10mg/l lead nitrate and 100mg/l vitamin C concurrently showed degenerated lamellae (Plate 22). The gills of *C.gariepinus* exposed to 15mg/l lead nitrate and 50mg/l vitamin C showed multiple curling of the lamellae (Plate 23).Gills exposed to concurrent 15mg/l lead and 100mg/l vitamin C showed severe destruction of the gill lamellae(Plate 24)

.Concurrent exposure to 5mg/l lead nitrate and 50mg/l vitamin C resulted in congested sinusoids and intact central vein in the liver section (Plate 25).Concurrent exposureto 5mg/l lead nitrate and 100mg/l vitamin C resulted in congested interlobular ducts and fibrosis of hepatocytes (Plate 26).Concurrent exposure to 15mg/l lead nitrate and 50mg/l vitamin C showed numerous necrotic foci in the liver with extensive cytoplasmic vacuolations and massive fatty degeneration (Plate 27). Concurrent exposure to 15 mg/l lead nitrate with 100mg/l vitamin C resulted in extensive destruction of the liver histoarchitecture shown in Plate 28 in form of extensive fatty degeneration of the hepatocytes and erosion of the interlobular ducts.

The stomach of *Clarias gariepinus* concurrentlyexposed to 5mg/l lead and 50mg/l vitamin showed loosened circular muscle and intact mucosa (Plate 29). That which was exposed to 5mg/l lead nitrate and 100mg/l vitamin C showed submucosal haemorrhages, intact muscularis and mucosa. (Plate 30). In the group exposed to 15mg/l lead nitrate and 50mg/l vitamin C,their stomach sections showed submucosal haemorrhages and cellular infiltration (plate 31), while that

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

concurrently exposed to 15 mg/l lead nitrate and 100mg/l vitamin Cshowed distorted mucosa, fibrosis of the muscularis mucosa,with the gastric lumen filled with inflammatory debris (Plate 32).

Formatted: Font color: Auto

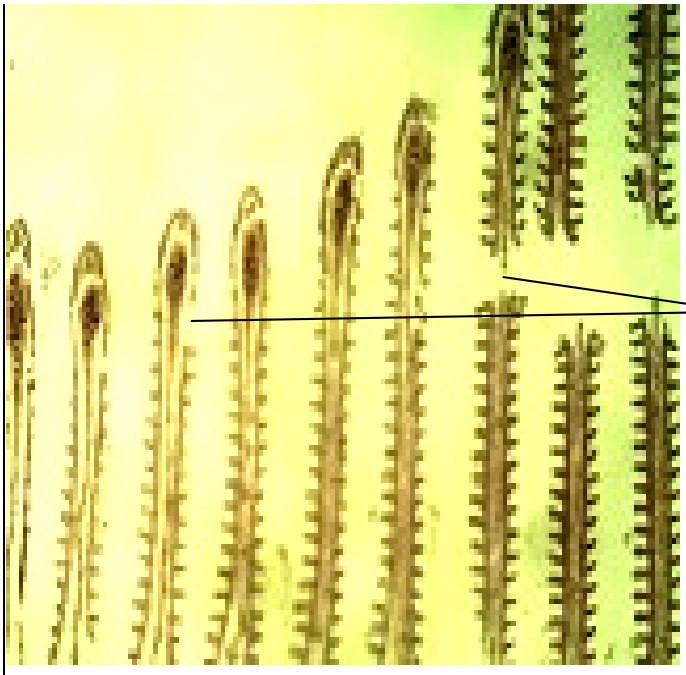

Plate 21: Gill exposed to concurrent 10mg/l lead and 50mg/l vitamin C showing minor distorted lamellae (D) H&E X 100.

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

Plate 22: Gill exposed to concurrent 10mg/l lead and 100mg/l vitamin C showing degenerated gill lamellae (DG). H&E X 100.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

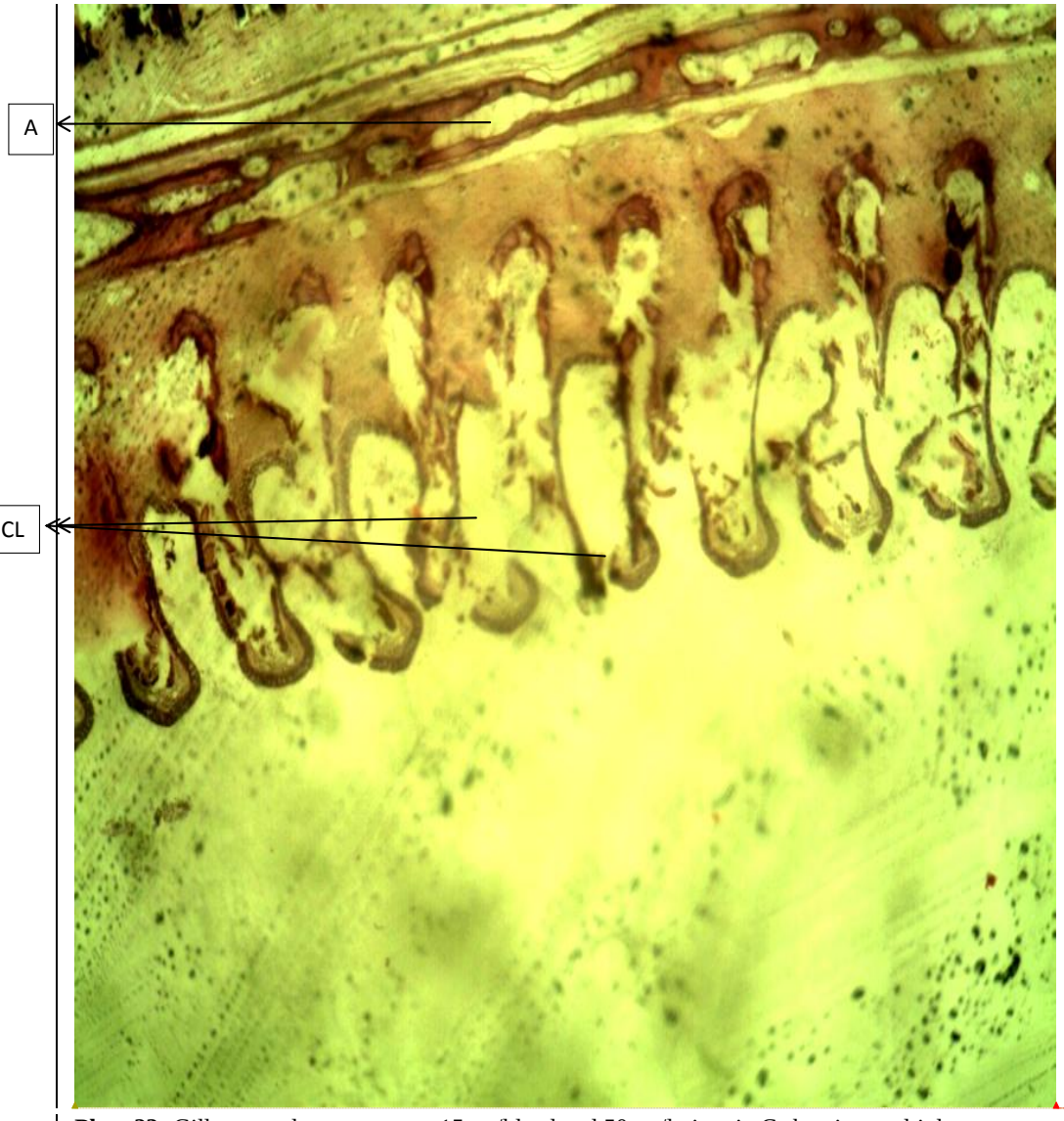

Plate 23: Gill exposed to concurrent 15mg/l lead and 50mg/l vitamin C showing multiple curlingof the lamellae (CL) and acellular bone (A) H&E X 100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

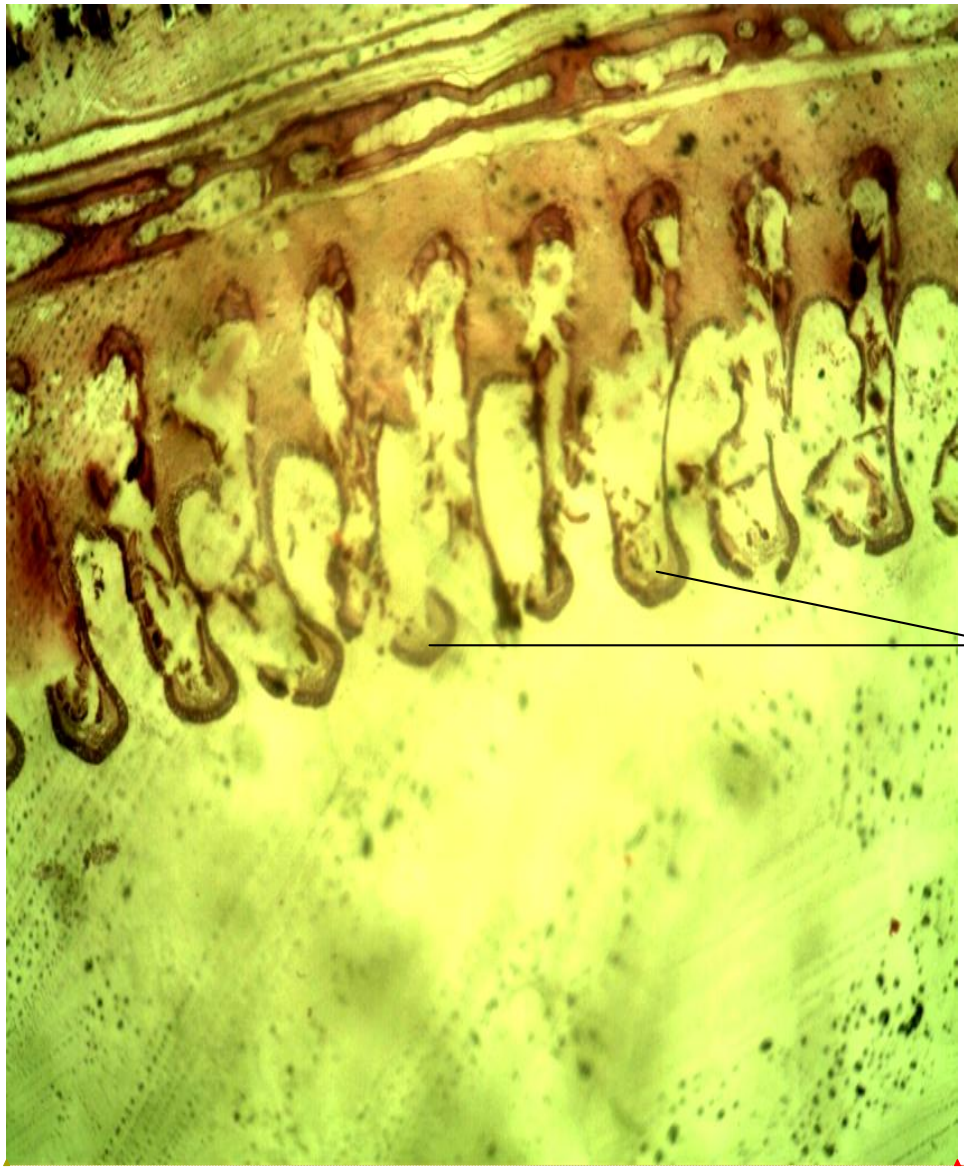

Plate 24: Gill exposed to concurrent 15mg/l lead and 100mg/l vitamin C showing severe Destroyed gill lamellae (SV). H&E X 100.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

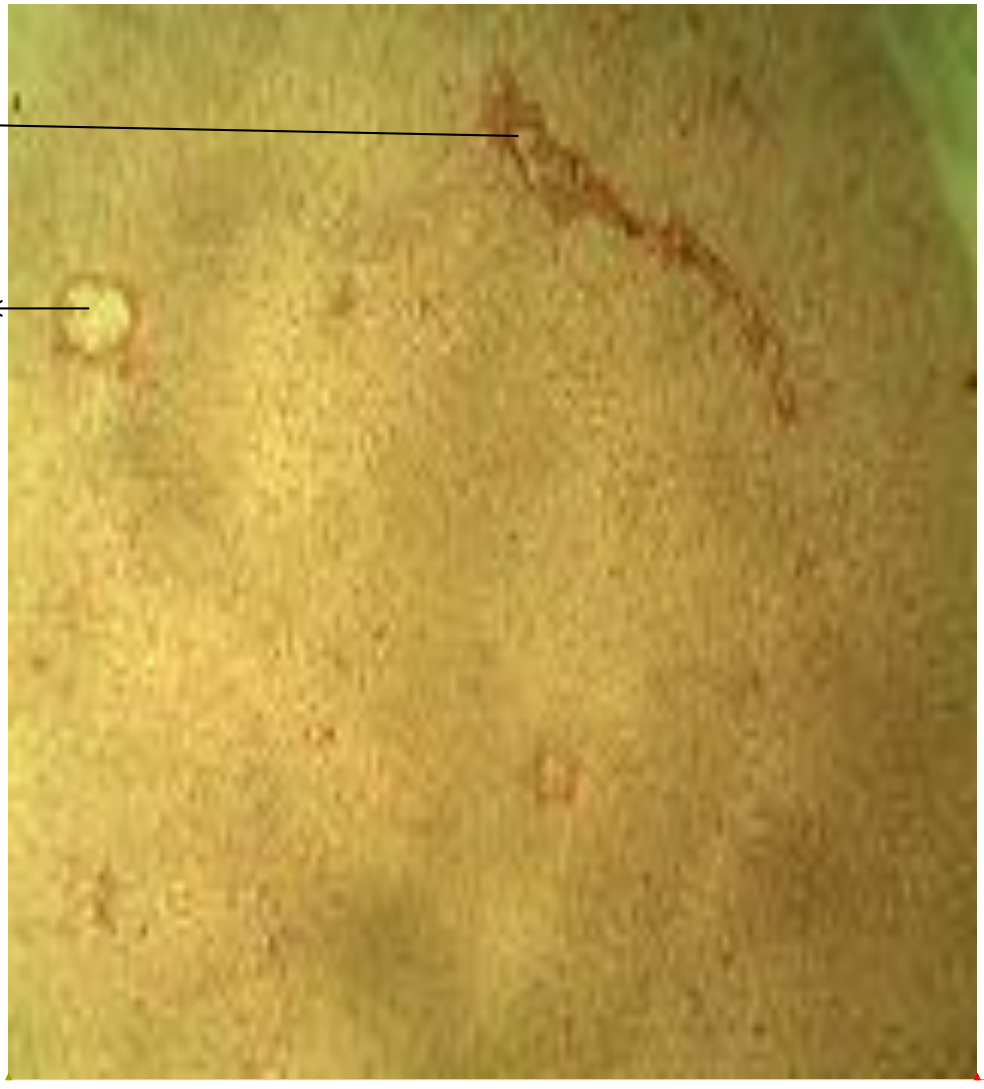

Plate 25: Liver exposed to concurrent 5mg/l lead and 50mg/l vitamin C showing intact centralvein (CV) and congested sinusoid (S) (black and white arrow).

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font color: Auto

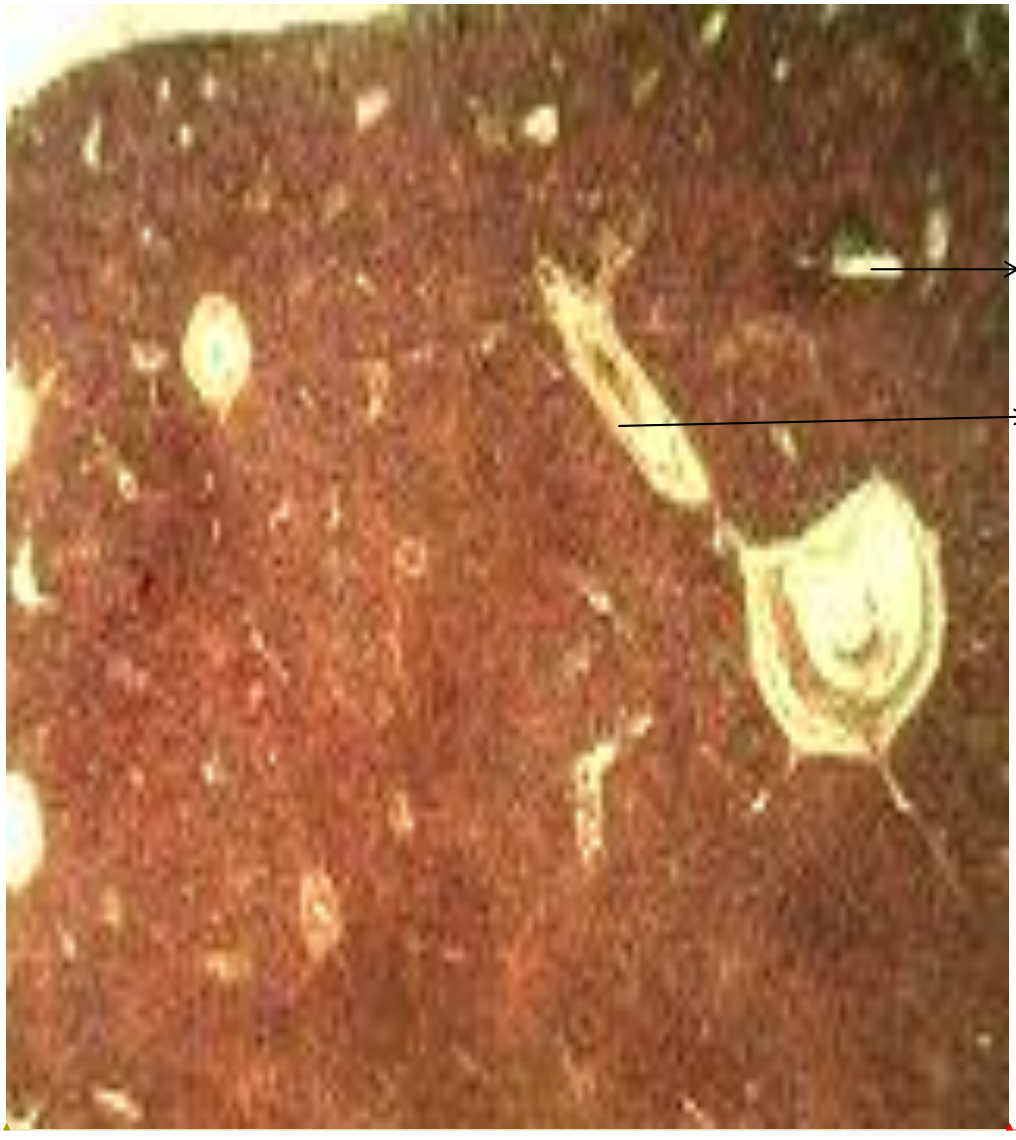

Plate 26: Liver exposed to concurrent 5mg/l lead and 100mg/l vitamin C showing intact centralvein and congested interlobular ducts(CI) and fibrosis of the hepatocytes (F)H & E X100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

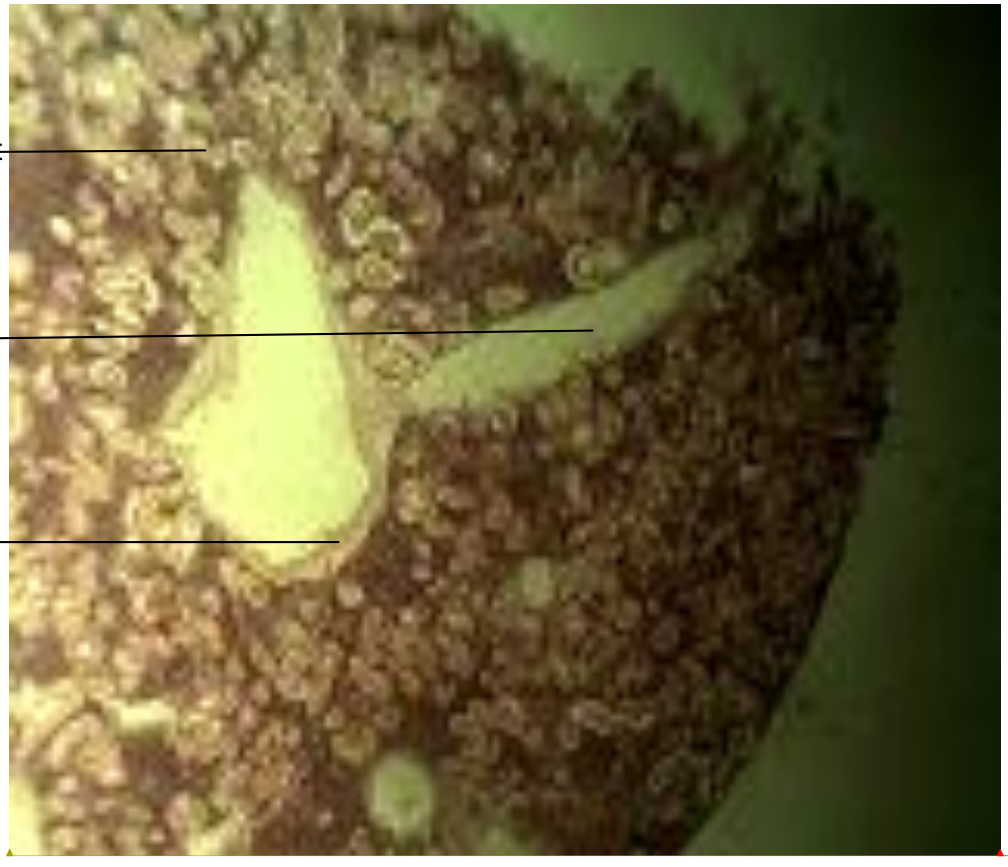

Plate 27: Liver exposed to concurrent 15mg/l lead and 50mg/l vitamin C showing cellular infiltrated central vein(CV) and congested interlobular duct (CI) and intact acini withmassive fatty degeneration (FD) of the hepatic tissues. H&E X100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

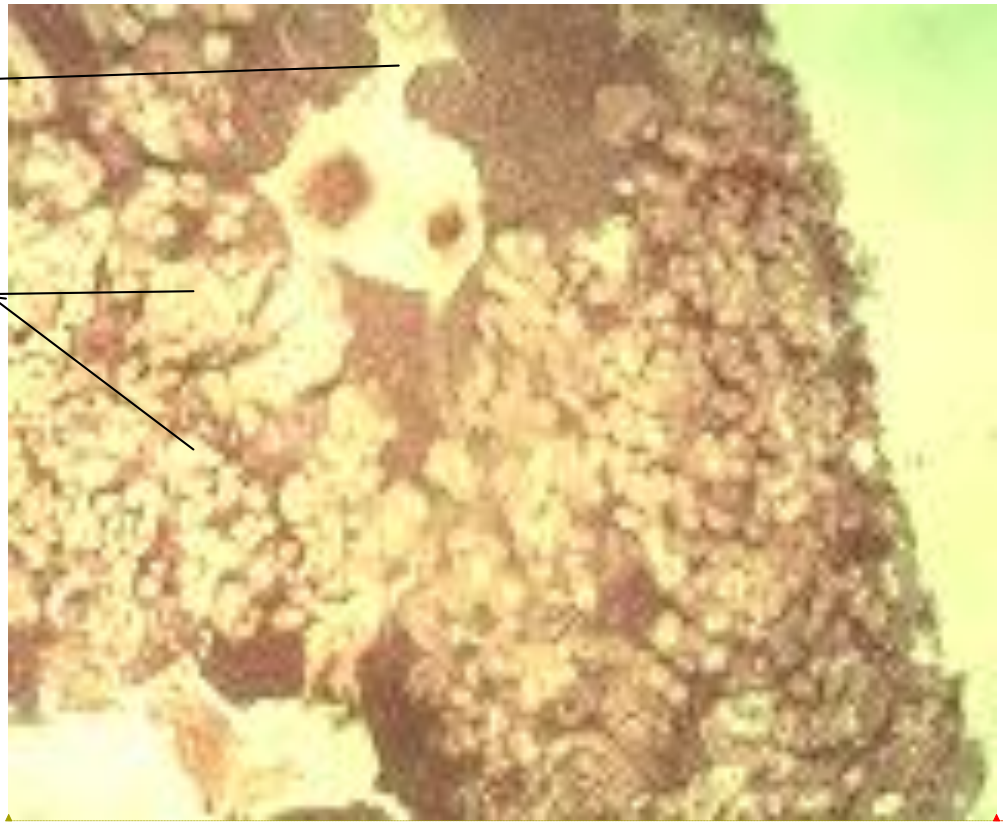

Plate 28: Liver exposed to concurrent 15mg/l lead and 100mg/l vitamin C showing extensive fatty degeneration of the hepatic tissues (FD), congestion and erosion of the interlobular duct (ID).H & E X100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

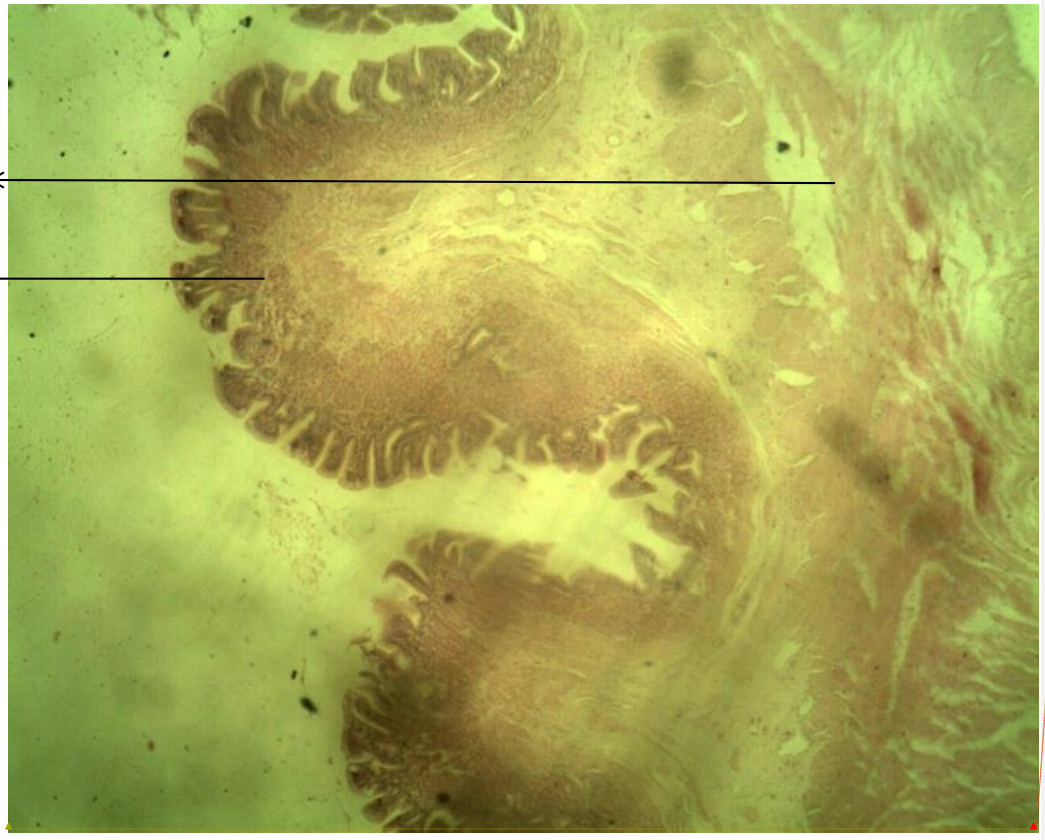

Plate 29: Stomach exposed to concurrent 5mg/l lead and 50mg/l vitamin C showing loosened inner circular muscle (CM) and intact mucosa (IC).H & E X100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

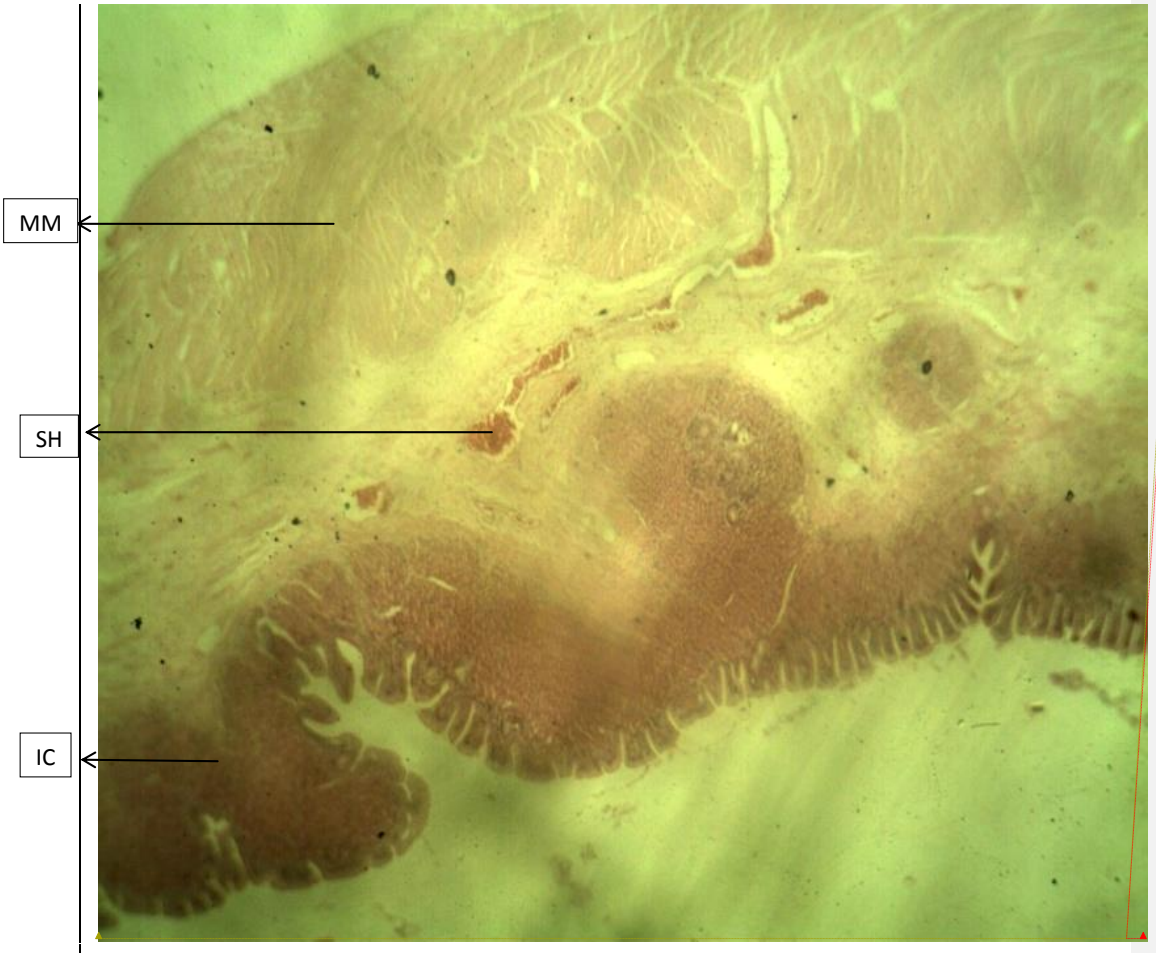

Plate 30: Stomach exposed to concurrent 5mg/l lead and 100mg/l vitamin C showing submucosal haemorrhages (SH), intact muscularis (MM) and intact mucosa (IC)

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto

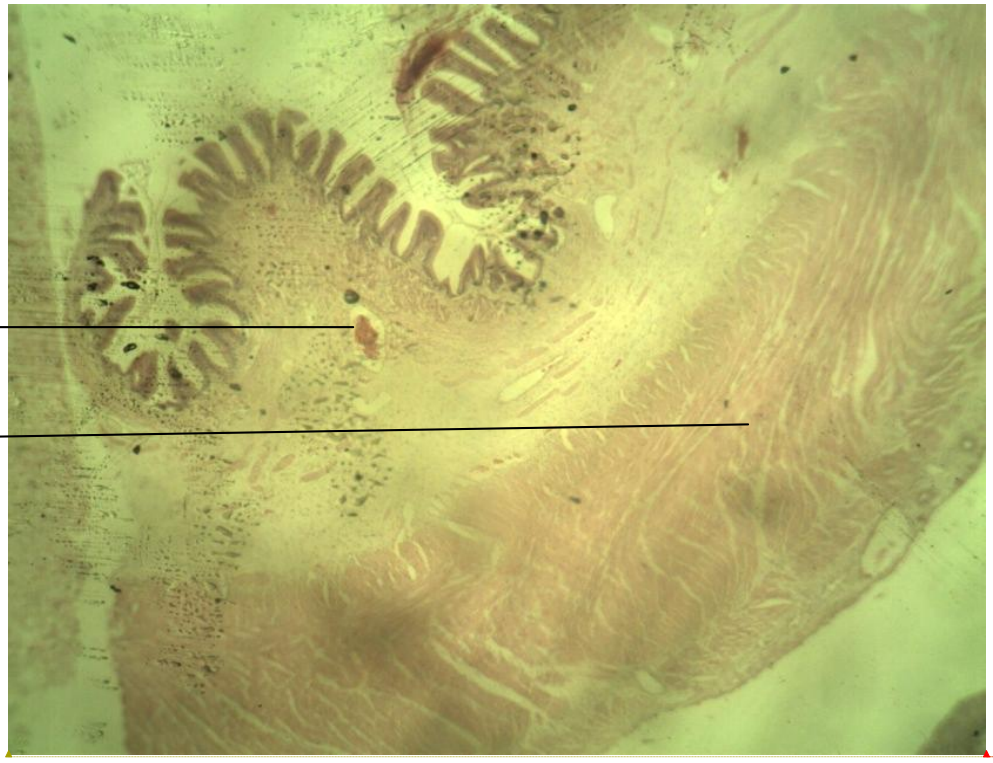

Plate 31: Stomach exposed to concurrent 15mg/l lead and 50mg/l vitamin C showing submucosal haemorrhages(SH) and necrotic muscularis mucosa (MM),H & E x100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

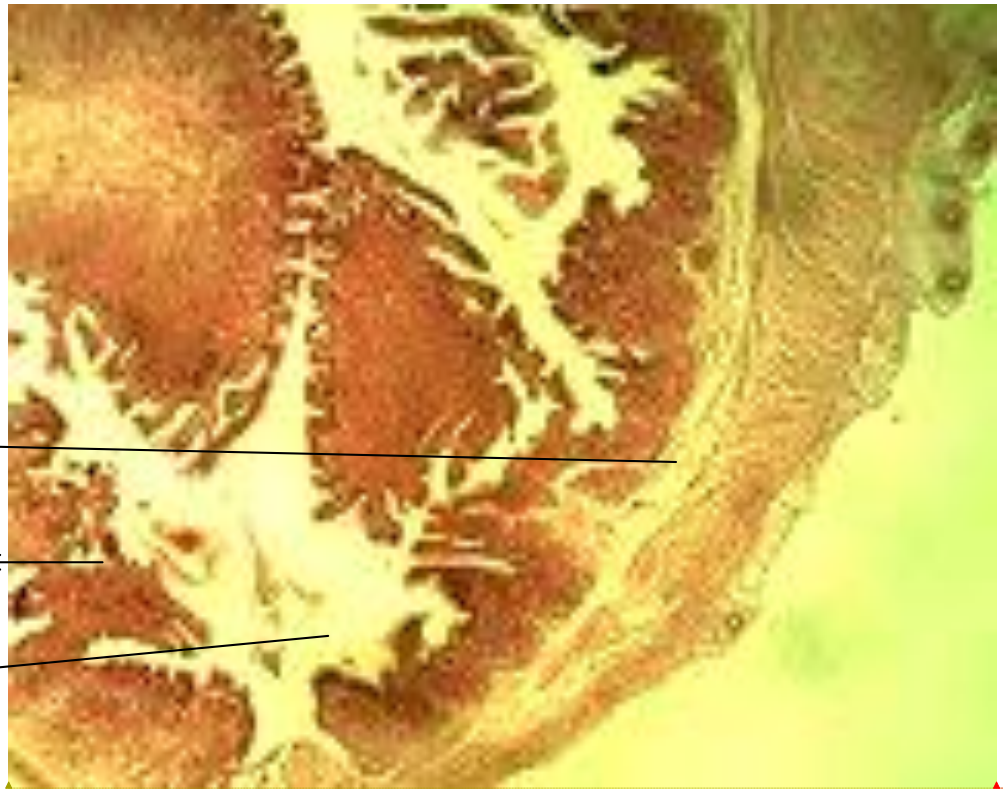

Plate 32: Stomach exposed to concurrent 15mg/l lead and 100mg/l vitamin C showing distorted mucosa (DM) with lumen filled with debris (LD) and fibrosis of the muscularis mucosae (MM). H &A x100

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Left

CHAPTER FIVE

DISCUSSION

5.1 DISCUSSION

The 48 hour LC_{50} of lead nitrate on *Clarias gariepinus* juveniles was 60mg/l. Singh and Manjeet (2015) recorded a 96 hour LC_{50} of 34.20 mg/l for lead nitrate in the fingerlings of fish *Labeo rohita.* Latif *et al.* (2013) recorded LC_{50} of 27.2mg/l for lead nitrate the fish *Labeo rohita*, while Abdullah *et al.* (2007) recorded LC_{50} of 32.70mg/l for lead nitrate in the fish *Labeo rohita*. Oluwatosin *et al.* (2018) recorded LC_{50} of 426mg/l for lead chloride in *Clarias gariepinus* juveniles. Hamed and Esmail (2012) reported LC_{50} of 2.62mg/l for lead nitrate in common carp *Cyprinuscarpio*. 96 hour LC_{50} of lead nitrate for *Clarias batrachus* was reported to be 378mg/l (Shamshun *et al.,* 2010). The LC_{50} of 57.5mg/l for lead nitrate was reported in *Clarias gariepinus* (Ikeogu *et al.*,2016). The variability in these acute toxicity value can be possibly explained by level of toxicity, species tested, fish age and undefined enzymatic defense response of fishes exposed to lead as well as duration of exposure (Dutta *et al.*,1996). In the same way, some toxicants which can cause detrimental effects to some organisms even at low concentrations may be less or more toxic to some other organisms at higher or same concentration (Shah and Altindu, 2005). This is attributed to the fact that several factors including differences in the test species, age, feeding habit, sex, composition of toxicant and experimental conditions under which the tests are performed (Witeska *et al.*,1993).Chronic toxicity may be lethal or sublethal and is caused by very low doses of a toxic compound or effluent over a

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

long period of time (Mager *et al.*,2011). Sublethal effects can occur at the biochemical, physiological or behavioural level, genotoxicity and change in growth rate (Goshling, 2004).

Lead is known to cause neurological, haematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes in animals.(Rout and Niak 2013). In this study *Clarias gariepinus* juveniles exposed to 5, 10 and 15mg/l of lead nitrate for 91 days manifested stress related behavior such as reduced feed intake, lethargy and coagulation of skin mucus in increasing concentrations when compared to the control. Mucous secretion on the skin and its coagulation all over the body surface observed by Oluwatosin, (2018) may be due to dysfunction of the pituitary endocrine gland under the toxic stress causing changes excessive secretion of mucus. Furthermore behaviorally Lal *et al.* (2013) reported vertical erect orientation of the fish with head up and tail down before death with exudation of mucus over the body of *Channa punctatus* exposed to lead chloride. Ascorbic acid has been reported to prevent fish from various stresses such as environmental stress, handling stress, pathogenic stress, transportation stress and osmotic stress (Koshio 2015).

Borane (2018) evaluated the effect of ascorbate on the lead induced alterations in the behavior of the fresh water fish, *Garra mullya* and concluded that the fish exposed to lead chloride and treated with ascorbic acid showed greater recovery than those exposed to only lead chloride.

Blood is an important medium in assessing the health status of animals. Both the physiological and pathological conditions of animals can be assessed by the evaluation of the haematological and biochemical analyses of the blood (Coles 1986). In this study haematological responses of the Catfish *Clarias gariepinus* exposed to varying concentrations of lead nitrate was significant decrease in RBC, Hb and PCV when compared to the control group. Decreased RBC count, Hb and

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

PCV in *Clarias gariepinus* after exposure to lead and cadmium was reported by Tawari-fufeyin*et al.*, (2008) andBorane and Zambare (2006). Ikeogu *et al.*, (2016) also reported decreased RBC count, Hb and Ht in *Clarias gariepinus* exposed to sub lethal concentrations of lead nitrate for 70 days. Adedeji (2009) reported decrease RBC count, Hb and PCV in *Clarias gariepinus* exposed to diazinon for 96 hours. The significant reduction in these parameters is an indication of severe anaemia caused by destruction of erythrocytes (kori-Siakpere *et al.*,2009) and or haemodilution (Adeyemo 2005) resulting from impaired osmoregulation across the gill epithelium and according to Okomoda *et al.* (2010) could be as a result of the destruction of intestinal cells, leading to impaired absorption of iron.Lead inhibits the synthesis of haeme in organisms and thus interfers with the effective utilization of iron.(Ademoroti 1995). The anaemia mostly found in lead toxicity is a microcytic, hypochromic anaemia. This is associated with the accumulation of iron in the mitochondria of the red blood cells precursors due to the inhibition of ferrochelatase by lead (Knollmann - Ritschel and Markowitz 2017).Lead causes haemoglobin oxidation which directly causes RBC lysis. This occurs due to inhibition of ALAD, which results in an ~~increased concentration~~increased concentration of substrate ALA in both blood and urine.The elevated ALA levels generate hydrogen peroxide and superoxide radicals and also interact with oxyhaemoglobin resulting in the generation of hydroxyl radicals (Patrick 2006).The prognosis of such oxidative stress is usually grave ~~for affected~~for affected cells and tissues.

Treatment of lead nitrate exposed *Clarias gariepinus* with Vitamin C showed a significant increase in the mean RBC, Hb and PCV of treated groups to values close to those of the ~~control groups~~control groups. There was no significant difference (P>0.05) between the 50mg/l Vitamin C and 100mg/l of vitamin C groups. This is similar to the findings of Adel (2009) where Vitamin C

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

enhanced the blood parameters in *Oreochromis niloticus* exposed to Ochratoxin which caused a significant decrease (P<0.05) in RBC, Hb and PCV.

Nourian *et al.*,(2018) studied the effects of lead(Pb) exposure on some blood biochemical indices in *Cyprinus carpio* and the potential alleviative effects of thiamine and reported that co-administration of thiamine with lead notably reversed the circulating biochemical biomarkers provoked by lead poisoning.

Studies on rats treated with lead manifested lowered levels of iron, resulting in impaired haeme formation. Treatment with 500 mg/day of vitamin C for one month in battery-manufacturing workers has been shown to reduce MDA concentration and nitrite levels, thereby improving antioxidant status, erythrocyte osmotic fragility and activities of endogenous antioxidant enzymes by scavenging the ROS generated due to high blood lead levels (Ganesh *et al*., 2016).

Xhyrel *et al*., (2016) reported that with increase in blood lead levels, RBC count, PCV and Hb level of albino rats; *Rattus norvegicus* reduced drastically but was ameliorated by treatment with vitamin C.

There was a significant increase in the total white blood cells (TWBC) in *Clarias gariepinus* exposed to varying concentrations of lead nitrate. Increase in TWBC is leucocytosis. This was also reported by Tawari-fufeyi *et al.*,(2008) in *Clarias gariepinus* exposed to lead nitrate and cadmium sulphate. Seyed and Mehdi (2013) reported leucocytosis in brown trout *Salmo trutta* exposed to water borne mercuric chloride.Ikeogu *et al.*(2016a) reported leucocytosis in *Clarias gariepinus* exposed to sublethal concentrations of lead nitrate. In contrast Al-Rudainy (2015) reported decreased TWBC termed Leucocytopaenia in *Mesopotamichthys sharpeyi* exposed to lead acetate. Leucocytopaenia was also reported in *Oreochromis niloticus* exposed to lead by Cogun and Salin (2013). According

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

to Neelima *et al.* (2015), the progressive increase in TWBC is as a result of the body trying to fight the foreign body(the toxicant lead nitrate).

Treatment of the lead nitrate exposed group with Vitamin C showed that there was no significant difference (P>0.05) between the TWBC of exposed and treated groups and between the 50mg/l vitamin C and 100 mg/l vitamin C. This implied that the leucocytes observed in the study were not significantly affected by the treatment with both doses of vitamin C.

The liver markers AST, ALT and ALP did not vary at day zero of the chronic experiment, showing that all the fish used in this study could be assumed to be of the same health status. Enzyme assay showed significant decrease (P<0.05) in AST, ALT and ALP levels in the lead nitrate exposed groups when compared to the control. Adedeji (2010) studied the acute effect of diazinon on blood plasma enzymes of *Clarias gariepinus* and reported significant decrease in the serum enzymes of the exposed fish when compared to the control. Ikeogu *et al.*(2016a) reported significant decrease in the ALT,AST and ALP of *Clarias gariepinus* exposed to lead nitrate.The reason for this major biochemical response to the effects of pollutants in fishes is the inhibition of enzymes (Michael *et al.*,1987).

Treatment of lead nitrate exposed group with vitamin C showed significant decrease in AST and ALT between the lead exposed group and the vitamin C treated group. Nourian *et al.* (2018) reported increase in ALT in *Cyprinus carpio* exposed to lead acetate. However co-administration of thiamine with lead resulted in increase in ALT. Ahmed *et al.* (2014) also reported increase in liver AST and ALT following treatment with vitamin C after cadmium exposure in *Oreochromis niloticus.* They concluded that vitamin C scavenges reactive oxygen species and render a protective effect

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

against cadmium toxicity. Youness *et al*.,(2016) discovered that liver damage caused by lead nitrate in mice exposed for four weeks was ameliorated by orange juice.

Blood urea nitrogen (BUN) measures the amount of urea nitrogen present in the blood after it breaks down proteins used by the cells. It is used as an assessment of kidney function. BUN helps to eliminate toxic substances and is formed in the liver, carried through the blood stream to the kidneys to be eliminated. The significantly high levels of urea in the fish exposed to lead nitrate is known as azotemia and is an indicator of poor renal function resulting from electrolyte imbalance. Azotemia was reported by Ude and Oti (2014) during exposure of *Clarias gariepinus* to acute concentrations of Delsate®.

Treatment of lead nitrate exposed group with vitamin C, showed a significant decrease in urea level between the lead exposed group and vitamin C treated groups. The 100mg/l vitamin C showed more significant decrease in urea level than the 50mg/l Vitamin C. Blood urea nitrogen is one indicator of kidney function. Urea is the principal end product of protein catabolism. Enhanced protein catabolism interprets the elevated levels of urea. Nourian *et al*. (2018) reported increased urea in *Cyprinus carpio* exposed to lead acetate but thiamine co-administration reversed this biochemical biomarker provoked by lead poisoning. However Ahmed *et al*. (2014) reported that urea level was not affected by vitamin C after exposure of *Oreochromis niloticus* to sublethal doses of cadmium.

There was a significant increase in the level of lead in the muscle of experimental groups when compared to the control groups. Bioaccumulation of lead was reported by Spokas *et al*.,(2006) in fat head minnow *Pimephales promelas* during chronic exposure to lead nitrate in aquarium water. They demonstrated significantly higher lead levels in the gills, skin and muscle of *pimephales*

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

promelas. Olmeda *et al.*,(2013) reported significantly higher lead levels in the frozen common sole *Solea vulgaris* during a study of risk assessment for the consumers of fish and shellfish in Andalusia Spain. The risk assessment performed indicated that fish and shellfish products were safe for consumption although a potential risk cannot be dismissed for regular or excessive consumers of common sole due to the high levels of lead detected in the examined samples. Nsofor *et al.*,(2014) reported that the concentration of lead in the shell fish *Macrobranchium rosenbergi* from River Niger Onitsha was found to be higher than WHO standard of 0.05mg/l in aquatic foods (WHO/FAO 1972). These findings support the possibility of food chain contamination.

There was a significant decrease in lead bioaccumulated fish muscle after treatment of the lead exposed group with vitamin C. there was a significant decrease between 50mg/l and 100mg/l vitamin C with 50mg/l vitamin C being more effective in reducing lead level in the muscle of the exposed fish. According to Monteiro *et al.* (2005), heavy metals accumulated in the tissues of fish catalyze redox reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress and therefore cause biochemical, molecular and morphological alterations in fish. Shahsavani *et al.*, (2017) reported that lead exposure caused a significant increase in lead content in kidney, liver, muscle, brain and gill of *Cyprinus Carpio* exposed to lead. They further reported that thiamine supplementation slightly decreased the augmented levels of lead in the muscle, brain and gill tissues which was not significantly different from that of control group. Vitamin C prevents cell damage by binding the free radicals and neutralizing its unpaired electron. It acts as a reducing agent to reverse oxidation in aqueous solutions.

The histopathological examination of the gills, liver and stomach of *Clarias gariepinus* showed that lead nitrate is toxic to these organs at sub lethal concentrations. Distorted lamellae,

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

necrotic primary and secondary lamellae, and severely damaged lamellae were observed in the exposed groups when compared to the control. This is because the gills have direct contact with the toxicant. Gill damage indicates impairment in the gaseous exchange efficiency of the gills (Obande *et al.*,2013) characterized by reduction in oxygen uptake and the resultant hypoxia. Ikeogu *et al.* (2016c) reported hyperaemic, oedematous and degenerated lamellae of gills of *Clarias gariepinus* exposed to sublethal concentrations of lead nitrate and crude oil. They concluded that impairment of the gill functions by the overall effect of the pathological changes in the gill of exposed fish will have grave consequences.The gill pathological changes in this study were also reported in *Clarias gariepinus* after exposure to kerosene oil (Gabriel *et al.*,2007), and Omitoyin (2006) who used paraquat on *Clarias gariepinus.* Histopathological alterations induced by chronic lead exposure for 91 days significantly decreased following 7 days of treatment with Vitamin C. Gill damage in the lead exposed group was inform of distortion of the gill filaments, fusion, curling and deformation of the gill lamellae but was significantly decreased by 50mg/l vitamin C treatment where no visible gill damage was observed.

Formatted: Font color: Auto

The liver of *Clarias gariepinus* exposed to varying concentrations of lead nitrate showed cytoplasmic vacuolations and pyknotic nuclei in the hepatocytes. The liver of the control group showed normal liver parenchymal cells (hepatocytes) arranged to form a lattice network. The cytoplasmic vacuolation of the hepatocytes had evidence of fatty degeneration as a result of lipid peroxidation. Lipid peroxidation is a biomarker of oxidative stress and is one of the most investigated consequences of ROS on lipid membranes. The generated free radical captures electrons from the lipids inside the cell membranes and damages the cell (Patrick, 2006) as evidenced by the ballooning degeneration of hepatocytes exposed to lead nitrate in this study.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Nsofor *et al.* (2014a) observed extensive hyperemia, oedematous sinusoids, apoptotic hepatocytes with pyknotic nuclei and widespread necrotic hepatocytes with mononuclear leucocyte infiltrations in the liver tissues of *Chrysichthys nigrodigitatus* –due to heavy metals in River Niger Nigeria. Ikeogu *et al.*,(2016b) observed hyperemia, leucocytic infiltrations, extensive necrosis, fibrosis, ballooning degenerations and varying degrees of destruction of histological architecture of the liver of *Clarias gariepinus* exposed to lead nitrate and crude oil. Currently liver histopathology is used mainly as a descriptive tool to assess the health status of fish exposed to toxicants (Guilio and Hinton 2008). Liver histopathology has been found to be a sensitive indicator of pollutions, stress and impaired fish health. Liver necrosis observed probably resulted from excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification. Such findings were recorded by Rahman *et al.*,(2002) in a test of diazinon on *Channa punctatus.* The inability of the fish to regenerate new liver cells may also have led to the necrosis (Obande *et al.*, 2013). The presence of mononuclear WBCs is likely due to an immune mechanism of the exposed fish, stimulated to fight against the toxicant.Elevated concentration of lead cause cytological degeneration in fish organs as well as heart, liver, and kidney dysfunction in man. (Benoof *et al.*, 2000).

Liver damage occurred in the lead exposed group in form of cytoplasmic vacuolations, congestion of sinusoids. Treatment with 50mg/l vitamin C showed liver with no visible damage while the 100mg/l vitamin C was reported to be ineffective in ameliorating hepatic toxicity induced by lead nitrate. This is because such a dose could elicit toxicity in the liver due to the pro-oxidant nature of vitamin C (Podmore*et al.*, 1998).The protective effect of vitamin C against lead induced oxidative stress could be by direct inhibition of lipid peroxidation or by scavenging free radicals or indirect through the enhancement of the activity of superoxide dismutase, the enzymatic free radicals scavengersin cells.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Praveen *et al*.(2015) reported reduced lipid peroxidation in tissues of *Channa punctatus* exposed to lead chloride supplemented with vitamin C.

In this study such cytological degenerations were also observed in the histology of the stomach of *Clarias gariepinus* exposed to varying concentration of lead nitrate in the form of haemorrhages in the mucosa, broken crypts and the degeneration of the columnar epithelial cells of the mucosa. Lead acetate induced histopathological changes in the stomach of Silver sailfin molly *Poecilia latipinna* was reported by Mobarak and Sharaf (2011) as irregularity, shrinkage and pyknosis of the epithelial cells as well as atrophy of the submucosa. Bais and Lokhande (2012) also reported disintegration of the epithelium and desquamation of the gastric mucosa of freshwater fish *Ophiocephalus striatus* exposed to cadmium chloride. Samantha *et al*., (2016) demonstrated degenerative changes in columnar epithelial cells, damage in gastric glands and mucosal folds in the stomach of *Anabas testudineus* under Almix exposure. Almix is a sulphonyl urea group type of herbicide containing 10.1% metsulfuron methyl 10.1% Chlorimuron ethyl and 79.80% adjuvants.

The stomach of the lead exposed group showed mucosal haemorrhages, broken crypts and degeneration of the epithelial mucosal cells. However the 5mg/l lead group treated with 50mg/l and 100mg/l vitamin C showed no lesions while the 15mg/l lead group treated with 50mg/l vitamin C showed necrosis of the mucosa.The 15mg/l lead group treated with 100mg/l vitamin C showed no lesion in the stomach section. This is because the therapeutic effect of vitamin C in gastritis is dose dependent as reported by Aditi and Graham (2012).

However, there was no significant difference between the pathology of fish exposed to lead nitrate and the group concurrently exposed to lead nitrate and vitamin C. The toxicity of lead nitrate could not be ameliorated by vitamin C when administered concurrently. This is similar to Shahsavani *et al.*

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

(2017) who reported that Vitamin C supplementation reduced the augmented concentration of lead in the muscle to the levels that were not significantly different from that of the unexposed group.

Davar *et al*. (2017) evaluated the possible ameliorative effects of vitamin C and thiamine on lead accumulation in kidney, liver, muscle, brain and gill of lead poisoned common carp and reported that neither thiamine nor vitamin C supplementation was effective in providing significant reduction of tissue lead among the thiamine and vitamin C supplemented groups.

Nandi *et al.* (2005) also demonstrated that co- treatments of ascorbic acid and arsenic inhibited lipid peroxidation in liver and kidney due to its antioxidativeproperty as ascorbic acid is known to inhibit oxidative damage to cellular membranes.

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

5.2 CONTRIBUTIONS TO KNOWLEDGE

The findings from this research revealed that lead nitrate is toxic to fish at sublethal concentrations. It also revealed that the antioxidant vitamin C can ameliorate the chronic effects of lead nitrate with 50mg/l dosage being better recommended than 100mg/l. This research also revealed that the combination of leadnitrate and vitamin C in the same tank did not result in the amelioration of the toxic effects of lead nitrate. Fish from heavy metal (lead) polluted waters can be made safe by treatment with 50mg/l vitamin C for 7 days.

5.3 CONCLUSION

It could be concluded from the findings of this research that lead is toxic to the liver, gills, stomach and blood of *Clarias gariepinus* atsublethal concentrations. It could also be inferred that lead will bioaccumulate in the muscle of exposed fish. It could be concluded that vitamin C can play therapeutic roles in lead toxicity in the African catfish *Clarias gariepinus*.

5.4 RECOMMENDATION:

Vitamin C at the dose of 50mg/l can be useful in the treatment of chronic lead toxicity in the African catfish *Clarias gariepinus*.

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Left

REFERENCES

Abdel Moneim, A. E. (2016). *Indigofera oblongifolia* prevents lead acetate-induced hepatotoxicity oxidative Stress, fibrosis and apoptosis in rats. *PLos one*, 11(7): 0158965.

Abdullah , S., Javad, M., and Javid, A. (2007). Studies on acute toxicology of metals to the fish *Labeo rohita, International Journals of Agricultural Biosciences*,9 : 333-337.

Adedeji, O.B., Adedeji, O.A., Adeyemo, O.K. and Agbede, S.A. (2008) Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*) *African Journal of Biotechnology*, 7(5): 651-654.

Adedeji, O.B. (2009). Effects of diazinon on blood parameters in the African catfish (*Clarias gariepinus*).*African Journal of Biotechnol*ogy, 8 (16):3940-3946.

Adedeji, O.B. (2010). Acute effect of diazinon on the blood plasma biochemistry in the African Catfish (*Clarias gariepinus*). *Journal of Clinical Medicine and Research*,2(1): 001-006.

Adel, M.E. (2009). The opposing effect of ascorbic acid (Vitamin C) on ochratoxin toxicity on Nile tilapia (*Oreochromis niloticus).Acta Polinica 2 : 18-22.*

Ademoroti, C.M.A. (1995). Pollution by heavy metalsIn *Environmental Chemistry and Toxicology*. Foludex press Ltd Ibadan, Nigeria:(pp171-186).

Adeyemo, O.K. (2005) Haematological and histopathological effects of cassava mill effluent in *Clarias gariepinus*. *African Journal of Biomedical Research* 8:179-183.

Formatted: Centered

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Aditi, A and Graham, D.Y.(2012) Vitamin C; gastritis and gastric disease : *A historical review and update. Digestive Diseases and Sciences* 57(10)2504-2515 DOI 10,1007/S/10620-012-22037 Dig.Dis.Sci

Ahamed, M., Verma, S., Kumar, A., & Siddiqui, M.K.(2005) Environmental exposure to lead and its correlation with biochemical indices in children. *Science of the Total Environment*, 346:48-55.

Ahamed, M., & Siddiqui, M.J. (2007).Environmental lead toxicity and nutritional factors.*Clinical Nutrition*,26: 400–408.

Ahmed, M. El- Gazzar, Khalid, E. Ashry &Yasser, S. El Sayed (2014). Physiological and oxidative biomarker in the fresh water nile tilapia *Oreochromis niloticus* exposed to Sublethal doses of Cadmium. *Alexandria Journal of Veterinary Science*, 40: 29-43.

Ajayi, G. O., Adeniyi, T. T., & Babayemi, D. O. (2009). Hepatoprotective and some haematological effects of *Allium sativum* and vitamin C in lead exposed Wistar rats. *International Journal of Medical Science*1:64-67.

Ajmal, M., Khan, M.A., & A.A. Nomani (1985). Distribution of heavy metals in plants and fish of the Yamuna River (India). *Environmental Monitoring Assessment Journal*, 5: 361-367.

Ajumobi, O.O., Tsofo, A., Yango, M., Aworrh, M.K., Anagbogu, I.N., Mohammed, A., UmarTsafe, N., Mohammed, S., Abdllahi, M., Davis, L., Idris, S., Poggensee, G., Nguku, P.Gitta, S., & Nsubuga, P.(2014). High concentration of blood lead levels among young children in Bagega community, Zamfara - Nigeria and the potential risk factor. *Pan African Medical Journal*, 18 (1):14.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Al-Attar, A.M. (2007). The influences of nickel exposure on selected physiological parameters and gill structure in the teleost fish, *Oreochromis niloticus* .*Journal of Biological Sciences*, 7: 77-85.

Al-awady, A.A. (2010). Cellular mechanisms of cadmium toxicity related to the homeostastis of essential metals. *Biometals*, 23(5) 877-896.

Al-awady, A.A. (2011). Concentration of some traces of metals in water, sediments and two Cyprinidae species in Al-masab Alamm, AL-nassiriya-Iraq. PhD Thesis, Women College of Science, Uril, Baghdad 11pp.

Alloway, B.J.(2013). Heavy metals in soils. Trace metals and metalloids in soils and their bioavailability 3rd edition, Springer Netherlands 614p.

Almeida, J.A., Novelli, E.L.B., Dal-Pai Silva, M., & Alves-Junior, R.(2001). Environmental cadmium exposure and metabolic responses of the Nile tilapia *Oreochromis niloticus. Environmental Pollution Journal*, 114:169-175.

Al-Rudainy A.J. (2015). Effect of sublethal exposure to lead acetate on haematological indices and growth rate of *Bunni mesopotamichthys sharpeyi*.*Advances in Animal & Veterinary Science*, 3(11): 569-573.

Al-Rudainy, A.J., Mustafa S.A.,& Abdulaziz, M.A.,(2015). Toxic effects of mercuric chloride on DNA damage, haematological parameters and histopathological changes in common carp (*Cyprinus carpio*). *Iraqi Journal of Veterinary Medicine*,38(2): 87-94.

AL-Saleh, I.,& Shinwari, N.(2002). Preliminary report on the levels of element in four fish species from the Arabian Gulf of Saudi Arabia. *Chemosphere*, 48 (7): 49-55.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font color: Auto

Alves, L.C., Wood, C.M. (2006). The chronic effects of dietary lead in freshwater juvenile rainbow trout (*Oncorhynchus mykiss*) fed elevated calcium diets. *Aquatic Toxicolology Journal*, 78:217-232.

Al-Weher, S.M. (2008). Levels of heavy metal Cd, Cu and Zn in three fish species collected from the northern Jordan valley, Jordan. *Jordan Journal of Biological Sciences* 1: 41-46.

Alya, A.,Ines, B.D.,Montassar, L.,Najoua, G.H.,& Saloua,E. F. (2015). Oxidative stress, biochemical alterations and hyperlipidaemia in female rats induced by lead chronic toxicity during puberty and post-puberty periods. *Iran Journal of Basic Medical Sciences*, 18:1034-1043.

Ambedkar, G.~~,~~ & Muniyan, M. ~~-~~(2011). Bioaccumulation of some heavy metals in the selected five freshwater fish from Kollidam River,~~–~~Tamilnadu, India. *Advanced Applied ~~Scientific Research~~Scientific Research Journal*, 2: 221-225.

Amiard, J.C., Amiard-Triquet, C., Barka, S., Pellerin, J., & Rainbow, P .S. (2006). Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. *Aquatic Toxicology Journal*, 76:160–202.

Aniladevi Kunjamma, K.P., Philip, B., Smitha, V., Bhanu, S.V.& Jose, J. (2008). Histopathological effects on *Oreochromis mossambicus* (Tilapia) exposed tochlorpyrifos. *Journal of Environmental Research and Developmental*, 2(4): 553-559

Anjali, Patel(2000).How does lead affect the Nervous system? http://serendip.brynmawr.edu/bb/neuro/neurooo/web/pate;.html retrieved 19/2/2014.

Annibald, A., Truzzi, C., Illuminati, S., & Scarponi, G. (2009). Recent sudden decrease of lead in Adriatic coastal seawater during the years 2000–2004 in parallel with the phasing out of leaded gasoline in Italy. *Marine Chemistry Journal*, 113:238–249.

Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted ...
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

APHA (2012) Standard methods for examination of water and waste water, 22nd Edition American Public health Association, Washington D.C USA.

Authman, M.M.N., Zaki, M.S., Khallaf, E.A & Abbas, H.H. (2015). Use of fish as bioindicator of the effects of heavy metals pollution. *Journal of Aquaculture Research and Development*, 6:328. Doi: 10.4172/2155-9546.1000328

Bais, U.E and Lokhande (2012). Effect of cadmium chloride on histopathological changes in fresh water fish *Ophiocephalus striabus. International Journal of Zoological Research*, 8: (23-32).

Baltova, S. and Velcheva, I. (2005). Some morphological and pathological modification of the blood cells from *Alburnus alburnus* in intoxication with heavy metals (Pb, Zn and Cd). *Bulgarian Journal of Agricultural Science*, 11: 577-582.

Bancroft, J.D and Stevens, A. (1997).*Theory and practice of histological techniques.* New York Longman Inc. U.S.A .

Beattie, A.D., Moore, M.R.,& Goldberg, A. (1972). Tetraethyl-lead poisoning. *Lancet*, 300:12–15.

Begovic, V., Nozic, D., Kupresanin, S., and Tarabar, D. (2008). Extreme gastric dilation caused by chronic lead poisoning: a case report. *World Journal of Gastroenterology*,14: 2599-2601.

Bellinger, D.C. (2004). Lead. *Pediatrics*, 113:1016–1022.

Benoff, S., Jcabo, A., & Hurley, I.R.(2000). Male fertility and environmental exposure to lead and cadmium. *Human Reproduction Update*,6: 107-121.

Berndtsson, R. (1993). Small-scale spatial patterns of bulk atmospheric deposition. *Journal of*

Formatted ... (repeated 50 times)

Environmental Quality, 22: 349–360

Bhattachrjee, C.R., Dey, S. & Goswami, P., (2003). Protective role of ascorbic acid against lead toxicity in blood of albino mice as revealed by uptake, lipid profiles and ultra-structure features of RBCS, *Bulletin of Environmental Contamination and Toxicology*, 70 (1):1189-1196.

Blazer, V.S., Facey, D.E., Fournie, L.A., Coutrney, J.W., & Summers, J.K. (1994). Macrophage aggregates as indicators of environmental stress. In: Modulators of Fish Immune Responses, Stolen, J.S. (Ed.). New Jersey, SOS Publications (pp: 169-185).

Boelsterli, U.A. (2003). Mechanistic Toxicology.London, Taylor and Francis.

Bokara, K. K., Brown, E., McCormick, R., Yallapragada, P., & Rajanna, S. (2008). Lead induced increase in antioxidant enzymes and lipid peroxidation products in developing rat brain. *Biometals* 21:9-16.

Bolognani Fantin, A.M., Trevisan, M.P., and Pederzoli, E.F. (1989). Preliminary studies on haematological parameters in *Carassius carassius (L).* var. *auratus* submitted to acute experimental lead pollution as pollution indices. *Studi Trentini di Scienze Naturali*, 65: 157-164.

Borane, V. R. (2018). Ascorbate effect on the lead induced alterations in the behavior of the fresh water fish, *Garra Mullya. International Journal of Recent Scientific Research*,9(4):25942-25944

Borane, V.R, Shinde, B.R and Zambare, S.P. (2013) Ascorbate effect on the lead chloride induced alteration in the protein contents of fresh water fish *Channa orientalis. Journal of environmental*

Formatted ... (×30)

Research and Development, 8(2) :268-272.

Borane, V.R and Zambare, S.P (2006). Protective role of ascorbic acid on the lead and cadmium induced alteration in total R.B.C. of the freshwater fish *Channa orientalis* in Aquaculture, New Delhi India. APH Publishing Corporation (pp 163)

Bradman, A., Eskenazi, B., Sutton, P., Athansoulis, M and Goldman, L.R. (2001) Iron deficiency associated with higher blood lead in children living in contaminated environments. *Environmental Health Perspective* 109 (10): 1079-1084. DOI 10.1289/ehp. 011091079.

Bressler, J., Kim, K.A., Chakraborti, T., and Goldstein, G. (1999).Molecular mechanisms of lead neurotoxicity. *Neurochemical Research*, 24:595–600.

Brodkin, E.,Copes, R., Mattman, A., Kennedy, J., Kling, R., & Yassi, A. (2007). Lead and mercury exposures: interpretation and action. *Canadian Medical Association Journal* 17:59-63.

Brønnum, J. and Hansen, E. (1998).Bly - anvendelse, problemer, den videre indsats [Lead – uses, problems, further activities].Environmental Projects. Danish Environmental Protection Agency, Copenhagen. (In Danish). Pp 377.

Butler, P.J., & Metcalfe, F.F (1983).Control of respiration and circulation. In J.C Rankin, T.J. Pitcherand R.T Duggan (eds). Control Processes in Fish Physiology, London, Croom and Helm.

Camargo, M.M.P., and Martinez, C.B.R. (2007). Histopathology of gills, kidney and liver of neotropical fish caged in an urban stream. *Neotropical Ichthyology*, 5(3): 327-336.

Campbell, P.G.C. (1995). Interactions between trace metals and organisms: A critique of the free-

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

ion activity model. In: Tessier, A. and D.R. Turner (Eds.) *Metal Speciation and Bioavailability in Aquatic Systems*,(pp 45-102) New York, John Wiley.

Canli, M., Ay, O., & Kalay, M. (1998). Levels of heavy metals (Cd, Pb, Cu, Cr and Ni) in tissues of *Cyprinus carpio*, *Barbus capito* and *Chondrostoma regium*from the Seyhan river Turkey. *Turkish Journal of Zoology*, 22: 149-157.

Chatterjee Chinamay, B., Mukhopadhyay, K. & Ghos, K. (1995). "Vitamin C" a potential savior against free radical induced oxidative damage. *Current Science*, 69(9): 747-751.

Cicik, B., Ayo, F., and Karayakar, F. (2004). Effects of lead and cadmium interactions on the metal accumulation in tissue and organs of the Nile Tilapia (*Oreochromis niloticus*). *Bulletin of Environmental Contamination and Toxicology* 72:141–148.

Cleveland, L.M., Minter, M.L., Cobb, K.A., Scott, A.A., & German, V.F. (2008) Lead hazards for pregnant women and children: Part 1 of a two-part article details how exposure happens, whom it affects, and the harm it can do. *American Journal of Nursing* 108:40-49.

Cogun, H.Y.& Sahin, M.(2013). The effect of lead and zeolite on haematological and some biochemical parameters in Nile fish (*Oreochromis niloticus*). *Current Progress in Biological Research, marina silvaopps. Intech open* Doi: 10. 5772/ 53076.

Coles, E.H. (1986) *Veterinary clinical pathology* (4th edition) Philadelphia, Saunders (110-155 Pp.) .

Cornelis, R. (2005).Handbook of elemental speciation II: Species in the environment, food, medicine & occupational health. Pp 24. Wiley.

Cory-Sletcha, D.A., and Pokora, M.J. (1995). Lead induced changes in muscarinic cholinergic sensitivity. *Neurotoxicology*.16:337-348.

Formatted ... (repeated)

Costa, L.G., and Fox, D.A. (1983). A selective decrease of cholinergic muscarine receptors in the visual cortex of adult rats following developmental lead exposure. *Brain Research* 276:259-266

Davar, S., Hasan, B.,& Kimiya, Nourian.(2017).Effect of thiamine and vitamin C on tissue lead accumulation following experimental lead poisoning in *Cyprinus carpio*. *Iranian Journal of Veterinary Science and Technology*, 9:1-3.

DeJonghe,W.R.A.,& Adams,F.C.(2006). Biogeochemical cycling of organic lead compounds. *Advanced Environmental Science and Technology Journal*, 17:561–594.

Deponte , M.(2013) Gluthhathione catalysis and the reaction mechanisms of gluthathione-dependent enzymes. *Biochimica et Biophysica Acta (BBA). General Subjects* 1830(5):3217-3266

Deutsche Gesellschaft fur Klinische cheime(1972) *Klinicka Biochemie*, 10:182-192.

Donaldson, W. E., and Knowles, S. O. (1993) Is lead toxicosis a reflection of altered fatty acid composition of membranes? *Comparative Biochemistry and Physiology Part C* 104:377–379.

Dooyema, C.A., Neri, A., Lo, Y.C., Durant, J., Dargan, P.I., Swarthout, T., Biya, O., Gidado, S.O., Haladu, S., Sani-Gwarzo, N., Nguku, P.M., Akpan, H., Idris, S., Bashir, A.M., & Brown, M.J. (2012). Outbreak of fatal childhood lead poisoning related to artisanal gold mining in Northwestern Nigeria. *Environmental Health Perspective*,120:601–607.

Dutta, H.M., Munshi, J.S.D., Roy, P.K., Singh, N.K. & Adhikari, S. (1996). Ultra structural changes in the respiratory lamellae of the catfish (*Heteropneustes fossillis*) after sub-lethal exposure to mellthion. *Journal of Environmental Pollution*. 92:329-341.

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Eaton,R.C., Di Domenico,R., & Nissanov, C.(1991). Role of the Mauthner cell in sensorimotor integration by the brain stem escape network. *Brain Behaviour Evolution Journal*, 37:272-285.

Eisler, R. (1988). Lead hazards to fish, wildlife, and invertebrates: a synoptic review, *Biological Report*. U.S. Department of the Interior, Fish and Wildlife Service, Laurel, Maryland 85:1-14.

El-Badawi, A.A., (2005). Effect of lead toxicity on some physiological aspects of Nile tilapia fish *Oreochromis niloticus. International Conference of Veterinary Research Division. National Research Council*. Cairo Egypt.249-277.

El-Nekeety, S., El-Kady, K., Soliman, M. S., Hassan, N. S., & Abdel-Wahab, M. A. (2009). Protective effect of *Aquilegia vulgaris* (L.) against lead acetate-induced oxidative stress in rats. *Food and Chemical Toxicology Journal*, 47:2209-2215.

Encyclopedia Britannica Lead/ Definition, Uses, Properties and Facts. https://www.britannica.com>science downloaded on 13/05/2020.

Enegide, C., Arome, D. and Solomon, A. (2013). A new method for determining acute toxicity in animal models. *Toxicology International*, 20(3):224-226 DOI 10.4103/0971 – 6580.121674.

EPA.(1986). Air quality criteria for lead. U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Research Triangle Park, NC, EPA600883028F.

Ercal, N., Gurer-Orhan, H., & Aykin-Burns, N. (2001). Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Current Topical Medical*

Formatted: No underline, Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto

Chemistry, 1: 529-539.

European Commission Report (2002) Heavy metals in waste. European Commission DG.ENV.E3.Project ENV.E.3/ETU/2000/0058. COWI A/S DenmarkP.11.Downloaded on 21/05/2020.

Fatima, M., & Usmani, N. (2013). Histopathology and bioaccumulation of heavy metals (Cr, Ni and Pb) in fish (*Channa striatus* and *Heteropneustes fossilis*) tissue: A study for toxicity and ecological impacts. *Pakistani Journal of Biological Sci*ences, 16: 412-420.

FHu, H., Shih, R., Rothenberg, S., & Schwartz, B.S. (2007).The epidemiology of lead toxicity in adults: measuring dose and consideration of other methodologic issues. *Environmental Health Perspectives*, 115:455–462.

Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: A review with recent updates. *Interdisciplinary Toxicolology Journal*, 5:47–58.

Flora, S.J.S. (2002). Nutritional components modify metal absorption, toxic response and chelation therapy. *Journal of Nutritional and Environmental Medicine*,12:53–67.

Flora, S.J.S., Flora, G.J.S., & Saxena, G. (2006). Environmental occurrence, health effects and management of lead poisoning. In: Cascas SB, Sordo J (eds) *Lead: chemistry, analytical aspects, environmental impacts and health effects.*(pp158–228). Netherlands, Elsevier Publication.

Flora, S.J.S. & Gupta R. (2007). Beneficial effects of *Centella asiatica* aqueous extract against arsenic-induced oxidative stress and essential metal status in rats. *Phytother Research*, 21**:** 980–988.

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

Flora, S.J., Mittal M. and Mehta, A., (2008). Heavy metals induced oxidative stress and its possible reversal by chelation therapy, *Indian Journal of Medical Research*, 128(1):501-523.

Flora, S.J.S. (2011). Arsenic induced oxidative stress and its reversibility. *Free Radical Biology and Medicine* , 51:257–281.

Fukumoto, K., Karai, I.,& Horiguchi, S. (1983). Effect of lead on erythrocyte membranes. *British Journal of Industrial Medicine*, 40**:** 220–223

Gabriel, U.U., Amakiri, E.U.& Ezeri, G.N.O. (2007). Hematology and Gill pathology of *Clarias gariepinus* exposed to refined petroleum oil, Kerosenne under laboratory conditions. *Journal of Animal and Veterinary Advances*, 6(3): 461-465.

Ganesh, H. G., Arun, J. P., Jyotsna, A. P., Mandakini, S, K., & Ajit, S. (2016). Biochemical effects of lead exposure on oxidative stress and antioxidant status of battery manufacturing workers of Western Maharashtra, India. *Journal of Basic Clinical Physiology and Pharmacology*,27:141-146.

Garcia-Medina, S., Razo-Estrada, A.C., Gomez-Olivan, L.M., Amaya-Chavez, A., Madrigal Bujaidar, E., & Galar-Martinez, M. (2010). Aluminium-induced oxidative stress in lymphocytes of common carp (*Cyprinus carpio*). *Fish Physiology and Biochemistry* 36:875-882.

Garza, A., Vega, R., & Soto, E. (2006).Cellular mechanisms of lead neurotoxicity. *Medical Science Monit*or, 12: RA57–RA65.

Goldstein, G.W (1990) Lead poisoning and brain cell function. Environmental Health Perspective 89:91-94 DOI: 10.1289/ehp.908991.

Gordon, N., Taylor, A.,& Benneth P.N (2002) Lead poisoning; case studies, *British Journal of Clinical Pharmacology*, 53(5): 451-458.

Gosling, E. (2004). Bivalve molluscs biology, ecology and culture. Fishing news books, library of congress cataloging-in- publication, data snails and fish pollution biomarkers in Lake Manzala and Laboratory : 78-100.

Grant, B.F., Seib, P.A., Liao, M..L.&Corpron, K.E.(1989). Polyphosphorylated L-ascorbic acid:A stable form of vitamin C for aquaculture feeds. *Journal of World Aquaculture Soc*iety, 20 (3):143-157.

Grant, L.D. (2008).Lead and compounds. *Environmental Toxicants (*pp 757–809) John Wiley & Sons, Inc.

Grant, L.D., & Davis, J.M. (1989). Effects of low-level lead exposure on paediatric neurobehavioural development: current findings and future directions. In: Smith MA, Grant LD, Sors AI (eds*) Lead exposure and child development: an international assessment*.(pp49–118.)Lancaster, MTP Press

Guillio Richard, T. & Hinton, David, E. (2008) The toxicology of fishes.(1st edition) pp 3-14.Florida CRC press, Taylor and Francis Group.

Gupta, R.C. (2012). *Veterinary toxicology: basic and clinical principles*, 2nd edition. (Pp 23-45) London, Elsevier.

Gurer, H.& Ercal, N. (2000). Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radical Biology and Medicine*,29(10):927- 945.

.

Gurer, H., Ozgunes, H., Saygin E. & Ercal, N., (2001). Antioxidant effect of taurine against lead

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

induced oxidative stress, *Archives of Environmental Contamination and Toxicology*, 4(2): 397- 402.

Halliwell, B.& Gutteridge, J.M.C.(2009).*Free radicals in biology and medicine* (2nd edition). Oxford, UK: Clarendon Press. (Pp.45-56)

Hamed, N. & Esmail, G. (2012).Determination of LC_{50} of lead nitrate and coppersulphate in common carp (*Cyprinus carpio*),*America European Journal of Toxicological sciences.*,4(2);60-63.

Hamer, D.H. (1986). Metallothionein. *Annual Review of Biochemistry*, 55: 913–951.

Hedayati, A. & Darabitabar, F. (2017). Lethal and sub-lethal impacts of lead on some hematological, biochemical and immunological indices in Caspian roach. *Pollution*, 3:21 27.

Higgins,T., Beutler, E.,& Doumas, B.T (2008). Measurement of blood haemoglobin In .C.A. Burtis, ER Ashwood & D.E Bruns (6th edition) *The fundamentals of clinical chemistry (*pp524-525) Saint Louis: MO: Sanders Elsevier.

Hilton, J.W., Cho, C.Y., Brown, R.G. and Slinger, S. J. (1978). Evaluation of the ascorbic acid status of rainbow trout (*Salmo gairdneri*). *Journal of Fisheries Research Board of Canada*, 34:2207-2210

Hinton, D.E., Baumann, P.C., Gardner, G.R., Hawkins, W.E., Hendricks, J.D., Murchelano, R.A. & Okihiro, M.S. (1992). Histopathologic biomarkers. In: Huggett, R.J., Kimerle, R.A., Mehrle J.R., Bergman, H.L.,(Editors)*Bio- markers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress*, 209Pp. Boca Raton Florida, Lewis Publishers.

Hodson, P.V., Blun, B.R., and Spry, D.J. (1977). Chronic toxicity of water-borne and dietary lead to rainbow trout(*Salmo gairdneri*) in lake Ontario water.*Water Research*,12:869-878.

Hodson, P.V., Blun, B.R., and Whittle, D.M. (1976) Biochemical monitoring of fish blood as an indicator of biologically available lead. *Thalassia Jugoslavica*, 16:389–396.

Holmgren, G.G.S., Meyer, M.W., Chaney, R.L., Daniels, R.B. (1993). Cadmium, lead, copper, and nickel in agricultural soils of the United States of America. *Journal of –Environmental Quality*, 22:335-348

Hontela , A.(2005). Adrenal toxicology: Environmental pollutants and HPI axis in *Biochemistry and Molecular Biology of fishes.* Pp 6.

Hontela , A., Dorval, J.,& Leblond, V.S. (2003).Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (Oncorhynchus mykiss) exposed *in vitro* to endosulfan, an organochlorine pesticide. *Aquatic Toxicology*-: 63 (3): 229-41.

Hoogeveen, J. T. (2000). Thermoconductometric- investigation of phosphatidylcholine in aqueous tertiary butanol solutions in the absence and presence of metal ions. In: Maniloff, J. Coleman J. R.; Miller, M. W. Effects of metals on cells, subcellular elements and macromolecule.(pp207-269)Springfield IL: Thomas.

Horiguchi,H.,Oguma, E &. Kayama, F. –(2011). Cadmium induces anaemia through Interdependent progress of hemolysis, body iron accumulation and insufficient erythropoietin production in rats. *Toxicological Sciences*, 122(1): 198-210.

Hultberg, B., Andersson, A. ,& Isaksson, A.(2001). Interaction of metals and thiols in cell damage and glutathione distribution: potentiation of mercury toxicity by dithiothreitol

*Toxicology,*156:93–100.

Ikeogu, C.F, Nsofor C.I, Igwilo, I.O &Ngene, A.A (2016a). Haematological and serological responses of *Clarias gariepinus* to sublethal concentrations of lead nitrate. *Journal of Pharmaceutical Science and Bioscientific Research,* 6(3) :442-446.

Ikeogu,C.F.,Nsofor,C.I.,Ikpeze,O.O.,Nwuba,L.A.,Ezeasor,D.N.&Nwaogu,I.C.(2016b). Histopathological alterations in gills of catfish and changes in aquarium pH, temperature and dissolved oxygen due to lead nitrate and crude oil. *Advances in Bioresearch,7(2):35-40.*

Ikeogu, C.F., Nsofor, C.I., Ikpeze, O.O Ezeasor, D.N..& Nwaogu, I.C.(2016c). Histopathological changes in liver of *Clarias gariepinus* fish exposed to sub-lethal concentrations of lead nitrate and crude oil. *Advances in Bioresearch,7(2):41-45.*

International Programme on Chemical Safety.(1995). Inorganic lead. *Environmental health criteria.* Pp 165 W.H.O., Geneva.

Jangid, A.P., John, P.J., Yadav, D., Mishra, S., &Sharma, P. (2012). Impact of chronic lead exposure on selected biological markers. *Indian Journal of Clinical Biochemistry*, 27: 83–89.

Jauncey, K., Soliman, A., & Roberts, R.J. (1985). Ascorbic acid requirements in relation to wound healing in the cultured tilapia, *Oreochromis niloticus* (Trewavas). *Aquaculture and Fisheries Management,* 16: 139-149.

Javed, M. (2005). Heavy metal contamination of freshwater fish and bed sediments in the river ravi stretch and related tributaries. *Pakistani Journal of Biological Sciences*, 8: 1337-1341.

Javed, M., and Usmani, N. (2011). Accumulation of heavy metals in fishes: A human health

concern. *International Journal of Environmental Sciences*,2: 659-670.

Jeong-Hyeon Kim. Hyeong-Min Byun, Eui-cheolchung., Han-Young chung, & OK-Nam Bae (2013). Loss of integrity, impairment of the blood brain barrier in heavy metal associated ischemic stroke. *Toxicology Research*, 29: 157-164.

Jezierska, B., & Witeska, M. (2001). Summary of metal-induced disturbances in fish organism In: *Metal Toxicity to Fish*. (.pp 214–243) Wydawnictvo Akademii Podlaskej, Siedlce.

Jobling, M. (1994).*Fish bioenergetics.* Pp 34.London Chapman & Hall.

Joshi, P. & Deep, H. (2002). Effect of lindane and malathion exposure to certain parameters in a fresh water teleost fish *Clarias batrachus. Pollution Research*, 21:55-57.

Kalia K, & Flora, S. J. (2005).Strategies for safe and effective therapeutic measures for chronic arsenicand lead poisoning. *Journal of Occupational Health*, 47:1–21.

Kamunde, C., Clayton, C.,& Wood, C.M. (2002): Waterborne vs. dietary copper uptake i rainbow trout and the effects of previous waterborne copper exposure. *American Journal of Physiology –Regulatory, Integrative and Comparative Physiology*, 283: R69–R78.

Kelly, S.A., Havrilla, C.H.M., Brady, T.C., Abramo, K.H., & Levin, E.D. (1998).Oxidative stress in toxicology: established mammalian and emerging piscine model systems. *Environmental Health Perspectives,* 106: 375–384.

Khan, I.A. (2000). Lead and Aroclor 1254 disrupt reproductive neuroendocrine function in Atlantic croaker. *Marine Environmental Research,* 50:119–123.

Khoshnood, Z., khodabandeh, S., Moghaddamm, S., Khorjestan S.M., (2011). Histopathological and pathomorphological effects of mercuric chloride on the gills of Persian sturgeon *Acipenser persicus,* fry. *International Journal of Nature Resort and Marine Science*, 1:23-32.

Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M., and Flick, R.W. (2007). Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences of the United States of America,* 104 (21): 8897–8901.

Knollman- Ritchel, B.F.C and Markowitz, M. (2017) Lead poisoning.Academic pathology.Published online DOI 10.1177/2374289517700160. Downloaded on 15/05/2020.

Kori-Siakpere, O., and Ubogu, E.O. (2008). Sublethal haematological effects of zinc on the freshwater fish, *Heteroclariassp*.(Osteichthyes:Clariidae),.*African Journal of Biotechnology*, 7: 2068-2073

.

Kori-Siakpere, O., Ogbe , M.G., and Ikomi, R.B. (2009). Haematological response of the African catfish, *Clarias gariepinus* to sublethal concentrations of potassium permanganate. *Scientific Research and Essay*, 4(5); 457-466

Koshio Shunsuke (2015). Immunotherapies targeting fish mucosal immunity-current knowledge and future perspective. *Frontiers in Immunology* 6:643.

Kruger, R., Pietrock, M., Meinelti, T., Yoshida, W., Steffens, T., & C. Steinberg, (1996) Distribution of macrophage centres in bream (Abramis brama L.) liver from the Oder river (Germany/Poland) within the nature reserve Unteres Odertal near the town of Schwedt.

Internationale Revue Gesamten Hydrobiologie Hydrographie, 81: 635-644.

Lal, V.F., Gaur, K.K. & Thakur, S. (2013). Bioassay evaluation of acute toxicity levels of lead chloride to *Channa punctatus* Bloch. *Asian Journal of Biological Sciences.* 8.1 :43-46.

Latif, A., Alim Sayyed, A.H., Igbal, F., Usman, K., Rauf, M. & Kaoser, R., (2013) Effect of copper sulphate and lead nitrate administered alone or in combination, on the histology of liver and kidney of *Labeo rohita*. *Pakistan Journal of Zoology*, 45(4):913-920.

Launer, C.A., Tiemeier, O.W., & Deyoe, C.W. (1978). Effects of dietary addition of vitamins C and D3 on growth and calcium and phosphorus content of pond-cultured channel catfish. *The Progressive Fish Culturist* 40(1):16-20.

Lawton, L. J., and Donaldson, W. E. (1991). Lead-induced tissue fatty acid alterations and lipid peroxidation. *Biological Trace Element Research,* 28:83–97.

Leggett, R. W. (1993). An age-specific kinetic model of lead metabolism in humans *Environmental Health Perspective,*101: 598–616.

Li, Y., & Lovell, T. (1985). Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. *Journal of Nutrition*, 115:123-131.

Lidsky, TI.,& Schneider, J.S. (2003). Lead neurotoxicity in children: basic mechanisms and clinical correlates. *Brain,* 126:5–19.

Lightner, D.V., Colvin, L.B., Brand, Cl, & Danald, D.A. (1977). Black Death, a disease syndrome of penaeid shrimp related to a dietary deficiency of ascorbic acid. *Proceedings of the World*

Mariculture Society,8:611-623.

Lim, C., & Lovell, R.T. (1978). Pathology of vitamin C deficiency syndrome in channel catfish. *Journal of. Nutrition*, 108: 1137-1146.

Livingstone, D.R. (2003). Oxidative stress in aquatic organisms in relation to pollution and agriculture. *Revue de Medecine Veterinaire*, 154: 427–430.

Locke, L.N., Bagley, G.E., & Irby, H.D. (1966). Acid-fast intranuclear inclusion bodies in the kidneys of mallards fed lead shot. *Bulletin of Wildlife Disease Association*, 2: 127–13.

Lovell, R.T. (1982). Elevated levels of vitamin C increase disease resistance in channel catfish. Reprint from Highlights of *Agricultural Research*,29 ,(1). Alabama Agricultural Experiment Station, Auburn University, Alabama.

Lovell, R.T., & Lim, C. (1978). Vitamin C in pond diets for channel catfish. *Transactions of the American Fisheries Society*, 107(2):321-325.

Luoma,S.N.,& Rainbow, P.S.(2008). Sources and cycles of trace metals. In: *MetalContamination in Aquatic Environments: Science and Lateral Management.* Cambridge. University Press (pp 47–66).

Lushchak, V.I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*, 1: 13–30.

Macintyre JE (1994) Dictionary of organometallic compounds Boca Raton, FLorida.CRC Press. ISBN 0412430606.

Mager, E.M., Brix, K.V., Gerdes, R.M., Ryan, A.C. & Grosell, M. (2011). Effects of water

chemistry on the chronic toxicity of lead to the Cladoceran, (*Ceriodaphnia dubia*) *Exotoxic and Environmental Safety*, 74.3: 238-243.

Mahajan, C.L.& Agrawal, N.K. (1980). The role of vitamin C in calcium uptake by fish. *Aquaculture*, 19:287-294.

Maheswaran, R., Devapaul, S., Muralidharan, B., Velmurugan, A., & Ignacimuthu, S. (2008) Haematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride. *International Journal of Integrative Biology*, 2: 49-54.

Malgortzata, W., Elzbieta, K., Monika & Malgorzata, O. (2010). Haematological changes in common carp (*Cyprinius carpio*) after short term lead exposure. *Polish Journal of Environmental Studies,* 19 (4):825-831

.

Martinez, C.B, Nagae, M.Y, Zaia, C.T. & Zaia D.A., (2004). Acute morphological and physiological effects of lead in the neotropical fish *Prochilodus lineatus*. *Brazilian Journal of Biology*, 64 (4): 797-807.

Mates, J. M (2000). Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology* 153:83–104.

Mayer, F.L., Mehrle, P.M., & Dwyer, W.P. (1978). Toxaphene: Chronic toxicity to fathead minnows and channel catfish. U.S. Environmental Protection Agency, *Ecological Research Series* EPA-600/3-77,069. 49 pp.

McGrath, S.P., Chang, A.C., Page, A.L., & Witter, E. (1994). Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and the United States. *Environmental Review*, 2:108–118.

.

Michael, M.I., Hilmy, A.M., EL-Domiaty N.A. &Wershara K. (1987). Serum transaminase activity and histophathological changes in *Clarias lazera* chronically exposed to lead nitrate. Comprehensive *Biochemistry & Physiology Journal*, 86: 255-262.

.

Mobarak, Y.M.S. & Sharaf, M.M.(2011). Lead acetate- induced histopathological changes in the gills and digestive system of silver sailfin methy (*Poecilia latipinla). International Journal of Zoological Research, 7(1): 1-18.*

Mohammed, A.A., Mohd Noor, M.H, Abdwahid, H., Mohd, Y., Mohd, S. and Mohd, A.R (2016) The detrimental effects of lead on human and animal health. *Veterinary World* 9 (6): 660-671 DOI: https://doi.org/10.14202/vetworld2016.660-671.

Monteiro, S.M, Mancera, J.M., Fontainhas-Fernandes, A.E & Souse, M. (2005). Copper induced alterations of biochemical parameters in the gill and plasma of *Oreochromis niloticus*. *Comprehensive Biochemistry &Physiology Journal*, 14: 375-383.

Moore, J., & Goyer, R.A. (1974). Induced-lead inclusion bodies: composition and probable role in lead metabolism. *Environmental Health Perspective*, 7:121–127.

Moulis, J.M. (2010). Cellular mechanism of cadmium toxicity related to the homeostasis of essential metals. *Bio-metals*, 23(5): 877-896

.

Mousa, M. A., & Khattab, Y. A., (2003). The counteracting effect of vitamin – C (L – ascorbic acid) on the physiological perturbations induced by ochratoxin intoxication in the African catfish (*Clarias gariepinus*). *Journal of Egypt Academy and Environmental Development*,4(1): 117 – 128

.

Myers, M.S., Johnson, L.L., Olson, O.P., Sther, C.M., Horness, B.H., Collier, T.K. & McCain, B.B. (1998). Toxicopathic hepatic lesions as biomarkers of chemical contaminant exposure and effects in marine bottom fish species from the Northeast Pacific Coast. USA. *Marine Pollution Bulletin*, 37:92-113

Najarnezhad, V., Aslani, M.R., & Balali-Mood, M. (2010). The therapeutic potientials of thiamine for treatment of experimentally induced sub-acute lead poisoning in sheep. *Comprehensive Clinical Pathology*, (19):69-73.

Nandi, D., Patra R.C & Swarup D. (2005). Effect of Cysteine, Methionine, Ascorbic acid and Thiamine on Arsenic-induced Oxidative stress and biochemical alteration in rats. *Toxicology* 211(1-2):26-35.

Neelima, P., Sunitha, K., Gopala, R.N., &Rao, J.C.S. (2015). Haematological alterations in *Cyprinus carpio* as biomarkers of cyper methrin toxicity. *International Journal of Current Research,* 7 (8):18864-18870.

Nesto, N.S., Romano, V., Moschino, M., & Da Ros, L. (2007).Bioaccumulation and biomarker responses of trace metals and micro-organic pollutants in mussels and fish from the Lagoon ofVenice, Italy. *Marine Pollution Bulletin*, 55: 469-484.

Newman N., Binns H., Karwowski M. and Lowry J. (2013) Recommendations on medical management of childhood lead exposure and poisoning. American Academy of Pediatrics. http://www.snohd.org/documentcentre/view/1997. Downloaded on 05/26/2020

Nicula, M., Negrea, I., Gergen, M., Harmanescu, I., Gogoasa, P., & Lunca, M. (2009). Mercury bioaccumulation in tissues of fresh water fish *Carassius auratus gibelio* (Silver

CrucianCarp) after chronic mercury intoxication.*Universitatea Stiinte Agricole Medicina Veterinara Iasi Lucrari Stiintifice*, 52: 676-679.

Nielsen, T. (1984). Atmospheric occurrence of organolead compounds. In: Grandjean, P. (ed) *Biological effects of organolead compounds*.(pp 43-62) Boca Raton, Florida, CRC Press,

Niki, E. (1991). Action of ascorbic acid as a scavenger of active and stable oxygen radicals *American Journal of Clinical Nutrition*, 54:1119-1124.

Nishida, Y. (2011): The chemical process of oxidative stress by copper (II) and iron (III) ions in several neurodegenerative disorders. *Monatshefte fur Chemie*,142, 375–384.

Noegrohati, S. (2006). Bioaccumulation dynamic of heavy metals in *Oreochromis niloticus* (predicted through a bioaccumulation model constructed based on biotic ligand model (BLM)).*Sri Noegrohati Bioaccumulation Dyn. Heavy*, 16: 29-40.

Nourian K, Shahsavani D, & Baghshani, H. (2018). Effect of lead exposure on some blood biochemical indices in *Cyprinus carpio*: Potential alleviative effect of Thiamine. *Comparative Clinical Pathology*, 28(1):189-194.

Nsofor, C.I., Igwilo, I.O., Ikpeze, O.O., Ikeogu, C.F., Umeoguagu, F.O. and Okonkwo, C.J. (2014a). Bioaccumulation of Heavy Metals in Shellfish *Macrobrachium rosenbergi* in Niger River at Onitsha.*International Journal of Agriculture and Biosciences*, 3 (1): 38-40.

Nsofor, C.I., Ikpeze, O.O., Ngenebo, U.C., Ikeogu, C.F. and Okonkwo, C.J. (2014b). Histopathological Alterations in the liver and kidney of the fish *Crysichthys nigrodigitatus* due to heavy metals in Niger River. *Journal of Natural Sciences Research* 4,(12):2224-3186.

Obande, R.A., Omeji, S. & Obilikwu, H. (2013). Acute toxicity of n-phosphonomethly glycine (Delsate)(R) on the African catfish *Clarias gariepinus* fingerlings. *Research Journal of Agriculture and Environmental management*, 2(10): 302-306.

Obasohan, E.E. (2008). Bioaccumulation of chromium, copper, manganese, nickel and lead in a freshwater cichlid, *Hemichromis fasciatus* from Ogba River in Benin City, Nigeria. *African Journal of General Agriculture*, 4: 141-152.

OECD. (1993). Risk Reduction Monograph No.1: Lead. OECD/GD (93) 67. OECD Environment Directorate, Paris. Cory-Slechta DA. Legacy of lead exposure: consequences for the central nervous system. *Head and Neck Surgery*, 114:224–226.

Okomoda, J., Ayuba; V.O. &Omeji; S. (2010). Haematological changes of *Clarias gariepinus* Fingerlings exposed to acute toxicity of formalin. PAT June, 6(1):92-101.Publication of Nasarawa state university, Keffi online copy available atwww.patnsukjournal.net/currentissue.

Olainike, K., Funmilola, A., Olufemi, B. & Olajide, O. (2008). Acute toxicity and blood profile of adult *Clarias gariepinus* exposed to lead nitrate. *International Journal of Hematology*, 4(2):1-9.

Olmeda, P. PLa, A., Hernandez, A.F., Barbier, F., Ayouni, L.,& Gil, F. (2013). Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environmental International*, 59: 63-72.

Olojo, E.A., Olurin, K.B., Mbaka, G & Oluwemimo, A.D. (2005).Histopathology of the gill and liver tissues of African catfish, *Clarias gariepinus* exposed to lead. *African Journalof*

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

Biotechnology,4:117-122.

Olowu, R.A., Ayejuyo, O.O., Adewuyi, G.O., Adejoro, I.A., Denloye, A.B., Babatunde, A.O.& Ogumdajo, A.L.(2010). Determination of heavy metals in fresh tissues, water and sediments from EPE and Badagry lagoon, Lagos, *Nigeria Journal of Chemistry*,7(1): 215-221.

Oluwatosin, E. A., Aladesanmi, O.T., Kosemani, O.E. & Adewusi, O.A. (2018). Effects of lead chloride on growth performance of *Clarias gariepinus* (Burchell,1822). *International Journal of Bioassays 7.5: 5638- 5644.*

Omitoyin, O.B., Ajani, K.E., & Fajimi, A.O.(2006). Toxicity of Gramoxone (paraquat) to juvenile African catfish, *Clarias gariepinus. World Journal of Zoology*, 1(1): 57-63.

Oseni , O.A., Odesanmi, E.O., Oloyede, O.I., Adebayo, O.D and Ogundare, M.A.B (2018) Antioxidant and hepatoprotective activities of *Carica papaya* (pawpaw leaf) and *Loranthus bengwensis* (cocoa mistletoes) against induced hepatoxicity in rats. *International Journal of Life Science And Specific Research.* 4(5):1974-1982 DOI: 10: 21276/ijlssr.2018.4.5.3

Palaniappan, P.L., Krishnakumar, N.,& Vadivelu, M.(2009). Bioaccumulation of lead and the influence of chelating agents in *Catla catla* fingerlings. *Environmental Chemistry Letter*, 7:51–54.

Paquin, P.R., Gorsuch, J.W., Apte, G.E., Batley, S., and Bowles, K.C. (2002). The biotic ligand model: A historical overview. *Comparative Biochemistry Physiology,Toxicology and Pharmacology*,133 : 3-35.

Partridge, B.L (1982).The structure and function of fish schools.*Scietific America*, 246:114-123.

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

Patel, M., Rogers, J.T., Pane, E.F., and Wood, C.M. (2006). Renal responses to acute leadwaterborne exposure in the freshwater rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, 80: 362–371.

Patrick, L. (2006). Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, a treatment. *Alternative Medical Review* 11:2–22.

Patterson, C. (1956). Age of meteorites and the earth.*Geochim Cosmochim Acta*, 10: 230–237.

Pearce, J. M. (2007). Burton's line in lead poisoning. *European Neurology*, 57:118–119.

Peters, N., Köhler, A., and Kranz, H. (1987). Liver pathology in fishes from the lower Elbe as a consequence of pollution. *Diseases of Aquatic Organisms*, 2: 87-97.

Philip, A.T., and Gerson, B. (1994). Lead poisoning--Part I. Incidence, etiology, and toxicokinetics. *Clinical Laboratory Medicine*, 14:423–444.

Pim de Voogt (2016) *Reviews of Environmental Contamination and Toxicology Springer* 238:48-51.

Piomelli, S. (2002).Childhood lead poisoning. *Pediatrics Clinics of North America*, 49:1285-1304.

Podmore, I.D., Griffiths, H.R., Herbert, K.E., Mistry, N., Mistry, P and Lunec, J. (1998) Vitamin C exhibits pro-oxidant properties. *Nature* 392,559.

Pourmoghaddas, H., & Shahryari, A. (2010). The concentration of lead, chromium, cadmium, nickel and mercury in three species of consuming fishes of Isfahan city. *Health System.Research*, 6: 30-35.

Pratap, H. B. & Wendelaar Bonga, S. E. (1990). Effects of water-borne cadmium on plasm

cortisol and glucose in the cichlid fish *Oreochromis mossambicus*. *Comparative Biochemistry and Physiology*, *95*(C): 313-317.

Praveen, K.S., Sohini, S. & Ashutosh,S.(2015).The effect of L-ascorbic acid supplementation on the immune response of Channa punctatus exposed to lead and cadmium. *International Journal of Applied Environmental Sciences* 10 (2): 545-557.

Radhaiah, V., & Jayantha, R.K. (1992). Fenvalerate toxicity to the liver in a freshwater teleost, *Tilapia mossambica*.*Comparative Physiology and Ecology*,17(2): 48-53.

Raghavan, S. R. (1981). Erythrocyte lead-binding protein after occupational exposure. II. Influence on lead inhibition of membrane Na1 2 K1-ATPase. *Journal of Environmental Health Toxicology*, **7:** 561–568.

Randall, D.J. (1970). The circulatory system. In D.J Randall (ed.). *Fish Physiology*, vol IV. The Nervous system, circulation and respiration. Pp 133-172 Academic press.

Rastogi, S.K. (2008). Renal effects of environmental and occupational lead exposure. *Industrial Journal of Occupational Environmental Medicine*, 12:103–106.

Rauf, A., Javed, M. & Ubaidullah, M. (2009). Heavy metal levels in three major carps (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) from the river Ravi, Pakistan. *Pakistani Veterinary* Journal, 29:24-26.

Reddy, K.J., Wang, L., & Gloss, S.P.(1995).Solubility and mobility of copper, zinc and lead in acidic environments. *Plant Soil*, 171:53–58.

Reed, B.E., Moore, R.E., Cline, S.R. (1995). Soil flushing of a sandy loam contaminated with Pb(II), PbSO4 (s), PbCO3 (3) or Pb-naphthalene: column results. *Journal of Soil*

Contamination, 4: 243-267.

Renner, R. (2010). Exposure on tap drinking water as an overlooked source of lead. *Environmental Health Perspective*, 118:A68–A74.

Rodriguez- Sanchez, V., Guzman-Moreno, J & Rodriguez- Gonzalez, V.(2017). Biosorption of lead phosphates by lead tolerant bacteria as a mechanism for lead immobilization. *World Journal of Microbiology and Biotechnology*. 33:150 https://doi.org/10.0007/S11274-017 -2314-6

Roesijadi, G.(1996). Metallothionein and its role in toxic metal regulation. *Comparative Biochemistry and Physiology*,113: 117–123.

Roganovic-Zafirova, D., Jordanova, S., Panov, M.& Velkova-Jordanoska, L. (2003). Hepatic capillariasis in the Mediterranean barbell (*Barbus meridionalis* petenyi Heck.) from lake Ohrid.*Folia Veterinary*, 47: 35-37.

Rout,P.C& Niak,B.N., (2013).Tracing out correlation between blood lead and haematological parameters in *Clarias batrachus*, Linn.during experimental plumbism. *International Journal of Current Research*,5(10):2770-2775.

Rubin, R.& Strayer,D.(2008).*Rubin's Pathology Clinicopathologic Foundations of Medicine* 5th edition, Lippincot, Williams and Wilkin 34Pp..

Ruby, S.M., Hull, R., & Anderson, P. (2000). Sublethal lead affects pituitary function of rainbow trout during exogenous vitellogenesis. *Archives of Environmental Contamination and Toxicology*, 38:46–51.

Saleh, H.A.,El-Aziz, G.A., El-Fark, M.M.,& El-Gohary, M. (2009). Effect of maternal lead exposure on craniofacial ossification in rat fetuses and the role of antioxidant therapy *Anatomia Histologia Embryologia*, 38:392–399.

Salman, N, M (2014).Haematological and histopathological effect of cadmium chloride on Mesopotamichthys *sharpeyi*. MSC thesis Veterinary Medicine College, Baghdad University. Pp 168.

Samanta P., Pal, S, Mukherjee A.K., Kole D. & Ghosh, A, R (2016). Histopathological study in stomach and intestine of *Anabas testudineus* under Almix(R) exposure. *Fisheries and Aquaculture Journal*, 7: 3 DOI:10.4172/2150-3508.1000177.

Sanders, T., Liu, Y., Buchne, V., & Tchounwou, P.B. (2009). Neurotoxic effects and biomarkers of lead exposure: a review. *Research of Environmental Health*, 24:15–45.

Sandnes, K., Ulgenes, Y., Braekkan, O.R., & Utne, F. (1984). The effect of ascorbic acid supplementation in brood stock feed on reproduction of rainbow trout (*Salmo gairdneri*). *Aquaculture.* 43:167-177.

Schulte, S., Miller, W.E., and Friedberg, F.R. (1994).*In vivo* exposure to lead does not influence muscarinic receptors in the frontal cortex of the mouse brain. *Toxicology*, 93: 99-112.

Sen, I., Shandil, A.& Shrivastava, V.S. (2011). Study for determination of heavy metals in fish speciesof the river Yamuna (Delhi) by inductively coupled plasma-optical emissions spectroscopy (ICP-OES). *Advances in Applied Science Research*, 2: 161-166.

Seyed Ehsan Mousavic & Mehdi Yousefian (2012). The alteration in the haematological parameters of endangered Caspian brown trout *Salmo trutta caspius* exposed to waterborne mercuric chloride. *Asian Journal of Animal Science* 6:154 -163.

Sevcikova, M., Modra, H ., Slaninova, A and Svobodova, Z. (2011). Metals as a cause of oxidative stress in fish: a review *Veterinarni Medicini* 56(11):537- 546.

Shafiq-ur-Rehman, S.,& Abdulla, M. (2003). Impacted lead on phospholipid metabolism in human erythrocyte membranes. *Bulletin of Journal of Occupational, Environmental Health and Toxicology*,:35.

Shah, S.L.& Altindu, A., (2005) Effect of heavy metal accommodation on the 96 hour LC_{50} values in Tench, *Tinca tinca*. *Turkish Journal of Veterinary & Animal Science*, 29:139-144.

Shahsavani, D., Baghshani, H. & Nourian, K. (2017). Effect of thiamine and Vitamin C on tissue lead accumulation following experimental lead poisoning in *Cyprinus carpio*. *Iranian Journal of Veterinary Science and Technology*, 9 (1):39-44.

Shamshun, N., Chandan, K., Kavita, M. Mamta, K.,(2010) Lead induced alteration in blood profile of air breathing catfish *Clarias batrachus*. *The Bioscan*: 2; 335-341.

Shatha, H. K., Nazarmuteb, Z. A., & Moayad, M. U. (2016). The effect of penicillamine in reducing the toxic effects of lead acetate on some blood parameters, liver functions and testicular tissue in male rats. *International Journal of Pharmaceutical Research*, 5:22-40.

Singh, B.O. & Manjeet, K. (2015). Determination of LC_{50} of Lead Nitrate for a fish, *Labeo rohita* (Hamilton- Buchanan). *International Research Journal of Biological Sciences*,4(8):23-26.

Sinicropi, M.S., Amantea, D., Caruso, A., & Saturnino, C. (2010). Chemical and biological properties of toxic metals and use of chelating agents for the pharmacological treatment of metal poisoning. *Archives of Toxicology*, 84:501–520.

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

Slaninova, A., Smutna, M, Modra, H.,& Svobodova, Z. (2009). A review: Oxidative stress in fish induced by pesticides. *Neuroendocrinology Letters*, 30, 2–12.

Smirnov, L.P., Sukhovskaya, I.V., &Nemova, N.N. (2005). Effects on environmental factors on low-molecular-weight peptides of fishes: A Review. *Russian Journal of Ecology*,3: 41-47.

Smith, J.F (2001) Lead alloys: Alloying, properties and applications in *Encyclopedia Of Materials Science And Technology*. Elsevier Ltd. ISBN 978-0-08-043152-9 https://www.sciencedirect.com. Downloaded on 05/20/2020.

Smith, J.R. (1994). Fish neurotoxicology. In L.J. Weber(ed.) Aquatic toxicology, vol 2. Pp 107-151. New York. Raven press.

Soliman A.K.,Jauncey, K.,&Toberts, R.J. (1986). The effect of dietary ascorbic acid supplementation on hatchability, survival rate and fry performance in *Oreochromis mossambicus* (Peters). *Aquaculture*. 59:197-208.

Spokas, E.G., Spur W Bernd, Smith, H., Kemp, F.W., & Bogden, J.D. (2006). Tissue lead concentration during chronic exposure of *Pimephales promelas* (Fathead minnow) to lead nitrate in aquarium water. *Environmental Science & Technology*, 40 (21) 6852- 6858.

Stohs, S.J and Bagchi, D (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radicals Biology and Medicine2*, 321-336.

Streit, B. (1998). Bioaccumulation of contaminants in fish. In: Braunbeck T, Hinton DE, Streit B (eds) *Fish ecotoxicology*. (Pp 353-387) Birkha¨ user, Basel.

Tabche, L.M., Martinez, C.M., & Sanchez-Hidlago, E. (1990).Comparative study of toxic lead effects on gill and haemoglobin of tilapia fish. *Journal of Applied Toxicol*ogy, 10:193-195.

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted ...
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

Tao, S., Wen, Y., Long, A., Dawson, R., Cao, J., & Xu, F. (2001). Simulation of acid-base condition and copper speciation in fish gill microenvironment. *Computers and Chemistry* 25:215–222.

Tawari-fufeyin P., Igetei J., & Okoidigun, E.M. (2008). Changes in the catfish (*Clarias gariepinus*) exposed to acute cadmium and lead poisoning. *Bioscience Research communication*, 20 (5): 271-276.

Taweel, A., Shuhaimi-Othman, M., and Ahmad, A.K. (2011). Heavy metals concentration in different organs of tilapia fish (*Oreochromis niloticus*) from selected areas of Bangi , Selangor, Malaysia. *African Journal of Biotechnology*, 10: 11562-11566

Thomas, P. (1990). Molecular and biochemical responses of fish to stressors and their potential use in environmental monitoring. In: S. M. Adams (ed.), *Biological indicators of stress infish*. American Fisheries Society, Bethesda, Maryland. Pp 57.

Thomas, P. & Juedes M.J. (1992). Influence of lead on the glutathione status of Atlantic croaker tissue. *Aquatic Toxicology*, 23: 11-30.

Thrall, M.A & Weiser M.G (2002). Haematology in Hendrix C.M. editor Laboratory procedures for Veterinary Technicians. 4th ed. ; P: 29-74 Missouri: Mosby inc.

Tilak, K.S., Koteswara, R., &Veeraiah, K. (2005). Effects of chlorpyrifos on histopathology of the fish Catla catla. *Journal of Ecotoxicological & Environmental Monitoring*, 15(2): 127-140.

Todd, A.C., Wetmur, J.G., Moline, J.M, Godbold, J.H., Lewin, S.M and Landrigen, P.I. (1

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

Unveiling the chronic toxicity of lead, an essential priority for environmental health. *Environmental Health perspective*, 104: 141-146.

Tolbert, B. M. (1979). Ascorbic acid metabolism and physiological function. *International Journal of Vitamin and Nutrition Research*, 19: 127-142.

Tong, S., and McMichael, A.J. (1999). The magnitude, persistence and public health significance of cognitive effects of environmental lead exposure in childhood. *Journal of Environmental Medicine*, 1:103-110.

Tucker, B.W., and J.E. Halver.(1984). Ascorbate-2-sulfate metabolism in fish. *Nutrition Reviews*., 41 (5)173-179.

Tulasi, S.J., Reddy, P.U.M., Rao, J.V. (1992). Accumulation of lead and effects on total lipids and lipid derivatives in the freshwater fish *Anabas testudineus* (Bioch). *Ecotoxicology and Environmental Safety*, 23:33–38.

Udeh,G.N. and Oti, E.E.(2014) Behavioural and some physico-chemical assessment of freshwater Catfish *Clarias gariepinus* exposed to acute concentrations of Delsate$^{(R)}$ herbicides (Glyphosate). *Journal of Aquatic Sciences*, 29(2A):275-283.

United State Environmental Protection Agency (USEPA) (2002). Fish and wildlife service. *Fish bulletin*, 76: 1-44.

USEPA (2000). Endocrine disruptor screening programme. Report to congress Washington, D.C. P.4.

Valko, M., Morris, H., and Cronin, M.T. (2005).Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*. 12:1161-1208.

Formatted ... (×30)

Vaya, J., and Aviram, M. (2000). Nutritional antioxidants mechanisms of action, analyses of activities and medical applications. *Current Medical Chemistry-Immunology Endocrine and Metabolic Agents* 1**:**99–117.

Verma, K and Belsare, S.D (2005).Superoxide dismutase level in fish tissues as indicator of Lead toxicity. In *Fisheries and Fish Toxicology* New Delhi, India.APH publishing 137p.

Vij, A.G. (2009). Hemopoietic, hemostatic and mutagenic effects of lead and possible prevention by zinc and vitamin C. *Al Ameen Journal of Medical Sciences*, 2: 27–36.

Vinodini, R. &Narayanan, M. (2008).Bioaccumulation of heavy metals in organs of fresh water fish, *Cyprinus carpio*. *International Journal of Environmental Science and Technology*, 5(2):179-182.

Vosylienė, M. Z., & Kazlauskienė, N. (2004). Evaluation of the Svede pond water effect on fish (after accidental discharge of the Kairiai dump filtrate into the environment).Protection and management of water bodies. *Proceedings of International Scientific Conference. Kaunas*, 219–223.

Wagner, M., Klein, C.L., Van Kooten, T.G., & Kirkpatrick, C.J. (1998). Mechanisms of cell activation by heavy metal ions. *Journal of Biomedical Materials Res*earch, 42: 443-452. DOI: 10.1002/ (sili) 1097-4636(19981205) 42:3<443: aid-jbm14>3.0.co;2-h

Wagstaff, D.J., & J.C. Street.(1971). Ascorbic acid deficiency and inductions of hepatic microsomal hydroxylative enzymes by organochlorine pesticides. *Toxicology and Applied Pharmacology*, 19:10-19.

Wahbi, O.M.& El-Greisy, Z.A. (2007). Comparative impact of different waste sources on the reproductive parameters and histology of gonads, liver and pituitary gland of *Siganus*

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 12 pt, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

rivaltus. *Journal of Applied Sciences Research*, 3(3): 236-244.

Wahli, T., Frischknecht, R., Sschmitt, M., Gabaudan, J., Verlhac, V., and W. Meier, F.J. (1995). A comparison of the effect of silicone coated ascorbic acid and ascorbyl phosphate on the course of icthyophthiriosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, 18:347-355.

Wahli, T., Meier, W., and Pfister, K. 1986). Ascorbic acid induced immune mediated decrease in mortality in *Ichthyophthrius multifiliis* infected rainbow-trout (*Salmo gairdneri*). *Acta Tropica*. 43:287-289.

Wahli, T., Streiff, K., & Meier, W. (1985). Influence of ascorbic acid on *Ichthyophthirius Multifiliis* infections in trout. *Bulletin of European Association of Fish Pathologists*, 5 (4): 86-87.

Waldron, H. A. (2006). The anemia of lead poisoning: a review. *British Journal of Industrial Medicine*, 23**:**83–100.

Wanasundara, P.K., & Shahidi, F. (2005). Antioxidants: Science, Technology, and Applications. *Bailey's Industrial Oil and Fat Products*: pp 45-90 John Wiley & Sons, Inc.

Wang, W.X., & Rainbow, P.S. (2007). Sub cellular partitioning and the prediction of cadmium toxicity to aquatic organisms. *Environmental Chemistry*, 3: 395-399.

Wang, W.X., and Rainbow, P.S. (2008). Comparative approaches to understand metal bioaccumulation in aquatic animals. ~~*Comparative Biochemistry*~~*Comparative Biochemistry*, *Physiology, Toxicology and Pharmacology*, 148: 315-323

Weber, D.N. (1993). Exposure to sub lethal levels of waterborne lead alters reproductive behavior patterns in fathead minnows (*Pimephales promelas*). *Neurotoxicology* 14(2–3):347–358.

Weber, L.J. and Gingerich, W.H. (1982). Hepatic toxicology of fishes. In: Weber, L.J. (Ed.), Aquatic toxicology, pp: 55-105. New York, Raven Press,

Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiology Review*, *77*: 591-625.

Wester, P.W., Van den Brandhof, J.H., Vos, E.J., & Ven der Ven, L.T.M. (2003). Identification of endocrine disruptive effects in the aquatic environment: A partial life cycle assay in Zebra fish. *Biological and Trace Elements Research*, 55**:**167–172.

WHO.(1989). Lead - environmental aspects.*Environmental Health Criteria*, 85**.**International Programmme on Chemical Safety.www.inchem.org/documents/ehc/ehc85.htm. Downloaded on 21/05/2020

Wilson, R. P. (1973). Absence of ascorbic acid synthesis in channel catfish *Ictalurus punctatus* and blue catfish *Ictalurus frucatus*. *Comparative Biochemistry and Physiology*, 468:635-638.

Wilson, R.P., & Poe, W.E. (1973).Impaired collagen formation in scorbutic channel catfish. *Journal of Nutrition*, 103:1359-1364.

Witeska, M., Jezierska, &. and Chaber, J., (1993). The influence of Cadmium on common carp embryos and larvae. *Aquaculture*, 129:129-132.

Wolke, R.E. (1992). Piscine macrophage aggregates: A review. *Annual Review of Fish Diseases*, 2: 91-108.

Formatted ... (repeated)

World Health Organization.(1972) Evaluation of Certain Food Additives And Contaminants Mercury, Lead And Cadmium.16th Report of the joint FAO/WHO experts committee on food additives. Technical Report Series NO 505.

World health organization (WHO) (1994): Glyphosate. Environmental Health criteria, publication 159, Geneva Switzerland.

Wu, H.Y., Xu, H.X., Hong, Y.B., Zhang, J.F and Wu, J.C. (2011) The use of biomarkers in the antioxidant responses of *Daphina magna* to the acute and chronic exposure to no 20 diesel oil and 2,4-dichlorophenol, Chemical Specification and Bioavailabilty, 23:2,80-87 DOI:10.3184/095422911X13027015402949.

Xhyrel, J. J., Nikko, L. G., & Marlo, C. P. (2016) Relative antioxidant efficacy of α-tocopherol and ascorbic acid on blood lead, haemoglobin and haematocrit level of lead-exposed *Rattus norvegicuss* (albino rat). *Der Pharmacia Lettre* 8:169-179.

Yamamoto,Y.,Sato,M.,& Ikeda, S. (1978). Existence of L-gulonolactone oxidase in some teleosts. *Bulletin of the Japanese Society for the Science of Fish*, 44:775-779.

Yiin, S. J., & Lin, T. H. (1998).Lead-catalyzed peroxidation of essential unsaturated fatty acids. *Biological Trace Elements and Research*, 50**:**167–172.

Youness, E. R, Mohammed, N., Abdel-Salam, O., Yassen, N., Khadrawy, Y.A., EL-Toukhy. S.E & Sleem. A.A. (2016) Novel neuroprotective and hepatoprotective effects of citric acid in acute malathion intoxication. *Asian Pacific Journal of Tropical Medicine*,*9*(12):1181-1194.

Zeynep, E. (2005). The effects of dietary lead exposure and ascorbic acid on performance, lipid peroxidation status and biochemical parameters of broilers. *Turkish Journal ofVeterinary*

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

andAnimal Science,29:1053-1059.

Zhao, S., Feng, C., Quan, W., Chen, J., Niu, X., and Shen, Z. (2012). Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. *Marine Pollution Bulletin*, 64: 1163-1171.

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: Centered

APPENDICES

1. Chemicals, Reagents, Drugs and Solutions

Chemicals and Reagents

Ehrlich Haematoxylin: Haematoxylin, glycerol, absolute alcohol, glacial acetic acid and ammonium/potassium alum.

Alcoholic eosin: Eosin (yellow), 70% alcohol and glacial acetic acid.

Scott tap water: Sodium bicarbonate and magnesium sulphate.

Acid alcohol: hydrochloric acid and 70% alcohol. Other chemicals include Xylene, Depex, (DPX), Mountant and Parrafin wax.

Drugs

Lead Nitrate

The Lead II nitrate ($Pb(NO_3)_2$) crystals was purchased from Chemisciences Laboratory Owerri. The white crystal powder was made by Guanghuna Chemical China. The molecular weight was 331.21. Batch No: 2040806 manufactured in 08 2014, to expire in 08 2019.

Ascorbic acid -(Vitamin C.)

The Vitamin C used was Kepro® Vitamin C (Ascorbic acid), a cream-colored powder in 100g sachet, a product of Kepro B.V Maag denburgstraat 17-7421 ZA Deventer-Holland. Each gram of the 100g sachet contains 1000mg of Vitamin C (Ascorbic acid). The batch number is 1632004, manufactured in 08 2016 and expiration date of 08 2018.

MSS 222

The anaesthetic used for immobilizing the experimental fish is Tricaine Methanesulfonate (MSS 222) brand-named Tricaine-S manufactured by Syndel laboratories.

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted: Font color: Auto

Solutions

Red blood cells (RBC), diluting fluid, total white blood cell (TWBC) diluting fluid, phosphate buffer, normal saline, distilled water, 70% alcohol, and 70% formal saline.

Instruments and Glass/Plastic wares

Instruments

Sensitive weighing balance, (Phocee'nne Mod.PH-SF 40),weighing balance(Kepro®),pH meter(pH test pH-107 RoHS), Dissolved oxygen meter (Lasany®), water thermometer (Deluxe) spectrophotometer (Buck Scientific M910 GC-ECD USA), 5ml syringes and 21G needles, micro haematocrit kit (Mariefeld Germany), microtone, tissue block, microscope, micro capillary tubes, plasticine, dissecting kit ,thick cotton gloves.

Glass/Plastic Wares

Beakers, pipettes, micropipette, test tubes, glass funnels, measuring cylinders, conical flasks, microscope slides, cover slips, blood collecting bottles, tissue sample bottles

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Formatted: No underline, Font color: Auto

Formatted: Font color: Auto

Table 10: Haematological values of *Clarias gariepinus* exposed to varying concentrations of Lead nitrate for 91 days and treated with 50 and 100mg/l Vitamin C for 7 days.

Parameters	Exposure to Lead nitrate	Treatment (days)	Experimental Groups			
			1A (0mg/l)	1B (5mg/l)	1C (10mg/l)	1D (15mg/l)
PCV (%)	0	-		26.33 ± 2.18	26.33 ±0.72	26.33 ± 1.20
	91	-	22.00 ± 2.00^{a}	19.00 ± 1.00^{b}	20.00 ±0.00^{b}	10.50 ± 0.50^{c}
		50mg/l VitC 7	24.00 ± 1.00^{a}	32.00 ± 1.00^{b}	34.00 ±1.00^{b}	32.00 ± 2.00^{b}
		100mg/l VitC 7	24.50 ± 2.50^{a}	29.50 ± 0.50^{b}	30.00±0.00^{b}	29.00 ± 1.00^{b}
RBCX 10^{6}(ml of blood)	0	-	2.82 ±0.13	3.07 ±0.55	3.39 ± 0.12	2.55 ±0.22
	91	-	2.98 ±0.61^{a}	2.89 ±0.06^{a}	1.98 ± 0.03^{b}	1.91±0.08^{b}
		50mg/l VitC 7	2.78 ±0.20^{a}	3.15 ±0.11^{b}	3.55 ± 0.06^{b}	2.83±0.13^{a}
		100mg/l VitC 7	3.50 ±0.52^{a}	3.12 ±0.11^{a}	2.90 ± 0.11^{b}	2.25±0.11^{b}
Hb(mg/dl)	0	-	6.80 ±0.36	7.50 ±0.87	7.70 ± 0.17	7.33 ±0.47
	91	-	6.75 ±0.95^{a}	6.45 ±1.65^{a}	4.65 ± 0.45^{b}	4.00 ± 0.20^{c}
		50mg/l VitC 7	7.45 ±0.23^{a}	10.85 ± 0.15^{b}	10.80 ±0.60^{b}	7.75 ± 0.05^{a}
		100mg/l VitC 7	9.60 ±0.30^{a}	7.35 ± 0.15^{b}	6.90 ±0.10^{b}	6.05 ± 0.55^{c}
TWBCX 10^{3}(ml of blood)	0	-	2.64 ±0.15	2.45 ± 0.27	2.65 ± 0.10	2.44 ± 0.22
	91	-	2.44 ±0.01^{a}	2.55 ± 0.24^{a}	2.51 ± 0.03^{a}	2.62 ± 0.07^{b}
		50mg/l VitC 7	2.34 ±0.01^{a}	2.78 ± 0.20^{b}	2.65 ± 0.07^{b}	2.49 ± 0.12^{c}
		100mg/l VitC 7	2.70 ±0.12^{a}	1.76 ± 0.07^{b}	2.29 ± 0.15^{c}	2.42 ± 0.03^{c}
DWBC(lymphocyte)	0	-	80.00 ± 1.16	76.67 ±1.33	78.00 ± 1.89	76.00 ± 2.31
	91	-	66.00 ± 4.00^{a}	62.00 ±0.00^{a}	72.00 ±2.00^{b}	68.00 ± 2.00^{a}
		50mg/l VitC 7	81.50 ± 1.50^{a}	76.00 ±2.00^{b}	79.00 ± 1.00^{b}	72.00 ± 2.00^{b}
		100mg/l VitC 7	77.50 ± 2.50^{a}	74.00 ±2.00^{a}	73.00 ±3.00^{a}	74.00 ± 0.00^{a}
Monocyte	0	-	12.00 ± 1.16	14.67 ±2.40	11.33 ±1.44	12.67 ± 1.67
	91	-	18.00 ± 0.00^{a}	23.00 ± 1.00^{b}	16.00 ± 0.00^{a}	17.00 ± 1.00^{a}
		50mg/l VitC 7	15.00 ± 1.00^{a}	17.00 ± 1.00^{a}	13.00 ± 1.00^{a}	15.00 ± 1.00^{a}
		100mg/l VitC 7	16.00 ± 1.00^{a}	21.00 ± 1.00^{b}	19.00 ± 1.00^{a}	16.00 ± 2.00^{a}
Neutrophil	0	-	8.00 ± 1.16	8.67 ± 2.40	8.00 ± 2.31	8.00 ± 2.31
	91	-	16.00 ± 4.00^{a}	17.00 ± 1.00^{a}	15.00 ± 1.00^{a}	11.00 ± 0.71^{a}
		50mg/l VitC 7	8.00 ± 1.00^{a}	9.00 ± 1.00^{a}	9.00 ± 1.00^{a}	15.00 ± 1.00^{b}
		100mg/l VitC 7	9.00 ± 1.00^{a}	9.00 ± 1.00^{a}	13.00 ± 1.00^{b}	10.00 ± 2.00^{a}

2. Tables 10-14

Table 10: Haematological values of *Clarias gariepinus* exposed to varying concentrations of Lead nitrate for 91 days and treated with 50 and 100mg/l Vitamin C for 7 days.

Parameters	Days of Exposure	Days of Post Treatment	Experimental Groups			
			1A 0mg/L	1B 5mg/L	1C 10mg/L	1D 15mg/L

Formatted: No underline, Font color: Auto
Formatted: Font color: Auto
Formatted: No underline, Font color: Auto
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted: Font color: Auto
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted ...
Formatted: Font: Font color: Auto
Formatted ...
Formatted: Font: Font color: Auto
Formatted ...
Formatted: Font: Font color: Auto
Formatted ...
Formatted: Font: Font color: Auto
Formatted ...
Formatted: Font: Font color: Auto
Formatted ...
Formatted: Font: Font color: Auto
Formatted ...
Formatted: Font: Font color: Auto
Formatted ...
Formatted: Font: Font color: Auto
Formatted ...
Formatted: Font: Font color: Auto

PCV (%)	0	-	25.67±1.20	26.33±2.18	26.33±0.72	26.33±1.20
	91	-	22.00±2.00[a]	19.00±1.00[a]	20.00±0.00[a]	10.50±0.50[a]
	50mg/L Vit. C	7	24.00±1.00[a]	32.00±1.00[b]	34.00±1.00[b]	32.00±2.00[b]
	100mg/L Vit. C	7	24.50±2.50[a]	29.50±0.50[b]	30.00±0.00[b]	29.00±1.00[b]
RBC X 10^6 (ml of blood)	0	-	2.82±0.13	3.07±0.55	3.39±0.12	2.55±0.22
	91	-	2.98±0.61[a]	2.89±0.06[a]	1.98±0.03[b]	1.91±0.08[b]
	50mg/L Vit. C	7	2.78±0.20[a]	3.15±0.11[a]	3.55±0.06[a]	2.83±0.13[a]
	100mg/L Vit. C	7	3.50±0.52[a]	3.12±0.11[b]	2.90±0.11[b]	2.25±0.11[c]
Hb (mg/dl)	0	-	6.80±0.36	7.50±0.87	7.70±0.17	7.33±0.47
	91	-	6.75±0.95[a]	6.45±1.65[a]	4.65±0.45[a]	4.00±0.20[b]
	50mg/L Vit. C	7	7.45±0.25[a]	10.85±0.15[b]	10.80±0.60[b]	7.75±0.05[a]
	100mg/L Vit. C	7	9.60±0.30[a]	7.35±0.15[a]	6.90±0.10[c]	6.05±0.55[c]
TWBC X 10^7 (ml of blood)	0	-	2.64±0.15	2.45±0.27	2.65±0.10	2.44±0.22
	91	-	2.44±0.01[a]	2.55±0.24[a]	2.51±0.03[a]	2.62±0.07[b]
	50mg/L Vit. C	7	2.34±0.01[a]	2.78±0.20[b]	2.65±0.07[b]	2.49±0.12[c]
	100mg/L Vit. C	7	2.70±0.12[a]	1.76±0.07[b]	2.29±0.15[c]	2.42±0.03[c]
DWBC (Lymphocyte)	0	-	80.00±1.16	76.67±1.33	78.00±1.89	76.00±2.31

Formatted ...

	91	–	66.00±4.00^{a}	62.00±0.00^{a}	72.00±2.00^{b}	68.00±2.00^{a}
	50mg/L Vit. C	7	81.50±1.50^{a}	76.00±2.00^{b}	79.00±1.00^{b}	72.00±2.00^{a}
	100mg/L Vit. C	7	77.50±2.50^{a}	74.00±2.00^{a}	73.00±3.00^{a}	74.00±0.00^{a}
Monocyte	0	–	12.00±1.16	14.67±2.40	11.33±1.44	12.67±1.67
	91	–	18.00±0.00^{a}	23.00±1.00^{b}	16.00±0.00^{a}	17.00±1.00^{a}
	50mg/L Vit. C	7	15.00±1.00^{a}	17.00±1.00^{a}	13.00±1.00^{a}	15.00±1.00^{a}
	100mg/L Vit. C	7	16.00±1.00^{a}	21.00±1.00^{b}	19.00±1.00^{a}	16.00±2.00^{a}
Neutrophil	0	–	8.00±1.16	8.67±2.40	8.00±2.31	8.00±2.31
	91	–	16.00±4.00^{a}	17.00±1.00^{a}	15.00±1.00^{a}	11.00±0.71^{b}
	50mg/L Vit. C	7	8.00±1.00^{a}	9.00±1.00^{a}	9.00±1.00^{a}	15.00±1.00^{b}
	100mg/L Vit. C	7	9.00±1.00^{a}	9.00±1.00^{a}	13.00±1.00^{a}	10.00±2.0^{a}

KEYS: Values with the same superscripts on the same columns are not significantly different

~~KEY: Values with the same superscripts on the same column are not significantly different~~

2. ,

Tables 10-14

Table 10: Haematological values of *Clarias gariepinus* exposed to varying concentrations of Lead nitrate for 91 days and treated with 50 and 100mg/l Vitamin C for 7 days.

Parameters	Days of Exposure	Days of Post Treatment	Experimental Groups			
			1A 0mg/L	1B 5mg/L	1C 10mg/L	1D 15mg/L
PCV (%)	0	-	25.67±1.20	26.33±2.18	26.33±0.72	26.33±1.20
	91	-	22.00±2.00^{a}	19.00±1.00^{a}	20.00±0.00^{a}	10.50±0.50^{a}
	50mg/L Vit. C	7	24.00±1.00^{a}	32.00±1.00^{b}	34.00±1.00^{b}	32.00±2.00^{b}
	100mg/L Vit. C	7	24.50±2.50^{a}	29.50±0.50^{b}	30.00±0.00^{b}	29.00±1.00^{b}
RBC X 10^{6} (ml of blood)	0	-	2.82±0.13	3.07±0.55	3.39±0.12	2.55±0.22
	91	-	2.98±0.61^{a}	2.89±0.06^{a}	1.98±0.03^{b}	1.91±0.08^{b}
	50mg/L Vit. C	7	2.78±0.20^{a}	3.15±0.11^{a}	3.55±0.06^{a}	2.83±0.13^{a}
	100mg/L Vit. C	7	3.50±0.52^{a}	3.12±0.11^{b}	2.90±0.11^{b}	2.25±0.11^{c}
Hb (mg/dl)	0	-	6.80±0.36	7.50±0.87	7.70±0.17	7.33±0.47
	91	-	6.75±0.95^{a}	6.45±1.65^{a}	4.65±0.45^{a}	4.00±0.20^{b}
	50mg/L Vit. C	7	7.45±0.25^{a}	10.85±0.15^{b}	10.80±0.60^{b}	7.75±0.05^{a}
	100mg/L Vit. C	7	9.60±0.30^{a}	7.35±0.15^{a}	6.90±0.10^{c}	6.05±0.55^{c}
TWBC X 10^{7} (ml of blood)	0	-	2.64±0.15	2.45±0.27	2.65±0.10	2.44±0.22
	91	-	2.44±0.01^{a}	2.55±0.24^{a}	2.51±0.03^{a}	2.62±0.07^{b}
	50mg/L	7	2.34±0.01^{a}	2.78±0.20^{b}	2.65±0.07^{b}	2.49±0.12^{c}

Formatted ...

	Vit. C 100mg/L Vit. C	7	2.70±0.12[a]	1.76±0.07[b]	2.29±0.15[c]	2.42±0.03[c]
DWBC (Lymphocyte)	0	-	80.00±1.16	76.67±1.33	78.00±1.89	76.00±2.31
	91	-	66.00±4.00[a]	62.00±0.00[a]	72.00±2.00[b]	68.00±2.00[a]
	50mg/L Vit. C	7	81.50±1.50[a]	76.00±2.00[b]	79.00±1.00[b]	72.00±2.00[a]
	100mg/L Vit. C	7	77.50±2.50[a]	74.00±2.00[a]	73.00±3.00[a]	74.00±0.00[a]
Monocyte	0	-	12.00±1.16	14.67±2.40	11.33±1.44	12.67±1.67
	91	-	18.00±0.00[a]	23.00±1.00[b]	16.00±0.00[a]	17.00±1.00[a]
	50mg/L Vit. C	7	15.00±1.00[a]	17.00±1.00[a]	13.00±1.00[a]	15.00±1.00[a]
	100mg/L Vit. C	7	16.00±1.00[a]	21.00±1.00[b]	19.00±1.00[a]	16.00±2.00[a]
Neutrophil	0	-	8.00±1.16	8.67±2.40	8.00±2.31	8.00±2.31
	91	-	16.00±4.00[a]	17.00±1.00[a]	15.00±1.00[a]	11.00±0.71[b]
	50mg/L Vit. C	7	8.00±1.00[a]	9.00±1.00[a]	9.00±1.00[a]	15.00±1.00[b]
	100mg/L Vit. C	7	9.00±1.00[a]	9.00±1.00[a]	13.00±1.00[a]	10.00±2.0[a]

KEY: Values with the same superscripts on the same column are not significantly different.

Formatted ...

Table 11: Mean values of liver enzymes (AST, ALT and ALP) and urea of *Clarias gariepinus* exposed to varying concentrations of lead nitrate for 91 days and treated with 50 mg/l and 100 mg/l Vitamin C for 7

Parameters	Days of Exposure	Days of Post Treatment	Experimental Groups			
			1A 0mg/L	1B 5mg/L	1C 10mg/L	1D 15mg/L
AST (%)	0	-	64.67±2.91	69.33±0.45	73.00±8.02	87.33±5.46
	91	-	67.00±0.00^{a}	36.50±5.50^{b}	55.00±4.00^{c}	53.00±1.00^{c}
	50mg/L Vit. C	7	60.50±1.50^{a}	32.50±1.50^{b}	37.00±1.00^{c}	50.00±3.00^{d}
	100mg/L Vit. C	7	123.00±3.00^{a}	159.00±1.00^{b}	115.00±6.00^{c}	125.50±5.50^{a}
ALT	0	-	24.33±5.36	19.33±0.05	17.67±3.18	25.33±5.92
	91	-	22.50±2.50^{a}	16.00±1.00^{b}	21.00±1.00^{a}	20.00±1.00^{a}
	50mg/L Vit. C	7	22.00±2.00^{a}	16.00±1.00^{b}	17.50±0.50^{b}	19.00±1.00^{c}
	100mg/L Vit. C	7	21.00±1.00^{a}	46.00±1.00^{b}	17.00±1.00^{c}	22.00±2.00^{a}
ALP	0	-	5.27±0.23	4.43±2.71	5.50±0.00	4.20±0.80
	91	-	5.00±1.00^{a}	8.00±0.00^{b}	16.50±0.50^{c}	4.00±1.00^{a}
	50mg/L Vit. C	7	4.00±1.00^{a}	5.00±1.00^{a}	6.00±1.00^{b}	4.00±1.00^{a}
	100mg/L Vit. C	7	8.00±1.00^{a}	15.00±1.00^{b}	10.00±1.00^{c}	12.00±2.00^{d}

UREA	**0**	**-**	**1.60±0.00**	**1.60±0.00**	**1.30±0.50**	**1.55±0.55**
	91	**-**	**1.90±0.20^{a}**	**16.00±1.00^{b}**	**22.50±2.50^{c}**	**23.50±3.50^{c}**
	50mg/L Vit. C	**7**	**1.85±0.15^{a}**	**3.50±0.50^{b}**	**3.60±0.10^{b}**	**4.30±0.30^{c}**
	100mg/L Vit. C	**7**	**1.70±0.20^{a}**	**1.75±0.05^{a}**	**3.33±0.25^{b}**	**3.60±0.00^{b}**

KEY: Values with the same superscripts on the same column are not significantly different.

TABLE 12: Mean values of lead concentration (ppm) in the muscles of *Clarias gariepinus* exposed to lead nitrate for 91days and treated with 50mg/l and 100mg/l Vitamin C.

Parameters	**Days post Administration**	**Experimental Groups**			
		1A (0mg/l)	**1B (5mg/l)**	**1C (10mg/l)**	**1D (15mg/l)**
Lead	0	0.19±0.09	0.18±0.04	0.14±0.05	0.091±0.01
	91	0.20±0.04^{a}	0.36±0.02^{b}	0.82±0.03^{c}	0.47±0.02^{b}

	Days post Administration	**1A (0mg/l)**	**1B (5mg/l)**	**1C (10mg/l)**	**1D (15mg/l)**
Lead	7(50mg/l vit C)	0.19±0.01^{a}	0.31±0.13^{b}	0.78±0.05^{c}	1.09±0.08^{d}
	7(100mg/l vit C)	0.17±0.01^{a}	0.59±0.04^{b}	0.00±0.00^{a}	0.00±0.00^{a}

Lead combined Exposure to lead nitrate and Vit.C 100mg/l		**2A**	**2B**	**2C**	**2D**

91days	0.19±0.09[a]	0.59±0.12[b]	0.46±0.18[b]	0.48±0.02[b]

KEY: Values with the same superscripts on the same column are not significantly different.

Table 13: Mean haematological values of *C. gariepinus* given concurrently exposed to lead nitrate and Vitamin C for 91days.

Parameters	Days of Admin. Of Tret.	Dosage of Vitamin C	Experimental Groups			
			2A 0mg/L	2B 5mg/L	2C 10mg/L	2D 15mg/L
PCV (%)	0	-	25.67±1.20	26.33±2.19	26.33±0.89	26.33±1.20
	91	50mg/L	22.00±2.00[a]	19.50±0.50[a]	27.50±2.20[b]	21.00±1.00[a]
	91	100mg/L	21.50±1.50[a]	18.00±2.00[a]	13.00±1.00[b]	16.50±1.50[c]
Hb (mg/dl)	0	-	6.80±0.36	7.50±0.87	7.70±0.21	7.33±0.47
	91	50mg/L	6.60±1.10[a]	5.70±0.20[b]	6.60±1.10[a]	6.65±0.75[a]
	91	100mg/L	7.55±1.25[a]	3.70±0.30[b]	5.70±0.20[c]	5.30±0.20[d]
RBC X 10^6 (—ml—of blood)	0	-	2.82±1.10	3.07±1.00	3.39±0.01	2.55±0.76
	91	50mg/L	2.48±0.04	1.96±0.06	2.92±0.50	2.92±1.21
	91	100mg/L	2.68±0.42	1.89±0.12	2.04±1.00	2.06±0.20
TWBC X 10^3 (ml of	0	-	2.64±0.15	2.45±0.27	2.65±0.13	2.44±0.23

blood)						
	91	50mg/L	2.44±0.10[a]	2.54±0.14[a]	2.05±0.42[b]	1.93±0.14[c]
	91	100mg/L	2.42±0.10[a]	2.15±0.03[a]	2.29±0.42 [a]	2.21±0.17[a]
Differential lymphocyte	0	-	80.00±1.16	76.67±1.33	78.00±2.31	76.00±2.31
	91	50mg/L	66.00±4.00[a]	63.00±3.00[a]	65.00±1.00[a]	77.00±1.00[b]
	91	100mg/L	73.00±9.00[a]	75.00±1.00[a]	73.00±1.00[a]	70.50±0.50[a]
Differential monocyte	0	-	12.00±1.16	14.67±2.40	11.33±1.77	1.77±12.67
	91	50mg/L	18.00±0.00[a]	16.00±2.00[a]	12.67±0.00[b]	15.00±1.00[c]
	91	100mg/L	16.00±0.00[a]	16.00±2.00[a]	15.00±0.77[a]	15.00±1.00[a]
Differential neutrophil	0	-	8.00±1.15	8.67±2.40	7.33±1.76	8.00±2.31
	91	50mg/L	11.00±1.00[a]	18.00±1.64[b]	15.00±1.50[c]	9.00±1.00[b]
	91	100mg/L	6.00±2.00[b]	15.00±1.00[b]	11.00±1.00[b]	14.00±2.00[b]

KEY: Values with same superscripts on the same column are not significantly different.

Table 14: Mean values of liver enzymes (AST, ALT, and ALP) and Urea of *Clarias gariepinus* concurrently exposed to lead nitrate and vitamin C for 91 days.

Parameters	**Days of exposure.**	**Dosage of Vitamin C**	**Experimental Groups**			
			2A	**2B**	**2C**	**2D**
			0mg/L	**5mg/L**	**10mg/L**	**15mg/L**
AST	0	-	64.67±2.91	69.33±8.45	73.00±8.02	87.33±5.46
	91	50mg/L	67.00±0.00[a]	82.00±0.10[b]	64.00±2.50[a]	117.50±3.50[c]

Formatted ...

	91	100mg/L	64.50±2.50[a]	94.00±0.00[b]	67.50±0.50[a]	80.50±3.50[c]
ALT	0	-	24.33±3.64	19.33±0.88	17.67±3.18	25.33±5.93
	91	50mg/L	23.50±1.50[a]	27.50±2.50[a]	22.50±2.00[a]	42.00±1.00[b]
	91	100mg/L	22.50±0.50[a]	25.00±0.00[a]	21.50±3.50[a]	32.50±1.50
ALP	0	-	5.27±0.23	4.43±1.17	5.50±0.00	4.20±0.81
	91	50mg/L	16.50±0.50[a]	8.00±0.00[a]	9.50±2.00[b]	8.50±2.50[a]
	91	100mg/L	8.50±2.50[a]	7.00±1.00[b]	6.00±2.00[b]	5.00±2.00[b]
UREA	0	-	1.60±0.00	1.60±0.00	1.30±1.50	1.55±0.55
	91	50mg/L	1.90±0.20[a]	13.00±0.50[b]	16.00±1.08[b]	16.50±6.50[b]
	91	100mg/L	2.05±0.25[b]	16.00±2.00[b]	13.00±5.00[b]	13.50±7.50[b]

KEY: Values with the same superscripts on the same column are not significantly different.

Formatted ...