THE EFFICACY OF RAW GARLIC (*ALLIUM SATIVUM*) BATH IN AMELIORATING CADMIUM AND LEAD TOXICITY IN CULTURED *CLARIAS GARIEPINUS* BURCHELL, 1822

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CERTIFICATION

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DEDICATION

This research work is dedicated to my daughter and little “Angel” Miss Agnes Chekwubeolise Awele Ojogbo and the loving memory of the Director of Veterinary Services Delta State, late Dr. Rekiya Pessu.

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ABSTRACT

Cadmium (Cd) and Lead (Pb) are toxic metals ubiquitous in the aquatic environment, inducing reactive oxygen species that cause stress, toxicity and mortality in fish. Organic source such as allicin extracted from garlic reversed this toxicity; however, there is a dearth of information on the use of raw organic source to reverse these conditions. This study was designed to explore the potentials of raw garlic (Gc) in ameliorating the toxic effects of these metals in cultured *Clarias gariepinus* (Cg).

Two hundred and forty apparently healthy Cg juveniles were exposed in four triplicate groups (20 per group/10 per sex) to garlic A; 8.0g /L, B; Cd 0.03mg/L, C; Pb 0.3mg/L, and D the control in static culture for 90 days. Also four hundred and eighty randomly-selected female Cg juveniles of 20 fish each were exposed to Cadmium alone, Cadmium + Gc1 and Gc1 or Gc2 only. Cadmium was replaced with lead and cadmium + lead in other experiments. Cadmium, lead, Gc1 and Gc2 were given at 64, 126, 0.65 and 0.87 mg/L, respectively. Exposure to metals was for six-hours followed by six-hours in freshwater before 12-hours garlic treatment for two consecutive days. Clinical signs for toxicity were monitored for 120-hours. Tissue concentration of metals, oxidative stress markers, gross and histopathology of tissues were also conducted using standard methods. Data were analysed using ANOVA and DMRT at $\alpha_{0.05}$.

Group A at forty-eight hours post-exposure manifested vertical positioning in 40.0% of fish. At day 30, scanty degenerate germinal cells were observed in group B while there were no visible lesion in the testis of group A fish at day 90. At day 90 female liver Pb concentration decreased in A (91.5%), increased in B (11.4%) and C (73.5%) groups relative to control. Also Cd, Pb and their combinations groups induced 60.0 – 90.0% vertical positioning and copious mucus secretion. There was also 5.0% mortality at six-hours exposure in Cd + Pb group. At 120-hours glucose decreased in Pb + Gc2 (7.9%), Pb (36.4%) and increased in Gc2 (9.8%) groups. At 120-hours intestinal manganese level increased in Cd + Gc1 (79.4%) and decreased in Cd (58.6%) while hydrogen peroxide level in the liver decreased in Cd + Gc1 (17.3%) and increased in Cd (16.4%) groups. Malondialdehyde level in the liver decreased in Pb + Gc2 (20.0%) and increased in Pb only (8.0%) while superoxide dismutase level increased in Gc2 (7.1%) groups. Spleenic congestion, goblet cell hyperplasia, neuronal necrosis of the brain, hepatocellular necrosis, hypochromasia, poikilocytosis, and rouleux formation were observed in all groups exposed to the metal(s). The severity of these lesions decreased with garlic bath

at 120-hours. Micronuclei were observed in Cd + Pb at six-hour post-exposure and Pb group at 120-hours.

Exposure to garlic reversed the deleterious effects of Cadmium and Lead, suggesting its efficacy in the detoxification of Cadmium and Lead toxicity in cultured fish. It is recommended that fish farmers use at least 0.65mg/l of raw garlic to reduce the lethal effects of these metals on farmed *Clarias gariepinus*.

Keywords: *Clarias gariepinus*, Reactive oxygen species, Cadmium and lead, Garlic toxicity treatment.

Word count: 498.

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ABBREVIATIONS AND TERMS

$US: United State Dollar

> Greater Than

< Less Than

AAS: Atomic Absorption Spectrophotometer

ALT: Alanine aminotransferase

ANOVA: Analysis of variance

APHA: American Public Health Association

As: Arsenic

AST: Aspartate aminotransferase

CAT: Catalase

Cd: Cadmium

Cr: Chromium

Cu: Copper

DDC: Diethyl dithiocarbamate

DDT: Dichlorodiphenyl trichloroethane

DMRT: Duncan multiple range test

DO: Dissolved oxygen

FAO: Food and Agricultural Organisation of the United Nations.

FGN: Federal Government

GPx: Glutathione peroxidase

GR: Glutathione reductase

GST: Glutathione S-transferase

H_2O_2: Hydrogen peroxide

Hb: Haemoglobin

K_2HPO_4; Di-potassium hydrogen orthophosphate

LPO: lipid peroxidation

MDA: Malondialdehyde

Meg/l: milliequivalent per litre

mg/g: Milligram per gram

mg: milligram

Mn: Manganese

MT:

NESREA: National environmental standard regulation and enforcement agency

Ni: Nickel

PAHs: Polyaromatic Hydrocarbons

Pb: Lead

PCBs: Polychlorinated Biphenyls

PCV: Pack cell volume

pH: Hydrogen ion

POPs: Persistent organic pollutants

ppm: Parts per million

QCA: Quimicaclinica applicada South Africa

RBC: Red blood cell

ROS: Reactive oxygen species

SOD: Superoxide dismutase

TBA: Thiobarbituric acid

TBARS: Thiobarbituric acid reactive substances

TCA: Trichloroacetic acid

UK: United Kingdom

WBC: White blood cell

WHO: World Health Organisation

Zn: Zinc

LIST OF SYMBOLS

Female	♀
Male	♂

CHAPTER ONE

INTRODUCTION

1.1 Background to study

1.1.1 Fish

Cold-blooded aquatic animals with backbones, gills, and fins are regarded as Fish. The shape of fish makes for efficient travel through water which are torpedo-shaped (fusiform) for most fishes, flattened and rounded, as in flounders, to vertical and angular, as in sea horses. The size of most fishes range from the pygmy goby, *Pandaka pygmaea*, of the Philippines, which reaches only 12mm long and about 1.5g in weight and is sexually mature at 6mm, to the whale shark, *Rhincodon typus*, which grows to 18m long and over 20 tons in weight; about 60% of the fish species live in marine waters, the remaining 40% are found in freshwater (Taiwo, 2005). Power, (1989), documented that fish are the most diverse group of vertebrates and can adapt to a wide range of environment. These findings were further supported by Flik and Verbost (1993) who reported that fish have well developed and sophisticated osmo- and ionoregulatory mechanisms that enable them to inhabit and thrive virtually in every aquatic environment.

1.1.2 Importance of fish

The Sahelian drought that occurred from 1971 to 1974 greatly decimated the cattle population which increased the importance of fish in developing countries because the price of livestock became prohibitive resulting in increased demand for fish as the main alternative animal protein source (Ladipo *et al.,* 1982). Ibe (1989) reported that the daily annual protein consumption in Nigeria is below the recommended level and noted that while annual protein in-take in North America, Western and Eastern Europe were estimated at 66g/caput/day, 39g/caput/day and 33g/caput/day, respectively, only 7.5g/caput/day was recorded for Nigeria; while, a minimum of 65g of protein/caput/day for an adult Nigeria out of which 28g should be of animal origin has been recommended. Tewe (1997) recorded 4.82g/caput/day intake of animal protein as against 35g/caput/day recommended by FAO. These figures represented a decline in protein of animal origin intake in Nigeria. In Nigerian about 40% of the total protein intake is of fish origin which is the cheapest source of animal protein (Atanda, 2007). West and Biney (1991) reported that fish provides about 40% percent of the protein intake for nearly two-thirds of the world's population. Fish has a higher efficiency of food conversion and edible flesh

content than pig, sheep and goats and cattle (Olufemi, 1998). The low cholesterol and fat content in fish muscles is one of the reasons for its recommendation in diets of patients suffering from high-blood pressure, diabetes and obesity (Akinyemi, 1998). Fish industry is a source of employment opportunities as well as income at household and national levels (FAO, 1996; Srivastava, 1988). Wester, *et al*., (1994) reported that fish may serve as an important biomarker for pollution due to its general characteristics of sensitivity, as it reflects change in the underlying causes and with the order of magnitude which is relevant, informative and specific for baseline condition, with a cause and effect relationship that could be easily reproducible and validated, and could be measured by non-invasive methods.

1.1.3 Importance of aquaculture

There is no single definition of aquaculture that is universally accepted, however, aquaculture as a term, is simply a large – scale husbandry or rearing of aquatic organisms for commercial purposes (Olufemi, 1998). Moses (1983) reported that the ocean yields about 82 percent of the world total fish output while 17 percent is supplied from inland waters. The rapid rise in human population, have created tremendous pressure on natural fish resources, which are on the decline (FAO, 1996). Small and large-scale fish farming is on the increase to make more fish available for the ever increasing human population (Satchell, 1991). The global production of farmed fish and shellfish has more than doubled in the past 15 years and it seems that growth is set to continue (Naylor *et al*., 2000). Abba (2011) in a memo to national fisheries development committee reported that Nigeria is a large fish consuming nation and the largest aquaculture producer in Africa with fisheries contribution to GDP of 4%, about $US1 billion out of the $US20 billion agriculture GDP with investment in commercial fish farming in Nigeria rapidly expanding at 25-33% per year. FAO (2006) reported that it is expected that the anticipated expansion of the consumer demand for fish and fishery products will predominantly be met by aquaculture, projected to account for 41 percent of global fish production in 2015, also total world demand for fish and fishery products is projected to expand to 179 million tonnes by 2015. Aquaculture challenges have resulted in an increase awareness of the consequences of interaction between intensive fish farming and the environment (Iwama, 1991).

1.1.4 Factors that influence fish performance

Factors that have influence on fish performance include nutrition, disease, environmental stress and pollutants (Lebelo *et al*., 2001). Agents that can cause diseases can be categorized into two broad groups: "Biotic" agents such as viruses, bacteria, rickettsia, protozoa, helminth, arhropods etc. and "Abiotic" agents such as heat and cold, water, nutrients, toxic substances etc (Olufemi, 2011). Toxic substances may cause abnormal behaviour and mortality, even when water quality is good and no infectious diseases are apparent (Taiwo, 2005). Stress impacts could be small and non visible effects, to severe increases in respiration and blood pressure, decreased reproductive performance, and increased susceptibility to disease which enventualy could result in mortality (Schreck *et al.,* 2001). The primary stress responses are the perception of an altered state by central nervous system and the release of stress hormones, cortisol and catecholamines into the blood circulation by endocrine system (Martinez-Porchas., *et al*., 2009). Primary stress responses trigger the sequential secondary response (e.g. increase in plasma glucose, lactate and heamatocrit and decrease in chloride, sodium and potassium) in teleosts (Mommsen *et al*., 1999; Barton, 2002). Tertiary responses are more of behavioural changes.

1.1.5 Toxic substances

Toxic substance includes all chemicals, radioactive materials and biologic wastes with harmful effects on living organisms, which could be an element or toxic and hazardous waste or discarded material that can pose long term risk to health and environment (Arowolo, 2008). Omoregie *et al*., (1994) reported that several physiological dysfunctions were significantly affected by toxicants and pollutants in fish. Residues of heavy metals in fish tissues captured from no industrial or agricultural activity area could be due to the deposition of heavy metals from the atmosphere and polluted air from nearby large cities (Ettler *et al*., 2005). Very low-levels of pollution may decrease the fecundity of fish populations, leading to a long-term decline and eventual extinction with no obvious signs of illness (Krishnani *et al*., 2003; Burger and Gochfeld, 2005). Heavy metals decrease the fecundity of fish populations, either indirectly via accumulation in the reproductive organs or directly by acting on sperm and ova (Rurangwa *et al.,* 1998). It has been documented that even very brief exposure period gives rise to cell death within the developing olfactory placode resulting in long-term deficits in olfaction (Blechinger *et al*., 2007). Ebrahimi and Taherianfard, (2011) documented concentration of heavy metals in tissues showed no significant differences between the two sexes or

between the two fish species from the different sampling sites, which could be due to a similar degree of accumulation in both sexes and species, as their feeding habits and treatments are similar. Cadmium (Cd) and lead (Pb) are the most abundant heavy toxic metal in the environment (Kumar *et al*., 2007). Aquatic organisms have been reported to accumulate heavy metals in their tissues several times higher than the ambient levels by absorption process through gills or by consumption of contaminated food and sediments (Malik *et al*., 2010). Adeyemo, (2005) reported lead levels in surface water ranging between 0.5 – 2.35mg/l (mean = 0. 76mg.l) and 1.5 – 2.20 mg/l (mean = 1.34mg/l) during dry and rainy season respectively, while lead content in fish ponds in Ibadan were higher than in surface water. Oze *et al*., (2006) reported that Qua-Iboe river had means of 0.3 ± 0.1mgl while whole fish bioaccumulation means was 25.58 ± 1.2mgg^{-1} for Lead (Pb) and means of 0.03 ± 0.01 while whole fish bioaccumulation means was 0.38 ± 0.06 for Cadmium. NESREA benchmark for maximium permissible limit of lead and cadmium in water are 0.05mgl^{-1} and 0.01mgl^{-1} respectively (FGN official gazette, 2009) while, WHO (1989) permissible level of Cd in water is 0.01 ppm.

1.1.6 Assessment of impact of natural and anthropogenic factors of freshwater ecosystem

The impact of natural and anthropogenic factors in the environment on physiological adaptation trends of animals and their reactive norms are becoming relevant investigations (Yakhnenko and Klimenlor, 2009). There are a number of ways that impacts and causal relationships of natural and anthropogenic factors can be investigated as summarized by Adams (2003). They are: (1) controlled laboratory exposures; (2) baseline microcosm and mesocosm exposures; (3) baseline observations of exposure and response including gradient studies at multiple levels of biological organisation; (4) mathematical simulation modelling; (5) statistical associations and approaches; (6) combinations of the previous approaches; and (7) weight and strength of evidence investigations.

1.1.7 Determination of health impairment in fish

This enhances the understanding of the pathogenesis and course of diseases for probable control, evaluation of the therapy and the prognoses requires better diagnostics (Rehulka, 1993). Adams, (2003), reported that health impairment could be more accurately determined from observations at several levels of biological organization including genetic, biochemical and histological.

1.1.7.1 Evaluation of blood parameters

Disease outbreaks is a major constraint to aquaculture production and trade, with a consequent effect on the industry's economic development (Yunxia *et al*., 2001). The evaluation of haematology can be the first indication of fish living under environmental stress arising from a change in water quality (Jimena *et al*., 2005). The measurement of specific physiological and biochemical alteration in the blood of fish exposed to short period of sublethal stressors could provide a sensitive method for predicting the effects of chronic exposure on survival, reproduction and growth (Nussey *et al*., 1990). Haematological parameters is frequently utilized for the detection of physiopathological changes in different stress conditions (Nussey *et al*., 1990). Stoskopf (1993) proposed evaluation of blood cells, blood biochemistry and hormones as a useful tool for the diagnosis of fish disease and was used to monitor the physiological status of fish. Blood analysis is crucial in many baselines of ichthyological research and fish farming and in the area of toxicology and environmental monitoring of changes in fishery management and diseases investigation (Adedeji *et al*., 2009).

1.1.8.2 Use of organ indices for health assessment

Condition indices could relate to life history patterns (reproduction, juvenile survival and migration) and ecological interactions (parasite load, social dominance, diet and density) of threatened or endangered species (Lazarus and Reddy, 1986). Organ indices and condition can be used as indicators of change in nutritional and energy status of fish (Adams, 1993). Fluctuation in condition factor values reflects the health condition of fish as well as their body protein and lipid contents (Weatherley and Gill, 1987). Khallaf *et al*., (2003) documented variation in condition factor with season and pollution. *'b'* value of length-weight relationship (LWR) and condition factor *k* are influenced by pollutants (Ojogbo *et al*., 2010). Ojogbo (2013) reported that condition factor (*K*) initial growth index (*a*) and growth index (*b*) are measurable indices for stress measurement in *Clarias gariepinus*.

1.1.7.3 Water quality

Water quality is defined as any characteristic of water whether physical, chemical or biological that affects the survival, reproduction and management of fish. The physical and chemical properties of water can directly and indirectly affect its quality and its suitability for fish survival and optimum production. Variation in physico-chemical characteristics influences the distribution and population of fish stock. The water quality of an aquatic environment is considered the main factor controlling the state of health

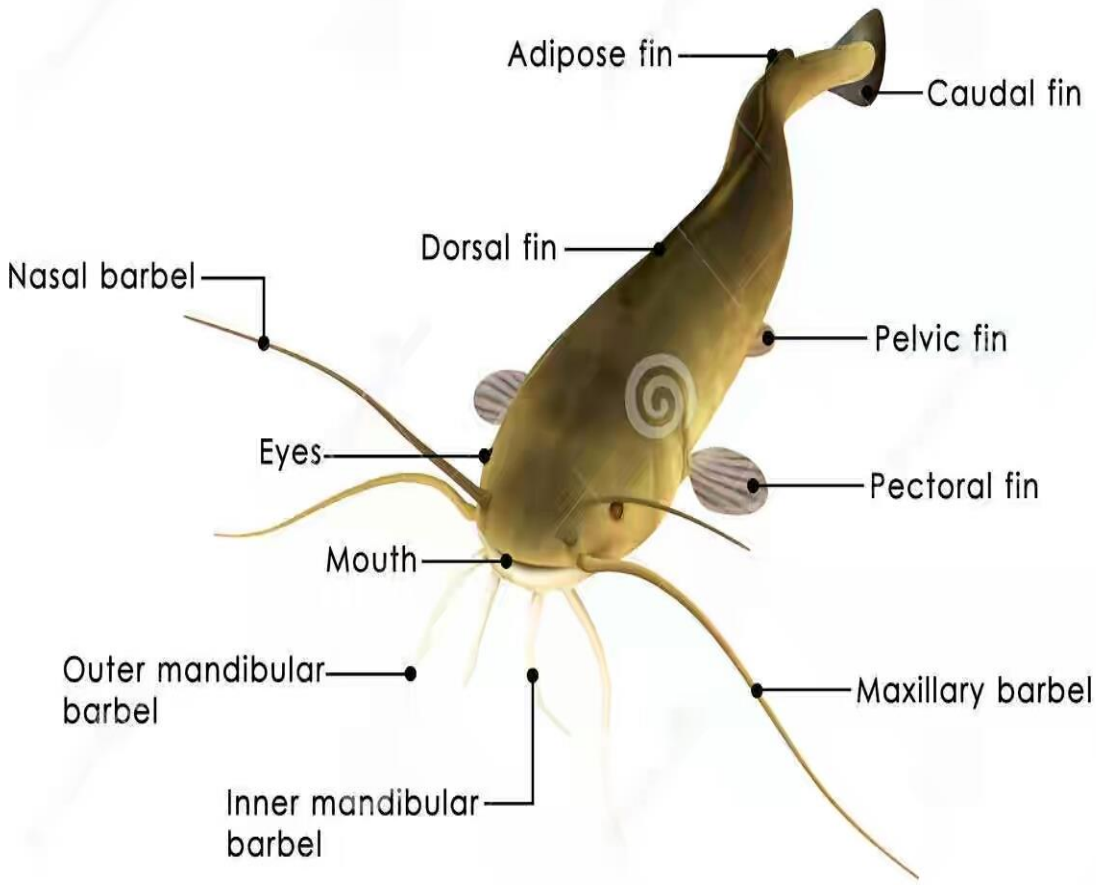
and disease in both cultured and wild fishes (Adeyemo, 2007). Toxicants affect water quality by physiological changes that is reflected in the changes in values of one or more of the haematological parameters (van Vuren, 1986).

1.1.8 Garlic

Plate 1.1: Garlic bulb

Lanzotti, (2006) reported that Allium is one of the largest and most important genus of the Alliaceae family, tt comprises of 450 species. Garlic also stands as the second most utilized supplement (Bongiorno *et al*., 2008). Garlic, the "spice of life" is unique among members of the plant kingdom (Verma *et al*., 2008). The bulbs of Garlic *A. sativum* are used both for medicinal and culinary purposes (Vallachira, 1998). The bulbs contain an acrid volatile oil (0.25%), starch, mucilage, albumin and sugar. Garlic containes high levels of Phosphorus, Potassium, Sulphur and Zinc. Moderate levels of Selenium, Vitamin A and C and low levels of Calcium, Magnesium, Sodium, Iron, Manganese and B-Complex vitamin. In addition, many compounds have been identified and isolated from Garlic extracts including 33 Sulfur compounds and 17 Amino Acids which include Alanine, Arginine, Aspartic Acid, Asparagine, Histidine, Proline, Serine, Threonine, Tryptophan and Valine (Agawal, 1998). The amount of minerals in the spices bulbs depends on the content of the respective minerals in the soil where the bulb is grown (Alejandra *et al.*, 2010). The following are sulfur-containing garlic's constituents that lower oxidative stress: Alliin, allicin, allixin, allyl polysulfides (APS), diallyl sulfide (DAS), diallyl disulfude (DADS), diallyl trisulfide (DATS), N-acetylcysteine (NAC), N-acetyl-S-allylcysteine (NASC), S-allylcysteine (SAC), S-allylmercaptocysteine (SAMC), S-ethylcysteine (SEC), S-methylcysteine (SMC), S-propylcysteine (SPC),1,2-vinyldithiin (1,2-DT) and thiacremonone. Galic is composed of total carbohydrate 73.03±0.03 (Otunola *et al*., 2010); Crude fibre, 3.05±0.15 (Okolo *et al*., 2012); Crude fat, 0.72±0.01 (Otunola *et al*., 2010); Ash, 1.33±0.04 (Odebunmi et al., 2010) and Crude protein, 16.55±0.01 (Okolo *et al*., 2012)

1.1.9 African catfish *Clarias gariepinus*



Source: Dreamstime.com

Plate 1.2: Labelled image of African catfish

Ancestral catfish originated before the break-up of Gondwanaland and then evolved in both the New and Old worlds (Bruton, 1996). The African catfish belongs to the genus *Clarias* which has six subgenera; *Clarias dinotopteroides, Clarias. brevicephaloides, Clarias platycephaloides, Clarias.* c*lariode), Clarias. anguilloclarias* and *Clarias clarias* (Teugels, 1982). *Clarias gariepinus* body is elongated with large, depressed and bony head, with small eyes. *Clarias gariepinus* occipital process is narrow and angular; gill openings wide; air-breathing labyrinthic organ arising from gill arches; first gill arch with 24 to 110 gillrakers; cleithrum pointed, narrow with longitudinal ridges and with sharpness. The mouth is terminal, large with four pairs of barbels present. The dorsal and anal fins are long while caudal fin rounded; without dorsal fin spine and adipose fin. Anterior edge of pectoral spine serrated. The colour varies from sandy-yellow through gray to olive with dark greenish-brown markings, belly white (FAO. © 2010-2012.). *Clarias gariepinus* belongs to the family clariidae which are air breathing non-scaly fresh water fish, valuable food of commercial importance (Marioghae, 1991). *Clarias gariepinus* has a wider distribution than any species in the clariids family (Ugwumba and Ugwumba, 2003). *Clarias gariepinus* constitutes a major catch of fisher folks particularly during the rainy season in most river in the southern part of the Nigeria (Moses, 1983). *Clarias gariepinus* have high survival rate under culture conditions, readily accept artificial feeds and high flesh quality (Nwadukwe and Ayinla, 1993).

1.1.10 Justification of study

In Nigeria, *Clarias gariepinus* is an indigenous fish occurring in freshwater throughout the country. It is suspected that apart from tilapia, *Clarias* is the most abundant cultivated fish species in Nigeria (Kori-Siakpere and Ubogu 2008). In Africa, especially Nigeria, the Catfish mostly cultured are *Clarias gariepinus, Heterobranchus bidorsalis* and their hybrids (Adewolu *et al.,* 2008). They are widely cultured owing to their high market price, fast growth rate and ability to withstand adverse pond conditions especially low oxygen content (Adewolu and Adeoti, 2010). Oladosu *et al*., (1993) also reported that inter-specific hybrid fishes transfer desirable traits between species, combine desirable trait of two species into a single group of fishes. Also the introduction of toxic metals in the environment is known to result in multiple changes in the internal dynamics of aquatic organisms even at sublethal level according to Al-Attar (2005). Cadmium (Cd) and lead (Pb) are the most abundant heavy toxic metal in the environment (Kumar *et al.,* 2007). Cadmium (Cd) most critical health effect is probably kidney damage (Jarup *et al.,*

1998); decreased cell mediated immunity (Kumar *et al.,* 2008), marked immunosuppression (Sovenyl and Szakolczal 1993) Lead toxic effects includes abnormal physiological activities (Larsson *et al*., 1985), biochemical processes (Daiz *et al*., 1986), reproduction, and growth Weis and Weis, (1989), stimulation of internal activities and plasma corticosteroid and glucose levels (Pratap and Wendelaar-Bonga, 1990), secondary stress responses (Martinez *et al*., 2004) and mortality (Mance, 1987). Free radicals and other reactive oxygen species (ROS) are incriminated in the pathogenesis of various metal toxicities by various authors as reviewed by Kumar and Singh (2010). Less attention has been directed to the use of natural biocompatible, biodegradable organic sources to reduce toxic metals in fish culture. Organic source such as allicin extracted from garlic reversed this toxicity; however, there is a dearth of information on the use of raw organic source to reverse these conditions. Acceptable method for the reduction of toxic elements such as cadmium in aquatic environments is needed (Osman *et al*., 2009).

1.1.11 Objective of study

In an attempt to add to the existing knowledge reviewed earlier, the main objective of this study is to identify some effects of raw garlic (*Allium sativum*), cadmium and lead on the physiology and pathology in African catfish *Clarias gariepinus* culture that could enable the development and eventual use of raw garlic (*Allium sativum*) homogenate in ameliorating the effects of sublethal and lethal concentration of cadmium and lead in fish. The specific objectives of this study are therefore:

1. To observe the clinical signs, gross pathology, pathological changes in tissues and determine metal/ions accumulation in the liver, including water quality in African catfish *Clarias gariepinus* exposed to raw garlic (*Allium sativum*); sublethal concentration of cadmium and lead.
2. To determine LD_{50}-96 hour's and LC_{100}-96 hour's cadmium and lead nitrate singularly for female *Clarias gariepinus*.
3. To determine optimum raw garlic concetration in apparently healthy female *Clarias gariepinus*.
4. To elucidate probable influence of raw garlic (*Allium sativum*) bath on clinical signs, gross and histopathological changes in *Clarias gariepinus* exposed to LC_{100}-96 hour's cadmium and lead nitrate in female *Clarias gariepinus*.

CHAPTER TWO

LITERATURE REVIEW: HEAVY METAL POLLUTION IN FRESHWATER AND FISH

2.1 Introduction

A major factor posing serious threat to the survival of aquatic organisms including fish is aquatic environment pollution by inorganic and organic chemicals (Simir and Ibrahim, 2008). Literatures abound of the deleterious effects of toxic metals on compromised growth, reproduction, altered behaviour and abnormal development in invertebrates, fish, amphibian, reptilian, avian and mammalian species (Lister and van Der Kraak, 2001). Heavy metals discharge into the aquatic environment from various sources even below permissible levels creates health hazards in aquatic organisms (Okocha and Adedeji, 2011). Untreated wastes discharge into rivers by many industries have been reported to be the main pollution source of many rivers in Nigeria (Alinnor, 2005). The consequences of pollution may be direct, through the toxic effect of a substance, or indirect as the impact becomes primarily benthic due to the sedimentation of food particles and faecal pellets under and around fish farms (Aure *et al.,* 1988; Holmer, 1991). China produced 70 percent of world farmed fish and since 1980s is threatened by pollution of rivers and coastal waters by discharge of huge quantities of wastewater from rapidly growing industries discharging pesticide, oil, mercury, lead, copper and agricultural runoffs, has caused massive die offs at fish farms leading to farmers to illegally mix veterinary drugs into their pond to try and keep their fish alive. (www.thefishiste.com/fishnews/7404/the-toxic-fish-farms-ofchina/print7/20/2008). In Nigeria the National Environmental Standards and Regulations Enforcement Agency (NESREA) is a major player in the prevention, management and control of pollution.

2.2 Pollution of the aquatic environment by heavy metals

Heavy metals are natural trace elements of the aquatic environment, but their levels have increased beyond their trace level due to industrial wastes, geochemical structure, agricultural and mining activities (Singh, *et al*., 2006; Sprocati *et al*., 2006). Bly *et al*., (1997) and Wedemeyer *et al*., (1984) documented the effects of adverse environment as a challenge to the ability of fish to maintain homeostasis. The ability of an organism to biotransform and excrete pollutants involves processes which may be highly reactive and toxic to the cell e.g. by covalent binding to DNA, RNA or protein (adults) or by the

production of reactive oxygen species (ROS) which are important factors in determining the effects of pollutant in the environment (Anders, 2008).

2.3 Haematological parameters in fish and toxic metals pollutants

Pollutants can induce hematological parameters (Ranzani-paiva and Silva-souza, 2004). Heamatological parameters are non-specific in their responses towards chemical stressors; however, toxic substances can significantly damage the haematological system of fish (Van der Oost *et al*., 2003). Blood parameters are considered patho-physiological indicators and are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari and Sarkar, 2004; Maheswaran *et al*., 2008). The haematological parameter in fish may be influenced by intrinsic factors such as sex, reproductive stages, age, size and health (Joshi, 1982; Nespolo and Rosenmann, 2002) and by external factors including seasonal dynamics, water temperature environmental quality, food, stress (Mahajan and Dheer, 1979; Rois *et al*., 2002). Abiotic factors include change in water temperature, pH, Oxygen concentration and water pollutants including pesticides, insecticides, (Meier *et al*., 1983; Lebelo *et al*., 2001), petroleum products and heavy metals (Witeska, 2005). Biotic factors include such change as predator pressure, parasitic invasion or strong competition with other organisms or among the fish in overcrowded areas and by human activities in relation to fish rearing, harvesting manipulation and transport, (Witeska, 2005). The change in the haematological parameters may be as a result of a disorder in erythrocyte cell membrane permeability and/or the result of the activation of protective mechanisms. The mechanisms may involve the release of erythrocyte from blood deposits and/or from haemopoetic tissues into the blood streams (Svodova *et al*., 1994). Ranzani Paiva and Godinho (1991) reported that blood parameters can change depending on the maturation of the gonads with fish exposed to pollution resulted in a significantly higher values of erythrocyte, PCV, Hb, MCH and MCHC than those from an unpolluted area. Hypoxic and diseased condition increased blood viscosity which could lead to a decreased blood flow and oxygen delivery to the tissues (Lebelo *et al*., 2001). Gabriel *et al*., (2004) documented that values of haematological parameters in the control except MCHC, MCH, neutrophils, monocyte, and thrombocytes were significantly ($p>0.05$) higher than in the exposed juvenile of African Catfish *C gariepinus.* Jimena *et al*., (2005) reported that fish exposed to pollution presented significantly higher values of erythrocytic, PCV, Hb, MCH and MCHC than those from an unpolluted area.

Haematocrit is used to determine the oxygen carrying capacity of blood (Larsson *et al*., 1985). The haematocrit reading is valuable in determining the effect of stressors on the health of fish (Munkittrick and Leatherland, 1983). Haematocrit abnormal values causes may include nutritional deficiencies, presence of disease-causing microorganism and other health aberrations (Anderson, 1990). Stress increased haematocrit, red blood cells count and volume and heamoglobin level in fish (Wendelaar-Bonga, 1997). Haematocrit values did not show significant difference in kutum subjected to captivity stress and in Jundia, *Rhamdia quelen* subjected to transport stress (Carneiro *et al*., 2009). Decrease in hematocrit values after stress was reported in *Tilapia zilli* and *Clarias gariepinus* (Gbore *et al*., 2006). Erythrocytes are produced in the haematopoietic tissue, which is situated in the spleen and head kidney (Heath, 1990). Wedemeyer *et al*., (1984) reported that the reduction in erythrocytes is due to haemodilution resulting from impaired osmoregulation across the gill epithelium and the destruction of the erythrocytes, thereby limiting erythrocytes synthesis. Jimena *et al*., (2005) reported that Hb concentration is a key parameter to distinguish between populations exposed to different environmental condition. The importance of Hb as a key parameter is in agreement with Saint – Paul, (1984) who suggested that the increase in Hb concentration could be a first indicator of an adaptational improvement in the oxygen transportation capacity of the blood. According to Blaxhall and Daisley (1973) the determination of haemoglobin can be a good indicator of anaemic conditions in fish. Cyriac *et al*., (1989) considered decreases in haemoglobin concentration as a contribution to haemodilution. The decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Grobler 1988; Wepener, 1990; Nussey, 1994). The significant decrease in the haemoglobin concentrations may also be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis (Reddy and Bashanihideen, 1989). The haemoglobin concentration progressive reduction may be attributed to depression/exhaustion of haemopoietic potential of the fish (Sawhney and Johal, 2000). Buckley *et al*., (1976) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport. Increase in number of circulating RBC is thought to be associated with release from reservoirs (spleen contraction) and even division of circulating cells in fish subjected to low oxygen tension (Murad *et al*., 1993)

A decrease in white blood cell count especially lymphocytes occurs in fish subjected to stress, the level of phagocytes sometimes increases with decrease activity of lymphocyte

and phagocytes (Elsaesser and Clem, 1986). White blood cells (WBC) count decrease mostly in lymphocytes due to heavy metal results in a compromised immune response in affected fish (Witeska 2005). White blood cells (WBC) count increase is attributable to stress conditions; which is more common under feral condition relative to the more conducive pond environment (Schreck *et al*., 1976).

2.4 Effect of heavy metals pollution on blood chemistry in fish

Aldham *et al.*, (1997) reported that serum enzymes, protein, lipid cholesterol, creatinine and glucose fluctuated in a positive correlation with water pollutant and are "biological markers" for contamination and stress. Khadiga *et al.*, (2003) reported that changes in certain biochemical parameter in fish blood have potential for detecting acute or chronic pollutant-induce change. The biochemical parameter of fish at prolonged exposure had insufficient variations indicating the development of a new homeostatic balance by fish to unfavorable environmental conditions (Adegun *et al*., 2012).

2.4.1 Protein

Plasma protein is important in the physiology of fish as they regulate many functions in blood (van Vuren, 1980). Plasma proteins are involved in blood clotting, maintenance of osmotic pressure and viscosity of blood of fish (Lepkovsky, 2012). According to Watson (2001), plasma proteins are essential with regards to fish nutrition and the defense of the body against viral infection. Diet may be a major influence in the variation of plasma/serum protein concentration (Badawi, 1971). Osmoregulatory dysfunction, hemodilution, or tissue damage in surrounding blood vessels have been reported to be indicative of high serum protein levels (Hille, 1982). Hypoxia activates the genes that are involved in protein synthesis, locomotive anaerobic ATP production, gluconeogenesis, and cell growth suppression in fish (Gracey *et al.*, 2001). In fish hypoxia may permanently modify certain species resulting in the organism new tolerances and domination in benthic communities leading to an overall reduction of biomass (Wu, 2002). There was lower total protein concentration in response to hypoxia in Nile tilapia (Kyoungju, 2004) in contrast to result of significant increase in total plasma protein of striped bass exposed to hypoxia (Lebelo *et al.*, 2001). Total protein, were significantly lower in catfish *Ictalurus punctatus* raised in intensive raceways (with low dissolved oxygen) then in pond population, (Warner and Whitney, 2006). Effective innate immune responses in fish is related to higher levels of serum protein, globulin and immunoblobin M (I_gM) (Wiegertjes *et al.*, 1996), while low level of plasma protein is associated with diseased fish (Wedemeyer *et al.*, 1984). Low level of plasma protein is associated with

starvation and depletion of energy stores (Lockhart and Metner, 1984; Cunjak, 1988) these processes may be due to decrease in protein synthesis or increase in protein catabolism in order to reduce energy utilization induced by low oxygen stress (Lutz and Nilsson 1997; Mazeaud *et al*., 1977). Elevated level of serum total protein may be due to kidney and gills damage which in turn leads to disturbance in osmoregulation (Gluth and Hanke, 1985). Wedemeyer and Yasutaka (1977) reported an increase in serum total protein in fishes exposed to lead. Alkahem *et al*., (1998) reported decreased protein level as an attribute to stress mediated mobilization of proteins to fulfil an increased energy demanded by fish to cope with toxicant exposure.

2.4.2 Plasma electrolytes

Plasma electrolytes (Na^+, Cl^-, Ca, K, P and Mg) were not found to differ significantly in response to acute hypoxia (Kyoungju, 2004). Sodium was significantly lower in catfish *Ictalurus punctatus* raised in intensive raceways (with low dissolved oxygen) than in pond population (Warner and Whitney, 2006). Low levels of chloride and sodium are considered as indices of secondary stress response as a consequence of the action of primary stress response. The low levels of chloride and sodium could be due to diffusive loss of small ions across gill membrane associated with the changes in gill mechanisms due to an increase in brachial blood flow and gill permeability (Barton, 2002). Wendelaar-Bonga (1997) reported chloride, sodium and calcium levels in the blood declined ($P > 0.05$) after captivity compared to the precaptivity values. Decrease in chloride and sodium levels in fish subjected to stressors was reported in Smallmouth bass (Carmichael *et al*., 1983) and Matrinxa Brycon cephalus (Carneiro and Urbinati 2002). A reduction in plasma electrolytes levels is an indication of osmoregulatory impairment in the experimental fish was recorded (Kori-Siakpere, 1996).

2.4.3 Aspartate and alanine Aminotransferases

The use of enzyme activities as sensitive biomarkers to onset of toxicity in fish has been documented (Gul *et al*., 2004). Transamination is important in assessing the state of the liver and some other organs (Verma *et al.,* 1981). The degree of enzyme activities is considered an indication of extent of organ damage (Younis *et al*., 2012). Increased plasma activity of AST may be indicative of degenerative changes in myocardium, skeletal muscle, kidney and brain (Adeogun *et al*., 2012). Increased plasma ALT and AST activities are biomaker of cytolysis as aminotransferases play vital roles in carbohydrates-protein metabolismin fish (Eze, 1983). ALT and AST elevated levels may

affect adversely amino acid metabolism (Kori-Siakpere *et al*., 2010). Abou-Hadeed. *et al*., (2008) reported a non-significant AST decrease with exposure to nickel. Khadiga *et al*., (2003) reported a lower aspartate aminotransferase in fish from waters with the highest metal concentration. Gluth and Hanke, (1985) reported that, liver damage due to accumulation of lead elevated the levels of SGOT and SGPT activities. Abbas (1994) recorded an increase in both aspartate and alanine transaminases of *O. niloticus* serum after exposure to lead.

2.4.4 Creatinine

Creatinine is a byproduct of creatine, which is a catalyst produced by the body to release stores of energy within muscle cells. Creatinine is not absorbed by the body but flows through the kidneys (glomerular filtration and proximal tubular secretion) to be eliminated within urine. Creatinine, were significantly lower in catfish *Ictalurus punctatus* raised in intensive raceways (with low dissolved oxygen) than in pond population, (Warner and Whitney, 2006). Temperature have no effect on creatinine (Farghaly *et al*., 1973).

2.4.5 Glucose

Most often, plasma or serum glucose level is used as an indicator of non-specific stress (Hunn and Greer, 1991). Environmental pollution may induce stress thereby enhancing glycogen breakdown in liver and consequently raise blood glucose level in fish (Diwan *et al*., 1979). Elevated glucose level may be due to the accumulation of heavy metals in the pancreatic islets that damage the insulin producing ß-cells as reported by Khanna and Gill (1975). Plasma cortisol and glucose are considered as primary and secondary responses to stress and indicators of the severity and duration of stress (Henrique *et al.*, 1998; Mazeaud *et al.*, 1977). Blood glucose is a sensitive and reliable indicator of environmental stress in fish (Nemcsok and Boross, 1982). Glucose, cortisol and haematocrit levels increased in Nile tilapia in response to hypoxia (Ishibashi *et al*., 2002). Glucose value was significantly higher in fish raised in intensive raceway than in pond population (Warner, 2006). Hyperglycemia was reported by Larsson *et al*., (1985) in freshwater species exposed to lead and cadmium.

2.5 Cadmium in fish

Anthropogenic activities have contributed to the entry of cadmium into human and animal food chain (WHO, 1992; Okada *et al.*, 1997; Kumar *et al.*, 2007). Cadmium has been reported to exert deleterious effects in terms of nephrotoxic, cytotoxic, genotoxic, immunotoxic and carcinogenic (ATSEMR, 1999; Lippmann. 2000 and Risso-de

Faverney, 2001). Cadmium exposure most important effect was renal tubular dysfunction, with increased excretion of protein, disturbance in the metabolism of bone minerals, (Murata, 1971). Fish can accumulate Cd in tissues to levels ten to one-thousand times higher than this level in ambient water (Fleischer *et al*., 1974). The effect of Cd on aquatic organisms may be affected by the presence of dissolved organic matter (Meinelt *et al*., 2007; Burnison *et al*., 2006). Elevated levels of Cd can induce respiratory or osmoregulatory dysfunctions (Zyadah and Abdel-Baky, 2000), lipid peroxidation, DNA damage, and glutathionylation of proteins (Stohs and Bagchi, 1990; Risso-de Faverney *et al*., 2001; Silvestre *et al*., 2006) in various tissues. Kumar *et al.*, (2005) reported that cadmium accumulation pattern in *Clarias batrachus* was in the following order: kidney> Liver> Gills. Cadmium induces various pathological changes in liver tissues including engorgement of blood vessels, congestion, vacuolar degeneration of hepatocytes, necrosis of pancreatic cells and fatty changes in the peripancreatic hepatocytes (Rani and Ramamurthi, 1989; Dangre *et al.,* 2010). The kidney is the principal target organ and is characterized by varying degree of renal damage in cadmium toxicity (Vesey, 2010). Gills are also reported to act as storehouse of cadmium in experimental studies (Fafioye *et al.,* 2004). Wong and Wong (2000) reported chloride cells as a prime target of cadmium toxicity, resulting into fish hypocalcemia. Other organs like intestine and gonads of fishes also appear susceptible for ill effects of cadmium toxicity (Taylor, 1983; Kumari and Ramkumar, 1997; Singh *et al.,* 2007; Kumar *et al*., 2007).

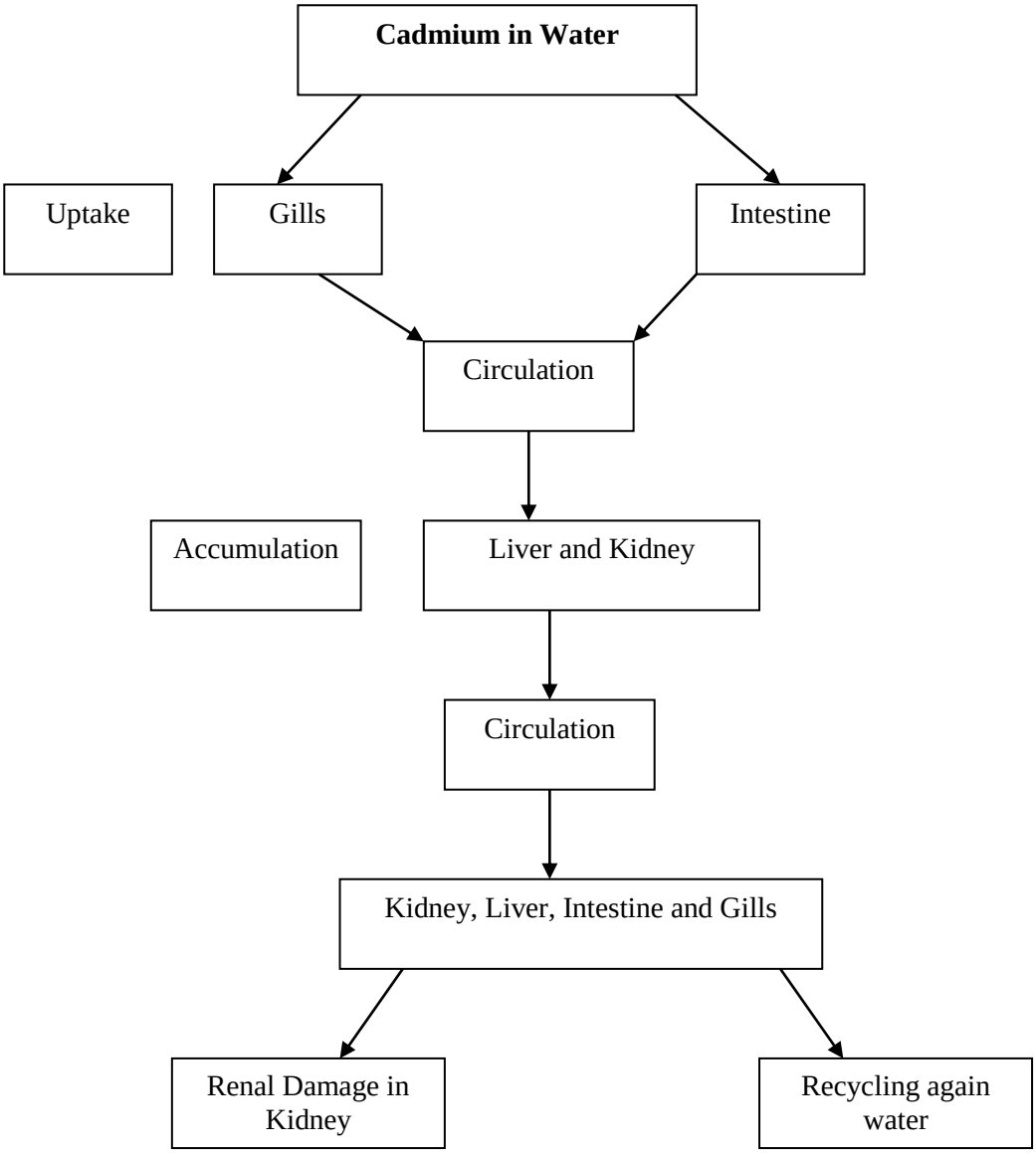


Figure 2.1: The cycle of cadmium in aquatic ecosystem

Source: Kumar and Singh, (2010)

2.6 Lead in fish

The natural concentration of lead in surface water has been estimated at 0. 02µg.L^{-1} and it rarely exceeds a few micrograms. L^{-1} (Martinez *et al.,* 2004). WHO, (2003) reported that the permissible level of Pb in water is 0.05mg/l. Most lead released into the environment find its way into the aquatic place as a result of direct input, atmospheric deposition and erosion due to rain water (Kalay and Canli 2000), and domestic sewage sludge (Katz,1982). Fish tissues uptake of lead is from aquatic plants, sediments and gasoline containing lead that leaks from fishery boats (Rashed, 2004). Adeyeye *et al*., (1996) detected lead in fish *Cyprinus carpio* and *Clarias gariepinus* tissues from an artificial pond. Heath (1987) suggested that there are more excretory routes than uptakes. Al-Nagaawy (2008) observed that, the accumulation of copper and lead metals was higher in the gills than in the muscles.

2.7 Garlic in fish culture

Garlic has several beneficial properties which includes antioxidant, antihypertensive, and antimicrobial (Sivam, 2001). Raw garlic is a source of antioxidant (Rahman *et al*., 2012); nutritional value (Jessus *et al*., 2002). *Allium sativum* has some constituents that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow (Jeorg and Lee, 1998). Garlic increases the welfare of fish, control of pathogens, especially bacteria and fungi (Corzo-Martinez *et al.,* 2007). Metwally (2009) reported that garlic (*Allium sativum*) in diet of Tilapia nilotica (*Oreochromis niloticus*), increased growth rate, decreased mortality rate and increased the antioxidant activity. *Allium sativum* is a growth promoter in *O. niloticus* (Diab *et al.,* 2002). *Allium sativum* was shown to have broad spectrum activities against bacterial agents (Abd-Elallatif and Ebraheem, 1996). Sahu *et al.,* (2007) showed that garlic, *Allium sativum*, powder in diet enhanced superoxide anion production, lysozyme, serum bactericidal, serum protein and albumin. Shalaby *et al*., (2006) reported that fish fed on diets containing *Allium sativum*, significantly increased erythrocyte count (RBC) and hemoglobin content and significantly decreased plasma glucose, Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities than control. Martins *et al.,* (2002), documented that addition of *Allium sativum* to fish diets increased erythrocytes number, hemoglobin content, hematocrit value, leucocytes, and thrombocytes. *Allium sativum* antimicrobial activities are not affected by acid environments of the digestive organ but was enhanced by the gastric juice (Lawson,

1996). Serum AST and ALT activities decreased significantly in the fish group fed on all levels of *Allium sativum* and chloramphenicol (Salah and Rogers, 1993).

2.8 Water quality in fish culture

One of the main factors which affect the quality of fish is the water quality (Fatima *et al*., 2008). The physical and chemical properties of water can directly and indirectly affect its quality and its suitability for fish survival and optimum production (Bly *et al.,* 1997) Water quality is important in aquaculture because water quality imbalances can cause stress, poor growth, and mortality of culture species (Boyd and Tucker, 1998). Different water sources have a different physical, chemical and biological characteristic (Saeed, 2000; Pulatsu *et al*., 2004) which subsequently affects the quality of the cultured fish (Ali, 2007). Due to the complex nature of water environment, prevention and control of diseases is difficult coupled with extraneous factors and natures physical changes which are difficult to manage (Okaeme, 2010). Water temperature is one of the most influencing environmental factors affecting pond water quality dynamics, metabolism and growth of fish (Boyd, 1990). Most fish species live in water but a few hibernate or can tolerate some level of wetness without necessarily living their entire life in a complete natural water environment (Okaeme, 2010). Erratic behavior and syndromes characteristic of infectious diseases may be produced by environmental stress due to abnormal levels of water quality parameters including nutritional deficiencies (Olufemi, 1998). Gabriel *et al*., (2008) demonstrated that water quality might affect the haematological parameters in free-living fish by an increased percentage of haematocrit and reduced numbers of lymphocytes and monocytes from the polluted site. Wedemeyer *et al*., (1984) documented the effects of adverse environment as a challenge to the ability of fish to maintain homeostasis. It has been reported that when the capability to maintain homeostasis is exceeded, there is an adverse effect on the hormonal status, immune function, and reproduction and also an elevated susceptibility to infectious diseases (Adams *et al*., 1989; Wendelaar-Bonga, 1997). Sudden changes specifically salinity and dissolved oxygen in any aquatic environment would compromise immune function in teleost fish by alteration of cytokine expression, namely, increase in TGF-B and decrease in IL-IB, suppression of phagocytosis, and deleterious changes in plasma chemistry and hematology (Kyoungju, 2004). pH is the measure of hydrogen ion in water. The best pH for pond fish culture is 7 to 9 (Swingle, 1961). High pH results from high rates of carbon

dioxide removal by phytoplankton for use in photosynthesis (Boyd and Tucker 1998). The ammonia formation depends on water pH, at higher pH, free toxic ammonia is released to critical levels (Boyd, 1990). Channel catfish can be cultured in water with total alkalinities as low as 5 to 10 mg/L (Murad and Boyd, 1990). Nevertheless, pond waters with less than 20 mg/L total alkalinity often have low abundance of phytoplankton because of low availability of carbon dioxide and removal of phosphate from water by acidic bottom soils (Thomaston and Zeller 1961). Calcium and magnesium ions are the source of water hardness, and is usually is recommended that water for food fish production should contain at least 50 mg/L total hardness (Boyd and Tucker 1998). Autotrophic activity increases pH through Co_2 absorption, while heterotrophic activity decreases pH through respiration, since the autotrophic and heterotrophic processes affect the measured variables in opposite ways (Boyd 1990; Saeed 2000; Ali 2007). A freshwater does not contain more than 1,000 mg/L TDS (Boyd, 2002). Channel catfish grow well in moderately saline water (Perry and Avault 1969). Plankton abundance and water quality tend to be more stable at moderate TDS, and the greater concentration of ions enhances osmoregulation and physiological condition in fish (Boyd and Tucker, 1998). Total hardness of drained water from agricultural activities in ponds were significantly lower when compared to that of the irrigation ponds water due to the higher photosynthetic activity in the agriculture drainage fish ponds water compared to the irrigation one; the high photosynthetic activity causes the release of carboxyl (OH^-) group which helps to bind Ca with the carbonate group (Co_3) to form $CaCo_3$ (Saeed, 2000; Ali, 2007). Total solids refer to any matter either suspended or dissolved in water. Everything that is retained by a filter is considered a suspended solid, while those that passed through are classified as dissolved solids, i.e. usually 0.45µ in size (American Public Health Association, 1995).

Table 2.1: Recommended Water Quality Requirement for the African Catfish (*Clarias gariepinus*)

Parameters	Recommended Level
Dissolved Oxygen	Not less than 4ppm
pH	6.5 – 8.5
Temperature	24^0c – 31^0c
Nitrate	Less than 250ppm
Nitrite	Less than 0.25ppm
Ammonia	Less than 0.05ppm
Water Hardness	50 – 300ppm
Alkalinity	50 – 200ppm
Turbidity	30cm dept

Source: Omitoyin (2007)

2.9 Histopathology

Histopathology study reveals the pathological changes in microscopic structure of the body tissue and also any peculiar type of alteration of cells that indicate the presence of the disease or the effect of toxic substance (Deore and Wagh, 2012). Histopathology is a useful tool in diagnosis of stressors at the individual animal level based on affected specific cell, tissues and organ types and these effects represents the cumulative effects of biochemical and physiological alterations and most times the actual injury to organism (Myer and Fournie, 2002). Histopathology is a useful biomarker for environmental contamination as documented by Jalaludeen *et al*., (2012). Accumulated heavy metals may cause morphological alterations in the tissues of fish (Monteiro *et al*., 2005). Histopathology tissue analysis can reveal the presence of contaminants at the microscopic level which may not be detected with the naked eye (Dural *et al*., 2006). Histopathological changes due to environmental stressors in animal tissues are indicators of prior exposure (Hinton and Lauren, 1990). The severity of tissue damage was found to be proportional to the exposure periods and concentration of the metals which was concentration and time dependent (Deore and Wagh 2012; Jalaludeen *et al*., 2012). Histopathological changes induced by stressors including toxic chemicals bacterial infection and cortisol injection in the gills include necrosis, lamellar fusion, hypertrophy and hyperplasia, excess mucus secretion and epithelial lifting of the outer layer of the lamellar epithelium (Smith, 2000) while Mcleay, (1973) reported atrophy of the interregnal tissue in the head of kidney of juvenile coho salmon. Lead nitrate caused in the liver, dilatation and congestion of the blood vessels, vacuolation of hepatic cells, proliferation of connective tissue and hepatic necrosis (Bothaina *et al*., 2012). Heavy metal exposure caused disintegration of mucosal epithelium, hypertrophied epithelial cells, goblet cells hyperplasia, sloughed off epithelial cells and mucous in the lumen, degeneration of the connective tissue of sub-mucosa and hyperemic blood vessels (Shawkat *et al*., 2010). Heavy metal exposure caused visible changes in centralobular area, cord disarray and connective tissues damage as well as focal necrosis in the liver of fish (Gbem *et al*., 2001). Olojo *et al.,* (2005) observed patchy degeneration and isolated degenerated cells around the parenchyma cells with progressive increase in fibrotic connective tissues and congestion of the sinuses at acute exposure and extensive necrosis of liver cells at prolonged exposure. Sastry and Gupta (1979) observed hypertrophy and degeneration of the villi in *H fossilis* exposed to cadmium. Lead caused liver damaged which decreased with garlic treatment (Sajitha, *et al*., 2010). Hepatic vacuolation is most

of the time associated with metal toxicity (Wolf and Wolfe, 2005). Hepatic vacuolation are indication of the presence of relative amount of hepatic glycogen and/or fat in the liver (Vanderberg *et al*., 1998). *Allium sativum* enables the liver to maintain its normal function by accelerating the regenerative capacity of its cells (Fazlolahzadeh *et al.*, 2011). In the intestine of fish, the degenerative changes in the tips of villi like hydropic degeneration, cloudy swelling and necrosis was possibly due to the fulfillment of extra energy requirement under the toxicity of cadmium chloride (Bais and Lokhande, 2012).

2.10 Oxidative stress and antioxidants

Heavy metal pollution of fish in aquatic environment leads to interactions between these metal and the fish which give rise to biochemical disturbances (Talas *et al.*, 2008). Heavy metal pollution, induce lipid peroxidation in aquatic organisms which is expressed by malondialdehyde (MDA) production (Draper *et al.*, 1993). Elevated concentrations of metals in fish tissues can induce redox reactions that generates free radicals, especially reactive oxygen species (ROS), e.g. singlet oxygen; superoxides; peroxides; hydroxyl radical; and hypochlorous acid (Dautremepuits, 2002). Free radicals and other reactive oxygen species (ROS) have been incriminated in the pathogenesis of various metal toxicities by various authors as reviewed by Kumar and Singh (2010). Free radicals are reactive molecules mainly derived from univalent reduction of oxygen that cause increase of numerous by-products through reactions with almost all the unsaturated bonds found in natural living cells (Nuriye *et., al* 1999). Cellular oxidative stress occurs when oxidants overwhelm antioxidant defense mechanisms (Li *et al.*, 2011). Basha and Rani (2003) reported that organisms have evolved a variety of mechanisms to protect themselves from the toxic effects of metals that cause oxidative stress. Blomhoff (2005), reported that with dietary oxidative stress in animals there was the development of compensatory induction of endogenous antioxidants. The impact of ROS are counteracted through various mechanisms including various antioxidant defense enzymes such as superoxide dismutases which catalyze the dismutation of superoxide radical to hydrogen peroxide, catalase acting on hydrogen peroxide, glutathione S-transferase family possessing detoxifying activities towards lipid hydroperoxides generated by organic pollutants such as heavy metals (Tjalkens *et al*., 1998). Antioxidant enzymes, glutathione system changes and induction of lipid peroxidation reflects the presence of heavy metals in the *Clarias gariepinus* may provide the basis for the use as biomarkers of oxidative stress in biomonitoring of aquatic pollution (Farombi *et al*.,

2007). Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) (Li *et al.*, 2011). Antioxidant response in fish can be influenced by natural environmental factors such as seasonal variations, oxygen saturation, feeding behavior and nutritional factors and age **(**Brucka-Jastrzębska**,** 2010**).** One of the most damaging effects of ROS and their products in cells is the peroxidation of membrane lipids, which can be indicated by MDA detection (Xue *et al*., 2009). Antioxidants are divided into four subgroups according to their effects: scavenging, quencher, repairing and chain breaking, inhibit lipid peroxidation (LPO) by preventing peroxidation chain reaction or by accumulating the reactive oxygen species (Nuriye *et al*., 1999). The antioxidant enzymes and lipid peroxidation products are biomarkers in environmental monitoring studies with high levels of MDA and low activity of SOD suggest a marked effect of possible fish species exposure to environmental stress (Nkwoji *et al*., 2014). Malondialdehyde (MDA) which are produced by lipid peroxidation (LPO) are due to free radical damage to membrane components of cells (Amin and Hashem 2012). GSH levels increased significantly in the liver, kidney and heart except the gills of fish in polluted river; also heavy metals accumulated in the tissues of fish may catalyze reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress (Farombi *et al*., 2007). Cadmium inhibit mitochondria electron transport, which results in an enhanced ROS formation, leading to peroxidative damage in the liver, kidney and gills (Dabas *et al.*, 2011). Lead induced a decrease in free radical scavenging enzymes and glutathione thereby enhance the production of free radicals (Sandhir *et al*., 1994). The oxidants produced by the toxicity of lead metabolites consumed a lot of antioxidant vitamin; garlic has enriched antioxidant properties (enriched with diallyl disulfides, diallyl polysulfides and their oxides like ajoene; which help destroy free radicals (Sajitha, *et al*., 2010). Fresh garlic extracts of contain antioxidant phytochemicals including water-soluble organosulphur compounds, lipid soluble organosulphur components and flavonoids, enhance the oxidant enzymes superoxide dismutase, catalase and glutathione peroxidase (Borek, 2001).

2.11 Erythrocyte Morphology

Fish erythrocyte morphology is more sensitive to various environmental agents than basic red blood parameters without distinct decrease in their values as recorded by different authors and reported by Witeska *et al.*, (2011). Nuclear anomalies such as

micronuclei, nuclear buds, irregular nucleus shape, binuclei or vacuolated nuclei are commonly considered indicators of genotoxicity (Ergene *et al*., 2007; Strunjak *et al*., 2009). Cadmium induced anemia is due to its adverse effect on iron uptake and metabolism and direct damage to erythrocytes (Gill and Epple 1993). Gill and Pant (1986) observed erythrocyte swelling, poikilocytosis, vacuolation, a mitosis, deformation and deterioration of cell membranes in *Barbus conchonius* exposed to chromium. Gill and Pant (1987) observed chromatin condensation, nuclear puffs and chromatin leakages in *Barbus conchonius* subjected to cadmium intoxication. Frequencies of nuclear anomalies such as irregular nucleus, shape, vaculation binuclei and micronuclei that indicate genotoxic effect often increase in fish exposed water pollution by various authors was reported by Witeska *et al*., (2011) which may also be influenced by season (Strunjak – Perovic *et al*., (2009). Witeska *et al*., (2006) observed in common carp sub lethally intoxicated with heavy metals that the frequency of erythrocyte anomalies was Pb ≥ Zn > Cd > Cu. Karuppasamy *et al*., (2005) reported increased fragility and rupture of erythrocyte membrane in *Channa punctatus* sub lethally exposed to cadmium.

2.12 Accumulation of metal/ions in tissues and water

Heavy metals like mercury, cadmium, arsenic and lead have no known role in biological systems (Sallam *et al*., 1999; Schmitt *et al*., 2005; Has-Schon *et al*., 2007). Most aquatic organisms have the ability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues (Osman *et al*., 2009). One of the principle of toxicology is that an increase in the animal exposure time results in identification of effects at lower concentration (Ensenbach and Nagel, 1997; Parrott *et al*., 2000). Most effects of metal interactions show synergistic or additive effects of metals but in some mixtures antagonistic effects may occur (Jezierska and Witeska, 2001). Fish ecological needs, size and age, their life cycle and life history, feeding habits and the season of capture were found to affect experimental results of tissues metal accumulation (Kime *et al*., 1996; Rurangwa *et al*., 1998). Heavy metals accumulation in a tissue is mainly dependent on water concentrations of metals and exposure period; however, other environmental factors such as salinity, pH, hardness and temperature do play significant influence in metal accumulation (Jeffree *et al*., 2006; Singh *et al*., 2006; Has-Schon *et al*., 2007). Fish surrounding environment includes water, sediments, suspended solids, and prey organisms (Ebrahimi and Taherianfard, 2011). Most chemicals achieve psuedo-equilibrium between toxicant concentration in the

exposure media and those of fish tissues within 30 days of exposure, however, for lipophilic chemicals, pseudo-equilibrium might be difficult to establish in this time frame (Veith *et al*., 1979). Jezierska and Witeska (2006) reported that interspecies differences in metal accumulation do exist and may be related to their living and feeding habits; accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions (water temperature, pH, hardness, salinity), and intrinsic factors (fish age, feeding habits). Accumulation of certain metals in fish may be altered in the presence of the others (Wicklund *et al*., 1988). Factors such as temperature, redox conditions (dissolved oxygen concentration), ionic strength, organic complexation, concentrations of metal that compete for uptake sites, pH, general physiologic behaviour, life cycle and life history also influence the extent of bioaccumulation of metals (Bryan, 1971; McKenna., *et al*., 1993). Aquatic organisms accumulate heavy metals in their tissues several times higher than the ambient levels by absorption process through gills or by consumption of contaminated food and sediments (Malik *et al.,* 2010). Excess metal concentration in an organism are actively excreted, compartmentalized in cells or tissues, or metabolically immobilized, however, some metal escape all these actions causing toxic and other adverse effects (Rand and Petrocelliet, 1985). Although the permissible level of Cd in fish muscles is 1.0 mg/g, (WHO, 1989) fish can accumulate Cd to levels ten to one-thousand times higher than its level in ambient water (Fleischer *et al*., 1974). The trend of accumulation of the metals in the Liver was Zn > Cu > Pb > As > Cd (Farombi *et al.,* 2007). Liver cadmium concentrations were positively correlated with liver protein concentrations which are largely a reflection of environmental contamination (Couture, and Ranjender 2003). Ekpo *et al*., (2008) documented that cadmium concentrations were in the range of 0.001 – 0.002mg/kg in the muscles, 0.004 – 0.006 mg/kg in the kidney and 0.002 – 0.004 mg/kg in the liver while that of the surrounding water was 0.001 mg/kg. Fish tissues uptake of lead is from aquatic plants, sediments and gasoline containing lead that leaks from fishery boats (Rashed, 2004). Lead accumulates in various organs such as the liver, kidneys and spleen, digestive tract and gills and bone. In lead pollution organs such as the gills and liver have been identified as the storage sites in *C. gariepinus* (Gbem *et al*., 2001). However, the main sites of these heavy metal uptake and accumulation are the gills and gastrointestinal tracts (Pantreath, 1973; Lovegroove and Eddy, 1982). The trend of accumulation of the metals in the liver was - Zn > Cu > Pb > As > Cd while the order of concentration of lead in the organs is as follows: Liver > Kidney > Gills > Heart (Farombi *et al*., 2007). The

trend of accumulation of the lead for fishes analysed from Escravos river was in order of the Gonad > Kidney > Brain > Fin > Muscle while from the Burutu river it was in the order of the Kidney> Gonad > Brain > Fin > Muscle (Ojogbo, 2006). Essential metal toxicity may occur when high amounts of metals that are essential are ingested and assimilated (Bryan, 1976; Alloway and Ayres, 1993) as a result, animal need to maintains a delicate balance of the body levels of the essential metals by mechanisms that integrates the various parameters of uptake, storage and excretion (Watanabe *et al.,* 1997). Srivastava and Agrawal (1983) have demonstrated the uptake of waterborne manganese, but the mineral is better absorbed via the dietary mode. Manganese acts either as an integral part of enzymes (private carboxylase, lipase) or as a cofactor for numerous enzymes involved in nitrogen, lipid and carbohydrate metabolism (Schlenk and Benson, 2001). Metals like manganese are cofactor in several enzyme systems in fish (Bury *et al*., 2003). Elevated concentration of bioactive metals may pose serious threats to normal metabolic processes (Ekeanyanwu *et al*., 2010). Calcium uptake of (Ca^{2+}) through the gills is continuous (Herrmann-Erlee and Flik, 1989). The contribution of extrabranchial calcium transport through the skin is considered to be small, and is reflected by the absolute numbers of ionocytes in gills and skin (Marshall *et al*., 1992; Perry *et al.* 1993). Medicinal plants possess therapeutic properties; exert beneficial pharmacological effects on the animal body, widely available in nature and eco-friendly (Rahman *et al*., 2009). The bulbs of Garlic (*A. sativum*) are used for medicinal and culinary purposes (Villachira, 1998). The consumption of garlic powder may decrease the accumulation of lipids in the liver, increase the excretion of total bile acids in faeces, and increase the antioxidant capacity in hamsters (Yaoling *et al.,* 1998).

2.13 Toxicity studies

Toxicity studies quantify an organism's response to a biologically active substance (Alderdice, 1976). The main goal in toxicity testing as reported by Adeyemo *et al*., (2007) is to predict, in combination with other environmental factors, with known accuracy, a concentration of a specific toxicant that will not harm an entire system. Mortality is used as criteria to the final response of an organism to the toxic effect of a particular toxicant (Kai Sun *et al*., 1995; Kazlauskiene and Vosyliene, 1999). On the basis of such acute toxicity tests, the sensitivity of various organisms and their developmental stages to contaminants can be compared (Kazlauskiene and Burba, 1997; Hussain *et al.,* 2011). Waterborne environmental contaminants have been shown to exert

pronounced effect on various aspects of fish physiology and sometimes may lead to large scale mortality (Ikeogu *et al*., 2013). The susceptibility of fish to a particular heavy metal is also a very important factor for LC50 values (Das and Banerjee, 1980). Both lethal and sub-lethal concentrations of heavy metals are indicator of the sensitivity of individual organisms across species (Velma *et al.*, 2009). Lethal and sub-lethal effects differ from metal to metal, from species to species, and from one experimental condition to another; development and use of toxicity tests provide data, which could be more effectively used in predictive toxicology and risk assessment (Zeynab *et al.*, 2012).

CHAPTER THREE

STATIC CULTURE OF *CLARIAS GARIEPINUS* WITH RAW GARLIC; NEAR ENVIRONMENTAL STANDARD LEVELS OF CADMIUM AND LEAD

3.1 Introduction

In studies by Adhikari and Sarkar, (2004); Maheswaran *et al*., (2008) blood parameters were considered patho-physiological indicators of the whole body and therefore were important in diagnosing the structural and functional status of fish exposed to toxicants. The evaluation of blood cells, blood biochemistry and hormones could be useful for the diagnosis of fish disease and to monitor the physiological status of fish (Stoskopf, 1993). Fish health could be better assessed by the evaluation of the interaction of intrisic and extrisic factor. There is a dearth of information on the use of raw garlic in fish culture since it has been documented that cooking and processing either reduced or completely destroyed some of its composition. Also there is a dearth of information on the influence of sublethal and near environmental standard levels of cadmum and lead on *Clarias gariepinus* in a prolonged static culture. The present study was aimed at evaluating holistically the singular influence of raw garlic, *Allium sativum*, near environmental standard levels of lead and cadmium on some clinical signs, peripheral blood cell and blood chemistry; water quality/chemical factors, gross and histopathology including liver levels of metals in *Clarias gariepinus* in static culture, an attempt to simulate some of their effects in a lotic aquatic ecosystem during dry season.

3.2 Methodology

All fish used in this study irrespective of weight and length were apparently healthy. Fishes were sexed visually using the shape of the urogenital papillae before blood collection and later confirmed by lethal method.

Garlic (*Allium savitum*) bulb apprently free from disease and pest was obtained from Bodija market in Ibadan. Garlic cloves were peeled, weighed, sliced and ground into a paste using distilled water and the homogenate at concetration of 8.0g/l (Rahman *et al*., 2009) was used. One hour (1hr) lapsed between preparation and use because garlic forms the active compound, allicin, steadily and in regular spurts rather than all at once so it was better to let it set for a minimuim of 15 minutes to 1 hour before using it in order to build up a greater amount of allicin. Allicin has a half-life in air of about 18 hours as it slowly deteriorates into other smelly, sulfurous compounds; adding allicin to water stabilizes it and preserves its antibiotic properties in water and extends its half-life to

about two months (Garlicoils,Pills&Extractswww.gourmetgarlicgardens.com/pill.htm, 2011). 1.0mg/l standard of lead and cadmium were prepared using 0.1599g of lead nitrate and 0.2282g of cadmium sulphate respectively made up to 1000ml with distilled water. 60ml of standard Lead nitrate solution in 200 litre of group water represents a dose of 0.3mg lead while 6mls of standard solutions of cadmium sulphate in 200litre of water represent a dose of 0.03mg/l cadmium.

African catfish *Clarias gariepinus* 75 day-old obtained from a commercial fish farm in Ibadan was used for the study in a static condition for 90 days. Before acclimatization, water for the culture was analysed to obtain the concentration of cadmium and lead. Also done was blood of six females and six males of *Clarias gariepinus* were pooled in two units for haematological and blood chemistry baseline data. Acclimation and treatments/exposure took place in prepared concrete tanks with white tarpaulin linings (used to line the tanks floor and side to avoid seepage and leakage). The tanks were in the same proximity with trees and plantains acting as shade from direct sunlight. The tanks were covered with a seined net to check water contamination/pollution by falling leaves from the surrounding trees and plantain. Before acclimatization the well water used was sampled and analysed for the levels of lead and cadmium. A total of 10 females and 10 males *Clarias gariepinus* were stocked in each of the 4 groups labeled group A, B, C, and CTL with 3 subunits (replicates) containing 200 litres of well water Group 4 is the control. Each group is in triplicate and there were no statistical significant differences in the stocking density of all the groups.

Acclimation was for 14 days. The 1st day of acclimatization the fish was not fed. Feeding started for each group the next day at 80gm/day ration, feed twice 9.00am and 5.00pm with a commercial floating feed of 2/3mm 49.00% crude protein The daily ration was increased to 120gm of 4.5mm 45% crude protein at day 60 of treatment /exposure to a day prior to sampling at day 90 of treatment/exposure (end of research). The study been static, fish was fed restrictively. The reason for this was that young catfish can experience a feed related problem known as “Ruptured Intestine Syndrome” (RIS) or “Open Belly Syndrome” which is caused by high feed loads (Hariati *et al*., 1994) and to reduce nutrient pollution.

At the end of acclimatization, study groups were subjected to the following respective treatments:

Group A: 8.0g raw garlic/l.

Group B: 0.03mgCd/L.

Group C: 0.3mgPb/L.

Group CTL: untreated/control

Sampling was done in the morning between 7.00am and 9.00am at an average ambient temperature of 26.3 ± 0.4^{0}c. Water temperature of each group and the ambient temperature were taken at 9.00am, 12.00noon and 4.00pm daily throughout the period of this study. A total of 27, 27, 29, and 26, males were sampled in groups A, B, C, and CTL respectively while a total of 26, 25, 25 and 26 females were sampled in groups A, B, C, and CTL respectively. These respective number of samples was depended on actual number sampled to obtain the effects of raw garlic (*Allium savitum)*, and sublethal doses of cadmium and lead on some heamatological and blood chemistry parameters, water quality, gross and histopathological studies including liver accumulation of metals at different exposure duration of culture.

Water samples were collected from the groups for water quality analysis at day 0, 7, 30, 60, 90, of treatment/exposure. Blood samples were collected by venipuncture of the caudal vein, which lies just ventral to the spinal cord, after anaesthesia with 0.2mg benzocaine dissolved in 5ml acetone in 4 liters of water (Adedeji and Adegbile, 2011).

Approximately 1 – 3 ml of blood was collected from each fish thereafter the fish was sacrificed by percussive stunning for pathological and liver accumulation of metals examination.

Appropriately labeled water samples of 1000ml (pooled into 2 units from each triplicate group) were transported to the laboratory in plastic container where they were kept in the refrigerator before water quality/chemical analysis using APHA (1995) standard methods and fish sampled at day 0, 7, 30, 60, 90 from each group after blood collection at the culture site and sacrificed by percussive stunning were transported in cool chain to the laboratory for the stated series of end points analysis: peripheral blood cells; blood chemistry parameters; water quality/chemical factors; gross and histopathology and metals in liver. Before the fish sampling water for dissolved oxygen examination was appropriately collected (pooled into 2 units from each triplicate group) and labelled were transported to the laboratory in plastic container where they were kept in the refrigerator before analysis using APHA (1995) standard methods.

3.2.1.1 Packed cell volume (PCV) determination

Three-quarter (¾) of microhaematocrit (heparinised) capillary tube was filled with blood from appropriately labelled whole blood in a heparinised tube by capillary action, one end of the tube was sealed with plastercine. The tube was spined at 1,200rpm in microhaematocrit centrifigue for 5 minutes (Jain, 1986). The PCV was read using microhaematocrit reader and expressed as a percentage of the total blood volume.

3.2.1.2 Haemoglobin concentration determination

The concentration of haemoglobin (Hb) was determined by cyanohaemoglobin method (Jain, 1986). Briefly 20µl blood was mixed with 4ml of modified drabkin's solution (potassium ferricyanide, 200mg, potassium cyanide, 50mg, potassium dihydrogen phosphate 140mg, volume made up to one litre with distilled water and pH adjusted to 7.0. This mixture was allowed to stand for 3 minutes before the haemoglobin concentration was read with Autospectrophotometer (spectrum lab 23A) at a wavelength of 540nm. The actual value of Hb concentration was extrapolated from a standard haemogblobin curve. For the solution to work perfectly well the drabkin's solution was kept in a dark cupboard after preparation before using.

3.2.1.3 Determination of total red blood (RBC) Counts

Total red blood cells and white blood cell counts were done manually. Differential WBC counts were done manually (Adedeji, *et al.*, 2009; Zinkl, 1986) because nucleated RBC prevents accurate enumeration using automated analysis (Huffman *et al*., 1997). By the use of Neubauer haemocytometer according to Jain (1986) and the number of cells were expressed as 10^{12} RBC per litre of blood and 10^{9} white blood cells per litre of blood respectively.

Procedure: 1ml of RBC diluting fluid (Daciers fluid) was taken into test tube. 0.05µl of whole blood was added into the Daciers fluid (99ml of 3 percent aqueous solution of sodium citrate, 1ml of 40 percent formaldehyde) to keep and preserve the shape of the cells. This was gently mixed and allowed to stand and counted at X40 magnification under the microscope.

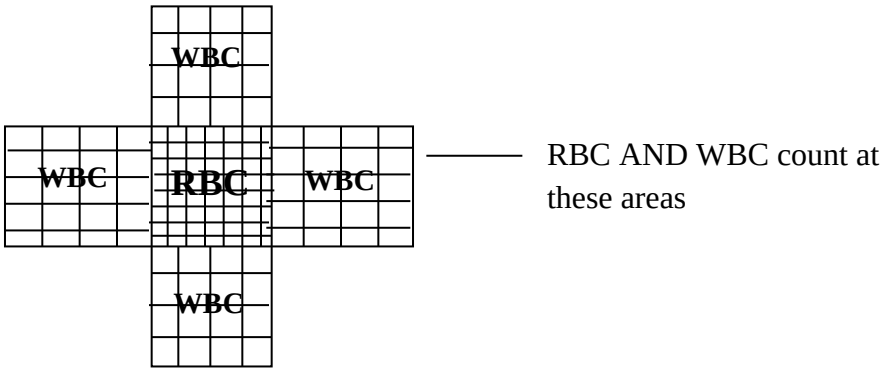


Figure 3.1: Diagrammatic illustration of Neubauer haemocytometer

3.2.1.4 Determination of white blood cell (WBC) counts

Procedure: 0.5ml of WBC diluting fluid (3 percent aqueous solution of acetic acid to which 1 percent gentian violet was added) was taken into a test tube. 25µl of whole blood was added into the fluid and gently mixed thoroughly and allowed to stand. It was counted at x40 magnification.

3.2.2.1 Preparation of plasma

After the determination of haematological parameters from the blood in the heparinised tubes, the remaining blood was spun down at 1200rpm for 1min to obtain plasma for total protein using RANDOX KIT (UK) and read with Autospectrophotometer (Spectrum lab 23A Spectrophotometer)

3.2.2.2 Preparation of Serum

Blood collected in plane test-tubes (non-heparinized) was allowed to clot at room temperature for 1 hour and spun at 1200rpm for 5minutes. The serum was harvested using sterile Pasteur pipette and divided into 4 parts dispensed in 0.5 aliquouts into scincillation vials (Fissons scientific apparatus) and the separated sera were stored at -20^0c for biochemical analysis.

3.2.2.3 Estimation of total protein

The estimation of total protein was done using Randox Kit®. Stock solution A and B were mixed to form the working solution. 3ml of distilled water was mixed with 3ml of Biuret (working solution) and was used as blank. 2.9ml of the working solution + 3mls of distilled water + 100µ of plasma sample formed sample solution; both the blank and sample solution were rocked gently and incubated at 37^0C for 10minutes. After incubation they were read using autospectrolab at the wavelength 540nm.

Total Protein (g/l) was calculated as:

$$\frac{\text{Optical Density of Sample}}{\text{Optical Density of Standard}} \times \frac{21.05}{1}$$

3.2.2.4 Estimation of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)

The estimation of ALT was done using Quimicaclinica applicada South Africa (QCA) Alanine aminotransferase Kit®. 1ml distroater and 1 drop of sabbaste in the kit was mixed and incubated at 37^0C for 5minutes using Gesell Scharft fur labortechnik water incubator to form solution A. 0.1ml of standard solution in the kit was mixed with

solution A and also incubated at 37^0C for 20minutes using Gesell Scharft fur labortechnik water incubator. These samples were read at 550 nm wavelength.

ALT (iu/L) was calculated using the following formular:

$$\frac{\text{Optical Density of Sample} \quad X \quad 30}{\text{Optical Density of Standard} \quad 1}$$

The estimation of AST

The same process as in estimation of ALT but the estimation of AST was done using Quimicaclinica applicada South Africa (QCA) Aspartate aminotransferase Kit®.

3.2.2.5 Estimation of glucose

The estimation of total glucose was done using Randox Kit® .0.10ml of Haparinised blood was deproteinised with Urinyl acetate (ml). 0.02ml of deproteinised blood and 0.2ml of reagent 1 were mixed gently and incubated at 20^0C for 25 minutes and read at set wavelength of 546nm.

Calculation = Optical Density of SampleX Standard Coefficient X 100ml = g/l.

3.2.2.6 Estimation of potassium and estimation of sodium

The Kit used for the estimation of potassium was Teco diagnostics California USA potassium Kit®. 10µl of serum + 1ml of potassium reagent provided in the kit were mixed properly and allowed to stand for 3 minutes at room temperature. It was thereafter, read at set wavelength of 500nm.

Potassium = Optical density of sample of sample X Standard

Standard = 4meg/l.

The Kit used for the estimation of sodium was Teco diagnostics California USA sodium Kit®. 50µl of serum sample was mixed vigorously with 1ml reagent for 3 minutes then centrifuged for 10 minutes. 50µl of the supernatant was added to 1ml acid reagent provided in the kit. 50µl of colour reagent provided was added and mixed thoroughly then read at set wavelength of 550nm and calculated as follows:

Sodium = Optical density of sample X Standard concentration.

Standard concentration given is 150meg/l.

3.2.2.7 Estimation of creatinine

Randox creatinine Kit® was used. 4ml of plasma and 6ml distilled water and 4ml of sulphuric acid and 2ml of sodium tungstate solution were mixed thoroughly and allowed

to stand for 3 minutes and centrifuged then to 5ml of supernatant was added 7.5ml of picrate solution then read at set wavelength of 540nm.

Creatinine calculation =

Optical density of sample X Standard coefficient. Standard coefficient is 96mg/l

3.2.3 Determination of Water quality

3.2.3.1 pH

Reagents:

Reagents of analytical grade, and deionised water were used for analysis.

Buffer: 4.02: 10.21g of solid Potassium hydrogen phthalate (dried at 105^0C for 1hr) was weighed and dissolved in 100ml deionised water then made up to 1000mL in a standard volumetric flask.

Buffer 9.22: 3.81g of Borax (sodium tetraborate) was weighed and dissolved in deionised water and made to 1000mL in a volumetric flask.

Procedure:

50ml of each of the water sample collected was poured to a 100ml beaker. The pH meter was standardized by alternately dipping the electrode into buffer solutions of pH 4.02 and pH 9.22. After standardization, the pH of the water sample was read to the nearest two decimal places using the pH meter.

3.2.3.2: Dissolved oxygen (DO)

Apparatus

Bottles: 250ml glass bottles with narrow neck and well-fitted ground-glass stoppers were used for sampling. These sampling bottles were cleaned with 5N sulphuric acid and then rinsed thoroughly with water; no soap or synthetic detergents were used for cleaning. These sampling bottles were subsequently kept clean by the acidic iodine solution of the Winkler procedure with no further treatment apart from thorough rinsing with distilled water before use.

Sampling:

The sampling bottles was held horizontally at the surface so that the water entered gently, without bubbling; as the bottle is filled, it was brought gradually to the vertical position

Apparatus: Conical flask, 50ml Burette, 100ml measuring cylinder.

Reagent:

Reagents of analytical grade, and deionised water were used for analysis.

Alkaline iodide solution: 400g of sodium hydroxide was dissolved in 560ml of water, after which 900g of sodium iodide was added and the solution kept hot until the iodide has completely dissolved. The solution was allowed to cool and diluted to 1litre. No iodine was liberated when 1ml was diluted to 50ml and acidified.

Alkaline iodide-azide solution: 1 litre of the alkaline iodide solution was mixed with 300ml of 2.5% sodium azide solution.

Sulphuric acid, diluted: 500ml of concentrated sulphuric acid, about 98%m/m (36N) was carefully added to 500ml water, stirring during the addition.

Manganese (II) sulphate solution: 500g of hydrated manganese (II) sulphate, $MnSO_4.5H_2O$, was dissolved in water, filtered and made up to 1 litre. No iodine was liberated when 1ml of the reagent was added to 50ml of acidified potassium iodide solution.

Potassium iodate, 0.025N. Standard volumetric solution: Potassium iodate salt was dried at 120^0C for 2hours, allowed to cool in adesiccators, 0.892g was weight out and dissolved in water and diluted to 1 litre. The solution was stored in a glass-stoppered bottle.

Sodium thiosulphate, approximately 0.025N: 6.3g of sodium thiosulphate pentahydrate $Na_2S_2O_8.5H_2O$ was dissolved in 1 litre of copper free water, with 1ml of chloroform to stabilize the solution and stored in a brown bottle. Sodium thiosulphate working solution: The 0.025N sodium thiosulphate solution was diluted to strength of 0.0125N.

For standardization, 10ml of 0.025N potassium iodate is measure by pipette into a conical flask containing 100ml water and 1ml of alkaline iodide solution is added, followed by 2ml of diluted sulphuric acid (1+1). The content was mixed thoroughly, and then titrated with the 0.0125N thiosulphate, adding 2ml of starch solution just before the end point.

Procedure:

At the site of sampling, to the water sample, 2ml of manganese (II) sulphate solution was added well below the surface of the liquid and 2ml of alkaline iodide solution was added at the surface with a 5ml syringe. The stopper of the oxygen bottle used for the water sampling was carefully replaced to avoid inclusion of air bubbles and the content was thoroughly mix by vigorously inverting and rotating the bottle several times. The sampled water was transported in a plastic container with iced cubes to the laboratory for

further analysis. In the laboratory 4ml of diluted sulphuric was added by pipette. The stopper was replaced and the content thoroughly mixed by rotation, the precipitate was allowed to stand for few minutes for the precipitate to dissolve. 100ml of the solution was measured into a conical flask and iodine immediately titrated with standard thiosulpate solution, using as indicator 2ml of starch solution added towards the end of the titration

Calculation:

Allowance was made in the formula given below for the slight displacement of sample by the manganese (II) sulphate and alkaline iodide reagents, which contain very little dissolved oxygen.

$$\text{Dissolved oxygen content} = \frac{\text{Volume of 0.0125N thiosulphate (ml)}}{\text{Volume titrated (ml)}} \times 101.6 \text{ in mg/L}$$

3.2.3.3: Total Solids

Apparatus:

Crucibles, Analytical balance, 500ml measuring cylinder.

Procedure:

300ml of a well-mixed sample was evaporated to dryness in the oven, in a previously ignited, cooled and weighed crucible. The residue was dried at 105^0C for 1 to 2 hours in the oven, cooled in a desiccators and weighed. The heating was repeated for 15 minutes and cooled until a constant weight was gotten. The increase in weight was express as milligrams of total solids per liter of sample.

3.2.4: Approximation of metal concentration in the treatment group

Apparatus:

Hot plate, 500ml beakers

Reagent:

Analytical reagents and deionized water were used for all analysis

Concentrated nitric acid.98%m/m, 1.52gcm^{-3}.

Procedure:

100ml of preserved water sample collected from each group were filtered through Whatman 0.45µm glass fibre filter and transferred to acid cleaned 250ml polypropylene bottles and then acidified with concentrated Nitric acid to pH not exceeding 2.0. This solution was concentrated to 10ml and heated on a hot plate for few hours. The concentrated extract was cooled and transferred into a 25ml standard flask, then made up

to mark with deionized water. Reagent blanks of 15ml 1:1 freshly prepared nitric acid and hydrogen peroxide were treated as above. These were done to check the reliability of the results from atomic absorption spectrophotometer reading was carried out in a similar way without each water sample.

Calculation

$$\text{Concentrate in µg/ml (ml)} = \frac{\text{instrument reading (µg /ml)} \times \text{extract volume}}{\text{Volume of water (ml)}}$$

3.2.5: Heavy metal determination in liver tissue

Reagent

1:1 HNO_3 – 500ml concentrated HNO_3 was mixed with equal volume of deionized water in a standard flask.

The concentrate of the standards was confirmed using flame atomic absorption spectrophotometer (FAAS).

Procedure

1.0g of tissue samples was weighed into a digestion tube; 20ml of 1:1 HNO_3 was added and heated on a water bath for 2 to 3 hours. The digest was filtered into a 20ml standard volumetric flask and made to mark with deionized water. The extract was analyzed for lead, cadmium, calcium, potassium and manganese using flame atomic absorption spectrophotometer, while calcium and potassium were analyzed using flame atomic emission spectrophotometer. Blank was carried out at every batch of sample without tissue.

Calculation

$$\text{Concentrate in µg/g} = \frac{\text{instrument reading (µg/ml)} \times \text{extract volume (ml)}}{\text{Weight of tissue (g)}}$$

3.2.6: Preparation of histopathologic slides

The sampled and appropriately labeled and sacrificed fish brought to the laboratory were subjected to the following procedure. The appropriate fish tissues samples were dissected out and appropriately labeled, and fixed in Bouin fluid for 6 hours then transferred to 10% buffered formalin for further fixing before been processed in automatic tissue processor, embedded in paraffin wax and sectioned at 5 microns on a rotary microtome mounted on glass slides. The stepwise protocol for the automatic tissue processor for

histological examination slide was as described by Akpokodje *et al*., (2005) Briefly the procedure is presented as follows:

Fixation: Fixation: This was the first process in the successful preparation of tissues histologically. The specimens were put in a sample bottle that contains the fixative 10 to 20 times the volume of the specimen. Fixation allows the tissues to form a mesh work that tends to hold the other cell constituents. The specimens were fixed for at least 24 hours before the commencement of dehydration. The next stage of tissue processing following fixation was dehydration. Dehydration basically the is process of removing the inherent water content of a given specimen of tissue in a gradual way considering osmotic dynamics. Dehydration was done by automated means using the automated tissue processor (Shandon-Elliot^{R})

A complete processing schedule was as follows:

70% Ethanol	1hr
80% Ethanol	1hr
90% Ethanol	1hr
95% Ethanol	1hr
95% Ethanol	1hr
100% Ethanol	1hr

At the end of processing schedule the tissues were totally dehydrated. The next stage is called clearing. Clearing involves the removal of the alcohol (Ethanol) that the tissues had bathed in and to initiate and complete a process that will make cells transparent at microscopic level and prepares the tissue for infiltration in molten paraffin wax.

The clearing processing was as follows

Xylene - 1hr

Infiltration: leaving xylene, the tissues were put into molten paraffin wax which serves as support to the tissues for subsequent stage of sectioning. Paraffin wax permeates into the tissues to fill up vacuoles that have been left by dehydration. Infiltration process was

1^{st} wax – 2hrs

2^{nd} wax – 2hrs – this was completed in a wax oven at a fairly high temperature before embedding.

Embedding: this was the positioning of the processed infiltrated tissues in molten paraffin wax within an enclosure called a mould. The tissues were carefully and

consciously positioned in the orientation they will be sectioned. The embedded tissues were left until the wax solidifies before sectioning.

Sectioning: Sectioning was done on a quint-essential piece of histological equipment called a micro tone which cuts only a very thin slice of the original tissue at a preset thickness e.g 4µ - this slice is what is called a section.

The produced sections, were floated out in a floating water bath. Satisfactory section(s) were picked up with frosted edge microscope glass slides. Specimens were labeled with a pencil, arranged in a slide carrier, put in oven and heated at 40^0c for 30 minute for dewaxing and to make the sections stick to the slide before staining.

Staining: The routine staining method was the H and E (Haematoxyline and Eosin). The H and E method was stepwise as follows:

- Dewaxed in Xylene 2 chambers for 15 mins each
- Hydrated in ethanol 100% for 30sec
- Hydrated in ethanol 100% for 30sec
- Hydrated in ethanol 80% for 30sec
- Hydrated in ethanol 70% for 3 30sec
- Water for 3mins
- Stained in haematoxylin for 15mins
- Washed off excess stain with water
- Differentiated in 1% acid alcohol for 3 – 5 secs
- Blue in water for 3 – 5mins
- Counterstained in 1% Eosin for 3 secs
- Dehydrated with Ethanol dipping

  70% Ethanol

  90% Ethanol

  100% Ethanol

The slides were left in the xylene until they were mounted using D.P.X which is a good transparent mountant that has a refractive index similar to that of glass. Sections were

stained with Haematoxylin and Eosin (Akpokodje *et al*., 2005) and examined with light microscope.

3. 3: Data analysis

All Data collected were coded and analyzed using the SPSS package version 17.0. Statistical data analysis was done using an independent sample t – test and one-way analysis of variance (ANOVA). The differences among the variables and groups were adjudged with Duncan multiple range tests (5% significant level). The *post hoc* comparison of means was carried out using Duncan's multiple range tests (Frank and Althoen, 1995).

3.4: Results and discussion

3.4.1: Lead and Cadmium level in well water

Table 3.1: The Mean±SEM of pooled water levels of Lead and Cadmium before acclimation of *Clarias gariepinus* in the static culture

Parameters	Level in well water used for experimental studies(mg/l)
Lead (Pb)	0.042 ± 0.01
Cadmium (Cd)	0.000 ± 0.00

n = 3

3.4.2: Haematological Parameters

3.4.2.1: Results of pooled blood parameters

Table 3.2: Effect of sex on the Mean ± SEM of pooled blood parameters of 75 day-old *Clarias gariepinus* before acclimation

	Freshwater Teleost *Clarias gariepinus*	
Parameters	♀females a^1 n = 2	♂ males a^2 n = 2
PCV (%)	25.50 ± 2.12	22.00 ± 1.41
Hb (g/dl)	8.05 ± 0.78	7.25 ± 0.35
RBC cellsx10^{12}/L	4.02 ± 0.30	2.68 ± 0.18
WBC cellx10^9/L	5.08 ± 0.25	5.65 ± 0.10
Total protein (g/l)	3.37 ± 0.05	3.27 ± 0.09
K^+(meg/l)	20.04 ±0.06	14.08 ± 1.31
Na^+(meg/l)	31.53 ± 0.88	23.55 ± 1.31*
Creatinine g/l	1.01 ± 001	1.00± 0.00
ALT (Int Units/l)	36.65 ± 1.34	35.10 ± 1.56
AST (Int Units/l)	64.00 ± 0.00	61.50 ± 2.12
Glucose(g/l)	46.02 ± 2.84	42.50 ± 0.71

[a1]fish *Clarias gariepinus* females. [a2]fish *Clarias gariepinus* males

*Significantly different at P≤ 0.05 (Student t-test).

3.4.2.2: Discussion on effect of sex and treatment on PCV%

Table 3.3: Effect of sex and treatment on PCV% Means ± SEM of *Clarias gariepinus* days 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/exp	A		B		C		CTL (CONTROL)	
osure days	♀$a^{1}{}_{/n}$	♂ $b^{1}{}_{/n}$	♀$a^{2}{}_{/n}$	♂$b^{2}{}_{/n}$	♀$a^{3}{}_{/n}$	♂$b^{3}{}_{/n}$	♀$a^{4}{}_{/n}$	♂$b^{4}{}_{/n}$
0(**after acclimation)**	19.33±0.67$^{a}{}_{/3}$	19.33±2.73$^{a}{}_{/3}$	20.00±4.00$^{a}{}_{/2}$	19.33±2.17$^{a}{}_{/3}$	20.50± 0.50$^{a}{}_{/2}$	19.50 ± 0.50$^{a}{}_{/2}$	21.67± 0.88$^{a}{}_{/3}$	19.33±0.68$^{a}{}_{/3}$
7	21.50±1.18 $^{a}{}_{/6}$	22.50±0.76$^{b}{}_{/6}$	16.50±0.77$^{b}{}_{/6}$	19.50 ± 1.29$^{c}{}_{/6}$	22.67 ± 0.88$^{a}{}_{/6}$	26.17 ± 0.60*$^{a}{}_{/6}$	23.00± 0.52$^{a}{}_{/6}$	21.00±0.52*$^{bc}{}_{/6}$
30	23.67±0.49$^{a}{}_{/6}$	23.00±0.58$^{a}{}_{/6}$	12.83±0.40$^{b}{}_{/6}$	16.17±0.31**$^{b}{}_{/6}$	13.33±0.49$^{b}{}_{/6}$	17.67.±0.42**$^{b}{}_{/6}$	23.67±0.33$^{a}{}_{6}$	21.67±0.84$^{a}{}_{/6}$
60	23.67± 0.33$^{b}{}_{/6}$	23.83±01.31$^{b}{}_{/6}$	39.00±0.37$^{a}{}_{/6}$	35.33±0.49**$^{a}{}_{/6}$	37.83 ± 0.48$^{a}{}_{/6}$	36.00±0.58*$^{a}{}_{/6}$	24.67 ± 0.49$^{b}{}_{/6}$	24.00 ±0.58$^{b}{}_{/6}$
90	27.33 ± 0.33$^{b}{}_{/6}$	25.67 ± 0.33* $^{b}{}_{/6}$	29.67 ±0.33$^{a}{}_{/6}$	22.00±0.58**$^{c}{}_{/6}$	26.17±0.31$^{b}{}_{/6}$	33.83±0.54**$^{a}{}_{/6}$	20.67 ± 0.94$^{c}{}_{/6}$	22.00± 0.45$^{c}{}_{/6}$

[a]fish *Clarias gariepinus* females. [b]fish *Clarias gariepinus* males. *Significantly different at P≤ 0.05 (Student t-test). ** Highly significantly different at P≤0.001 (Student t.test). Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil
Means of same sex with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n= number of sample.

At 75 day-old the females had a higher value of 25.50 ± 2.12% than the males with 22.00 ± 1.41% (Table 3.2).

At day 0 after 14 days' acclimation in A, the females and males had similar values of 19.33±0.67% and 19.33±2.73% respectively; In B, the females had a higher non-significant value of 20.00±4.00% than the males with 19.33±2.17%; In C, the females had a non-significant higher value of 20.50± 0.50% than the males with 19.50 ± 0.71%; While in CTL, the females had a higher non-significant value of 21.67± 0.88% than the males with 19.33±0.68% (Table 3.3).

At day 7 of treatment/exposure, in A treated with 8.0g raw garlic/l, the females had a lower non-significant value of 21.50±1.18% than the males with 22.50±2.88%; In B treated with 0.03mgCd/l the females had a lower non-significant value of 16.50±0.77 % than the males with 19.50 ± 1.29%; in C treated with 0.30mgPb/l, the females had a lower significant value of 22.67 ± 0.88% than the males with 26.17 ± 0.60 %; and in CTL with no treatment the females had a higher significant ($p \leq 0.05$) value of 23.00± 0.52 % than the males with 21.00±0.52% (Table 3.3).

At day 30 of treatment, the females treated with 8.0g raw garlic/l, in A, the females and males had similar values of 23.67±0.49% and 23.00±0.58% respectively; In B treated with 0.03mgCd/l, the females had a lower highly significant ($p \leq 0.001$) value of 12.83±0.40 % than the males with 16.17±0.31%.; In C treated with 0.30mgPb/l, the females had a lower highly significant ($p \leq 0.001$) value of 13.33±0.49 % than the males with 17.67.±0.42% ; While in CTL with no treatment the females had a higher non-significant value of 23.67±0.33% than the males with 21.67±0.84 % (Table 3.3).

At day 60, 8.0g raw garlic/l treatment/exposure, in A, the females and males had similar values of 23.67± 0.33 % and 23.83±01.31 % respectively; In B treated with 0.03mgCd/l, the females had a higher highly significant ($p \leq 0.001$) value of 39.00±0.37 % than the males with 35.33±0.49%.; in C treated with 0.30mgPb/l, the females had a higher significant ($p \leq 0.05$) value of 37.83 ± 0.48 % than the males with 36.00±0.58%; While in CTL with no treatment (control), the females and males had similar values of 24.67 ± 0.49 % and 24.00 ±0.58 % respectively. (Table 3.3).

At day 90 of treatment /exposure, in A treated with 8.0g raw garlic/l, the females had a higher significant ($p \leq 0.05$) value of 27.33 ± 0.33% than the males with 25.67 ± 0.33%; In B treated with 0.03mgCd/l, the females had a higher highly significant ($p \leq 0.001$) value of 29.67 ±0.33% than the males with 22.00±0.58%; In C treated with 0.3mgPb/l, the females had a lower significant ($p \leq 0.001$) value of 26.17±0.31 % than the males with

33.83±0.54%; In CTL with no treatment (control), the females had a lower non-significant value of 20.67 ± 0.94% than the males with 22.00± 0.45% (Table 3.3).

3.4.2.3: Discussions on effect of sex and treatment on PCV%

At day 0 after acclimatisation, PCV decreased in female and male. The decrease showed no significant difference in all the groups. This decrease may be due to stress caused by the confinement and probably the Pb concentration in the well water used for the culture. PCV was higher in the female. This finding is in agreement with the findings of Gbore *et al*., (2006) who reported decreased in haematocrit values after stress in *Tilapia zilli* and *Clarias gariepinus*.

8.0g raw garlic/l treatment increased PCV at day 7 in both the females and males. PCV was not expected to increase significantly within 7 days. It thus appears that the observed stress condition (40.0% vertical positioning) during the first 2 days of the experiment may have been due to garlic with the resultant manifestation of increase in PCV in both the females and males. This is in agreement with Larsson *et al*., (1985) that increase in haematocrit reduces oxygen delivery to tissues. At day 7 the females may have undergone adaptation syndrome as reported by Ellis (1981) that fish under stressful conditions undergo a general adaptation syndrome. The fish coped physiologically by the manifestation of a non-significant increase at day 30 and 60 before PCV increased $P \geq 0.05$ in males; increased significantly ($p \leq 0.05$) in females. 8.0g raw garlic/l thus increased PCV in both the females and males. This is in agreement with Martins *et al.,* (2002), that addition of *Allium sativum* to fish diets increased the hematocrit value.

0.03mgCd/l treatment/exposure shows at day 7 a decreased PCV in the females and an increase in the males. These results suggest that the females were the first to respond to stress associated with the treatment/exposure. At day 7 adaptation syndrome may have manifested as a decreased PCV. These decreases are suggestive of anaemia in the females and stress in males that experienced PCV increase within 7 days. At day 30, PCV decreased in both females and males which may be indicative of coping strategy in the females and males but a further and enduring coping strategy in the females. At the day 60, PCV increased in both females and males. These may be indicative of probably failure of the developed coping strategy. At day 90, PCV decreased in both the females and males. These may be indicative of a developed coping strategy. These observed frequent changes in coping strategy which is consequent upon changes in PCV values may affect immune responses and increase the susceptibility to infection and disease. Osman *et al*., (2009) reported that the exposure of Tilapia *Oreochromis niloticus to* 15

ppm cadmium significantly decreased the packed cell volume (PCV) in fish after 15 and 45 days of exposure to cadmium comparing with control group and Jimena *et al.*, (2005) reported that fish exposed to pollution presented significantly higher values of PCV, than those from an unpolluted area.

0.30mgPb/l treatment/exposure shows PCV decreased in the females and increased in the males at day 7. At day 7 the females may have undergone adaptation syndrome as reported by Ellis (1981) that fish under stressful conditions undergo a general adaptation syndrome. The adaptation syndrome may have manifested as decreased PCV. These decreases are suggestive of anaemia as a coping strategy in the female but physiological in the males that experienced PCV increase within 7 days which may be due to increase stress level. At day 30, PCV decreased in both the females and males which may be indicative coping strategy in the females and males but a further and more enduring coping strategy in the females. At day 60, PCV increased in both the females and males with the females. These may be indicative of failure of the developed coping strategy. At day 90, PCV decreased in both the females and males. These may be indicative of a developed coping strategy. These observed frequent changes in coping strategy which is consequent upon changes in PCV values may affect immune responses and increase the susceptibility to infection and disease. These findings at day 30 and 90 is in agreement with the findings of Gbore *et al*., (2006) who reported decreased in haematocrit values after stress in *Tilapia zilli* and *Clarias gariepinus.*

The fish that had no treatment/exposures (control) shows that the PCV increased non-significantly in the females and males at day 7. This may mean that at day 7 the females and males were not under any detrimental stressful conditions. At day 30, there was non-significant increase in PCV in the females and males. At day 60, PCV increased $P \geq 0.05$ in females and increased significantly ($p \leq 0.05$) in males. At day 90, PCV decreased in both the females and males. These may be indicative of probably failure of the developed coping strategy at day 60. These observed change at day 90 in coping strategy which is consequent upon decrease in PCV values may affect immune responses and increase the susceptibility to infection and disease.

At day 7 PCV increased significantly in females and males in A; Decreased ($p \leq 0.05$) in female and increased $P \geq 0.05$ in males in group 2; Increased significantly in females and males in group 3; increased $P \geq 0.05$ in females and males in control. The non-significant difference of the females in A, C, and the males in groups A, B with the control may mean that 0.03mgCd/l decreased significantly ($p \leq 0.05$) PCV in females whereas

0.3mgPb/l decreased significantly ($p \leq 0.05$) PCV in males. At day 30 PCV increased $P \geq 0.05$ in females and males in group A; decreased P≥005 in female and decreased significantly ($p \leq 0.05$) in males in B; Decreased significantly ($p \leq 0.05$) in females and males in group C; increased $P \geq 0.05$ in females and males in CTL. PCV in females and males increased significantly ($p \leq 0.05$) in A, declined significantly ($p \leq 0.05$) in B and C than CTL may mean that 0.03mgCd/l and 0.3mgPb/l decreased significantly ($p \leq 0.05$) PCV in females and males whereas 8.0g raw garlic/l increased PCV.

At day 60, PCV increased $P \geq 0.05$ in females and males in A; Increased significantly ($p \leq 0.05$) in female and male in B; increased $P \geq 0.05$ in females increased significantly ($p \leq 0.05$) in female and male in C and increased significantly ($p \leq 0.05$) in males in CTL. PCV in females and males increased significantly ($p \leq 0.05$) in B, C declined significantly ($p \leq 0.05$) in groups A and control may mean that 0.03mgCd/l and 0.3mgPb/l increased significantly ($p \leq 0.05$) PCV in females and males whereas 8.0g raw garlic/l decreased PCV. At day 90, PCV increased significantly ($p \leq 0.05$) in female and increased $P \geq 0.05$ in males in A; decreased significantly ($p \leq 0.05$) in female and male in B; decreased significantly ($p \leq 0.05$) in female and male in C; decreased significantly ($p \leq 0.05$) in females and males in control. PCV in males in A, C were higher significantly ($p \leq 0.05$) than control and B it thus appears 0.03mgCd/l had no effect on males PCV whereas 8.0g raw garlic/l and 0.3mgPb/l increased significantly ($p \leq 0.05$) in males. PCV in males in groups A, B and C were higher significantly ($p \leq 0.05$) than control, it thus appears 8.0g raw garlic/l, 0.03mgCd/l and 0.3mgPb/l increased significantly ($p \leq 0.05$) in males.

It appeared that females were the first to be affected negatively by stress factors while the negative effects in males occurred later. Garlic increased PCV in females and males except at day 30 and 90 of treatment/exposure, respectively.

Garlic provided a stable aquatic environment, increase and prolonged copping for stress by increasing PCV values with age. Lead, cadmium and control resulted in frequent changes (biphasic) in PCV which could be detrimental to the immune system in both females and males.

3.4.2.4: Result of effect of sex and treatment on Hb (g/dl)

Table 3.4: Effect of sex and treatment on Hb (g/dl) Means ± SEM of *Clarias gariepinus* days 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/exposure days	Group A		Group B		Group C		CTL (Control) Group	
	♀a^1/n	♂ b^1 /n	♀a^2 /n	♂b^2 /n	♀a^3 /n	♂b^3/n	♀a^4 /n	♂b^4 /n
0(**after acclimation)**	6.10 ± 0.60^{b}/3	6.40 ± 0.99^{ab}/3	6.55 ± 1.35^{ab}/2	6.33 ± 0.72^{ab}/3	8.90 ± 0.00^{a}/2	7.85 ± 0.05*/2	7.23±0.67^{ab}/3	6.43±0.27^{ab}/3
7	6.50 ± 0.74 ^{ab}/6	6.92 ± 0.58 ^{b}/6	5.40 ± 0.43^{bc}/6	7.47 ± 0.77*^{b}/6	7.67± 0.47 ^{a}/6	8.90 ± 0.58 ^{a}/6	4.72±0.17 ^{c}/6	9.03±0.13** ^{a}/6
30	4.62±0.26 ^{a}/6	6.42 ± 0.35* ^{a}/6	3.15 ± 0.21^{c}/6	4.42 ±0.90*^{b}/6	2.93 ±0.22 ^{c}/6	4.98 ± 0.25**^{b}/6	3.80±0.19^{b}/6	4.83±0.23*^{b}/6
60	7.43 ± 0.31 ^{c}/6	7.10 ± 0.40 ^{b}/6	11.75±0.17 ^{a}/6	10.75 ± 0.08 ^{a}/6	7.42 ± 0.16 ^{c}/6	10.90±0.14^{a}/6	9.70 ± 0.19 ^{b}/6	7.17 ± 0.21** ^{b}/6
90	2.32 ± 0.07 ^{b}/6	1.90 ± 0.07* ^{b}/6	2.35 ±0.09 ^{b}/6	1.82 ±0.12* ^{b}/6	3.17.±0.04^{a}/6	3.45 ±0.05*^{a} /6	1.53 ± 0.03 ^{c}/6	1.35 ± 0.08^{c}/6

^{a}fish *Clarias gariepinus* females. ^{b}fish *Clarias gariepinus* males. *Significantly different at P≤ 0.05 (Student t-test). ** Highly significantly different at P≤0.001 (Student t.test). Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil
Means of same sex with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n= number of sample.

Table 3.2 shows that at 75 day-old, the females had a higher value of 8.05 ± 0.78g/dl than the males with 7.25 ± 0.35g/dl.

Table 3.4 shows that after 14 days acclimation, in group A, the females had a lower non-significant value of 6.10 ± 0.60g/dl than the males with 6.40 ± 0.99g/dl; In group B the females had a higher non-significant value of 6.55± 1.35g/dl than the males with 6.33 ± 0.72g/dl; In group C the females had a higher significant (P≤ 0.05) value of 8.90 ± 0.00g/dl than the males with 7.85 ± 0.05g/dl; and in CTL group, the females had higher non-significant value of 7.23±0.67g/dl than the males with 6.43±0.27g/dl.

Table 3.4 shows that at day 7 of treatment/exposure, in group A treated with 8.0g/l raw garlic, the females had a lower non-significant value of 6.50 ± 0.74dg/dl than the males with 6.92 ± 0.58g/dl. In group B treated with 0.03g/dl cadmium, the females had a lower significant (P≤ 0.05) value of 5.40 ± 0.43g/dl than the males with 7.47 ± 0.77g/dl; In group C treated with 0.30mg/l lead, the females had a lower non-significant value of 7.67± 0.47dg/l than the males with 8.90 ± 0.58g/dl; In CTL group, with no treatment, the females had a lower highly significant (p≤0.001) value of 4.72±0.17g/dl than the males with 9.03±0.13g/dl.

Table 3.4 shows that at day 30 of treatment/exposure, in group A treated with 8.0g/l raw garlic, the females had a lower highly significant (p≤0.001) value of 4.62±0.26g/dl than the males with 6.42 ± 0.35g/dl; In group B treated with 0.03mg/l cadmium, the females had a lower significant (p≤0.05) value of 3.15 ± 0.21g/dl than the males with 4.42 ±0.90g/dl; In group C treated with 0.30mg/l lead, the females had a lower highly significant (p≤0.001) value of 2.93 ±0.22g/dl than the males with 4.98 ± 0.25g/dl; And in CTL group with no treatment, the females had a lower significant (p≤0.05) value of 3.80±0.19g/dl than the males with 4.83±0.23g/dl.

Table 3.4 shows that at day 60 of treatment/exposure, in group A treated with 8.0g/l raw garlic, the females had a higher non-significant value of 7.43 ± 0.31g/dl than the males with 7.10 ± 0.40g/dl; In group B treated with 0.03mg/l cadmium, the females had a higher significant (p≤0.05) value of 11.75±0.17g/dl than the males with 10.75 ± 0.08g/dl; In group C treated with 0.30mg/l lead, the females had a lower highly significant (p≤0.001) value of 7.30 ± 0.54g/dl than the males with 10.90±0.14g/dl; And in CTL group with no treatment, the females had a higher highly significant (p≤0.001) value of 9.70 ± 0.19g/dl than the males with 7.17 ± 0.21g/dl.

Table 3.4 shows that at day 90 (6 months old) of treatment/exposure, in group A treated with 8.0g/l raw garlic, the females had higher significant ($p \leq 0.05$) value of 2.32 ± 0.07g/dl than the males with 1.90 ± 0.07g/dl; In group B treated with 0.03mg/l cadmium, the females had a higher significant ($p \leq 0.05$) value of 2.35 ±0.09g/dl than the males with 1.82 ±0.12g/dl; In group C treated with 0.30mg/l lead, the females had a lower significant ($p \leq 0.05$) value of 3.17.±0.04g/dl than the males with 3.45 ±0.05g/dl; And in CTL group with no treatment, the females had a higher non-significant value of 1.53 ± 0.03g/dl than the males with 1.35 ± 0.08g/dl.

3.4.2.5: Discussions on result of effect of sex and treatment on Hb (g/dl)

At day 0 after acclimatisation, Hb decreased in female and male. The decrease showed no significant difference in all the groups. This decrease may be due to stress caused by the confinement and probably the Pb concentration in the well water used for the culture. Hb was higher in the female. This finding is in agreement that decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Grobler 1988; Wepener, 1990; Nussey, 1994). The observed increase in female and male in group C may be due to the lower number of fish sampled.

Fish treated with 8.0g raw garlic/l treatment, Hb changes were non- significant $P \geq 0.05$ at day 7 and 60 in females in male at day 7, 30 and 60. The non- significant change in both the females and males could be interpreted as physiological since 8.0g raw garlic/l may not be as potent as fish fed on diets containing 40g *Allium sativum* that significantly had higher Hb than in control (Shalaby *et al.*, 2006) more so when it has been documented by Wendelaar-Bonga, (1997) that increase in haemoglobin level is usually in fish subjected to stress. However, the significant ($p \leq 0.05$) decrease at day 30 in the female suggests that they could have been the observed "hangers" at the 2nd day of treatment due to increase in stress level. The stress could probably be due to the high level of manganese in garlic. However, at day 30 based on the coping ability of the female in an environment with the side effects reversed and at day 90 in females and males Hb decreased. The decreased Hb may be as a result of haemodilution to reduce the concentration of the garlic (probably manganese) that may be responsible for stress level observed as "hangers" at the 2nd of treatment and probably a decrease in the synthesis of haemoglobin. The findings are in agreement with studies by Cyriac *et al.*, (1989) that decreases in haemoglobin concentration resulted to haemodilution which is a mechanism that reduces the

concentration of the pollutants; Reddy and Bashanihideen, (1989) reported that significant decrease in the haemoglobin concentrations may also be due to a decrease in the rate of haemoglobin synthesis. Also the decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Grobler 1988; Wepener, 1990; Nussey, 1994). At day 60 Hb increased in both the females and males. This result suggests that the reduced activity experienced at day 30 may have resulted in reduce absorption and/or increased excretion of garlic based on adaptation syndrome in response to the stress level at day 7 may be detrimental. As a result, the fish may have undergone adaptation syndrome by increasing the absorption of garlic in culture water which consequently increased Hb concentration to improve in the oxygen transportation capacity of the blood in both the females and males. The findings are in agreement with studies by Shalaby *et al.*, (2006) that hemoglobin content in fish fed on diets containing 40g *Allium sativum* were significantly higher than in control; Saint – Paul, (1984) who suggested that the increase in Hb concentration could be a first indicator of an adaptational improvement in the oxygen transportation capacity of the blood. At day 90 Hb decreased in both the males and females. The decreased Hb at day 90 in both the females and males may be as a result of prolonged static culture which may have resulted in low dissolved oxygen (DO) and reduced activity as a coping strategy. The findings are in agreement with studies that decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Grobler 1988; Wepener, 1990; Nussey, 1994). These results at day 7, 30, 60 and 90 may mean that there is a cyclic adaptational syndrome and garlic provided a stabilized environment for both the females and males to respond the same way.

Fish treated with 0.03mgCd/l, at day 7 and 30, Hb decreased significantly ($P \leq 0.05$) in the females and increased $P \geq 0.05$ in the males. The findings which appear to be a prolonged decrease may be as a result of haemodilution to reduce the concentration of the cadmium and probably a decrease in the synthesis of haemoglobin. The findings are in agreement with studies by Cyriac *et al*., (1989) that decreases in haemoglobin concentration resulted to haemodilution which is a mechanism that reduces the concentration of the pollutants; Reddy and Bashanihideen, (1989) that significant decrease in the haemoglobin concentrations may also be due to a decrease in the rate of synthesis of haemoglobin. Also progressive reduction in haemoglobin content may also

be attributed to depression/exhaustion of haemopoietic potential of the fish (Sawhney and Johal, 2000). Buckley *et al*., (1976) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and could be ascribed as pathological conditions in fishes exposed to toxicants. The findings are in agreement with studies that decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Grobler 1988; Wepener, 1990; Nussey, 1994). At day 60, 0.03mgCd/l increased significantly ($P \leq 0.05$) Hb in both the females and males. These results suggest that the reduced activity experienced at day 7 and 30 which may be probably due to reduced absorption and/or increased excretion of cadmium based on adaptation syndrome may have become overwhelmed by the chemical cue from cadmium resulting in increased absorption of cadmium in culture water which consequently increased Hb concentration to improve in the oxygen transportation capacity of the blood in both the females and males. Saint – Paul, (1984) suggested that the increase in Hb concentration could be a first indicator of an adaptational improvement in the oxygen transportation capacity of the blood. At day 90, 0.03mgCd/l decreased Hb in both the females and the males. This may be an adaptation syndrome of haemodilution to reduce the concentration of the cadmium and probably a decrease in the synthesis of haemoglobin. The findings are in agreement with studies by Cyriac *et al*., (1989) that decreases in haemoglobin concentration resulted to haemodilution which is a mechanism that reduces the concentration of the pollutants; Reddy and Bashanihideen, (1989) that significant decrease in the haemoglobin concentrations may also be due to a decrease in the rate of haemoglobin synthesis; The progressive reduction in haemoglobin content may also be attributed to depression/exhaustion of haemopoietic potential of the fish (Sawhney and Johal, 2000); Buckley *et al*., (1976) that prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia could be ascribed as pathological conditions in fishes exposed to toxicants.

Fish treated with 0.30mgPb/l at day 7, Hb decreased significantly ($P \leq 0.05$) in the females and increased $P \geq 0.05$ Hb in the males. The decrease in the haemoglobin concentrations in the females may be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis as stated by Reddy and Bashanihideen, (1989). The decrease in the females is in agreement with Annune and Ahuma, (1998) that haemoglobin decreased following exposure of *C. gariepinus* to sublethal concentrations of copper and lead. The increased $P \geq 0.05$ Hb in

the males may be due to an adaptational improvement in the oxygen transportation capacity of the blood as reported by Saint – Paul, (1984). These differences in response within the same treatment may due to trait and competition for feed and feed resources, probably may be responsible for the findings of the decrease of Hb in the females coupled with a lower value in the females than the males. The females may be more conscious and less aggressive as a coping strategy than the males and also may mean that the females being more conscious of the treatment had a reduced activity. The findings are in agreement with studies that shows decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Grobler 1988; Wepener, 1990; Nussey, 1994). At day 30, 0.30mgPb/l decreased significantly ($P \leq 0.05$) Hb in both the females and the males. The decrease in the females is in agreement with Annune and Ahuma (1998) that haemoglobin decreased following exposure of *C. gariepinus* to sublethal concentrations of copper and lead. The lower highly significant ($p \leq 0.001$) values in the females than the males may mean that females are reactive while the males proactive. At day 60, 0.30mgPb/l increased significantly ($P \leq 0.05$) Hb in both the females and males. These results show a uniform response which may suggest that with increase duration of culture with no culture water change, both the females and males responses may be the same (increase or decrease). These results also suggest that the reduced activity experienced at day 30 may be detrimental on the fish resulting in adaptation syndrome which consequently increased Hb concentration to improve the oxygen transportation capacity of the blood in both the females and males in agreement with Saint – Paul, (1984) who suggested that the increase in Hb concentration could be a first indicator of an adaptational improvement in the oxygen transportation capacity of the blood. The increase is not in agreement with Annune and Ahuma (1998) that haemoglobin decreased following exposure of *C. gariepinus* to sublethal concentrations of copper and lead. This may mean that the effect of sublethal concentrations of lead on Hb in *C. gariepinus* may depend on the duration of exposure. The lower highly significant ($p \leq 0.001$) values in the females than the males may mean that females are reactive while the males proactive which may have a reproductive process implication. At day 90, 0.30mgPb/l decreased significantly ($P \leq 0.05$) Hb in both the females and the males. This result shows a uniform response which is in agreement with the findings of uniform response at day 60 that suggest that with increase duration of culture with no culture water change, both the females and males responses may be the same (increase or

decrease). The decrease in the haemoglobin concentrations in the females and males may be due to collapse of adaptational improvement in the oxygen transportation capacity of the blood at day 60 due probably to increased stress in the static culture that may have resulted to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis as stated by Reddy and Bashanihideen, (1989). The lower significant ($p \leq 0.05$) values in the females than the males may mean that females are still reactive while the males proactive which may have a reproductive process implication. It therefore appears both the females and males respond to stress induced by lead by lower Hb in the females while the response of the males may be an increase or decrease depending on time of exposure. Also females Hb concentration are more negatively affected than the males.

Group without treatment (control), at day 7 Hb decreased significantly ($P \leq 0.05$) in the females and increased significantly ($P \leq 0.05$) Hb in the males. The decrease in the haemoglobin concentrations in the females may also be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis as stated by Reddy and Bashanihideen, (1989). The increased Hb in the males may be due to an adaptational improvement in the oxygen transportation capacity of the blood as reported by Saint – Paul, (1984). These differences in response within the same treatment may due to trait and competition for feed and feed resources resulting in decrease of Hb in the females coupled with a lower highly significant ($p \leq 0.001$) value in the females than the males. The females may be more conscious and less aggressive as a coping strategy than the males and also may mean that the females been more conscious of the treatment had a reduced activity. The findings are in agreement with studies that decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Grobler 1988; Wepener, 1990; Nussey, 1994). At day 30 Hb decreased significantly ($P \leq 0.05$) in both the females and the males. These results show a uniform response. The decrease in the haemoglobin concentrations in the females and males may be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis as stated by Reddy and Bashanihideen, (1989). The females may be more conscious and less aggressive in the fresh culture water as a coping strategy than the males and also may mean that the females been more conscious of the treatment had a reduced activity. The findings are in agreement with studies that decreases in haemoglobin concentration signifies that the fish's ability to

provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Grobler 1988; Wepener, 1990; Nussey, 1994). At day 60 Hb increased significantly ($P \leq 0.05$) in both the females and males. These result shows a uniform response which may suggest that with increase duration of culture with no culture water change, both the females and males responses may be the same (increase or decrease). The result also suggests that the reduced activity experienced at day 30 may be detrimental on the fish resulting in adaptation syndrome which consequently increased Hb concentration to improve the oxygen transportation capacity of the blood in both the females and males in agreement with Saint – Paul, (1984) who suggested that the increase in Hb concentration could be a first indicator of an adaptational improvement in the oxygen transportation capacity of the blood. The higher highly significant ($p \leq 0.001$) values in the females than the males may mean that females are proactive while the males reactive. This may be a pointer that the males respond late to stress than females in a macro environment. At day 90, there were decreased significantly ($P \leq 0.05$) Hb in both the females and the males. These results show a uniform response which is in agreement with the findings of uniform response at day 60 that suggest that with increase duration of culture with probably no sex related induced toxicant both the females and males' responses may be the same (increase or decrease in Hb). The decrease in the haemoglobin concentrations in the females and males may be due collapse of adaptational improvement in the oxygen transportation capacity of the blood at day 60 due probably to increased stress in the static culture that may have resulted to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis as stated by Reddy and Bashanihideen, (1989).

3.4.2.6: Result of effect of sex and treatment on RBCcellsx10^{12}/l

Table 3.5: Effect of sex and treatment on RBCcellsx10^{12}/l Means ± SEM of *Clarias gariepinus* days 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/exposure days	Group A		Group B		Group C		CTL (Control) Group	
	♀a^1/n	♂ b^1 /n	♀a^2 /n	♂b^2 /n	♀a^3 /n	♂b^3/n	♀a^4 /n	♂b^4 /n
0(**after acclimation)**	7.49 ± 0.92^{a}/3	7.96 ± 0.93^{a} /3	7.75 ± 0.89^{a}/2	7.96 ± 0.58^{a} /3	9.43 ± 0.01^{a}/2	8.33 ± 0.26^{a}/2	7.89 ± 0.40^{a}/3	8.23 ± 0.19^{a} /3
7	7.33 ± 0.50 ^{ab}/6	7.20 ± 0.61^{c}/6	5.99 ± 0.37 ^{c}/6	8.41 ± 0.75*^{bc} /6	7.70 ± 0.39^{a}/6	10.30± 0.36* ^{a}/6	5.71 ± 0.17 ^{c}/6	9.46±0.14**^{ab}/6
30	5.37 ± 0.24 ^{b}/6	8.26 ± 0.30**^{a}/6	4.90 ± 0.21 ^{ab}/6	5.83± 0.20*^{c} /6	4.57 ± 0.33^{b}/6	6.12 ± 0.19*^{bc}/6	5.47 ± 0.27 ^{a}/6	6.77±0.26*^{b}/6
60	8.18 ± 0.16 ^{d}/6	8.73 ± 0.44^{b}/6	12.63±0.21^{ab}/6	11.42 ± 0.15*^{a} /6	13.20 ± 0.28^{a} /6	11.88 ± 0.19*^{a}/6	10.13 ± 0.32 ^{c}/6	8.08 ± 0.23**^{b}/6
90	10.05 ± 0.28$^{a/}$6	8.92 ± 1.19**^{b}/6	8.78 ± 0.36 ^{b}/6	7.25 ± 0.20*^{c}/6	10.45±0.20 ^{a}/6	11.32 ±0.16*^{a}/6	6.25 ± 0.18 ^{c}/6	7.03 ± 0.21*^{c}/6

^{a}fish *Clarias gariepinus* females. ^{b}fish *Clarias gariepinus* males. *Significantly different at P≤ 0.05 (Student t-test). ** Highly significantly different at P≤0.001 (Student t.test). Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil

Means of same sex with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n= number of sample.

Table 3.2 shows that at 75 day-old, the females had a higher value of 4.02 ± 0.30cellsX10^{12}/l than the males with 2.68 ± 0.18cellsX10^{12}/l.

Table 3.5 shows that after 14 days acclimation, in group A, the females had a lower non-significant value of 7.49±0.92cellsX10^{12} than the males with 7.96 ± 0.93cellsX10^{12}/l; In group B, the females had a lower non-significant value of 7.75 ± 0.89cellsX10^{12}/l than the males with 7.96 ± 0.58cellsX10^{12}/l; In group C, the females had a higher value of 9.43 ± 0.01 cellsX10^{12}/l than the males with 8.33 ± 0.26cellsX10^{12}/l; In CTL group, the females had a lower non-significant value of 7.89 ± 0.40cellsX10^{12}/l than the males with 8.23 ± 0.19cellsX10^{12}/l.

Table 3.5 shows that at day 7 of treatment/exposure, in group A treated with 8.0g/l raw garlic, the females had a higher non-significant value of 7.33 ± 0.50 cellsX10^{12}/l than the males with 7.20 ± 0.61 cellsX10^{12}/l; In group B treated with 0.03mg/l cadmium, the females had a lower significant ($p \leq 0.05$) value of 5.99 ± 0.37 cellsX10^{12}/l than the males with 8.41 ± 0.75cellsX10^{12}/l; In group C treated with 0.3mg/l of lead, the females had a lower significant ($p \leq 0.05$) value of 7.70 ± 0.39 cellsX10^{12}/l than the males with 10.30± 0.36cellsX10^{12}/l; in CTL group with no treatment, the females had a lower highly significant ($p \leq 0.001$) value of 5.71 ± 0.17 cellsX10^{12}/l than the males with 9.46±0.14cellsX10^{12}/l.

Table 3.5 Shows that at day 30 of treatment/exposure, in group 1 treated with 8.0g/l raw garlic, the females had a lower highly significant ($p \leq 0.001$) value of 5.37 ± 0.24 cellsX10^{12}/l than the males with 8.26 ± 0.30cellsX10^{12}/l; In group B treated with 0.03mg/l cadmium, the females had a lower significant($p \leq 0.05$) value of 4.90 ± 0.21 cellsX10^{12}/l than the males with 5.83± 0.20cellsX10^{12}/l; In group C treated with 0.30mg/l lead, the females had a lower significant ($p \leq 0.05$) value of 4.57 ± 0.33cellsX10^{12}/l than the males with 6.12 ± 0.19cellsX10^{12}/l; In CTL group with no treatment, the females had lower significant ($p \leq 0.05$) value of 5.47 ± 0.27 cellsX10^{12}/l than the males with 6.77±0.26cellsX10^{12}/l.

Table 3.5 shows that at day 60 of treatment/exposure, In group A treated with 8.0g/l raw garlic, the females had a lower significant value of 8.18 ± 0.16 cellsX10^{12}/l than the males with 8.73 ± 0.44 cellsX10^{12}/l; In group B treated with 0.03mg/l cadmium the females had a higher significant ($p \leq 0.05$) value of 12.63±0.21cellsX10^{12}/l than the males with 11.42 ± 0.15cellsX10^{12}/l; In group C treated with 0.30mg/l lead, the females had a higher significant ($p \leq 0.05$) value of 13.20 ± 0.28cellsX10^{12}/l than the males with 11.88 ±

0.19cellsX10^{12}/l; In CTL group with no treatment, the females had a higher highly significant ($p \leq 0.001$) value of 10.13 ± 0.32 cellsX10^{12}/l than the males with 8.08 ± 0.23cellsX10^{12}/l..

Table 3.5 shows that at day 90 of treatment/exposure, in group A treated with 8.0g/l raw garlic, the females had a higher highly significant ($p \leq 0.001$) value of 10.05 ± 0.28 cellsX10^{12}/l than the males with 8.92 ± 1.19cellsX10^{12}/l; In group B treated with 0.03mg/l cadmium, the females had a higher significant ($p \leq 0.05$) value of 8.78 ± 0.36 cellsX10^{12}/l than the males with 7.25 ± 0.20cellsX10^{12}/l; In group C treated with 0.30mg/l cadmium, the females had a lower significant ($p \leq 0.05$) value of 10.45±0.20 cellsX10^{12}/l than the males with 11.32 ±0.16cellsX10^{12}/l; In CTL group with no treatment, the females had a lower significant ($p \leq 0.05$) value of 6.25 ± 0.18 cellsX10^{12}/l than the males with 7.03 ± 0.21cellsX10^{12}/l.

3.4.2.7: Discussions on effect of sex and treatment on RBC cells x10^{12}/l

At day 0 after acclimatisation, RBC increased in female and male. The increased showed no significant difference in all the groups. This decrease may be due to stress caused by the confinement and probably the Pb concentration in the well water used for the culture. RBC was higher in the female. This finding is in agreement that an increase in RBC count is associated with fish subjected to low oxygen tension (Murad *et al.,* 1993).

Fish treated with 8.0g raw garlic/l, females had at day 7 a non-significant decrease; at day 30 a significant decrease; at day 60 significant increase and at day 90 significant increase. The males had at day 7 a non-significant decrease; at day 30 a non-significant increase; at day 60 a non-significant increase and at day 90 a significant decrease. These results may mean that 8.0mg/l significantly increased RBC in the females but in the males had no significant effect at day 30 and 60 but significantly decreased RBC after a prolonged exposure at day 90. These results may mean that 8.0g raw garlic /l provided an environment that supports the physiological development of both the females and the males after day 30.

Fish treated with 0.03mgCd/l, females had at day 7 a significant decrease, at day 30 a non-significant decrease at day 60 a significant increase and at day 90 a significant decrease. The males had at day 7 a non-significant decrease at day 30 a non-significant decrease at day 60 a significant increase and at day 90 a significant decrease. RBC results show that 0.03mg/l cadmium during a short duration exposure decreased significantly ($p \leq 0.05$) in the females and caused a non-significant decrease in the males.

However, with increased exposure time there was a further non-significant decrease in both the females and the males. With further exposure as seen at day 60 both the females and the males had a significant increase but subsequently had a significant decrease at day 90. These suggest that 0.03mg/l cadmium increase of RBC is temporal and after a prolong exposure in both females and the males.

Fish treated with 0.30mg/l lead, females had at day 7 a significant decrease; at day 30 a significant decrease; at day 60 a significant increase; at day 90 significant decrease. The males had at day 7 significant increase; at day 30, a significant decrease, at day 60, a significant increase and at day 90, a non- significant decrease. These results show that the males had more bout of increases than the females. These may suggest that the females are more negatively affected 0.30mg/l lead.

Fish with no treatment (control), females had at day 7 a significant decrease, at day 30 a non-significant decrease, at day 60 a significant increase and at day 90 a significant decrease. The males had at day 7 a significant increase, at day 30 a significant decrease, at day 60 a significant increase and at day 90 a significant decrease. These results show that at day 7 after the 14-day acclimation, the females and males had significant decrease and increase respectively. At day 30 females had a non-significant decrease and the males a significant decrease. At day 60, both females and males may have undergone adaptational syndrome with a significant ($p \leq 0.05$) increase in RBC count. This adaptation syndrome failed at day 90 of the experiment with a significant decrease in RBC count.

3.4.2.8: Results of effect of sex and treatment on WBC cellx10^9/l

Table 3.6: Effect of sex and treatment on WBC cellx10^9/l Means ± SEM of *Clarias gariepinus* day 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/exposure days	Group A		Group B		Group C		CTL (Control) Group	
	♀$a^1{}_{/n}$	♂ $b^1{}_{/n}$	♀$a^2{}_{/n}$	♂$b^2{}_{/n}$	♀$a^3{}_{/n}$	♂$b^3{}_{/n}$	♀$a^4{}_{/n}$	♂$b^4{}_{/n}$
0(after acclimation)	6.53 ± 0.27 $^{a}{}_{/3}$	6.13±0.53$^{ab}{}_{/3}$	8.60±0.40$^{a}{}_{/2}$	8.53±0.99$^{a}{}_{/3}$	7.90± 2.50$^{a}{}_{/2}$	8.05 ± 0.05$^{a}{}_{/2}$	7.00±0.57$^{a}{}_{/3}$	7.00± 0.40$^{ab}{}_{/3}$
7	7.50±0.43$^{a}{}_{/6}$	7.83±0.41$^{a}{}_{/6}$	5.50 ± 0.43$^{b}{}_{/6}$	7.95 ± 1.20 $^{a}{}_{/6}$	8.10 ± 0.62$^{a}{}_{/6}$	9.20 ± 0.58 $^{a}{}_{/6}$	4.50±0.43$^{b}{}_{/6}$	10.10± 0.37** $^{a}{}_{/6}$
30	2.85 ± 0.21$^{ab}{}_{/6}$	7.00 ± 0.31**$^{a}{}_{/6}$	3.52 ±0.14$^{a}{}_{/6}$	5.23 ± 0.35*$^{b}{}_{/6}$	2.60 ± 0.31$^{b}{}_{/6}$	4.87 ± 0.19** $^{b}{}_{/6}$	3.50±0.34$^{a}{}_{/6}$	4.53± 0.31$^{b}{}_{/6}$
60	7.63 ± 0.17 $^{d}{}_{/6}$	7.53 ± 0.44$^{c}{}_{/6}$	11.85±0.29 $^{b}{}_{/6}$	10.63 ± 0.19* $^{b}{}_{/6}$	21.27 ± 0.40 $^{a}{}_{/6}$	12.03±0.24** $^{a}{}_{/6}$	8.95 ± 0.18$^{c}{}_{/6}$	7.13 ± 0.19**$^{c}{}_{/6}$
90	8.40 ± 0.30 $^{b}{}_{/6}$	6.45 ± 0.13*$^{a}{}_{/6}$	6.25 ± 0.54$^{c}{}_{/6}$	6.33 ± 0.23 $^{a}{}_{/6}$	9.82 ± 0.17$^{a}{}_{/6}$	5.05 ±0.22**$^{b}{}_{/6}$	6.40 ± 0.23 $^{c}{}_{/6}$	7.10 ± 0.44$^{a}{}_{/6}$

[a]fish *Clarias gariepinus* females. [b]fish *Clarias gariepinus* males. *Significantly different at P≤ 0.05 (Student t-test). ** Highly significantly different at P≤0.001 (Student t.test). Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil
Means of same sex with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n= number of sample.

Table 3.2 shows that at 75 day-old females had a lower value of 5.08 ± 0.25 cellsX10^9/l than males with 5.65 ± 0.10cellsX10^9/l

Table 3.6 shows that at 0 days in group A treated with 8.0g/l raw garlic the females had a higher non-significant value of 6.53 ± 0.27 cellsX10^9/l than the males with 6.13±0.53cellsX10^9/l; In group B treated with 0.03mg/l cadmium the females had a higher non-significant value of 8.60±0.40cellsX10^9/l than the males with 8.53±0.99cellsX10^9/l; In group C treated with 0.30mg/l lead, the females had a lower value of 7.90± 2.50cellsX10^9/l than the males with 8.05 ± 0.05cellsX10^9/l; In CTL group with no treatment the females and males had similar values of 7.00±0.57 cellsX10^9/l and 7.00± 0.40 cellsX10^9/l respectively.

Table 3.6 shows that at 7 days in group A treated with 8.0g/l raw garlic the females had a lower non-significant value of 7.50 ± 0.43cellsX10^9/l than the males with 7.83±0.41cellsX10^9/l; In group B treated with 0.03mg/l cadmium, the females had a lower non-significant value of 5.50 ± 0.43 cellsX10^9/l than the males with 7.95 ± 1.20 cellsX10^9/l; In group C treated with 0.30mg/l lead, the females had a lower non-significant value of 8.10 ± 0.62 cellsX10^9/l than the males with 9.20 ± 0.58 cellsX10^9/l; In CTL group with no treatment the females lower highly significant ($p \leq 0.001$) values of 4.50±0.43 cellsX10^9/l than males with 10.10± 0.37 cellsX10^9/l.

Table 3.6 shows that at day 30 of treatment/exposure, in group A treated with 8.0g/l raw garlic, the females had a lower highly significant ($p \leq 0.001$) value of 2.85 ± 0.21cellsX10^9/l than the males with 7.00 ± 0.31cellsX10^9/l; In group B treated with 0.03mg/l cadmium, the females had a lower significant ($p \leq 0.05$) value of 3.52 ±0.14cellsX10^9/l than the males with 5.23 ± 0.35cellsX10^9/l; In group C treated with 0.3mg/l lead, the females had a lower highly significant ($p \leq 0.001$) value of 2.60 ± 0.31cellsX10^9/l than the males with 4.87 ± 0.19cellsX10^9/l; In CTL group with no treatment the females had a lower non-significant value of 3.50±0.34cellsX10^9/l than the males with 4.53± 0.31cellsX10^9/l.

Table 3.6 shows that at day 60 of treatment/exposure, in group A treated with 8.0g/l raw garlic, the females had a higher non-significant value 7.63 ± 0.17cellsX10^9/l than the males with 7.53 ± 0.44cellsX10^9/l; In group B treated with 0.03mg/l the females had a higher significant ($p \leq 0.05$) value of 11.85±0.29cellsX10^9/l than the males with 10.63 ± 0.19cellsX10^9/l; In group C treated with 0.3mg/l lead, the females had a higher highly significant ($p \leq 0.001$) value of 21.27 ± 0.40 cellsX10^9/l than the males with

12.03±0.24cellsX10^9/l; In CTL group with no treatment, the females had a higher highly significant ($p \leq 0.001$) value of 8.95 ± 0.18 cellsX10^9/l than the males with 7.13 ± 0.19cellsX10^9/l.

Table 3.6 shows that at day 90 of treatment/exposure, in group A treated with 8.0g raw garlic/l, the females had a higher significant ($p \leq 0.05$) value of 8.40 ± 0.30 cellsX10^9/l than the males with 6.45 ± 0.13cellsX10^9/l; In group B treated with 0.03mg/l cadmium the females had a lower non-significant value of 6.25 ± 0.54 cellsX10^9/l than the males with 6.33 ± 0.23 cellsX10^9/l; In group C 3 treated with 0.30mg/l lead, the females had a higher highly significant value of 9.82 ± 0.17 cellsX10^9/l than the males with 5.05 ±0.22cellsX10^9/l; In CTL group with no treatment, the females had a non-significant lower value of 6.40 ± 0.23 cellsX10^9/l than the males with 7.10 ± 0.44 cellsX10^9/l.

3.4.2.9: Discussions on effect of sex and treatment on WBC cellx10^9/l

At day 0 after acclimatisation, WBC increased in female and male. The increase showed no significant difference in all the groups. This increase may be due to stress caused by the confinement and probably the Pb concentration in the well water used for the culture. WBC was higher in the female. This may be to increase the innate immune response. This is in agreement with This is in agreement with the findings of Wendelaar-Bonga (1997). that postulated that in disease diagnostics, a higher WBC count is usually positively correlated to the immune system stimulation for defense against diseases and environmental stressors.

Fish treated/exposed to 8.0g/l garlic, females at day 7 had a significant increase, at day 30 a non-significant decrease at day 60 a significant increase and at 90 non-significant increases. The males had at day 7 a significant increase, at day 30 a non-significant decrease, at day 60 a non-significant increase and at day 90 a non-significant decrease. These results show that both the females and males had a significant increase at day 7 and subsequently at day 30, 60 and 90 no significant changes in the WBC counts. These may mean that 8.0g raw garlic/l effect on WBC was an initial significant increase and stabilized the WBC count with prolong exposure in both the females and the males. Fish treated with 0.03mg/l cadmium, females had at 7 a significant decrease, at day 30 a significant decrease, at day 60 a significant increase and at day 90 a significant decrease. The males had at day 7 a non-significant decrease at day 30 a significant decrease, at day 60 a significant increase and at day 90 a significant decrease. These results show that 0.03 mg/l cadmium enhances a significant increase in both the females and the males at

day 60. It may mean that cadmium practically decreased WBC count in both females and males.

Fish treated exposed to 0.3mg/l lead, females had at day 7 non-significant increase, at day 30 a significant decrease, at day 60 a significant increase and at day 90 a significant decrease. The males had at day 7 non-significant increase; at day 30 significant decrease; at day 60 significant increase; at day 90 a significant decrease. These results show that both the females and males had biphasic uniform responses. The females had significant ($p \leq 0.05$) increase at day 60.

Fish with no treatment, females had at day 7 a significant decrease, at day 30 a non-significant decrease, at day 60 a significant increase and at day 90 a significant decrease. The males had at day 7 a significant increase, at day 30 a significant decrease, at day 60 a significant increase and at day 90 a non-significant decrease. These results shows that at day 7 after the 14-day acclimation, the females had a non-significant decrease while the males had a significant increase while at day 30 the females fish had a non-significant decrease and the males had a significant decrease. It may mean that males responded to an acute water change with a significant decrease in WBC count. Both the females and males may have undergone adaptational syndrome and with significant ($p \leq 0.05$) WBC count increase this adaptational syndrome failed at day 90 of the experiment. These results may suggest that the culture environment practically decreased WBC count.

3.4.3: Blood chemistry Parameters

3.4.3.1: Results of Effect of sex and treatment on Total protein (g/l)

Table 3.7: Effect of sex and treatment on Total protein (g/l) Means ± SEM of *Clarias gariepinus* day's 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/exposure days	Group A		Group B		Group C		CTL (Control) Group	
	♂$a^{1}{}_{/n}$	♀$b^{1}{}_{/n}$	♂$a^{2}{}_{/n}$	♀$b^{2}{}_{/n}$	♂$a^{3}{}_{/n}$	♀$b^{3}{}_{/n}$	♂$a^{4}{}_{/n}$	♀$b^{4}{}_{/n}$
0 (after acclimation)	3.33 ±2.73$^{a}{}_{/3}$	3.46 ±0.07$^{a}{}_{/3}$	3.33 ± 0.12$^{a/}{}_{3}$	3.50±0.50$^{a}{}_{/2}$	3.05±0.05$^{a}{}_{/2}$	4.00 ±0.40$^{a}{}_{/2}$	3.17±0.09$^{a}{}_{/3}$	3.53±0.38$^{a}{}_{/3}$
7	3.50±0.37 $^{a}{}_{/6}$	3.60 ±0.41 $^{a}{}_{/6}$	3.45 ±0.37 $^{a}{}_{/6}$	3.75 ±0.36 $^{a}{}_{/6}$	3.75 ±0.37 $^{a}{}_{/6}$	3.30 ±0.37 $^{a}{}_{/6}$	3.60 ± 0.45 $^{a}{}_{/6}$	3.80 ±0.38 $^{a}{}_{/6}$
30	3.00 ±0.03$^{c}{}_{/6}$	2.18±0.03$^{**\ d}{}_{/6}$	4.17±0.04 $^{b}{}_{/6}$	3.22 ±0.03$^{**\ b}{}_{/6}$	4.85 ±0.07 $^{a}{}_{/6}$	2.87 ± 0.10$^{**\ c}{}_{/6}$	4.93 ± 0.04 $^{a}{}_{/6}$	3.93±0.10$^{**\ a}{}_{/6}$
60	4.02 ±0.05 $^{b}{}_{/6}$	3.23 ±0.04$^{**\ d}{}_{/6}$	4.22 ±0.05 $^{a}{}_{/6}$	4.53 ±0.04$^{*\ a}{}_{/6}$	3.47 ±0.03 $^{c}{}_{/6}$	4.02 ±0.15$^{*\ b}{}_{/6}$	3.13 ±0.04 $^{d}{}_{/6}$	3.52 ±0.03$^{**\ c}{}_{/6}$
90	4.13 ±0.04 $^{bc}{}_{/6}$	3.83 ±0.04$^{*\ b}{}_{/6}$	5.22 ± 0.05 $^{a}{}_{/6}$	4.03 ±0.04$^{**\ a}{}_{/6}$	3.68 ±0.05 $^{c}{}_{/6}$	3.23 ±0.05$^{**\ c}{}_{/6}$	4.28 ± 0.05 $^{b}{}_{/6}$	3.03±0.05$^{**\ d}{}_{/6}$

[a]fish *Clarias gariepinus* females. [b]fish *Clarias gariepinus* males. *Significantly different at P≤ 0.05 (Student t-test). ** Highly significantly different at P≤0.001 (Student t.test). Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil

Means of same sex with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n= number of sample.

At 75 day-old the females had a higher non-significant value of 3.37±0.05g/l than the males with 3.270 ± 0.09g/l. (Table 3.2).

Table 3.7 shows at day 7, in group A, the females had a higher non-significant value of 3.60 ±0.4g/l than the males with 3.50±0.37 g/l; In group B the females had a higher non-significant value of 3.75 ±0.36g/l than the males with 3.45 ±0.37g/l; In group C the females had a lower non-significant value of 3.30 ±0.37 g/l than the males with 3.75 ±0.37 g/l; In CTL group, the females had a higher non-significant value of 3.80 ±0.38g/l than the males with 3.60 ± 0.45g/l.

Table 3.7 shows at day 30, in group A the females had a lower highly significant ($p \leq 0.001$) value of 2.18±0.03g/l than the males with 3.00 ±0.03g/l; In group B the females had a lower highly significant ($p \leq 0.001$) value of 3.22 ±0.03g/l than the males with 4.17±0.04g/l; In group C the females had a lower highly significant ($p \leq 0.001$) value of 2.87 ± 0.10g/l than the males with 4.85 ±0.07g/l; In CTL group, the females had a lower highly significant ($p \leq 0.001$) value of 3.93 ±0.10g/l than the males with 4.93 ± 0.04g/l.

Table 3.7 shows at day 60 in group A the females had a lower highly significant ($p \leq 0.001$) value of 3.23 ±0.04g/l than the males with 4.02 ±0.05g/l; In group B the females had a higher significant ($p \leq 0.05$) value of 4.53 ±0.04g/l than the males with 4.22 ±0.05g/l; In group C the females had a higher significant ($p \leq 0.05$) value of 4.02 ±0.15g/l than the males with 3.47 ±0.03g/l; In CTL group, the females had a higher highly significant ($p \leq 0.001$) value of 3.52 ±0.03g/l than the males with 3.13 ±0.04 g/l.

Table 3.7 shows at day 90 In group A the females had a lower significant ($p \leq 0.05$) value of 3.83 ±0.04g/l than the males with 4.13 ±0.04g/l; In group B the females had a lower highly significant ($p \leq 0.001$) value of 4.03 ±0.04g/l than the males with 5.22 ± 0.05g/l; In group C, the females had a lower highly significant ($p \leq 0.001$) value of 3.23 ±0.05g/l than the males with 3.68 ±0.05g/l; In CTL group, the females had a lower highly significant ($p \leq 0.001$) value of 3.03±0.10g/l than the males with 4.28±0.13g/l.

3.4.3.2: Discussions on effect of sex and treatment on total protein (g/l)

At day 0 after acclimatisation, total protein increased in female and male. The increased showed no significant difference in all the groups. This increased may be due to stress caused by the confinement and probably the Pb concentration in the well water used for the culture. total protein was higher in the female. This finding is in agreement that

osmoregulatory dysfunction, hemodilution, or tissue damage in surrounding blood vessels have been reported to be indicative of high serum protein levels (Hille, 1982).
Fish treated with 8.0g raw garlic/l, the females had at day 7 a non-significant increase, at day 30 a significant decrease, at day 60 a significant increase and at day 90 a non-significant increase in total protein levels. These results may mean that 8.0g raw garlic/l, decreased significantly protein at day 30 unlike the males with a non-significant decrease. These may mean that the males had a more stable protein synthesis and catabolism. However, with increase in exposure at day 60, protein level increased in both the females and the males, and increased non-significantly at day 90 probably due to stabilizing effect of garlic.

Fish treated/exposed to 0.03mgCd/l, at day 7 there was a non-significant increase in both the females and males, at day 30 there was a significant decrease and increase in females and males respectively, at day 60, there was a significant increase in the females and a non-significant increase in the males, and at day 90 there was a non-significant decrease in the females and a significant increase in the males. These results showed that both the females and the males with increased exposure time had a non-uniform biphasic response probably due to an unstable environment which may have elicited different coping strategy in both the females and the males which was not enduring.

Fish treated/exposed to 0.30mgPb/l, the females had at day 7 a non-significant decrease; at day 30 a non-significant decrease; at day 60 a significant increase; at day 90 a non-significant increase. The males had at day 7, an increase; at day 30 an increase; at day 60 a decrease; at day 90 an increase. These results may suggest that 0.30mgPb/l once it disrupted the coping strategy of the females the females could not recover after a prolong exposure while in the males the coping strategy was developed much later at day 60 which was not sustained at day 90.

Fish with no treatment (control), at day 7 there was a non-significant increase in both the females and the males. At day 30 there was a non-significant increase in the females and a significant increase in the males at day 60 and 90. The females and males had a non-significant and significant ($p \leq 0.05$) decrease and increase respectively. These results may suggest that during a short period of exposure, to stress, both the females and males had a non-significant decrease in protein levels. However, with duration of exposure, the females responded by having a non-significant decrease and the males a significant

increase. The males had at day 30 a significant increase, this may mean that the males responded to stress earlier than the females.

3.4.3.3: Results of effect of sex and treatment on Potassium (K^+) meg/l

Table 3.8: Effect of sex and treatment on Potassium (K^+) meg/l Means ± SEM of *Clarias gariepinus* days 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/exposure days	Group A		Group B		Group C		CTL (Control) Group	
	♂$a^1{}_{/n}$	♀$b^1{}_{/n}$	♂$a^2{}_{/n}$	♀$b^2{}_{/n}$	♂$a^3{}_{/n}$	♀$b^3{}_{/n}$	♂$a^4{}_{/n}$	♀$b^4{}_{/n}$
0(after acclimation)	25.67 ± 1.87$^{a}{}_{/3}$	27.00±1.00$^{a}{}_{/3}$	22.33±3.67$^{a}{}_{/3}$	25.00 ±5.00 $^{a}{}_{/2}$	24.50 ±0.50$^{a}{}_{/2}$	33.00 ±5.00$^{a}{}_{/2}$	25.00 ±0.58$^{a}{}_{/3}$	27.67 ±2.19$^{a}{}_{/3}$
7	29.00 ±0.58$^{ab}{}_{/6}$	28.00±1.83$^{ab}{}_{/6}$	24.00±0.9$^{b/}{}_{6}$	26.50 ±0.76 $^{b}{}_{/6}$	27.50 ±2.88$^{ab}{}_{/6}$	24.50 ±1.18 $^{b}{}_{/6}$	30.00 ±2.27$^{a}{}_{/6}$	31.00 ±0.58$^{a}{}_{/6}$
30	24.83 ±0.83$^{b}{}_{/6}$	20.67±0.33$^{*c}{}_{/6}$	32.00±0.58 $^{a}{}_{/6}$	44.83 ±1.19$^{*a}{}_{/6}$	30.33 ±0.61$^{a}{}_{/6}$	22.50 ±0.56$^{**b}{}_{/6}$	31.17 ±0.17 $^{a}{}_{/6}$	30.17 ±0.17$^{*a}{}_{/6}$
60	30.50 ±0.56 $^{b}{}_{/6}$	26.67±0.76$^{*d}{}_{/6}$	39.00 ±0.52 $^{a}{}_{/6}$	41.00±0.68$^{*a}{}_{/6}$	28.00 ±0.82$^{c}{}_{/6}$	32.83 ±0.60$^{* b}{}_{/6}$	26.17 ±0.79^{c}	28.83 ±0.60$^{*c}{}_{/6}$
90	26.50 ±0.56$^{c}{}_{/6}$	29.00±0.68$^{*b}{}_{/6}$	39.17 ±0.31$^{a}{}_{/6}$	36.17 ±0.30$^{**a}{}_{/6}$	28.83 ±0.60$^{b}{}_{/6}$	26.17 ±0.79$^{*c}{}_{/6}$	27.67 ±0.57$^{bc}{}_{/6}$	25.33 ±0.92$^{c}{}_{/6}$

[a]fish *Clarias gariepinus* females. [b]fish *Clarias gariepinus* males. *Significantly different at P≤ 0.05 (Student t-test). ** Highly significantly different at P≤0.001 (Student t.test). Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil

Means of same sex with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n= number of sample.

At 75 day-old, the females had a non-significant value of 20.04 ±0.06meg/l. than the males with 14.08 ± 1.31meg/l (Table 3.2).

At day 0 after acclimation, in group A, the females had a higher non-significant value of 27.00±1.00meg/l than the males with 25.67 ± 1.87meg/l; In group B, the females had a higher non-significant value of 25.00 ±5.00meg/l than the males with 22.33±3.67meg/l; In group C, the females had a higher value of 33.00 ± 5.00meg/l than the males with 24.50 ± 0.50meg/l; In CTL group, the females had a higher non-significant value of 27.67 ± 2.19meg/l than the males with 25.00±0.58 meg/l. (Table 3.8)

At day 7 of exposure/treatment in group A, the females had a lower non-significant value of 28.00±1.83meg/l than the males with 29.00 ±0.58meg/l; In group B, the females had a higher non-significant value of 26.50 ±0.76meg/l than the males with 24.00±0.97meg/l; In group C, the females and males had similar values of 26.50 ±1.61meg/l and 26.67±1.76meg/l respectively; In CTL group, the females had a higher non-significant value of 31.00 ±0.58meg/l than the males with 30.00 ±2.27meg/l. (Table 3.8)

At day 30 of treatment/exposure in group A, the females had a lower significant ($p \leq 0.05$) value of 20.67±0.33meg/l than the males with 24.83±2.04 meg/l; In group B, the females had a higher significant ($p \leq 0.05$) values of 44.83 ±1.19meg/l than the males with 32.00±0.58meg/l; In group C the females had a lower highly significant ($p \leq 0.001$) value of 22.50 ±0.56meg/l than the males with 30.33 ±0.61meg/l; In CTL group, the females had a lower significant ($p \leq 0.05$) values of 30.17 ±0.17meg/l than the males with 31.17 ±0.17meg/l. (Table 3.8).

At day 60 of treatment/exposure in group A, the females had a lower significant ($p \leq 0.05$) value of 26.67±0.76meg/l than the males with 30.50 ±0.56meg/l; In group B, the females had a higher significant ($p \leq 0.05$) value of 41.00±0.68meg/l than the males with 39.00 ±0.52meg/l; In group C, the females had a higher significant ($p \leq 0.05$) value of 32.83 ±0.60meg/l than the males with 28.00 ±0.82meg/l; In CTL group, the females had a higher significant ($p \leq 0.05$) value of 28.83 ±0.60meg/l than the males with 26.17 ±0.79meg/l. (Table 3.8)

At day 90 of treatment/exposure in group A, the females had a higher significant ($p \leq 0.05$) value of 29.00±0.68meg/l than the males with 26.50 ±0.56meg/l; In group B, the females had a lower highly significant ($p \leq 0.001$) value of 36.17 ±0.30meg/l than the males with 39.17 ±0.31meg/l; In group C, the females had a lower significant ($p \leq 0.05$) value of 26.17 ±0.79meg/l than the males with 28.83 ±0.60meg/l; In CTL group, the females had a lower non-significant value of 25.33 ±0.92meg/l than the males with 27.67 ±0.57meg/l. (Table 3.8)

3.4.3.4: Results of effect of sex and treatment on Sodium (Na^+) meg/l

Table 3.9: Effect of sex and treatment on Sodium (Na^+) meg/l Means ± SEM of *Clarias gariepinus* day 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment days	Group A		Group B		Group C		CTL (Control) Group	
	♂$a^1{}_{/n}$	♀$b^1{}_{/n}$	♂$a^2{}_{/n}$	♀$b^2{}_{/n}$	♂$a^3{}_{/n}$	♀$b^3{}_{/n}$	♂$a^4{}_{/n}$	♀$b^4{}_{/n}$
0(after acclimation)	34.67 ± 6.57$^{a}{}_{/3}$	41.00 ±2.08$^{a}{}_{/3}$	38.67±3.33$^{a}{}_{/3}$	39.50 ±5.50$^{a}{}_{/2}$	26.50±0.50$^{a}{}_{/2}$	45.00 ±1.00$^{*a}{}_{/2}$	36.67 ±1.67$^{a}{}_{/3}$	41.67 ±2.03$^{a}{}_{/3}$
7	35.00 ±1.39$^{a}{}_{/6}$	35.00±0.43$^{a}{}_{/6}$	29.00 ±0.58$^{b}{}_{/6}$	35.00 ±0.58$^{**a}{}_{/6}$	35.67 ±0.61$^{a}{}_{/6}$	28.50 ±1.61$^{*a}{}_{/6}$	36.00 ±2.70$^{a}{}_{/6}$	37.33 ±2.11$^{b}{}_{/6}$
30	30.17 ±0.60$^{a}{}_{/6}$	28.00±6.62$^{b}{}_{/6}$	38.00 ±0.58$^{a}{}_{/6}$	44.83 ±1.19$^{*a}{}_{/6}$	28.50 ±0.43$^{b}{}_{/6}$	25.88 ±0.48$^{*c}{}_{/6}$	27.67 ±0.33$^{b}{}_{/6}$	26.83 ±0.31$^{bc}{}_{/6}$
60	48.33±0.76$^{b}{}_{/6}$	38.50 ±0.56$^{**d}{}_{/6}$	65.17±0.54$^{a}{}_{/6}$	68.50 ±0.85$^{*a}{}_{/6}$	38.33 ±0.56$^{a}{}_{/6}$	50.17 ±0.70$^{**c}{}_{/6}$	38.83 ±0.70$^{a}{}_{/6}$	40.83 ±0.70$^{*c}{}_{/6}$
90	37.50 ±0.56$^{c}{}_{/6}$	45.33 ±0.67$^{**b}{}_{/6}$	67.83 ±0.70$^{a}{}_{/6}$	62.83 ±0.70$^{*a}{}_{/6}$	42.17±0.95$^{b}{}_{/6}$	40.17 ±0.48$^{c}{}_{/6}$	39.17 ±0.31$^{c}{}_{/6}$	35.67 ±0.61$^{*d}{}_{/6}$

[a]fish *Clarias gariepinus* females. [b]fish *Clarias gariepinus* males. *Significantly different at P≤ 0.05 (Student t-test). ** Highly significantly different at P≤0.001 (Student t.test). Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil

Means of same sex with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n= number of sample.

At 75 day-old, females had higher significant (P≤ 0.05) value of 31.53 ± 0.88meg/l. than males with 23.55 ± 1.31meg/l (Table 3.2)

At day 0 after acclimation in group A, the females had a higher non-significant value of 41.00 ± 2.08meg/l than the males with 34.67 ± 6.57meg/l; In group B, the females had a higher non-significant value of 39.50 ± 5.50meg/l than the males with 38.67±3.33meg/l; In group C, the females had a higher (p≤0.05) value of 45.00 ±1.00meg/l than the males with 26.50±0.50meg/l; In CTL group, females had a higher non-significant value of 41.67 ±2.03meg/l than the males with 36.67 ±1.67meg/l. (Table 3.9)

At day 7 of treatment/exposure in group A, the females and males had similar values of 35.00±0.43 meg/l and 35.00 ±1.39meg/l respectively; In group B, the females had a higher highly significant (p≤0.001) value of 35.00 ±0.58 meg/l than the males with 29.00 ±0.58meg/l; In group C, the females had a lower significant (p≤0.05) value of 28.50 ±1.61meg/l than the males with 35.67 ±0.61meg/l; In CTL group, the females had a higher non-significant value of 37.33 ±2.11meg/l than the males with 36.00 ±2.70meg/l (Table 3.9).

At day 30 of treatment/exposure in group A, the females had a non-significant lower value of 28.00±6.6meg/l than the males with 30.17 ±0.60meg/l; In group B, the females had a higher significant (p≤0.05) value of 44.83 ±1.19meg/l than the males with 38.00 ±0.58meg/l; In group C, the females had a lower significant (p≤0.05) value of 25.88 ±0.48meg/l than the males with 28.50 ±0.43meg/l; In CTL group, the females had a lower non-significant value of 26.83 ±0.31meg/l than the males with 27.67 ±0.33meg/l. (Table 3.9)

At day 60 of treatment/exposure in group A, the females had a lower highly significant (p≤0.001) value of 38.50 ±0.56meg/l than the males with 48.33±0.76meg/l; In group B, the females had a higher significant (p≤0.05) value of 68.50 ±0.85meg/l than the males with 65.17±0.54 meg/l; In group C, the females had a higher highly significant (p≤0.001) value of 50.17 ±0.70meg/l than the males with 38.33 ±0.56meg/l; In CTL group, the females had a higher significant (p≤0.05) value of 40.83 ±0.70meg/l than the males with 38.83 ±0.70meg/l. (Table 3.9)

At day 90 treatment/exposure in group A, the females had a higher highly significant (p≤0.001) value of 45.33 ±0.67meg/l than the males with 37.50 ±0.56meg/l; In group B, the females had a lower significant (p≤0.05) value of 62.83 ±0.70meg/l than the males

with 67.83 ±0.70meg/l; In group C, the females had a lower significant ($p \leq 0.05$) value of 40.17 ±0.48meg/l than the males with 42.17±0.95meg/l; In CTL group, the females had a lower significant ($p \leq 0.05$) value of 35.67 ±0.61meg/l than the males with 39.17 ±0.31meg/l. (Table 3.9).

3.4.3.5: Discussions on effect of sex and treatment on potassium and sodium

At day 0 after acclimatisation, potassium and sodium increased in female and male. The increased showed no significant difference in all the groups. This increased may be due to stress caused by the confinement and probably the Pb concentration in the well water used for the culture. Potassium and sodium was higher in the female.

Fish treated with 8.0g raw garlic/l at day 7 there was an increase of potassium in both the females which was non-significant, and in the males which was significant. At day 7, sodium decreased in both the females which were significant and in the males which was non-significant. These results may suggest that 8.0g raw garlic/l during a short period of exposure, increased potassium non-significantly and decreased sodium significantly in the females, while in the males, increased potassium significantly and decreased sodium non- significantly. These may suggest a proper working of the chloride balance of both potassium and sodium. At day 30, potassium decreased significantly in the females and the males had a non-significant decrease. Also at day 30, sodium decreased in the females which was significant and the males there was a non-significant decrease. These results also suggest that with increased exposure time (at day 30), 8.0g raw garlic/l decreased potassium and sodium significantly in the females but in the males had a non-significant decrease in both potassium and sodium. This may also suggest a proper chloride ion balance by garlic. At day 60, sodium increased significantly in both the females and the males. While potassium also increased significantly in both the females and the males. This also suggests a proper chloride ion balance by garlic. At day 90, potassium increase was non-significant in the females and a non-significant decrease in the males. Also sodium increase in the females was significant while in the males there was a significant decrease. This may suggest an imbalance of potassium and sodium in both females and the males at day 90. These results suggest that 8.0g raw garlic/l provided an environment that balanced potassium and sodium until day 60 but it failed though not severely at day 90.

Fish treated/exposed to 0.03mgCd/l, at day 7 there was decrease of sodium which was significant in the females and in the males an increase which was significant. Potassium

increased in both the females which was non-significant and the males which was non-significant. These results suggest that there was no proper functioning of the chloride balance mechanism which appears to be more severe in the females with a significant decrease in sodium and a non-significant increase in potassium. At day 30, potassium increased non-significantly in the females and significantly in the males. While sodium increased significantly in both the females and males. These results may suggest that there was a malfunction of the chloride balance mechanism in the females. At day 60, potassium and sodium increased significantly in both the females and the males. It does appear that after a prolonged exposure, 0.03mgCd/l increase in the level of potassium and sodium becomes significant probably due to adaptation syndrome may be to establish a balance. At day 90, potassium decrease in the females significantly and the males had a non-significant increase.

Fish treated/exposed to 0.30mgPb/l, at day 7, there were significant increase in the females and an increase in the males in sodium. Potassium decreased significantly in the females and increased in the males. These results suggest that at day 7 which is a short period of exposure 0.30mgPb/l caused an imbalance of electrolyte in the females. The short period did not affect the males. This may also suggest that the females were more affected by lead in the short exposure duration. At day 30, the females had a non-significant increase and the males an increase in potassium. The females had a non-significant decrease and the males a significant decrease in sodium. These results suggest that there was an electrolyte imbalance in both the females and the males. At day 60, the females had a significant increase and the males a significant increase in sodium. The females had a significant increase and the males a significant decrease in potassium. These results also suggest that there was an electrolyte balance in the females and an imbalance on the males. At day 90, the females had a significant decrease and the males a significant increase in potassium whereas the females had a significant decrease and the males a significant increase in sodium. These results suggest that there was a balance of electrolyte. These results suggest that 0.30mgPb/l caused an imbalance of electrolyte in the females during a short duration exposure which could persist but probably due to adaptation syndrome, the females and males that had also an imbalance after the females, had a balance electrolyte after prolongs exposure.

Fish with no treatment (control), at day 7 there was significant increase of potassium in both the females and the males. Sodium non-significantly decreased in the females and

increased in the males. These results suggest that no balance was achieved but however the imbalance is more severe in the females than in the males. At day 30, in both the females and the males, potassium and sodium decreased non-significantly. These results suggest that a balance was achieved in both the females and the males. At day 60, sodium increased significantly in both the females and males while potassium decreased non-significantly in the females and decreased significantly in the males. These results may suggest that balance achieved in both the females and the males developed at day 30 failed at day 60. At day 90, sodium decreased non-significantly in the females and increased non- significantly in the males. Also potassium non-significantly decreased in the females and increased non-significantly in the males. These results suggest that a balance was established.

3.4.3.6: Results of effect of sex and treatment on Creatinine g/l

Table 3.10: Effect of sex and treatment on Creatinine g/l Means ± SEM of *Clarias gariepinus* days 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/expos	Group A		Group B		Group C		CTL (Control) Group	
ure days	♂$a^{1}{}_{/n}$	♀$b^{1}{}_{/n}$	♂$a^{2}{}_{/n}$	♀$b^{2}{}_{/n}$	♂$a^{3}{}_{/n}$	♀$b^{3}{}_{/n}$	♂$a^{4}{}_{/n}$	♀$b^{4}{}_{/n}$
0(**after acclimation)**	1.08± 0.06$^{a}{}_{/3}$	1.14± 0.03$^{a}{}_{/3}$	1.12 ±0.03$^{a}{}_{/3}$	1.09±0.04$^{a}{}_{/2}$	1.17±0.08$^{a}{}_{/2}$	1.11 ±0.01$^{a}{}_{/2}$	1.09 ±0.04$^{a}{}_{/3}$	1.14 ±0.04$^{a}{}_{/3}$
7	1.64 ±0.21$^{a}{}_{/6}$	1.62±0.23 $^{a}{}_{/6}$	1.50±0.21 $^{a}{}_{/6}$	1.78 ±0.21 $^{a}{}_{/6}$	1.11 ±0.37$^{a}{}_{/6}$	1.16 ±0.37 $^{a}{}_{/6}$	1.22 ±0.37$^{a}{}_{/6}$	1.23±0.37 $^{a}{}_{/6}$
30	1.11 ±0.00$^{c}{}_{/6}$	1.03 ±0.02$^{*\,a}{}_{/6}$	1.20±0.01$^{b}{}_{/6}$	1.15 ±0.02$^{c}{}_{/6}$	2.10±0.03$^{a}{}_{/6}$	1.13±0.02$^{**ab}{}_{/6}$	2.07 ±0.02$^{a}{}_{/6}$	1.07 ±0.02$^{**bc}{}_{/6}$
60	1.18 ±0.02$^{a}{}_{/6}$	1.05±0.02$^{*d}{}_{/6}$	1.17±0.04$^{ab}{}_{/6}$	2.05 ±0.02$^{**a}{}_{/6}$	1.10±0.00$^{bc}{}_{/6}$	1.20±0.00$^{**b}{}_{/6}$	1.07 ±0.02$^{c}{}_{/6}$	1.13 ±0.02$^{*c}{}_{/6}$
90	1.12 ±0.03$^{b}{}_{/6}$	1.06±0.02$^{b}{}_{/6}$	2.10±0.03$^{a}{}_{/6}$	1.91 ±0.17$^{a}{}_{/6}$	2.12 ±0.01$^{a}{}_{/6}$	2.10 ±2.05$^{a}{}_{/6}$	2.09 ±0.02$^{a}{}_{/6}$	2.06±0.02$^{a}{}_{/6}$

[a]fish *Clarias gariepinus* females. [b]fish *Clarias gariepinus* males. *Significantly different at P≤ 0.05 (Student t-test). ** Highly significantly different at P≤0.001 (Student t.test). Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil

Means of same sex with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n= number of sample.

At 75 day-old the females and males had similar value of 1.01 ± 00g/l and 1.00±0.00g/ respectively (Table 3.2)

Table 3.10 shows at day 0 after 14-day acclimation, in group A, the females had a higher non-significant value of 1.14± 0.03g/l than the males with 1.08± 0.06g/l; In group B, the females had a lower non-significant value of 1.09±0.04g/l than the males with 1.12 ±0.03g/l; In group C, the females had a lower value of 1.11 ±0.01g/l than the males with 1.17±0.08g/l; In CTL group, females had a higher non-significant value of 1.14 ±0.04g/l than the males with 1.09 ±0.04g/l.

Table 3.10 shows that at day 7 of treatment/exposure in group A, the females and males had similar values of 1.62±0.23g/l and 1.64 ± 0.21g/l respectively; In group B, the females had a higher non-significant value of 1.78 ±0.21g/l than the males with 1.50±0.21g/l; In group C, the females and males had similar values of 1.16 ±0.37g/l and 1.11 ±0.37g/l respectively; In CTL group, the females and males had similar values of 1.23±0.37g/l and 1.22 ±0.37g/l respectively.

Table 3.10 shows that at day 30 of treatment/exposure in group A, the females had a lower significant ($p \leq 0.005$) value of 1.03 ±0.02g/l than the males with 1.11 ±0.00g/l; In group B, the females had a lower non-significant value of 1.15 ±0.02g/l than the males with 1.20±0.01g/l; In group C, the females had a lower highly significant ($p \leq 0.001$) value of 1.13±0.02g/l than the males with 2.10±0.03g/l; In CTL group, the females had a lower highly significant ($p \leq 0.001$) value of 1.07 ± 0.02g/l than the males with 2.07 ±0.02 g/l.

Table 3.10 shows that at day 60 of treatment/exposure in group A, the females had a lower significant ($p \leq 0.05$) value of 1.05±0.02g/l than the males with 1.18 ±0.02g/l; In group B, the females had a higher highly significant ($p \leq 0.01$) value of 2.05 ±0.02g/l than the males with 1.17±0.04g/l; In group C, the females had a higher highly significant ($p \leq 0.01$) value of 1.20±0.00g/l than the males with 1.10±0.00 g/l; In CTL group, the females had a higher significant ($p \leq 0.05$) value of 1.13 ±0.02g/l than the males with 1.07 ±0.02g/l.

Table 3.10 shows that at day 90 of treatment/exposure, in group A, the females had a lower non-significant value of 1.06±0.02g/l than the males with 1.12 ±0.03g/l; In group B, the females had a lower non- significant value of 1.91 ±0.17g/l than the males with 2.10±0.03g/l; In group C, the females and males had similar values of 2.10 ±2.05 g/l and 2.12 ±0.01g/l respectively; In CTL group, the females and males had similar values of 2.06±0.02g/l and 2.09 ±0.02 g/l respectively.

3.4.3.7: Discussions on effect of sex and treatment on Creatinine g/l

At day 0 after acclimatisation, Creatinine increased in female and male. The increased showed no significant difference in all the groups. This increased may be due to stress caused by the confinement and probably the Pb concentration in the well water used for the culture. Creatinine was higher in the male.

Fish treated/exposed to 8.0g raw garlic/l, at day 7 the females and males had significantly increased creatinine may be due to significant increase in manganese from garlic in water. At day 30, 60 and 90 the females and males had non-significant changes. These results suggest that 8.0g/l garlic stabilized the creatinine levels in the females and males.

Fish treated/exposed to 0.03mgCd/l, at day 7, at day 7 the females and males had a significantly increased creatinine. At day 30 females had significant decrease and males had a non-significant decrease. At day 60 the females had a significant ($p \leq 0.05$) increase and the males a non-significant decrease. At day 90, the females had a non-significant increase and the males a significant ($p \leq 0.05$) increase. These results suggest that during a short duration exposure 0.03mgCd/l have a significant effect on both the females and males. However, with increased exposure time, the females and males had different non-significant or significant biphasic responses at the same exposure time. These may mean unstable environment. It also appears that the effect of 0.03mgCd/l in the females was earlier in females and males but continued in the males.

Fish treated/exposed to 0.30mgPb/l, at day 7, the females and males had a non-significant increase and decrease respectively this may mean that 0.30mgPb/l had no significant effects. At day 30 the females had a non-significant increase and the males significant ($p \leq 0.05$) increase; at day 60, the females had a non- significant increase and the males significant ($p \leq 0.05$) decrease. At day 90, the females and males had significant ($p \leq 0.001$) increases. These results suggest that 0.30mgPb/l significantly increased creatinine in the females and males.

Fish with no treatment (control), at day 7 and 30, the females and males had had a non-significant increase. These may suggest that there was no change in the creatinine levels of females and males. At day 30, the females had a non-significant decrease while the males had a significant ($p \leq 0.05$) increase. At day 60, the females had a non-significant increase while the males had a significant decrease. At day 90, the females and males had a significant ($p \leq 0.05$) increase. The responses were biphasic (increase followed by a

decrease) an evidence of frequent adjustments which is detrimental. These responses by the males may mean that the ability to cope was lower than in the females.

3.4.3.8: Results of effect of sex and treatment on Glucose g/l

Table 3.11: Effect of sex and treatment on Glucose g/l Means ± SEM of *Clarias gariepinus* days 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/expos	Group A		Group B		Group C		CTL (Control) Group	
days	♂$a^{1}{}_{/n}$	♀$b^{1}{}_{/n}$	♂$a^{2}{}_{/n}$	♀$b^{2}{}_{/n}$	♂$a^{3}{}_{/n}$	♀$b^{3}{}_{/n}$	♂$a^{4}{}_{/n}$	♀$b^{4}{}_{/n}$
0(**after acclimati**	68.67 ± 6.57$^{a}{}_{/3}$	64.67 ± 10.48$^{a}{}_{/3}$	70.67±0.67$^{a}{}_{/3}$	74.00 ±1.00$^{a}{}_{/2}$	69.50±1.50$^{a}{}_{/2}$	71.00±1.00$^{a}{}_{/2}$	68.33±6.89$^{a}{}_{/3}$	62.33 ±5.90$^{a}{}_{/3}$
7	49.00 ±0.58$^{a}{}_{/6}$	50.00 ±0.97$^{ab}{}_{/6}$	58.00 ±1.83$^{a}{}_{/6}$	59.50 ±2.01$^{a}{}_{/6}$	51.00±8.50$^{a}{}_{/6}$	47.50 ±5.60$^{b}{}_{/6}$	50.17 ±0.70$^{a}{}_{/6}$	46.00 ±4.49$^{b}{}_{/6}$
30	46.17±0.65$^{b}{}_{/6}$	45.50 ±0.56$^{c}{}_{/6}$	46.33 ±0.56$^{b}{}_{/6}$	49.17 ±1.15$^{b}{}_{/6}$	60.33 ±1.31$^{a}{}_{/6}$	59.67 ±1.05$^{a}{}_{/6}$	38.83 ±0.91$^{c}{}_{/6}$	37.83 ±0.91$^{d}{}_{/6}$
60	65.83 ±0.54$^{a}{}_{/6}$	54.83±0.54$^{**c}{}_{/6}$	68.00 ±0.26$^{a}{}_{/6}$	78.00±0.26$^{**a}{}_{/6}$	53.83 ±1.45$^{b}{}_{/6}$	65.00 ±1.57$^{**b}{}_{/6}$	52.67 ±0.49$^{b}{}_{/6}$	56.67 ±0.49$^{**c}{}_{/6}$
90	57.00 ±0.45$^{c}{}_{/6}$	67.33 ±0.50$^{**d}{}_{/6}$	80.17 ±0.17$^{a}{}_{/6}$	76.67 ±0.82$^{**b}{}_{/6}$	79.83 ±0.54$^{a}{}_{/6}$	81.83 ±0.65$^{*a}{}_{/6}$	73.67 ±0.56$^{b}{}_{/6}$	71.17 ±0.79$^{*c}{}_{/6}$

[a]fish *Clarias gariepinus* females. [b]fish *Clarias gariepinus* males. *Significantly different at P≤ 0.05 (Student t-test). ** Highly significantly different at P≤0.001 (Student t.test). Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil

Means of same sex with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n= number of sample.

At 75 day-old the females had a higher non-significant value of 46.02 ± 2.84g/l than the males with 42.50 ± 0.71g/l (Table 3.2)

Table 3.11 shows at day 0 after acclimation in group A, the females had a lower non-significant value of 64.67 ± 10.48g/l than the males with 68.67 ± 6.57g/l; In group B, the females had a higher non-significant value of 74.00 ±1.00g/l than the males with 70.67±0.67g/l; In group C, the females and males had similar value of 71.00±1.00g/l and 69.50±1.50g/l respectively; In CTL group, the females had a lower non-significant value of 62.33 ±5.90g/l than the males with 68.33±6.89g/l.

Table 3.11 shows at day 7 of treatment/exposure fish in group A with 8.0g/l raw garlic treatment, the females had a higher non-significant value of 50.00 ±0.97g/l than the males with 49.00 ±0.58 g/l than the males with 49.00±1.14g/l. In group B the females had a higher non-significant value of 59.50 ±2.01g/l than the males with 58.00 ±1.83g/l. In group C the females had a lower non-significant value of 47.50 ±5.60g/l than the males with 51.00±8.50 g/l.; In group C the females had lower non-significant value of 46.00 ±4.49 g/l than the males with 50.17 ±0.70 g/l.

Table 3.11 shows at day 30 of treatment/exposure, fish in group 1 with 8.0g/l raw garlic treatment in group A, the females had a lower non-significant value of 45.50 ±0.56g/l than the males with 46.17±0.65g/l; I3 group B the females had a higher non-significant value of 49.17 ±1.15g/l than the males with 46.33 ±0.56g/l; In group 4, the females had a lower highly significant ($p \leq 0.001$) value of 59.67 ±1.05g/l than the males with 60.33 ±1.31g/l.; In CTL group, the females had a lower non-significant value of 37.83 ±0.91g/l than the males with 38.83 ±0.91g/l.

Table 3.11 shows at day 60 of treatment/exposure, fish in group 1 with 8.0g/l raw garlic treatment in group A, the females had a lower highly significant ($p \leq 0.001$) value of 54.83±0.54g/l than the males with 65.83±0.54g/l; In group 2, the females had a higher highly significant ($p \leq 0.001$) value of 78.00±0.26g/l than the males with 68.00±0.26g/l; In group C, the females had a higher highly significant ($p \leq 0.001$) value of 65.00 ±1.57g/l than the males with 53.83 ±1.45g/l; In CTL group, the females had a higher significant ($p \leq 0.05$) value of 56.67 ±0.49g/l than the males with 52.67 ±0.49g/l.

Table 3.11 shows at day 90 of treatment/exposure, fish in group 1 with 8.0g/l raw garlic treatment in group A, the females had a higher highly significant ($p \leq 0.001$) value of

67.33 ±0.50g/l than the males with 57.00 ±0.45g/l; In group B, the females had a lower highly significant ($p \leq 0.001$) value of 76.67 ± 0.82g/l than the males with 80.17±0.17g/l; In group C, the females had a higher significant ($p \leq 0.05$) value of 81.83±0.65g/l than the males with 79.83±0.54g/l; In CTL group, the females had a lower significant ($p \leq 0.05$) value of 71.17±0.79g/l than the males with 73.67±0.56 g/l.

3.4.3.9: Discussions on effect of sex and treatment on Glucose g/l

At day 0 after acclimatisation, glucose increased in female and male. The increased showed no significant difference in all the groups. This increased may be due to stress caused by the confinement and probably the Pb concentration in the well water used for the culture. This is in agreement that Environmental pollution may induce stress thereby enhancing glycogen breakdown in liver and consequently raise blood glucose level in fish (Diwan *et al*., 1979). Glucose was higher in the female.

Fish treated with 8.0g raw garlic/l, at day 7 there was a significant decrease in both the females and males. At day 30, both the females and males had a non-significant decrease. At day 60, there was a significant increase in both the females and males. At day 90 the females had a significant increase and the males a significant decrease. These results suggest that 8.0g raw garlic/l decreased glucose in the blood during a short duration in the females and males and maintained the level for period with increased exposure time. However, with a further exposure time, the females and males coping strategy failed in the females at the 60 but male recovered at day 90 with a significant decrease These results may suggest that 8.0g raw garlic/l in decreasing glucose level is not elastic.

Fish treated/exposed to 0.03mgCd/l, at day 7 and 30 there were significant decrease in glucose in both the females and the males, at day 60 there was a significant increase in glucose in both the females and the males while at day 90, there was a non-significant decrease in glucose in the females and a significant increase in the males. These results may suggest that 0.03mgCd/l decreased significantly glucose in the early stage of exposure but with increased exposure time with no evidence of a stable coping strategy developed, in both the females and males 0.03mgCd/l increased significantly glucose in both the females and males which was also not stable resulting in a non-significant decrease in the females and significant increase in the males. These may suggest that 0.03mgCd/l did not support the development of a stable coping strategy in both the females and the males and also it increased the level of glucose more in the males.

Fish treated/exposed to 0.30mgPb/l, females and males had at day 7 a significant decrease; At day 30, females had significant increase while the males had non-significant increase; At day 60, females and males had a non-significant increase and decrease respectively and at day 90, females and males had significant increases. These result may mean that 0.30mgPb/l during a short period of exposure both the females and the males had uniform response as seen at day 7. However, at day 30 and 60 females and males do not have uniform responses probably due to sex related effect of lead and/or the different coping strategy by both the females and the males. It appears that the females and males did not cope in the environment after prolong exposure as seen at day 90 when there was a significant increase.

For fish with no treatment (control) at day 7, there was a significant decrease in glucose in both females and males. At day 30 of the experiment, both the females and the males had a non-significant decrease. At day 60 and 90 there were a significant increase in both the females and the males. These results suggest that in an environment with no known sex related induced stressors, at the early stages of exposure, both the females and the males' response may be uniform. The increase in both female and male at day 60 and 90 may mean increased level of stress.

The increases obtained in the groups is due to increased stress levels. This was supported by findings that glycolsis as response to stress and transformation to glucose for energy requirement (Tahmina et al., 2015) and increasing of glucose level is due to high secretion of hormones like catecholamines, glucocorticords and that lead to increasing of glycolysis resulting to high glucose level in blood (Hussein and Nadim, 2003). The fluactuations is due to attempts of the fish to adapt (Ellis, 1986).

3.4.4: Water quality

3.4.4.1: Result of effect of treatment on pH

Table 3.12: Effect of treatment on pH Means ± SEM of *Clarias gariepinus* day's 0 after 14 day's acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Temperature/Exposure (days)	Groups A	B	C	CTL (Control)
0	7.06±0.03^{a}	7.05±0.05^{a}	7.08±0.02^{a}	7.05±0.01^{a}
7	6.95±0.05^{b}	6.75 ± 0.05^{a}	6.95±0.05^{b}	6.79±0.03^{ab}
30	7.50±0.00^{d}	6.30±0.00^{a}	7.00±0.00^{c}	6.47±0.04^{b}
60	6.70±0.00^{c}	6.20±0.00^{a}	6.40±0.00^{b}	6.41±0.08^{b}
90	7.60±0.00^{c}	6.00±0.00^{a}	6.02±0.01^{a}	6.10±0.01^{b}

Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil.
Means with the same letter on the same row are not significantly different according to DMRT at P≥0.05.
n = 2

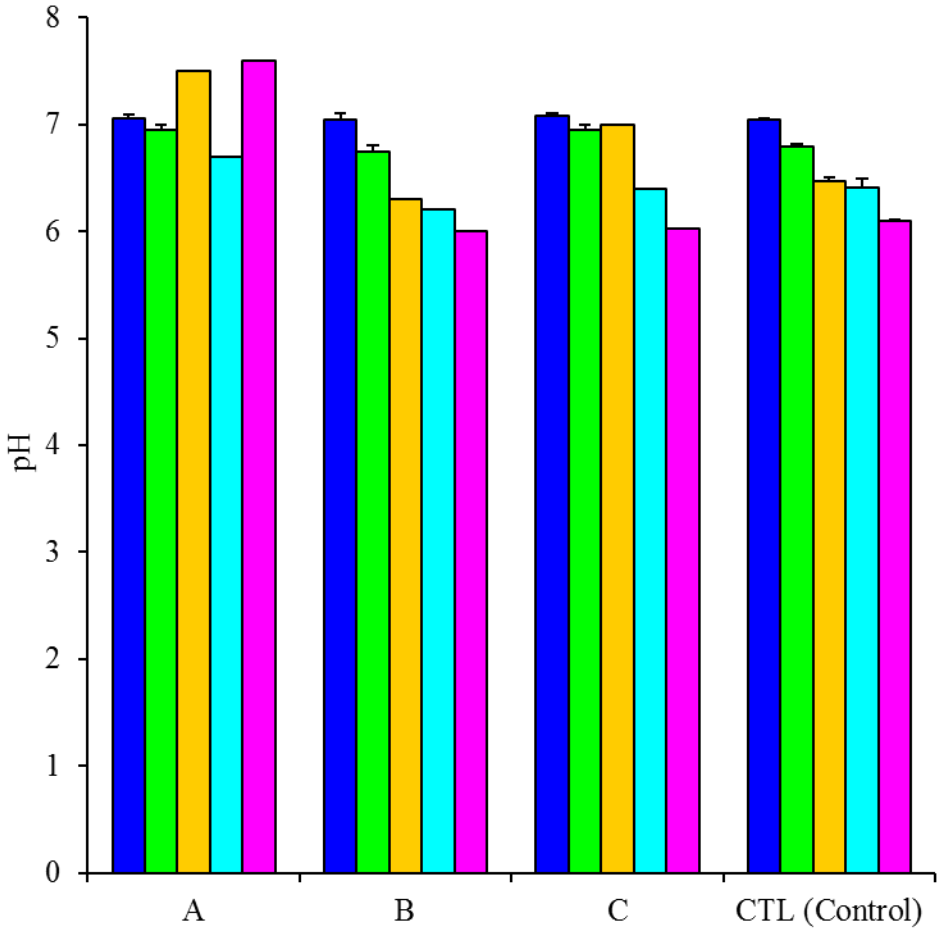


Figure 3.2: Effect of duration of treatment on pH in water

pH in group A, at day 0, was 7.06±0.03, at day 7, was 6.95±0.05, at day 30, was 7.50±0.00, at day 60, was 6.70±0.00, and at day 90, was 7.60±0.00. pH in group B, at day 0, was 7.05±0.05, at day 7, was 6. 6.75 ± 0.05, at day 30, was 6.30±0.00, at day 60, was 6.20±0.00, and at day 90, was 6.00±0.00. pH in group C, at day 0, was 7.08±0.02, at day 7, was 6.95±0.05, at day 30, was 7.00±0.00, at day 60, was 6.40±0.00, and at day 90, was 6.02±0.01. pH in CTL (control) group at day 0, was 7.05±0.01, at day 7, was 6.79±0.03, at day 30, was 6.47±0.04, at day 60, was 6.41±0.08, and at day 90, was 6.10±0.01. (Table 3.12)

Table 3.12 showed pH in group A treated with 8.0g raw garlic/l order of significant ($P \leq 0.05$) difference in value with days of exposure was $90 < 30 < 0 < 7 < 60$. pH in group B treated with 0.03mgCd/l order of significant ($P \leq 0.05$) difference in values with days of exposure was $0 < 7 < 30 < 60 < 90$. pH in group C treated with 0.30mgPb/l order of significant ($P \leq 0.05$) difference in values with days of exposure was $0 < 7 < 60 < 90$ there was no significant ($P \geq 0.05$) in day 0 and 30 and day 7 and 30. In CTL (control) group with no treatment, the order of significant ($P \leq 0.05$) difference in values with days of exposure was $0 < 7 < 30, 60 < 90$.

Table 3.12 also showed the differences at different days of exposure in different groups that the order of significant ($P \leq 0.05$) differences in values at day 0, there was no significant ($P \geq 0.05$) difference; at day 7, group A, C < B with no significant ($P \geq 0.05$) with control groups, at day 30, group A < C< CTLl < B; at day 60, group A < C, CTL< B2, whereas at day 90, group A < CTL < B, C.

3.4.4.2: Discussions on effect of treatment on pH

Water culture in group A, treated with 8.0g raw garlic/l had a range of 6.70 to 7.60. At day 7 there was significant ($p \leq 0.05$) decrease, at day 30 there was significant ($p \leq 0.05$) increase, at day 60 there was significant ($p \leq 0.05$) decrease and at day 90 there was significant ($p \leq 0.05$) increase. These results showed significant fluctuations of decrease and increase. These fluctuations could have been due to increased and decreased level of contaminants.

Water culture in group B, treated with 0.03mgCd/l had a range of 6.00 to 7.10. At day 7 there was a significant ($p \leq 0.05$) decrease, at day 30 there was significant ($p \leq 0.05$) decrease at day 60 there was a non- significant ($p \geq 0.05$) decrease while at day 90 there was a significant ($p \leq 0.05$) decrease. These results showed that 0.03mgCd/l decreased

significantly water culture pH with increase duration of treatment. These could have been due to increased level of contaminants with increased exposure time.

Water culture in group C, treated with 0.30mgPb/l treatment had a range of 6.01 to 7.09. At day 7 there was a significant ($p \leq 0.05$) decrease, at day 30 there was a non-significant ($p \geq 0.05$) increase while at both day 60 and 90 there were significant ($p \leq 0.05$) decreases. These results suggest that 0.30mgPb/l decreased pH significantly ($p \leq 0.05$) with increased exposure time. These could have been due to increased level of contaminants with increased exposure time.

Water culture in CTL (control) group, with no treatment had a range of pH 6.09 to 7.06. At day 7 there was a significant ($p \leq 0.05$) decrease, at day 30 and 60 there was a non- significant ($p \geq 0.05$) decrease, at day 90 there was a significant ($p \leq 0.05$) decrease. The significant decreases at day 7 and 90 may be due to increased level of contaminants with increased exposure time.

The pH lower range of culture water of groups was not within the recommended lower range for *Clarias gariepinus* culture of 6.5 (Omitoyin, 2007) except in group 1. At day 90 pH increased and decreased significantly ($p \leq 0.05$) in group 1 and in groups 2, 3 and control, respectively.

3.4.4.3: Result of effect of treatment on Total solids (mg/l)

Table 3.13: Effect of treatment on Total solids (mg/l) Means ± SEM of *Clarias gariepinus* days 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/ exposure days	Groups			
	Group A	Group B	Group C	CTL (Control)
0	606.82±8.19^{ab}	647.50±10.50^{b}	599.00±9.00^{a}	620.45±16.85^{ab}
7	590.00±10.00^{a}	719.00 ± 9.00^{c}	741.50±11.50^{c}	668.50±14.50^{b}
30	617.50±7.50^{a}	631.00±11.00^{a}	681.50±11.50^{b}	621.00±7.00^{a}
60	592.00±11.00^{a}	1081.00±19.00^{d}	861.50±9.50^{c}	764.00±26.00^{b}
90	772.50±25.50^{a}	2691.00±21.00^{d}	1750.00±30.00^{c}	1108.00±68.00^{b}

Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil.
Means with the same letter on the same row are not significantly different according to DMRT at P≥0.05.
n = 2.

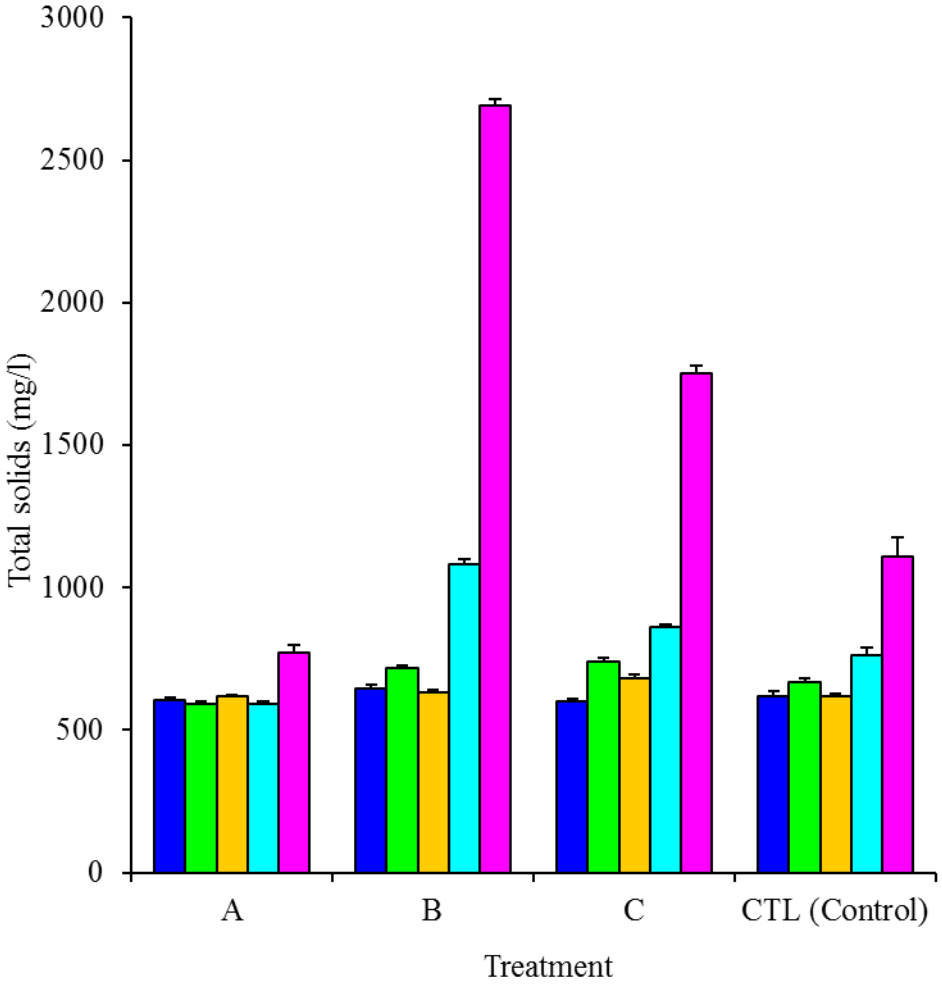


Figure 3.3: Effect of duration of treatment on total solids in water

Day 0
7 days exposure
30 days exposure
60 days exposure
90 days exposure

Treatment
A : 8.0g raw garlic/l
B : 0.03 cadmium (Cd)/l
C : 0.3 Lead (PbNO3)2/l
CTL : Nil

Total solids in group A, at day 0, was 606.82±8.19mg/l; at day 7, was 590.00±10.00 mg/l; at day 30, was 617.50±7.50mg/l; at day 60, was 592.00±11.00 mg/l, and at day 90, was 772.50±25.50 mg/l. Total solids in group B, at day 0, was 647.50±10.50mg/l; at day 7, was 719.00 ± 9.00 mg/l; at day 30, was 631.00±11.00 mg/l; at day 60, was 1081.00±19.00 mg/l, and at day 90, was 2691.00±21.00 mg/l. Total solids in group C, at day 0, was 599.00±9.00 mg/l; at day 7, was 741.50±11.50 mg/l; at day 30, was 681.50±11.50 mg/l; at day 60, was 861.50±9.50 mg/l and at day 90, was 1750.00±30.00 mg/l. Total solids in CTL (control) group, at day 0, was 620.45±16.85mg/l; at day 7, was 668.50±14.50 mg/l; at day 30, was 621.00±7.00 mg/l; at day 60, was 764.00±26.00 mg/l and at day 90, was 1108.00±68.00 mg/l. (Table 3.13)

Table 3.13 showed total solids level in various groups at different days of exposure. At day 0, only group B and C was significantly ($P \leq 0.05$) different in order of group B < C. The order of significant ($P \leq 0.05$) differences was at day 7 group B, C < CTL < A; at day 30, group C < A, B, CTL; at day 60 and 90 group 2 < C< CTL < A.

3.4.4.4: Discussion of effect of treatment on Total solids (mg/l)

Water culture in group A, treated with 8.0g raw garlic/l, had a range of 590.00-772.50mg/l. At day 7, there was significant ($p \leq 0.05$) decrease, at day 30, there was non-significant increase, at day 60 there was a non-significant decrease while at day 90 there was a significant increase. These results may mean that 8.0g raw garlic/l, acutely decreased significantly total solids in the culture water and with increase duration of treatment stabilised total solids in the water, due to the complexation of solids by garlic however with prolonged treatment increased significantly total solids in the water probably due to failure of the complexation of solids by garlic.

Water culture in group B, treated with 0.03mgCd/l had a range of 631.00-2091.00mg/l. At day 7, there was a non- significant decrease, at day 30 there was a significant decrease, at day 60 and 90, there were significant ($p \leq 0.05$) increases. These mean that 0.03mgCd/l at the early stages of treatment decreased total solids probably due to its non-bioavailability and may be its uptake. However, with increase duration of treatment, it increased the level of total solids probably due to its bioavailability, interaction with other solids by making them soluble and/or excretion by fish.

Water culture in group C, treated with 0.30mgPb/l had a range of 599.00-1750.00 mg/l. At day 7, there was a significant ($p \leq 0.05$) increase, at day 30, there was a significant ($p \leq 0.05$) decrease, at day 60 there was significant ($p \leq 0.05$) increase while at day 90 there

was a significant ($p \leq 0.05$) increase. These mean that 0.30mgPb/l increased the total solids culture water with increase duration of culture. These may be due to increase bioavailability and /or excretion by the fish. The decrease at day 30 may be due to uptake by fish or other organism in the culture system.

Water culture in group CTL (control), with no treatment had a range of 413-683.00mg/l. At day 7 there was a significant ($p \leq 0.05$) increase, at day 30, there was a significant ($p \leq 0.05$) decrease, at day 60 there was a significant increase while at day 90 there was a significant ($p \leq 0.05$) decrease. These mean that in a non- sex related induced stressors, in a aquatic environment, there may be a bout of increase and decrease which may be due to increase uptake and increase excretion based on unstable environmental adaptation by *Clarias gariepinus* therein.

3.4.4.5: Result of effect of treatment on Dissolved oxygen (DO) mg/l

Table 3.14: Effect of treatment on Dissolved oxygen (DO) mg/l Means ± SEM of *Clarias gariepinus* days 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in culture groups

Treatment/exposure days	Groups			
	Group A	Group B	Group C	CTL (Control)
0	4.39±0.28[a]	4.99±0.02 [b]	4.71±0.09 [ab]	4.46±0.04 [ab]
7	2.70±0.03 [a]	3.13 ± 0.03 [b]	3.24±0.03 [bc]	3.38±0.08 [c]
30	3.11±0.01 [c]	2.86±0.00 [b]	2.23±0.01 [a]	2.89±0.03 [b]
60	1.97±0.06 [c]	1.39±0.01 [b]	0.84±0.02 [a]	1.37±0.04 [b]
90	1.88±0.01 [b]	1.32±0.10 [a]	1.30±0.01 [a]	1.37±0.03 [a]

Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil.
Means with the same letter on the same row are not significantly different according to DMRT at P≥0.05.
n= 2

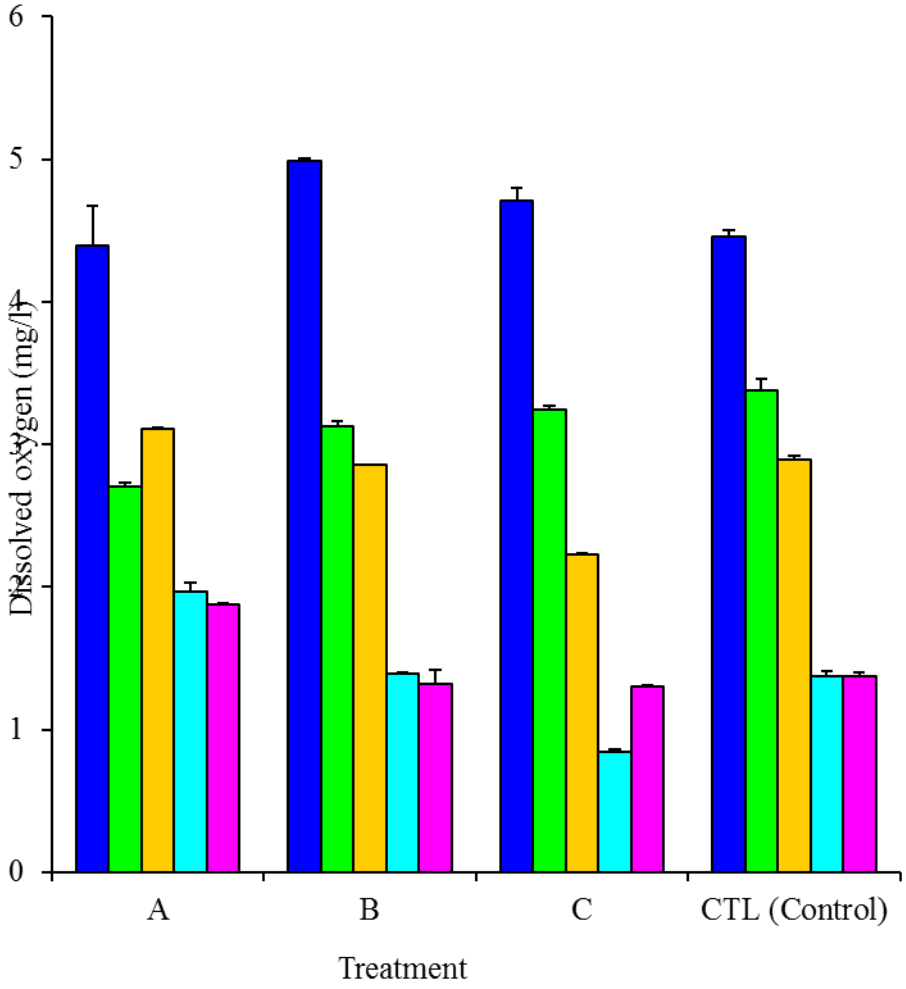


Figure 3.4: Effect of duration of treatment on Dissolved oxygen in water

- Day 0
- 7 days exposure
- 30 days exposure
- 60 days exposure
- 90 days exposure

Treatment

A : 8.0g raw garlic/l
B : 0.03 cadmium (Cd)/l
C : 0.3 Lead $(PbNO_3)_2$/l
CTL : Nil

DO in group A, at day 0, was 4.39±0.28mg/l; at day 7, was 2.70±0.03 mg/l; at day 30, was 3.10±0.01mg/l; at day 60, was 1.97±0.06mg/l, and at day 90, was 1.88±0.01mg/l. DO in group B, at day 0, was 4.99±0.02mg/l; at day 7, was 3.13 ± 0.03mg/l; at day 30, was 2.86±0.00mg/l; at day 60, was 1.39±0.01mg/l, and at day 90, was 1.32±0.10mg/l. DO in group C, at day 0, was 4.71±0.09mg/l; at day 7, was 3.24±0.03mg/l; at day 30, was 2.23±0.01 mg/l; at day 60, was 0.84±0.02mg/l and at day 90, was 1.30±0.01 mg/l. DO in CTL (control) group, at day 0, was 4.46±0.04 mg/l; at day 7, was 3.38±0.08mg/l; at day 30, was 2.89±0.03mg/l; at day 60, was 1.37±0.04mg/l and at day 90, was 1.37±0.03mg/l. (Table 3.14).

Table 3.14 showed dissolved oxygen (DO) level in various groups at different days of exposure. At day 0, only group 1 and 2 was significantly ($P \leq 0.05$) different in order of group B < A. The order of significant ($P \leq 0.05$) differences was at day 7 group CTL < B < A. There was no significant ($P \geq 0.05$) difference in group 3 and control. The order of significant ($P \leq 0.05$) was at day 30 and 60 group A < B, control < C; at day 90 group A < B, C, CTL.

3.4.4.6: Discussion on effect of treatment on Dissolved oxygen (DO) mg/l

Water culture in group A, treated with of 8.0g raw garlic/l, had a range of 1.88-3.13mg/l. At day 7, there was a significant ($p \leq 0.05$) decrease, at day 30 there was a significant ($p \leq 0.05$) increase at day 60 there was a significant ($p \leq 0.05$) decrease while at day 90 there was a non- significant decrease. These mean that 8.0g raw garlic/l, acutely decreased DO. However, with increase in duration of treatment there was a significant increase which may be due to the production of oxygen by photosynthetic plants in the culture water that may be favoured by garlic as a substrate for them. However, at day 60 there was a significant decrease with a non- significant decrease at day 90. These non-significant decreases at day 90 may mean that photosynthetic plants may have improved their oxygen production due to the little rain shower that occurred at day 87 of the study which may have agitated the water and improved the dissolve oxygen. The range of 1.88-3.13mg/l may mean a reduced devolution of oxygen in the culture system due to 8.0g raw garlic/l.

Water culture in group B, treated with 0.03mgCd/l had a range of 1.32-4.99 mg/l. At day 7 there was a significant ($p \leq 0.05$) decrease, at day 30, there was a significant ($p \leq 0.05$) decrease, at day 60 there was a significant ($p \leq 0.05$) decrease while at day 90 there was a non-significant decrease. These results showed that 0.03mgCd/l significantly decreased

dissolved oxygen with increase duration of treatment in culture water. The range of 1.32-4.99 mg/l means an increased devolution of oxygen in the culture system due to 0.03mgCd/l.

Water culture in group C, treated 0.30mgPb/l had a range of 0.84-6.25mg/l. At day 7, there was a significant ($p \leq 0.05$) decrease, at day 30, there was a significant ($p \leq 0.05$) decrease, at day 60, there was a significant ($p \leq 0.05$) decrease and at day 90, there was a significant ($p \leq 0.05$) increase. These results showed that 0.30mgPb/l significantly decreased dissolved oxygen with increase duration of treatment in culture water until at day 90. The increase at day 90 may mean that photosynthetic plants may have improved their oxygen production due to the little rain shower that occurred at day 87 of the study which may have agitated the water and improved the dissolve oxygen. The significant ($p \leq 0.05$) increase at day 90 suggests that photosynthetic plants responded better to the little rain shower agitation in a more contaminated treatment probably due to higher level of starvation. The range of 0.84-6.25mg/l means an increased devolution of oxygen in the culture system due to 0.03mgPb/l.

The DO level in all the groups were below the recommended standard level, this made the treatments unfavourable after acclimation to the end of the experiment with no mortality recorded probably because the fish are small in size and African catfish are obligate air breathers and therefore more tolerant of low DO levels. However, the rate of devolution of DO was lower in groups treated with garlic. Generally, concentrations below 5 mg/L may adversely affect the functioning and survival of biological communities and below 2 mg/L may lead to the death of most fish (Chapman, 1997).

3.4.4.7: Effect of treatment on Cadmium (mg/L)

Table 3.15: Effect of treatment on Cadmium (mg/L) Means ± SEM of *Clarias gariepinus* day 0 after 14 days acclimation before treatment/exposure, and day 7, 30, 60 and 90 of treatment/exposure in B

Treatment/exposure days	Cadmium
0	0.00 ± 0.00
7	0.00±0.00
30	0.04±0.01 [a]
60	0.01±0.01 [a]
90	0.01±0.01 [a]

Treatments: B: 0.03mgCd/l.
Means with the same letter on the same row are not significantly different according to DMRT at P≥0.05.
n = 2

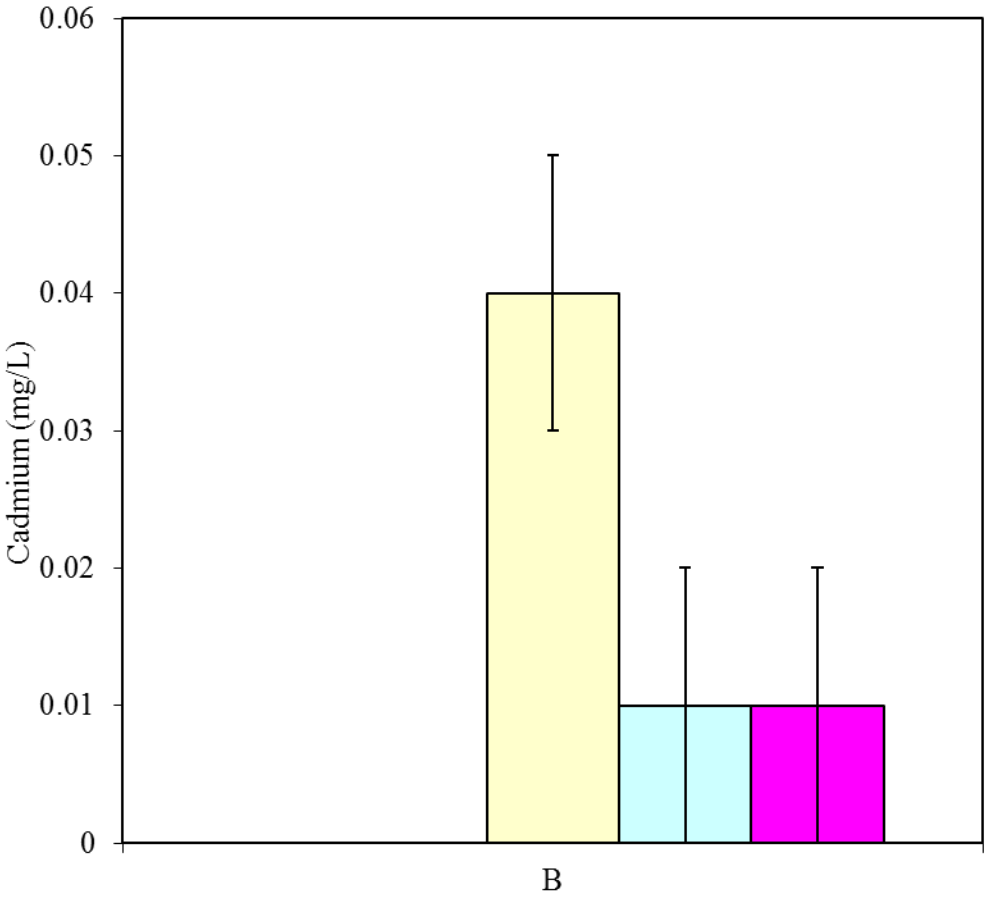


Figure 3.5: Effect of duration of exposure on Cadmium in water

Cadmium in group B, at day 0, was 0.00 ± 0.00mg/l; at day 7, was 0.00 ± 0.00mg/l; at day 30, was 0.04±0.01mg/l; at day 60, was 0.01±0.01mg/l, and at day 90, was 0.01±0.01mg/l. (Table 3.15).

Table Table 3.15 showed the level of Cadmium (Cd) in group 2 at different days of exposure. The order of significant ($P \leq 0.05$) difference was day day 0, 7 < 60, 90 < 30.

3.4.4.8: Discussion of effect of treatment on Cadmium (mg/L)

Water culture in group B, treated with 0.03mgCd/l had a range of 0.000-0.042mg/l. The concentration of cadmium in the water before acclimation was not detectable. At day 0 and 7 cadmium was not detectable probably due to low solubility and concentration. However, at day 30, the significant increase obtained may probably be due to increased solubility and/or excretion into water by fish. At day 60 and 90, since the pH was lower than day 30, it is espected that solubility should be higher therefore, the significant increases in cadmium water may be due to increased uptake and/or decreased excretion by fish not due to decreased solubility.

3.4.5: Gross and histopathology

3.4.5.1: Results of gross and histopathology

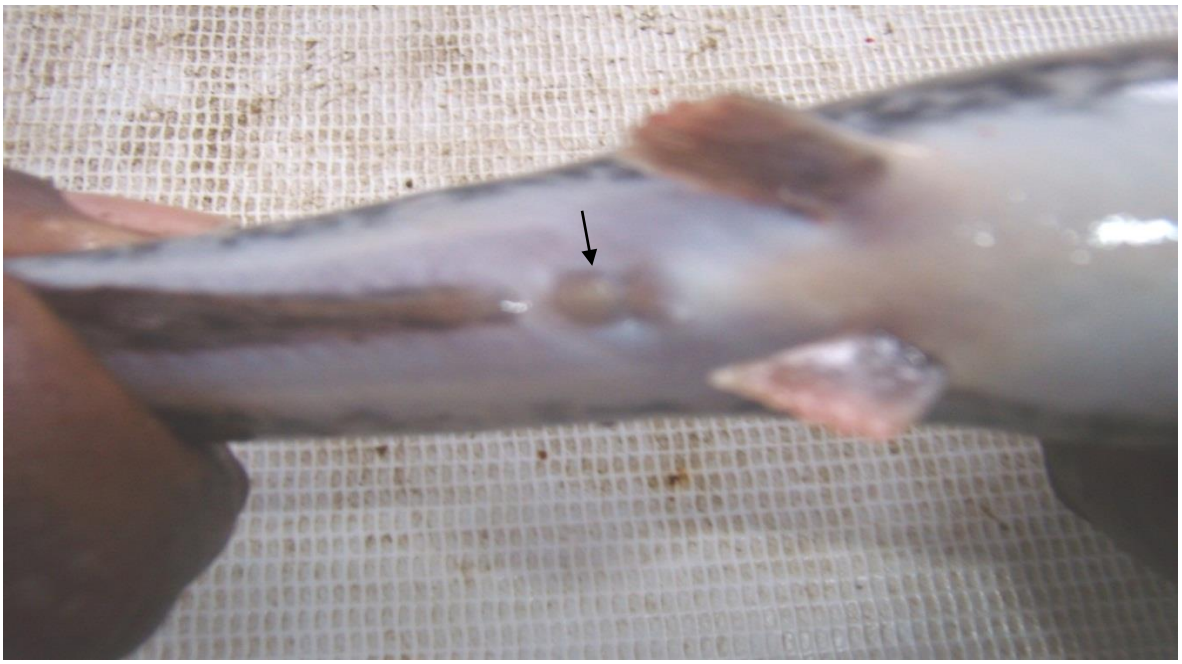


Plate 3.1: Females *Clarias gariepinus* blunt urogenital pappila (black arrow).

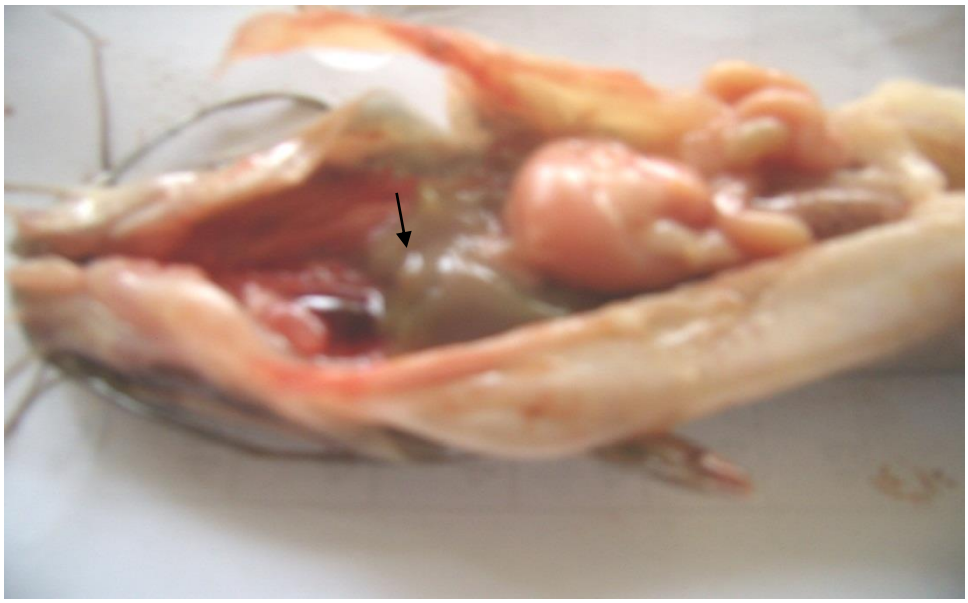


Plate 3.2: Diffuse greenish discolouration of the liver on day 30 (black arrow) Treatments: 8.0g/l raw garlic

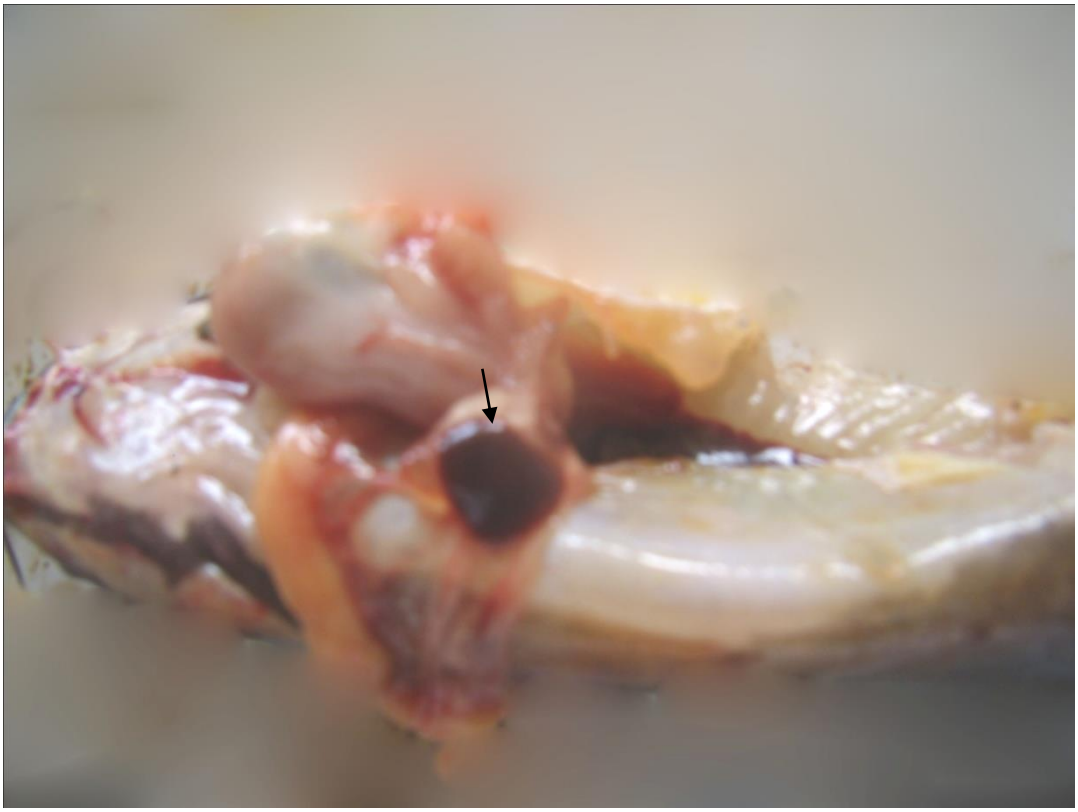


Plate 3.3: Enlarged spleen on day 30 (black arrow)
Treatment: 0.03mg/l cadmium

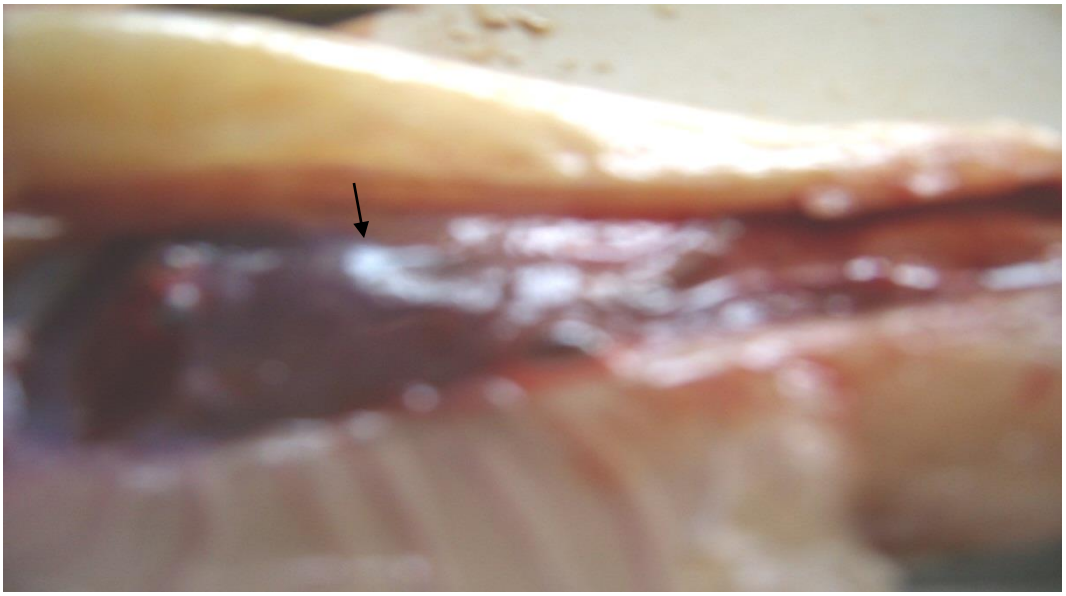


Plate 3.4: Enlarged kidney on day 30 (black arrow)
Treatment: 0.3mg/l lead

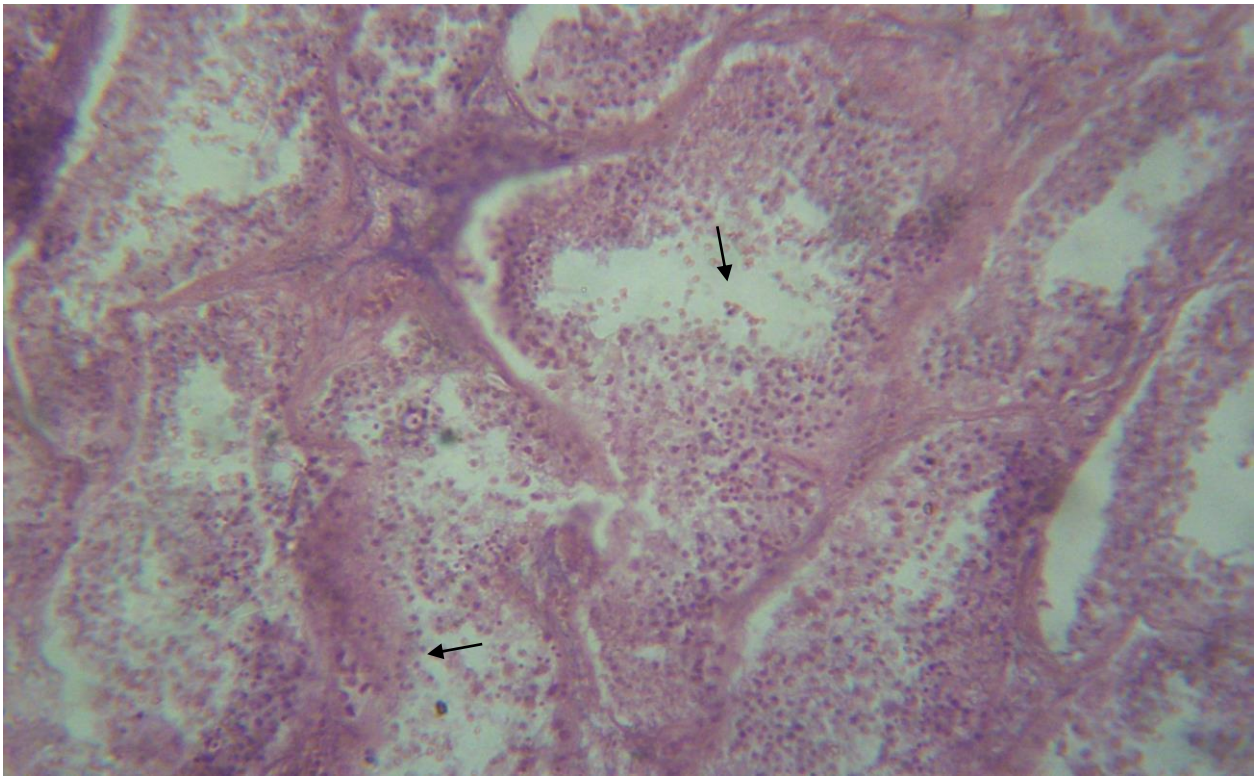


Plate 3.5: Seminiferous tubules with scanty (spent) germinal cells in the testis (arrow) on day 30 HEx 400. Treatment: 0.03mgCd/l

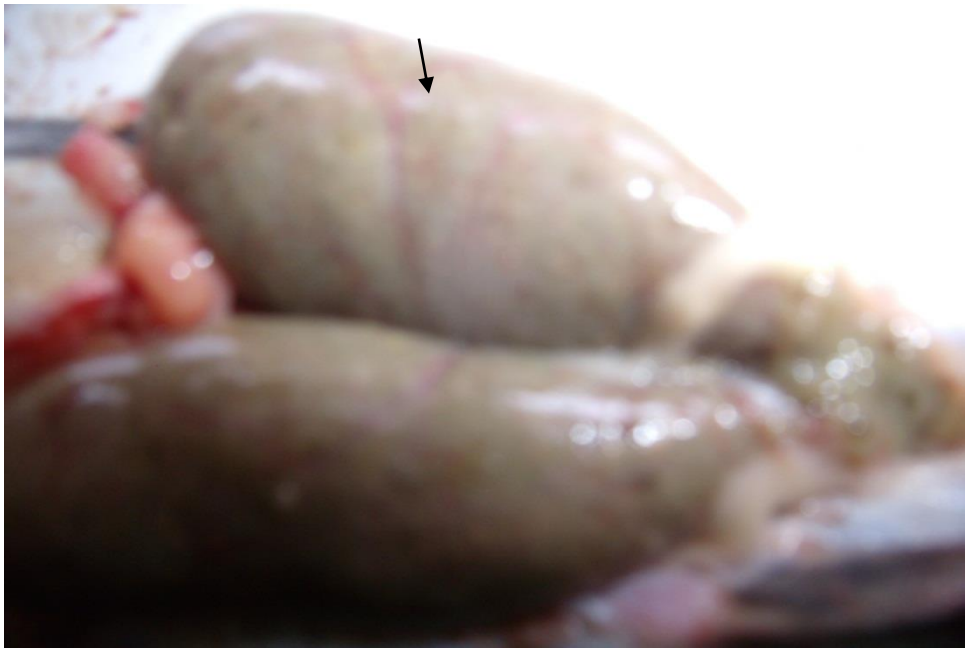


Plate 3.6: Females *Clarias gariepinus* with gravid ovary on day 60 (arrow). Treatment: 8.0 raw garlic/l at day 60

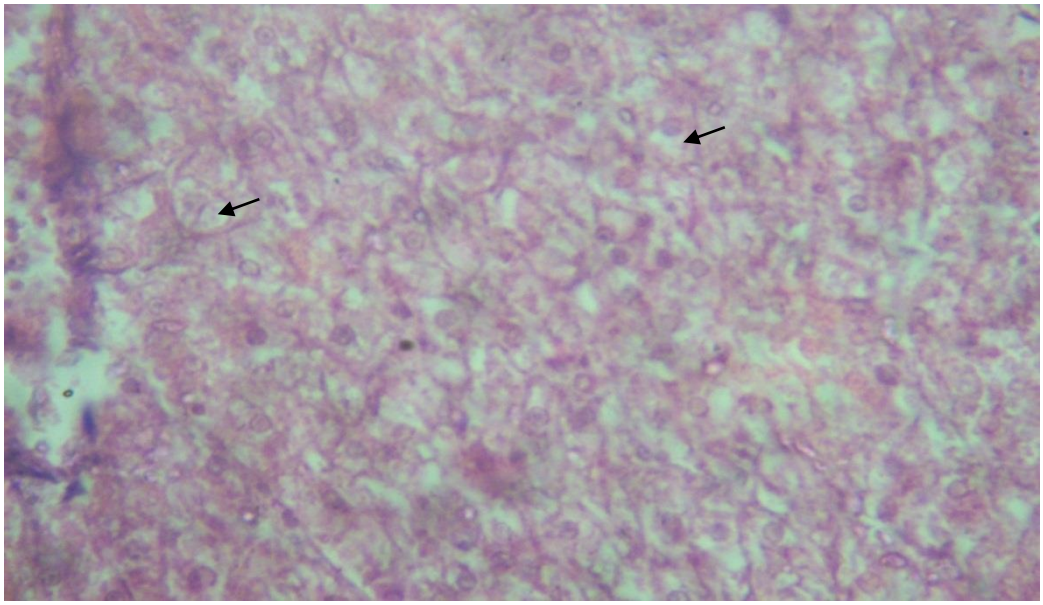


Plate 3.7: Severe diffuse hepatocellular degeneneration (swelling) on day 90 (arrows). HE x400.
Treatment: 0.3mg/l lead

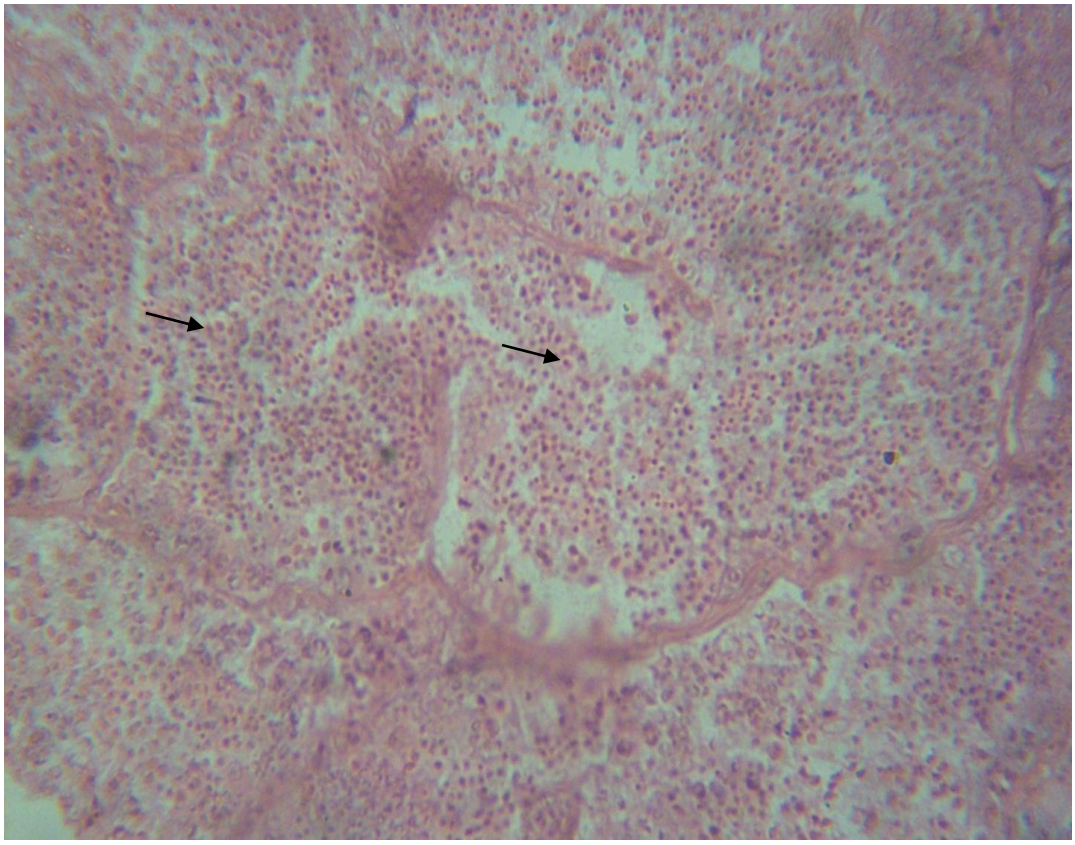


Plate 3.8: Normal tubules and stages of spermatic cells in the testis on day 90 (arrows) HE x400.
Treatment: 8.0g/l raw garlic

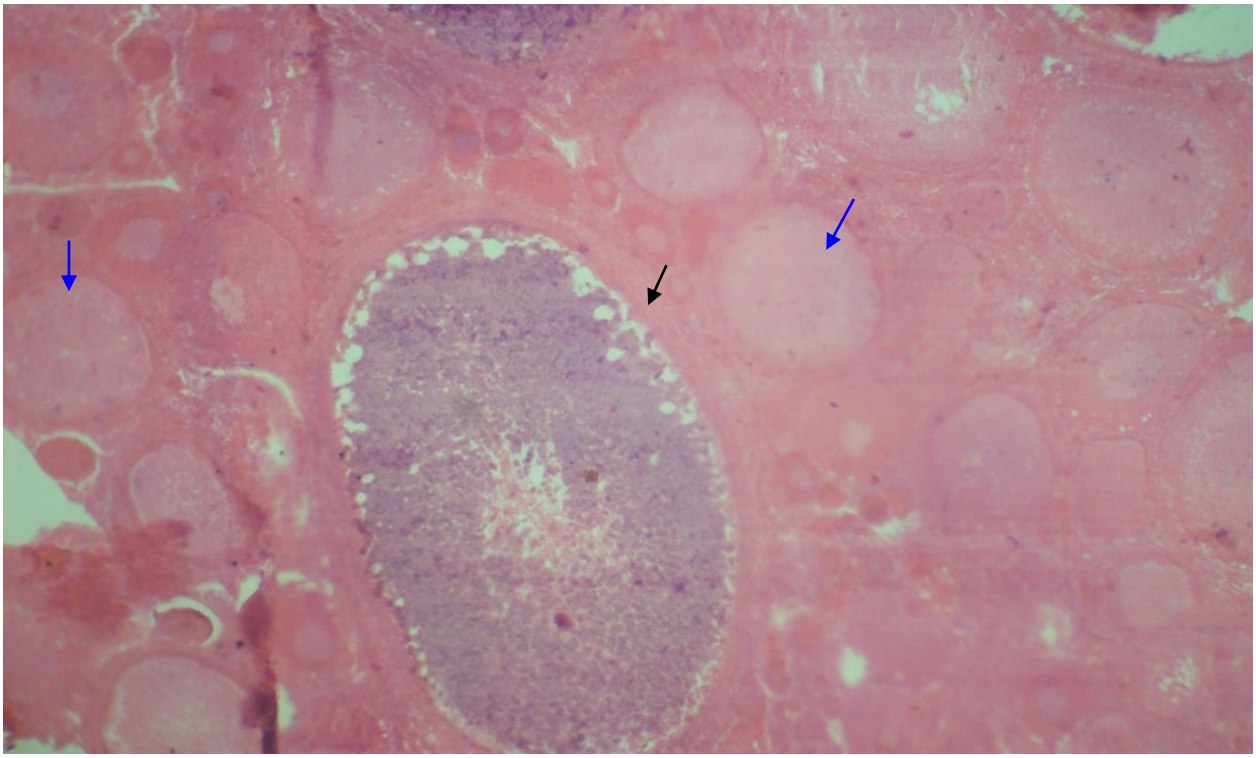


Plate 3.9: Different oval stages in the ovary, mature (black arrow) and immature (blue arrow) on day 90 (HE x400).

Treatment: 8.0g/l raw garlic

3.4.5.2: Discussions on clinical signs, gross and histopathology

The *Clarias gariepinus* females and males had blunt and pointed urogenital papillae, respectively. At day 2 and 3 few fish treated with garlic in group 1 had reduced appetite, 40% swimming near the water surface in form of vertical positioning (‘hangers’) with no mortality, may be due to stress caused by the high level of manganese in garlic but returned to normal as in at the fifth day oftreatment/exposure. These mean that 8.0g raw garlic/l concentration may be high and toxic for young *Clarias gariepinus*. Throughout the study fish exposed to 8.0g raw garlic/l after day 3 and those exposed to 0.03mgCd/l, 0.30mgPb/l and the control had good appetite probably due to the restricted feeding. *Clarias gariepinus* exposed to 8.0g raw garlic/l and the control at the end of the study had less slimy body than *Clarias gariepinus* exposed 0.03mgCd/l and 0.30mgPb/l. The trend of slimy body was in the order 8.0g raw garlic/l < control < 0.03mgCd/l < 0.30mgPb/l. These mean that dissolved oxygen need deficiency is more in the same order. Yellowish discoloration of the ventral part of the skin was observed at day 30 which disappeared in group 2 and 3 at the end of the study. These mean that yellowish discoloration may be an indication of acute toxicity of 0.03mgCd/l and 0.30mgPb/l. The trend of yellowish discoloration was in order 0.03mgCd/l < 0.30mgPb/l. In *Clarias gariepinus* for the production of mucus on the skin was 8.0g raw garlic/l < control < 0.30mgPb/l < 0.03mgCd/l. These mean that dissolved oxygen need deficiency is more in the same order. Post mortem examination for *Clarias gariepinus*, exposed to 8.0g raw garlic/l revealed apparently normal diffused greenish discoloration of the liver at day 30. This mean garlic was easily absorbed in high concentration and the detoxification may be slow because of the apparent greenish discolorationof the liver to be at day 30. At day 30 the ovaries were apparently normal however at day 60 which was equivalent to 5months old *Clarias gariepinus*, the ovary became gravid and at day 90 different ova stages were observed. This may mean 8.0g raw garlic/l enhanced the reproductive processes. Also the kidney at 90 was apparently normal. This apparently normal gross pathology was due to garlic. These are in agreement with Lawson, (1996) who reported that *Allium sativum* helps the liver to maintain its normal function by accelerating the regenerative capacity of its cells. In addition to its direct intragastric effects, its antimicrobials are not affected by acid environments but rather gastric juice enhances the antimicrobial activity of *Allium sativum* constituents (Fortunator, 1990).

Post mortem examination for *Clarias gariepinus*, exposed to no treatments (control) revealed various mild changes. Post mortem examination for *Clarias gariepinus*, exposed to 0.03mgCd/l; 0.30mgPb/l revealed various degree of inflamed and enlarged bile duct, enlarged spleen, kidney and liver. Histopathological changes for fish exposed to 8.0g raw garlic/l at day 90, which is the end of the study revealed mild diffused hepatic vacuolations at day 90, no visible lesion in the kidney and testis. Histopathological changes for fish exposed to 0.03mgCd/l; 0.30mgPb/l; no treatments (control) revealed various degree of histopathological changes. These changes included severe and marked mucosal erosion by the treatment of 0.3mgPb/l and 0.03Cd/l at day 90, severe diffuse hepatic vacuolations by 0.3mg/l lead treatment at day 90, mild diffuse vacuolation of hepatocytes by 8.0g/l raw garlic, 0.03mg/l cadmium, 0.3mg/l lead treatment at day 90, no visible lesion in the kidney by 8.0g/l raw garlic, mild interstitial congestion, seminiferous tubules with scanty (spent) germinal cells by 0.03mgCd/l at day 30, necrotic material interspersed with germinal cells in the seminiferous tubular lumen mild interstitial congestion by 0.3mgPb/l treatment at day 90. These pathological changes seen in the liver are in agreement with the findings by various authors that Lead nitrate exerted some histological effects on the hepatic tissue in the form vacuolation of hepatic cells (Teh *et al*., 1997; Wolf and Wolfe, 2005; Bothaina *et al*., 2012;). Cadmium exposure leads to liver degenerative changes (Jalaludeen *et al*., 2012); the liver showed conspicuous changes in centralobular area, cord disarray and connective tissues damage as well as focal necrosis (Gbem *et al*., 2001). Mcleay, (1973) reported atrophy of the interregnal tissue in the head of kidney of juvenile coho salmon due to cadmium exposure. Cadmium exposure leads caused severe damage and disorganization of tubules, glomerular edema and necrosis (Jalaludeen *et al*., 2012). In the intestine the common histopathological changes under heavy metal exposure were disintegration of mucosal epithelium, hypertrophied epithelial cells, increased number of goblet cells, sloughed off epithelial cells and mucous in the lumen, degeneration of the connective tissue of sub-mucosa and hyperemic blood vessels (Shawkat *et al*., 2010). Sastry and Gupta (1979) observed degeneration of hypertrophied villi in *H fossilis* exposed to cadmium.

The pathological changes severity increased with exposure duration in this study and this is in agreement with (Deore and Wagh 2012; Jalaludeen *et al*., 2012). 8.0g raw garlic/l exposure showed no visible lesion in gross pathology and histopathology is in agreement

with Fazlolahzadeh., *et al.*, (2011); *Lawson*, (1996); Fortunator, (1990); Faisal (2003). Histopathological changes induced by 0.03mgCd/l and 0.3mgPb/l are in agreement withseveral authurs including Shawkat *et al*., 2010); (Bothaina *et al*., 2012). The reproductive disturbances by 0.03mgCd/l and 0.3mgPb/l is in agreement with Rurangwa *et al.*, (1998); Kime, (1995); Kime *et al*., (2001); Song *et al*., (2002); Drevnick and Sandheinrich, (2003); Webb *et al*., (2006); Hinck *et al*., (2007) as cited by Ebrahimi and Taherianfard, (2011).

3.4.6: Liver tissue accumulation of metals

3.4.6.1: Result of effect of sex on liver tissue accumulation of Lead

Table 3.16: Effect of sex on liver tissue accumulation of Lead (Pb) means ± SEM (µg/g) of *Clarias gariepinus* at day 30, 60 and 90 of Treatment/exposure in culture groups

Treatment/exposure days	Group A		Group B		Group C		CTL (Control) Group	
	♂ a^1	♀ b^1	♂ a^2	♀ b^2	♂ a^3	♀ b^3	♂ a^4	♀ b^4
30	0.35 ± 0.01^{b}	$1.03\pm 0.03^{**b}$	0.43 ± 0.03^{b}	$1.11\pm0.03^{**b}$	0.57 ± 0.05^{a}	$1.66\pm0.07^{**a}$	0.34 ± 0.02^{b}	$1.06\pm0.02^{**b}$
60	0.24 ± 0.02^{d}	$0.56\pm 0.10^{*c}$	0.41 ± 0.01^{b}	$2.09\pm0.15^{** b}$	0.63 ± 0.01^{a}	$3.48\pm0.14^{**a}$	0.36 ± 0.01^{c}	$1.74\pm0.14^{**b}$
90	0.07 ± 0.02^{c}	$0.26\pm0.02^{**d}$	0.59 ± 0.03^{b}	$3.41\pm0.12^{**b}$	1.42 ± 0.10^{a}	$5.31\pm0.09^{**a}$	0.49 ± 0.02^{b}	$3.06\pm0.03^{**c}$

[a]fish *Clarias gariepinus* males. [b]fish *Clarias gariepinus* females. Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l. CTL: Nil. Means with the same letter on the same row are not significantly different according to DMRT at $P\geq0.05$. n = 6

Table 3.16 shows liver accumulation of lead by the females and males. At day 30; in group A the females had highly significantly (P≤0.001) higher value of 1.03± 0.03µg/g than the males with 0.35±0.01µg/g; In group B the females had highly significantly (P≤0.001) higher value of 1.11±0.03µg/g than the males with 0.43±0.03µg/g; In group C the females had highly significantly (P≤0.001) higher value of 1.66±0.07µg/g than the males with 0.57±0.05µg/g; In CTL (control) group the females had highly significantly (P≤0.001) higher value of 1.06±0.02µg/g than the males with 0.34±0.02µg/g.

At day 60; in group A the females had significantly (P≤0.05) higher value of 0.56± 0.10µg/g than the males with 0.24±0.02µg/g; In group B the females had highly significantly (P≤0.001) higher value of 2.09±0.15µg/g than the males with 0.41±0.01µg/g; In group C the females had highly significantly (P≤0.001) higher value of 3.48±0.14µg/g than the males with 0.63±0.01µg/g; In CTL (control) group the females had highly significantly (P≤0.001) higher value of 1.74±0.14µg/g than the males with 0.36±0.01µg/g.

At day 90; In group A the females had significantly (P≤0.05) higher value of 0.26±0.02µg/g than the males with 0.07±0.02µg/g; In group B the females had highly significantly (P≤0.001) higher value of 3.41 ±0.12µg/g than the males with 0.59±0.03µg/g; In group C the females had highly significantly (P≤0.001) higher value of 5.31±0.09µg/g than the males with 1.42±0.10µg/g; In CTL (control) group the females had highly significantly (P≤0.001) higher value of 3.06±0.03µg/g than the males with 0.49±0.02µg/g.

Table 3.16 also shows Liver levels of Lead in various groups. Lead (Pb) levels in group A treated with 8.0g raw garlic/L order of significant ($P \leq 0.05$) difference with days of exposure was 90 < 60 < 30 for female and male. Lead (Pb) levels in group B treated with 0.03mgCd/L order of significant ($P \leq 0.05$) difference with days of exposure in female was 30 < 60 < 90 whereas in male was 60, 30 < 90. There was no significant ($P \geq 0.05$) difference at day 30 and 60. Lead (Pb) levels in group C treated with 0.3mgPb/L order of significant ($P \leq 0.05$) difference with days of exposure in female was 30 < 60 < 90 whereas in male was 30, 60 < 90. There was no significant ($P \geq 0.05$) difference at day 30 and 60. Lead (Pb) levels in CTL (control) group with no treatment order of significant ($P \leq 0.05$) differences with days of exposure in female was 30 < 60 < 90 whereas in male was 30, 60 < 90. There was no significant (P≥0.05) difference at day 30 and 60.

Table 3.16 showed result of comparative study of the order of significant ($P \leq 0.05$) differences in Liver levels of Lead (Pb) of sex across the groups at day 30, 60 and 90 of

exposure. At day 30, the order of significant ($P \leq 0.05$) differences was group C < A, C, CTL in female and male. At day 60, the order of significant ($P \leq 0.05$) differences was in female group C < B, CTL < A whereas in male was group C < B < CTL < A. At day 90, the order of significant ($P \leq 0.05$) differences was in female group C < B < CTL < A whereas in male was group C < B, CTL < A.

3.4.6.2: Discussions on effect of sex on liver tissue accumulation of Lead

Treatment with 8.0g raw garlic/l showed a non-significant decrease in the liver of both males and females and could probably be by garlic complexation with lead in water reducing its availability in its ionic state for easier uptake and while in the liver also the complexation by component of garlic increases its excretion. Females accumulated more significantly lead than males.

Treatment with 0.03mgCd/l resulted in a lower significant decrease at day 60. This could be due to uptake by the fish but the levels returned to a non-significant level at day 90 probably because that there was an increase bioavailability of lead with increased exposure time. At day 60 the liver of the females had lower non-significant higher means than the males probably due to the fact that cadmium which affects males more, increased acutely the uptake and subsequently reduced the complexation and excretion of lead in males than the females but with prolonged exposure, the females accumulated a non-significantly higher lead than the males. Lead liver accumulation increased non-significantly with exposure time in both males and females due to bioaccumulation and reduced excretion.

The treatment with 0.03mgPb/l showed that there were significant increases of lead in water with increased exposure due to increase bioavailability. The males also exhibited a lower non-significant mean than the females with exposure time. This may mean that the females accumulated lead easily than the males. Liver accumulation of lead decreased with exposure time with both males and females.

Control (CTL) group showed that there was a non-significant increase in liver accumulation of lead in both the males and females with exposure time.

3.4.6.3: Result of effect of sex on liver tissue accumulation of Cadmium

Table 3.17: Effect of sex on liver tissue accumulation of Cadmium (Cd) means ± SEM (µg/g) of *Clarias gariepinus* at day 30, 60 and 90 of Treatment/exposure in culture groups

Treatment/exposure days	Group A		Group B		Group C		CTL (Control)	
	♂ a^1	♀ b^1	♂ a^2	♀ b^2	♂ a^3	♀ b^3	♂ a^4	♀ b^4
30	0.00±0.00^{c}	0.00±0.00^{c}	4.24±0.29^{a}	1.25±0.24**^{b}	0.84±0.15^{b}	1.64±0.03*^{a}	0.47±0.19^{bc}	1.06±0.02*^{b}
60	0.00±0.00^{d}	0.00±0.00^{d}	9.23±0.32^{a}	4.89±0.16**^{a}	3.04±0.06^{b}	1.41±0.05**^{b}	1.75±0.21^{c}	1.07±0.03*^{c}
90	0.00±0.00^{d}	0.00±0.00^{d}	12.64±0.44^{a}	5.55±0.13**^{a}	3.48±0.10^{b}	2.15±0.07**^{b}	2.29±0.07^{c}	1.90±0.06*^{c}

[a]fish *Clarias gariepinus* males. [b]fish *Clarias gariepinus* females. Treatments: A: 8.0g raw garlic/l. B: 0.03mgCd/l. C: 0.30mgPb/l.
CTL: Nil. Means with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n = 6.

Table 3.17: Shows liver accumulation of cadmium by the females and males. At day 30; in group A, cadmium was not detected in female and male; In group B, the females had highly significantly (P≤0.001) lower value of 1.25±0.24µg/g than the males with 4.24±0.29µg/g; In group C the females had significantly (P≤0.05) higher value of 1.64±0.03µg/g than the males with 0.84±0.15µg/g; In CTL (control) group, the females had significantly (P≤0.05) higher value of 1.06±0.02µg/g than the males with 0.47±0.19µg/g.

At day 60; in group A, cadmium was not detected in female and male; In group B, the females had highly significantly (P≤0.001) lower value of 4.89±0.16µg/g than the males with 9.23±0.32µg/g; In group C, the females had highly significantly (P≤0.001) lower value of 1.41±0.05µg/g than the males with 3.04±0.06µg/g; In CTL (control) group, the females had significantly (P≤0.05) lower value of 1.07±0.03µg/g than the males with 1.75±0.21µg/g.

At day 90; in group A, cadmium was not detected in female and male; In group B, the females had highly significantly (P≤0.001) lower value of 5.55±0.13µg/g than the males with 12.64±0.44µg/g; In group C, the females had highly significantly (P≤0.001) lower value of 2.15±0.07µg/g than the males with 3.48±0.10µg/g; In CTL (control) group the females had significantly (P≤0.05) lower value of 1.90±0.06µg/g than the males with 2.29±0.07µg/g.

Table 3.17 also showed liver level of cadmium in various groups. Cadmium (Cd) levels in group A, treated with 8.0g raw garlic/l order of significant ($P \leq 0.05$) difference showed no significant differences at day 30, 60 and 90 in female and male. Cadmium (Cd) levels in group B, treated with 0.03mgCd/L order of significant ($P \leq 0.05$) differences was $30 < 60 < 90$ in female and male. Cadmium (Cd) levels in group C treated with 0.3mgPb/l, order of significant ($P \leq 0.05$) differences in female was $60 < 30 < 90$ whereas in male was $30 < 60 < 90$. Cadmium (Cd) levels in control group with no treatment order of significant ($P \leq 0.05$) differences in female 60, $30 < 90$ whereas in male was $30 < 60 < 90$. There was no significant ($P \geq 0.05$) difference at day 30 and 60 in female.

Table 3.17 showed the result of comparative study of Liver levels of Cadmium (Cd) across the groups at day 30, 60 and 90. At day 30, the order of significant ($P \leq 0.05$) differences was in female group A < B, CTL < C whereas in male was group A< C < B. There was no significant ($P \geq 0.05$) difference in group A and CTL, group C and CTL. At day 60, the order of significant ($P \leq 0.05$) difference was in female group A< CTL < C< B in female and male. At day 90, the order of significant ($P \leq 0.05$) difference was group A< CTL < C< B in female and male.

3.4.6.4: Discussions on effect of sex on liver tissue accumulation of Cadmium

Cadmium levels in group A with 8.0g raw garlic/l treatment, at day 60, the males' accumulated lower cadmium while at 90, the males' accumulated higher but non-significant levels of cadmium than the females. This could probably be due to the differential accumulation of cadmium. There was a non-significant decrease in the females. This could be due to low excretion of cadmium however it appeared that garlic in the medium assisted in making the females to excrete cadmium more than the males.

Treatment with 0.03mgCd/l in group B, the males accumulated cadmium higher although non-significantly than the females which increased with exposure time may mean that males' accumulated cadmium the liver more than females and excretion did not take place during the exposure duration probably due to the low excretion rate of cadmium

Treatment with 0.3mgPb/l in group C, cadmium levels were higher though non-significant in the males than the females at day 60 and 90 appeared to be because of the increased levels of contaminants (addition of 0.3mgPb/l), there probably was preferential uptake and accumulation of lead by the females as a result the male preferential bioaccumulation of cadmium was higher than the females. With regard to accumulation and excretion, the males had a non-significant decrease at day 90 while the females had a non-significant increase. This probably could be because with a higher accumulation of lead in the females compared with that of the males at day 90, the excretion of cadmium was low in the females resulting in a non-significant increase. It appeared that males excreted lead more easily than the females while the females excrete cadmium more easily than the males.

Control (CTL) group with no treatment at day 60, probably because the level of cadmium in water was low 0.00mg/L before acclimatisation, the males' accumulated lower but non-significant levels of cadmium than the females while at day 90 of exposure, with increase exposure time and level of cadmium in water increased solubility due probably decrease pH, the males accumulated more although non-significantly than the females. In comparism with accumulation in both the males and the females with increase time of exposure there was non-significant increase and decrease respectively. This appears to indicate that at low level of cadmium in water, the females accumulate cadmium faster and also excretes it faster than the males.

Present results indicate that garlic is effective in reducing Cd accumulation in the liver of *C.* gariepinus. Particulate organic matter can scavenge metal from water and help to reduce

metal from fish. These results are in agreement with Santschi (1988) as reported by (Osman *et al*., 2009) that any agent that can remove Cd from water helps to reduce the bioaccumulation of this metal in fish. Cd accumulation in liver, gills and musculature of fish exposed to Cd alone was higher than that of garlic. These results suggest that garlic could chelate Cd ions producing a stable complex, thus reducing the chance for metal uptake by tissues and excretion of Cd would reduced the metal burden in tissues.

CHAPTER FOUR

DETERMINATION OF LETHAL CONCENTRATION (LD_{50}-96 HR) AND (LC_{100}-96 HR) OF CADMIUM NITRATE ($Cd(NO_3)_2$) AND LEAD NITRATE ($Pb(NO_3)_2$) IN FEMALE AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

4.1 Introduction

The 96-hr L_D50 values of fish vary from species to species and from metal to metal as documented by various authors as reported by Zeynab *et al*., (2012). The result in chapter 3 of this thesis revealed significant differences in responses in female and male *Clarias gariepinus* to cadmium and lead including garlic exposure. Therefore, the purpose of this study was to determine L_D50 of $Cd(NO_3)_2$, and Pb $(NO_3)_2$ in female *Clarias gariepinus* fish species, from exposure to different Cd and Pb concentrations during wet season.

4.2 Methodology

Ninety apparently normal 11 weeks old *Clarias gariepinus* females were purchased from a commercial fish farm in Ibadan. The Mean and standard error of mean of lengths and weights for acute cadmium (Cd) testing was approximately 13.67 ± 0.06cm and 21.81±0.30g respectively. The Mean and standard error of mean of lengths and weights for acute lead (Pb) testing was approximately 13.41 ± 0.06cm and 20.20±0.26g respectively. Prior to toxicity testing, the fishes were acclimatized for 14 days in a fish rearing facility with approximately 12hour light: 12hour dark. They were fed with fish feeds to satiation at 10.00 hours and 17.00 hours respectively and the water was renewed every other day.

Stock solutions of metal were prepared with deionized water. A 96-hour daily static renewal acute toxicity was conducted following the methods described by Adeyemo *et al*., 2007. Acclimatized fish were randomly stocked at six (6) fish per group (A, B, C, D and E) in 20 liters of non-chlorinated water based on the concentrations of cadmium and lead nitrate. For cadmium toxicity testing female were exposed to 0, 22, 32, 42 and 52mg/l. For Lead toxicity fish were exposed to 0.0, 72, 82, 92 and 102mg/l. The experiment was set up in triplicates. Fish allotted to group A served as the control. Fish were observed at 2 hours' intervals for the first 12 hours after and were observed at 6 hours' interval for mortality. During the toxicity test, the fishes were not fed. The numbers of dead fish were counted daily and removed immediately from each group. The concentration of each heavy metal that caused 50% mortality in fish for 96hour was taken as the LD50 value, calculated by method described by Adeyemo *et al.*, (2008).

4.3: Results

Table 4.1: Mortalities of Female Africa Catfish *Clarias gariepinus* recorded at different concentration mg/l of cadmium (Cd) during 96hr

	CD Concentration mg/l				
Duration (hr)	Control	22	32	42	52
24	0	0	0	0	1
48	0	0	0	1	1
72	0	0	1	2	2
96	0	1	2	2	2

Table 4.2: Mortalities of Female Africa Catfish *Clarias gariepinus* recorded at different concentration mg/l of Lead (Pb) during 96hr

	CD Concentration mg/l				
Duration (hr)	Control	72	82	92	102
24	0	0	0	0	0
48	0	0	0	1	1
72	0	0	0	1	2
96	0	1	3	3	3

Table 4.3: 96 hours' Cadmium LD 50 determination in female *Clarias gariepinus* based on Arithmetic method

Concentration mg/l	Concentration difference	Number alone	Number dead	Mean mortality	Mean mortality dose difference
0	-	6	0	-	0
22	22	5	1	0.5	11.0
32	10	3	3	2	20.0
42	10	1	5	4	40.0
52	10	0	6	5.5	55.0
					126.0

$$LD50 = LC100 - \frac{\sum \text{mean mortality x concentration difference}}{\text{No. of organism in a group}}$$

$$= 52 - \frac{126}{6}$$

$$= 52-21$$

31.0mg/l

Table 4.4: 96 hours Lead LD 50 determination in female *Clarias gariepinus* based on Arithmetic method

Concentration mg/l	Concentration difference	Number alone	Number dead	Mean mortality	Mean mortality dose difference
0	-	6	0	-	0
72	72	5	1	0.5	36.0
82	10	3	3	2.0	20.0
92	10	1	5	4.0	40.0
102	10	0	6	5.5	55.0 151.0

$$LD50 = LC100 - \frac{\sum \text{mean mortality x concentration difference}}{\text{No. of organism in a group}}$$

$$= 102.0 - \frac{151}{6}$$

$$= 102.0 - 25.2$$

76.8mg/l

4.4 Discussion

This study was done to assess the sensitivity of the female *Clarias gariepinus* to cadmium, and lead through determination of acute 96-h LC50 values induced from exposure to different concentrations of the introduced heavy metals. The results obtained implies that female *Clarias gariepinus* appears to be more tolerant of the toxicant burden of lead than the toxicant burden of cadmium. It was found that in the control groups mortality was zero during the test.

4.5 Conclusion

The 50% lethal concentrations (LD50-96h) of $Cd(NO_3)_2$ and Pb $(NO_3)_2$ for female *Clarias gariepinus* were 31.0, and 76.8mg/l respectively. Also 96-h LC100 of $Cd(NO_3)_2$ and Pb $(NO_3)_2$ was 52 and 102mg/l, respectively. The Mean and standard error of mean of lengths and weights for acute cadmium (Cd) testing was approximately 13.67 ± 0.06 and 21.81±0.30g, respectively. The Mean and standard error of mean of lengths and weights for acute lead (Pb) testing was approximately 13.41 ± 0.06cm and 20.20±0.26g respectively.

CHAPTER FIVE

EVALUATION OF MAXIMIUM EFFECTIVE SAFE THERAPEUTIC CONCENTRATION (OPTIMUM) OF RAW GARLIC (*Allium sativum)* IN FEMALE *Clarias gariepinus* CULTURE

5.1 Introduction

Adams, (2003), reported that health impairment could be more accurately determined from observations at several levels of biological organization including genetic, biochemical and histological. The results from chapter 3 of this thesis that showed 8g raw garlic/l acutely caused severe stress with no mortality probably due to low stocking density including significant differences in responses between female and male within the same macro-enviroment. Also there is a dearth of information on the use of raw garlic in the culture of *Clarias gariepinus.* Therefore, this study, goal was to evaluate the least toxicity concentration of garlic with maximium effective benefit of raw garlic homogenate in the culture of female Clarias *gariepinus* using water quality, length- weight relationship including condition indices and histological results.

5.2 Methodology

The garlic cloves were purchased from the Bodija market, in Ibadan. 144 apparently normal 12 weeks old *Clarias gariepinus* of females were purchased from a commercial fish farm in Ibadan. The mean±SEM weights and lengths for the acute toxicity testing was approximately 25.87±0.65g and 15.52±0.26cm respectively. Prior to the acute toxicity testing, the fishes were acclimatized for 14 days under a fish rearing facility with approximately 12-hour light: 12-hour dark. They were fed with fish feed to satiation at 10.00 hours and 17.00 hours respectively and the water was renewed every other day.

A 7 day daily static renewal acute toxicity was conducted to assess the optimum concentration of raw garlic in apparently healthy female *Clarias gariepinus* using histopathological changes as indices of toxicity in accordance with the method as described by Adedapo *et al*., (2009) in addition to water quality changes, length –weight relationship and condition factor parameters. Ninety-six (96) acclamatised female *Clarias gariepinus* were randomly stocked at twelve (12) fish alloted A, B, C, D, E, F, G, H, in 20 liters of non chlorinated water. The groups were exposed to following graded doses of raw garlic homogenate 5 (A), 10 (B), 20 (C), 30 (D), 40 (E), 50 (F) and 60 (G) mg/l, respectively. Fish allotted to group H served as the control. The experiment was set up in duplicates. Fish were

observed at regular interval for changes in behavior. Fish were not fed during the toxicity test. On day 7 from each group, culture water, fish length and weight value, brain and liver tissues were collected from sacrificed fish to obtain changes in water quality, length and weight relationship including condition factor *k* parameters and for histopathology, respectively.

5.2.1: Determination of length-weight relationship parameters and condition factor

Fish standard length, and total length were obtained as described by Anderson and Gutreuter (1983). The body weight was measured to the nearest gram (0.01) using a weighing balance. The relationship of length and weight was calculated using the formular

$W = aL^b$ (Ricker, 1975).

A logarithmic transformation of this formular gives:

Log W = log a + b log L

Where:

W = weight of the fish (g)

L = Total length of the fish (cm)

a = constant (intercept)

b = slope (exponent)

The parameters a (initial growth coefficient) and b (growth coefficient) of the weight – length relationship were estimated by the least – square method from logarithmically transformed data.

Condition factor *K* was calculated using the following formular:

$K = 100\ W/L^3$ (Hile 1936)

Where K - Condition Factor

W - Weight of Fish (g)

L - Total Length of Fish (cm)

Condition factor *K* was also calculated for each set of sample and test of significance of condition factor *K* in relation to each group was done.

5.2.2: Determination of water quality and metal parameters

pH, total solids, dissolved oxygen, approximation of calcium, magnisium and potassium were determined including preparation of tissues for histopathological studies were by the methods described in chapter three of this thesis.

5.2.2.1 Alkalinity

Apparatus:

50ml class A burette, 100ml measuring cylinder, 25ml pipette, conical flask, 1L standard flask.

Reagents:

Reagents of analytical grade, and deionised water were used for analysis.

Sodium Carbonate, 0.1N Standard volumetric solution: 5.300g of anhydrous sodium carbonate, previously dried for 1 hour at 250^0C to 300^0C was dissolved in deionised water and diluted to 1 litre in a volumetric flask.

Hydrochloric Acid, 0.1N Standard Volumetric Solution: 10ml of concentrated hydrochloric acid, about 36% m/m (HN) was added to deionised water and diluted to 1litre. The solution was standardized against the 0.1N sodium carbonate using methyl orange indicator.

Methyl orange indicator solution: 1g of methyl orange was dissolved in deionised water and diluted to 1 litre.

Phenolphthalein indicator solution: 1g of Phenolphthalein was dissolved in 100ml ethanol (90%v/v) and 100ml of water, with stirring.

Procedure:

Alkalinity to phenolphthalein: 100ml of the water sample was placed in a conical flask over a white surface. 2 to3 drops of phenolphthalein indicator were added. If sample turns pink, it was then titrated with 0.1N HCL until the pink colour was just discharged. If no colour was produced, the alkalinity to phenolphthalein was zero.

Total Alkalinity: Few drops of methyl orange indicator solution was added to the same sample to which phenolphthalein was added, If the sample turns yellow, it was titrated with the standard acid until the first perceptible colour towards orange takes place. If the sample is orange without the addition of acid, the total alkalinity is zero. Difficulty experienced in detecting the end point was reduced by placing a second 100ml of sample with the same amount of indicator in a similar container alongside that in which the titration is being carried out, and comparing the colours.

Calculation:

Alkalinity for 100ml sample as mg $CaCO_3$/L

= volume of 0.1N hydrochloric acid (ml) x 50

5.2.2.2: Chloride

Apparatus: Conical flask, 50ml Burette, 100ml measuring cylinder.

Reagent:

Reagents of analytical grade, and deionised water were used for analysis.

Hydroquinone solution: 1.0g of hydroquinone was dissolved in 100ml of water. The solution is freshly prepared when needed.

Nitric acid, approximately 0.05N: 3.2ml concentrated nitric acid; about 70% m/m (16N) was diluted to 1 litre.

Sodium hydroxide, approximately 0.05N: 2g of Sodium hydroxide was dissolved in water and diluted to 1 litre.

Mercury (II) nitrate standard solution: 5.04g of mercury (II) nitrate, $Hg(NO_3)_2.H_2O$, was dissolved in 50ml of water containing 0.5ml of concentrate nitric acid, about 70%m/m (16N). Diluted to 1 liter and filtered. This solution was standardized against 25ml of the sodium chloride standard solution, and adjusted so that

$$1.0ml \equiv 1.0mg\ CL^-$$

Sodium chloride standard solution: 1.648g of sodium chloride, dried at 140^0 C was dissolved in water and diluted to 1 liter, so that

$$1.0ml \equiv 1.0mg\ CL^-$$

Indicator solution: 0.5g of sym-diphenylcarbazone and 0.05g of bromophenol blue was dissolved in 100ml of ethanol (90%v/v), and stored in a brown bottle.

Procedure:

A measured volume (V) of sample containing not more than 20mg of chloride was placed in a conical flask, and diluted or evaporated down as necessary to about 50ml. Chromate and iron (III) ions present in excess of 10mg/L was masked by adding 5ml of hydroquinone solution. 5 to 10 drops of indicator solution were added. when a blue, blue-violet, or red colour developed, 0.05N nitric acid was added drop wise until the colour changed to yellow and 1ml in excess was added. When a yellow or orange colour developed, 0.05N sodium hydroxide was added until the colour change to blue-violet; then 0.05N nitric acid was added until the colour changes to yellow and then 1ml in excess is added. The solution was titrated with mercury (II) nitrate standard solution (Note 1). Blank correction was carried out by titrating 50ml of water by the same procedure.

Calculation:

If V_1 and V_2 were respectively the volumes of mercury (II) nitrate solution required by the sample and blank, then:

$$\text{Chloride, } CL^- \text{mg/L} = \frac{(V_1 - V_2) \times 1000}{V}$$

5.2.2.3: Total hardness

Apparatus: Conical flask, 100ml and 10ml measuring cylinder, 50ml burette.

Reagents:

Sodium Hydroxide 1.0N: 40.0g of sodium hydroxide was dissolved in 1000ml of water.

Buffer (pH 10): 143ml of concentrated ammonium hydroxide (NH_4OH) was added to 16.9g of ammonium chloride (NH_4CL) and diluted to 250ml with water.

Erichrome Black T Indicator: 0.2g of the solid indicator was dissolved in 15ml of triethanolamine plus 5ml of absolute ethanol.

Solid Calcon indicator

Standard EDTA Titrant, 0.01M. 3.723g of sodium ethylaminetetraacetate dehydrate $Na_2H_2EDTA.\ 2H_2O$ which had been dried at 80^0 C for 1hour was accurately weighed and dissolved in warm water. It was then diluted to 1000ml. The solution was standardized against standard calcium solution. The solution was stored in a polyethylene bottle.

Standard calcium solution. 1.000g anhydrous calcium carbonate $CaCO_3$, previously dried at 105^0 C, was weighed into a 250ml conical flask. By placing a small funnel at the neck of the flask, 1:1 HCL was gradually added until all $CaCO_3$ had dissolved. 200ml water was then added and content was boiled for few minutes to expel CO_2. Cooled, and few drops of methyl red indicator added, the color was adjusted to the intermediate orange by adding 3N NH_4OH or 1:1 HCL as required. The solution was quantitatively transferred and diluted to 1000ml in a standard flask.

$$1.0\text{ml} \equiv 1.00\text{mg } CaCO_3$$

Sodium sulphide inhibitor: 5.0g of sodium sulphide, $Na_2S.\ 9H_2O$ was dissolved in 100ml of water and stored in an air tight container.

Procedure:

Total Hardness: To 50ml of sample in a conical flask, 2ml of pH 10 buffer was added followed by 2 drops of Eriochrome Black T, then titrated with 0.01M EDTA from wine-red to blue color end point. The absence of a sharp end-point color change in the titration mean that the indicator had deteriorated or that 1ml of inhibitor must be added.

Calcium Hardness: To 50ml sample in a conical flask, 2ml of NaOH solution or a volume sufficient to produce a pH of 12 to 13 was added followed by the addition of 0.1g of Calcon indicator and stirred, then titrated with 0.01M EDTA with continuous stirring to a blue end-

point. For hard water samples or samples with alkalinity greater than 300mg $CaCO_3$/L, smaller volume of sample was taken and diluted to 50ml before titration. While for samples with low hardness 100ml was taken for analysis. Blank was ran in a similar way as samples, and blank values where subtracted from that of sample.

Calculation:

$$\text{Total hardness as mg } CaCO_3/L = \frac{A \times B \times 1000}{\text{ml sample}}$$

Where

A = ml titrant for sample

B = mg $CaCo_3$ equivalent to 1.0ml EDTA titrant

5.3 Results

Table 5.1: Length - weight relationship and condition factor *K* parameters means±SD of female *Clarias gareipinus* culture exposed to graded concentration (mg/l) of raw garlic

Parameter mg/l	5mg/l	10mg/l	20mg/l	30mg/l	40mg/l	50mg/l	60mg/l	Control
K	1.03 ± 0.14^{abc}	0.91 ± 0.10^{d}	0.99 ± 0.07^{bcd}	1.05 ± 0.14^{ab}	1.08 ± 0.18^{ab}	0.92 ± 0.09^{cd}	1.12 ± 0.11^{a}	0.79 ± 0.11^{e}
A	+0.28	-2.23	-3.86	+0.45	+0.19	+0.16	+0.65	-0.31
B	1.00	3.17	4.70	0.81	1.03	1.03	0.64	1.41
r^2	0.71	0.47	0.91	0.86	0.90	0.61	0.63	0.47

Means with the same letter on the same row are not significantly different (DMRT at $P\geq 0.05$). Number of sample (n) = 12.

Table 5.2: Water quality/essential metals parameters means ±SEM of *Clarias gareipinus* culture exposed to graded concentration (mg/l) of raw garlic during wet season

Parameters (mg/l)	Concentration (mg/l)							
	5	10	20	30	40	50	60	Control
Ph	6.02±0.01^{f}	6.05±0.01^{e}	6.14±0.01^{b}	6.16±0.01^{a}	6.10±0.01^{d}	5.97±0.01^{g}	6.02±0.01^{f}	6.13±0.01^{c}
Alkalinity(mg/l)	91.00±0.100^{d}	81.50±1.50^{e}	111.00±1.00^{b}	91.00±0.100^{d}	101.50±1.50^{c}	111.50±1.50^{b}	91.00±1.00^{d}	121.50±1.50^{a}
Chloride(mg/l)	1.17±0.01^{f}	1.84±0.01^{e}	2.22±0.01^{b}	2.13±0.01^{d}	2.11±0.01^{d}	2.19±0.01^{c}	2.31±0.01^{a}	2.21±0.01^{b}
Total hardness ($MgCaCo_3$mg/l)	114.50±1.50^{d}	123.50±1.50^{a}	119.00±1.00^{bc}	115.50±0.50^{d}	122.50±0.50^{a}	120.50±0.50^{a}	117.50±0.50^{ab}	116.50±0.50^{cd}
Calcium(mg/l)	43.30±0.10^{e}	45.20±0.10^{b}	45.60±0.10^{a}	44.70±0.10^{c}	41.45±0.15^{f}	43.70±0.20^{d}	45.95±0.15^{a}	45.00±0.10^{bc}
Magnesium(mg/l)	2.87±0.02^{c}	3.59±0.02^{a}	1.81±0.02^{fg}	1.68±0.01^{g}	3.32±0.02^{b}	2.95±0.15^{c}	1.96±0.14^{ef}	2.17±0.01^{de}
TDS(mg/l)	162.00±1.00^{e}	167.00±1.00^{b}	172.00±1.00^{a}	163.00±0.50^{cde}	162.50±0.50^{de}	161.50±0.50^{e}	164.50±0.50^{cd}	165.50±0.50^{cd}
DO(mg/l)	4.68±0.02^{e}	5.20±0.02^{c}	4.15±0.02^{f}	5.89±0.12^{b}	5.13±0.02^{c}	4.98±0.02^{d}	6.18±0.02^{a}	5.99±0.02^{b}
Potassium(mg/L)	0.26±0.01^{b}	1.90±0.01^{a}	0.12±0.01^{c}	0.14±0.01^{c}	0.12±0.02^{c}	0.23±0.02^{b}	0.24±0.02^{b}	0.12±0.02^{c}

TDS: Total dissolved solids. DO: Dissolved oxygen. Means with the same letter on the same row are not significantly different according to DMRT at P≥0.05 n=2

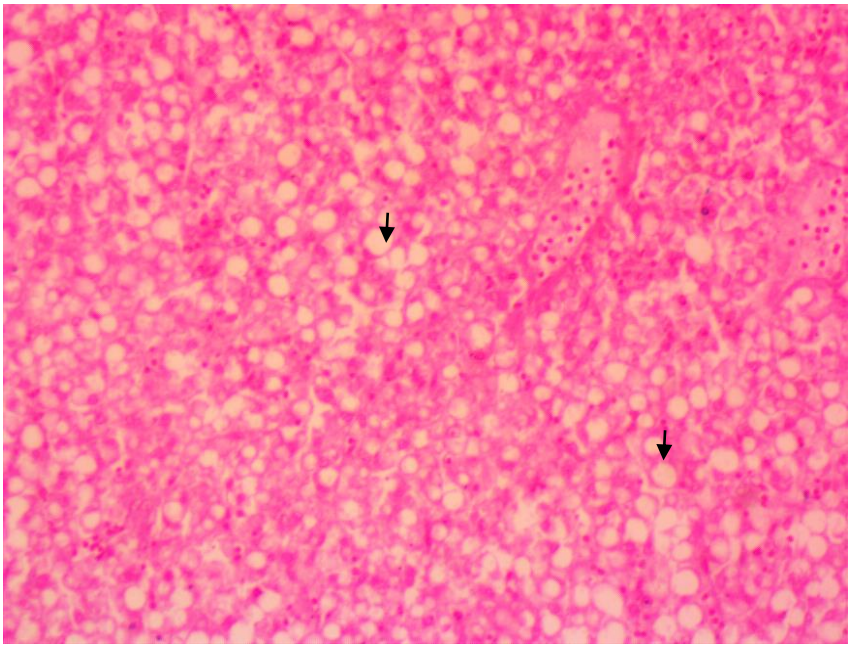


Plate 5.1: Normal hepatocytes with fat accumulation in cytoplasm in the liver (arrows). HE x100.
Treatment: Nil (Control)

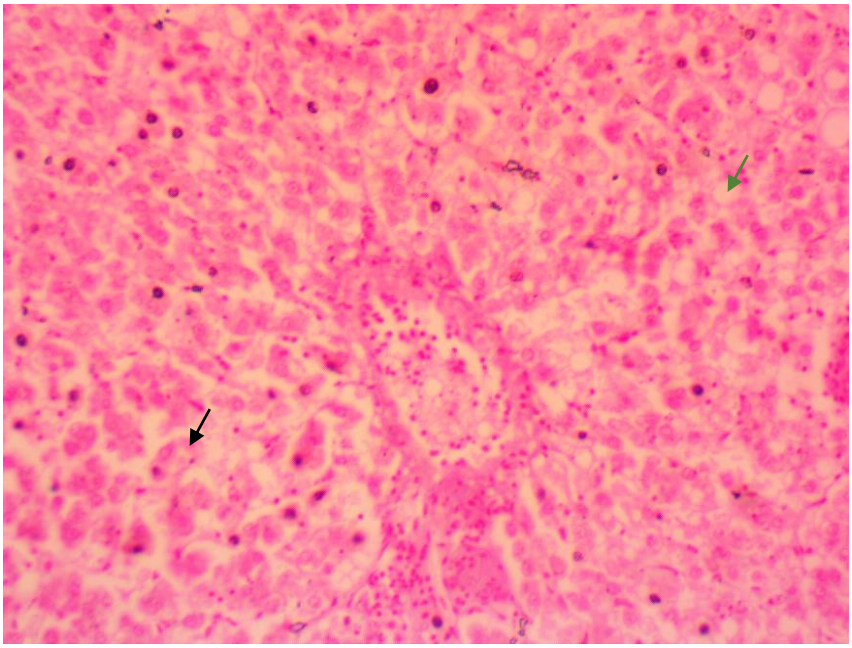


Plate 5.2: Liver hepatocellular degeneration (green arrow) and necrosis (black arrow). HE x400. Treatment: 10mg/l raw garlic

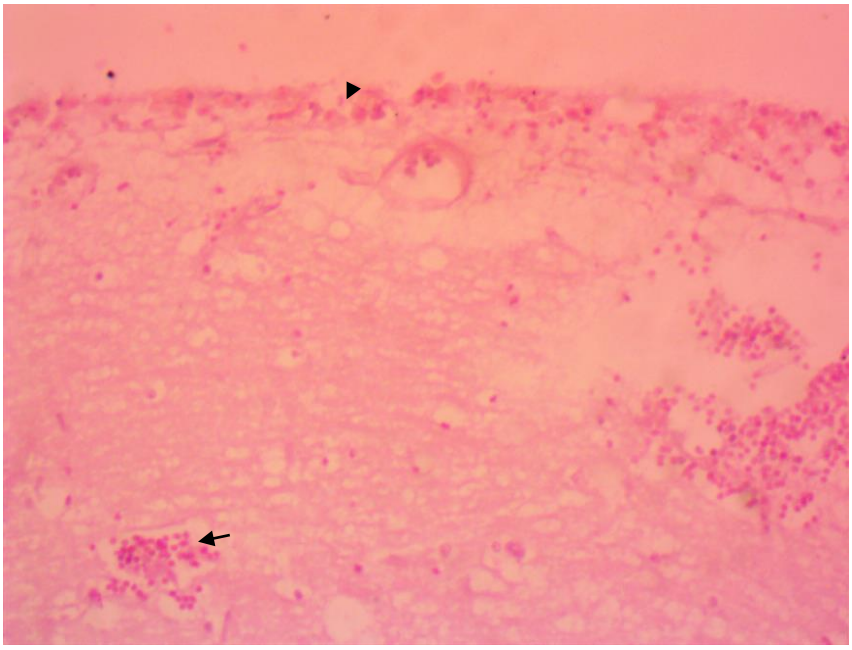


Plate 5.3: Brain with cellular infiltrates in meninges (arrowhead) and vasculitis (arrow). HE x 400. Treatment: 10mg/l raw garlic

5.4 Discussion

5.4.1: Length- Weight Relationship (LWR) and condition factor

Table 5.1 shows the LWR and condition factor, Ojogbo (2013) reported that condition factor (K) initial growth index (a) and growth index (b) are measurable indices for stress in *Clarias gariepinus*. The Table shows that, 5mg/l, 10mg/l and 20mg/l produced a satisfactory response for the duration of 7days due to relatively good condition factor compared to the control although significantly lower and the negative initial growth index which is lower in 10mg/l and 20mg/l, indicative of lower stress level and higher growth index than control. Other parameters need to be considered to enable the selection of optimal concentration for female as 5mg/l had increased stress level which could be considered beneficial with a higher condition factor and a lower growth index.

5.4.2: Water quality parameters

Table 5.2 shows the values of water quality in this study.

pH values for all concentration were all above the recommended value of 6.5-8.5 for catfish production (Omitoyin, 2007). However, these were all within the acceptable range. These may therefore mean that other parameter consideration would be needed to obtain the concentration that had the best "fit" pH.

Alkalinity 5-10mg/l is recommended (Omitoyin, 2007). However, alkalinity of 50-200ppm is acceptable in pond production, to support abundance of planktons. However, for the purpose of the test, because there was no room for plankton growth, the lower the total alkalinity value the better best "fit". As a result, 5mg/l, 10mg/l, 20mg/l, 30mg/l and 40mg/l could be acceptable as "fit".

Chloride value of less than 5mg/l is acceptable. For fish production, it means all concentration including control are acceptable. Total hardness of 50-300mg/l is recommended. Again, all concentration produced total hardness that was acceptable for *Clarias gariepinus*. However, the concentration selected at alkalinity still remained selected. Calcium and magnesium had no standard upper limits, so the concentration earlier selected stays. Total dissolved solids, less than 200mg/l is recommended (http://fisheryfarming.blogspot.com.ng/search?q=TDS, 2015).

All concentration produced values higher than 100mg/l, these may mean that the lower the total dissolved value the better. As a result of the "fit" concentration, 5mg/l was now selected although some other concentrations had non significantly different value as the 5mg/l. 10mg/l may still be accepted as "fit" because it had a significantly lower value than 20mg/l which has now been eliminated as it had the highest TDS. Dissolved oxygen not less than 4mg/l is

recommended (Omitoyin, 2007). All concentration had above the recommended value. So 5mg/l and 10mg/l still remained "fit" for treatment. Potassium level of 1 or 2mg/l is recommended. Potassium and sodium enhances osmoregulation. It appears that the higher the Potassium in water better the water quality. All concentration produced lower values of potassium then recommended value. However, 5mg/l had the highest value of potassium. There were no significant $p \leq 0.05$ differences with 50mg/l and 60mg/l. 10mg/l produced the second highest value of potassium.

Based on the above results and discussion, 5mg/l or 10mg/l could be said to be best "fit" for treatment in *Clarias gariepinus* culture. Other parameters such as histopathology could be considered for the evaluation.

5.4.3: Histopathological studies

Histological studies show in control group at day 7 kidney, gut, liver and brain were normal. Liver had normal hepatocytes with fat accumulation. Kidney showed normal hemopietic cells and excretory tubules while the brain had normal white matter.

For exposure to 5mg/l raw garlic at day 7 kidney, gut, liver and brain were normal with no visible lesion. Brain (cerebellum) had normal pukinje cells. Liver had normal hepatocytes with depleted fat stores.

For exposure to 10mg/l raw garlic at day 7, liver had no fatty vacuoles, mild hepatocellular atrophy and sinusoidal congestion. Gut showed mild villi and glandular atrophy. Kidney had hemopoietic tissue hypoplasia ('punched out spaces'). Brain showed moderate non-suppurative meningitis and vasculitis, mild granule cell hypoplasia and cellular infiltrates in meninges and vasculitis

For exposure to 20 to 60mg/l raw garlic at day 7 the histopathological changes varied in severity been more severed in 30 and 40mg/l than 50 and 60mg/l of raw garlic which included in the brain mild necrosis of neurons, purkinje fiber, cerebellum, neuropil.; and moderate demyelination of nerve fibres and gliosis.

5.5: Conclusion

The use of water quality, Histopothological changes and length-weight relative indices including condition factor proved adequate for the evaluation of safety of raw garlic. The least toxicant (safest) concentration in female with mean±SEM body weight and length of 25.87±0.65g and 15.52±0.26cm respectively was 5mg/l raw garlic.

CHAPTER SIX

RESPONSES OF FEMALE AFRICAN CATFISH (*Clarias gariepinus*) TO LETHAL CONCENTRATIONS (LC_{100}-96 HR) OF CADMIUM NITRATE ($Cd(NO3)_2$) AND LEAD NITRATE ($Pb(NO3)_2$) TOXICITY IN RAW GARLIC BATH TREATMENT

6.1: Introduction

Mass fish kill due to acute heavy metal toxicities have been documented by several authors. It is very rare that only one toxic element at a time is released into the aquatic ecosystem (Kumar and Singh, 2010). High concentrations of metals in fish tissues can lead to redox reactions generating free radicals, especially reactive oxygen species (ROS), e.g. singlet oxygen; superoxides; peroxides; hydroxyl radical; and hypochlorous acid (Dautremepuits, 2002). Secombes (1996) reported that free radicals are used by the immune system to kill pathogens and that excess production of free radicals that occurs during chronic infections may be harmful to nearby cells. Most of the heavy metals interacts with each other and are also influenced by ions. Cadmium and lead are the most common heavy metals in the aquatic ecosystem. There is a dearth of information on the use of raw garlic in treatment of acute toxicities of heavy metals. The current study therefore aims to observe Clinical Signs/ behavioural changes and determine the responses of blood biochemistry, water quality, oxidative stress parameter markers including levels of metals accumulation and pathology to acute lethal toxicities of cadmium and lead singularly and co-jointly with raw garlic in order to provide management of cadmium and lead toxicity recommendation in the culture of African catfish (*Clarias gariepinus*).

6.2: Methodology.

The garlic cloves were purchased from the Bodija market, in Ibadan. 480 apparently normal 12 weeks old *Clarias gariepinus* of females were purchased from a commercial fish farm in Ibadan. The mean±SEM weights was approximately 26.87 ± 0.10g with a minimium and maximium weight of 23 and 31g respectively. Prior to the acute toxicity testing, the fishes were acclimatized for 14 days under a fish rearing facility with approximately 12hour light: 12hour dark. They were fed with fish feeds to satiation at 10.00 hours and 17.00 hours and the water was renewed every other day.

The fish were exposed to LC_{100}-96 hours of cadmium nitrate and lead nitrate by extrapolation of the obtained LC_{100}-96 hours of cadmium nitrate and lead nitrate in chapter four of this thesis to induce lethal toxicity singularly and jointly. Exposure was to 64 mg cadmium /L and 126 mg lead/l. Six-hour exposure achieved induced lethal toxicities with 60-90% vertical

column positioning in the culture tanks. The optimum garlic concentration ("Best fit") for apparently healthy female obtained from the previous study on determination of acute minimum toxicant concentration (optimum) from the previous study on determination of acute minimum toxicant (optimium) concentration of garlic in female *Clarias gariepinus* of body weight 25.87g was 5mg/L. By simple extrapolation, the best fit for apparently healthy *Clarias gariepinus* of body weight 27.0 used in this study was approximated to be 5.2mg raw garlic /l. To treat cadmium toxicity in female with more sensitivity to cadmium, a ratio of 1:8 optimum garlic concentrations was proposed because the fish are already more stressed than lead. The ratio of 1:8 was 0.65mg raw garlic/L. To treat lead toxicity in female that is less sensitive to lead toxicity, a ratio of 1:6 of "best fit" garlic concentration for apparently healthy female was also proposed which was approximated to be 0.87mg/L. For the combination of lead and cadmium treatment it was proposed that the lower treatment concentration be used because it was expected that it could be the concentration of garlic that may induce the least toxicity in the culture. As a result, to treat the combination of cadmium and lead toxicity 0.65mg/L was used. Garlic only subgroups in exposure with cadmium and cadmium + lead was with 0.65 mg/l whereas in lead subgroups was 0.87mg/L. All controls were not treated with garlic or exposed to the metals sepaerately or jointly. Treatment was done on day 1 and 2 for 12 hours after 6 and 12hr water renewal respectively at 16 – 1700 hours to enhance stabilization and abate the stress of water renewal before treatment. Observance of clinical signs/behavioural changes, blood chemistry, gross lesions, post mortem and histopatholgical examination including values of water quality (pH, alkalinity, total hardness, calcium and total solids) and metals (cadmium, lead, potassium, calcium and manganese) in water, in gills, intestines, blood and livers were obtained by methods previously described in chapters three and five of this thesis. Liver total protein, superoxide dismutase (SOD) activity, glutathione (GSH) level, glutathione peroxidase (GPX) activity, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were assayed using standard methods. At day 3, water was renewed and the experiment was terminated at day 6 with improved response to treatment in exposed groups and ammonia build up in the negative control groups.

6.2.1: Erythrocyte morphology examination

A drop of blood was poured on the clean glass slide, and then a spreader was used to evenly spread the blood at angle 45^0 to make a very good thin blood smear. This was allowed to dry under room temperature, then fixed with methanol (Analar grade) and allowed to dry; it was

then immersed in giemsa stain solution for 45min. After staining, it was allowed to dry, then viewed under the microscope at x100 magnification using Leica Galen III Microscope to identify erythrocyte and record erythrocyte morphology. Thin blood smear stained with Giemsa was as described by Jain (1986) and Kelly (1984).

6.2.2: Biochemical assays

6.2.2.1 Preparation of tissues for biochemical assays

The *Clarias gareipinus* with different treatments were sacrificed by stunning and excised livers were quickly rinsed in ice-cold 1.15% KCl after which they were blotted and weighed. The livers were then minced with scissors in 4 volumes of ice-cold 0.1M phosphate buffer, pH 7.4, and homogenized using a Tefflon homogenizer. The resulting homogenates were centrifuged at 10,000rpm at 4°C for 10minutes. The supernatant was collected and processed for biochemical estimations.

Reagents:

1. Homogenizing buffer (0.1M Phosphate buffer, pH 7.4)

   35.822g of Na_2HPO_4 (BDH Chemical Limited, England) and 15.603g of NaH_2PO_4 were dissolved in 900ml of distilled water. The pH was adjusted to 7.4 and then made up to 1 liter with distilled water.

2. 1.15% Potassium Chloride

   1.15g of potassium chloride (BDH Chemical Limited, England) was dissolved in distilled water and made up to 1000ml and stored at 4°c.

6.2.2.2: Determination of protein concentration in liver

The protein concentrations of the various samples were determined by means of the Biuret method as described by Gornal *et al*. (1949) with a slight modification.

Liver supernatant were made with distilled water. The serum and the post mitochondrial fractions of the liver supernatants were diluted 100 times with distilled water. This was done to reduce the level of protein in the samples to the sensitivity range of Biuret method. 1ml of the diluted sample was taken and added to 3ml of Biuret reagent in triplicate. The mixture was incubated at room temperature for 30 minutes after which the absorbance was read at 540nm using distilled water as blank. The protein content of the samples was usually extrapolated from the standard curve and multiplied by 100 to get the actual amount in the fraction.

The procedure is the colorimetric determination of H_2O_2. Different amount of H_2O_2, ranging from 10 to 100 μmoles was taken in small test tubes and 2ml of dichromate/acetic acid was

added to each. Addition of the reagent instantaneously produced an unstable blue precipitate of perchromic acid. Subsequent heating for 10 minutes in a boiling water bath changed the colour of the solution to stable green due to formation of chromic acetate. After cooling at room temperature, the volume of the reaction mixture was made to 3ml and the optical density measured with a spectrophotometer at 570nm. The concentrations of the standard were plotted against absorbance.

6.2.2.3 Determination of superoxide dismutase (SOD) activity

The activity profile of SOD in the homogenates was determined by the method of Misra and Fridovich (1972)**.**

Principle is based on the ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for dismutase. Superoxide (O^{-}_{2}) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O^{-}_{2} introduced increased with increasing pH (Valerino and Mc Cormack, 1971) and also increased with increasing concentration of epinephrine. These results led to the proposal that autoxidation of epinephrine proceeds by at least two distinct pathways, only one of which is a free radical chain reaction involving superoxide (O^{-}_{2}) radical and hence inhibitable by SOD.

Reagents

1. 0.05M Potassium Phosphate buffer (pH 7.8).

6.97g of K_2HPO_4 and 1.36g KH_2 PO_4 were dissolved in 900ml of distilled water and the volume made up to 1 litre. The pH was adjusted to 7.8.

2. 0.05M Carbonate buffer (pH 10.2)

14.3g of Na_2CO_3 $10H_2O$ and 4.2g of $NaHO_3$ were dissolved in 900ml of distilled water and then made up to 1 litre. The pH was adjusted to 10.2.

3. 0.3mM Adrenaline

0.0137g of adrenaline (epinephrine) was dissolved in 200ml-distilled water and then made up to 250ml. This solution was prepared afresh.

Procedure

1ml of sample was diluted in 9ml of distilled water to make a 1 in 10 dilutions. An aliquot of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference

cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

Calculation

Increase in absorbance per minute = $\frac{A_3 - A_0}{2.5}$

Where, A_0 = absorbance after seconds

A_3 = absorbance 150 seconds

% inhibition = $\frac{\text{increase in absorbance for substrate X } 100}{\text{increase in absorbance of blank}}$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.

6.2.2.4 Estimation of reduced glutathione (GSH) level

The method of Beutler *et al*., (1963) was followed in estimating the level of reduced glutathione (GSH).

The principle is based on the reduced form of glutathione that comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable (yellow) colour when 5′,5′ – dithiobis - (2-nitrobenzoic acid, DTNB) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412nm. Reduced glutathione is proportional to the absorbance at 412.

Reagents

GSH working standard

1.40mg GSH was dissolved in 0.1M phosphate buffer, pH 7.4, and made up to 100ml with the same.

2. 0.1M Phosphate buffer (pH 7.4)

a) First 0.1M K_2HPO_4 $12H_2O$ (MW=) was prepared by dissolving 0.992g in 200ml of distilled water.

b) 0.1M KH_2PO_4. $2H_2O$ (MW=156.03) was prepared by dissolving 1.946g in 200ml of distilled water.

Finally, 0.1M phosphate buffer was prepared by adding 200ml of (a) to 100ml of (b) and the pH adjusted to 7.4 with drops of concentrated HCl or NaOH as the case may be.

3. Ellman Reagent [5′, 5′-Dithiobis- (2-nitrobenzoate) DTNB]

a) This was prepared by dissolving 0.04g of Ellman reagent in 0.1M Phosphate buffer and made up to 200ml.

4. Precipitating Solution

4% of sulphosalicyclic acid ($C_7H_6O_6S.2H_2O$ Mol. Wt. 254.22) was prepared by dissolving 4.8g of sulphosalicyclic acid in 120ml of distilled water. This reagent is stable for approximately three weeks at 40C.

Calibration of GSH standard curve procedure involves

serial dilutions of the GSH working standard. The protocol for this preparation is in the appendix. To each was added 4.5ml of Ellman reagent. GSH was proportional to the absorbance at 412nm (as well as at 430nm using colorimeter). The readings were taken before five minutes. This is because the colour is stable for at least 5 minutes after the addition of Ellman reagent. After 10 minutes of standing, there is frequently a loss of 1 to 2% of the colour. However, an additional delay of 5 – 15 minutes will result in only a small error. Each sample was prepared in duplicate. A graph of optical density against concentration was plotted.

6.2.2.5: Estimation of GSH level

0.5ml of sample was placed into the tubes and 0.5ml of the precipitating solution was mixed with sample. The mixture was centrifuge at 4,000rpm for 5minutes. Take 0.5ml of the supernatant and put in another test tube, add 4.5ml of Ellman's reagent to the supernatant in the tube. Read at 412nm against blank as distill water.

A blank was prepared with 2ml of the 0.1M phosphate buffer, 3ml of diluted precipitating solution (3parts to 2 parts of distilled water), 1ml of the above mixture is added to 4.5ml Ellman's reagent. The optical density was measured at 412nm. GSH was proportional to the absorbance at that wavelength and the estimate was obtained from the GSH standard curve.

6.2.2.6 Assessment of lipid peroxidation (MDA)

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale (1970).

The principle is on the basis that under acidic condition, malondialdehyde (MDA) produced from the peroxidation of fatty acid membranes and food products react with the chromogenic reagent, 2-thiobarbituric acid (TBA) to yield a pink coloured complex with maximum absorbance at 532nm and fluorescence at 553nm. The pink chromophore is readily extractable into organic solvents such as butanol.

Reagents used are:

1. 30% Trichloroacetic acid (TCA)

6g of TCA (CCl3COOH) was dissolved in distilled water and made up to 20ml.

2. 0.75% Thiobarbituric acid (TBA)

This was prepared by dissolving 0.15g of TBA in 0.1M HCl and made up to 20ml.

3. 0.15M Tris-KCl buffer (pH 7.4)

1.15g of KCl and 2.36g of Tris base were dissolved separately in distilled water and made up to 100ml. The pH was then adjusted to 7.4.

The procedure was as follw:

1.6ml of Tris-KCl buffer was mixed with an aliquot of 0.4ml of the test sample (i.e Post mitochondrial supernatant) to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a water bath for 45 minutes at 80^{o}C. This was then cooled in ice and centrifuged at 3000rpm. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532nm. The MDA level was calculated according to the method of Adam-Vizi and Seregi (1982). Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x 10^5 $M^{-1}Cm^{-1}$.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance x volume of mixture}}{E_{532nm} \text{ x volume of sample x mg protein}}$$

6.2.2.7 Hydrogen peroxide generation

Hydrogen peroxide generation is as described by wolff´s (1994) method

Preparation of reagent (FOX-1)

1) 100µmol/L Xylenol orange (760.6)
   0.00375g of xylenol orange in 50mls 0f distilled water
2) 250mMol/L ammonium ferrous sulfate (MW = 392.14)
   0.01g of ammonium ferrous sulfate in 100mls of distilled water
3) 100Mm/L sorbitol (MW = 182.2)
   0.91g of sorbitol in 50mls of distilled water
4) 250mMol/L $H_2 SO_4$
   1ml of 1M H_2SO_4 is made up to 40mls with distilled water.
5) SAMPLE

50µl of sample was vortexed and incubated at room temperature for a minimum of 30minutes. Absorbance was taken at 560nm.

6.2.2.8 Glutathione peroxidase assay (GPx)

Reagents

1. Sodium azide (NaN3; 10mM)

0.0325g of sodium nitrite was dissolved in 50 mls of distilled water

2. Reduced glutathione (GSH 4mM)

0.0123g of reduced GSH was dissolved in 100ml of phosphate buffer

3. Hydrogen peroxide (H2O2; 2.5mM)

28µL of hydrogen peroxide was dissolved in 100mls of distilled water.

4. Tricholoroacetic acid (TCA, 10%)

2g of TCA was dissolved in 20 ml of distilled water

5. Di-potassium hydrogen orthophosphate (K2HPO4; 0.3M)

5.23g of di-potassium hydrogen orthophosphate was dissolved in 100mls of distilled water

6. 5'-5'-dithiobis-(2-dinitrobenzoic acid) DTNB

0.04 of DTNB was dissolved in 100mls of phosphate buffer.

7. Phosphate buffer

0.992g of K2HPO4 and 1.946g of KH2PO4 were dissolved with 200ml of distilled water and adjusted to pH of 7.4 according to Rotruck *et al*., (1973).

The whole reaction mixture was incubated at 37oC for 3 minutes after which 0.5ml of TCA was added and thereafter centrifuged at 3000rpm for 5 minutes. To 1ml of each of the supernatants, 2mls of K_2HPO_4 and 1ml of DTNB was added and the absorbance was read at 412 nm against a blank. Glutathione peroxidase activity was observed by plotting the standard curve and the concentration of the remaining GSH was extrapolated from the curve.

GSH consumed = 245.34 – GSH remaining

Glutathione peroxidase activity = H_20_2 CONSUMED

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mg PROTEIN

6.3: Data Analysis

Data were analysed using ANOVA, DMRTwith level of significace accepted as ($p \leq 0.05$)

6.4 Results and Discussion

6.4.1 Results of Clinical Signs

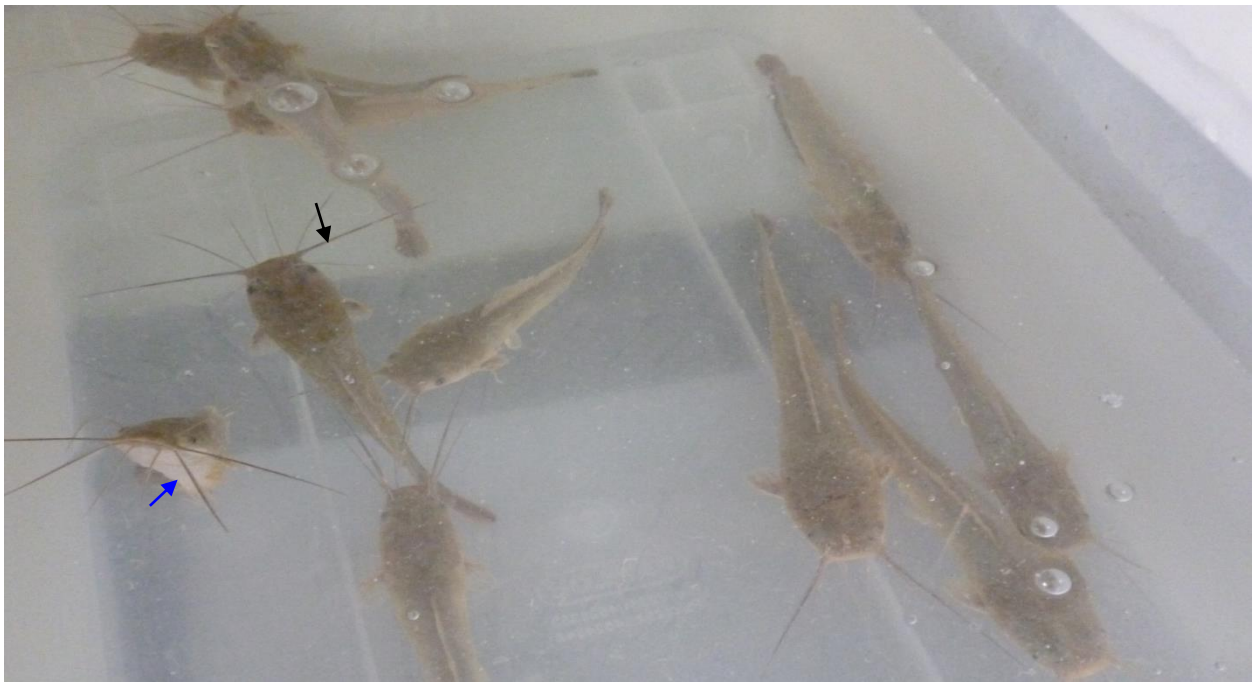

Plate 6.1: *C gariepinus* with extended barbels (black arrow) and vertical positioning (blue arrow) after exposure to Cd (64mg/L) for 6hr.

Upon exposure of fish to cadmium, lead, and cadmium + lead, the observed responses were increased violent, swift and uncoordinated swimming with abrupt stoppage before another bout, loss of shoal formation and "Head hitting". These responses reached a peak at about 3 hours of exposure to cadmium + lead, 4 – 5 hours post exposure in exposure to cadmium only, lead only. After the attainment of these peak, the swimming activities declined at about 6 hours to 40 – 60% post exposure. Vertical positioning under or on the surface of the water in the tank with little or no response to minor agitation, decreased opercula beat/min, and horizontally positioned barbel. At about 4 hours of cadmium + lead exposure the first mortality was recorded. Copious mucus secretion appeared at about 3 hours of exposure which increased with increase in duration of exposure. The degree of mucus secretion in order of severity were exposure to cadmium + lead > lead > cadmium. There was no mucus secretion in control groups.

Responses to feeding varied with exposure to cadmium and lead separately and co-jointly, at 8hr after water renewal at 6hr all exposed groups did not respond to feeding. Control groups responded to feeding and were feed to satiation. At 6 hours of exposure water renewal was done, 3 hours later, (9 hour of exposure) fish exposed especially in cadmium + lead, appeared a little more stable with horizontal/semi lateral position under water and erratic swimming on agitation. Mucus secretion was more severe than at 6 hours of exposure without water renewal in all metal exposed groups.

Fish exposed to cadmium only appeared a little more stable with 40 – 50% vertical position with minimal movement on agitation. Exposure to lead only appeared stable, 20 – 40% vertical position, lateral movement at the bottom of the tank responds well to minimal agitation. In garlic only and control groups, swimming were normal and no flacking observed.

At day 2 all groups exposed to cadmium, lead only and combination with or without 12-hour garlic treatment exhibited short duration, vertical position under water column. All exposed groups with garlic treatment were off feed while all exposed groups with no garlic treatment had improved appetite. At day 2, exposure cadmium only had 10% exhibiting tumescence. The group that were not exposed, but had garlic treatment had a lower appetite than the control group.

At day 3, 48 hours after 12 hours' water renewal before 12-hour garlic treatments, female exposed to 6-hour cadmium + garlic 12 hour consecutive treatments were active with 30% exhibiting tumescence and mild flaking. Exposure to cadmium only were active with 40%

exhibiting tumescence with no flaking. Treatment with garlic only and control groups were active with normal behavior.

Exposure to lead only and lead treated for 2 consecutive days of 12 hours' garlic were active. Exposure to cadmium + lead + garlic treatment, cadmium + lead both developed with tumescence and garlic only and control groups were all active. All groups exposed and treated with garlic had varied improved appetite which was however, lower than all groups exposed with no garlic treatment. All groups not exposed but treated with garlic had improved appetite which was lower than the control.

At day 6, exposure to cadmium and treated with garlic in all subgroups including female with tumescent were all active. The size of tumescent did not regress in all groups. Exposure to cadmium only in all subgroups including fish with tumescence were all active, size of tumescence increased and were most of the time crowded at the corner of the tank. Exposure to cadmium but treated with garlic were active and evenly distributed with shoal formation. Exposure to lead and treated with/without garlic treatments, garlic only treatment and in control were all active and evenly distributed on tanks. The control group had high ammonia smell. Exposure to cadmium + lead with/without garlic treatments including those with tumescence, garlic only treatment and in control were all active and evenly distributed in tanks.

All control groups were active and evenly distributed with shoal formation with high ammonia odour necessitate the termination of the experiment to avoid the introduction of ammonia toxicity.

6.4.2 Discussion of clinical Signs

Upon exposure the behavioural alterations observed were mechanism of an attempt to avoid toxicity and is in agreement with several authors' findings including Olufemi (1998). The more stable state and increased mucus secretion observed 3hr after the first water renewal than at 6hr post exposure showed that water renewal was therapeutic; it reduced the severity of toxicity but not mucus secretion which may be an indication that that the effects of cadmium and lead on skin was not abated even with water renewal due probably to the skin accumulation in the skin.

However, on day 6 (120hr post first garlic treatment) the increased severe slimmy body and hyperplasia of goblet cells in exposed and untreated may not necessary be an indication of higher ameliorative effect but higher degree of irreversible structural destruction and an over whelmed goblet cell by heavy metals in exposed fish that was untreated. Garlic reduced the

severity of copious secretions in metal exposed groups and acted as a treatment for the skin erosions and ulcerations caused by the metals.

6.4.3 Results of Blood chemistry

Table 6.1: Effect of garlic treatments Means ± SEM on blood chemistry levels at 12hr, 24hr, 48hr and 120hr after 6hr exposure to lethal dose of Cadmium

Parameter	Hours of experiment/hr of garlic treatment	Cadmium + garlic	Cadmium only	Garlic only	Control
Total protein (g/l)	6	3.12 ± 0.64^{b}	3.14 ± 0.03^{b}	4.31 ± 0.05^{a}	4.23 ± 0.09^{a}
	24/12	6.11 ± 0.08^{a}	3.74 ± 0.04^{c}	5.14 ± 0.06^{b}	5.00 ± 0.12^{b}
	48/12	7.29 ± 0.02^{a}	6.10 ± 0.01^{b}	6.04 ± 0.16^{b}	7.57 ± 0.14^{a}
	120	2.43 ± 0.17^{c}	3.16 ± 0.06^{b}	4.80 ± 0.23^{a}	4.45 ± 0.13^{a}
K^{+}(meg/l)	6	31.25 ± 0.48^{b}	31.50 ± 0.29^{b}	55.00 ± 2.38^{a}	53.50 ± 2.99^{a}
	24/12	59.25 ± 0.48^{a}	37.75 ± 0.48^{d}	49.25 ± 0.48^{c}	53.25 ± 1.11^{b}
	48/12	69.75 ± 0.63^{b}	62.75 ± 0.95^{c}	60.25 ± 18^{c}	75.00 ± 1.47^{a}
	120	23.50 ± 1.50^{c}	30.25 ± 0.85^{b}	46.00 ± 2.27^{a}	42.00 ± 1.29^{a}
Na^{+}(meg/l)	6	41.00 ± 0.71^{b}	4.25 ± 0.25^{b}	63.50 ± 2.26^{a}	62.50 ± 2.60^{a}
	24/12	70.25 ± 0.63^{a}	45.50 ± 0.66^{d}	56.75 ± 0.48^{b}	53.25 ± 1.11^{c}
	48/12	84.75 ± 0.25^{a}	72.75 ± 0.48^{b}	711.25 ± 2.78^{b}	87.75 ± 1.32^{a}
	120	30.75 ± 2.18^{c}	41.00 ± 1.58^{b}	59.00 ± 2.27^{a}	54.75 ± 1.25^{a}
Creatinine g/l	6	1.09 ± 0.01^{b}	1.07 ± 0.01^{b}	1.23 ± 0.02^{a}	1.23 ± 0.02^{a}
	24/12	3.97 ± 0.06^{a}	1.19 ± 0.01^{d}	2.23 ± 0.02^{b}	2.09 ± 0.04^{c}
	48/12	4.20 ± 0.11^{a}	3.19 ± 0.11^{b}	3.04 ± 0.17^{b}	4.44 ± 0.07^{a}
	120	1.18 ± 0.07^{b}	1.10 ± 0.02^{b}	1.61 ± 0.08^{a}	1.47 ± 0.06^{a}
ALT (Int Units/l)	6	44.75 ± 0.63^{b}	44.75 ± 0.25^{b}	71.00 ± 1.68^{a}	71.00 ± 1.85^{a}
	24/12	70.00 ± 0.91^{a}	45.50 ± 0.50^{d}	56.50 ± 0.29^{b}	50.25 ± 1.11^{c}
	48/12	84.25 ± 0.48^{b}	73.00 ± 0.41^{c}	73.50 ± 2.18^{c}	89.00 ± 0.91^{a}
	120	34.75 ± 2.46^{b}	40.25 ± 2.56^{b}	60.75 ± 2.92^{a}	55.25 ± 2.47^{a}
AST (Int Units/l)	6	34.25 ± 0.48^{b}	32.75 ± 0.48^{b}	64.00 ± 1.68^{a}	63.75 ± 1.49^{a}
	24/12	60.00 ± 0.71^{a}	34.25 ± 0.75^{d}	45.25 ± 0.25^{b}	41.75 v 1.03^{c}
	48/12	79.50 ± 0.65^{b}	72.00 ± 0.41^{c}	71.25 ± 1.87^{c}	81.50 ± 0.65^{a}
	120	27.50 ± 2.47^{b}	32.25 ± 0.85^{b}	48.50 ± 2.50^{a}	43.25 ± 2.50^{a}
Glucose(g/l)	6	41.00 ± 0.71^{b}	41.00 ± 0.41^{b}	68.50 ± 1.32^{a}	69.75 ± 0.85^{a}
	24/12	68.75 ± 0.48^{a}	43.75 ± 0.95^{c}	55.50 0.29^{b}	40.50 ± 1.32^{d}
	48/12	79.50 ± 0.65^{a}	72.00 ± 0.41^{b}	71.25 ± 1.89^{b}	81.50 ± 0.65^{a}
	120	30.00 ± 2.17^{d}	40.00 ± 0.82^{c}	60.75 ± 3.20^{a}	52.75 ± 2.78^{b}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.2: Effect of garlic treatments Means ± SEM on blood chemistry levels at 12hr, 24hr, 48hr and 120hr after 6hr exposure to lethal dose of Lead

Parameter	Hours of experiment	Lead + garlic	Lead only	Garlic only	Control
Total protein (g/l)	6	3.35 ± 0.05^{b}	3.36 ± 0.05^{b}	4.24 ± 0.08^{a}	4.24 ± 0.11^{a}
	24/12	4.53 ± 0.03^{c}	5.42 ± 0.09^{a}	4.89 ± 0.13^{b}	5.19 ± 0.04^{a}
	48/12	4.21 ± 0.01^{d}	8.25 ± 0.02^{a}	5.85 ± 0.15^{c}	7.69 ± 0.18^{b}
	120	4.34 ± 0.04^{b}	3.35 ± 0.03^{c}	4.91 ± 1.16^{a}	4.56 ± 0.05^{b}
K^{+}(meg/l)	6	39.25 ± 0.48^{b}	39.25 ± 0.48^{b}	54.00 ± 0.91^{a}	54.25 ± 0.85^{a}
	24/12	45.50 ± 0.29^{c}	54.75 ± 0.95^{a}	43.00 ± 1.23^{c}	49.00 ± 0.41^{b}
	48/12	39.75 ± 0.95^{d}	82.25 ± 0.85^{a}	58.00 ± 2.04^{c}	72.50 ± 2.90^{d}
	120	42.00 ± 0.48^{c}	32.75 ± 1.18^{d}	48.25 ± 0.29^{a}	45.50 ± 1.54^{b}
Na^{+}(meg/l)	6	48.50 ± 0.65^{b}	48.75 ± 0.95^{b}	62.00 ± 0.91^{a}	61.75 ± 0.95^{a}
	24/12	56.50 ± 0.29^{b}	67.75 ± 1.11^{a}	52.00 ± 1.08^{c}	56.75 ± 0.48^{b}
	48/12	52.00 ± 0.41^{a}	96.25 ± 0.25^{a}	66.25 ± 3.18^{c}	85.50 ± 2.10^{b}
	120	53.75 ± 0.63^{c}	42.75 ± 0.48^{d}	60.25 ± 1.55^{a}	57.00 ± 0.41^{b}
Creatinine g/l	6	1.17 ± 0.02^{a}	1.17 ± 0.02^{a}	1.19 ± 0.03^{a}	1.21 ± 0.03^{a}
	24/12	2.12 ± 0.01^{bc}	2.31 ± 0.04^{a}	2.04 ± 0.05^{c}	2.22 ± 0.02^{b}
	48/12	1.40 ± 0.00^{d}	6.45 ± 0.03^{a}	2.75 ± 0.17^{c}	4.66 ± 0.07^{b}
	120	1.43 ± 0.02^{b}	1.20 ± 0.01^{c}	1.55 ± 0.05^{a}	1.21 ± 0.11^{c}
ALT (Int Units/l)	6	44.00 ± 0.41^{b}	43.75 ± 0.63^{b}	73.75 ± 0.63^{a}	72.50 ± 1.04^{a}
	24/12	55.50 ± 0.29^{b}	72.75 ± 1.11^{a}	50.00 ± 1.23^{c}	56.25 ± 0.25^{b}
	48/12	54.75 ± 0.75^{d}	95.25 ± 0.25^{a}	69.00 ± 2.42^{c}	88.75 ± 1.49^{b}
	120	52.25 ± 1.93^{c}	37.25 ± 1.11^{d}	63.75 ± 1.49^{a}	57.50 ± 0.65^{d}
AST (Int Units/l)	6	31.25 ± 0.48^{b}	31.75 ± 0.63^{b}	66.50 ± 0.65^{a}	63.25 ± 1.18^{a}
	24/12	44.50 ± 0.29^{bc}	63.00 ± 1.08^{a}	42.00 ± 1.23^{c}	44.75 ± 0.25^{b}
	48/12	42.00 ± 0.41^{d}	83.75 ± 0.25^{a}	60.00 ± 1.47^{c}	77.50 ± 2.23^{b}
	120	42.25 ± 0.85^{b}	30.50 ± 0.50^{c}	46.75 ± 0.75^{a}	45.00 ± 0.71^{a}
Glucose(g/l)	6	38.75 ± 0.63^{b}	38.75 ± 0.63^{b}	68.75 ± 1.44^{a}	69.00 ± 1.78^{a}
	24/12	52.50 ± 0.29^{b}	67.75 ± 1.11^{a}	47.50 ± 2.87^{c}	55.50 ± 0.29^{b}
	48/12	52.25 ± 0.25^{d}	85.00 ± 0.41^{a}	67.00 ± 2.42^{c}	77.75 ± 2.02^{b}
	120	49.25 ± 1.38^{c}	34.00 ± 0.41^{d}	58.75 ± 0.48^{a}	53.50 ± 0.96^{b}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.3: Effect of garlic treatments Means ± SEM on blood chemistry levels at 12hr, 24hr, 48hr and 120hr after 6hr exposure to lethal dose of Cadmium + Lead

Parameter	Hours of experiment	Cadmium + Lead + garlic	Cadmium + Lead only	Garlic only	Control
Total protein (g/l)	6	3.22 ± 0.02^{b}	3.21 ± 0.02^{b}	4.10 ± 0.06^{a}	4.18 ± 0.06^{a}
	24/12	5.02 ± 0.03^{ab}	4.21 ± 0.01^{c}	4.71 ± 0.23^{d}	5.21 ± 0.04^{a}
	48/12	6.15 ± 0.03^{c}	7.47 ± 0.03^{b}	6.12 ± 0.16^{c}	7.84 ± 0.06^{a}
	120	2.85 ± 0.09^{c}	2.32 ± 0.03^{d}	4.91 ± 0.27^{a}	4.40 ± 0.10^{b}
K^{+}(meg/l)	6	33.75 ± 0.48^{b}	33.75 ± 0.48^{b}	52.25 ± 1.03^{a}	53.25 ± 0.25^{a}
	24/12	43.50 ± 0.29^{b}	41.75 ± 0.25^{b}	41.75 ± 2.02^{b}	49.00 ± 0.58^{a}
	48/12	58.75 ± 0.48^{c}	75.00 ± 0.41^{a}	64.00 ± 1.23^{b}	73.50 ± 0.65^{a}
	120	26.25 ± 0.85^{c}	20.75 ± 0.48^{d}	48.75 ± 1.89^{a}	42.25 ± 1.03^{b}
Na^{+}(meg/l)	6	43.25 ± 0.63^{b}	34.75 ± 10.10^{b}	60.25 ± 1.11^{a}	61.00 ± 0.41^{a}
	24/12	51.50 ± 0.29^{bc}	54.00 ± 0.41^{ab}	49.00 ± 2.55^{c}	56.75 ± 0.48^{a}
	48/12	71.00 ± 0.41^{c}	86.25 ± 0.25^{a}	75.00 ± 1.73^{b}	86.25 ± 1.03^{a}
	120	30.25 ± 0.85^{c}	24.00 ± 0.82^{d}	63.25 ± 3.12^{a}	54.50 ± 1.04^{b}
Creatinine g/l	6	1.09 ± 0.01^{d}	1.10 ± 0.01^{b}	1.17 ± 0.01^{a}	1.19 ± 0.02^{a}
	24/12	2.08 ± 0.01^{ab}	2.13 ± 0.01^{ab}	1.80 ± 0.24^{b}	2.24 ± 0.03^{a}
	48/12	4.04 ± 0.03^{b}	4.27 ± 0.02^{b}	3.02 ± 0.24^{c}	4.04 ± 0.05^{a}
	120	1.12 ± 0.04^{b}	0.98 ± 0.01^{b}	1.55 ± 0.10^{a}	1.47 ± 0.02^{a}
ALT (Int Units/l)	6	35.50 ± 0.65^{b}	35.25 ± 0.48^{b}	71.75 ± 1.18^{a}	71.75 ± 1.65^{a}
	24/12	49.50 ± 0.65^{b}	50.25 ± 0.25^{b}	48.50 ± 1.71^{d}	57.25 ± 0.75^{a}
	48/12	74.75 ± 0.63^{c}	87.25 ± 0.48^{b}	75.50 ± 1.19^{c}	91.50 ± 0.87^{a}
	120	28.75 ± 1.10^{c}	22.50 ± 1.04^{c}	66.50 ± 3.93^{a}	56.50 ± 0.87^{b}
AST (Int Units/l)	6	47.00 ± 0.41^{b}	46.25 ± 0.48^{b}	64.75 ± 1.18^{a}	65.50 ± 2.02^{a}
	24/12	41.50 ± 0.65^{b}	42.25 ± 0.25^{b}	39.50 ± 2.63^{b}	47.00 ± 0.71^{a}
	48/12	63.25 ± 0.48^{c}	75.75 ± 0.25^{b}	62.75 ± 1.60^{c}	80.00 ± 0.82^{a}
	120	24.25 ± 1.03^{c}	17.15 ± 0.85^{d}	51.25 ± 3.82^{a}	43.75 ± 0.63^{b}
Glucose(g/l)	6	44.75 ± 0.48^{b}	44.50 ± 0.29^{b}	67.50 ± 0.29^{a}	67.75 ± 1.11^{a}
	24/12	49.50 ± 0.50^{bc}	52.50 ± 0.29^{b}	48.00 ± 2.16^{c}	56.25 ± 0.75^{a}
	48/12	71.00 ± 0.41^{b}	79.25 ± 0.48^{a}	72.25 ± 1.80^{b}	80.25 ± 0.25^{a}
	120	27.25 ± 1.11^{b}	20.75 ± 1.11^{c}	58.25 ± 2.32^{a}	54.50 ± 1.04^{a}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Tables 6.1 to 6.3 blood chemistry values. At 6 hours in female exposed to cadmium, total protein, potassium, sodium, creatinine, ALT, AST and glucose significantly ($p \leq 0.05$) decreased compared to control.

At 24 hours after 12-hour garlic treatment, compared to control, total protein significantly ($p \leq 0.05$) increased and decreased in cadmium + garlic and cadmium only respectively. There was no significant ($p \geq 0.05$) difference in garlic only and control. Potassium significantly ($p \leq 0.05$) increased and decreased in cadmium + garlic, cadmium only and garlic only respectively. Potassium level was significantly ($p \leq 0.05$) higher in garlic only than cadmium only. Sodium and creatinine, ALT and AST significantly ($p \leq 0.05$) increased and decreased in cadmium + garlic only and cadmium only respectively sodium level was significantly ($p \leq 0.05$) higher in cadmium + garlic than garlic only. Glucose compared to control significantly ($p \leq 0.05$) increased in all groups. The order of significant ($p \leq 0.05$) increase was cadmium + garlic > garlic only > cadmium only.

At 48 hours after 2 consecutive days of 12-hour garlic treatments, total protein compared to control significantly ($p \leq 0.05$) decreased in cadmium only and garlic only. There were no significant ($p \geq 0.05$) differences in cadmium + garlic and control, cadmium only and garlic only. Potassium, ALT and AST, compared to control significantly ($p \leq 0.05$) decreased in all groups. The values in all groups. The values in cadmium only and garlic only was significantly ($p \leq 0.05$) lower than the cadmium + garlic. Cadmium only and garlic only showed no significant ($p \geq 0.05$) differences. Sodium, creatinine and glucose compared to control significantly ($p \leq 0.05$) decreased in cadmium only and garlic only. There were no significant ($p \geq 0.05$) differences in cadmium only and garlic only.

At 120 hours of the experiment with 2 days 12-hour consecutive garlic treatment, total protein, potassium and sodium compared to control significantly ($p \leq 0.05$) decreased in cadmium + garlic and cadmium only. The values in cadmium + garlic was significantly ($p \leq 0.05$) lower than in cadmium only. There was no significant ($p \leq 0.05$) difference in garlic and control. Creatinine, ALT, AST and sodium compared to control significantly decreased in cadmium + garlic and cadmium only. There was no significant ($p \geq 0.05$) difference in cadmium + garlic and cadmium only, garlic only and control. Glucose compared to control significantly decreased and increased in cadmium + garlic, cadmium only and garlic only respectively. The value in cadmium + garlic was significantly ($p \leq 0.05$) lower than cadmium only.

6 hours in female exposed to lead compared to control, total protein, potassium, sodium, ALT, AST and glucose significantly ($p \leq 0.05$) decreased. Creatinine showed no significant ($p \geq 0.05$) differences in all groups.

At 24 hours after 12 hours of garlic treatment, total protein compared to control significantly ($p \leq 0.05$) decreased in lead + garlic and garlic only. The value in lead + garlic was significantly ($p \leq 0.05$) lower than in garlic only. There was no significant ($p \geq 0.05$) difference in lead only and control. Potassium compared to control significantly ($p \leq 0.05$) decreased and increased in lead + garlic, garlic only and lead only respectively. There was no significant ($p \geq 0.05$) differences in lead + garlic and garlic only. Sodium, ALT and glucose compared to control significantly ($p \leq 0.05$) decreased and increased in lead + garlic, garlic and lead only respectively. The value in lead + garlic was significantly ($p \leq 0.05$) higher than in garlic only. Creatinine and AST compared to control significantly ($p \leq 0.05$) decreased and increased in garlic only and lead only respectively. There was no significant ($p \geq 0.05$) differences in lead + garlic and garlic only, lead + garlic and control.

At 48 hours after 2 days consecutive 12-hour garlic treatment, total protein, potassium, sodium, creatinine, ALT, AST and glucose compared to control, significantly ($p \leq 0.05$) decreased and increased in lead + garlic, garlic only and lead only. The values in lead + garlic was significantly ($p \leq 0.05$) lower than garlic only.

At 120hours of the experiment, total protein compared to control significantly ($p \leq 0.05$) decreased and increased in lead only and garlic only respectively. There was no significant ($p \geq 0.05$) difference in lead + garlic and control. Potassium and sodium compared to control significantly ($p \leq 0.05$) decreased and increased lead + garlic and lead only and garlic only respectively. The value in lead + garlic was significantly ($p \leq 0.05$) higher than lead only. Creatinine compared to control significantly ($p \leq 0.05$) increased in lead + garlic and garlic only. The value was significantly ($p \leq 0.05$) higher in garlic only than lead + garlic. There was no significant ($p \geq 0.05$) difference in lead only and control. ALT and glucose compared to control significantly ($p \leq 0.05$) decreased and increased in lead + garlic, lead only and garlic only respectively. The value was significantly ($p \leq 0.05$) higher in lead + garlic and lead only. The value was significantly ($p \leq 0.05$) higher in lead + garlic than in lead only. There was no significant ($p \geq 0.05$) difference in garlic only and control. AST compared to control significantly ($p \leq 0.05$) decreased in lead + garlic and lead only. The value was significantly ($p \leq 0.05$) higher in lead + garlic than in lead only. There was no significant ($p \geq 0.05$) difference in garlic only and control.

At 6 hours in female exposed to cadmium + lead, total protein, potassium, sodium, creatinine, ALT, AST and glucose compared to control significantly ($p \leq 0.05$) decreased.

At 24 hours after 12 hour of garlic treatment, total protein compared to control significantly ($p \leq 0.05$) decreased in cadmium + lead and garlic only. The value was significantly ($p \leq 0.05$) higher in garlic only than cadmium + lead. There was no significant ($p \geq 0.05$) difference between cadmium + lead + garlic and control. Potassium compared to control, significantly ($p \leq 0.05$) decreased in all groups. There was no significant ($p \geq 0.05$) difference in the groups. Sodium compared to control, significantly ($p \leq 0.05$) decreased in cadmium + lead + garlic and garlic only. The value was not significantly ($p \geq 0.05$) different in cadmium + lead + garlic, garlic only, cadmium + lead, control, cadmium + lead + garlic, cadmium + lead. Creatinine compared to control significantly ($p \leq 0.05$) decreased in garlic only. There was no significant ($p \geq 0.05$) difference in cadmium + lead + garlic, cadmium + lead, control. ALT and AST to control, significantly ($p \leq 0.05$) decreased in all groups with no significant ($p \geq 0.05$) differences. Glucose compared to control significantly ($p \leq 0.05$) decreased in all groups. the order of significant ($p \leq 0.05$) decrease was garlic only > cadmium + lead. There was no significant ($p \geq 0.05$) differences in cadmium + lead + garlic, garlic only; cadmium + lead + garlic, cadmium + lead.

At 48 hours of 2 days consecutive 12-hour garlic treatment, female exposed to 6-hour cadmium + lead, total protein, ALT and AST compared to control significantly ($p \leq 0.05$) decreased in all groups. The values in cadmium + lead + garlic was significantly ($p \leq 0.05$) lower than cadmium + lead. Potassium and sodium compared to control significantly ($p \leq 0.05$) decreased in cadmium + lead + garlic and garlic only. The value was significantly ($p \leq 0.05$) lower in cadmium + lead + garlic than garlic only. There was no significant ($p \geq 0.05$) difference in cadmium + lead and control. Creatinine compared to control significantly ($p \leq 0.05$) decreased in all groups. the order of significant ($p \leq 0.05$) decrease was garlic only > cadmium + lead + garlic, cadmium + lead. There was no significant ($p \leq 0.05$) difference in cadmium + lead + garlic and cadmium + lead garlic and cadmium + lead. Glucose compared to control significantly ($p \leq 0.05$) decreased in cadmium + lead + garlic and garlic only. There was no significant ($p \geq 0.05$) difference in cadmium + lead + garlic, garlic only, cadmium + lead, control.

At 120 hours in exposure to 6-hour cadmium + lead, total protein, potassium, sodium, creatinine, ALT, AST and glucose compared to control significantly ($p \leq 0.05$) decreased in cadmium + lead + garlic and cadmium + lead groups. Relative to control, total protein,

potassium and sodium significantly ($p \leq 0.05$) increased whereas creatinine, ALT, AST and glucose were not significantly ($p \geq 0.05$) different in garlic only groups.

6.4.4 Discussion on Blood chemistry

The result show that at 6 hours of cadmium exposure, total protein, potassium, sodium ALT, AST and glucose ($P \geq 0.05$) were different with control. The result shows that at 6 hours of lead exposure total protein, potassium, sodium, ALT, AST, glucose was significantly ($P \leq 0.05$) lower than control whereas creatinine was not significantly ($P \geq 0.05$) different from the control. The results also show that in exposure to cadmium + lead, total protein, potassium, sodium creatinine, ALT, AST and glucose were significantly ($P \leq 0.05$) lower than control. These results are in agreement with Stoskopf (1993) that the evaluation of blood cells, blood biochemistry and hormones could be useful for the diagnosis of fish disease and to monitor the physiological status of fish. Kyoungju (2004) reported a lower total protein concentration in nile Tilapia in response to hypoxia (low dissolved oxygen). However, this is not in agreement with the findings in this study whereby dissolved oxygen at 6 hour of exposure to cadmium dissolved oxygen was higher significantly ($P \leq 0.05$) than control, yet there was lower total protein. Also, exposure to cadmium + lead that resulted in mortality just before the end of 6-hour exposure dissolved oxygen was significantly ($P \leq 0.05$) higher than control probably due to reduced activities induced by the metals. It may therefore be said that lower total protein was not due to low dissolved oxygen in exposure to cadmium, lead and cadmium + lead. It may have been due to damage in the tissues such as the gills and accessory organs involved in uptake of oxygen. Lower level of plasma protein has been associated with diseased fish (Wedemeyer *et al*., 1984) and also associated with starvation and depletion of energy stores (Lockharl *et al*., 1984; Cunjak 1988). It means cadmium; lead and cadmium + lead cause ill health. The mechanism of producing the low protein level according to Lutz and Nilson 1997; Mazeaud *et al*., 1971 may be due to decreased protein synthesis or increased in protein catabolism in order to reduce energy utilization induced by low oxygen stress. In this regard, with a significantly higher DO than control, the low oxygen stress may mean low oxygen in tissue because of damaged oxygen uptake mechanisms. The water quality and metals in water of concern in monitoring cadmium induced acute toxicity could be indicated by significant ($P \leq 0.05$) decrease in pH, alkalinity and lead with no significant ($P \geq 0.05$) change in potassium and increase cadmium and manganese. For lead it could be indicated by significant decrease in pH, no change in alkalinity and magnesium, significant ($P \leq 0.05$) increase in lead and cadmium with a significant decrease in manganese. For cadmium + lead that caused mortality was a significant ($P \leq 0.05$) decrease in pH, no

change in alkalinity, significant (P≤0.05) increase in ammonia, cadmium, lead and manganese. The decrease in electrolytes, exposure to cadmium, lead and cadmium + lead is in agreement with Warner and Whitney (2006) that reported significantly lower sodium. in *Ictalurus punctatus* raised in intensive raceways (with low dissolved oxygen) than in pond population. This reason had earlier been advanced when discussing total protein. Also, Kori-Siakpere (1988) reported that reduction of plasma electrolytes levels was an indication of osmoregulatory impairment in experimental fish.

Transaminases (ALT and AST) enzymes significantly (P≤0.05) decrease and is in agreement with Khadiga *et al*., 2003 that AST was suppressed in fish caught from water with the highest metal concentration. For creatinine, it appears for the effect of cadmium and lead singularly, lead had no effect which could be used as biomarker for cadmium toxicity. Cadmium and lead effects may be the suppression of organs involved in creatinine synthesis. It therefore means that acute cadmium suppressed creatinine production. Also, acutely lead had no effect. In cadmium + lead there was suppression of creatinine production which resulted in a decline and is an indication of decrease in the mass of muscle. Glucose level significant decrease may be due to high level of the metals which was suppressive and the reason could be related to the explanation of the effects in ALT and AST.Lead and cadmium + lead exposure resulted in a lower level of activities with an attendant consumption of lower dissolved oxygen in the water. Lead significantly (P≤0.05) decrease in pH, alkalinity, no significant change in chloride, total hardness, magnesium and significant (P≤0.05) but increased cadmium, lead and manganese.

For cadmium + lead the significant (P≤0.05) decrease in pH, alkalinity, DO and significant (P≤0.05) increase in Cd, Pb, and Mn at 6 hours post exposure may be due to the high concentration that is yet to to be taken up and/or be transformed. The effect on pH and alkalinity may be due to increased acidity whereas for DO it may be due to increased erratic activities induced by the metals. At 24 hours of the experiment, the effect of 12-hour garlic bath in cadmium exposure showed significantly (P≤0.05) higher total protein, potassium, sodium, creatinine ALT, AST and glucose than control, these results show a higher value than the values at 6 hour of experiment. It means garlic increased these values by antagonizing the effects of these metals. The result is in agreement with the findings of Metwally (2009) that total protein in blood serum significantly (P≤0.05) increased in *Tilapia nilotica* fed with garlic. The increase in serum total protein in albino rats treated with *Allium sativum* oil could be attributed to increase in immunoglobulin level and total globulin concentration. It thus appears that with the initial significant (P≤0.05) decrease in total

protein at 6 hours, the garlic increase of total protein at 24 hours after garlic bath is beneficial to increase the innate immune response. This explanation could be extended to the increase in potassium, sodium, creatinine, ALT, AST and glucose. The findings by some authors including Hille (1982) that high serum protein level is an indicative of osmoregulatory dysfunction, haemodilution or tissue damage of surrounding blood vessels may be for chronic exposure not acute exposure.

At 24 hour of experiment the effect of 12-hour water renewal in cadmium exposure showed significantly ($P \leq 0.05$) lower total protein, potassium, sodium, creatinine, ALT and AST. These values significant ($P \leq 0.05$) decrease was not reversed by water renewal. The value of glucose was significantly ($P \leq 0.05$) higher than control. These mean that glucose is very sensitive to change in the environment. In acute toxicity of cadmium an increase in glucose level may be indication of recovery.

In general, in all the three groups of cadmium only, lead only and cadmium + lead with no exposure but bathed with garlic for 12 hours, there was significant ($P \leq 0.05$) decrease in total protein, potassium, sodium, creatinine and glucose. They are in agreement with the findings that plasma glucose, ALT, AST significantly decreased with increasing levels of *Allium Sativum* (Shalaby *et al*., 2006; Salah and Rogers 1993).

The reduction of plasma electrolyte levels is an indication of osmoregulatory impairment in experimental fish (Kori-Siakperie, 1996) may be relevant to chronic exposure. It appears that garlic decreased significantly sodium and potassium which may be compensatory without loss of sodium and potassium balance making it beneficial.

At 24 hour of the experiment, the effect of 12-hour garlic bath in lead exposure showed significant ($P \leq 0.05$) decrease in total protein and potassium. There was no significant ($P \geq 0.05$) difference in sodium, creatinine, ALT, AST and glucose. These means that garlic was beneficial with increase in innate immune response (increased protein, decreased potassium but fall to decrease sodium resulting in probably and imbalance in the ions, but increased the levels of sodium, creatinine, ALT, AST and glucose to similar value of control.

At 24 hour of experiment, the effect of 12-hour water renewal only in lead only exposure showed no significant ($P \geq 0.05$) difference in total protein, significant ($P \leq 0.05$) increase in potassium, sodium, creatinine, ALT, AST and glucose. It means that after the 6-hour exposure, water renewal could not improve innate immune response, but increased electrolyte (without loss of balance) increased level of renal and organ damage and stress.

At 24 hour of experiment, the effects of 12-hour garlic bath in cadmium + lead exposure showed no-significant ($P \geq 0.05$) difference in total protein and creatinine, significant ($P \geq 0.05$)

decrease in potassium, sodium, ALT, AST and glucose compared with control. The total protein result may be due to potentiation of cadmium by garlic to antagonize lead. For potassium and sodium garlic potentiated lead to antagonize cadmium. For creatinine, ALT, AST and glucose garlic potentiated lead to enhance competitive antagonism of cadmium and lead. These may mean that garlic improve the level of protein from the 6 hour very low level thereby enhancing innate immune response still significantly decreasing potassium and sodium as an osmoregulatory compensatory mechanism, regeneration of renal function by non significant difference in creatinine level and significant ($P \leq 0.05$) rduction in organs damages with a significant ($P \leq 0.05$) lower level of ALT, and AST enzymes activities including lower stress levels indicated by lower glucose level compared to control.

Generally, garlic decreases the effects of cadmium, lead and cadmium + lead toxicity. Water renewal could not abate cadmium, lead and cadmium + lead toxicity.

At 48 hour of the experiment after 2 days consecutive 12-hour water renewal exposure to cadmium, there was significant ($P \leq 0.05$) decrease in total protein which may mean decreased innate immune response is detrimental, whereas the significant ($P \leq 0.05$) decrease in potassium, sodium creatinine, ALT, AST, and glucose are beneficial. It means water renewal reduced cadmium toxicity.

Generally, in the 3 groups of cadmium only, lead only and cadmium + lead bathed in 2 consecutive days of 12 hour non optimum garlic concentration showed significant ($P \leq 0.05$) decrease in total protein which was detrimental, potassium, sodium, creatinine, ALT, AST and glucose which were beneficial. The decreased total protein may be due to lower non optimum concentration of garlic and/or short duration of garlic bath.

At 48 hour of experiment after 2 days consecutive 12-hour garlic bath of lead exposure the effects of significant ($P \leq 0.05$) decrease in total protein was detrimental, potassium, sodium, creatinine, ALT, AST, glucose is beneficial. At 48 hour of experiment after 2 days of consecutive 12 hours' water renewal in exposure to lead the effects of significant ($P \leq 0.05$) increase in total protein was detrimental, potassium and sodium was beneficial, creatinine, ALT, AST and glucose was detrimental. It means water renewal was not able to abate lead toxicity in female.

At 48 hours of experiment after 2 days consecutive 12-hour garlic bath in exposure to cadmium + lead effects significant ($P \leq 0.05$) decrease in total protein, was detrimental, potassium, sodium creatinine, ALT, AST and glucose was beneficial. The effect on total protein, potassium, sodium, creatinine, ALT, AST and glucose may be due to potentiation of lead by garlic to antagonize cadmium.

At 48 hour of experiment after 2 days consecutive 12-hour water renewal in exposure to cadmium + lead effects of significant (P≤0.05) decrease in total protein, significant (P≤0.05) increase in potassium, sodium, significant (P≤0.05) decrease in creatinine, ALT and AST and the non significant (P≥0.05) difference in glucose were beneficial. These effects may be due to non competitive antagonism of cadmium by lead.

At 120 hour of experiment, exposure to cadmium with 2 days consecutive 12-hour garlic bath, after 6-hour cadmium exposure effect of significant (P≤0.05) decrease in total protein, potassium, sodium, creatinine, ALT, AST and glucose is beneficial.

At 120 hour of experiment, exposure to cadmium with 3 days' consecutive water renewal effects of significant (P≤0.05) decrease in total protein, potassium, sodium, creatinine, ALT, AST and glucose was beneficial. However, the value of potassium, sodium and glucose in garlic bath was significantly (P≤0.05) lower than with water renewal which may means more benefit in the decrease of cadmium toxicity with garlic bath than water renewals only.

Generally, at 120 hours in 3 groups of cadmium only, lead only and cadmium + lead not exposed to metals but bathed in 2 consecutive days of 12 hour non optimum garlic concentration showed varied responses. It showed in summary, significant (P≤0.05) increase in total protein, potassium, sodium, creatinine, ALT, AST and glucose. It means that the effect of garlic is still on after the stoppage of bath. At 120 hour of experiment after 2 days consecutive 12-hour garlic bath in exposure to lead, the effects of significant (P≤0.05) increase in total protein, significant (P≤0.05) decrease in potassium, sodium, ALT, AST and glucose were beneficial whereas the significant increase in creatinine was detrimental. It means however that there was a decrease in toxicity.

At 120 hour of experiment after 3 days consecutive 12-hour water renewal in exposure to lead, the effects of significant (P≤0.05) decrease in total protein, was detrimental, whereas the significant (P≤0.05) decrease in potassium, sodium, ALT, AST and glucose including the non significant (P≥0.05) difference in creatinine were beneficial. It appears that garlic bath and water renewal decreased toxicity of lead suggestive that total protein, potassium, sodium creatinine ALT, AST, and glucose may not be very sensitive in the assessment of lead toxicity probably after treatment had started.

At 120 hour of experiment after 2 days consecutive 12-hour garlic bath at day 1 and 2, in female exposed to cadmium + lead effects of significant (P≤0.05) decrease in total protein, potassium, sodium, creatinine, ALT, AST, and glucose were beneficial as a compensatory mechanism.

At 120 hour of experiment after 3 days consecutive 12-hour water renewal at day 1, 2 and 3 in female exposed to cadmium + lead effects of significant ($P \leq 0.05$) decrease in total protein, potassium, sodium, creatinine, ALT, AST and glucose in absolute value appear detrimental but it appears as a compensatory mechanism which is beneficial.

The values in garlic bath were significantly ($P \leq 0.05$) higher than with only water renewal in total protein, potassium, sodium, AST and glucose indicative of more effect in cadmium + lead toxicity decrease. It appears therefore that the treatment of cadmium + lead may require more days of garlic bath. In garlic bath, the effects on total protein, creatinine, ALT, AST, and glucose was garlic acted as a catalyst/substrate competitive antagonism of cadmium and lead whereas for potassium and sodium, garlic potentiated cadmium to non competitively antagonize lead. In water renewal, for total protein, potassium, sodium and creatinine cadmium competitively antagonized lead whereas for ALT, AST and glucose lead competitively antagonised cadmium.

Comparatively exposure to cadmium for 6 hours and treated in 12-hour garlic bath at day 1 and 2 of experiment shows that the comparative order of significant ($P \leq 0.05$) decrease at 6 hours, 24 hours, 48 hours and 120 hours in total protein, potassium, sodium, creatinine, ALT, AST and glucose was 48 hours > 24 hours > 6 hours > 120 hours. It means garlic increased the parameters with the first and second bath but the effects weaned at 120 hours. The values of total protein, potassium, sodium, creatinine ALT, AST and glucose were significantly ($P \leq 0.05$) higher at 24 hours than control. It appears that 48 hours after the second garlic bath, the values of total protein, sodium, creatinine and glucose showed no significant ($P \geq 0.05$) difference with control whereas there was significant ($P \leq 0.05$) decrease in ALT, AST and Potassium compared to control. It means the first garlic bath effect could have been regarded as side effect whereas the second bath had beneficial effects in the reduction of cadmium toxicity. However, at 120 hour of the experiment, value of total protein, potassium, sodium, creatinine, ALT, AST and glucose were significantly ($P \leq 0.05$) lower than control which were also significantly ($P \leq 0.05$) lower than the values at 6 hour of exposure which may suggest that the use of absolute of lower creatinine, ALT, AST and glucose as beneficial effects may not be absolutely correct. It may be considered with other factors/parameters to determine if it is beneficial, adaptational/ or compensatory. Also the significant ($P \leq 0.05$) decrease in total protein, as detrimental should also be considered. However, using these blood biochemical parameters could suggest that cadmium toxicity in female, is tractable and may require garlic bath beyond 2 times to sustain the levels of these parameters.

Exposure to cadmium for 6 hours and water renewal for 3 consecutive days shows comparative order of significant (P≤0.05) decrease at 6 hours, 24 hours, 48 hours and 120 hours for total protein, potassium and sodium was 48 hours > 24 hours > 6 hours, 120 hours, for creatinine was 48 hours > 24 hours > 120 hours, 6 hours for ALT and AST was 48 hours > 24 hours > 6 hours > 120 hours whereas for glucose was 48 hours > 24 hours > 6 hours, 120 hours. It means water renewal had different effects on different parameters. For total protein, potassium and sodium, it means first, and second water renewals increased progressively these parameters whereas the third decreased having been left for 3 days without renewal decreased the values non-significantly to the 6-hour value. The value of total protein, potassium creatinine, ALT and AST were significantly (P≤0.05) lower than control whereas the value of glucose was significantly (P≤0.05) higher than control at 24 hours. It means water renewal was therapeutic by increasing these values. It may mean that increased activity may be the first sign of recovery. The second water renewal resulted in the values of these parameters at 48 hours still significantly (P≤0.05) lower than control including glucose. It means the increased activity with increased glucose declined which means water renewal could still not significantly reduce cadmium toxicity. The values of these parameters at 120 hours were below the values at 48 hours and still significantly (P≤0.05) lower than control and not significantly (P≥0.05) different from the 6 hour values for total protein, potassium, sodium, creatinine, and glucose whereas was significantly (P≤0.05) lower than 6 hours' values. It means the third water renewal could not improve on reduction of toxicity values at 48 hours. It does agree with the findings with garlic bath that further treatment maybe needed including water renewal.

Generally, with garlic only in three groups of cadmium, lead and cadmium + lead for 12 hour at day 1 and 2 of the experiment shows that the order of significant (P≤0.05) decrease for total protein was 48 hours > 24 hours, 120 hours > 6 hours, for potassium and sodium was 48 hours > 6 hours, 120 hours > 24 hours for creatinine was 48 hours > 24 hours > 6 hours there was no significant (P≥0.05) difference in 48 hours and 120 hours,120 hour and 6 hour, for ALT was 48 hour > 120 hours > 24 hours with no significant (P≥0.05) difference in 48 hours and 6 hours, 6 hours and 120 hours, for AST was 6 hours, 48 hours > 120 hours > 24 hours, for glucose was 48 hours, 6 hours > 120 hours > 24 hours. It means garlic bath had different effects for the different parameters at different duration of bath.

For total protein, the first and second bath increased significantly total protein. However, at 120 hours, it significantly (P≤0.05) decreased to the value at 24 hours. The value of total protein, was significantly (P≤0.05) lower than control at 24 hours of the first treatment, at 48

hours of the second garlic bath was still significantly (P≤0.05) lower than control whereas at 120 hours was significantly (P≤0.05) higher than control. It appears the effect of garlic bath on total protein was a decreased effect initially but increased effect later compared with control.

For potassium, and sodium, garlic decreased their value significantly (P≤0.05) with the first garlic bath whereas the second bath significantly (P≤0.05) increased their value. However, at 120 hours, their values declined significantly to the values at 6 hours, which was significantly higher than control. Potassium and sodium were significantly (P≤0.05) lower than control at 24 hours and 48 hours. It may be due to lower non optimum concentration use and the duration of bath. However, at 120 hours of the experiment, their value was significantly (P≤0.05) higher than control. It may mean that garlic effect weaned for creatinine garlic the first and second bath significantly (P≤0.05) increased creatinine whereas the values at 120 hours was not significantly (P≥0.05) different in 6 hours and 48 hours' values. The values at 24 hours and 48 hours were significantly (P≤0.05) lower than control whereas at 120 hours was not significantly (P≥0.05) different from control. It appears that garlic effect weaned at 120 hours. For ALT, the first garlic bath decreased significantly (P≤0.05) ALT whereas the second increased significantly (P≤0.05) which declined significantly (P≤0.05) at 120 hours. The values of ALT were significantly (P≤0.05) lower than control at 24 hours and 48 hours whereas was significantly (P≤0.05) higher than control at 120 hours. It means at 120 hours, garlic effect weaned. For AST, the first garlic bath decreased significantly (P≤0.05) AST whereas the second bath increased significantly (P≤0.05) AST whereas at 120 hours, it was significantly (P≤0.05) lower than the 48 hours' value. The value of AST at 24 hours and 48 hours was significantly (P≤0.05) lower than control whereas at 120 hours the value of AST was not significantly (P≥0.05) different from control. It means also that the effect of garlic weaned at 120 hours. For glucose, the first garlic bath decreased significantly (P≤0.05) glucose, the second bath increased significantly (P≤0.05) glucose whereas at 120 hours, the values significantly (P≤0.05) decreased. The value of glucose at 24 hours and 48 hours was significantly (P≤0.05) lower than control whereas at 120 hours was significantly (P≤0.05) higher than control. It means the effect of decreased glucose weaned at 120 hours. It appears in female garlic decrease of total protein, potassium, sodium, creatinine, ALT, AST and glucose weaned within a period of three days after the last bath in apparently healthy fish.

Generally, in the three control groups of cadmium, lead and cadmium + lead, with 3 consecutive days (day 1, 2 and 3) of 12 hours' water renewal order of significant (P≤0.05) decrease at 6 hours, 24 hours, 48 hours and 120 hours for total protein and creatinine was 48

hours > 24 hours > 120 hours, 6 hours, for potassium, sodium and glucose was 48 hours > 6 hours > 24 hours, 120 hours, for ALT and AST was 48 hours > 6 hours > 120 hours, 24 hours. For total protein and creatinine, the first water renewal significantly (P≤0.05) increased their values; the second water renewal increased their values whereas the third water renewal effect at 120 hours (6^{th} day) was a significant (P≤0.05) decrease to a value that showed no significant (P≤0.05) difference with the value at 6 hours. It means water renewal significantly (P≤0.05) increased total protein and creatinine. For potassium, sodium and glucose, the first water renewal decreased significantly (P≤0.05) their values, the second increased significantly (P≤0.05) their values while the third water renewal at the 3^{rd} day resulted at 120 hours (at day 6) significant (P≤0.05) decrease in their value which was not significantly (P≥0.05) different with the value at 24 hours. For ALT and AST, the first water renewal decreased significantly (P≤0.05) their values the second water renewal increased significantly (P≤0.05) their values whereas the 3^{rd} water renewal at 120 hours significantly (P≤0.05) decreased their values. These means that water renewal was therapeutic. It appears in apparently healthy female, first water renewal, which significantly (P≤0.05) increased total protein, creatinine, decreased potassium, sodium, glucose, ALT and AST may be preferable than a consecutive second and third water renewals. It may also mean that in apparently healthy female, increased total protein and creatinine, decreased potassium, sodium, glucose, ALT and AST may indicate good health.

Exposure to lead for 6 hours and treated in 12 hours garlic bath at day 1 and 2 of experiment shows that the comparative order of significant (P≤0.05) decrease at 6 hours, 24 hours, 48 hours and 120 hours in total protein was 24 hours > 120 hours > 48 hours > 6 hours, for potassium was 24 hours > 120 hours > 48 hours, 6 hours, for sodium was 24 hours > 120 hours > 48 hours >6 hours, for creatinine was 24 hours > 120 hours, 48 hours > 6 hours, for ALT was 24 hours, 120 hours, 48 hours > 6 hours, for AST was 24 hours > 120 hours, 48 hours > 6 hours, for glucose was 24 hours, 48 hours > 120 hours > 6 hours for total protein, it mean the first garlic bath significantly (P≤0.05) increased its value, the second significantly (P≤0.05) decreased its value which at 120 hours significantly (P≤0.05) increased its value. The value of total protein was significantly (P≤0.05) lower than control at 24 hour and 48 hours whereas it was not significantly (P≥0.05) different with control at 120 hours of the experiment. It means garlic decreased total protein in female and it effect weaned at 120 hours of the experiment. For potassium, it shows that the first garlic bath significantly (P≤0.05) increased it value, the second bath significantly (P≤0.05) decreased its value whereas at 120 hours there was a significant (P≤0.05) increase in its value. The value of

potassium was significantly (P≤0.05) lower than control at 24 hours and 48 hours whereas it showed no significant (P≥0.05) difference at 120 hours. It may mean that garlic decreased potassium whose effect weaned at 120 hours. For sodium, the first garlic bath significantly (P≤0.05) increased its value, the second bath significantly (P≤0.05) decreased its value which at 120 hours showed a significant (P≤0.05) increase. The value of sodium not significantly (P≥0.05) different with control at 24 hours, but was significantly (P≤0.05) lower than control at 48 hours which at 120 hours was significantly (P≤0.05) lower than control. It means garlic needed more garlic bath to effect a decrease in sodium whose effect lasted till 6 days of the experiment. For creatinine, it shows that the first garlic bath significantly (P≤0.05) increased its values, the second significantly (P≤0.05) decreased its value which at 120 hours showed a no significant (P≥0.05) difference at 24 hours with control, at 48 hours was significantly (P≤0.05) lower than control which at 120 hours was significantly (P≤0.05) higher than control. It means garlic decreased creatinine which effect weaned at 120 hours. For ALT, it shows that the first bath significantly (P≤0.05) increased its value, the second bath value showed no significant (P≤0.05) difference with value at 48 hours and 120 hours and 24 hours. The value of ALT at 24 hours showed no significant (P≥0.05) difference with control, at 48 hours was significantly (P≤0.05) lower than control while at 120 hours was also significantly (P≤0.05) lower than control. It means garlic significantly (P≤0.05) decreased ALT which continued at 120 hours of the experiment. For AST, it shows that the first garlic bath significantly (P≤0.05) increased its value, the second bath significantly (P≤0.05) decreased its value which was not significantly different (P≥0.05) from the 120 hours' value. The value of AST at 24 hours was not significantly (P≥0.05) different with control, at 48 hour and 120 hours was significantly (P≤0.05) lower than control. It means garlic significantly (P≤0.05) decreased AST whose effect was obtained after the second garlic bath and continued till 120 hours of the experiment. For glucose, the first garlic bath increased glucose, the second bath resulted in a non-significant (P≥0.05) decrease in value which became significant (P≤0.05) decrease at 120 hours. The value of glucose at 24 hours was not significantly (P≥0.05) different from control, at 48 hours and 120 hours it was significantly (P≤0.05) lower than control. It means garlic decreased glucose level after the second garlic bath which remained effective at 120 hours. It means in lead toxicity, garlic significantly decreased total protein, potassium and the effect weaned at 120 hours, sodium, creatinine, ALT, AST and glucose which remained effective at 120 hours of the experiment.

Exposure to lead for 6 hours with 3 days (day 1, 2 and 3) consecutive 12 hours' water renewal show that the comparative order of significant (P≤0.05) decrease at 6 hours, 24

hours, 48 hours and 120 hours for total protein, creatinine, and AST was 48 hours > 24 hours > 6 hours, 120 hours, for potassium, sodium and glucose was 48 hours > 24 hours > 6 hours > 120 hours and for ALT was 48 hours > 24 hours > 6 hours > 120 hours. For total protein, creatinine and AST, it means that the first and second water renewal significantly ($P \leq 0.05$) increased their values while the third water renewal at day 3 produced a significantly ($P \leq 0.05$) decreased value at 120 hours. The value of total protein was not significantly ($P \geq 0.05$) different from control at 24 hours, at 48 hours, it was significantly ($P \leq 0.05$) higher than control whereas at 120 hours, it was significantly ($P \leq 0.05$) lower than control. It means the first water renewal had no effect while the second increased total protein above control which is considered detrimental probably due to the effect of lead toxicity becoming effective and water renewal could not abate it. However, at 120 hours after the water renewal at day 3, total protein value declined probably due to adatatunal syndrome/adjustment which was still detrimental. The value of creatinine was significantly ($P \leq 0.05$) higher than control at 24 hours, at 48 hours it was significantly ($P \leq 0.05$) higher than control which may also be due to the effect of lead manifesting, while at 120 hours it showed no significant ($P \geq 0.05$) difference with control. It may mean that water renewal at these stages do decrease creatinine. The value of AST was not significantly ($P \geq 0.05$) different from control at 24 hours, at 48 hours it was significantly ($P \leq 0.05$) higher than control whereas at 120 hours it was significantly ($P \leq 0.05$) lower than control. It means lead toxicity with regards to AST was delayed till 48 hours when water renewal reduced its effects at 120 hours. For potassium, sodium and glucose, it shows that the first and second water renewals progressively and significantly ($P \leq 0.05$) increased their values. However, at 120 hours after the 3rd water renewal at day 3, their values significantly declined. The value of potassium, sodium and glucose were significantly ($P \leq 0.05$) higher than control at 24 hours, and 48 hours which mean water renewal had no positive effects on these parameters. However, after 3 days of the last water renewal at 120 hours, their values became significantly ($P \leq 0.05$) lower than control which is adaptational although detrimental could be considered slightly beneficial. For ALT, it shows that the first and second water renewal progressively and significantly ($P \leq 0.05$) increase its value while the third water renewal significantly decreased its value. The value of ALT at 24 hours and 48 hours was significantly ($P \leq 0.05$) higher than control while at 120 hours it was significantly ($P \leq 0.05$) lower than control. It means that in lead toxicity in female there was a delay in its effects on total protein, potassium, sodium, ALT, AST and glucose except creatinine that appear to be very sensitive. Also, water renewal could reduce the effects of lead toxicity in female at 120 hours.

Exposure to cadmium + lead and treated in 12-hour garlic bath at day 1 and 2 of experiment show that order of comparative significant ($P \leq 0.05$) decrease at 6 hours, 24 hours, 48 hours and 120 hours for total protein, potassium, sodium, ALT and glucose was 48 hours > 24 hours > 6 hours > 120 hours, for creatinine was 48 hours > 24 hours > 120 hours > 6 hours for AST was 48 hours > 6 hours > 24 hours > 120 hours. For total protein, potassium, sodium, ALT and glucose it shows that the first and second garlic bath progressively and significantly ($P \leq 0.05$) increased their values while at 120 hours the values significantly declined to a level that was significantly ($P \leq 0.05$) lower than their values at 6 hours. The value of total protein, at 24 hours was not significantly ($P \geq 0.05$) different from control, at 48 hours it was significantly ($P \leq 0.05$) lower than control while at 120 hours it was also significantly ($P \leq 0.05$) lower than control. It means that garlic although significantly increased total protein it was still not able to meet the control level which means more days of garlic bath to achieve it. This means garlic decreased total protein with increased garlic bath. The values of potassium, sodium, ALT and glucose were significantly ($P \leq 0.05$) lower than control at 24 hours. It means that despite garlic significant ($P \leq 0.05$) increase in these values it was significantly lower than control at 24 hours and 48 hours. However, it was still significantly ($P \leq 0.05$) lower than control at 120 hours. It means the toxic level may have been reduced marginally and probably may require longer period of garlic bath. For creatinine, it still shows that garlic progressively and significantly ($P \leq 0.05$) increased creatinine at 24 hours and 48 hours. However, there was a significant ($P \leq 0.05$) decrease at 120 hours. The value of creatinine was not significantly ($P \geq 0.05$) different from control at 24 hours, significantly ($P \leq 0.05$) lower than control at 48 hours and 120 hours. It means creatinine is sensitive and a decrease could be used as a sign of recovery which was obtained at 48 hours. For AST, it shows that first garlic bath significantly ($P \leq 0.05$) decreased its value, the second significantly ($P \leq 0.05$) increased its value while at 120 hours there was a significant ($P \leq 0.05$) decrease. The value of AST was significantly ($P \leq 0.05$) lower at 24 hours, 48 hours and 120 hours than control. It means cadmium + lead toxicity in garlic bath, garlic increased significantly total protein, potassium, sodium, creatinine, ALT, AST and glucose but failed to attain the control values. It may require more days of garlic bath.

Exposure to cadmium + lead for 6 hours and had water renewed for 3 consecutive days (day 1, 2, and 3) shows comparative order of significant ($P \leq 0.05$) decrease at 6 hours, 24 hours, 48 hours and 120 hours for total protein, potassium, creatinine, ALT and glucose was 48 hours > 24 hours > 6 hours > 120 hours, for sodium was 48 hours > 24 hours > 6 hours, 120 hours with no significant ($P \geq 0.05$) difference between values of 6 hours and 120 hours, for AST

was 48 hours > 6 hours > 24 hours > 120 hours. For total protein, potassium, creatinine, ALT and glucose it shows that the first and second water renewal significantly ($P \leq 0.05$) increased their values whereas the third water renewal at day 3 produced a significantly ($P \leq 0.05$) lower value at 120 hours which was significantly ($P \leq 0.05$) lower than the 6 hours' value. The values at 24 hours of total protein, potassium, ALT and glucose were significantly ($P \leq 0.05$) lower than control whereas creatinine value was not significantly ($P \geq 0.05$) different from control. It means the first water renewal increase of these values could not get to the control value at 24 hours. At 48 hours, the values of total protein, ALT was significantly ($P \leq 0.05$) lower than control, however the value of potassium and glucose was not significantly ($P \geq 0.05$) different from control including the significantly ($P \leq 0.05$) lower value of creatinine than control at 48 hours shows reduced toxicity by water renewal. At 120 hours after the third water renewal at day 3, the value of total protein, potassium, ALT, creatinine and glucose were significantly ($P \leq 0.05$) lower than control but being that these values were significantly lower than the level at 6 hours it may mean more level of toxicity. For sodium, it shows that the first and second water renewal progressively and significantly ($P \leq 0.05$) increased its value at 24 hours and 48 hours. At 120 hours, its value was significantly ($P \leq 0.05$) decreased below the level at 6 hours of the experiment. It values at 24 was significantly ($P \leq 0.05$) lower than control. At 48 hours, its value was not significantly ($P \geq 0.05$) different from control. At 120 hours its value was significantly ($P \leq 0.05$) lower than control. For AST it shows that the first water renewal significantly ($P \leq 0.05$) decreased its value, the second water renewal significantly increased its value while the third water renewal at day 3 produced at 120 hours a significantly ($P \leq 0.05$) decreased value. The value of AST at 24 hours, 48 hours and 120 hours were significantly ($P \leq 0.05$) lower than control. It means at 48 hours the second water renewal decreased cadmium + lead toxicity in female which was not sustained at 120 hours. It may mean that more days of water renewal may be required to sustain the decreased toxicity at 48 hours after the second water renewal.

6.4.5 Results of metals in water

Table 6.4: Effect of garlic treatments Means ± SEM of water metal levels at 12hr and 24hr after 6hr exposure to lethal dose of Cadmium

Parameter	Hours of experiment	Cadmium + garlic	Cadmium only	Garlic only	Control
Lead mg/l	6	0.67 ± 0.01^{a}	0.67 ± 0.01^{a}	0.66 ± 0.04^{b}	0.66 ± 0.03^{b}
	24	0.33 ± 0.01^{a}	0.29 ± 0.01^{a}	0.20 ± 0.04^{b}	0.37 ± 0.01^{a}
	120	0.29 ± 0.01^{b}	0.39 ± 0.01^{a}	0.23 ± 0.01^{c}	0.02 ± 0.01^{d}
Cadmium mg/l	6	76.75 ± 0.45^{a}	76.80 ± 1.20^{a}	0.10 ± 0.00^{b}	0.10 ± 0.00^{b}
	24	0.71 ± 0.01^{b}	0.77 ± 0.01^{a}	0.32 ± 0.00^{c}	0.34 ± 0.01^{c}
	120	0.13 ± 0.01^{b}	0.41 ± 0.0^{a}	0.03 ± 0.00^{c}	0.13 ± 0.01^{b}
Manganese mg/l	6	0.15 ± 0.01^{a}	0.15 ± 0.01^{a}	0.12 ± 0.01^{b}	0.12 ± 0.00^{b}
	24	0.02 ± 0.61^{b}	0.39 ± 0.01^{a}	0.06 ± 0.02^{b}	0.03 ± 0.01^{b}
	120	0.14 ± 0.62^{b}	0.22 ± 0.02^{a}	0.24 ± 0.01^{a}	0.18 ± 0.02^{ab}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$.

Table 6.5: Effect of garlic treatments Means ± SEM of water metal levels at 12hr and 24hr after 6hr exposure to lethal dose of Lead

Parameter	Hour of experiment	Lead + garlic	Lead only	Garlic only	Control
Lead mg/l	6	111.50 ± 3.50^{a}	112.00 ± 1.00^{a}	0.62 ± 0.03^{b}	0.66 ± 0.02^{b}
	24	4.44 ± 0.06^{a}	1.22 ± 0.03^{b}	0.11 ± 0.01^{d}	0.37 ± 0.02^{e}
	120	0.49 ± 0.04^{a}	0.13 ± 0.03^{c}	0.26 ± 0.02^{b}	0.03 ± 0.01^{c}
Cadmium mg/l	6	0.50 ± 0.03^{a}	0.49 ± 0.01^{a}	0.10 ± 0.00^{b}	0.09 ± 0.01^{b}
	24	0.15 ± 0.01^{b}	0.11 ± 0.01^{c}	0.14 ± 0.01^{b}	0.32 ± 0.01^{a}
	120	0.23 ± 0.02^{a}	0.17 ± 0.06^{b}	0.18 ± 0.01^{b}	$0.04 \pm 0.03a^{b}$
Manganese mg/l	6	0.60 ± 0.01^{b}	0.67 ± 0.01^{b}	0.12 ± 0.01^{a}	0.11 ± 0.01^{a}
	24	1.72 ± 0.05^{a}	0.06 ± 0.01^{b}	0.07 ± 0.01^{b}	0.03 ± 0.00^{d}
	120	0.21 ± 0.02^{a}	0.19 ± 0.05^{a}	0.27 ± 0.04^{a}	0.19 ± 0.02^{a}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$.

Table 6.6: Effect of garlic treatments Means ± SEM of water metal levels at 12hr and 24hr after 6hr exposure to lethal dose of Cadmium + Lead

Parameter	Hour of experiment	Cadmium + lead + garlic	Cadmium + lead only	Garlic only	Control
Lead mg/l	6	102.00 ± 2.00^{a}	102.50 ± 0.50^{a}	0.66 ± 0.02^{b}	0.65 ± 0.01^{b}
	24	0.94 ± 0.02^{b}	1.56 ± 0.03^{a}	0.19 ± 0.01^{d}	0.37 ± 0.01^{c}
	120	0.38 ± 0.03^{b}	0.76 ± 0.62^{a}	0.23 ± 0.00^{c}	0.03 ± 0.01^{d}
Cadmium mg/l	6	86.95 ± 1.05^{a}	87.20 ± 1.10^{a}	0.10 ± 0.01^{b}	0.09 ± 0.00^{b}
	24	0.48 ± 0.01^{a}	0.44 ± 0.01^{a}	0.26 ± 0.03^{c}	0.36 ± 0.02^{b}
	120	0.15 ± 0.01^{ab}	0.17 ± 0.01^{a}	0.03 ± 0.00^{d}	0.11 ± 0.03^{b}
Manganese mg/l	6	0.63 ± 0.02^{a}	0.63 ± 0.01^{a}	0.12 ± 0.00^{b}	0.12 ± 0.01^{b}
	24	0.15 ± 0.01^{a}	0.11 ± 0.01^{b}	0.06 ± 0.01^{c}	0.04 ± 0.01^{c}
	120	0.16 ± 0.01^{c}	0.31 ± 0.01^{a}	0.24 ± 0.01^{b}	0.18 ± 0.01^{c}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$.

Tables 6.4 to 6.6 shows the results of the values of metals in water. At 6 hours of cadmium exposure, cadmium and manganese levels were significantly (P≤0.05) higher than in garlic only and control groups. Lead levels were significantly (P≤0.05) lower than in garlic only and control groups.

At 6 hours of lead exposure, lead and cadmium levels were significantly (P≤0.05) higher than in garlic only and control groups. Manganese levels were significantly (P≤0.05) lower than in garlic only and control groups. At 6 hours of cadmium + lead exposure, cadmium, lead and manganese levels were significantly (P≤0.05) higher than in garlic only and control groups.

At 24 hours after 12 hours of garlic treatment off 6 hours' cadmium exposed female, lead level order of significant (P≤0.05) decrease were garlic only > cadmium only, cadmium + garlic, control. Cadmium level order of significant (P≤0.05) decrease were garlic only, control > cadmium + garlic > cadmium only. Manganese level order of significant (P≤0.05) decrease were cadmium + garlic, control, garlic only > cadmium only.

At 24 hours after 12 hours' garlic treatments of 6 hours' lead exposed female, lead level order of significant (P≤0.05) decrease were garlic only > control > lead only > lead + garlic. Cadmium level order of significant (P≤0.05) decrease were lead only > garlic only, lead + garlic > control. Manganese level order of significant (P≤0.05) decrease were control > lead only, garlic only > lead + garlic. At 24 hours after 12 hours' garlic treatment of 6 hours' cadmium + lead exposed female, lead level order of significant (P≤0.05) decrease were garlic only > control > cadmium + lead + garlic > cadmium +_lead. Cadmium level order of significant (P≤0.05) decrease were garlic only > control > cadmium + lead, cadmium + lead + garlic. Manganese level order of significant (P≤0.05) decrease were control, garlic only > cadmium + lead > cadmium + lead + garlic.

At 120 hours after 2 days consecutive 12-hour garlic treatments, of 6-hour cadmium exposed female, lead level order of significant (P≤0.05) decrease were control > garlic only > cadmium + garlic > cadmium only. Cadmium level order of significant (P≤0.05) decrease were garlic only > cadmium + garlic, control > cadmium only. Manganese level order of significant (P≤0.05) decrease were cadmium only, garlic only > cadmium + garlic. There were no significant (P≥0.05) difference in garlic only, cadmium only, control; cadmium + garlic, control. At 120 hours after 2 days consecutive 12-hour garlic treatment, of 6 hours' lead exposed female, lead level order significant (P≤0.05) decrease were control, lead only > garlic only > lead + garlic.

Cadmium level order of significant (P≤0.05) decrease were garlic only > lead + garlic, lead only. There were no significant (P≥0.05) difference in lead + garlic, lead only, control; garlic only, control. Manganese level showed no significant (P≥0.05) difference in all groups.

At 120 hours after 2 days consecutive 12 hours' garlic treatments of 6-hour cadmium + lead exposed female, lead level order of significant (P≤0.05) decrease were control > garlic only > cadmium + lead + garlic > cadmium + garlic.

Cadmium level order of significant (P≤0.05) decrease were garlic only > control > cadmium + lead. There were no significant (P≥0.05) difference in cadmium + lead + garlic, control; cadmium + lead + garlic, cadmium + lead. Manganese level order of significant (P≤0.05) decrease were cadmium + lead + garlic, control > garlic only > cadmium + lead.

6.4.6 Results of metals/ions in gills, intestines, blood and liver

Table 6.7: Effect of garlic treatments Means ± SEM of gill metal levels at 12 and 120hr after 6hr exposure to lethal dose of Cadmium

Parameter	Hours of experiment	Cadmium + garlic	Cadmium only	Garlic only	Control
Pb	6	10.03 ± 0.65^{a}	9.99 ± 0.04^{a}	7.38 ± 0.69^{b}	7.38 ± 0.05^{b}
	120	1.66 ± 0.02^{c}	7.38 ± 0.04^{a}	0.58 ± 0.18^{d}	2.00 ± 0.04^{b}
Cd	6	8.95 ± 0.01^{a}	8.95 ± 0.01^{a}	5.96 ± 0.01^{b}	5.96 ± 0.01^{b}
	120	1.56 ± 0.01^{b}	4.11 ± 0.01^{a}	1.84 ± 0.11^{b}	3.15 ± 0.80^{a}
Ca	6	6.36 ± 0.01^{b}	6.35 ± 0.01^{b}	7.39 ± 0.01^{a}	7.38 ± 0.01^{a}
	120	18.30 ± 0.01^{b}	21.79 ± 0.04^{ab}	19.80 ± 1.28^{ab}	23.30 ± 2.51^{a}
Mn	6	8.86 ± 0.01^{b}	8.86 ± 0.01^{b}	13.00 ± 0.19^{a}	13.00 ± 0.116^{a}
	120	5.40 ± 0.01^{ab}	3.35 ± 0.02^{c}	4.03 ± 0.97^{c}	6.92 ± 0.66^{a}
K	6	12.40 ± 0.04^{b}	12.48 ± 0.05^{b}	18.47 ± 0.75^{a}	18.43 ± 0.48^{a}
	120	26.42 ± 0.02^{a}	27.47 ± 0.03^{a}	26.29 ± 2.49^{a}	35.40 ± 5.32^{a}

Means sex with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.8: Effect of garlic treatments Means ± SEM of gill metal levels at 12 and 120hr after 6hr exposure to lethal dose of Lead

Parameter	Hours of experiment	Lead + garlic	Lead only	Garlic only	Control
Pb	6	23.53 ± 0.09^{a}	23.43 ± 0.13^{a}	7.43 ± 0.11^{b}	7.45 ± 0.06^{b}
	120	6.58 ± 0.08^{b}	13.00 ± 0.29^{a}	0.48 ± 0.06^{d}	2.01 ± 0.06^{c}
Cd	6	5.52 ± 0.01^{b}	5.52 ± 0.01^{b}	5.95 ± 0.01^{a}	5.94 ± 0.01^{a}
	120	1.27 ± 0.01^{a}	2.75 ± 0.06^{a}	1.59 ± 0.26^{a}	2.47 ± 0.85^{a}
Ca	6	8.35 ± 0.01^{a}	8.35 ± 0.01^{a}	7.40 ± 0.01^{b}	7.40 ± 0.01^{b}
	120	13.54 ± 0.03^{b}	7.51 ± 0.04^{c}	13.60 ± 0.49^{b}	20.08 ± 2.75^{a}
Mn	6	8.73 ± 0.01^{b}	8.73 ± 0.01^{b}	12.98 ± 0.48^{a}	13.00 ± 0.07^{a}
	120	1.86 ± 0.04^{d}	8.50 ± 0.03^{a}	5.04 ± 0.25^{a}	6.78 ± 0.22^{b}
K	6	10.00 ± 0.08^{b}	9.98 ± 0.09^{b}	18.48 ± 0.09^{a}	18.48 ± 0.05^{a}
	120	25.84 ± 0.09^{b}	36.40 ± 0.15^{a}	25.93 ± 0.87^{b}	29.73 ± 4.99^{ab}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.9: Effect of garlic treatments Means ± SEM of gill metal levels at 12 and 120hr after 6hr exposure to lethal dose of Cadmium + lead

Parameter	Hours of experiment	Cadmium + lead + garlic	Cadmium + lead only	Garlic only	Control
Pb	6	147.05 ± 1.65^{a}	147.35 ± 1.05^{a}	7.40 ± 0.14^{b}	7.43 ± 0.09^{b}
	120	9.63 ± 0.12^{a}	3.88 ± 0.03^{b}	0.45 ± 0.19^{d}	2.00 ± 0.06^{c}
Cd	6	10.30 ± 0.13^{a}	10.30 ± 0.16^{a}	5.94 ± 0.03^{b}	5.94 ± 0.02^{b}
	120	2.84 ± 0.07^{a}	1.06 ± 0.02^{b}	1.84 ± 0.13^{ab}	3.13 ± 0.83^{a}
Ca	6	7.15 ± 0.01^{b}	7.17 ± 0.14^{b}	7.40 ± 0.01^{a}	7.40 ± 0.01^{a}
	120	15.59 ± 0.10^{b}	13.31 ± 0.03^{b}	20.63 ± 1.37^{a}	22.80 ± 2.70^{a}
Mn	6	75.18 ± 0.82^{a}	75.30 ± 0.86^{a}	12.90 ± 0.11^{b}	13.04 ± 0.06^{b}
	120	7.95 ± 0.48^{a}	5.57 ± 0.31^{b}	4.84 ± 1.14^{b}	6.53 ± 0.46^{ab}
K	6	8.82 ± 0.04^{b}	8.81 ± 0.05^{b}	18.48 ± 0.05^{a}	18.45 ± 0.67^{a}
	120	16.36 ± 0.03^{b}	16.38 ± 0.02^{b}	29.66 ± 2.78^{a}	31.83 ± 3.96^{a}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.10: Effect of garlic of treatments Means ± SEM of blood metal and ions levels at 12 and 120hr after 6hr exposure to lethal dose of Cadmium

Parameter	Hours of experiment	Cadmium + garlic	Cadmium only	Garlic only	Control
Pb µg/ml	6	35.38 ± 0.48^{b}	35.38 ± 0.38^{b}	43.45 ± 0.25^{a}	43.25 ± 0.32^{a}
	120	36.74 ± 0.05^{a}	22.29 ± 0.04^{c}	34.33 ± 0.75^{a}	26.20 ± 2.05^{b}
Cd µg/ml	6	20.33 ± 0.18^{a}	20.20 ± 0.13^{a}	13.73 ± 0.44^{b}	13.75 ± 0.32^{b}
	120	9.31 ± 0.02^{b}	12.65 ± 1.59^{a}	8.30 ± 0.01^{b}	6.03 ± 0.11^{c}
Ca µg/ml	6	0.07 ± 0.01^{b}	0.07 ± 0.06^{b}	0.10 ± 0.01^{a}	0.10 ± 0.00^{a}
	120	0.14 ± 0.01^{a}	0.12 ± 0.01^{a}	0.17 ± 0.04^{a}	0.11 ± 0.01^{a}
Mn µg/ml	6	521.50 ± 3.62^{a}	518.75 ± 2.50^{a}	301.50 ± 16.49^{b}	309.25 ± 12.80^{b}
	120	12.06 ± 0.02^{a}	8.17 ± 0.02^{b}	7.19 ± 0.42^{b}	5.81 ± 0.62^{c}
K µg/ml	6	0.19 ± 0.02^{a}	0.19 ± 0.02^{a}	0.15 ± 0.01^{b}	0.15 ± 0.02^{b}
	120	0.14 ± 0.01^{c}	0.18 ± 0.01^{bc}	0.22 ± 0.02^{ab}	0.26 ± 0.03^{a}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.11: Effect of garlic treatments Means ± SEM of blood metal and ions levels at 12 and 120hr after 6hr exposure to lethal dose of Lead

Parameter		Lead + garlic	Lead only	Garlic only	Control
Pb µg/ml	6	55.70 ± 1.13^{a}	55.80 ± 0.15^{a}	43.45 ± 0.33^{b}	43.33 ± 0.30^{b}
	120	26.25 ± 0.47^{b}	22.43 ± 0.51^{c}	35.27 ± 0.46^{a}	26.13 ± 1.72^{b}
Cd µg/ml	6	14.65 ± 0.03^{a}	14.59 ± 0.10^{a}	13.58 ± 0.27^{b}	13.60 ± 0.37^{b}
	120	5.49 ± 0.07^{c}	8.13 ± 0.02^{b}	9.91 ± 0.51^{a}	5.67 ± 0.81^{c}
Ca µg/ml	6	0.11 ± 0.01^{a}	0.12 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}
	120	0.07 ± 0.00^{c}	0.06 ± 0.00^{c}	0.39 ± 0.02^{a}	0.11 ± 0.01^{b}
Mn µg/ml	6	1178.25 ± 5.38^{a}	1148.75 ± 10.53^{a}	291.50 ± 8.69^{b}	302.75 ± 11.15^{b}
	120	10.40 ± 0.07^{a}	7.53 ± 0.04^{b}	7.05 ± 0.12^{b}	5.85 ± 0.60^{c}
K µg/ml	6	0.09 ± 0.00^{b}	0.09 ± 0.00^{b}	0.15 ± 0.00^{a}	0.15 ± 0.01^{a}
	120	0.18 ± 0.01^{b}	0.23 ± 0.01^{b}	0.43 ± 0.08^{a}	0.25 ± 0.02^{b}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.12: Effect of garlic treatments Means ± SEM of blood metal and ions levels at 12 and 120hr after 6hr exposure to lethal dose of, Cadmium + Lead.

Parameter	Hours of experiment	Cadmium + lead + garlic	Cadmium + lead only	Garlic only	Control
Pb µg/ml	6	49.76 ± 0.09^{a}	49.70 ± 0.17^{a}	43.35 ± 0.24^{b}	43.38 ± 0.34^{b}
	120	31.48 ± 0.51^{a}	25.39 ± 0.54^{b}	31.62 ± 2.15^{a}	26.61 ± 1.58^{b}
Cd µg/ml	6	14.80 ± 0.09^{a}	14.73 ± 0.14^{a}	13.65 ± 0.35^{b}	13.53 ± 0.28^{b}
	120	4.78 ± 0.31^{b}	8.64 ± 0.29^{a}	8.33 ± 0.09^{a}	3.52 ± 2.00^{b}
Ca µg/ml	6	0.04 ± 0.01^{b}	0.04 ± 0.00^{b}	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}
	120	0.04 ± 0.01^{d}	0.07 ± 0.01^{c}	0.22 ± 0.01^{a}	0.12 ± 0.01^{b}
Mn µg/ml	60	263.50 ± 2.60^{b}	265.00 ± 4.10^{b}	299.25 ± 8.38^{a}	295.75 ± 7.84^{a}
	120	9.22 ± 0.28^{a}	6.75 ± 0.41^{b}	6.48 ± 0.09^{b}	5.85 ± 0.41^{b}
K µg/ml	6	0.16 ± 0.01^{a}	0.16 ± 0.01^{a}	0.15 ± 0.01^{a}	0.15 ± 0.01^{a}
	120	0.18 ± 0.01^{b}	0.12 ± 0.01^{c}	0.20 ± 0.02^{b}	0.26 ± 0.02^{a}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.13: Effect of garlic treatments Means ± SEM of liver metal and ions levels at 12 and 120hr after 6hr exposure to lethal dose of Cadmium

Parameter	Hours of experiment	Cadmium + garlic	Cadmium only	Garlic only	Control
Pb µg/g	6	137.75 ± 1.44^{a}	138.01 ± 0.82^{a}	57.41 ± 0.04^{b}	57.44 ± 0.03^{b}
	120	16.24 ± 0.10^{c}	23.82 ± 0.10^{a}	3.67 ± 0.06^{d}	18.20 ± 0.08^{b}
Cd µg/g	6	56.69 ± 0.04^{a}	56.71 ± 0.03^{a}	49.64 ± 0.02^{b}	49.65 ± 0.02^{b}
	120	10.36 ± 0.06^{b}	15.67 ± 0.06^{a}	5.30 ± 0.10^{c}	10.23 ± 0.09^{b}
Ca µg/g	6	4.34 ± 0.01^{b}	$4.34\ 0.01^{b}$	13.27 ± 0.01^{a}	13.27 ± 0.01^{a}
	120	19.47 ± 0.11^{d}	28.98 ± 0.09^{b}	27.68 ± 0.14^{c}	36.32 ± 0.48^{a}
Mn µg/g	6	71.79 ± 0.04^{a}	71.76 ± 0.01^{a}	29.78 ± 0.03^{b}	29.78 ± 0.02^{b}
	120	3.25 ± 0.02^{d}	5.53 ± 0.06^{c}	59.88 ± 0.48^{a}	18.14 ± 0.22^{b}
K µg/g	6	9.46 ± 0.01^{b}	9.46 ± 0.01^{b}	27.60 ± 0.04^{a}	26.32 ± 1.28^{a}
	120	37.86 ± 0.22^{a}	44.00 ± 0.42^{c}	65.60 ± 0.58^{b}	82.93 ± 0.93^{a}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.14: Effect of garlic treatments Means ± SEM of liver metal and ions levels at 12 and 120hr after 6hr exposure to lethal dose of Lead

Parameter	Hours of experiment	Lead + garlic	Lead only	Garlic only	Control
Pb µg/g	6	84.15 ± 0.02^{a}	84.14 ± 0.02^{a}	57.39 ± 0.01^{b}	57.41 ± 0.03^{b}
	120	71.2 ± 0.09^{a}	82.81 ± 0.14^{a}	2.72 ± 0.11^{c}	18.27 ± 0.05^{b}
Cd µg/g	6	55.16 ± 0.02^{a}	55.13 ± 0.01^{a}	44.62 ± 0.02^{b}	49.63 ± 0.02^{b}
	120	2.78 ± 0.64^{d}	6.36 ± 0.21^{b}	4.02 ± 0.15^{c}	10.32 ± 0.04^{a}
Ca µg/g	6	10.28 ± 0.02^{b}	10.28 ± 0.02^{b}	13.29 ± 0.02^{a}	13.29 ± 0.01^{a}
	120	17.58 ± 0.27^{d}	25.38 ± 0.10^{b}	23.06 ± 0.58^{c}	36.91 ± 0.13^{a}
Mn µg/g	6	16.46 ± 0.02^{b}	16.47 ± 0.02^{b}	29.77 ± 0.02^{a}	29.77 ± 0.03^{a}
	120	5.64 ± 0.09^{d}	179.29 ± 1.22^{a}	77.00 ± 2.05^{b}	18.27 ± 0.06^{c}
K µg/g	6	13.40 ± 0.00^{b}	13.41 ± 0.01^{b}	27.58 ± 0.04^{a}	27.61 ± 0.04^{a}
	120	45.05 ± 0.38^{c}	45.82 ± 0.75^{c}	59.53 ± 0.54^{b}	84.03 ± 1.12^{a}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.15: Effect of garlic treatments Means ± SEM of liver metal and ions levels at 12 and 120hr after 6hr exposure to lethal dose of Cadmium + Lead

Parameter	Hours of experiment	Cadmium + lead + garlic	Cadmium + lead only	Garlic only	Control
Pb µg/g	6	17.61 ± 0.04^{b}	17.63 ± 0.05^{b}	57.44 ± 0.03^{a}	57.42 ± 0.03^{a}
	120	4.13 ± 0.09^{c}	9.46 ± 0.04^{b}	3.66 ± 0.06^{d}	18.25 ± 0.03^{a}
Cd µg/g	6	45.86 ± 0.05^{b}	45.90 ± 0.05^{b}	49.65 ± 0.02^{a}	49.64 ± 0.02^{a}
	120	3.62 ± 0.08^{d}	6.68 ± 0.08^{b}	5.28 ± 0.09^{c}	10.24 ± 0.08^{a}
Ca µg/g	6	9.810 ± 0.02^{b}	10.82 ± 0.02^{b}	13.28 ± 0.01^{a}	13.29 ± 0.02^{a}
	120	17.63 ± 0.45^{c}	27.04 ± 0.32^{b}	27.64 ± 0.14^{b}	36.31 ± 0.45^{a}
Mn µg/g	6	1122.75 ± 1.70^{b}	1180.50 ± 0.34^{a}	29.78 ± 0.26^{c}	29.78 ± 0.26^{c}
	120	92.50 ± 2.40^{a}	67.70 ± 0.83^{b}	59.85 ± 0.47^{c}	18.09 ± 0.14^{d}
K µg/g	6	15.56 ± 0.02^{c}	16.57 ± 0.01^{b}	27.60 ± 0.04^{a}	27.60 ± 0.03^{a}
	120	30.39 ± 0.79^{c}	29.00 ± 0.36^{c}	65.67 ± 0.56^{b}	83.32 ± 1.13^{a}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$. n = 5

Table 6.16: Effect of garlic treatments Means ± SEM of intestine metals and ions level at 12 and 120hr after 6hr exposure to lethal dose of Cadmium

Parameter	Hours of experiment	Cadmium + garlic	Cadmium only	Garlic only	Control
Pb µg/g	6	7.80 ± 0.01^{b}	7.79 ± 0.01^{b}	75.62 ± 0.15^{a}	75.56 ± 0.11^{a}
	120	26.20 ± 0.56^{c}	48.35 ± 1.29^{a}	8.29 ± 0.26^{d}	29.73 ± 1.27^{b}
Cd µg/g	6	6.99 ± 0.11^{c}	6.99 ± 0.01^{c}	71.58 ± 0.17^{a}	70.77 ± 0.40^{b}
	120	17.74 ± 3.70^{b}	25.42 ± 0.60^{a}	10.40 ± 0.71^{c}	10.85 ± 0.34^{c}
Ca µg/g	6	3.76 ± 0.02^{b}	3.77 ± 0.02^{b}	4.84 ± 0.01^{a}	4.84 ± 0.01^{a}
	120	9.94 ± 0.48^{b}	15.88 ± 0.56^{a}	15.79 ± 0.50^{a}	10.07 ± 0.61^{b}
Mn µg/g	6	10.51 ± 0.09^{b}	10.50 ± 0.09^{b}	86.03 ± 0.19^{a}	86.00 ± 0.27^{a}
	120	76.65 ± 3.60^{a}	17.71 ± 0.75^{d}	34.40 ± 2.68^{c}	42.73 ± 1.71^{b}
K µg/g	6	8.32 ± 0.11^{b}	8.33 ± 0.01^{b}	11.45 ± 0.06^{a}	11.43 ± 0.09^{a}
	120	9.70 ± 0.46^{a}	119.07 ± 0.93^{a}	16.65 ± 0.81^{b}	12.30 ± 0.61^{c}
K µg/g	6	9.55 ± 0.02^{b}	9.55 ± 0.01^{b}	11.40 ± 0.07^{a}	11.48 ± 0.05^{a}
	120	15.40 ± 0.52^{c}	17.28 ± 0.54^{b}	23.17 ± 0.41^{a}	11.67 ± 6.51^{d}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$. n = 5

Table 6.17: Effect of garlic treatments Means ± SEM of intestine metals and ions level at 12 and 120hr after 6hr exposure to lethal dose of Lead

Parameter	Hours of experiment	Lead+ garlic	Lead only	Garlic only	Control
Pb µg/g	6	11.34 ± 0.02^{b}	11.33 ± 0.02^{b}	75.56 ± 0.08^{a}	75.64 ± 0.13^{a}
	120	15.28 ± 0.60^{b}	13.33 ± 0.40^{b}	5.80 ± 1.58^{c}	30.67 ± 0.58^{a}
Cd µg/g	6	5.73 ± 0.01^{b}	5.73 ± 0.01^{b}	71.24 ± 0.45^{a}	71.60 ± 0.18^{a}
	120	12.82 ± 0.38^{b}	9.92 ± 0.39^{c}	11.63 ± 0.69^{b}	20.09 ± 0.67^{a}
Ca µg/g	6	6.23 ± 0.01^{a}	6.23 ± 0.01^{a}	4.83 ± 0.01^{b}	4.34 ± 0.01^{c}
	120	5.37 ± 0.21^{d}	7.54 ± 0.22^{c}	17.89 ± 0.59^{a}	9.88 ± 0.41^{b}
Mn µg/g	6	16.15 ± 0.02^{b}	16.15 ± 0.02^{b}	86.25 ± 0.07^{a}	86.13 ± 0.22^{a}
	120	26.77 ± 0.92^{c}	37.70 ± 1.06^{b}	58.28 ± 2.08^{a}	40.40 ± 1.72^{b}
K µg/g	6	9.55 ± 0.02^{b}	9.55 ± 0.01^{b}	11.40 ± 0.07^{a}	11.48 ± 0.05^{a}
	120	15.40 ± 0.52^{c}	17.28 ± 0.54^{b}	23.17 ± 0.41^{a}	11.67 ± 6.51^{d}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$. n = 5

Table 6.18: Effect of garlic treatments Means ± SEM of intestine metals and ions level at 12 and 120hr after 6hr exposure to lethal dose of Cadmium + Lead

Parameter	Hours of experiment	Cadmium + lead + garlic	Cadmium + lead only	Garlic only	Control
Pb µg/g	6	731.75 ± 3.13^{a}	732.78 ± 3.13^{a}	75.59 ± 0.09^{b}	75.63 ± 0.15^{b}
	120	16.65 ± 0.28^{c}	21.39 ± 0.04^{b}	8.49 ± 0.16^{d}	30.25 ± 0.85^{a}
Cd µg/g	6	86.01 ± 1.24^{a}	86.01 ± 1.24^{a}	71.02 ± 0.35^{b}	71.53 ± 0.14^{b}
	120	9.58 ± 0.16^{b}	8.47 ± 0.02^{c}	10.54 ± 0.13^{a}	10.35 ± 0.60^{a}
Ca µg/g	6	5.36 ± 0.01^{b}	6.36 ± 0.01^{a}	4.84 ± 0.01^{c}	4.84 ± 0.01^{c}
	120	10.81 ± 0.19^{b}	9.05 ± 0.01^{c}	14.57 ± 0.42^{c}	10.43 ± 0.32^{b}
Mn µg/g	6	1217.25 ± 1.97^{a}	1217.25 ± 1.97^{a}	86.20 ± 0.18^{b}	86.20 ± 0.14^{b}
	120	70.58 ± 0.99^{a}	54.88 ± 0.05^{b}	34.98 ± 1.31^{d}	43.00 ± 1.31^{c}
K µg/g	6	10.63 ± 0.32^{b}	8.85 ± 0.32^{b}	11.42 ± 0.06^{a}	11.43 ± 0.07^{a}
	120	10.54 ± 0.17^{c}	12.00 ± 0.01^{b}	15.24 ± 0.61^{a}	11.99 ± 0.43^{b}

Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$. n = 5

Tables 6.7 to 6.18 shows the values of metal/ions in the gill, blood, liver and intestine. At 6 hour of cadmium exposure in cadmium + garlic and cadmium only groups, lead levels were significantly ($P \leq 0.05$) higher than in garlic only and control groups. Cadmium levels were significantly ($P \leq 0.05$) higher than in garlic only and control groups. Calcium levels were significantly ($P \leq 0.05$) lower than in garlic only and control groups. Manganese levels were significantly ($P \leq 0.05$) lower than garlic only and control groups. Potassium levels were significantly ($P \leq 0.05$) lower than in garlic only and control.

At 6 hours of lead exposure in lead + garlic and lead only, lead, calcium levels were significantly ($P \leq 0.05$) higher than in garlic only and controls groups. Cadmium, manganese, and potassium levels were significantly ($P \leq 0.05$) lower than garlic only and controls groups.

At 6-hour exposure of cadmium + lead, lead, cadmium and manganese levels were significantly ($P \leq 0.05$) higher than in garlic only and control groups. Calcium and potassium levels were significantly ($P \leq 0.05$) lower than in garlic only and control groups.

At 120 hours of 2 days consecutive 12-hour garlic treatment, in cadmium exposure, lead level order of significant ($P \leq 0.05$) decrease were garlic only > cadmium + garlic > control > cadmium only. Cadmium level order of significant ($P \leq 0.05$) decrease were cadmium + garlic, garlic only > control, cadmium only.

Calcium level order of significant ($P \leq 0.05$) decrease was cadmium + garlic > control. There was no significant ($P \geq 0.05$) difference in cadmium only, garlic only. Manganese level order of significant ($P \leq 0.05$) decrease was cadmium only > control. There were no significant ($P \geq 0.05$) difference in cadmium + garlic, control; cadmium + garlic, garlic only; cadmium only, garlic only. Potassium showed no significant ($P \geq 0.05$) difference in all groups.

At 120 hours of 2 days consecutive 12-hour garlic treatment in lead exposure, lead levels order of significant ($P \leq 0.05$) decrease were garlic only > control > lead + garlic > lead only.

Cadmium level showed no significant ($P \geq 0.05$) difference in all groups. Calcium levels order of significant ($P \leq 0.05$) decrease were lead only > lead + garlic, garlic only > control. Manganese levels order of significant ($P \leq 0.05$) decrease were lead + garlic > garlic only > control > lead only. Potassium levels order of significant ($P \leq 0.05$) decrease were garlic only, lead + garlic > lead only. There was no significant ($P \geq 0.05$) difference in garlic only, lead only, control; lead + garlic, control.

At 120 hours of 2 days consecutive 12-hour garlic treatment, in cadmium + lead exposure, lead levels order of significant ($P \leq 0.05$) decrease were garlic only > control > cadmium + lead only > cadmium + lead + garlic. Cadmium levels order of significant ($P \leq 0.05$) decrease

were cadmium + lead > control. There were no significant (P≥0.05) difference in garlic only, cadmium + lead; garlic only, control, cadmium + lead + garlic. Calcium levels order of significant (P≤0.05) decrease were cadmium + lead + garlic, cadmium + lead > garlic only, control. Manganese levels order of significant (P≤0.05) decrease were garlic only, cadmium + lead > cadmium + lead + garlic, control. There were no significant (P≥0.05) difference in cadmium + lead + garlic and control. Potassium levels order of significant (P≤0.05) decrease were cadmium + lead + garlic, cadmium + lead > garlic only, control.

At 6 hours of cadmium exposure in cadmium + garlic and cadmium only groups, lead levels were significantly (P≤0.05) lower than in garlic only and control groups. Cadmium levels were significantly (P≤0.05) higher than in garlic only and control groups. Calcium levels were significantly (P≤0.05) lower than in garlic only and control groups. Manganese and potassium levels were significantly (P≤0.05) higher than in garlic only and control groups.

At 6 hours of lead exposure in lead + garlic and lead only groups, lead, cadmium and manganese levels were significantly (P≤0.05) higher than in garlic only and control groups. Calcium levels were not significantly (P≥0.05) difference in all groups. Potassium levels were significantly (P≤0.05) lower than in garlic only and control groups. At 6 hours of cadmium + lead exposure in cadmium + lead + garlic and cadmium + lead only groups, lead, cadmium was significantly (P≤0.05) higher than in garlic only and control groups. Calcium and manganese levels were significantly (P≤0.05) lower than in garlic only and control groups. Potassium levels were not significantly (P≤0.05) different in all groups.

At 120 hours of 2 days consecutive 12 hours' garlic treatments, in cadmium exposure, lead levels order of significant (P≤0.05) decrease were cadmium + garlic > control > garlic only, cadmium only. There was no significant (P≤0.05) difference in garlic only and cadmium only groups. Cadmium levels order of significant (P≤0.05) decrease were control > garlic only, cadmium only > cadmium + garlic. There was no significant difference in garlic only and cadmium only. Calcium levels showed no significant (P≥0.05) difference in all groups.

Manganese levels order of significant (P≤0.05) decrease were control > garlic only, cadmium only > cadmium + garlic. There was no significant (P≥0.05) difference in garlic only and cadmium only. Potassium levels order of significant (P≤0.05) decrease were cadmium + garlic > garlic only, control. There were no significant (P≥0.05) difference in cadmium + garlic, cadmium only; garlic only, cadmium only; garlic only, control.

At 120 hours of 2 days consecutive 12 hours' garlic treatments, in lead exposure, lead levels order of significant (P≤0.05) decrease were lead + garlic > control, lead only > garlic only. There was no significant (P≥0.05) difference in lead only and control groups.

Cadmium levels order of significant (P≤0.05) decrease were lead only, control > lead + garlic > garlic only. There was no significant (P≥0.05) difference in lead only and control groups. Calcium levels order of significant (P≤0.05) decrease were lead only, lead + garlic > control > garlic only. There was no significant (P≥0.05) difference in lead + garlic and lead only groups. Manganese levels order of significant (P≤0.05) decrease were control > garlic only, lead + garlic > lead only. There was no significant (P≥0.05) difference in garlic only and lead + garlic. Potassium levels order of significant (P≤0.05) decrease were garlic only > lead + garlic, lead only, control groups. There was no significant (P≥0.05) difference in lead + garlic, lead only and control groups.

At 120 hours of 2 days consecutive 12 hours' garlic treatments, in cadmium + lead exposed females, lead levels order of significant (P≤0.05) decrease were cadmium + lead, control > cadmium + lead + garlic, garlic only. There was no significant (P≥0.05) difference in cadmium + lead, control; cadmium + lead + garlic, garlic only groups. Cadmium levels order of significant (P≤0.05) decrease was control, cadmium + lead + garlic > garlic only, cadmium + lead. There was no significant (P≥0.05) difference in cadmium + lead + garlic, control; garlic only, cadmium + lead groups. Calcium levels order of significant (P≤0.05) decrease were cadmium + lead + garlic > cadmium + lead > control > garlic only. Manganese levels order of significant (P≤0.05) decrease was control, garlic only, cadmium + lead > cadmium + lead + garlic. There were no significant (P≥0.05) difference in cadmium + lead, garlic only and control. Potassium levels order of significant (P≤0.05) decrease were cadmium + lead > cadmium + lead + garlic, garlic only > control. There was no significant (P≥0.05) difference in cadmium + lead + garlic and garlic only groups.

At 6 hour of cadmium exposure in cadmium + garlic and cadmium only groups, lead, cadmium, calcium, manganese and potassium levels were significantly (P≤0.05) lower than in garlic only and control groups. At 6 hour of lead exposure in lead + garlic and lead only groups, lead, cadmium, manganese and potassium levels were significantly (P≤0.05) lower than in garlic only and control groups. Calcium levels were significantly (P≤0.05) higher than in garlic only and control groups. At 6 hour of cadmium + lead + garlic group and cadmium + leaad groups, lead, cadmium, calcium and manganese were significantly (P≤0.05) higher than

in garlic only and control groups. Potassium levels were significantly (P≤0.05) lower than in garlic only and control groups.

At 120 hours of 2 days consecutive 12-hour garlic treatment, in cadmium exposed female, lead levels order of significant (P≤0.05) decrease were garlic only > cadmium + garlic > control > cadmium only. Cadmium levels order of significant (P≤0.05) decrease were garlic only > cadmium + garlic > control > cadmium only. Calcium levels order of significant (P≤0.05) decrease were cadmium + garlic, control > garlic only, cadmium only. Manganese levels order of significant (P≤0.05) decrease were cadmium only > garlic only > control > cadmium + garlic. Potassium levels order of significant (P≤0.05) decrease were control > garlic only > cadmium only, cadmium + garlic. There were no significant (P≥0.05) difference in cadmium + garlic and cadmium only.

At 120 hours of 2 days consecutive 12-hour garlic treatments, in lead exposure, lead levels of significant (P≤0.05) decrease were garlic only > lead only, lead + garlic > control. There was no significant (P≥0.05) difference in lead only and lead + garlic. Cadmium levels of significant (P≤0.05) decrease were lead only > garlic only, lead + garlic > control. There was no significant (P≥0.05) difference in lead only and garlic only groups. Calcium levels of significant (P≤0.05) decrease were lead only > lead + garlic > control > garlic only. Manganese levels order of significant (P≤0.05) decrease were lead only > lead + garlic, control > garlic only. There was no significant (P≥0.05) difference in lead + garlic and control groups. Potassium levels order of significant (P≤0.05) decrease were control > lead only > lead + garlic > garlic only.

At 120 hours of 2 days consecutive 12-hour garlic treatment, in cadmium + lead, lead levels order of significant (P≤0.05) decrease were garlic only > cadmium + lead + garlic> lead only > control. Cadmium levels order of significant (P≤0.05) decrease were cadmium + lead > cadmium + lead + cadmium + garlic, garlic only > control. There was no significant (P≥0.05) difference in cadmium + lead + garlic and garlic only. Calcium levels order of significant (P≤0.05) decrease were cadmium + lead > control, cadmium + lead + garlic > garlic only. There was no significant (P≥0.05) difference in cadmium + lead + garlic and control. Manganese levels order of significant (P≤0.05) decrease were garlic only > control > cadmium + lead > cadmium + lead + garlic. Potassium levels order of significant (P≤0.05) decrease were cadmium + lead + garlic > control, cadmium + lead > garlic only. There was no significant (P≥0.05) difference in cadmium + lead and control groups.

At 6 hour of cadmium exposure in cadmium + garlic and cadmium only groups, lead, cadmium and manganese levels were significantly (P≤0.05) higher than in garlic only and control groups. Calcium and potassium levels were significantly (P≤0.05) lower than in garlic only and control groups. At 6 hour of lead exposure in lead + garlic and lead only groups, lead and cadmium levels were significantly (P≤0.05) higher than in garlic only and control groups. Calcium, manganese and potassium levels were significantly (P≤0.05) lower than in garlic only and control groups. At 6 hours of cadmium + lead exposure in cadmium + lead + garlic and cadmium + lead groups, lead, cadmium, and potassium levels were significantly (P≤0.05) lower than in garlic only and control groups. Manganese levels were significantly (P≤0.05) higher than in garlic only and control groups.

At 120 hours of 2 days consecutive 12-hour garlic treatments, in cadmium exposure, lead levels order of significant (P≤0.05) decrease were garlic only > cadmium + garlic > control > cadmium only. Cadmium levels order of significant (P≤0.05) decrease were garlic only > control, cadmium + garlic > cadmium only. Calcium levels order of significant (P≤0.05) decrease were cadmium + garlic > garlic only > cadmium only > control. Manganese levels order of significant (P≤0.05) decrease were cadmium + garlic > cadmium only > control > garlic only. Potassium levels order of significant (P≤0.05) decrease were cadmium only > garlic only > cadmium + garlic, control. There were no significant (P≥0.05) difference in cadmium + garlic, control.

At 120 hours of 2 days consecutive 12-hour garlic treatments, in lead exposure, lead order of significant (P≤0.05) decrease were garlic only > lead only > control > lead + garlic. Cadmium order of significant (P≤0.05) decrease were lead only > garlic only > control > lead + garlic.

Calcium levels order of significant (P≤0.05) decrease were lead only > garlic only > lead + garlic > control. Manganese levels order of significant (P≤0.05) decrease were lead only > control > garlic only > lead + garlic. Potassium levels order of significant (P≤0.05) decrease were lead only, lead + garlic > garlic only > control. There were no significant (P≥0.05) difference in lead + garlic, lead only.

At 120 hours of 2 days consecutive 12 hours' garlic treatments in cadmium + lead exposed female, lead levels order of significant (P≤0.05) decrease were garlic only > cadmium + lead + garlic > cadmium + lead > control. Cadmium levels order of significant (P≤0.05) decrease were cadmium + lead + garlic > garlic only > cadmium + lead > control. Calcium levels order of significant (P≤0.05) decrease were cadmium + lead + garlic > cadmium + lead, garlic only > cont0rol. There were no significant (P≥0.05) difference in garlic only, cadmium + lead.

Manganese level order of significant (P≤0.05) decrease were control > garlic only > cadmium + lead > cadmium + lead + garlic. Potassium level of order of significant (P≤0.05) decrease were cadmium + lead + garlic, cadmium + lead > garlic only > control.

6.4.7 Discussion on metals and ions in water, gills, intestines, blood and liver

At 6 hour of exposure to cadmium showed a significant (P≤0.05) decrease in lead, a non significant (P≥0.05) difference in potassium and significantly (P≤0.05) higher cadmium, calcium and manganese than control in water. The decreased levels may have been due to either increased absorption and/or decreased bioavailability whereas the increased level may have been due to increased excretion and/or increased bioavailability.

At 6 hours, the significant decrease in lead by cadmium showed also a significant (P≤0.05) increase in the gills, blood and liver whereas there was a significant (P≤0.05) decrease in the intestine than control. It means the significant (P≤0.05) decrease in lead may be due to increased absorption of lead influenced by cadmium in the gill, blood and liver. The significantly higher value of cadmium in water in cadmium exposure showed also significant (P≤0.05) increase in gills, liver and blood with a significant (P≤0.05) decrease in the intestine than control. It means the significant (P≤0.05) increase in cadmium may be due to the high concentration added to the water. There was a significant (P≤0.05) increase in calcium in water of cadmium exposure than control, also shows significant (P≤0.05) decrease in the gills, intestine, liver and blood. It means cadmium exposure significantly increased the excretion of calcium from these organs into the water. The significant (P≤0.05) increase of manganese in the water in cadmium exposure than control also shows significant (P≤0.05) decrease in the gills and intestines whereas there was a significant (P≤0.05) increase in the liver and blood. It means cadmium increased significantly the excretion of manganese in the gills and intestine. It appears manganese may be absorbed through other system more actively than the gills and intestine probably through the skin for uptake into the liver and blood. The non significant (P≥0.05) difference of potassium in the water of cadmium exposure with control including a significant (P≤0.05) decrease in gills, intestine, and liver whereas there was a significant (P≤0.05) increase in the blood. It means the non significant difference may be due to a redistributive effect of cadmium only not uptake or excretion of potassium from accumulation level, (gills, intestine and liver) to circulatory system (blood).

At 6 hour of exposure with lead, shows significant (P≤0.05) increase in lead levels in water than control. It also shows significant (P≤0.05) increase in the gills, liver and blood and significant (P≤0.05) decrease in intestine. It may mean the significant (P≤0.05) increase of

lead in water may be due to the high concentration added to the water. It also may mean that the intestine does not accumulate lead but may be an excretory route for lead. At 6 hours, the significant ($P \leq 0.05$) increase in cadmium in lead exposure in water than control also shows significant ($P \leq 0.05$) decrease in the gills, and intestine whereas there was a significant ($P \leq 0.05$) increase in the liver and blood than control. It means lead significantly ($P \leq 0.05$) increased the excretion of cadmium in gills and intestine into the water for absorption through other system probably the skin to the blood and liver and/or displacement of cadmium from these tissues due to the higher concentration (diffusion). At 6 hours the significant ($P \leq 0.05$) increase in calcium in water in lead exposure than control also shows, significant ($P \leq 0.05$) decrease in gills, intestine, liver and blood. It means lead significantly ($P \leq 0.05$) increased the excretion of calcium from the gills, intestine, liver and blood into the water which resulted in the significantly increased levels in water. These may mean higher lead toxicity in these tissues. At 6 hours, the significantly ($P \leq 0.05$) higher manganese levels in water than control also shows significant ($P \leq 0.05$) decrease in the gills, intestine and blood and significant ($P \leq 0.05$) increase in the blood than control. It means lead increased the excretion of manganese from the gills and intestine and liver into the water and increased its uptake through other means probably through the skin into the blood. At 6 hours the significantly ($P \leq 0.05$) higher levels of potassium in water than control also shows significantly ($P \leq 0.05$) decreased level in the gills, intestine, liver and blood. It means lead may have increased significantly ($P \leq 0.05$) the excretion of potassium from the gills, intestine, liver and blood into the water.

At 6 hour of exposure to cadmium + lead showed a significant ($P \leq 0.05$) increase in lead in water, gills, intestine, and blood whereas there was a significant ($P \geq 0.05$) decrease in the liver than control. It may mean that the increase in water may be due to the high concentration of lead in water and uptake by the gills, intestine and blood through diffusion process. The significant ($P \leq 0.05$) decrease in the liver may be due to the effects of high level cadmium and lead that resulted in high level inducement of metal-binding protein in the liver to reduce the level by the conversion to non-polar forms and/or that liver do not accumulate cadmium fast as other tissues. At 6 hours, the significant ($P \leq 0.05$) increase in cadmium in water, gills, intestine and blood maybe due to the high concentration of the cadmium in water. The significantly ($P \leq 0.05$) lower value in the liver maybe due to the effects of cadmium + lead that probably induced metal-binding protein in the liver to reduce their levels by converting cadmium to some non-polar form and/or that liver do not accumulate cadmium fast as other

tissues. At 6 hours, calcium level in water was significantly (P≤0.05) higher than control. It also shows a significant (P≤0.05) decrease in gills, blood and liver whereas there was a significant (P≤0.05) increase in the intestine. It means cadmium + lead increased the excretion of calcium in the gill, blood and liver into the water and redistribution to the intestine for accumulation and/excretion. At 6-hour manganese level in water was significantly (P≤0.05) higher than control. It also shows a significant (P≤0.05) increase in the gills, intestine, and liver, whereas there was a significant (P≤0.05) decrease in the blood. It means, cadmium + lead increased the bioavailability and solubility of manganese in water, increased its absorption in the gills, intestine and liver. It appears the blood may be more of excretory/detoxification system of manganese. At 6 hours the significantly (P≤0.05) higher potassium level in water than control also show, significant (P≤0.05) decrease in gills, intestines and liver with no significant (P≥0.05) difference in the blood compared with control. It means cadmium + lead increased the excretion of potassium in these organ into the water.

Exposure to 6-hour cadmium, at 120 hour of experiment after a 12 hour three days consecutive (day 1, 2 and 3) water renewal, lead levels in water was significantly (P≤0.05) higher than control in water, gill, blood, liver and intestine. These result may be due to the association of cadmium and lead whereby the high concentration of cadmium resulted in increased sulubilty and bioavailability of lead in water and uptake into gill, blood, liver and intestine. It means water renewal could not reduce the accumulation of lead cadmium lethal level exposure in other words could not reduce effects of cadmium toxicity The influence at 6 hour of cadmium exposure in cadmium levels was increased cadmium levels in the water, liver, intestine and blood than control with a non significant difference in the gills with the control. It means water renewal could not abate the effects of cadmium toxicity. However, it may also mean that water renewal had tremendous positive effect in depurating cadmium in the gills which could be very benefitial in oxygen uptake. Calcium level in water, gills and blood showed no significant (P≥0.05) difference with control. Also, calcium levels were significantly (P≤0.05) higher than control in intestine and significantly (P≤0.05) lower value in liver. At 6 hours the influence of cadmium on calcium value was increase in water level and decreased in gill, intestine, liver and blood levels. At 120hr of the experiment, calcium values in water was non significantly (P≥0.05) different from garlic bath in water level, gill and blood and significantly (P≤0.05) higher in intestine and liver. It may mean that the lower the deviation from control in calcium in water, gills and blood the higher deviations from

control in the intestines and liver the higher the toxicity of cadmium. At 6hr, manganese values in water was significantly (P≤0.05) higher than control. Also, the values were significantly (P≤0.05) lower than control in the gills, intestines, liver and blood. At 120hr hours, the influence of cadmium was increase levels in water, liver and blood and decrease in gills and intestine. It means the influence of water renewal was an increase in manganese in the gills, intestine, liver and blood which do not appears to be signs of reduced toxicity of cadmium. At 120hr potassium value in water was significantly (P≤0.05) lower than control. Also, potassium was significantly (P≤0.05) higher than control in the intestine, significantly (P≤0.05) lower than control in the liver and blood including no significant (P≥0.05) difference with control in the gills. At 6 hours the influence of cadmium was significant (P≤0.05) decrease in water, liver, intestine and gills and a significantly (P≤0.05) higher value in the blood. It means water renewal influence was increased potassium in the intestine and gills. It means water renewal marginally reduced the toxicity of cadmium.

Generally, in garlic only bath in the three groups of cadmium, lead and cadmium + lead for 12 hours at day 1 and 2 and water renewal for 3 consecutive days of (day 1, 2 and 3) before the garlic bath, at 120 hours in apparently healthy female *C gariepinus*, lead levels were significantly (P≤0.05) lower in gills, intestine, liver and blood than control. It may mean that garlic increased the excretion of lead in their organ into the water which increased significantly compared to control but the value was lower than the value at 6 hour of the experiment. Cadmium level in water was significantly (P≤0.05) lower than control. Also, cadmium level was significantly (P≤0.05) lower in the gills, intestine, liver and blood than control. It means garlic decreased the level of cadmium in gill, intestine, liver and blood. Calcium was not significantly (P≥0.05) different from control level in water, gills and blood whereas it was significantly (P≤0.05) higher in the intestine and significantly (P≤0.05) lower in the liver. It means garlic increased the concentration of calcium in the intestine probably to reduce the effect in the intestine and uptake through the intestine. Manganese level in water was not significantly (P≥0.05) different from the level in control. Also, manganese level was significantly (P≤0.05) lower than control in the gills and intestine whereas it was significantly (P≤0.05) higher in the liver and blood. It means garlic had redistributive effect for manganese which favours more concentration in the liver and blood. Potassium level in water was not significantly (P≥0.05) different with control in water, gills and blood whereas potassium was significantly (P≤0.05) lower in the intestine and liver. These effects of garlic may not be

optimal because the concentration used was non optimal concentration for treatment of toxicity.

Exposure to 6-hour lead at 12 hour of the experiment after a 12 hour three days consecutive (day 1, 2 and 3) water renewal water levels of lead was significantly ($P \leq 0.05$) higher than control. Also, lead was significantly ($P \leq 0.05$) higher in the gills, and liver whereas lead levels was significantly ($P \leq 0.05$) lower in the intestine and blood. It may mean there was a decrease in lead levels in intestine and blood and also a decreased uptake of lead in water. It may also mean that the effect of lead toxicity may be mostly in liver, gills and skin (high concentration of lead in water). However, these decreases may have reduced the toxicity of lead marginally. Cadmium level was not significantly ($P \geq 0.05$) different from control level in water and gills but significantly ($P \leq 0.05$) lower in the intestine and liver and significantly ($P \leq 0.05$) higher in the blood. The result may mean that the association of cadmium and lead, higher concentration of lead do not make cadmium more soluble and increased bioavailability. Also higher concentration of lead resulted probably in the redistribution of cadmium from the intestine and liver to the blood. Calcium water level was significantly ($P \leq 0.05$) lower than control. Also, calcium level was significantly ($P \leq 0.05$) lower than the control in the gills, intestine, liver and blood. The decrease in the level calcium in water, giils, liver and intestine may mean the depletion of calcium in these media and tissues by lead. Calcium binds in tissues to prevent the binding of metal in the tissues, therefore, the lower levels were not beneficial, which may mean that water renewal could not abate lead toxicity. Manganese level in water was not significantly ($P \geq 0.05$) different from control which was beneficial. It appears that water renewal reduced lead toxicity marginally. Manganese appears to be a sensitive marker for lead toxicity. Potassium was significantly ($P \leq 0.05$) lower than control in water level which at 6 hours was significantly higher. It may mean the lower value than control the more the reduction in toxicity and beneficial. Also, potassium was significantly ($P \leq 0.05$) lower than control in the liver and intestine whereas it showed no significant ($P \geq 0.05$) difference with control in the gills and blood. The significant lower level in the liver and intestine are detrimental but however their values were higher than the value at 6 hours which was an improvement. It means garlic reduced toxicity but may have required more days of garlic bath.

Exposure to 6-hour lead at 120 hour of the experiment after a 12 hour three days consecutive (day 1, 2 and 3) water renewal before the 12-hour garlic bath at day 1 and 2, water levels of lead was not significantly ($P \geq 0.05$) different from control. This result may mean that the high

concentration at 6hr post exposure have been either taken up by the fish and/or have been converted to non polar forms by garlic. Lead levels was however significantly ($P \leq 0.05$) higher than control in the gills and liver and significantly ($P \leq 0.05$) lower in the intestine and non significantly ($P \geq 0.05$) different in blood. The values in the liver at 120 hours was not significantly ($P \geq 0.05$) different from the value at 6 hours. The value in the gill at 120 hours was significantly ($P \leq 0.05$) lower than the value at 6 hours. It means the garlic bath could not decrease lead levels in the liver, which means lead effect could still be in the liver. It also may mean that garlic reduced lead toxicity marginally more than with water renewal only. Cadmium water level was not significantly ($P \geq 0.05$) different from control with its level lower than 6-hour level. It means garlic reduced cadmium level in water due to its low concentration. Also, cadmium level was significantly ($P \leq 0.05$) higher in the liver. The value in the liver was not different from the value at 6 hours. These may mean that the high concentration of lead influenced the accumulation of cadmium in the liver probably in a complex form that garlic could not affect. The value in the blood was lower than the value at 6 hours. It means garlic decreased cadmium in the blood easily. Cadmium level was significantly ($P \leq 0.05$) lower than control in the intestine, the value was higher than the value at 6 hours which may mean that the intestine maybe more of an excretory route with the effects of cadmium also being pronounced. The value in the gills showed non significant ($P \geq 0.05$) difference with control which may mean that the gills under low level of cadmium may be more of uptake rather than accumulative tissues as shown by the value at 120 hours been lower than the value at 6 hours. Garlic could not decrease cadmium level in the liver and it increased cadmium level in the intestine. It means may be garlic decreased cadmium toxicity in female to a lesser extent than lead toxicity despite a lower cadmium concentration. Calcium water levels was significantly ($P \leq 0.05$) lower than control whose value may mean increased uptake from water to the tissues. The value was higher than the value at 6 hours, which may also mean that garlic increase the solubility and/or bioavailability of calcium. However, with calcium levels been significantly ($P \leq 0.05$) lower than control in the gills, intestine, liver and blood, indicative of depleted stores of calcium by lead, it may mean that the effect of lead toxicity has not abated with decreased calcium levels in all tissues. The values of calcium in gills, intestine, blood and liver were higher at 120 hours than 6 hours. It means garlic at 120 hour reduced lead toxicity with increased calcium levels in the organ. Manganese levels in water was not significantly ($P \geq 0.05$) different from control, which is beneficial. The value was higher than the value at 6 hours. It means garlic increased the level

of manganese in water. Also, manganese level was not significantly (P≥0.05) different with control in the intestine, which is beneficial, significantly (P≤0.05) lower in the gills which is beneficial as the value was lower than the value at 6 hours, significantly (P≤0.05) higher in blood and liver. The value in the liver and blood was lower than the 6-hour value which was beneficial. It means garlic reduced the toxicity of lead marginaly. It appears that manganese level could be a marker for lead toxicity which is dependent on tissue. Potassium level in water was significantly (P≤0.05) lower than control which was opposite effect at 6 hours. It means water renewal reduced the toxicity of lead. The value of potassium at 120 hours was higher than 6-hour value. Also, the value of potassium was not significantly (P≥0.05) different with control in gills, and blood which was beneficial significantly (P≤0.05) higher in intestines, significantly (P≤0.05) lower in the liver. The value in the liver was higher than the values at 6 hours was an improvement. The value in the intestine was higher than the value at 6 hours which was beneficial. It appears that the lower the degree of deviation above the control the less level of toxicity. It means garlic was therapeutic but less effective in reducing lead toxicity.

Exposed to 6-hour cadmium + lead at 120 hour of the experiment after a 12 hour three days consecutive (day 1, 2 and 3) water renewal water levels of lead was significantly (P≤0.05) higher than control but significantly (P≤0.05) lower than without garlic bath. It means garlic bath though with a higher value than control significantly decreased lead in water probably through increased absorption or detoxification. This could only be ascertained by the levels of lead in tissues. Lead levels was significantly (P≤0.05) higher in the intestine, and liver which was significantly lower than without garlic bath except water renewal. It means garlic bath decreased lead in the liver and intestine. The higher significant value in the blood and gills than control and with water renewal only may mean that garlic may not be effective in detoxication or excretion of lead in cadmium + lead toxicity through the blood and gills. So the effects of lead in cadmium + lead toxicity would be mostly in the gills and blood but rather in the gills probably as an excretory route which maybe responsible for the higher level in water than control. It thus appears therefore that garlic bath reduced lead toxicity more effectively than water renewal. Cadmium levels showed no significant (P≥0.05) difference with control. Cadmium levels was significantly (P≤0.05) lower in the intestine, and liver with no significant (P≥0.05) different with control. It means garlic bath reduced the effect of cadmium more than lead in the cadmium + lead toxicity. However, with only water renewal values in the gills, intestine was significantly (P≤0.05) lower than with garlic bath and

significantly (P≤0.05) higher in blood than with garlic bath. Also, the water levels with water renewal was not significantly (P≥0.05) different from garlic bath although higher in value. It may mean that blood levels of cadmium maybe of more significance in the assessment of cadmium + lead toxicity. Water levels of calcium was significantly (P≤0.05) higher than control and with water renewal which is an added advantage to decrease the uptake of cadmium and lead through the skin. It may mean that garlic increased the solubility of calcium in water. Also, calcium levels were significantly (P≤0.05) lower than control and water renewal in blood, and liver which could be regarded as deplection of calcium stores by the metals leading to high level of induced toxicity. In the blood, calcium was significantly (P≤0.05) higher in value than in water renewal although no significant difference with control in the intestine to block the site for uptake of cadmium + lead and reduce toxicity whereas in the gills there was no significant (P≥0.05) difference with water renewal but they were significantly (P≤0.05) lower than control. It means garlic reduced toxicity of cadmium + lead by these mechanisms. Manganese level in water was non significantly (P≥0.05) different with control that was significantly (P≤0.05) lower than in water renewal. It means the lower manganese level was beneficial. Also, manganese level was significantly (P≤0.05) higher than in control and water renewal in the intestine, liver, gills, and blood which appear to be detrimental but was beneficial as it represented lower values in blood, liver, gills and intestines compared to the values at 6 hours. Potassium values in water was significantly (P≤0.05) lower than control and with water renewal was opposite effect at 6 hours. The value was higher than the value at 6 hours which could be regarded for all beneficial. It means water levels of potassium may not be sensitive except very acute toxicity of cadmium + lead. Potassium level was significantly (P≤0.05) lower than control and significantly (P≤0.05) higher than water renewal in blood, significantly (P≤0.05) lower than control and water renewal with no significant (P≥0.05) difference in the intestine, significantly (P≤0.05) lower than control with no significant (P≥0.05) difference from water renewal in liver and gills. It means marginal reduction of toxicity was achieved by both garlic and water renewal.

6.4.8 Results of water quality

Table 6.19: Effect of garlic treatments Means ± SEM of water quality parameters at 6, 24 and 120hr after 6hr exposure to lethal dose of Cadmium

Parameter	Hours of experiment	Cadmium + garlic	Cadmium only	Garlic only	Control
pH	6	5.88 ± 0.03^{b}	5.83 ± 0.10^{b}	6.47 ± 0.01^{a}	6.57 ± 0.8^{a}
	24	6.62 ± 0.02^{a}	6.27 ± 0.02^{b}	6.42 ± 0.02^{ab}	6.75 ± 0.22a^{a}
	120	6.80 ± 0.01^{b}	7.03 ± 0.02^{a}	6.4 ± 0.03^{c}	6.77 ± 0.02^{b}
ALK. mg/l	6	40.15 ± 0.15^{b}	40.35 ± 0.25^{b}	53.50 ± 1.50^{a}	52.50 ± 0.50^{a}
	24	53.00 ± 1.00^{c}	71.50 ± 1.50^{b}	73.00 ± 1.00^{b}	90.00 ± 1.00^{a}
	120	142.00 ± 2.00^{a}	131.50 ± 1.50^{b}	114.00 ± 1.00^{c}	139.00 ± 1.50^{a}
Chloride mg/l	6	2.86 ± 0.03^{a}	2.92 ± 0.85^{a}	1.90 ± 0.01^{b}	1.88 ± 0.02^{b}
	24	2.27 ± 0.03^{d}	3.17 ± 0.02^{c}	5.73 ± 0.02^{b}	7.34 ± 0.02^{a}
	120	1.84 ± 0.01^{c}	1.57 ± 0.02^{d}	2.32 ± 0.01^{b}	3.74 ± 0.01^{a}
TH mg/l	6	92.50 ± 0.50^{a}	94.00 ± 1.00^{a}	37.80 ± 0.20^{b}	38.05 ± 0.05^{b}
	24	39.00 ± 1.00^{a}	31.50 ± 1.50^{b}	35.00 ± 1.00^{ab}	36.65 ± 0.85^{a}
	120	64.00 ± 2.00^{a}	51.10 ± 0.90^{c}	52.00 ± 2.00^{b}	43.00 ± 1.00^{d}
Calcium mg/l	6	34.80 ± 0.20^{a}	35.35 ± 0.65^{a}	14.10 ± 0.10^{b}	14.25 ± 0.05^{b}
	24	14.70 ± 0.10^{a}	10.95 ± 0.15^{d}	11.60 ± 0.10^{c}	12.10 ± 0.10^{b}
	120	23.30 ± 0.60^{a}	19.00 ± 0.60^{b}	17.85 ± 0.45^{b}	18.60 ± 0.60^{b}
Magnesium mg/l	6	2.32 ± 0.01^{a}	2.34 ± 0.01^{a}	2.04 ± 0.06^{b}	1.94 ± 0.02^{b}
	24	0.74 ± 0.06^{d}	1.12 ± 0.01^{c}	3.11 ± 0.01^{a}	2.16 ± 0.01^{b}
	120	2.18 ± 0.02^{a}	2.13 ± 0.03^{a}	2.15 ± 0.04^{a}	1.85 ± 0.02^{b}
TDS mg/l	6	135 ± 1.00^{a}	134 ± 1.00^{a}	76.05 ± 0.05^{b}	76.20 ± 0.10^{b}
	24	104.50 ± 1.50^{b}	87.00 ± 1.00^{d}	96.00 ± 1.00^{c}	112.00 ± 3.00^{a}
	120	142.00 ± 1.00^{ab}	137.00 ± 1.00^{b}	145.00 ± 2.00^{b}	167.00 ± 1.50^{a}
Ammonia mg/l	6	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}	0.11 ± 0.00^{a}	0.12 ± 0.01^{a}
	24	2.86 ± 0.03^{c}	4.36 ± 0.25^{b}	4.41 ± 0.03^{b}	6.45 ± 0.02^{a}
	120	3.70 ± 0.02^{d}	4.09 ± 0.02^{c}	5.93 ± 0.03^{b}	7.15 ± 0.03^{a}
DO mg/l	6	4.97 ± 0.01^{a}	5.31 ± 0.32^{a}	2.55 ± 0.41^{b}	2.49 ± 0.35^{b}
	24	4.13 ± 0.03^{a}	3.78 ± 0.02^{c}	3.18 ± 0.02^{d}	3.87 ± 0.03^{b}
	120	3.69 ± 0.02^{a}	3.13 ± 0.01^{b}	3.41 ± 0.02^{a}	2.75 ± 0.01^{b}
Potassium mg/l	6	0.12 ± 0.01^{a}	0.13 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}
	24	1.83 ± 0.01^{d}	2.16 ± 0.01^{c}	2.69 ± 0.01^{b}	3.53 ± 0.01^{a}
	120	2.75 ± 0.01^{d}	3.00 ± 0.02^{c}	3.69 ± 0.02^{b}	5.80 ± 0.02a

ALK – Alkalinity; H – Total Hardness; TDS - Total Dissolved Solids; DO – Dissolved Oxygen
Means with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n = 2

Table 6.20: Effect of garlic treatments Means ± SEM of water quality parameters at 6, 24 and 120hr after 6hr exposure to lethal dose of Lead

Parameter	Hours of experiment	Lead + garlic	Lead only	Garlic only	Control
pH	6	4.75 ± 0.04^{b}	4.76 ± 0.04^{b}	6.56 ± 0.96^{a}	6.49 ± 6.01^{a}
	24	6.80 ± 0.02^{a}	6.40 ± 0.02^{c}	6.64 ± 0.03^{b}	6.68 ± 0.03^{b}
	120	7.09 ± 0.02^{a}	6.88 ± 0.02^{b}	6.48 ± 0.02^{d}	6.68 ± 0.03^{c}
ALK. mg/l	6	48.15 ± 0.15^{a}	48.20 ± 0.30^{a}	48.50 ± 6.50^{a}	49.00 ± 1.00^{a}
	24	57.00 ± 1.00^{b}	56.00 ± 14.00^{b}	76.00 ± 1.00^{ab}	91.00 ± 1.00^{a}
	120	117.50 ± 2.50^{b}	99.50 ± 4.50^{c}	122.50 ± 2.50^{b}	137.50 ± 2.50^{a}
Chloride mg/l	6	2.86 ± 0.03^{a}	2.88 ± 0.02^{a}	1.88 ± 0.01^{b}	1.91 ± 0.02^{b}
	24	2.20 ± 0.01^{a}	3.13 ± 0.01^{c}	5.75 ± 0.02^{b}	7.44 ± 0.01^{a}
	120	1.79 ± 0.02^{c}	1.65 ± 0.02^{d}	2.34 ± 0.01^{b}	3.68 ± 0.01^{a}
TH mg/l	6	39.50 ± 0.50^{a}	39.35 ± 0.75^{a}	34.50 ± 0.50^{b}	35.25 ± 0.25^{b}
	24	39.05 ± 1.05^{a}	35.25 ± 0.85^{b}	34.20 ± 0.80^{c}	37.05 ± 1.05^{b}
	120	57.00 ± 1.00^{a}	47.90 ± 0.30^{c}	53.60 ± 0.60^{b}	45.50 ± 0.50^{c}
Calcium mg/l	6	14.40 ± 0.20^{a}	14.50 ± 0.20^{a}	12.50 ± 0.30^{b}	13.20 ± 0.40^{b}
	24	13.45 ± 0.15^{a}	11.60 ± 0.10^{b}	10.50 ± 0.10^{c}	11.95 ± 0.05^{b}
	120	18.20 ± 0.40^{b}	17.85 ± 0.55^{bc}	15.70 ± 0.60^{c}	22.05 ± 0.65^{a}
Magnesium mg/l	6	1.55 ± 0.02 ^{a}	1.55 ± 0.01^{a}	1.80 ± 0.21^{a}	1.77 ± 0.03^{a}
	24	1.52 ± 0.01^{d}	2.10 ± 0.01^{c}	3.18 ± 0.01^{a}	2.57 ± 0.01^{b}
	120	2.28 ± 0.02^{a}	1.71 ± 0.02^{c}	2.16 ± 0.03^{b}	2.20 ± 0.02^{ab}
TDS mg/l	6	141.5 ± 1.15^{a}	141.0 ± 1.00^{a}	75.50 ± 0.50^{b}	76.20 ± 0.10^{b}
	24	87.50 ± 1.50^{b}	82.00 ± 1.00^{b}	94.50 ± 6.50^{ab}	104.00 ± 1.00^{a}
	120	136.00 ± 1.00^{c}	126.50 ± 1.50^{d}	154.50 ± 0.50^{b}	165.50 ± 1.50^{a}
Ammonia mg/l	6	0.12 ± 0.02^{a}	0.13 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}
	24	2.09 ± 0.03^{d}	3.86 ± 0.02^{c}	4.18 ± 0.03^{b}	7.25 ± 0.04^{a}
	120	3.86 ± 0.02^{d}	4.12 ± 0.02^{c}	6.19 ± 0.02^{b}	7.12 ± 0.02^{a}
DO mg/l	6	4.90 ± 0.09^{a}	4.90 ± 0.07^{a}	2.82 ± 0.19^{b}	2.77 ± 0.13^{b}
	24	3.77 ± 0.05^{a}	3.67 ± 0.03^{a}	3.23 ± 0.03^{b}	3.28 ± 0.08^{b}
	120	3.13 ± 0.02^{b}	2.93 ± 0.02^{d}	3.46 ± 0.02^{a}	2.99 ± 0.02^{c}
Potassium mg/l	6	0.21 ± 0.01^{a}	0.20 ± 0.01^{a}	0.12 ± 0.01^{b}	0.14 ± 0.02^{b}
	24	1.83 ± 0.02^{d}	2.15 ± 0.01^{c}	2.64 ± 0.01^{b}	3.57 ± 0.02^{a}
	120	2.68 ± 0.02^{d}	3.00 ± 0.03^{c}	3.68 ± 0.02^{b}	5.78 ± 0.02^{a}

ALK – Alkalinity; TH – Total Hardness; TDS - Total Dissolved Solids; DO – Dissolved Oxygen
Means with the same letter on the same row are not significantly different according to DMRT at P≥0.05. n = 2

Table 6.21: Effect of garlic treatments Means ± SEM of water quality parameters at 6, 24 and 120hr after 6hr exposure to lethal dose of Cadmium + Lead.

Parameter	Hours of experiment	Cadmium + lead + garlic	Cadmium + lead only	Garlic only	Control
pH	6	4.91 ± 0.01^{b}	4.91 ± 0.02^{b}	6.51 ± 0.45^{a}	6.47 ± 0.01^{a}
	24	6.65 ± 0.02^{b}	6.58 ± 0.02^{c}	6.60 ± 0.01^{bc}	6.84 ± 0.02^{a}
	120	7.07 ± 0.03^{a}	6.88 ± 0.03^{b}	6.47 ± 0.03^{c}	6.88 ± 0.03^{b}
ALK. mg/l	6	42.50 ± 0.50^{a}	43.00 ± 1.00^{a}	47.50 ± 7.5^{a}	54.00 ± 1.00^{a}
	24	41.50 ± 0.50^{d}	61.00 ± 1.00^{c}	74.50 ± 0.50^{b}	101.00 ± 1.00^{a}
	120	121.50 ± 1.50^{b}	116.50 ± 1.50^{b}	116.50 ± 1.50^{b}	139.00 ± 1.00^{a}
Chloride mg/l	6	2.96 ± 0.20^{a}	2.97 ± 0.10^{a}	1.89 ± 0.20^{b}	1.88 ± 0.20^{b}
	24	3.15 ± 0.52^{c}	3.17 ± 0.01^{c}	5.78 ± 0.15^{b}	7.37 ± 0.15^{a}
	120	1.63 ± 0.01^{c}	1.64 ± 0.01^{c}	2.25 ± 0.01^{b}	3.57 ± 0.01^{a}
TH mg/l	6	86.50 ± 1.10^{a}	87.05 ± 0.95^{a}	35.50 ± 0.50^{b}	38.15 ± 0.15^{b}
	24	39.10 ± 1.10^{b}	36.30 ± 0.70^{bc}	33.15 ± 0.75^{c}	45.00 ± 0.80^{a}
	120	59.10 ± 1.10^{a}	53.65 ± 1.05^{b}	51.65 ± 0.65^{b}	55.15 ± 0.95^{b}
Calcium mg/l	6	33.85 ± 0.65^{a}	34.55 ± 0.45^{a}	13.45 ± 0.15^{b}	14.25 ± 0.05^{b}
	24	13.50 ± 0.15^{a}	12.40 ± 0.10^{c}	9.97 ± 0.02^{d}	13.50 ± 0.10^{b}
	120	22.15 ± 0.75^{a}	18.90 ± 0.80^{b}	18.90 ± 0.35^{b}	19.55 ± 0.60^{b}
Magnesium mg/l	6	4.19 ± 0.84^{a}	4.15 ± 0.03^{a}	1.77 ± 0.04^{b}	2.03 ± 0.07^{b}
	24	1.53 ± 0.01^{d}	1.75 ± 0.01^{c}	3.19 ± 0.01^{a}	2.62 ± 0.02^{b}
	120	2.01 ± 0.02^{c}	2.93 ± 0.05^{a}	2.16 ± 0.02^{b}	2.23 ± 0.03^{b}
TDS mg/l	6	204.50 ± 4.50^{a}	208.50 ± 3.50^{a}	75.00 ± 1.00^{b}	76.00 ± 0.00^{b}
	24	85.00 ± 1.00^{b}	89.00 ± 1.00^{b}	98.00 ± 1.00^{a}	101.50 ± 1.50^{a}
	120	131.00 ± 1.00^{c}	141.50 ± 1.50^{b}	168.50 ± 1.50^{a}	164.50 ± 1.50^{a}
Ammonia mg/l	6	0.13 ± 0.01^{a}	0.14 ± 0.01^{a}	0.11 ± 0.01^{b}	0.11 ± 0.01^{b}
	24	1.26 ± 0.03^{d}	3.39 ± 0.02^{c}	4.50 ± 0.03^{b}	6.63 ± 0.03^{a}
	120*	3.73 ± 0.03^{d}	4.04 ± 0.06^{c}	6.2 ± 0.03^{b}	7.11 ± 0.02^{a}
DO mg/l	6	4.68 ± 0.05^{a}	4.68 ± 0.02^{a}	2.84 ± 0.17^{b}	2.52 ± 0.38^{b}
	24	3.81 ± 0.03^{a}	3.91 ± 0.03^{a}	3.16 ± 0.03^{b}	3.15 ± 0.04^{b}
	120	2.68 ± 0.02^{c}	2.94 ± 0.03^{b}	3.16 ± 0.04^{a}	3.02 ± 0.02^{b}
Potassium mg/l	6	0.21 ± 0.02^{a}	0.21 ± 0.01^{a}	0.11 ± 0.01^{b}	0.11 ± 0.01^{b}
	24	1.86 ± 0.02^{a}	2.27 ± 0.02^{a}	4.17 ± 1.49^{a}	3.59 ± 0.01^{a}
	120	2.66 ± 0.03^{d}	3.13 ± 0.03^{c}	4.25 ± 0.03^{b}	6.46 ± 0.02^{a}

ALK – Alkalinity; TH – Total Hardness; TDS - Total Dissolved Solids; DO – Dissolved Oxygen
Means with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$. n = 2

Tables 6.19 to 6.21 shows the results of water quality. At 6 hour of cadmium exposure in cadmium + garlic and cadmium only groups, pH was significantly (P≤0.05) lower than in garlic only and control groups. Alkalinity was significantly (P≤0.05) lower than in garlic only and control groups. Chloride was significantly (P≤0.05) higher than in garlic only and control groups. Total hardness was significantly (P≤0.05) higher than garlic only and control groups. Calcium was significantly (P≤0.05) higher than in garlic only and control. Magnesium was significantly (P≤0.05) higher than in garlic only and control groups. Total dissolved solids were significantly (P≤0.05) higher than garlic only and control groups. Ammonia was not significantly (P≥0.05) different with the garlic only and control. Dissolved Oxygen was significantly (P≤0.05) higher than in garlic only and control groups. Potassium was not significantly (P≥0.05) different with the garlic only and control (Table 6.12).

At 6 hours of lead exposure in lead + garlic and lead only, pH was significantly (P≤0.05) lower than in garlic only and control groups. Alkalinity was not significantly (P≥0.05) different with the garlic only and control. Chloride was significantly (P≤0.05) higher than in garlic only and control groups. Total hardness was significantly (P≤0.05) higher than garlic only and control groups. Calcium was significantly (P≤0.05) higher than in garlic only and control. Magnesium was not significantly (P≥0.05) different with the garlic only and control. Total dissolved solids were significantly (P≤0.05) higher than garlic only and control groups. Ammonia was not significantly (P≥0.05) different with the garlic only and control. Dissolved Oxygen was significantly (P≤0.05) higher than in garlic only and control groups. Potassium was significantly (P≤0.05) higher than garlic only and control groups

At 6 hours of exposure in cadmium + lead + garlic and cadmium + lead only, pH was significantly (P≤0.05) lower than in garlic only and control groups. Alkalinity was not significantly (P≥0.05) different with the garlic only and control. Chloride was significantly (P≤0.05) higher than in garlic only and control groups. Total hardness was significantly (P≤0.05) higher than garlic only and control groups. Calcium was significantly (P≤0.05) higher than in garlic only and control. Magnesium was not significantly (P≥0.05) different with the garlic only and control. Total dissolved solids were significantly (P≤0.05) higher than garlic only and control groups. Ammonia was significantly (P≤0.05) higher than in garlic only and control. Dissolved Oxygen was significantly (P≤0.05) higher than in garlic only and control groups. Potassium was significantly (P≤0.05) higher than garlic only and control groups (Table 6.12).

At day 2 (24 hours of the experiment) of cadmium exposure in cadmium + garlic, cadmium only, garlic only and control groups, pH in cadmium only was significantly (P≤0.05) lower than control group. Alkalinity was significantly (P≤0.05) lower in all groups than control groups. Chloride was significantly (P≤0.05) lower in all groups than control groups. Total hardness was significantly (P≤0.05) lower in cadmium only than control groups. Calcium was significantly (P≤0.05) higher in cadmium + garlic and lower in cadmium only and garlic only than control. Magnesium was significantly (P≤0.05) higher in garlic only and lower in cadmium + garlic and cadmium only than control. Total dissolved solids were significantly (P≤0.05) lower in all groups than control groups. Ammonia was significantly (P≤0.05) lower in all group than control. Dissolved Oxygen was significantly (P≤0.05) higher in cadmium + garlic and lower in cadmium only and garlic only than control. Potassium was significantly (P≤0.05) lower in all group than control (Table 6.12).

At day 2 (24 hours of the experiment) of lead exposure in lead + garlic, lead only, garlic only and control groups pH was significantly (P≤0.05) higher in lead + garlic and lower in lead only than control groups. Alkalinity was significantly (P≤0.05) lower in lead + garlic and lead only than control groups Chloride was significantly (P≤0.05) lower in lead only and garlic only than control groups. Total hardness was significantly (P≤0.05) higher in lead + garlic and lower in garlic only than control groups. Calcium was significantly (P≤0.05) higher than in lead + garlic and lower in lead + garlic than control. Magnesium was significantly (P≤0.05) higher than in garlic only and lower in lead + garlic and lead only than control. Total dissolved solids were significantly (P≤0.05) lower in lead + garlic and lead only than control groups. Ammonia was significantly (P≤0.05) lower in lead + garlic, lead only and garlic only than control groups. Dissolved Oxygen was significantly (P≤0.05) higher than in lead + garlic and lead only than control groups. Potassium was significantly (P≤0.05) lower in all groups than control (Table 6.12).

At day 2 (24 hours of the experiment) exposure in cadmium + lead + garlic, cadmium + lead only, garlic only and control groups, pH was significantly (P≤0.05) lower in all exposure groups than control group. Alkalinity was significantly (P≤0.05) lower in all exposure groups than control group. Chloride was significantly (P≤0.05) lower in all exposure groups than control group. Total hardness was significantly (P≤0.05) lower in all exposure groups than control group. Calcium was significantly (P≤0.05) higher in cadmium + lead + garlic and lower in cadmium + lead only and garlic only than control. Magnesium was significantly (P≤0.05) higher in garlic only and lower in cadmium + lead + garlic and cadmium + lead only

than control. Total dissolved solids were significantly (P≤0.05) lower in cadmium + lead + garlic and cadmium + lead only than control groups. Ammonia was significantly (P≤0.05) lower in all groups than control. Dissolved Oxygen was significantly (P≤0.05) higher in cadmium + lead + garlic and cadmium + lead only than control. Potassium in all groups was not significantly (P≥0.05) different with control (Table 6.12).

At day 6 (120 hours post exposure) of cadmium exposure in cadmium + garlic, cadmium only, garlic only and control groups, pH in cadmium only was significantly (P≤0.05) higher and lower in garlic only than control group. Alkalinity was significantly (P≤0.05) lower in cadmium only and garlic only than control groups. Chloride was significantly (P≤0.05) lower in all groups than control groups. Total hardness was significantly (P≤0.05) higher all exposed than control groups. Calcium was significantly (P≤0.05) higher in cadmium + garlic than control. Magnesium was significantly (P≤0.05) higher in all groups than control groups. Total dissolved solids were significantly (P≤0.05) lower in cadmium only and garlic only than control groups. Ammonia was significantly (P≤0.05) lower in all group than control. Dissolved Oxygen was significantly (P≤0.05) higher in cadmium + garlic and garlic only than control. Potassium was significantly (P≤0.05) lower in all group than control (Table 6.12).

At day 6 (120 hours of the experiment) of lead exposure in lead + garlic, lead only, garlic only and control groups pH was significantly (P≤0.05) higher in lead + garlic and lead only whereas it was lower in garlic only than control groups. Alkalinity was significantly (P≤0.05) lower in all exposed than control groups. Chloride was significantly (P≤0.05) lower in all exposed than control groups. Total hardness was significantly (P≤0.05) higher in lead + garlic and garlic only than control groups. Calcium was significantly (P≤0.05) lower in all exposed than control groups. Magnesium was significantly (P≤0.05) lower than in lead only than control. Total dissolved solids were significantly (P≤0.05) lower in all exposed than control groups. Ammonia was significantly (P≤0.05) lower in all exposed than control groups. Dissolved Oxygen was significantly (P≤0.05) higher in lead + garlic and garlic only whereas it was lower in lead only than control groups. Potassium was significantly (P≤0.05) lower in all groups than control (Table 6.12).

At 120 hours in cadmium + lead + garlic, cadmium + lead only, garlic only and control groups, pH was significantly (P≤0.05) higher in cadmium + lead + garlic and lower garlic only than control group. Alkalinity was significantly (P≤0.05) lower in all exposure groups than control group. Chloride was significantly (P≤0.05) lower in all exposure groups than control group. Total hardness was significantly (P≤0.05) higher in cadmium + lead + garlic

than control group. Calcium was significantly (P≤0.05) higher in cadmium + lead + garlic than control. Magnesium was significantly (P≤0.05) lower in cadmium + lead + garlic whereas it was higher in cadmium + lead than control. Total dissolved solids were significantly (P≤0.05) lower in cadmium + lead + garlic and cadmium + lead only than control groups. Ammonia was significantly (P≤0.05) lower in all groups than control. Dissolved Oxygen was significantly (P≤0.05) lower in cadmium + lead + garlic whereas it was higher in garlic only than control. Potassium in all groups was significantly (P≤0.05) lower than control (Table 6.12).

6.4.9 Discussion on water quality

At 6-hr cadmium exposure, pH decreased significantly (p≤0.05) than control. The second day of sampling, after 16hr water renewal, 12hr garlic treatment, pH showed no significant (p≤0.05) differences with Cd exposed group with no treatment, garlic only treated group and the control. However, pH value in Cd exposed group only was significantly lower than the control group. These may mean that 12hr garlic treatment had no significant effect on pH while Cd still had effect despite water renewal.

At 120hr after 24hr water renewal at day 3, the 12hr garlic treated group at day 2 showed that pH was not significantly (p≤0.05) different with control group whose values were significantly lower than Cd only exposed group. The garlic only group had also showed lower and higher significant (p≤0.05) values than Cd only and Cd + garlic and control groups respectively. These may mean that garlic decreased pH in healthy fish while in Cd exposure garlic treatment had no effect. In Cd only exposure water change increased pH. In comparison of effect of exposure and treatment with days of samplings, the order of decreasing significant (p≤0.05) difference of pH was 120hr > 24hr > 6hr, and no significant (p≤0.05) differences with Cd and garlic treatment respectively. For Cd exposure and no treatment was 120hr > 24hr > 6hr. For with no cadmium exposure and garlic treatment was 120hr > 6hr, 24hr. For with no Cd exposure and no garlic treatment (control) there was no significant (p≤0.05) difference.

These may mean that in Cd exposure + garlic treatment, pH increased with increased culture duration of treatment. In Cd exposure with no garlic treatment pH increased with increased water renewal. In no Cd exposure with garlic treatment only, pH, first treatment had no effect the second treatment increased significantly (p≤0.05) In control group with no cadmium exposure and no garlic treatment, first and second water renewal had no significant (p≤0.05) effect.

At 6hr cadmium exposure, total hardness significantly ($p \leq 0.05$) increased than control. The second day of sampling after 16hr water renewal, 12hr garlic treatment, total hardness showed no significant ($p \geq 0.05$) difference with garlic only treated group which showed no significant ($p \geq 0.05$) difference with cadmium only exposed group and control (no exposure, no treatment). The garlic only treated group showed a higher significant ($p \leq 0.05$) value than cadmium only exposed group. These may mean in the female, the garlic had effect on healthy fish and in exposed group, garlic increased total hardness significantly in exposed.

At day 6, after 24hr water renewal at day 3, 12hr garlic treated group at day 2 showed in female culture that total hardness was significantly ($p \leq 0.005$) higher than all groups with garlic only treated group higher than both only cadmium exposed with no treatment and control groups. Also only cadmium exposed group had the lowest value. It may mean that garlic significantly increased total hardness in both apparently healthy and sick cadmium exposed fish, with more effect in sick fish. In comparison of effect of exposure and treatment with days of sampling the order of decreasing significant ($p \leq 0.005$) difference of total hardness in cadmium + garlic was 6hr >120hr > 24hr. It may mean that the first water renewal decreased total hardness while the second water renewal increased total hardness. For cadmium exposure and no garlic treatment the order of decreasing significant difference was 6hr >120hr > 24hr. It may mean that the first water renewal decreased total hardness while the second water renewal increased total hardness.

For garlic treatment and no cadmium exposure the order of decreasing significant difference was 120hr > 6hr, 24hr. These may mean the first treatment had no effect while the second treatment increased total hardness. In control group with no cadmium exposure the order of decreasing significant ($p \leq 0.05$) difference of total hardness was 120hr > 6hr, 24hr. It may mean that the first water change had no effect while the second water renewal increased total hardness.

At 6hr cadmium exposure, ammonium was not significantly ($p \geq 0.05$) different in exposed groups and control groups. This may mean that cadmium during a short duration had no effect on ammonia level. The second day of sampling after 16hr water renewal, 12hr garlic treatment, ammonia showed the lowest significant ($p \leq 0.05$) value than all groups. The control group had the highest level of ammonia. The control group had the highest level of ammonia. There was no significant ($p \geq 0.005$) difference in group exposed to cadmium only and garlic only. This may mean garlic decreased ammonia. The ammonia level in control group was due to increased food intake compared to garlic only. Also the higher ammonia in group with Cd

cadmium exposure only than cadmium exposure and garlic treatment was due to increased feed intake in cadmium only exposed group than cadmium exposed group and garlic treatment. The decrease in ammonia in garlic treated groups was due to decreased feed intake and excretion of nitrogenous waste.

At 120hr after 24hr water renewal at day 3, 12hr garlic treated group at day 2 showed that ammonia was significantly ($p \leq 0.05$) different in all groups with the highest level in control group and least in cadmium exposed group treated with garlic. However, feed intake was higher in cadmium exposed group with garlic treatment than cadmium exposed group only. These may mean garlic decreased ammonia in exposed group with increased appetite. While apparently healthy group garlic decreased ammonia due to reduced feed intake and excretion of nitrogenous waste.

In comparison of effect of exposure and treatment hours, the order of decreasing significant ($p \leq 0.005$) differences of ammonia in cadmium exposed and treated with garlic group was 120hr > 24hr > 6hr. This may mean that ammonia increased with increased duration of garlic treatment.

In cadmium exposed group with no garlic treatment the order of decreasing significant ($p \leq 0.005$) difference of ammonia was 24hr, 120hr > 6hr. It may mean that the first water renewal increased ammonia while the second water renewal had no effect. For garlic treatment only was 120hr > 24hr > 6hr. It may mean that ammonia increased with increased duration of garlic treatment.

In control group with no cadmium exposure, the order of decreasing significant ($p \leq 0.005$) difference of ammonia was 120hr >24hr > 6hr. It may mean that ammonia increased with increased water renewal.

At 6hr cadmium exposure, dissolved oxygen (DO) was significantly ($p \leq 0.05$) lower than the control groups. These may mean that the decreased activity in cadmium exposed group decreased dissolved oxygen uptake in these groups than the control due probably to increased chemical oxygen demand (COD). The second day of sampling after 16hr water renewal, 12hr garlic treatment, DO showed highest significant ($p \leq 0.05$) value than all groups while the group with only garlic treatment had the higher significant ($p \leq 0.05$) value than control group. It may mean that in apparently healthy fish, garlic decreased the devolution of oxygen with increased activity (effective oxygen utilization) while in cadmium exposed group increased the devolution of oxygen. The decrease of DO in cadmium exposed group with no garlic

treatment may be due to increased foraging activity which is caused by cadmium chemical cue and/or inceased COD.

At day 6 after 24hr water renewal at day 3, 12hr garlic treated group at day 2, showed DO was significantly higher than Cd and control group which was not significantly ($p \geq 0.005$) different with garlic only treated group. The reason had earlier been explained in the previous paragraph. The control had a lowest significant ($p \leq 0.005$) value than all groups which may mean that activity of fish is highest.

In comparison of effect of exposure and treatment with days of sampling the order of decreasing significant ($p \leq 0.05$) differences of DO in cadmium exposed and treated with garlic group was 6hr > 24hr > 120hr. It may mean that DO decrease with increased duration of garlic treatment which may be due to increased activity. In cadmium exposed group with no garlic treatment the order of decreasing significant ($p \leq 0.005$) difference of DO was 6hr > 24hr > 120hr. This may mean that water renewal decreased DO through increased activity. In garlic only treated group the order was 6hr > 24hr > 120hr. This may mean DO decreased with increased duration of garlic treatment probably due to increased activities and appetite. In the control group with no cadmium exposure and no garlic treatment, the order was 24hr > 6hr. There were no significant differences at 24hr and 120hr; 6hr and 120hr. It means first water renewal increased DO with probably decreased activity. The second water renewal had no effect. These may mean frequent water change may not affect DO positively in apparently healthy fish.

At 6hrs of lead exposure, pH was lower significantly ($p \leq 0.05$) than control. This may mean that lead had a negative effect on pH. The 24hr sampling after12hr garlic treatment, pH was highest significantly ($p \leq 0.05$) than all groups. There was no significant ($p \geq 0.05$) difference in garlic only treated group and control group. It may mean that in apparently healthy fish culture 12hr treatment of garlic had no effect on pH while in lead exposed groups and bathed with garlic, garlic may have been responsible for the increased pH.

In comparison of effects of lead exposure and treatment hours, order of decreasing significant ($p \leq 0.05$) differences was 120hr > 24hr > 6hr. It may mean that pH increased with increased duration of garlic treatment. Lead only exposure with no treatment the order was 120hr > 24hr > 6hr. It may mean that with water renewal, pH increased. Garlic only treatment with no lead exposure, the order was not significantly ($p \geq 0.05$) different in all groups. These may mean that garlic had no effect in apparently healthy fish culture pH. In control, the order was

120hr, 24hr > 6hr. It may mean that the 1st water renewal increased pH while the 2nd water renewal had no effect on pH.

At 6hr of lead exposure, total hardness was higher significantly ($p \leq 0.005$) than control. It may mean that lead increased total hardness. The second day of sampling after 16hr water renewal, 12hr garlic treatment, total hardness in lead exposed group with garlic treatment had the highest significantly ($p \leq 0.05$) different value than all groups. The garlic only treated group had a lower significant value than control. These may mean that in apparent healthy fish, garlic decreased total hardness while in lead exposed group with garlic treatment increased significantly total hardness.

At 120hr after 24hr water renewal at 72hr, 12hr garlic treated group at day 2 had total hardness that was lower significantly ($p \leq 0.05$) than garlic only treated group but higher significantly ($p \leq 0.05$) than lead exposed group with no treatment and control. Garlic only treated group was also higher significantly than control. These may mean that garlic increased total hardness in both apparently healthy and lead exposed female.

In comparison of effects of cadmium exposure and treatment with days of sampling, the order of decreasing significant ($p \leq 0.05$) differences of total hardness in lead exposure and garlic treatment was 120hr > 6hr, 24hr. This may mean that total hardness only significantly increases after the second treatment with garlic. In lead exposure and no garlic treatment the order was 120hr > 6hr > 24hr. This may mean that the firstt water renewal decreased total hardness while the second water renewal increased total hardness. In garlic only treated group the order was 120hr > 6hr, 24hr. It may mean that the first had no effect while the second treatment increased total hardness. In control group with no lead exposure and no garlic treatment the order was 120hr > 24hr, 6hr. This may mean that the first water renewal had no effect on water hardness while the second renewal increased water hardness.

At 6hr of lead exposure, ammonia showed no significant ($p \geq 0.05$) differences in all groups. This may mean that lead did not affect the excretion of nitrogenous waste because during the said period there was complete loss of appetite and they were not fed. At 24hr of sampling after 16hr water renewal and 12hr garlic treatment, ammonia showed the lowest significant ($p \leq 0.05$) value than all groups. The control group had the highest ammonia level. It may mean that garlic decreased ammonia in both lead exposed group and apparently healthyfish, probably due to reduced metabolic activities and feed intake.

In comparison of effect of exposure and treatment duration, the order of decreasing significant ($p \leq 0.05$) differences in ammonia in lead exposed group treated with garlic group

was 120hr > 24hr > 6hr. This may mean that the generation of ammonia increased with increased garlic treatment duration probably due to increased activities in feeding and metabolism. In lead exposed group with no garlic treatment, the order of ammonia was 120hr > 24hr > 6hr. This may mean that ammonia increased with increased water renewal probably also due to increased activities and metabolism. In garlic only treated group the order was 120hr > 24hr > 6hr which also may be due to increased activities with increased garlic treatment duration. In control group with no lead exposure and no garlic treatment, the order was 120hr > 24hr > 6hr. This may be due to increased activities and metabolism.

At 6 hr of lead exposure, dissolved oxygen (DO) was significantly ($p \leq 0.05$) higher than control groups. This may be due to decreased activities that require oxygen due to the toxicity of lead. At 24hr of sampling after 16hr water renewal, 12hr garlic treatment, DO showed higher significant ($p \leq 0.05$) values in exposed groups than garlic only treated group and control both with no significant ($p \geq 0.05$) differences. Lead exposed group and lead exposed group with no garlic treatment had no significant ($p \geq 0.05$) differences in DO levels. The higher DO may be due to the reduced activities. These may also mean that garlic had no effect on dissolved oxygen in both apparently healthy and lead exposed fish.

At 120hr after 24hr water renewal 72hr, 12hr garlic treated group at day 2 showed DO in apparently healthy fish was higher significantly ($p \leq 0.05$) than lead exposed group with no garlic treatment and control but lower significantly ($p \leq 0.05$) than garlic only treated group. These may mean garlic increased DO. These increases may be due to increase in phytoplankton and not due to decreased activities as feed intake increased when compared to the quantity consumed at 72hr. In the control, the lower value may be due to increased activities as feed intake was still higher than garlic only treated group. In comparison of effect of exposure and treatment with days of sampling order of decreasing significant ($p \leq 0.05$) differences of DO in lead exposure and treated with garlic was 6hr > 24hr > 120hr. It may mean that dissolved oxygen decreased with increased duration of garlic treatment. In lead exposed group with no garlic treatment the order was 6hr > 24hr > 120hr. These may also mean that water renewal decreased DO with increase in number of renewal. In garlic only treated group the order was 120hr > 6hr. There was no significant ($p \geq 0.05$) differences at 120hr and 24hr; 6hr and 24hr. It may therefore mean that the first garlic treatment was effective in increasing DO while the second treatment had no effect. In control group with no lead exposure and no garlic treatment the order was 24hr > 6hr. There was no significant

($p \geq 0.05$) differences at 24hr and 120hr; 6hr and 120hr. It may mean that the first water renewal increased dissolved oxygen while the second had no effect.

At 6hr the combination of cadmium and lead, pH was lower significantly ($p \leq 0.05$) than control. The second day of sampling after 16hr water renewal, 12hr garlic treatment pH was higher significantly ($p \leq 0.005$) in control than all groups. Cd + Pb exposed group treated with garlic had higher significant ($p \leq 0.05$) value than Cd + Pb exposed group with no garlic treatment. These may mean that garlic increased pH in Cd + Pb exposed group and decreased pH in apparently healthy fish. At 120hr, pH after water renewal at 72hr, with previous 12hr garlic consecutive 2 days treated showed the highest significant ($p \leq 0.05$) value than all groups. There was no significant ($p \geq 0.05$) differences in control and Cd + Pb exposed group with no garlic treatment. It may mean that garlic increased pH in Cd + Pb exposed groups and decreased pH in apparently healthy fish. In comparison of effects of exposure and treatment within days of sampling, pH order of decreasing significant ($p \leq 0.05$) differences in Cd + Pb exposed with no garlic treatment order was 120hr > 24hr > 6hr. It may mean that pH increased with water renewal. In garlic only treated group, the pH order showed no significant difference. It may mean that garlic treatment had no effect on pH. In control the order was 24hr, 120hr > 6hr. It may mean that the first water renewal increased pH while the second had no effect in culture of female.

At 6hrs, the combination of cadmium and lead, total hardness was higher significantly ($p \geq 0.05$) than control. The second day of sampling after 16hrs water renewal, 12hrs garlic treatment, total hardness showed no significant ($p \geq 0.05$) difference with Cd + Pb exposed group with no garlic treatment. The garlic only treated group was significantly ($p \leq 0.05$) lower than control. It may mean that garlic had no effect on Cd + Pb exposed groups but in apparently healthy fish, garlic decreased total hardness.

At 120hr after water renewal at 72hr, 12 hr garlic treated group at day 1 and 2 of the experiment showed that total hardness in Cd + Pb exposed with garlic treatment had higher significant ($p \leq 0.05$) value than Cd + Pb exposed group with no garlic treatment. The garlic only treated group and control group had no significant ($p \geq 0.05$) difference. It may mean that garlic increased total hardness in Cd + Pb + garlic treatment. In comparison of effect of exposure and treatment with days of sampling order of decreasing significant ($p \leq 0.05$) differences of total hardness was 6hr > 120hr > 24hr. It may mean that the first garlic treatment decreased whereas the second treatment increased total hardness. In Cd + Pb

exposed group with no garlic treatment, the order was 6hr > 120hr > 24hr. It means the first water renewal decreased total hardness while the second water renewal increased total hardness. In garlic only treated group, the order in female and male was 120hr > 6hr, 24hr. It may mean that the first treatment had no effect while the second treatment increased total hardness. In control group with no Cd + Pb exposure and no treatment the order was 120hr > 24hr > 6hr. It may mean that in apparently healthy fish, total hardness increased significantly ($p \leq 0.05$) with increased water renewal.

At 6hr, cadmium and lead combination, ammonia was higher significantly ($p \leq 0.05$) than control. This may mean that Cd + Pb increased ammonia by the increase in excretion of nitrogenous waste. The second day of sampling after 16hr water renewal, 12hr garlic treatment ammonia showed the lowest significant ($p \leq 0.05$) value than in all groups. The control group had the highest significant ($p \leq 0.05$) level of ammonia. Cd + Pb + garlic had a lower significant ($p \leq 0.05$) value than Cd + Pb with no garlic treatment. It may mean that garlic decreased ammonia in Cd + Pb exposed group and in apparently healthy probably due to decreased feed intake and excretion of nitrogenous waste. At 120hr, after water renewal at day 72hr, garlic treated group showed the same trend as in second day of sampling.

In comparison, of effects of exposure and treatment with days of sampling the order of decreasing significant ($p \leq 0.05$) differences of ammonia in Cd + Pb + garlic was 120hr > 24hr > 6hr. It means ammonia production increased with increase garlic treatment may be due to increased feed intake, increased physical activities and excretion of nitrogenous waste. In Cd + Pb exposed group and no garlic treatment the order was 120hr > 24hr > 6hr. It may mean that ammonia production increased with increase water renewal due to increased feed intake, increased physical activities and excretion of nitrogenous waste. In garlic only treated group, the order was 120hr > 24hr > 6hr. It may mean that ammonia increased with increased garlic treatment due to increased feed intake and excretion of nitrogenous waste. In control, the order was 120hr > 24hr > 6hr. It also followed the same trend as in garlic only treated groups.

At 6hr in the combination of Cadmium (Cd) and lead (Pb), dissolved oxygen (DO) was higher significantly ($p \leq 0.05$) than control groups. This may be due to decreased activities that required dissolved oxygen than control. The second day of sampling after 16hr water renewal 12hr garlic treatment showed DO was significantly higher in Cd + Pb + garlic and Cd + Pb than garlic only treated group and control group. There was no significant ($p \geq 0.05$) difference in Cd + Pb + garlic and Cd + Pb; garlic only and control. It may mean that garlic had no effect on DO in Cd and Pb exposure and apparently healthy fish. The higher values in Cd +

Pb exposed groups may be due to decrease in activities that required dissolved oxygen than control and garlic treated groups.

At 120hr, after 72hr water renewal at 72hr of the experiment, with 12hr garlic treatment for two consecutive days at 6hr after a 6hr exposure at day 1 and 12hr later at day 2 showed DO was lowest significantly ($p \leq 0.05$) in Cd + Pb + garlic treatment than all groups. Garlic only treated group was highest significantly ($p \leq 0.05$) than all groups. It may mean that garlic decreased DO in Cd + Pb exposed group due to increased activities that require DO such as increased feed intake than Cd + Pb with no treatment group. In apparently healthy fish, garlic treatment resulted in a lower feed (reduced metabolism) intake than control which may have been the reason for a higher DO than control.

In comparison of effects of exposure and treatment with days of sampling the order of decreasing significant ($p \leq 0.05$) difference in DO in Cd + Pb exposed and garlic treated group was 6hr > 24hr > 120hr. It may mean that DO decreases with increased garlic treatment in apparently healthy fish whereas in Cd + Pb exposed group the two garlic interventions appear to be benefifial. In Cd + Pb exposed group with no garlic treatment, the order was 6hr > 24hr > 120hr. This may mean that DO decrease with the first water renewal while the second water renewal had no effect. In garlic only treated group, the order was not significantly ($p \geq 0.05$) different. It may mean that with the treatment in apparent health, garlic had no effect due to the reduced activity, low optimum concentration used, frequency and short treatment of water change to allow for emergence of phytoplankton and zooplankton to enhance oxygen production. In control group with no Cd and Pb exposure and no garlic treatment, the order showed no significant ($p \geq 0.05$) difference due probably to reasons earlier adduced in garlic only treated group.

6.4.10: Results of Oxidative stress markers

Table 6.22: Effect of garlic treatments Means ± SEM of on oxidative stress markers levels at 6 and 120hr after 6hr exposure to lethal dose of Cadmium

Parameter	Hours of experiment	Cadmium+ garlic	Cadmium only (64mg/l)	Garlic only (0.65mg/l)	Control
Total Protein mg/g	6	0.09 ± 0.00^{ab}	0.09 ± 0.00^{a}	0.08 ± 0.00^{bc}	0.08 ± 0.00^{c}
	120	0.09 ± 0.00^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}
GSHμmol/g tissues	6	53.82 ± 0.05^{a}	53.79 ± 0.01^{a}	54.16 ± 0.66^{a}	54.16 ± 0.67^{a}
	120	50.95 ± 0.20^{b}	54.35 ± 0.55^{a}	51.85 ± 0.28^{b}	54.85 ± 0.23^{a}
H_20_2(μmole/mg protein	6	8.05 ± 0.02^{b}	8.05 ± 0.01^{b}	12.34 ± 0.07^{a}	12.08 ± 0.24^{a}
	120	8.85 ± 0.45^{c}	12.45 ± 0.09^{a}	10.95 ± 0.28^{b}	10.70 ± 0.70^{b}
GPxunit/mg protein	6	2643.90 ± 2.07^{b}	2641.77 ± 2.44^{b}	2717.24 ± 0.24^{a}	2717.20 ± 0.27^{a}
	120	2426.58 ± 35.37^{b}	2365.70 ± 6.30^{b}	2613.34 ± 20.11^{a}	2669.19 ± 30.42^{a}
MDAμmole/mg protein	6	3.20 ± 0.28^{b}	3.38 ± 0.29^{b}	4.45 ± 0.10^{a}	4.39 ± 0.07^{a}
	120	2.20 ± 0.06^{b}	3.82 ± 0.00^{a}	0.30 ± 0.01^{c}	0.38 ± 0.04^{c}
SODUnits/mg protein	6	729.76 ± 0.65^{b}	729.94 ± 0.57^{b}	781.11 ± 2.05^{a}	781.84 ± 1.25^{a}
	120	691.61 ± 5.53^{c}	712.88 ± 4.27^{bc}	765.34 ± 5.60^{a}	730.65 ± 11.30^{b}

Means of same sex with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.23: Effect of garlic treatments Means ± SEM of on oxidative stress markers levels at 6 and 120hr after 6hr exposure to lethal dose of Lead

Parameter	Hours of experiment	Lead+ garlic	Lead only (126mg/l)	Garlic only (0.87mg/l)	Control
Total Protein mg/g	6	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.80 ± 0.00^{b}	0.80 ± 0.00^{b}
	120	0.9 ± 0.00^{a}	$0.9\ 0.00^{a}$	0.9 ± 0.00^{a}	0.9 ± 0.00^{a}
GSHμmol/g tissues	6	57.05 ± 0.02^{a}	57.05 ± 0.02^{a}	53.69 ± 0.68^{b}	54.37 ± 0.63^{b}
	120	54.29 ± 0.31^{b}	56.10 ± 0.19^{a}	51.20 ± 0.49^{c}	54.65 ± 0.22^{b}
H_20_2(μmole/mg protein	6	14.22 ± 0.08^{a}	14.22 ± 0.05^{a}	12.08 ± 2.41^{b}	12.32 ± 0.08^{b}
	120	10.90 ± 0.25^{a}	11.30 ± 0.12^{a}	$9.35\ 0.19^{b}$	10.75 ± 0.72^{a}
GPxunit/mg protein	6	2364.65 ± 0.74^{b}	2363.28 ± 0.07^{b}	2717.24 ± 0.24^{a}	2717.20 ± 0.26^{a}
	120	2554.91 ± 13.82^{b}	2341.74 ± 8.51^{c}	2647.85 ± 19.27^{a}	2685.61 ± 20.17^{a}
MDAμmole/mg protein	6	11.82 ± 0.05^{a}	11.76 ± 0.02^{a}	4.45 ± 0.10^{b}	4.44 ± 0.06^{b}
	120	0.60 ± 0.01^{c}	0.81 ± 0.01^{a}	0.25 ± 0.02^{d}	0.75 ± 0.03^{b}
SODUnits/mg protein	6	660.87 ± 0.36^{b}	660.87 ± 0.36^{b}	782.68 ± 1.40^{a}	781.84 ± 1.25^{a}
	120	716.86 ± 0.07^{c}	682.00 ± 0.01^{d}	781.04 ± 0.02^{a}	729.14 ± 0.03^{b}

Means of same sex with the same letter on the same row are not significantly different according to DMRT at $P \geq 0.05$

Table 6.24: Effect of garlic treatments Means ± SEM of on oxidative stress markers levels at 6 and 120hr after 6hr exposure to lethal dose of Cadmium + Lead

Parameter	Hours of experiment	Cadmium + lead + garlic	Cadmium (64mg/l) + lead (126mg/l) only	Garlic only (0.65mg/l)	Control
Total Protein mg/g	6	0.09 ± 0.00^{a}	0.09 ± 0.00^{a}	0.08 ± 0.00^{b}	0.08 ± 0.00^{b}
	120	0.9 ± 0.00^{a}	$0.9\ 0.00^{a}$	0.9 ± 0.00^{a}	0.9 ± 0.00^{a}
GSHμmol/g tissues	6	51.77 ± 0.02^{b}	51.78 ± 0.02^{b}	54.31 ± 0.61^{a}	53.65 ± 0.65^{a}
	120	55.10 ± 0.27^{a}	55.10 ± 0.67^{a}	53.05 ± 0.18^{b}	53.70 ± 0.49^{b}
H_20_2(μmole/mg protein	6	11.74 ± 0.01^{b}	11.74 ± 0.01^{b}	12.10 ± 0.26^{ab}	12.31 ± 0.11^{a}
	120	10.95 ± 0.20^{ab}	12.10 ± 0.06^{a}	11.05 ± 0.34^{ab}	10.75 ± 0.72^{b}
GPxunit/mg protein	6	2473.62 ± 2.24^{b}	2476.47 ± 1.99^{b}	2717.03 ± 0.20^{a}	2717.06 ± 0.29^{a}
	120	2532.48 ± 35.53^{b}	2458.04 ± 8.68^{c}	2569.39 ± 18.19^{b}	2669.19 ± 20.11^{a}
MDAμmole/mg protein	6	4.98 ± 0.03^{a}	4.98 ± 0.03^{a}	4.45 ± 0.10^{b}	4.40 ± 0.08^{b}
	120	0.15 ± 0.63^{c}	0.40 ± 0.00^{a}	0.30 ± 0.01^{b}	0.41 ± 0.04^{a}
SODUnits/mg protein	6	702.61 ± 1.85^{b}	702.61 ± 185^{b}	781.71 ± 1.59^{a}	781.84 ± 1.25^{a}
	120	733.99 ± 3.00^{b}	729.90 ± 1.71^{b}	759.69 ± 6.21^{a}	730.65 ± 11.30^{b}

Means with the same letter on the same row are not significantly different according to DMRT at P≥0.05

Tables 22 to 23 shows the results of oxidative markers. At 6 hours of Cadmium exposure, liver total protein was significantly (P≤0.05) higher than in garlic only and control. GSH showed no significant (P≥0.05) difference in all groups. H_20_2, GPX, MDA and SOD were significantly (p≤0.05) lower than in garlic and control groups.

At 6 hours of lead exposure liver total protein, GSH, H_20_2 and MDA were significantly (P≤0.05) higher than in garlic only and control groups. GPX and SOD were significantly (P≤0.05) lower than in garlic only and control groups. At 6 hours of cadmium + lead exposure liver, total protein and MDA were significantly (P≤0.05) higher than in garlic only and control groups. GSH, H_20_2, GPX and SOD were significantly (P≤0.05) lower than in garlic only and control groups.

At 120 hours after 2 days consecutive 12-hour garlic treatments of 6-hour exposure to cadmium, liver total protein showed no significant (P≥0.05) difference in all groups. GSH order of significant (P≤0.05) decrease was cadmium + garlic, > garlic only > control and cadmium only. There was no significant (P≥0.05) difference in control and cadmium only, garlic only control. H_20_2 order of significant (P≤0.05) decrease were cadmium + garlic > control, garlic only > cadmium only. GPX order of significant (P≤0.05) decrease was cadmium only, cadmium + garlic > garlic only, control. MDA order of significant (P≤0.05) decrease were garlic only, control > cadmium + garlic > cadmium only. There was no significant (P≥0.05) difference in garlic only and control. SOD order of significant (P≤0.05) decrease were cadmium + garlic > garlic only. There was no significant P≥0.05) difference in cadmium + garlic, cadmium only; cadmium only and control.

At 120 hours of 2 days consecutive 12-hour garlic treatment of 6 hours' exposure to lead in female liver, total protein showed no significant (P≥0.05) difference in all groups. GSH order of significant (P≤0.05) decrease were garlic only > lead + garlic, control > lead only. H_20_2 order of significant (P≤0.05) decrease were garlic only > control, lead + garlic, lead only. There were no significant (P≥0.05) difference in lead + garlic, lead only. GPX order of significant (P≤0.05) decrease were lead only > lead + garlic > garlic only, control. There was no significant (P≥0.05) difference in garlic only and control. MDA order of significant (P≤0.05) decrease were garlic only > lead + garlic > control > lead only. SOD order of significant (P≤0.05) decrease were lead only > lead + garlic > control > garlic only.

At 120 hours of 2 days consecutive 12-hour garlic treatments of 6-hour exposure to cadmium + lead exposure, in female liver, total protein showed no significant (P≥0.05) difference in all groups. GSH order of significant (P≤0.05) decrease was garlic only, control. > cadmium +

lead, cadmium + lead + garlic. There were no significant (P≥0.05) difference in cadmium + lead + garlic, cadmium + lead; and garlic only and control. H_20_2 order of significant (P≤0.05) decrease was control > cadmium + lead. There were no significant (P≥0.05) difference in cadmium + lead + garlic, cadmium + lead; cadmium + lead + garlic, garlic only; garlic only, control. GPX order of significant (P≤0.05) decrease were cadmium + lead > cadmium + lead + garlic, garlic only > control. MDA order of significant (P≤0.05) decrease were cadmium + lead + garlic > garlic only > cadmium + lead, control. There was no significant (P≥0.05) difference in cadmium + lead, control. SOD order of significant (P≤0.05) decrease was cadmium + lead, cadmium + lead + garlic, control > garlic only.

6.4.11 Discussion on Oxidative stress markers

At 6-hour exposure of cadmium in the liver there were significant (P≤0.05) decrease in H_20_2, GPX, MDA and SOD. The significant (P≤0.05) decrease in GPX and SOD levels was in agreement with the findings of Ahmet *et al*., (2013). However, the findings that MDA significantly (P≤0.05) decreased were not in agreement with Ahmet *et al*., (2013) that reported that MDA was increased by cadmium. It appears therefore that in a very highly cadmium polluted environment, the effect on the mitochondrial enzyme activities was damage (Lacroix and Hontela, 2004) resulting in decreased production of H_20_2 and MDA rather than an inhibitory effect (Dabas *et al*., 2011) that leads to an enhancement in ROS (H_20_2) which enhance peroxidase damage in the liver (increased MDA). The non significant (P≥0.05) difference in GSH (non enzymatic antioxidant) may mean that acutely high level of pollutions by cadmium had no effect on non enzymatic antioxidant.

At 6 hour exposed to lead show in the liver significant (P≤0.05) increase in total protein, GSH, H_20_2 and MDA. Also, significant (P≤0.05) decreases in GPX and SOD. The significant increase in H_20_2 may be due to inhibition of delta aminolevulinic acid dehydrate by lead (Farant and Wigbaseline, 1982) which leads to the accumulation of delta aminolevulinic acid which is a potential endogenous source of free radicals (Hermeh – Lima 1995). Also, the significant (P≤0.05) increase in MDA maybe due to the findings by Adonaylo and Otieza 1999) that direct interaction of lead with biological membrane induces lipid peroxidation (increase in MDA) in presence of Fe^{2-}. The significant (P≤0.05) increase in GSH maybe due to induction of non enzymatic antioxidant due to the higher level of lead to help destroy the free radicals which is not in agreement with the findings of Sandhir *et al*., (1994) that lead decreased gluthatione (GSH) but in agreement that lead decreased free radical scavenging enzymes. The non significant difference in the value of GSH in control in exposure to

cadmium, that became significantly ($P \leq 0.05$) higher than control in exposure to lead may also be due to water renewal that abated the lethal effect of 6hr exposure, which probably damaged the mitochondria to an inhibitory effect (Dabas *et al*., 2011) that leads to an enhancement in ROS (H_20_2) which enhance peroxidase damage in the liver (increased MDA) which GSH increase in polluted environment had also been reported by Faroumbi *et al*., (2007) and high level of pollution by lead as reported by Nkwoji *et al*., (2014) increase the levels of antioxidants indicates presence of pollutants in the environment. It appears that GSH increase is compensatory mechanism to combat the high free radical production. The significant ($P \leq 0.05$) increase in total protein in cadmium exposure may be due to modification in the enzyme structure by the interaction with enzyme cofactors or possible induction of stress proteins as reported by Ahmet, *et al*., (2013). The same explanation may be given for the significant ($P \leq 0.05$) increase in total protein in exposure to lead. GSH increase in polluted environment had also been reported by Faroumbi *et al*., (2007).

Exposure to cadmium + lead at 6 hour showed significant ($P \leq 0.05$) increase in total protein, and MDA than control and significant ($P \leq 0.05$) decrease in GSH, H_20_2, GPX and SOD. These effects may be due to the interaction of cadmium and lead. It appears the result in total protein may be due to non competitive antagonism of lead by cadmium, it may thereby mean that lead was more toxic than cadmium in female. The effects on GHS may be due to competitive antagonism of cadmium and lead. The effects on H_20_2 may be due to antagonism of lead by cadmium. The effect on GPX may be due to non competitive antagonism of cadmium by lead. The effect on MDA may be due to non competitive antagonism of cadmium by lead. The effect on SOD may be due to non competitive antagonism of cadmium by lead. There was mortality in cadmium + lead group. Comparatively the order of significant ($P \leq 0.05$) decrease for total protein was lead > cadmium+ lead > cadmium, GSH was lead > cadmium > cadmium + lead, H_20_2 was lead > cadmium + lead > cadmium, GPX was cadmium > cadmium + lead > lead, MDA was lead > cadmium + lead > cadmium, SOD was cadmium > cadmium + lead > lead. It may mean that the mortality in cadmium + lead may have been caused by the decreased levels of GSH, H_20_2, GPX and SOD; and increased level of total protein and MDA. The decreasing level of H_20_2 may be suggestive that it may not be critical. This may be in aggrement with the findings of Secombes (1996), that free radicals are used by the immune system to kill pathogens and that excess production of free radicals that occurs during chronic infections may be harmful to nearby cells.

At 120 hours, cadmium exposed group, with 3 consecutive days (day 1, 2 and 3) water renewal, before 12hour garlic bath at day 1 and 2, shows a non significant ($P \leq 0.05$) difference in total protein with the control, significant ($P \leq 0.05$) decrease in H_20_2, GPX, SOD and significant ($P \leq 0.05$) increase in MDA and GSH than control. In fish without garlic bath there was a no significant ($P \geq 0.05$) difference in total protein, and SOD, significant ($P \leq 0.05$) decrease in and GPX and increase in GSH than control. In garlic only group without exposure to metals, there was no significant ($P \geq 0.05$) difference in total protein, GSH, H_20_2, GPX and MDA whereas SOD significantly ($P \leq 0.05$) increased than control. It means that garlic decreased GSH as the value was significantly ($P \leq 0.05$) lower than the values at 6 hours. In garlic only group, GSH showed significant ($P \leq 0.05$difference with control. These may also confirm that garlic decreased GSH value in exposure to cadmium and bathed with garlic. For H_20_2, in cadmium exposure and bathed with garlic there was a significant ($P \leq 0.05$) decrease whereas in exposure to cadmium with only water renewal there was a significant ($P \leq 0.05$) increase in H_20_2 compared to control. However, in only garlic bath, there was no significant ($P \geq 0.05$) difference with control. It means garlic decreased H_20_2 in cadmium toxicity, water renewal increased H_20_2 whereas garlic only had no effects probably due to the low non optimum concentration. The value with garlic bath after exposure was non significantly ($P \geq 0.05$) different with the value at 6 hours, the value with only water renewal was highly significantly ($P \leq 0.05$) higher than the value at 6 hours. The value with garlic bath only was significantly ($P \leq 0.05$) lower than the value at 6 hours whereas the control value was not significantly ($P \geq 0.05$) different from the value at 6 hours. It may mean that the significant decrease in hydrogen peroxide (H_20_2) in exposure with garlic bath was due to non enzymatic antioxidant (Vitamin C, Vitamin E, Selenium, zinc and manganese) in garlic. The non significant ($P \geq 0.05$) difference in total protein, GSH, GPX and MDA with control in garlic only bath may be due to lower optimum concentration used in apparently healthy fish. For GPX, it may mean that garlic bath and water renewal in cadmium exposed female was not able to increase their values significantly. The value of GPX in garlic bath after exposure was higher than with water renewal only. The value in garlic bath was higher significantly ($P \leq 0.05$) whereas in water renewal it was highly significantly ($P \leq 0.01$) lower than the value at 6 hours. It may mean garlic bath marginally reduced oxidation stress than with water renewal. For MDA in cadmium exposure, values were significantly ($P \leq 0.05$) lower in garlic bath than water renewal although both were significantly ($P \leq 0.05$) higher than control. It may mean that despite the decreased MDA in garlic bath than water renewal, oxidation stress still

persists but was lower in garlic bath. The value in garlic bath was significantly ($P \leq 0.05$) lower than the value at 6 hours (decreased oxidation stress) whereas in water renewal, the value increased but was not significantly ($P \geq 0.05$) different with the value at 6 hours (no effect). The effect of decrease MDA in cadmium exposure may be due to garlic effect in H_20_2 earlier explained. The increase in MDA in water renewal may be due to water renewal acting as a repairer of the cadmium damaged liver resulted in inhibitory effects on the mitochondrial enzyme activities (Dabas *et al.*, 2011) that enhanced MDA production. For SOD in cadmium exposure, value was significantly ($P \leq 0.05$) lower in both with garlic bath and water renewals than control. Both values were not significantly ($P \geq 0.05$) different although the value with garlic bath was lower than with water renewal. The values with garlic bath and water renewal was significantly ($P \leq 0.05$) lower than the value at 6 hours, which may mean that oxidative stress was more than at 6-hour post exposure despite attempts to reduce it. However, garlic bath reduced oxidative stress more than with water renewal.

Generally, with non optimum garlic concentration bath in apparently healthy unexposed female, there was no significant ($P \geq 0.05$) difference in total protein, significant ($P \leq 0.05$) increase in SOD, significant ($P \leq 0.05$) decrease in H_20_2, GSH, GPX, and MDA than control. It means garlic decreased oxidation stress with the lower non optimum concentration in the three subgroups of cadmium only, lead only and cadmium + lead.

At 120-hour, lead exposed group shows no significant ($P \geq 0.05$) difference in garlic bath and control in total protein and GPX. Total protein values at 120 hours was significantly ($P \leq 0.05$) lower than the value at 6 hours, it may mean garlic and water renewal significantly decreased protein in the liver which was beneficial, GSH level in garlic bath was significantly lower than water renewal. The value with garlic bath was not significantly different relative to control whereas the value with water renewal was significantly ($P \leq 0.05$) higher than control. The value at 120 hours in garlic bath was significantly ($P \leq 0.05$) lower than 6-hour value whereas the value in water renewal was not significantly different from 6-hour value. The decreased GSH value at 120 hours in garlic bath is in agreement with the findings of Donaldson (1991) and Klanns-Dieter (1983) that the organic sulphites of garlic can scavenge the OH^- radical and regenerate the vitamins and sulphates by a process of recycling. The non significant ($P \leq 0.05$) difference in the level of H_20_2 in garlic bath, with bath water renewal and control may mean that garlic had no effect on H_20_2 in exposure to lead. However, the significant lower value in only garlic bath relative to control may be in agreement with the findings of Sajitha *et al*., (2010) that garlic significant decrease of H_20_2 maybe due to action

of garlic as a detoxifying agent and antioxidant, scavenging free radicals as well as an independent action of removal of lead salts as lead sulphides. The values of H_20_2 in garlic bath and water renewal at 120 hours was highly significantly ($P\leq0.01$) lower than the value at 6 hours. GPX levels was not significantly different in control and garlic only, the value with garlic bath was significantly ($P\leq0.05$) higher than the value with water renewal. This mean that garlic bath reduced oxidative stress more than with water renewal. The value at 120 hours in garlic bath was significantly ($P\leq0.05$) higher than at 6 hours whereas the value in water renewal showed no significant ($P\geq0.05$) difference with the value at 6 hours. It may mean that garlic significantly elevated GPX level. The reason for decrease of GPX by lead may be due to consumption by high level of prooxidants produced by Lead. MDA level was significantly ($P\leq0.05$) higher than in control, in garlic bath and garlic only groups. However, the values in garlic bath was significantly ($P\leq0.05$) lower than in water renewal which was significantly ($P\leq0.05$) lower than control. It may mean that oxidative stress till persist in lead only group. The values at 120 hours in garlic bath and water renewal was highly significantly ($P\leq0.01$) lower than the values at 6 hours. It may mean that water renewal also significantly decreases MDA. The significant decrease in MDA may be due to the high level of enzymatic antioxidants (vitamin C, E, etc) in garlic and is in agreement with Nuriye *et al*., (1999) that antioxidants inhibits lipid peroxidation (LPO) by preventing peroxidation chain or by accumulating the reaction oxygen. Also, supplementation of dietary Vitamin E and vitamin C etc lowered lipid peroxidation, protein oxidation and the incidence of various morbidities or mortalities, induced catalase activity and reduce the oxidation stress in rat (Amin and Hashem 2012). SOD level was significantly ($P\leq0.05$) lower than control in garlic bath and water renewal which mean oxidative stress still exist. However, the value in garlic bath was significantly ($P\leq0.05$) higher than in water renewal which mean garlic bath reduced oxidative stress of lead toxicity more than water renewal. The value at 120 hours was highly significantly ($P\leq0.05$) higher than the value at 6 hours which may also mean garlic bath increased SOD level thereby decreasing oxidative stress caused by lead. The value at 120 hours in water renewal was significantly ($P\leq0.05$) higher than the value at 6 hours, it means water renewal also reduced oxidative stress.

Exposure to cadmium + lead at 120 hour showed significant ($P\geq0.05$) difference in garlic bath water renewal with control. The effect in garlic bath was due to garlic acting as a quencher (Nurije *et al*., 1999) of cadmium + lead reaction/interaction on protein synthesis in the liver. The water renewal may also have acted as a quencher of cadmium + lead

reaction/interaction on protein synthesis in the liver. GSH value was significantly (P≤0.05) higher with/out garlic bath. The value at 120 lower in with/out garlic bath water significantly (P≤0.05) higher than the values at 6 hours. It means both did not reduce oxidative stress but are in the process of combating it. This may may be due to water renewal that abated the on the mitochondria, damaging effect of 6hr exposure, to an inhibitory effect (Dabas *et al*., 2011) that leads to an enhancement in ROS (H_20_2) which enhance peroxidase damage in the liver (increased MDA) which increase GSH in polluted environment had also been reported by Faroumbi *et al*., (2007) and high level of pollution by lead as reported by Nkwoji *et al*., (2014) increase the levels of antioxidants indicates presence of pollutants in the environment.. H_20_2 values in garlic bath was not significantly (P≥0.05) different from control and water renewal whereas water renewal value was significantly (P≤0.05) higher than control. It may mean garlic bath reduced oxidative stress whereas water renewal could not. The value at 120 hours in garlic bath was highly significantly (P≤0.001) lower than the value at 6 hours whereas the value in water renewal was highly significantly (P≤0.001) higher than the value at 6 hours. It means garlic bath decreased H_20_2 whereas water renewal increased H_20_2. The significant lower value in garlic bath may be in agreement with the findings of Sajitha *et al*., (2010) that garlic significant decrease of H_20_2 maybe due to action of garlic as a detoxifying agent and antioxidant, scavenging free radicals as well as an independent action of removal of lead salts as lead sulphides. The increase in H_20_2. may be due to water renewal that abated the on the mitochondria, damaging effect of 6hr exposure, to an inhibitory effect (Dabas *et al*., 2011) that leads to an enhancement in ROS (H_20_2) which enhance peroxidase damage in the liver (increased MDA). GPX values in garlic bath and water renewal was significantly (P≤0.05) lower than control but the value in garlic bath was significantly (P≤0.05) higher than in water renewal. It may mean oxidative stress still persists but garlic bath reduced oxidative stress more than water renewal. The value at 120 hours was higher than the value at 6 hours in garlic bath was whereas the value at 120 hours was lower than the value at 6 hours' water renewal. It may mean that garlic bath increased GPX level. MDA values in garlic bath was significantly (P≤0.05) lower than in water renewal and in control. It may mean that garlic reduce significantly oxidative stress. The value at 120 hours in garlic bath water renewal at 120 hours was highly significantly (P≤0.001) lower than the value at 6 hours. It may mean water renewal. reduced significantly oxidative stress, probably due to the no significant difference with control. SOD value in garlic bath and water renewal was showed no significant difference from control. This may mean that oxidative stress has been

reduced significantly, probably due to the no significant difference with control and garlic bath have no effect on SOD. The value at 120 hours in both garlic bath and water renewal was significantly ($P \leq 0.05$) higher than the value at 6 hours which may indicate lower oxidative stress. At 120 hours, the effects on GSH in garlic bath may be due to potentiation of Lead by garlic to competitively antagonism of cadmium, in water renewals, may be due to non competitive antagonism of cadmium by lead. H_20_2 in garlic bath may be due to potentiation of cadmium to competitively antagonize lead. In water renewal was due to competitive antagonism of lead by cadmium. GPX in garlic bath may be due to competitive synergy by cadmium and Lead. In water renewal, may be due to competitive synergy by cadmium and Lead. MDA in garlic bath may be due to non competitive synergy of cadmium and lead. In water renewal may be due to non competitive antagonism of cadmium by Lead. SOD in garlic bath may be due to may be due to non competitive antagonism of cadmium by lead. In water renewal, it may be due to may be due to non competitive antagonism of cadmium by lead. At 120 hour compared to 6 hour values, in garlic bath and waterrenewal GSH values was significantly higher. H_20_2 was significantly ($P \leq 0.05$) lower and higher in garlic bath and water renewal respectively. GPX value was higher in garlic bath but lower with no significant ($P \geq 0.05$) difference inwaterrenewal. MDA was highly significantly ($P \leq 0.001$) lower in both garlic bath and water renewal. SOD value was highly significantly ($P \leq 0.001$) lower.

The order of significant ($P \leq 0.05$) decrease across cadmium, lead and cadmium + lead groups for total protein was non significant difference. It may mean that garlic bath had significant effects than others. For GSH it was cadmium, lead + garlic > cadmium + lead + garlic > cadmium + garlic. It may mean that garlic had more effect in decreasing GSH in lead. H_20_2 and GPX was cadmium + lead + garlic, lead + garlic > cadmium + garlic. There was no significant difference in cadmium + lead + garlic, and lead + garlic. It means garlic decreases GPX and H_20_2 more in cadmium exposure. MDA was cadmium + garlic > lead + garlic > cadmium + lead + garlic. It means garlic decreased MDA more in cadmium + lead + garlic. SOD was cadmium + lead + garlic > lead + garlic > cadmium + garlic. It may mean that garlic decreased SOD more in cadmium + garlic. For water treatment only, total protein, showed no difference. GHS was cadmium + lead > cadmium, lead. It means it increased GSH more. H_20_2 was cadmium > cadmium + lead > lead. It may mean it increased H_20_2 more in cadmium. GPX was cadmium + lead > cadmium, lead. It may mean it increased GPX more in cadmium + lead. MDA was cadmium > lead > cadmium + lead. It means it increased MDA

more. SOD was cadmium + lead > cadmium > lead. It may mean it increased SOD in cadmium + lead more.

Generally, Blomhoff (2005) reported that when animals are exposed to a dietary oxidative stress, they react with compasatory induction of endogeneous antioxidants which is in agreement with these findings. Garlic bath in addition to water renewal reduced the toxicity (oxidative stress) of cadmium, lead and cadmium + lead than water renewal only. In cadmium only exposure, response to garlic treatment relative to cadmium only exposed group with no garlic bath could be indicated by probably the induced endogeneous antioxidants with garlic bath such as GPX and SOD which were not significantly different with cadmium exposed female with no garlic bath may be due to their very fast consumption of the oxidants generated by cadmium that resulted in significant decrease in H_2O_2 and MDA at 120 hours. Response to garlic treatment in lead only exposure relative to lead only exposed group with no garlic bath could be indicated by probably the induced endogeneous antioxidants with garlic bath such as GSH, GPX and SOD significant increase which resulted in significant decrease in MDA with no effect on H_2O_2. The no effect on the generation of H_2O_2 may mean that in lead toxicity H_2O_2 level may not be critical and/or that the H_2O_2 boosted the immune system in support of GPX and SOD to decrease MDA which appears to be more important antioxidant in lead toxicity. This may be in aggrement with the findings of Secombes (1996), that free radicals are used by the immune system to kill pathogens and that excess production of free radicals that occurs during chronic infections may be harmful to nearby cells. Respose to garlic treatment in cadmium + lead only exposure relative to cadmium + lead only exposed group with no garlic bath could be indicated by the induced endogeneous antioxidants with garlic bath such as GPX and SOD which were not significantly different with cadmium + lead exposure with no garlic bath may be due to their very fast consumption of the oxidants generated by cadmium + lead resulting in significant derease in MDA. H_2O_2 was not significantly different in garlic bath and without garlic bath.

At 6-hour exposure, in their combination, cadmium and lead were synergistic in their action of increase in total protein, GPX, and SOD whereas their action of decrease was antagonistic on GSH, H_2O_2 and MDA. At 120-hour, in their combination, cadmium and lead with garlic treatment were synergistic in their action of no effect in total protein and SOD; decrease in GPX, whereas their antagonistic action was of no effect H_2O_2, increased GSH and decreased MDA. At 120-hour, in their combination, cadmium and lead with no garlic treatment were synergistic in their action of no effect in total protein; decrease in GPX whereas their

antagonistic action was of no effect on SOD and MDA; and an increase in GSH and H_2O_2. These results shows that cadmium may be more toxic than lead singularly and in their combination their interaction is dependent on exposure duration, treatment and parameter assayed.

6.4.12 Result of Erythrocyte Morphology

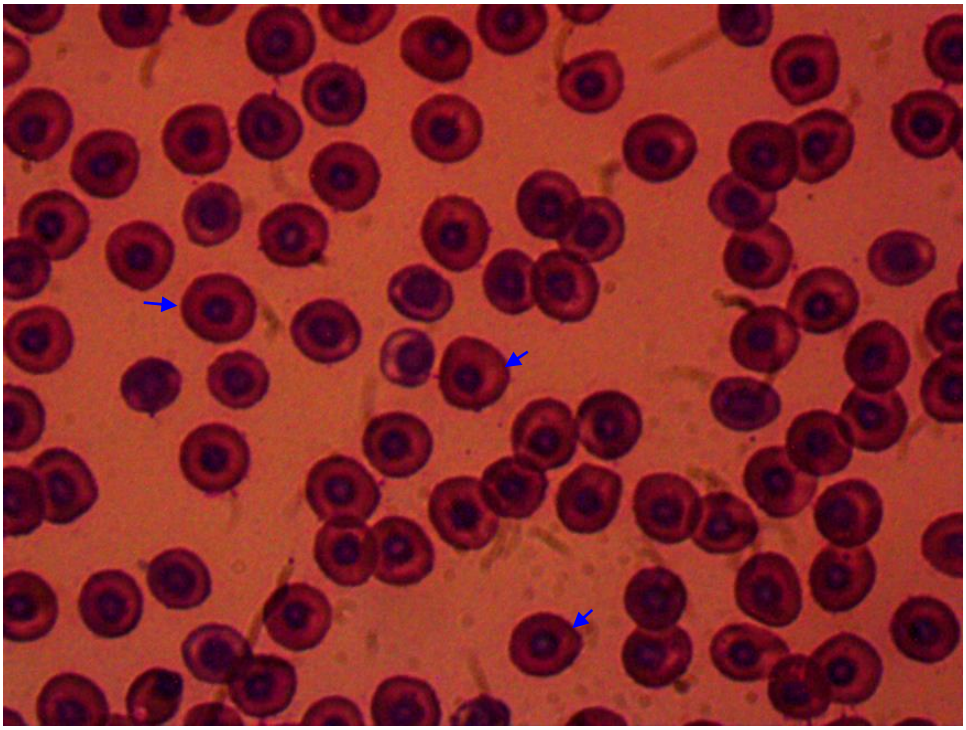

Plate 6.2: Erythrocytes (arrows) with normal shapes and sizes in control groups at 6hr of exposure. Giemsa x1000

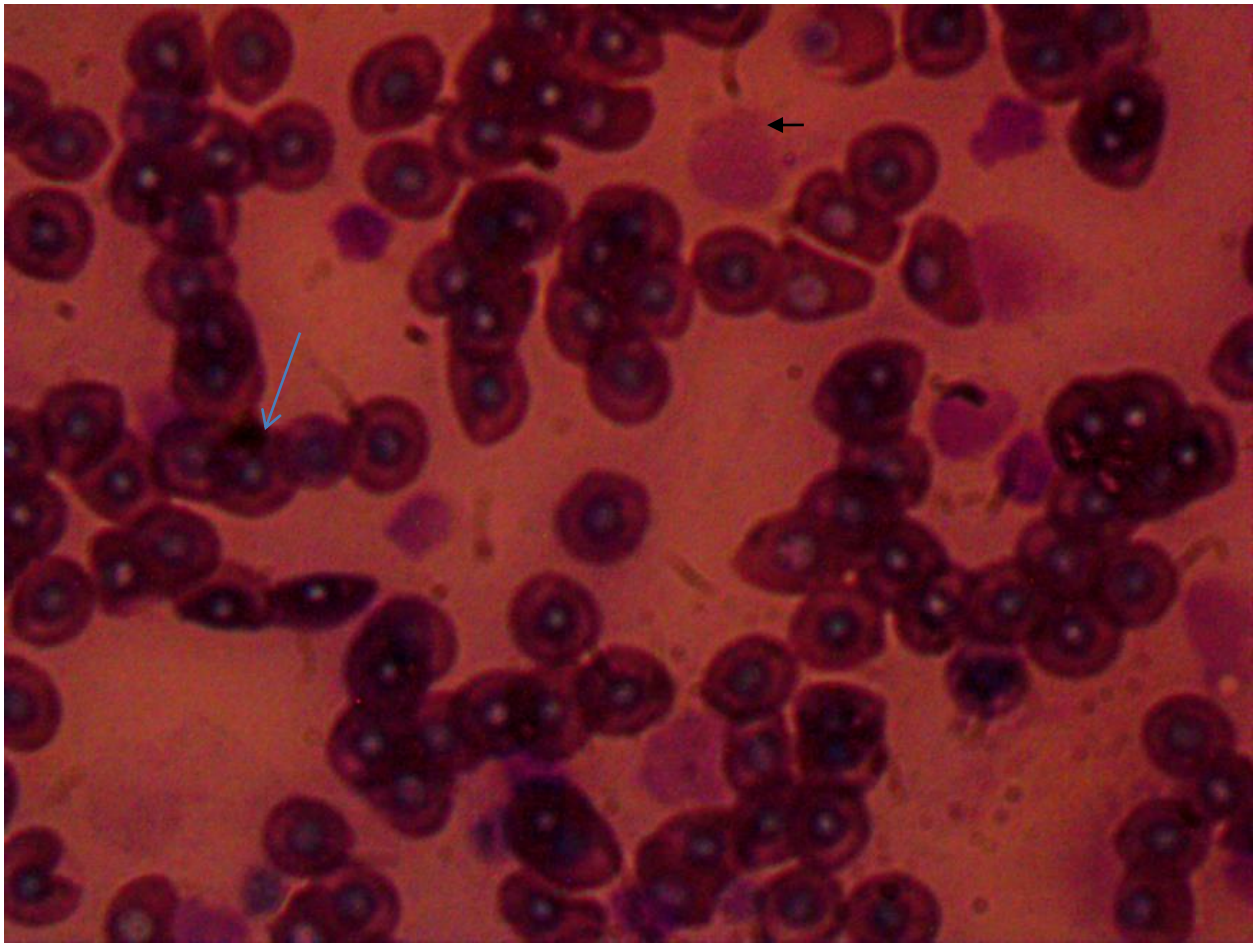

Plate 6.3: Few smudge cells (black arrow) and rouleux formation of erythrocytes (blue arrow) in exposure to Cd + Pb for 6hr of exposure. Giemsa x1000

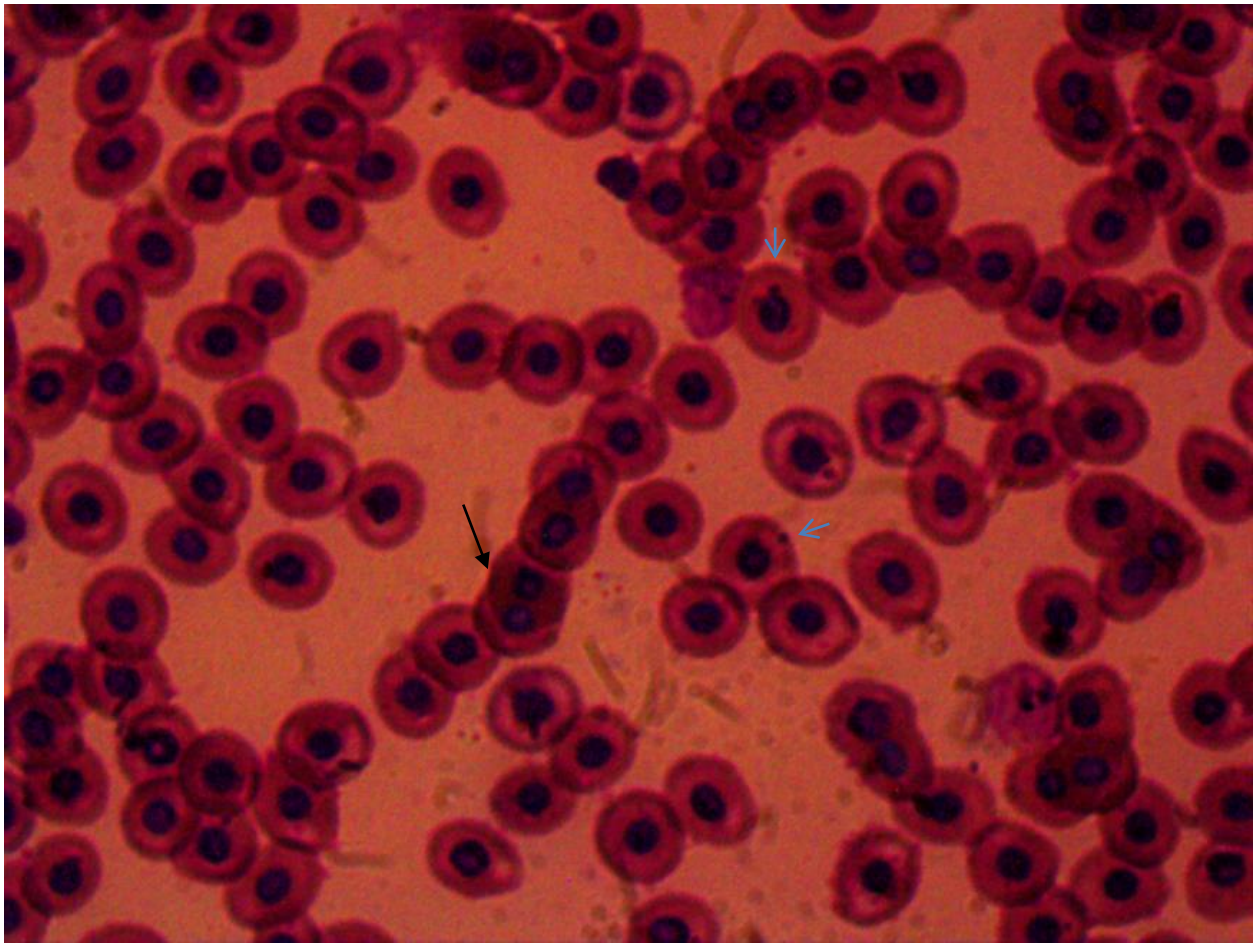

Plate 6.4: Erythrocytes with micronuclei (blue arrow), rouleux formation (black arrow), poikilocytosis (polygonal shapes) and smudge cells in exposure to Cd + Pb for Giemsa x1000

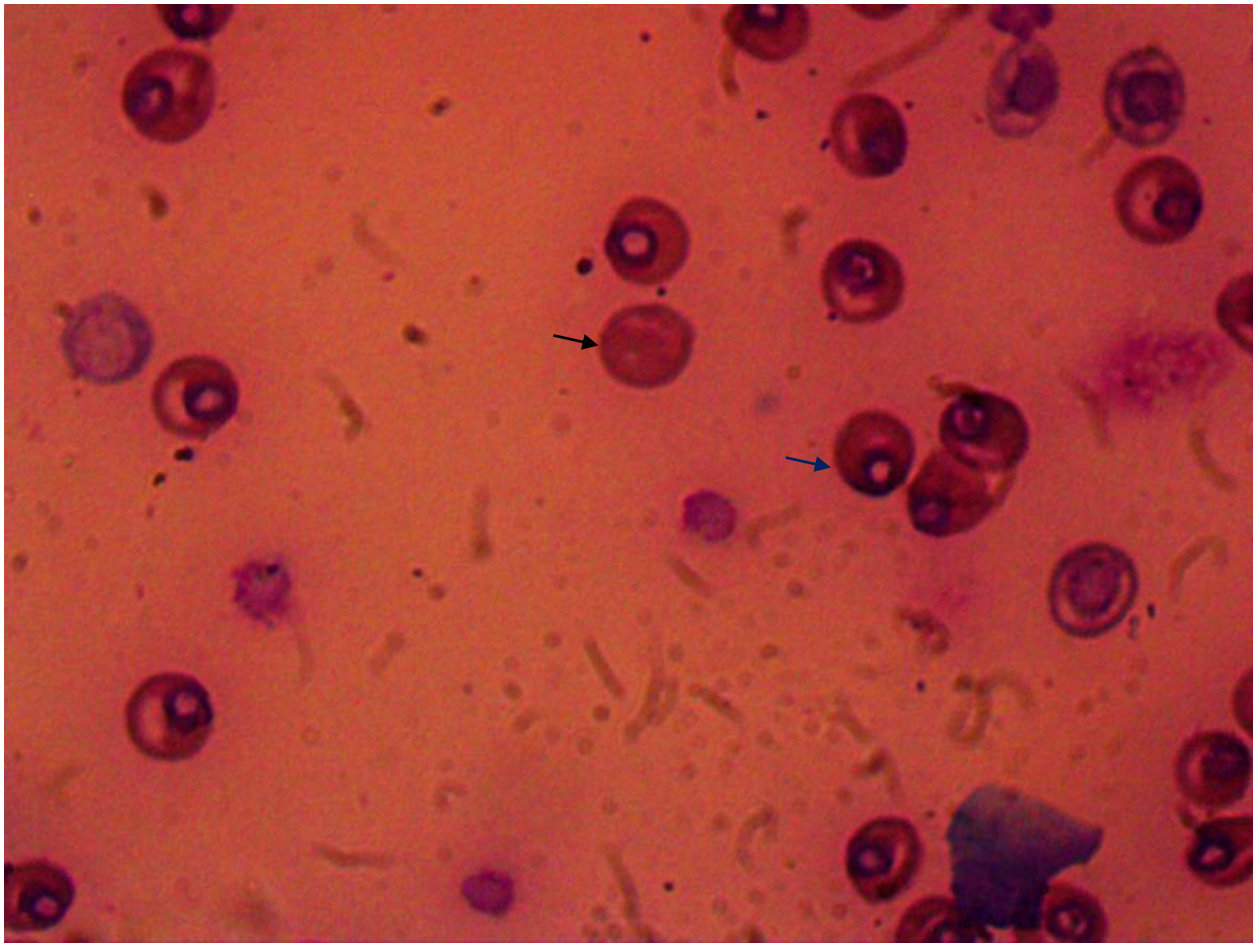

Plate 6.5: Eccentrocytes (blue arrow) and smudge cells (black arrow) in exposure to Pb at 24hr post exposure. Giemsa x1000

At 6 hours of cadmium exposure, there were 10 – 18% smudge cells with few rouleux formation and cytoplasmic vacoulation. In lead exposure there were 5 – 10% smudged cells, rouleaux formation, cytoplasmic vacoulation and moderate eccentrocyte. In unexposed groups (garlic only and control), erythrocytes had normal shape and size with no degeneration, less than 3% smudge cells and few immature erythrocytes with basophilic cytoplasm with 2 nuclei. In cadmium + lead exposed female were about 3 micronuclei, roleaux formation, pikilocytosis, polygonal shaped and 20 – 30% smudge cells.

At day 2 exposure to cadmium + 12-hour garlic treatment has moderate rouleax formation, 10 – 15% smudge cells and few binucleated erythrocytes. At day 2, exposure to cadmium had 80% smudge cells, passed nuclei membrane severe hypochromasia and macrocytosis. At day 2, exposure to lead + garlic had mild hypochromasia, 40 – 55% smudge cells and mild poikilocytosis. At day 2 exposure to lead had 70 – 80% smudge cells and moderate poikilocytosis. At day 2, exposure to cadmium + lead + garlic had 20 – 25% smudge cells, moderate hypochromasia and mild anisocytosis. At day 2, exposure to cadmium + lead had moderate rouleax formation, 50 – 80% smudge cells, moderate poikilocytosis (flamed shape) severe cytoplasmic vacoulation. In general, female exposed to garlic only had 5 – 10% smudge cells, mild poikilocytosis (sickled to polygonal). In control groups had 30 – 40% smudge cells, hypochromasia, mild poikilocytosis and anisocytosis.

At day 6 in cadmium exposure with no further garlic treatment after day 2 had 5% smudge cells with normal size and shape of erythrocytes. At day 6 exposure to cadmium had 10 – 15% smudge cells with normal shape and size of erythrocytes. At day 6 exposure to lead had 5 – 10% smudge cells, moderate piokilocytosis (polygonal), jagged nuclear membrane and micronuclei. At day 6 exposure to lead with no further garlic treatment after day 2 had 10 – 20% smudge cells and normal shape of erythrocytes. At day 6 exposure to cadmium + lead with no further garlic treatment after day 2 had 10 – 20% smudge cells, clumping of erythrocytes. At day 6 exposure to cadmium + lead had less than 5% smudge cells, moderate hypochromasia, few ecentrocyte and macrocytosis (responsive effect). At day 6 in all garlic only treated groups had 5 – 15% smudge cells and mild piokilocytosis (Drepanocyte). At day 6 female on all control groups had 10 – 20% smudge cells, moderate poikilocytosis, mild hypochromasia and few eccentrocytes.

6.4.13 Discussion on Erythrocyte Morphology

The erythrocyte anomalies observed in exposed fish were in agreement with the findings of Witeska *et al*., (2011) that frequencies of nuclear anomalies such as irregular nucleus, shape,

vaculation binuclei and micronuclei that indicate genotoxic effect often increase in fish exposed. However, it appears that the severity of these anomalie in lethal toxicity is also dependent on the offending metals(s) and duration because for just 6 hours' exposure showed severity in these anomalies in cadmium + lead with induced micronuclei formation which was induced by lead at 120hours which is indicative of genotoxic effect. Also toxicity of metals to *Clarias gariepinus* erythrocyte range (according to the frequency of erythrocyte anomalies) in female Cd + lead > Pb > Cd.

6.4.14 Results of gross pathology

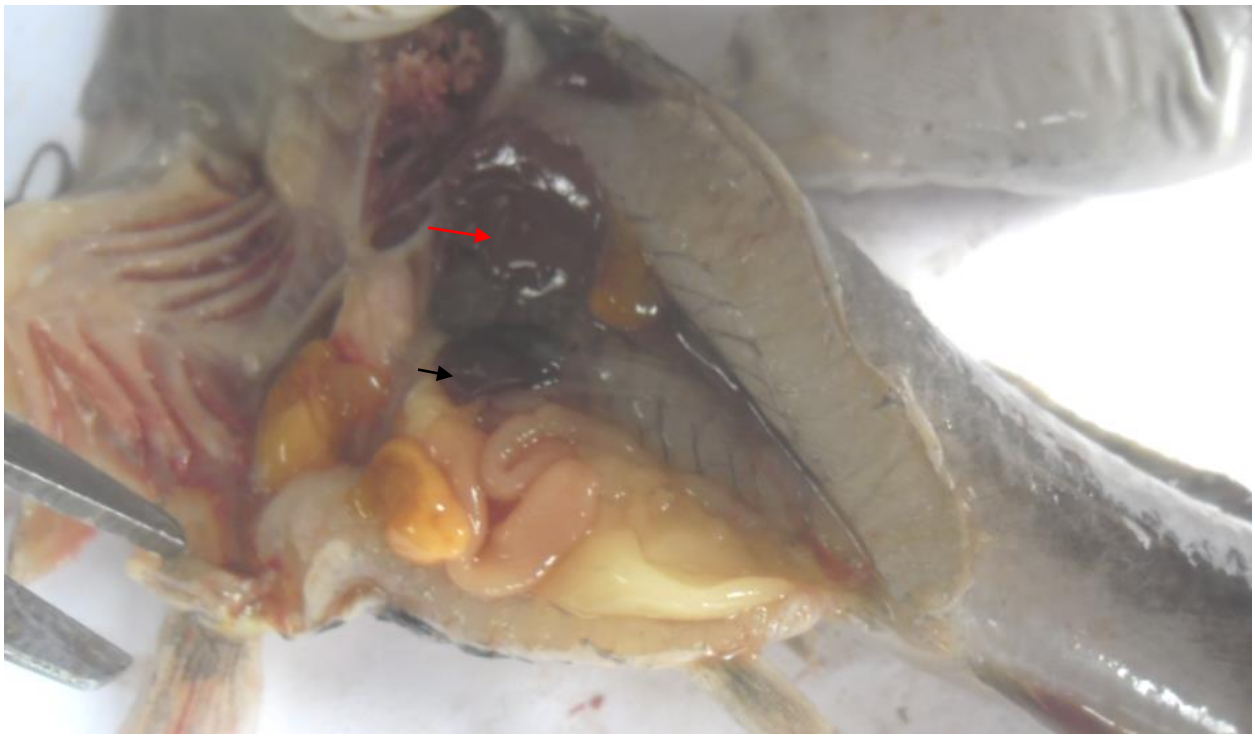

Plate 6.6: Congested spleen (black arrow) and friable kidney (red arrow) in fish exposed to Pb at 24hr post exposure

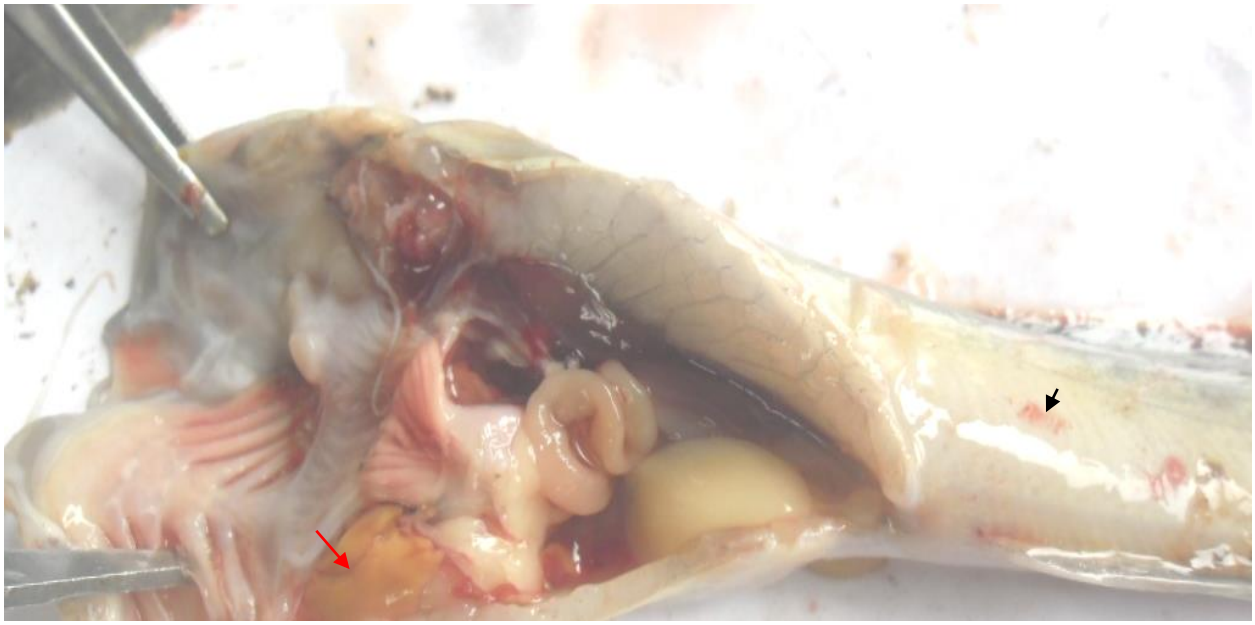

Plate 6.7: Focal cutaneous petechiation (black arrow), and pale liver (red arrow) in fish exposed to Pb at 120hr post exposure

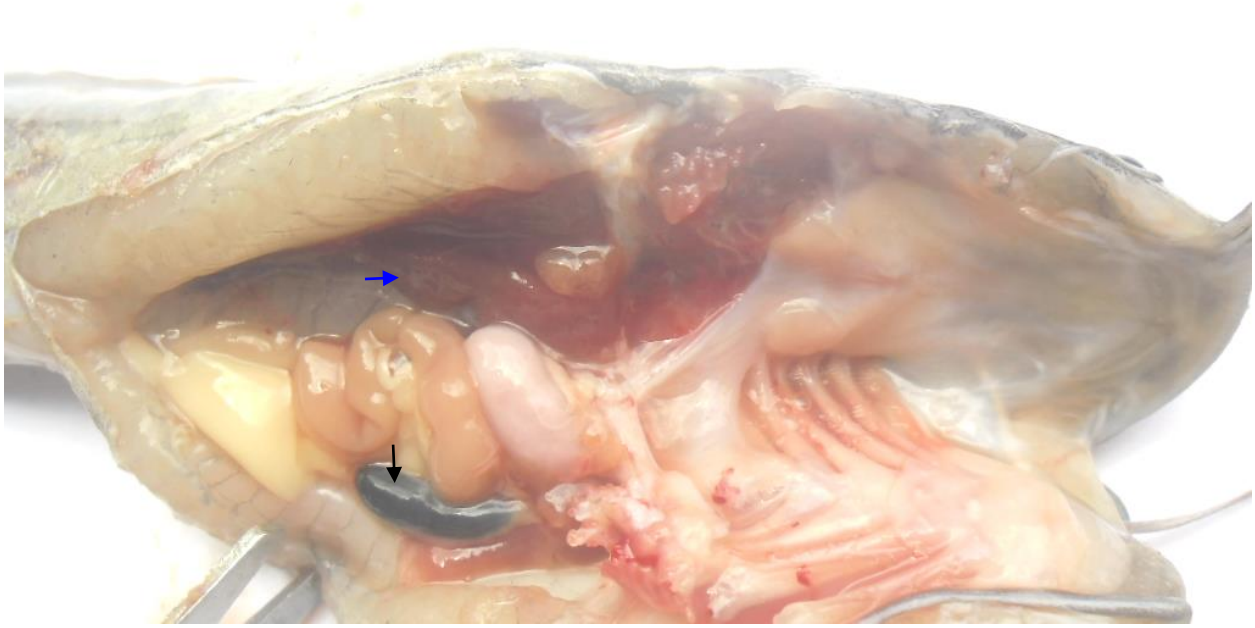

Plate 6.8: Engorged gall bladder (black arrow) and congested kidney (blue arrow) in fish exposed to Pb at 120hr post exposure

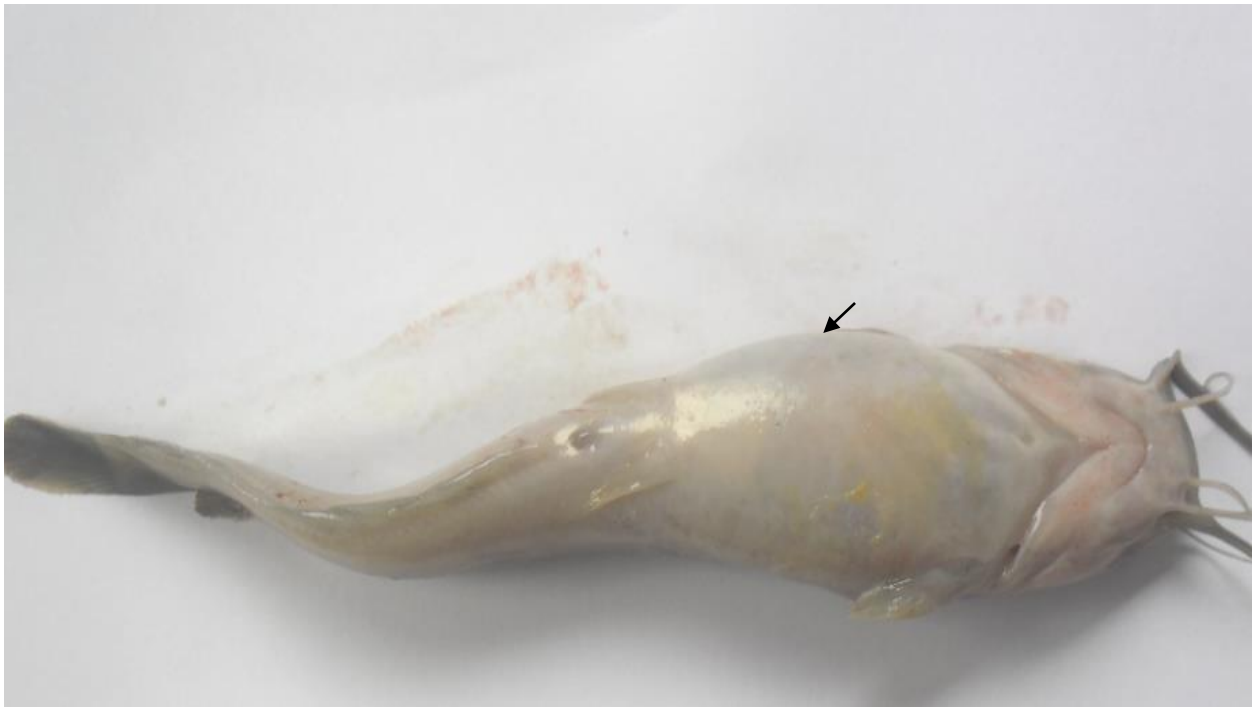

Plate 6.9: Tumescence (arrow) of the fish exposed to Cd + Pb + garlic at 120hr post exposure.

Plate 6.10: Cutaneous ulcer (arrow) and haemorrhages on the fish skin exposed to Cd + Pb at 120hr post exposure

Plate 6.11: Fish (Control) with normal cutaneous appearance at 144hr of experiment

At 6 hour of exposure to cadmium, lead and cadmium + lead was marked spleenic congestion. Garlic and control groups had no visible lesion

At day 2, exposure to cadmium + garlic had cutaneous swelling (ventrally), petechiation of ventral mandible and left pectoral fin and babel and friable liver. Exposure to cadmium without garlic treatment had generalized cutaneous petechiation, moderate slimy skin and moderate tumescent. Exposure to lead with garlic treatment showed congested spleen, enlarged kidney and friable liver. Exposure to lead with no garlic treatment had ventral opercula hyperemia and moderate slimy skin. Exposure to cadmium + lead + garlic had no visible lesion. Exposure to cadmium + lead had severe slimy skin and congested kidney. Generaly in garlic only and control groups had no visible lesion.

At day 72hr, 48 hours after 2 consecutive days of 12-hour water renewal, after 6 hour of cadmium exposure + 2 days consecutive 12-hour garlic treatment had moderate tumescence and skin erosion, pale gills and liver. Exposure to cadmium had moderate slimy skin, skin petechiation, severe tumescence and pale gills and liver. Exposure to lead with 2 days consecutive 12 hours' garlic treatment had moderate slimy skin and no visible lesion. Exposure to lead had severe slimy skin, engorged gall bladder, congested spleen and friable kidney. Exposure to cadmium + lead with 2 days consecutive 12-hour garlic treatment had moderate slimy skin, pale gills and congested visceral. Exposure to cadmium + lead had mild slimy skin, friable liver and kidney and congested spleen. Generaly in garlic only and control groups had no visible lesion.

At 120hr post exposure to cadmium and treated with garlic at day 1 and 2, fish had tumescence with moderate skin erosion and ulcer, pale liver and congested spleen. Exposure to cadmiumwithout garlic treatment had severe tumescence, severe skin erosion, moderate slimy skin, congested spleen and pale liver. Exposure to lead had mild slimy skin, focal cutaneous petechiation, skin erosion (ventral abdomen) and yellowish liver. Exposure to lead but treated with garlic and control groups had no visible lesion. Exposure to cadmium + lead and treated with garlic at day 1 and 2 had mild tumescence, mild slimy skin, and congested spleen. Exposure to cadmium + lead had moderate tumescence congested spleen.

Generaly in garlic only and control groups had no visible lesion.

6.4.15 Results of histopathology

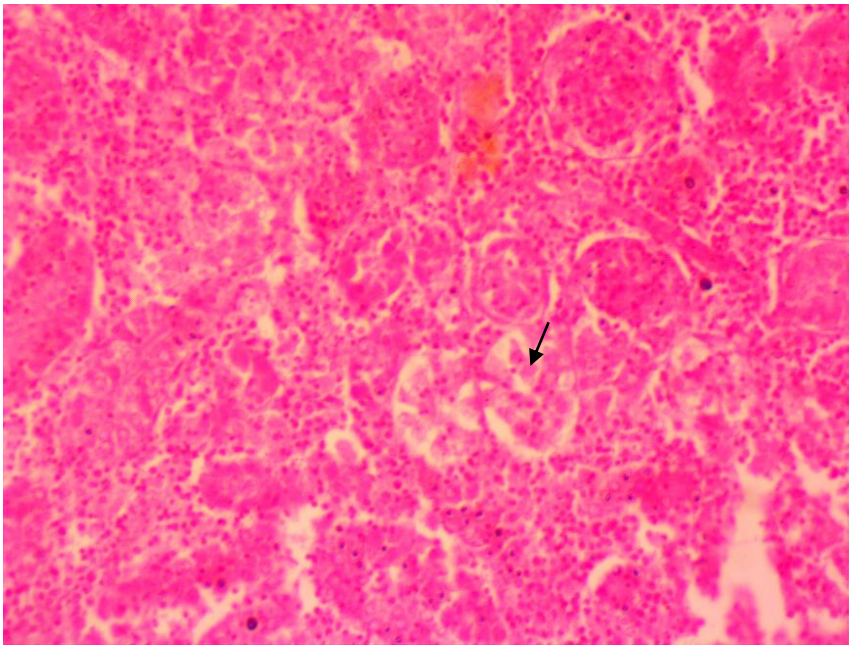

Plate 6.12: Focus of tubular epithelia degeneration (arrow) in the kidney from fish exposed to Cd+Pb for 6 hours. HE x100

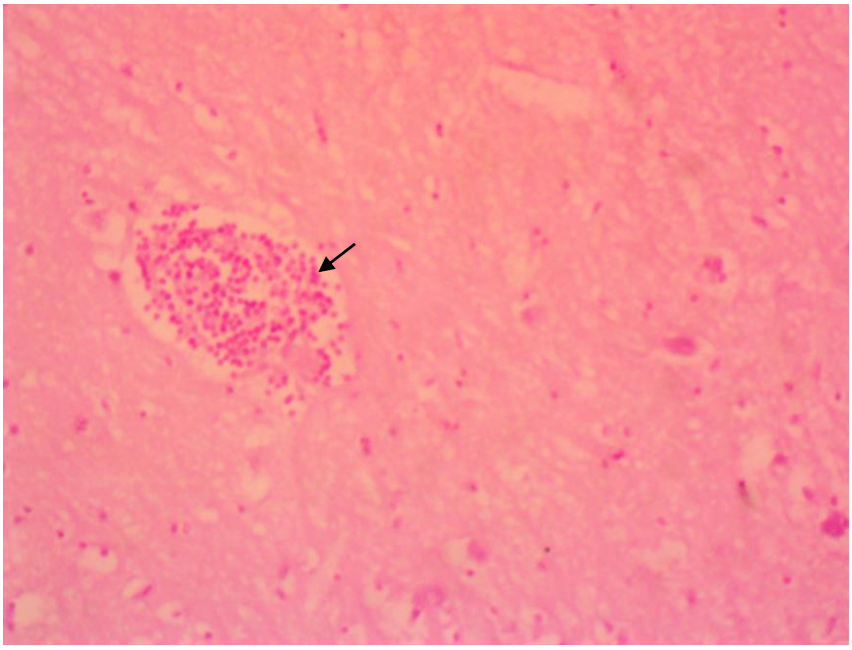

Plate 6.13: Vasculitis (arrow head) in the brain from fish exposed to Cd + Pb for 6 hours HE x100

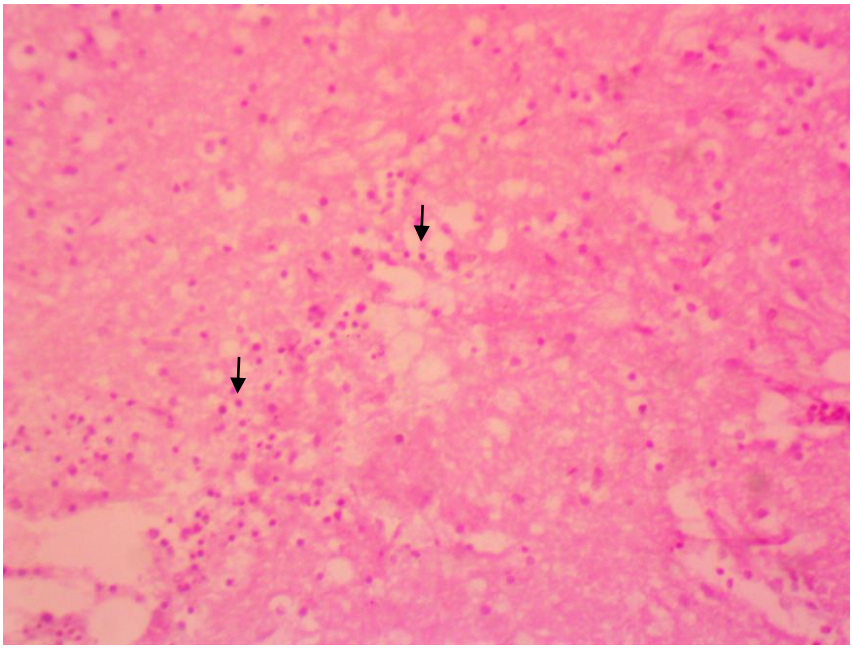

Plate 6.14: Diffuse gliosis (arrows) in the brain from fish exposed to Cd for 6 hours. HE x100

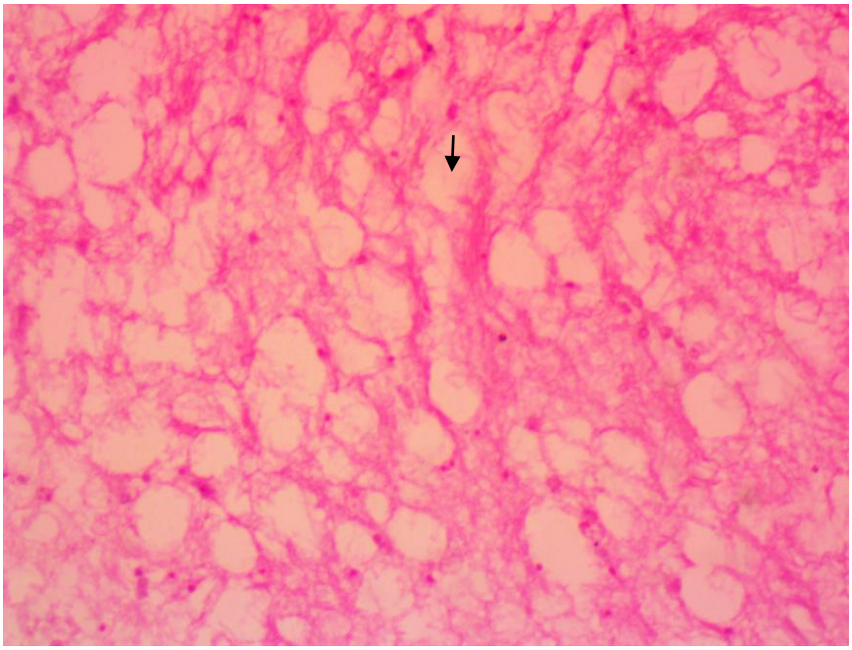

Plate 6.15: Diffuse irregular vacuolation (demyelination) in the brain from fish exposed to Pb for 6 hours. HE x400

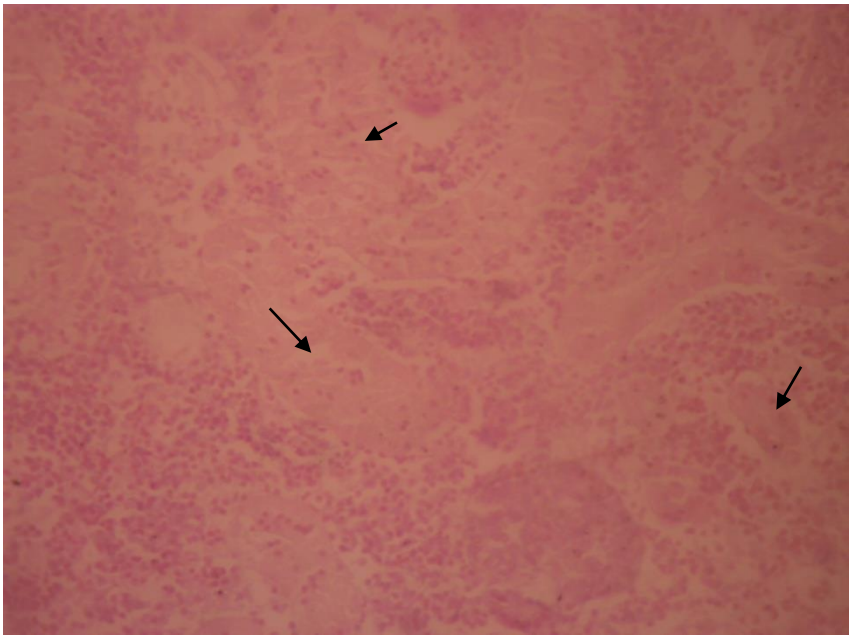

Plate 6.16: Diffuse tubular coagulation necrosis (arrows) with disrupted basement membrane and mild haemopoietic tissue hypoplasia in the kidney from fish exposed to Cd at 120hr post exposure. HE x100

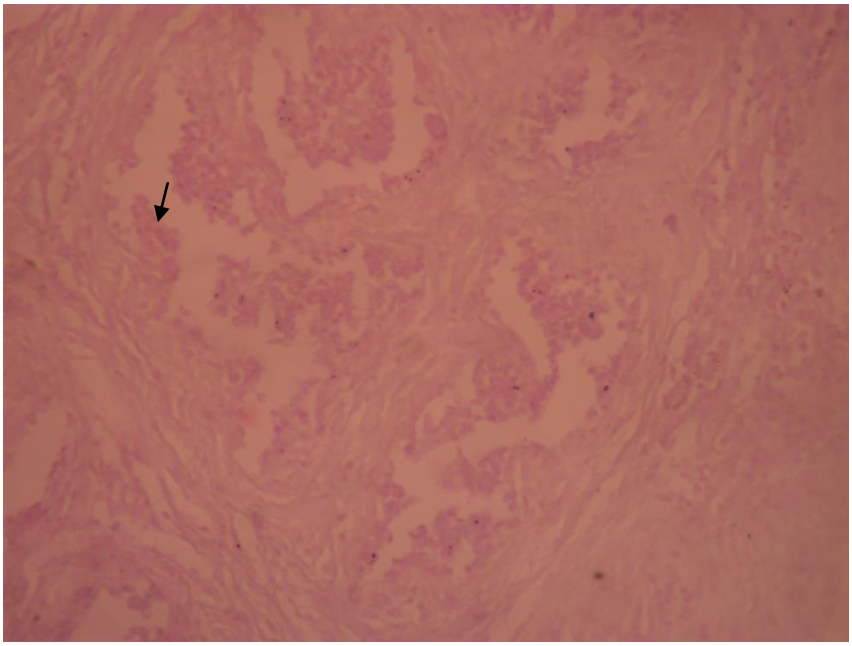

Plate 6.17: Severe villi atrophy (arrow) and denudation of enterocytes in the pyloric caeca from fish exposed to Cd+Pb at 120hr post exposure. HE x400

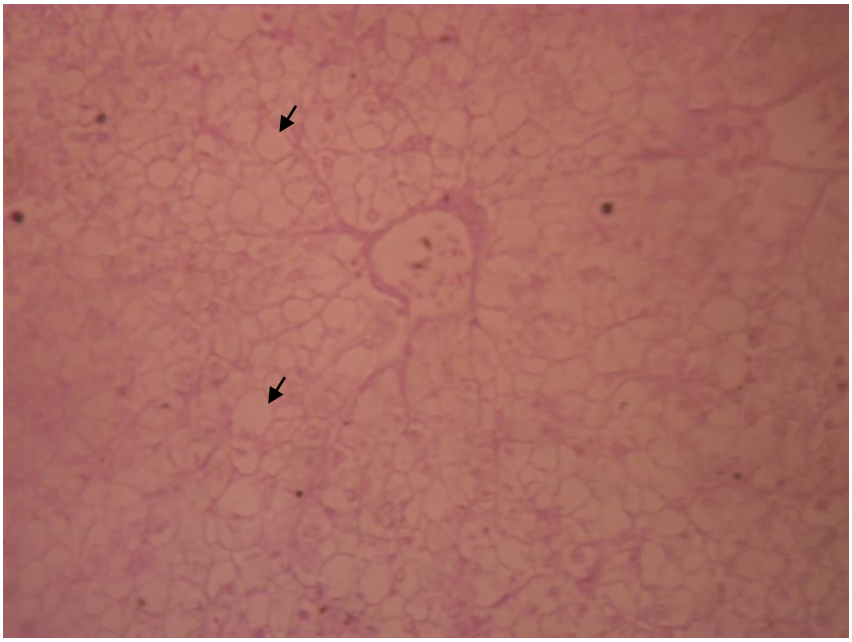

Plate 6.18: Severe centrilobular degeneration (swelling) of hepatocytes (arrows) in the liver from fish exposed to Pb at 120hr post exposure. HE x400

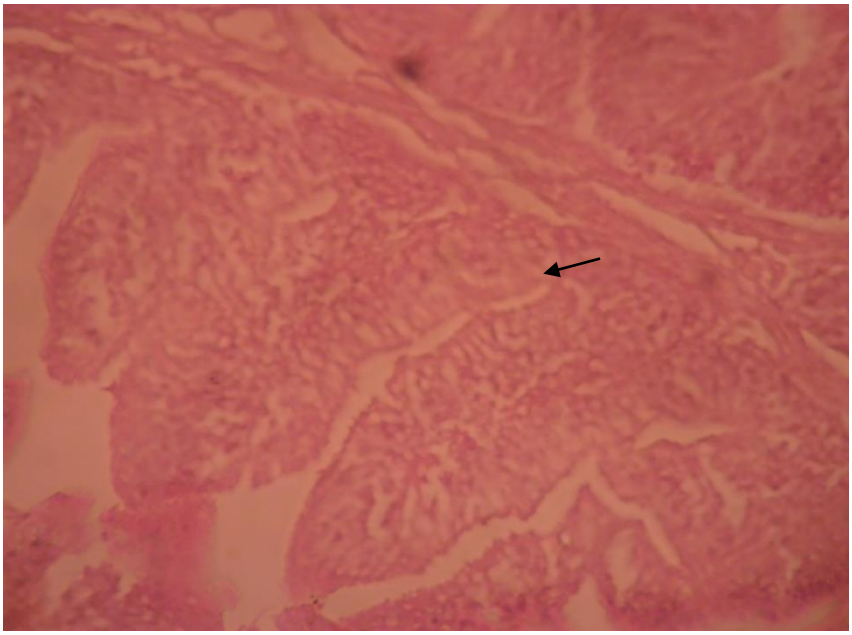

Plate 6.19: Marked cryptal hyperplasia (arrow) of the intestine from fish exposed Pb + 0.87mg/L garlic at 120hr post exposure. HE x400

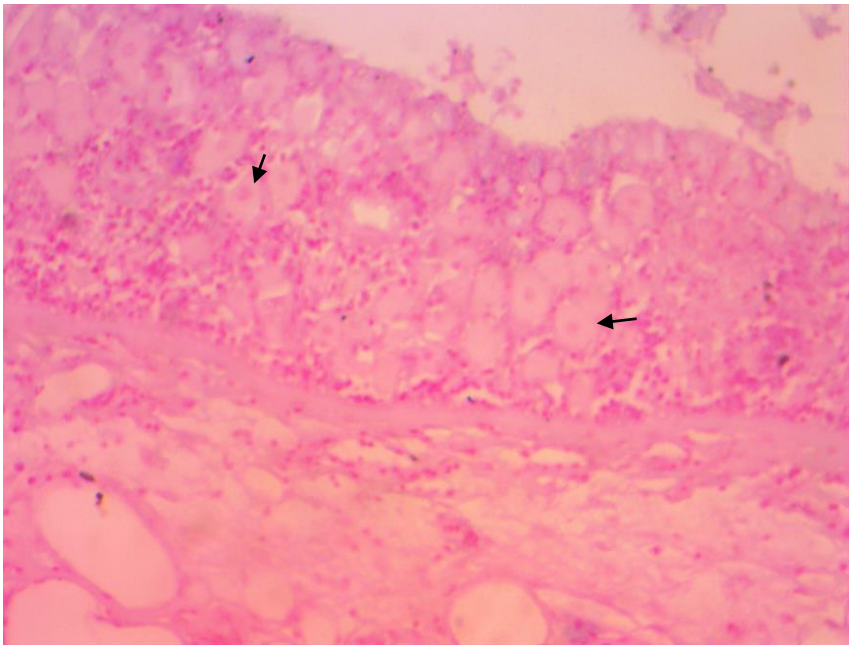

Plate 6.20: Ectodermal and alarm cells (arrows) in fish exposed to garlic (0.87mg/L) for 120hr. HE x400

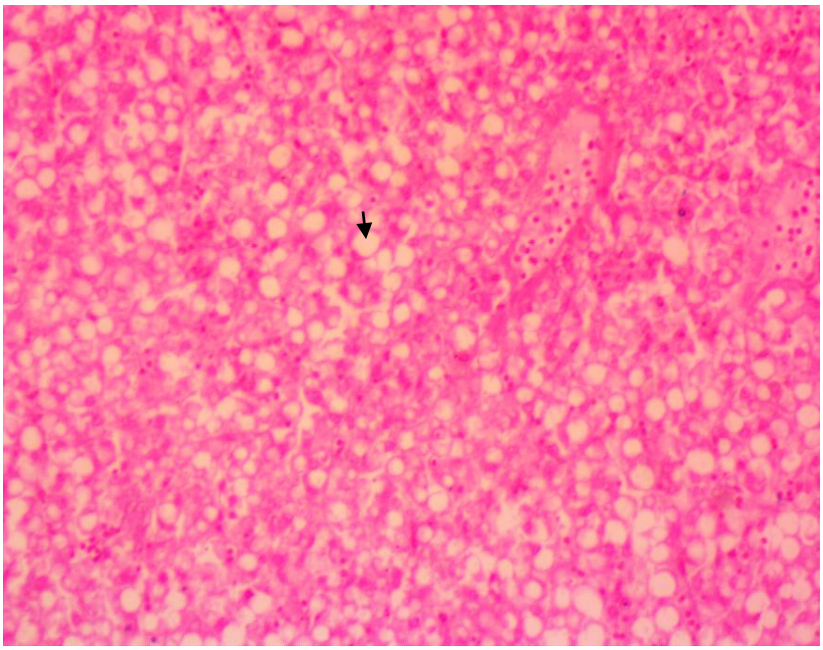

Plate 6.21: Hepatocytes with fat accumulation in cytoplasm (arrow) of the liver in control fish at 144hr of the experiment. HE x100

At 6 hours of cadmium exposure there were in the liver hepatocellular swelling and degeneration, chromatolysis in the brain. At 6 hour of lead exposure there were moderate hyperplasia of secondary lamellae of the gills, moderate degeneration of hepatocytes and alteration of sinusoids in the liver, moderate vascular endothelial hyperplasia and focal neuronal neurosis in the brain. There is no visible lesion in the kidney. At 6 hour of cadmium + lead exposure there were goblet hyperplasia, moderate neuronal necrosis in the brain, moderate hepatocellular swelling in the liver. There was no visible lesion in intestine. At 6 hours in unexposed groups (garlic only and control) there were no visible lesion in skin, gills, intestine, liver, kidney, pancreas, spleen and brain no visible lesion in intestine and pancreas.

At 24hr post exposure to lead, there were severe hyperplasia of vascular endothelium and lamellae of the gills, moderate hypertrophy of alarm cells and severe hyperplasia of goblet cells, marked diffused degeneration and focal necrosis of hepatocytes, including vascular congestion of severe neuronal necrosis of the neuropil of the brain. There was no visible lesion in the stomach, intestine and pancreas.

At 24hr post exposure to cadmium + lead + garlic exposure had moderate congestion of vascular spaces and hyperplasia of pillar cells in the gills, severe goblet cells hyperplasia and hyperkeratosis of the skin, severe diffused hepatocellular swelling (degeneration) with hyperplasia of sinusoidal capillaries of endothelium in the liver, moderate vascular congestion in the neutropil and moderate chromatolysis (degeneration of neurons) in the brain, and severe diffused hyperplasia of vascular endothelium of the spleen. There was no visible lesion in the heart.

At 24hr post exposure to cadmium + lead exposure were moderate diffused hepatocellular swelling of the liver, moderate neuronal necrosis, moderate pillar cell hypertrophy of the gills, moderate goblet cells hyperplasia and moderate renal tubular and diffused epithelial necrosis. No visible lesion in pancreas. At day 2 in general, garlic only treated groups were no visible lesion in the gills, liver, kidney, intestine, pancreas, spleen and brain. In the skin were mild goblet cell hyperplasia. At day 2 in general, control groups had no visible lesion in skin, intestine, liver, kidney, brain pancreas and spleen.

At 72hr post exposure to cadmium + 2 days 12 hours' consecutive garlic treatments at day 1 and 2, were mild hyperplasia of secondary lamella in the gills, mild goblet hyperplasia in the skin, mild hepatocellular and swelling in the liver.

At 72hr post exposure to cadmium were moderate hyperplasia of pillar cells and mild hyperplasia of secondary lamellae in the gills, moderate goblet hyperplasia, diffused

centrilobular hepatocellular swelling with coagulative necrosis and kuffer cell hyperplasia in the liver, patchy or multi focal tubular epithelial and coagulative necrosis of posterior kidney and neuronal necrosis (angulated and red nuclei) diffused glyosis and vasculities in the brain. There is no visible lesion in intestine and heart muscle.

In exposure to lead + garlic were hypertrophy of alarm cells in the skin, mild goblet hyperplasia in the intestine, mild hepatocellular degeneration in the liver. There is no visible lesion in the heart muscle, gills and brain.

At 72hr post exposure to lead were moderate hypertrophy of alarm cells and goblet cells hyperplasia in the skin, moderate lamellar hyperplasia and congestion of vascular species in the gills, mild vascular congestion, focal neuronal necrosis and neurophagia (glia cells eating up dead neurons) in the brain, severe diffused hepatocellular degeneration, in tubular necrosis and vascular endothelia, hyperplasia in the kidney and severe goblet hyperplasia and sloughing of villi. There is no visible lesion in pancreas.

At 72hr post exposure to cadmium + lead + 2 days consecutive 12-hour garlic treatment were goblet hyperplasia, and epidermal cell degeneration in the skin, lamellar hyperplasia in the gills, sloughed of enterocytes and matting of villi in the intestine, mild hepatocellular necrosis and atrophy.

At 72hr post exposure to cadmium + lead was loss of enterocytes, erosion and blunting of villi in the intestine, severe lamellar, hyperplasia in gills, thinning of epidemics and exocytosis including hyperkeratosis in the skin, severe hepatocellular necrosis and degeneration in the liver, moderate necrosis in the brain, extensive renal tubular epithelia degeneration and necrosis in the kidney.

In general, treatment with garlic only had at day 3 with 2 days' consecutive garlic treatments no visible lesion in gills, liver, intestine, kidney, brain pancreas and brain. In the skin was mild goblet hyperplasia. In control groups there was no visible gill, liver, intestine, brain and skin.

At 120hr post exposure to cadmium with no further garlic treatment after day 2 had moderate secondary lamellar hyperplasia with cellular infiltrate arch in the gill, moderate epidermal hyperplasia and goblet hyperplasia in skin. At 120hr post exposure to cadmium had severe demyelination of nerve fibre and neuropil in the brain, moderate hepatocellular necrosis in the liver, moderate tubular necrosis in the kidney, secondary lamellar and pillar cells hyperplasia and severe epidermal and goblet hyperplasia in the skin. At 120hr post exposure to lead with no further garlic treatment after after day 2 had mild hepatocellular swelling and

degeneration in the liver, mild alarm cells hypertrophy in the skin, capillary and pillar cell hypertrophy in the gills. There is no visible lesion in spleen and intestine. At 120hr post exposure to lead had moderate focal neuronal necrosis and mild vascular congestion in the brain, severe diffused hepatocellular degeneration in the liver, moderate lamellar and pillar cell hyperplasia in the gills, moderate hypertrophy of alarm cells and goblet cells in the skin. There is no visible lesion intestine. At 120hr post exposure to cadmium + lead with no further garlic treatment after day 2 had mild hepatocellular swelling in the liver, and mild goblet hyperplasia. There is no visible lesion in intestine and pancreas. At 120hr post exposure, exposure to cadmium + lead had moderate hepatocellular degeneration in the liver, moderate goblet hyperplasia in the intestine. There is no visible lesion in the kidney. At 120hr, garlic only group and control group had no visible lesion in gill, intestine, liver, kidney, brain and pancreas.

6.4.16 Discussion on gross and histopathology

Histopathology observed were in agreement with several authors including Jalaludeen *et al*., (2012); Rani and Ramamurthi, (1989) and Smith, (2000), In exposed fish treated with garlic, the severity of pathology was less which is in agreement with the findings of Sajitha, *et al*., (2010) that lead toxins actually damaged the liver, but there was improvement of condition by the treatment with garlic.

CHAPTER SEVEN

SUMMARY, CONCLUSION AND RECOMMENDATION

7.1: Summary

This study was carried out using *Clarias gariepinus* which is of commercial importance and widely accepted in Nigeria (Olaifa *et al*., 2004). They are widely cultured owing to their high market price, fast growth rate and ability to withstand adverse pond conditions especially low oxygen content (Adewolu and Adeoti, 2010).

The first part of this study was necessitated due to a dearth of information on the use of raw garlic homogenate in fish culture. Yet garlic has several beneficial effects including antioxidant, antihypertensive, and antimicrobial properties (Sivam, 2001). Garlic is listed as "generally recognized as safe" (GRAS) by the U.S., however, cooking and processing methods of garlic (*Allium sativum*) cloves even in the oven of a gas chromatograph, at moderate temperatures, lower raw garlic (*Allium sativum*) benefecial properties and leading to rapid decomposition of unstable thiosulphinates. Raw garlic homogenate is easy in its preparation and would add knowledge to the increasing use of alternative medicine in aquaculture which is at the rudimentary stage in Nigeria. Based on the above submission, this first part of the study investigated the clinical/pathological changes and determine metal/ions accumulation in the tissues and included water quality assessment in African catfish *Clarias gariepinus* exposed to: 8gm/l raw garlic (*Allium sativum*); near environmental standard (sublethal) concentration of cadmium (0.03mg/l) and lead (0.3mg/l) seperately for 90 days in a static culture. This study showed that 8.0mg/l raw garlic caused reversible detrimental stress, provided a stable environment, increased and prolonged the coping ability for stress and improved wellness of both the female and males *Clarias gariepinus*. 0.03mg/lCd and 0.3mg/lPb provided unstable environment with biphasic coping ability for the female and male *Clarias gariepinus* at day 7, 30, 60 and 90. These adjustments provided by 0.03mg/lCd and 0.3mg/lPb may negatively affect the growth and feeding habit including immunity of *Clarias gariepinus*. With regards to gross/histopathological conditions, there were no visible lesions observed at day 7, 30 and 60 in fish on 8.0mg/l raw garlic and the control group. 8.0mg/l raw garlic remarkably improved the female. 0.03mg/lCd and 0.3mg/lPb caused remarkably abnormal changes which increased with duration of exposures. The female responded to stress earlier than male. With regards to water quality and accumulation of metals/ions in The liver liver levels on day 90 of Lead decreased in fish on 8.0mg/l raw garlic and

increased in fish on 0.03mg/lCd, 0.3mg/lPb and in control whereas and an opposite effect on pH was obtained. Females significantly accumulated more lead than males in all groups.

Study two determined the LD_{50}-96 hours and LC_{100}-96 hours' cadmium and lead nitrate in apparently healthy *Clarias gariepinus* female. The significant differences in responses of female and male *Clarias gariepinus* in the same macroenviroment observed in study one also surports this study. It was found that in the control groups mortality was zero during the test. The 50% lethal concentrations (LD50-96h) of $Cd(NO_3)_2$ and Pb $(NO_3)_2$ were 31.0mg/l, and 76.8mg/l respectively. Also LC_{100}-96 hours of $Cd(NO_3)_2$ and Pb $(NO_3)_2$ was 52 and 102mg/l respectively. The Mean and standard error of mean of lengths and weights for acute cadmium (Cd) testing was approximately 13.67 ± 0.06 and 21.81±0.30g respectively. The Mean and standard error of mean of lengths and weights for acute lead (Pb) testing was approximately 13.41 ± 0.06cm and 20.20±0.26g respectively.

Study three evaluated the least (safest) concentration of garlic homogenate (*Allium sativum*) for apparently healthy female as a baseline or in polluted environment guide since there is a dearth of information on the appropriate concentration of raw garlic for fish. The significant toxicity of 8.0g raw garlic/l on day 2 and 3 of exposure observed in study one also surports this study. It was found that the least toxicant (safest) concentration of raw garlic in apparently healthy fish with mean±SEM body weight and length of 25.87±0.65g and 15.52±0.26cm respectively was 5mg/l raw garlic.

Study four investigated the effects of raw garlic homogenate (*Allium sativum*) in female exposed to 64.0mg/l (LC_{100}-96 hours') cadmium treated with 0.65mg raw garlic/L (Cd/Cd + G1) and 126mg/L (LC_{100}-96 hours') lead treated 0.87mg/L raw garlic (Pb/Pb + G2) singularly and jointly treated with 0.65mg/L raw garlic (Cd + Pb/Cd + Pb + G1) in apparently healthy *Clarias gariepinus*. The obtained results from study one, two, three and the fact that pollution of the aquatic environment by inorganic and organic chemicals is a major factor posing serious threat to the survival of aquatic organisms including fish (Simir and Ibrahim, 2008) including that less attention has been directed to the use of organic/natural biocompatible, biodegradable antioxidants to reduce toxic metals in fish culture also surport this study. At 6hr, Cd + Pb caused mortality including copious mucus secretion and vertical positioning. Marked gross and histopathological changes in all tissues examined were cuased by Cd only and Pb only. Also at 6h of exposure to all metal had similar effects of reducing on blood parameters except creatinine level in fish

on Pb only exposure that showed no significant difference from control. These may be due to suppression of synthesis of these parameters and/or direct damage to the organs responsible for their synthesis. With regard to water quality, all exposure to metal had similar effect of decreasing pH, and increasing chloride, total hardness, total dissolved solids, calcium and DO. Magnesium increased in Cd + Pb and Cd only while Pb only had no effect. Alkalinity decreased in Cd only whereas it was not affected by Cd + Pb and Pb only; ammonia increased in Cd + Pb whereas was not affected by Cd only and Pb only. Potassium increased in Cd + Pb and in Pb only while Cd only did not affect it. Metal/ion levels in water showed similar trend though with varied degree of increase. Manganese was increased by Cd + Pb and Cd only whereas Pb only decreased manganese in the water. Metals/ion levels in tissues showed accumulation that was dependent on the tissues type and the metal(s) involved. With exposure to Cd only, the level of lead increased in the gills and liver but decreased in the blood and intestine. In Pb only exposure, the level of Pb increased in the gill, blood and liver but decreased in the intestine. With exposure to Cd + Pb, the level of lead increased in the gill, blood and intestine but decreased in the liver. High or low levels of metals/ions in the gill, blood, liver or intestine may not be diagnostic of low or high pathology. Liver oxidative markers showed variation which were dependent on metal(s) exposure. Mortality and genotoxicity in Cd + Pb may have been caused by the higher levels of total dissolved solids and cadmium, in water; higher levels of cadmium and Lead in the gill; lower ALT and GSH activity and moderate necrosis of the brain. Increase in GPX and SOD are natural mechanism to combat acute oxidative stress. At acute lethal level of exposure to Cadmium and Lead, GSH activity is decreases. This may indicate an overwhelmed antioxidant mechanism. At sublethal level GSH activity is proportionally to the of oxidative stress level. This may indicate a responcisive reaction to combact the effect of prooxidants. It appears that SOD is more easily induced than GPX and a higher level of SOD may be an indication lower level of oxidative stress. The severity of gross, histopathological and morphological lesions varied and decreasing with garlic bath at 120 hours post exposure. At 120 hours post exposure (day 6 of the study) it appears that the effect of garlic was influenced by the metal(s) type and the parameters measured.

7.2: Conclusion

The exposure to 8.0g garlic/l caused reversible toxicity for 48 hours which reversed thereafter. 0.03mgCd/l 0.3mgPb/l in static culture was deleterious. 0.87mg raw garlic/l reversed the toxicity of 126mgLead/l, 0.65mg raw garlic/l reversed the toxicity of 64 mg

Cadmium/l and 64 mg Cadmium/l + 126 mg Lead/l suggesting its efficacy in the detoxification of Cadmium and Lead toxicity in female *Clarias gariepinus*. Cadmium involved toxicity was more tractible to treat than lead. The concentrations of 0.65mg raw garlic/l and 0.87mg raw garlic/l improved wellness although 0.87mg raw garlic/l had more effect due to its higher concentration in apparently healthy female *Clarias gariepinus*.

7.3: Recommendation

It is recommended that fish farmers should use at least 0.65mg/l of raw garlic between 18.00 and 19.00 hour for a duration of twelve hours for two consecutive days after six hours' water renewal to reduce the lethal toxicity effects of Cadmium and Lead on farmed female *Clarias gariepinus*.

Further study on dosage regime of the optimum concentration of raw garlic in the female to enable its use as prophylasis and the effects of raw garlic on metal toxicity on other commercially viable fishes should be investigated.

REFERENCES

Abba, Y. A. 2011. Fisheries society of Nigeria (FISON) president memo to National Fisheries Development Committee Royalton Hotel Area 2, Section, Garki Abuja 8th March.

Abbas, H. H. 1994. Effect of lead on some physiological and biochemical aspects of Nile tilapia (O. niloticus). M.Sc. Thesis, faculty of Science, Cairo University, Egypt.

Abd-Elallatif, A. and Ebraheem, K. 1996. Studies on the effects of *Hibiscus subdariff*, *Allium sativum* and *Negella sativa* on some bacterial isolates of chickens. Fac. Vet. Med. Assut. Univ. Egypt, 17, 245 - 51.

Adams, S. A., Shepard, K. L., Greeley Jr, M. S., Jimenez, B. D., Ryon, M. G., Shugart, L. R. and Mc Carthy, J. F. 1989. The use of Bioindicators for Assessing the effects of pollutant stress on fish. *Marine Environmental Research* 28: 459 - 464.

Adams, S.M. 1993. A qualitative health assessment index for rapid evaluation of fish condition in the baseline. *Transaction of American fisheries society* 122: 63-73.

Adedapo, A. A., Mogbojuri, O. M. and Emikpe B. O. 2009. Safety evaluation of aqueous extract of the leaves of *Moriga oleifera* in rats. *Journal of Medicinal Plants Research* 3 (8): 586 – 591

Adedeji, O. B. and Adegbile, A. F. 2011. Comparative Haematological Parameters of the Bagrid Catfish (Chrysichthys Nigrodigitatus) and the African Catfish (*Clarias gariepinus*) from Asejire Dam in Southwestern Nigeria. *Journal of Applied Sciences Research* 7(7): 1042-1046.

Adedeji, O. B., Adedeji, O. A., Adeyemo, O. K. and Agbede, S. A. 2009. Acute effects of Diazinon on Blood parameters. In: The African Catfish (*Clarias gariepinus*). *The Internet Journal of Hematology 5*: *No. 2.*

Adeogun, A. O., Onocha, P. A. and Oladoyinbo, S. O. 2012. Variation in plasma biochemical parameters of *Clarias gariepinus* exposed to sub-lethal concentration of the methanolic extracts of *Raphia Hooker. Trop Vet* 30 (1): 17 – 31.

Adewolu, M. A. and Adeoti, A. J. 2010. Effect of mixed feeding schedules with varying dietary crude protein levels on the growth and feed utilization of *Clarias gariepinus* (Burchell, 1822) fingerlings. *J. Fish. Aquat. Sci*., 5: 304-310.

Adeyemo, O. K. 2005. Bioconcentration and Toxicological effects of lead on African catfish (*Clarias gariepinus* Buchell 1822). A thesis in the department of veterinary public health and preventive medicine, Doctor of philosophy, University of Ibadan.

Adeyemo, O. K. 2007. Haematological Profile of *Clarias gariepinus* (Burchell, 1822) Exposed to Lead. *Turk. J. Fish. Aquat Sci.* 7: 163-169

Adeyemo, O. K., Ajani F., Adedeji. O. B. and Ajiboye O. O. 2008. Acute Toxicity and Blood Profile of Adult *Clarias gariepinus* exposed to Lead Nitrate. *The Internet Journal of Haematology* 4: No.2.

Adeyeye, I. E., Akinyugba, N. J., Fesobi, M. E. and Tenade, O. V. 1996. Determination of some metals in *Clarias gariepinus* (cuvier and vallenciennes) *Cyprinus carpio (L)* fishes in polyculture fresh water pond and their environment. *Environ Int* 10: 90-98

Adham, K. K., Abu-Shabana, A., Abdel-maguid, N. and Moneim, A. 1997. Environmental stress in Lake Marijut and Physiological response of *Tilapia zilli* Gerv. *Journal of environmental science and health* part A 32 (9 & 10): 2585-2598.

Adhikari, S. and Sarkar, B. 2004. Effects of cypermehrin and carbofuran on certain haematological parameters and prediction of their recovery in fresh water teleost, *Labeo rohita* (Ham). *Ecotoxicol. Environ Safety* 58: 220-226.

Adonaylo, V. N and Otieza, P. I. 1999. Pb^{2+} promotes lipid peroxidation and alteration in membrane physical properties. *Toxicology*. 132:19–32.

Agawal, K.C.1998. Therapeutic Actions of Garlic Constituents. *Medical Research Reviews* 16 (1): 111-124.

Ahmet, T., Gonca, A., Muhammed, A., Ertan, O., Saltuk, B., Ceyhun, A. U., Harun, A., Fikret, Ç. and Yavuz, S. S. 2013. Effects of Humic Acid on Liver and Kidney Toxicity Induced by Cadmium in Brown Trout (*Salmo trutta fario*, L) *Turk. J. Fish. Aquat. Sci.* 13: 621- 627

Akinyemi, O. 1998. Sustainable Management of Nigeria's Fisheries in the 21st century. Faculty lecture series No. 7, Faculty of Agric and Forestry, University of Ibadan, 27p.

Akpokodje, J. O., Mpama, I. C. and Ladokun A. O. 2005. A brief protocol for Histological Slides Preparation. Dept Veterinary pathology, University of Ibadan. pp8.

Al-Attar, A. M. 2005. Biochemical effects of short-term cadmium exposure in the freshwater fish, *Orechromis niloticus. J Biol Sci*. 5(3):260-5.

Alderdice, D. 1967. The detection and measurement of water pollution-biological assays. Canadian Fisheries Report (9): 33-9.

Alejandra, C., Ana, C. S., Marta, C. and Mar, V. (2010) A Comprehensive Survey of Garlic Functionality: In Garlic Consumption and Health Editors: M. Pacurar and G Krejei pp1-60 ISBN: 978-1-60741-642-5 Nova Sciuence Publishers, Inc.

Ali, A. N. 2007. Ecological study on some water characteristics used in fish farms and their relation to fish productivity. Ph. D. Thesis. Al-Azhar Unive. Fac Sci. Chemistry dept. Egypt.

Alinnor, I. J. 2005. Assessment of elemental contaminants in water and fish samples from Aba River. *Environ Monit Assess*. 102:15–25.

Alkahem, H. F., Al.-Akel, S. A. and Ahmed Z. 1998. Carbohydrate metabolism and ethological responses of the cichlid fish, *Oreochromis niloticus* exposed to trichlorfon. *Geobios* 25. 10 – 16.

Alloway, B. J. and Ayres, D. C 1993. Chemical principles of Environmental Pollution. Blackie Academic U.K., Pp 140-149.

Al-Nagaawy, A. M. 2008. Accumulation and elimination of copper and lead from *O niloticus* fingerlings and consequent influence on their tissue residue and some biochemical parameters. 8th international symposium on tilapia.

Amin, K. A. and Hashem. S. K. 2012. Catfish (*Clarias gariepinus*): antioxidant defense and role of alpha – tocopherol. *BMC Veterinary Research* 8:45 doi:10.1186/1746-6148. 8 - 45.

Anders, G. 2008. Environmetnal Toxicology and Ecotoxicology. http://www.uib.no/mbi/research/goksogr.html retrieved on 12/1/2008.

Anderson, D. P. 1990. Immunological indicators, effects of environmental stress on immune protection and disease outbreaks. In Adams S. M. (ed), Biological indicators of stress in fish. *American fisheries symposium* Bethesda Maryland 8: 38-50.

Anderson, R. O and Gutreuter S. J. 1983. Length, weight, and associated structural indices. In: Niekseb LA, Johnson DL, editors. Fisheries techniques. Bethesda, MD: *American Fisheries Society.* 283–300.

Annune, P. A., and Ahuma H. 1998. Haematological change in mudfish *Clarias gariepinus* exposed to sublethal concentrations of copper and lead. *J. Aquat. Sci.* 13: 33 – 36.

APHA, American Public Health Association 1995. Standard methods for the examination of water and waste water 23rd Ed; 1268pp.

Arowolo, R. O. A. 2008. Toxic wastes and pollution: A paper delivered at veterinary council of Nigeria (VCN) professional continuing education seminar on 26th November 2008 at Zodiac Hotels Enugu, Nigeria.

Atanda, A. N. 2007. Freshwater fish seed resources in Nigeria. Bondad-REanteso, M.G. (Ed.), Assessment of freshwater fish seed resources for sustainable aquaculture. FAO Fisheries Technical Paper No. 501. Rome, FAO 628P: 361-380.

ATSEMR, 1999. Toxicol. Profile of Cadmium. Agency for Toxic Substances and Drug Registrar, Atlanta, GA. US Deptt. of Health and Human Services.

Aure, J., Ervik, A., Johannessen, P. and Ordemann, T. 1988. Recipient Pavirkning fra fiskeoppdrett i Saltvann (The Environmental Effects of sea water fish farms). Fisken Og Havet I, *lnst. Mar. Res*, Norway, 94pp (In Norwegian, abstract and legends to figure in English).

Badawi, H. K. 1971. Eletrophoretic studies of serum protein of four tilapia species (pisces). *Journal of marine biology* 8: 21.

Bais, U. E and Lokhande, M.V. 2012. Effect of Cadmium Chloride on Histopathological Changes in the Freshwater Fish *Ophiocephalus striatus* (*Channa*). *International Journal of Zoological Research*, 8: 23-32.

Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular references to changes in circulating corticosteroids. *Integ Comp Biol* 42: 517-525.

Basha, P. S and Rani A. U. 2003. Cadmium induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). *Ecotoxicol. and Environ. Safety.* 56 (2): 218-221.

Beutler, E., Durgun, O. and Kelly, B. M. 1963. Improved method for the determination of blood glutathione *J, Lab, Clin, Med.* 51: 882 – 8.

Blaxhall, P. and Daisley, K. W. 1973. Routine Haematological methods of use with fish blood *J. Fish Biol* 5: 771-781.

Blechinger, S. R. and Kusch R. C. 2007. Brief embryonic cadmium exposure induces a stress response and cell death in the developing olfactory system followed by long-term olfactory deficits in juvenile zebrafish. *Toxicology and Applied Pharmacology* 224 (1): 72-80.

Blomhoff, R. 2005. Dietary antioxidants and cardiovascular disease. *Curr Opin Lipidol* 16: 47–54.

Bly, J. E., Quiniou, S. M. and Clem L. W. 1997. Environmental Effect of fish Immune mechanisms. *Fish vaccinology development in Biological standardization* 90: 33-43.

Bongiorno, P., Patrick, B., Fratellone, M. and LoGiudice, P. 2008. Potential Health Benefits of Garlic (*Allium Sativum*): A Narrative Review. *Journal of Complementary and Integrative Medicine* 5 (1): Art. 1.

Borek, C. 2001. Antioxidant health effects of aged garlic extract *Nutr.,* 131: 1010 S – 1015 S

Bothaina, M. K., Imam A., Mekkawy A., Ahmed S., Harabawy, A. and Abdel S. 2012. Effect of Lead Nitrate on the Liver of the Cichlid Fish (*Oreochromis niloticus*): A Light Microscope Study. *Pakistan Journal of Biological Sciences* 15: 854-862.

Boyd, C. E. 1990. Water Quality in ponds for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama.

Boyd, C. E. 2002. Standardize terminology for low-salinity shrimp culture. *Global Aquaculture Advocate* 5 (5): 58-59.

Boyd, C. E. and Tucker, C. S. 1998. Pond Aquaculture Water Quality Management. Kluwer Academic Publishers, Boston, Massachusetts.

Brucka-Jastrzębska, E. 2010. The Effect of Aquatic Cadmium and Lead Pollution on Lipid Peroxidation and Superoxide Dismutase Activity in Freshwater Fish. *Polish J. of Environ. Stud.* Vol. 19, No. 6 1139-1150.

Bruton, M. N. 1996. Alternative life-history strategies of catfishes. *Aquatic Living Resources* 9: 35-41.

Bryan, G. W. 1976. Some effect of Heavy Metal tolerance in aquatic organism, In: Lockwood A.P.M(ed). Effects of pollutants on aquatic organisms. Cambridge University Press Cambridge England, pp7

Bryan, G.W. 1971. The effect of heavy metals (other than mercury) on marine and estuarine organisms. *Progr R Soc London Ser B* 177: 389-410.

Buckley, J. A., Whitmore, C. M. and Matsuda, R. I. 1976. Changes in blood chemistry and blood cell morphology in coho salmon, *Oncorhynchus kisutch* following exposure to sublethal levels of total residual chlorine in municipal wastewater. *J. Fisheries Res. Board, Canada.* 33: 776 - 782.

Burger, J. and Gochfeld, M. 2005. Heavy metals in commercial fish in New Jersey. *Environtal Research* 99 (3), 403-412.

Burnison, B. K. T., Meinelt, R. C., Playle, M., Pietrock, A. W. and Steinberg C. E. W. 2006. Cadmium accumulation in zebrafish (*Danio rerio*) embryos is modulated by dissolved organic matter. *Aquat. Toxicol.,* 79: 180–191.

Bury, N. R., Walker, P. A and Glover, C. N 2003. Nutritive metal uptake in teleost fish. *Journal of Experimental Biology*, 206: 11 – 23.

Carmichael, G. J., Wedemeyer, G. A., McCraren, J. P. and Millard, J. L. (1983). Physiological effects of handling and hauling stress on smallmouth bass. *Prog. Fish-Cult*. 45: 110–113.

Carneiro, A. P. M., Pascoal, L. A. F., Wantanabe, P. H., Santos, I. B., Lopes, J. M. and Arruda, J. C. B. 2009. Babassa meal in finishing broiler fodder: Performance, carcass yield and economical evaluation. *Ciencia Anim. Bras*., 10 (1): 40 – 47.

Carneiro, P. C. F. and Urbinati, E. C., 2002. Transport stress in Matrinxã, *Brycon cephalus* (Teleostei: Characidae), at different densities. *Aqua Int* 10: 221–229.

Chapman, P. M., Allen H. E. and Z'Graggen M. N. 1996. Evaluation of bioaccumulation factors in regulating metals. *Environ Sci Technol* 30 (10): 448A- 452A.

Corzo-Martinex, M., Corzo, N. and Villamiel, M. 2007. Biological Properties of Onions and Garlic. *Trends in Food Science and Technology* 18: 609-625.

Couture, P. and Rajender, P. K. 2003. Impairment of metabolic capacities in copper and cadmium contaminated wild yellow perch (Perca flavescens). *Aquatic Toxicology* 64 (1): 107-120.

Cunjak, R. A. 1988. Physiological consequences of overwintering in stream: the cost of acculimitisation? *Can J. Fish Aquat. Sci* 45: 443-452.

Cyriac, P. J., Anthony, A. and Nambison P. N. K. 1989. Haemoglobin and haematocrit values in the fish *Oreochromis mossambicus* (Peters) after short term exposure to copper and mercury. *Bulletin of Environmental Contamination and Toxicology.* 43: 315 - 320.

Dabas, A., Nagpure, N. S., Kumar, R., Kushwaha, B., Kumar, P. and Lakra, W.S. 2012. Assessment of tissue-specific effect of cadmium on antioxidant defense system and lipid peroxidation in freshwater murrel, *Channa punctatus*. *Fish Physiology and Biochemistry* 38: 469-82. doi: 10.1007/s10695-011-9527-7.

Dabas, A., Nagpure, N. S., Kumar, R., Kushwaha, B., Kumar, P. and Lakra, W.S. 2012. Assessment of tissue-specific effect of cadmium on antioxidant defense system and lipid peroxidation in freshwater murrel, *Channa punctatus*. *Fish Physiology and Biochemistry* 38: 469-82. doi: 10.1007/s10695-011-9527-7.

Dangre, A. J., Manning, S. and Brouwer A. 2010. Effects of cadmium on hypoxia-induced expression of hemoglobin and erythropoietin in larval sheepshead minnow, *Cyprinodon variegates. Aquatic Toxicol* 99 (2): 168-175.

Das, K. K. and Banerjee, S. K. 1980. Cadmium toxicity in fishes. *Hydrobiologia* 75 (2):117- 21.

Dautremepuits, C., Betoulle S., and Vernet, G. 2002. Antioxidant response modulated by copper in healthy orparasitized carp (*Cyprinus carpio* L.) by Ptychobothrium sp.(Cestoda). *Bioch. Bioph. A* 1573: 4.

Deore, S. V. and Wagh S. B. 2012. Heavy metal induced histopathological alterations in liver of Channa gachua (Ham). *Journal of Experimental Sciences* 3 (3): 35-38.

Diab, A. S., EL-Nagar, G. O. and Abd-el-Hady, Y. M. 2002. Evaluation of *Nigella sativa* L (black seeds; baraka), *Allium sativum* (garlic) and Biogen as feed additives on growth performance and immunostimulants of *O. niloticus* fingerlings. *Suez Canal Vet. Med. J* 7: 45-75.

Diaz, L. S. Roa, A., Gacia C. B., Acero, A. and Navas G. 1986. Length-weight relationship of dermesal fishes from upper continertal slope off Colombia. *The ICLARM Quarterly* 23 (3): 23-25.

Diwan, A. D., Hingorani, H. G. and Chandrasekhram, N. N. 1979. Levels of blood glucose and tissue glycogen in two live fish exposed to industrial effluents. *Bulletin of Environmental Contamination and Toxicology*, 21: 269-272.

Donaldson, E. M., 1981. The pituitary – international axis as indicator of stress in fish. In A. D. Pickering (ed). Stress and fish academic press, London.

Draper, H. H., Squires, E. J., Mahmooch, H., Wu, J., Agarwal, S. and Handley, M. 1993. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. *Free Radical Biology and Medicine* 15: 353–363. doi: 10.1016/0891-5849(93)90035-S.

Drevnick, P. E. and Sandheinrich, M. B. 2003. Effects of dietary methylmercury on reproductive endocrinology of fathead minnows. *Environmental science & technology* 37(19): 4390-4396.

Dural, M., Goksu, M. Z., Ozak, A. A. and Derici, B. 2006. Bioaccumulation of some heavy metals in different tissues of dicentrarchus labrax l, 1758, sparus aurata l, 1758 and mugil cephalus l, 1758 from the camlik lagoon of the eastern coast of mediterranean (turkey). *Environmental Monitoring and Assessment*, 118 (1-3): 65-74.

Ebrahimi, M.1. and Taherianfard, M. 2011. The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River *Iranian Journal of Fisheries Sciences* 10 (1): 13-24.

Ekeanyanwu, C. R., Ogbuinyi C. A. and Etienajirhevwe O. F. 2010. Trace Metals Distribution in FishTissues, Bottom Sediments and Water from Okumeshi River in Delta State, Nigeria *Ethiopian Journal of Environmental Studies and Management* 3 (3):12-17.

Ekpo, K. E., Asia, I. O., Amayo, K. O. and Jegede, D. A., 2008. Determination of lead, cadmium and mercury in surrounding water and organs of some species of fish from Ikpoba river in Benin city. *Nigeria International Journal of Physical Sciences* Vol. 3 (11): 289-292.

Ellis, A. E., 1981. Stress and the modulation of defense mechanisms in fish In: stress and fish. Pickering, A D (ed). Academic press London, pp 147 – 171.

Ellis, A. E., 1986. The function of teleost fish, lymphocytes in relation to inflammation. *International journal of tissues reactions* 8 (4): 263-270.

Ellsaesser C. F. and Clem L. W. 1986. Hematological and immunological changes in channel cat fish stressed by handling and transport. *J Fish Biol* 28: 511-521.

Ensenbach, U. and Nagel, R. 1997. Toxicity of Binary Chemical Mixtures: Effects of Reproduction of Zebrafish (*Brachydanio Rerio*. *Arch.*) *Environ Contain Toxicol* 32 (2): 204-210.

Ergenes, C. T., Celika, K. N., Kaya F. and Karahan A. 2007. Monitoring of nuclear abnormalities in peripheral erythrocytes of three fish species from the Goksu Delta (Turkey); genotoxic damage in relation to water pollution. *Ecotoxicology* 16: 385.

Ettler, V., Johan, Z., Baronnet, A., Jankovsky, F., Gilles, C., Mihaljevic, M. and Sebek, O. 2005. Mineralogy of air-pollution-control residues from a secondary lead smelter: Environmental implications. *Environmental science & technology*, 39 (23): 9309-9316.

Eze, L. C. 1983. Isoniazid Inhibition of liver glutamic oxaloacetic transaminase from goat (*Carpa hercus*). *Inter J. Biochem* 15: 13 – 16.

Fafioye, O. O., Adebisi, A. A. and Fagade, S. O. 2004. Toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. *African J. Biotechnol*. 3 (11): 627-630.

Faisal, A. S. R. 2003. Adverse effects of some antimicrobial agents used in fish. Cairo: Cairo University, Faculty of Veterinary Medicine. 238p. [Ph. D. Thesis].

FAO, 1996. Fisheries and aquaculture in Sub-Saharan Africa: Situation and outlook in 1996. FAO fisheries circular No. 922FIPP/C922, Rome, Italy. http://www.fao.org/docrep/006/ w3839e /W3839e00.htm

FAO, 2006. The State of World Fisheries and Aquaculture 2006, Rome, pp. 162.

FAO, © 2010-2012. Cultured Aquatic Species Information Programme. Clarias gariepinus. Cultured Aquatic Species InformationProgramme . Text by Pouomogne, V. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated 1 January 2010. http://www.fao.org/fishery/culturedspecies/Clarias_gariepinus/en Retrived 28/7/2012

Farant, J. P., and Wigbaseline D. C. 1982. Biomonitoring of lead exposure with ALAD activity ratios. *Int Arch Occup Environ Health*, 51: 15–24.

Farghaly, A. M., Ezzat, A. A., and Shabane, M.B. 1973. Effect of temperature and salimenting changes on the blood characteristics of *Tilapia Zilli*. In Egyptian littoral lakes. *Comparative Biochemistry and physiology* 46 (1) 183 – 193.

Farombi, E. O., Adelowo, O. A. and Ajimoko, Y. R. 2007. Biomarkers of Oxidative Stress and Heavy Metal Levels as Indicators of Environmental Pollution in African Cat Fish (*Clarias gariepinus)* from Nigeria Ogun River. *Int J Environ Res Public Health* 4 (2): 158–165.

Fatima, A. E., Nema, A., Ali, Mohamed, M. Z., and Olfat, A. R., 2008. Impacts of different water resources on the ecological parameters and the quality of Tilapia production at El-Abbasa fish farms in Egypt. 8th international symposium on tilapia in aquaculture.

Fazlolahzadeh, F., Keramati, K., Nazifi, S., Shirian, S., and Seifi, S., 2011. Effect of Garlic (Allium sativum) on Hematological Parameters and Plasma Activities

of ALT and AST of Rainbow trout in Temperature Stress. *Australian Journal of Basic and Applied Sciences* 5 (9): 84-90.

FGN, Federal Republic of Nigeria Official Gazette (2009): National Environmental (Chemical, Pharmaceutical, soap and Detergent Manufacturing Industries) Regulations.68 (96) 1343

Fleischer, M., Sarafim A. F., Fasset D. W., Hammond P., Shacklette H. T., Nisbet I. C. T. and Epstein S. 1974. Environmental impact of cadmium. *Environ. Health* 253.

Flick, G., Van Rijis, J. H. and Wendelaar Bonga, S. E. 1987. Evidence for high-affinity Ca+2-ATPase activity andATP driven Ca+2 -ansport in membrane preparations of the gill epithelium of the cichlid fish *Oreochromis mossambicus*. *J. Experi. Biol*. 119: 335-347.

Flik, G. and Verbost, P. M., 1993. Calcium transport in fish gills and intestine *J. exp. Bio.* 184: 17–29.

Fortunator, M. N. 1990. On the activity of the phytoncides from garlic in the human organism upon per oral administration. *Farmakal- Toksikal* 18: 43-46.

Frank, H. and Althoen, S. C. 1995. Statistics: Concepts and Applications. Cambridge low perice edition, Cambridge University Press, Cambridge.

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

Gabriel, U. U., Ezeri, G. N. O., and Opabunmi O. O. 2004. Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822). *African journal of biotechnology* 3 (9): 463-467.

Garlic Oils, Pills & Extracts www.gourmetgarlicgardens.com/pill.htm retrived on 7/6/2011.

Gbem, T. T., Balogun J. K., Lawal F. A. and Annune P. A. 2001. Trace metal accumulation in *Clarias gerieppnus* exposed to sub lethal levels of tannery effluent. *Sci total environ.* 271: 1 – 9.

Gbore, F. A., Oginni O., Adewole, A. M. and Aladetan, J. O. 2006. The effect of transportation and handling stress on haematology and plasma biochemistry in fingerlings of *Clarias gariepinus* and *Tilapia zilli*. *World J Agri Sci* 2: 208-212.

Gill, T. S. and Pant J. C. 1986. Chromatin condensation in erythrocyte of fish following exposure to cadmium. *Bull Environ. Contam Toxicol* 36: 199.

Gill, T. S. and Epple, A. 1993. Stress-related changes in hematological profile of American eel, *Anguilla rostrata. Ecotoxicol. Environ. Saf* 25: 227-235.

Gluth, G. and Hanke, W. 1985. A comparison of physiological changes in Carp; *Cyprinus carpio* induced by several pollutants of sublethal concentrations. I-The dependency on exposure time. *Ecotoxicology and Environmental Safety*, 9: 179-188.

Gornal, A. G., Bardawill J. C. and David M. M., 1949. Determination of serum proteins by means of Biuret reaction. *J. Biol. Chem* 177: 751-766.

Gracey, A. Y., Troll, J. V. and Somero, G. N. 2001. Hypoxia-induced gene expression profiling in euryoxic fish *Gillichthys mirabilis*. *Proc Natl Acad Sci* USA 98 (4): 1993-8.

Grobler, E. 1988. Die Effek van Atrasien, Sink in Yster op die Hematologie en Suurstofverbruik van *Tilapia sparrmanii* (Cichlidae). M.Sc. Thesis, Rand Afrikaans University, Johannesburg, South Africa.

Gul, S., Belge-Kurutas, E., Yildiz, E., Suhan, A. and Doran, F. 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (cyprinidae) living in seyhamdam lake, Turkey. *Environ. Int* 30: 605 – 609.

Hariati, A. M., Machiels, M.A.M., Verdegem, M.C.J. and Boon, J.H. 1994. The prevalence of ruptured intestine syndrome in African catfish *Clarias gariepinus* (Burchell 1822) fed different rations when between 3 and 5 weeks old. *Aquaculture* 125 (1-2): 11-16.

Has-Schon, E., Bogut, I., Kralik, G., Bogut, S., Horvatic, J., and Cacic, I. 2007. Heavy metal concentration in fish tissues inhabiting waters of "busko blato" reservoar (bosnia and herzegovina). *Environmental Monitoring & Assessment* 54 (1): 75-83.

Hattingh, W. H. J. 1995. Heparin and ethylenediamine tetra-acetate as anticoagulants for fish blood. *European Journal of Physiology* 355 (4): 347-352.

Heath, A. G. 1987. Water pollution and fish physiology. CRC Press Inc. Boca Raton, Florida, USA.

Heath, A.G. 1990. Water Pollution and Fish Physiology. Second Edition. CRC Press Inc., Florida p. 359.

Henrique, M., Gomes M. F. and Gouillou, M. 1998. Influence of supplementation of practical diets with vitamin C on growth and response to hypoxic stress of seabram, *Sparus aurata*. *Aquaculture* 161: 415-425.

Hermes-Lima, M. 1995. How do Ca^{2+} and 5-aminolevulinic acid derived oxyradical promote injury to isolated mitochondria. *Free Radic Biol Med* 19: 381–390.

Herrmann-Erlee, M. P. M. and Flik, G. 1989. Bone: Comparative studies on endocrine involvement in bone metabolism. In *Vertebrate Endocrinology: Fundamentals and Biomedical Implications*, vol. 3 (ed. P. K. T. Pang and M. P. Schreibman), pp. 211–243. New York: Academic Press.

Hile, R., 1936. Age and growth of the cisco Leucichthys artedi (Le Sueur), in the lakes of the north-eastern highlands, Wisconsin. *Bull. U.S. Bureau Fish.* 48: 211–317.

Hille, S., 1982. A literature review of the blood chemistry of rainbow trout, *Salmo gairdneri* Rich. *J. Fish. Biol* 20: 535-69.

Hinck, J. E., Blazer, V. S., Denslow, N. D., Myers, M. S., Gross, T. S. and Tillitt, D. E. 2007. Biomarkers of contaminant exposure in northern pike (esox lucius) from the yukon river basin, Alaska. *Archives of environmental contamination and toxicology*, 52 (4): 549-562.

Hinton, D., and Lauren, D.J. 1990. Integrative Histopathological approaches to detecting effects of environmental stressors on fishes. American fisheries society symposium 8:51-66, in Biological indicators of stress in fish. Adams M.ed.

Holmer, M. 1991. Impact of Aquaculture on Surrounding Sediments; generation of organic rich sediments. In: de pauw, N., Joyce J (Eds), Aquaculture and the environment. European Aquaculture Society Special Publications No. 16, Gent, Belgium, pp 155-175.

Huffman, P. A., Arkoosh, M. R. and Casillas, E. 1997. Characteristics of peripheral blood cells from rainbow trout evaluated by particle counter, image analysis, and hemocytometric techniques. *Journal of Aquatic Animal Health* 9: 239-248.

Hunn, J. B. and Greer I. E. 1991. Influence of sampling on the blood chemistry of Atlantic salmon. *Prog. Fish Cult* 53: 184-7.

Hussain, S. M., Javed M., Javid A., Javid, T. and Hussain, N. 2011. Growth responses of *Catla catla, Labeo rohita and Cirrhina mrigala* during chronic exposure of iron. *Pakistan J. Agric. Sci* 48: 225–230.

Hussein, A. J. and Nadim, C. 2003. Bio-accumulation of zinc and cadmiumin freshwater fishes. *Indian J. Fish*., 50 (1): 53-65, 2003.

Ibe, S. N. 1989. Structural Adjustment Programme and Self Sufficiency in Animal production. Pro of National Conf. on Impact of Nigerian Agric and Rural Life. NISER, Ibadan.

Ikeogu, C. F., Nsofor, C. I. and Igwilo, I. O. 2013. Toxicity of lead nitrate and crude oil on the growth of the African catfish *Clarias gariepinus*. *Inter J Agri Biosci* 2 (6): 337-339.

Ishibashi, Y., Ekawa, H., Hirata H. and Kumai, H. 2002. Stress response and energy metabolism in various tissues of Nile tilapia *Oreochromis niloticus* exposed to hypoxic condition. *Fisheries science* 68: 1374-1383.

Iwama, G. K. 1991. Interaction between aquaculture and the environment. *Crit. Rev. Environ. Coutr* 21: 177-216.

Jain, N.C., 1986. Veterinary Hematology 4th edition published by Lea and Febiger, philadephia USA p. 36-556.

Jaluludeen, M. D., Arunachalam, M., Raja, M., Nandagopal, S., Showkat, A. B., Sundar S. and Palanimuthus D. 2012. Histopathology of the Gill, Liver and Kidney Tissue of the freshwater fish Tilapia mossambica exposed to Cadmium Sulphat. *IJABR* 2 (4): 572 – 578

Järup, L., Berglund, M., linder, G., Nordberg, G. and Vahter, M. 1998. Health effects of cadmium exposure – a review of the literature and a risk estimate. *Scandain J. Work Environ. Health.* 24 (1):52p.

Jeffree, R. A., Warnau, M., Oberhansli, F. and Teyssie, J. L. (2006). Bioaccumulation of heavy metals and radionuclides from seawater by encased embryos of the spotted dogfish scyliorhinus canicula. *Marine Pollution Bulletin* 52 (10): 1278-1286.

Jeorg, H. G. and Lee, Y. W. 1998. Protective effect of daily sulfide on Nitrosodimethylamine- induced immunosuppression in mice. *Cancer Lett.*, 11: 73-9.

Jessus, O., Alberto, C., Alejandro, R. M., Angeles, E. and Jose M. 2002. Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the celluler innate immune response of gillhead seabream, *Sparus aurata* L. *J. Veterinary immunology and immunopathology* 85: 41-50.

Jezierska, B. and Malgorzata, W. 2006. The metal uptake and accumulation in fish living in polluted waters *I. Twardowska et al.*, (*eds.*), *Soil and. Water Pollution Monitoring, Protection and Remediation* 3–23.

Jezierska, B. and Witeska, M. 2001. Metal Toxicity to Fish, *Wydawnictwo Akademii Podlaskiej, Siedlce* 318 pp.

Jimena, C., Daniel, A. W., Andrea, C. H. and Maria de los, A. B. 2005. Haematological parameters in neotropical fish, *Corydoras paleatus* (Jenyns 1842) (Pisces, Callichthyidae) captured from pristine and polluted water. *Hydrobiologia* 537: 25-33

Joshi, B. D. 1982. Circannual fluctuations in some blood components of the fish *Rita rita*, in relation to certain eco-physiological conditions. *Uttar Pradesh journal of Zoology* 2: 62-66.

Kai Sun, G.F., Krause, L.F., Mayer, J. and Mark, R. 1995. Predicting chronic lethality of chemicals to fishes from acute toxicity test data: Theory of accelerated life testing. *Environ. Toxicol. Chem* 14: 1745–175.

Kalay, M. and Canli, M. 2000. Elimination of Essential (Cu, Zn) and non-essential (CD, Pb) metals from Tissue of a freshwater fish *Tilapia zilli*. *Turk J* 24: 429-436.

Karuppasamy, R., Subathra, S. and Puvaneswari, S. 2005. Haematological responses to exposure to sublethal concentration of cadmium in air breathing fish, *Channa punctatus* (Bloch). *J. Environ. Biol* 26: 123-8.

Katz, S.A. 1982. Determination of Heavy Metal in Sewage Sludge. Specialist periodical reports. *Environmental Chemistry* Vol. 3 Royal Society of Chemistry Barlington House London WI VOBN pp 78-80.

Kazlauskiene, N. and Burba, A. 1997. Investigations of the effect of heavy metal mixtures on hydrobionts. *Ekologija* 2: 7–11.

Kazlauskiene, N. and Vosyliene, M. Z. 1999. Peculiarities of the physiological responses of rainbow trout *Oncorhynchus mykiss* to copper. *Acta Zool. Litu.*, 9: 56–71.

Kelly, W. R. 1984. Veterinary Clinical Diagnosis. Third edition published by Bailliete Tindall. London Pp. 312-335.

Khadiga, G. A., Hania, M. I., Sherifa, S. H. and Ramadan A. S. 2003. Blood chemistry of Nile tilapia, Oreochromis niloticus (Linnaeus 1757) under the impact of water pollution. *Aquatic ecology* 36: 549-557.

Khallaf, E. A., Galal, M. and Authman, M. 2003. The biology of *Oreochromis niloticus* in a polluted canal. *Ecotoxicol* 12: 405-416.

Kime, D. E. 1995. The effects of pollution on reproduction in fish. *Reviews in Fish Biology and Fisheries*, 5(1): 52-96.

Kime, D. E., Ebrahimi, M., Nysten, K., Roelants, I., Moore, H. D. M. and Ollevier, F. 1996. Use of computer assisted sperm analysis (casa) for monitoring the effects of pollution on sperm quality of fish; application to effects of heavy metals. *Aquatic Toxicology,* 36(1): 223-237.

Klanns-Dieter, A. 1983. Sulfur content of free radicals. In: Nygaard O.F, Simic M.G, editors. Radioprotectors and Anticarcinogenes. New York: Academic Press, pp. 23–42.

Kori – Siakpere, O., Ikomi, R. B. and Ogbe, M. G. 2010. Variations in alamine aminotransferase and aspartate aminotransferase activities in African catfish: *Clarias gariepinius* (1822) at different sub-lethal concentration of potassium permanganate. *Sci. Res Essays* 5 (12): 1501 – 1505.

Kori-Siakpere, O. 1996. Some alterations in haematological parameters in *Clarias isheriensis* (Sydenham) exposed to sublethal concentration of waterborne lead. *Biores Comm.* 8(2): 93 - 98.

Kori-Siakpere, O. and Ubogu, E. O. 2008. Sublethal haematological effects of zinc on the freshwater fish, *Heteroclarias* sp. (Osteichthyes: Clariidae. *African Journal of Biotechnology* 7 (12): 2068-2073.

Krishnani, K. K., Azad, I. S., Kailasam, M., Thirunavukkarasu, A. R., Gupta, B. P., Joseph, K. O. and Muralidhar, M. 2003. Acute toxicity of some heavy metals to lates calcarifer fry with a note on its histopathological manifestations. *Journal of Environmental Science and Health, Part C,* 38 (4): 645-655.

Kumar, P. and Singh, A. 2010. Cadmium toxicity in fish: An overview. *GERF bulletin of Bioscience* 1 (1): 41-47.

Kumar, P., Prasad, Y., Dhama, K. and Nandi, D. 2007. *In vitro* lymphoproliferative responses of peripheral blood mononuclear cells from different *in vivo* treated catfish (*Claris batrachus*). *Indian J. Comparative Microbiol., Immunol. Infectious Disease*. 27 (1&2): 27-31.

Kumar, P., Prasad, Y., Ranjan, R., Swarup, D., Pattanaik, A.K. and Patra, R.C. 2008. Accumulation Pattern of Cadmium in Tissues of Indian Catfish *Clarias batrachus. Animal Nutrition. and Feed Technol* 8 (1): 115-119.

Kumar, P., Prasad, Y., Swarup, D., Patra, R.C. and Nandi, D. 2005. Bioaccumulation of cadmium in fresh water indian catfish Clarias batrachus. *Proceedings of aninternational conference held in Lucknow (India),* 14-17th November 2005. pp: 45

Kumari, S.A. and Ramkumar, N.S. 1997. Effect of water pollution on histology of intestine of two fresh water fishes from Hussainsagar Lake (A.P.). *Indian J. Environ. and Toxicol* 7 (2): 68-70.

Kyoungju, C. 2004. Modulation of immune function parameters in fish causes by sudden environmental changes. A dissertation submitted to the graduate Faculty of North Carolina State University U.S.A pp. 144.

Lacroix, A. and Hontela, A. 2004. A comparative assessment of the adrenotoxic effects of cadmium in two teleost species, rainbow trout, *Oncorhynchus mykiss*, and yellow perch, Perca flavescens. *Aquatic Toxicology* 67: 13–21.

Ladipo, O. O., Sonaike, O.O. and Oludimu, O.L. 1982. A statistical investigation of fish in Nigeria. Proceedings of the 2nd Annual Conference of fisheries society of Nigeria (FISON), Calabar, 25th -27th, January.

Lanzotti, V. 2006. The Analysis of Onions and Garlic. *Journal of Chromatography* 1112: 3-22.

Larsson, A., Haux, C. and Sjobeck, M. L. 1985. Fish physiology and metal pollution: results and experiences from laboratory and baseline studies. *Ecotoxicol. Environm. Safety* 9: 250-281.

Lawson, L. D. 1996. The composition and chemistry of garlic cloves and processed garlic. In: Koch HP, Lawson LD. Eds. Garlic. The science and the therapeutic application of *Allium sativum* and related species. Baltimore: Williams & Wilkins, pp: 37-107.

Lazarus, R. J., Rami, P. S., and Reddy, P.M. 1986. On the length-weight relationship of the little-known lesser spiny eel, *Macrognathus aculeatus* (Bloch) (Pisces: Mastacembelidae) Biomedical and Life Sciences Proceedings: *Animal Sciences* 90: (4) 423-427.

Lebelo, S. L., Saunders, D. K. and Crawford, T. G. 2001. Observations on Blood Viscosity in Striped Bass, *Morone saxatilis* (Walbaum) Associated with Fish Hatchery Conditions. *Transactions of the Kansas Academy of Science* 104 (3 & 4):183-194.

Lepokovsky, S. 1926. The Distribution of serum and plasma proteins in fish.www.jbc.org retrieved on august 17th 2012.

Li, Z. H., Zlabek, V., Velisek, J., Grabic, R., Machova, J., Kolarova, J., Li, P. and Randak, T. 2011. Acute toxicity of carbamazepine to juvenile rainbow trout (*Oncorhynchus mykiss*): Effects on antioxidant responses, hematological parameters and hepatic EROD. *Ecotoxicology and Environmental Safety*, 74: 319–327.

Lippmann, M. 2000. Human exposures and their health effects. In: *Environmental Toxicants* (2nd Edition) Wiley Intersciences. USA. pp-824-829

Lister, A. and Van Der Kraak, G. 2001. Endocrine disruption: why is it so complicated? *Water Qual Res J Can* 36: 175–190.

Lockhart, W. L. and Metner, D. A. 1984. Fish Serum Chemistry as a pathological tool. In contaminant effects on fisheries. Edited by Cairs, V.W., Hodsom, P.V. and Nriagu, J.O. Wiley, New York. Pp. 73-83.

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

Lutz, P. L. and Nilsson, G. E. 1997 contrasting strategies for anoxic brain survival: Glycolysis up or down. *Journal of experimental biology* 200: 411-419.

Mahajan, C. C. and Dheer, J.S. 1979. Seasonal variation in blood constituents of an air-breathes fish *Charina punctatus* (bloch). *Journal of Fish Biology* 14: 413-417.

Maheswaran, R., Devapaul, A., Velmurugan, B. and Ignacimuthu, S. 2008. Haematological studies of freshwater fish, *Clarias batrachus* (L.) exposed to mercuric chloride. *Inter. J. Integr. Biol.* 2 (1): 49 - 54.

Malik, N., Biswas, A. K., Qureshi, T. A., Borana, K. and Virha, R. 2010. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. *Environmental Monitoring and Assessment,* 160: 267-276.

Mance, G. 1987. Pollution threat of heavy metals in aquatic environment, Elsevie 363 pp London.

Marioghae, I. E. 1991. Cultivable fish Pp. 3–10. In Ayinla O.A (ed). Proceedings of the fish seed propagation course. ARAC, 14th – 28th August, 1991. ARAC Aluu, Port Harcourt, Nigeria 106pp.

Marshall, W. S., Bryson, S. E. and Wood, C. M. 1992. Calcium transport by isolated skin of rainbow trout. *J. exp. Biol.* 166: 297–316.

Martinez, C. B. R., Nagae, M. Y., Zaia, C. T. and Zaia, D. A .M. 2004. Acute morphological and physiological effects of lead in Neotropical fish *Prochilodus lineatus..Braz. J. Biol* 64 (4): 797-807.

Martinez-Porchas, M., Martinez-Cordova, L. R. and Ramos-Enriquez, R. 2009. Cortisol and glucose: Reliable indicators of stress?. *Pan Am J Aquat Sci* 4: 158-178.

Martins M L., Moraes F. R., Miyazaki D. M., Brum C. D., Onaka E. M., Fenerick Jr. J. and Bozzo, F. R. 2002. Alternative treatment for *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae) infection in cultivated pacu, *Piaractus mesopotamicus* (Osteichthyes: Characidae) in Brazil and its haematological effects. *Parasite* 9: 17180.

Mazeaud, M., Mazeaud, F. and Donaldson, E. 1977. Primary and Secondary effects of stress in fish. Some new data with a general review. *Transaction of the American fisheries society* 106: 201-212.

McKenna, J. M., Chaney, R. L. and Williams, F. M. 1993. The effect of cadmium and zinc interactions on the accumulation and tissue distribution of zinc and cadmium in lettuce and spinach. *Environ Pollut* 79: 113-120.

McLeay, D. J. 1973. Effects of a 12-hr and 25-day exposure to kraft pulp mill effluent on the blood and tissues of juvenile Coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Board Can.* 30: 390–400.

Meier, P. G., Fook D. C. and Lagler, K. F. 1983. Organochlorie pesticide residues in rice paddies in Malaysia 1981 *Bulletin of Environment contaminants and toxicology* 30: 351-357.

Meinelt, T., B. K., Burnison, M., Pietrock, E., Zwirnmann, A. and Steinberg, C. E. W. 2007. Cadmium uptake rates in freshly hatched zebrafish (Danio rerio) larvae in the presence of two dissolved organic matter (DOM) isolates. *J. Appl. Ichthyol* 1–2.

Metwally, M. A. A. 2009. Effect of Garlic *(Allium sativum*) on some antioxidant activities in Tilapia Nilotica *(Orechromis niloticus*). *World journal of fish and marine sciences* 1 (1): 56-64.

Misra, H. P. and Fridovich, I. 1972. The role of superoxide anoin in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem*. 217: 3170-3175.

Mommsen, T.P., Vijayan, M. M. and Moon, T.W. 1999. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. *Rev Fish Biol Fisher* 9: 211–268.

Monteiro, S. M., Mancera, J. M., Fontainhas F. A. and Sousa, M. 2005. Copper induced alterations of biochemical parameter in the gill and plasma of Oreochromis niloticus. *Comp. Biochem. Physiol C* 141: 375-383.

Moses, B. S. 1983. Introduction to Tropical fisheries, University Press Ibadan, 117pp.

Munkittrick, K. R. and Leatherland, J. F. 1983. Haematocrit values in feral goldfish, *Carasius auratus* L., as indicators of the health of the population. *J. Fish Biol*. 23: 153 - 161.

Murad, A., Everills, A. and Housten, A., 1993. Division of goldgish erytrocytes in circulation. *Canadian journal of zoology* 71: 2190-2198.

Murad, H. and Boyd, C.E. 1990. Production of sunfish (Lepomis spp.) and channel catfish *(Ictalurus punctatus)* in acidified ponds. *Aquaculture* 94: 381-388.

Murata, I. 1971. Chronic entero-osteo-nephropathy Cadmium. *Nippon Ishikai Zasshi* 65: 15.

Myers, M. S. and Fournie, J. W. 2002. Histopathological biomarkers as integrators of anthrogenenic and environmental stressors. Pages 221-287 (ERL, GB1139) in S. M. Adams, editor Biological indicators of Aquatic Ecosystem Stress. *American fisheries society, BetheSEMa*, M. D.

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C. M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. 2000. Effect of aquaculture on world fish supplies. *Nature* 405: 1017-1024.

Nemcsok, J. and Boross, L. 1982. Comparative studies on the sensitivity of different fish species to metal pollution. *Acta Biologica Academiae Scientiarum Hungaricae*, 33 (1): 23-27. 1

Nespolo, R. F. and Rosemann, M. 2002. Intraspecfic allometry of hematological parameters in *Basilichthys australis*. *Journal of fish biology* 60: 1358-1362.

Nkwoji, J, A., Doherty, F. J., Renner K. O., Igbo J. K., and Ajani G. E., 2014. Spatiotemporal Variations in Hepatosomatic Index and Antioxidantenzyme Activity in Brackish Water Fish Species in the Lagos Coastal Zone. *Journal of environment and human* 1 (1): 18.

Nuriye, M., Birgül, I., Levent, E. and Fuat, G. 1999. The Effect of Fish Oil on Liver and Plasma MDA and Antioxidant Status of Rats. *Tr. J. of Medical Sciences* 29: 1–6

Nussey, G. 1994. The effect of copper on blood coagulation and general haematology of *Oreochromis mossambicus* (Cichlidae). M.Sc. Thesis, Rand Afrikaans University, Johannesburg, South Africa.

Nussey, G., van Vuren, J. H. J., Du Preez, H. H. 1990. Effects of copper on haematology and osmoregulation of the Mozambique tilapia, *Oreochromis mossambicus* (Cichlidae). *Comp. Biochem. Physiol* 111(C): 369 - 380.

Nwadukwe, F. O. and Ayinla O. A. 1993. The effects of brood fish rearing period and seasons on spawn weight and hatching rates of *Clarias gariepinus* (Burchell) and *Heterobranchus longifillis* (Val). *Nig. Inst. Oceanogr. And Mar. Res. Tech. Pap.* 90: 15pp.

Odebunmi, E. O., Oluwaniyi, O. O. and Bashiru M. O. 2010. Comparative Proximate Analysis of Some Food Condiments. *Journal of Applied Sciences Research*, 6 (3): 272-274.

Ojogbo, A. I. 2013. Effects of induced stress on morphometric indices of young African catfish Clarias gariepinus (Burchell 1822) in static culture. Fellowship diploma college of Veterinary surgeons Nigeria (FCVSN) thesis 145pp.

Ojogbo, A. I. 2006. Lead Levels and Condition Factor K of the mullet *Liza grandisquamis* (valencienaces 1836) from Warri waters, Delta State, Nigeria. MVSc Dissertation University of Ibadan 166pp.

Okada, I. A., Sakuma, A. M., Maio, F. D., Dovidemskas, S. and Zenebon, O. 1997. Evaluation of lead and cadmium in milk due to environmental contamination in Paraiba Valley region of South Eastern Brazil. *Revista-de-Saude-Publication.* 31: 140-143.

Okaeme, A. N. 2010. Managing aquatic environment for best practices in fish farming: A paper delivered at Veterinary Council of Nigeria (VCN) professional continuing education seminar on 8thjuly at Nelrose hotel, Asaba, Delta state.

Okocha, R. C. and Adedeji O. B. 2011. Overview of Cadmium Toxicity in Fish. *J. Appl. Sci. Res* 7 (7): 1195-1207

Okolo, S. C., Olajide, O. O., Idowu, D. I., Adebiyi, A. B., Ikokoh, P. P. and Orishadipe, A.T. 2012 Comparative Proximate Studies on Some Nigerian Food Supplements *Annals of Biological Research* 3 (2): 773-779.

Oladosu, G. A., Ayinka O. A., Adeyemo, A. A., Yakubu, A. F. and Ajani, A. A. 1993. A comparative study of the reproductive capacity of the African catfish species *Heterobranchus bidorsalis* (Geoffrey), *Clarias gariepinus* (Burchell) and their hybrid "Heteroclarias". African Regional Aquaculture Centres Technical paper 92: 1-5

Olaifa, F. G., Olaifa A. K.and Onwude, T. E. 2004. Lethal and sub lethal effects of copper to the African Cat fish (Clarias gariepinus) *African. J. Biomed Research* 7: 65–70

Olojo, E. A., Olorun, K. B., Mbaka G. and Oluwemimo A. D. 2005. Histopathology of gill and liver tissues of African Catfish *Clarias gareipnus* exposed to Lead. *African J. Biotech* 4 (1): 117 – 122

Olufemi, B. E. 1998. Fish Husbandry and Medicine: The experience of a tropical Veterinarian. An Inaugural Lecture delivers at the University of Ibadan, Ibadan. September 17. pp. 34.

Olufemi, B. E. 2011. Epidemiology: Some basic concepts and definitions: a lecture delivered to 1st year fellowship students of College of Veterinary Surgeon Nigerian (CVSN) course VMD 811

Omitoyin, B. O. 2007. Introduction to fish farming in Nigeria, Ibadan. University Press Publishing House, University of Ibadan, pp52.

Omoregie, E., Eseyin, T. G. and Ofojekwu, P. C. 1994. Chronic effects of formalin on erythrocyte counts and plasma glucose of the Nile Tilapia *Oreochromis niloticus. Asian Fisheries Science* 7: 1 - 6.

Osman, H. A., Taghreed M., Ibrahim, B., Ali, A. T. and Derwa., H. I. M. 2009. Baseline Application of Humic Acid Against the Effect of Cadmium Pollution on Cultured Tilapia *Oreochromis niloticus*. *World Applied Sciences Journal* 6 (11): 1569-1575.

Otunola, G. A., Oyelola, B., Oloyede, A., Oladiji., T. and Afolayan, A. J. 2010. Comparative analysis of the chemical composition of three spices – *Allium sativum* L. *Zingiber officinale* Rose. and *Capsicum frutescens* L. commonly consumed in Nigeria. *African Journal of Biotechnology* 9 (41): 6927-6931.

Oze, G. R., Oze, C., Anunuso, C., Ogukwe, H. N. and Okorie, K. 2006. Heavy metal pollution of fish of Qua-Iboe River Estuary: Possible implications for Neurotoxicity. *The internet journal of Toxicology* 3 (1).

Pantreath, R. J. 1973. The accumulation and retention of 65Zn and 54Mn by Plaice, *Pleuronectes Platessa* L. *Mar. Biol. Ecol* 12: 1-8

Parrott, J. L., Fodor, G. G. and Blunt, B. R. 2000. Fathead Minnow Lifecycle Exposure to Ethinyl Estradiol. Cannadian Technical Report of Fisheries and Aquatic Sciences No. 2331, p.60.

Pelgrom, S. M. G. J., Lamers, L. P. M., Lock, R. A. C., Balm, P. H. M. and Wendelaar Bonga, S. E. 1995. Interactions between copper and cadmium modify metal organ distribution in mature tilapia, *Oreochromis mossambicus*. *Environ. Pollut* 90: 415–423.

Perry, W. G., Jr. and Avault, J. W. Jr. 1969. Experiments on the culture of blue, channel, and white catfish in brackishwater ponds. Proceedings Annual Conference of the Southeastern Association of Game and Fish Commissioners 23: 592-605.

Power, D. A. 1989. Fish as model systems. *Science* 246: 352-358.

Pratap, H. B. and Wenderlaar Bonga, S. E. 1990. Effects of waterborn cadmium on plasma cortisol and glucose in Cichlid fish *Oreochromis mossambicus. Comp Biochem. Physiol* 90 C: 313-317.

Pulatsu, S, F., Rad, G., Koksal, F., Aydm, A. C. K. and Topcu, A. 2004. The Impact of Rainbow Trout Farm Effluents on Water Quality of Karasu Stream, Turkey. *Turkish J. fisheries and aquatic Sci.* 4: 09-15

Rahman, M. M., Fazlic, V. and Saad, N. W. 2012. Antioxidant properties of raw garlic *(Allium sativum)* extract *International Food Research Journal* 19 (2): 589-591

Rahman, T., Akanda, M. M., Rahman, R. M. M. and Chowdhury, M. B. R., 2009. Evaluation of the efficacies of selected antibiotics and medicinal plants on common bacterial fish pathogens. *J. Bangladesh Agril Univ* 7 (1): 163-168.

Rand, G. M. and Petrocelli, S.R. (Eds.) 1985. Fundamentals of Aquatic Toxicology. Methods and Applications. Hemisphere, New York, pp. 374-415.

Rani, U. A. and Ramamurthi, R. 1989. Histopathological alteration in the liver of freshwater teleost *Tilapiamossambica* in response to cadmium toxicity. .*Ecotoxicol. Environ. Saf.* 17 (2): 221-216.

Ranzani Paiva, M. J. T. and Godinho, H. M. 1991 caracterusticas sanguineas da pirapitinga do sul, *Brycon sp* sob condicoes experimentais de criacoa intesiva. *Brazilian journal veterinary research animal science* 28: 141-153.

Ranzani-Paiva, M. J. T., and Silva-souza, A. T. 2004. Hematology of Brazilian fish. In: Ranzani-Paiva, M. J.T.; Takemoto, R. M.; Lizama, M. de los A. P. (Ed.). Sanity of the aquatic organisms. São Paulo: Varela. p. 89-120.

Rashed, M. N. 2004. Cadmium and lead levels in fish (*Tilapia nilotica*) Tissues as Biological Indicator for lake water pollution. *Journal Environmental Monitoring and Assessment* 68 (1).

Reddy, P. M. and Bashamohideen, M. 1989. Fenvalerate and cypermethrin induced changes in the haematological parameters of *Cyprinus carpio. Acta Hydrochim. Hydrobiologia* 17: 101 - 107.

Rehulka, J. 1993. Erythrodermatitis of Carp, *Cyprinus carpio* (t.): an Electrophoretic Study of Blood Serum Protein Fraction Levels. *Acta Vet. Brno*, 60: 1993: 187-197.

Ricker, W. E. 1975. Computation and Interpretation of Biological Statistics of Fish Populations. Bulletin of the Fisheries Research Board of Canada 191: 1-382.120.

Risso-de Faverney, C., Devaux, A., Lafaurie, M. J., Girard, P., Bailly, B. and Rahmani, R. 2001. Cadmium induces apoptosis and genotoxicity in rainbow trout hepatocytes through generation of reactive oxygen species. *Aquatic Toxicology* 53: 65–76

Rois, F. S., Kalinin, A. L. and. Rantin, F. T. 2002. The effects of long-term food deprivation on respiration and hematology of neotropical fish *Hoplias malabaricus. journal of fish biology* 61: 85-95.

Rotruck, J. T., Pope, H. E., Ganther, A. B., Swanson, A. B., Hafeman, D. G. and Hoekstra, W. G. 1973. Biochemical role as a component of glutathione peroxide. *J. Science* 179: 588 – 590.

Rurangwa, E., Roelants, I., Huyskens, G., Ebrahimi, M., Kime, D. E. and Ollevier, F. 1998. The minimum acceptable spermatozoa to egg ratio for artificial insemination and the effects of heavy metal pollutants on sperm motility and fertilization ability. In the african catfish (*clarias gariepinus*, burchell 1822). *Journal of Fish Biology* 53: 402-413.

Saeed, M. S. 2000. A study on factors affecting fish production from certain fish farms in the delta. M. Sc. Thesis. Ins. Environmental studies and Research. Ain Shams Univ. Egypt

Sahu, S., Das, B. K., Mishra, B. K., Pradhan, J. and Sarangi, N. 2007. Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Journal of Applied Ichthyology* 3 (1): 80-86.

Saint-Paul, U. 1984. Physiological adaptation to hypoxia of a neotropical characoid fish *Colossoma macropomum*, serrasalmidae *Environmental biology of fish* 11: 53-62.

Sajitha, G. R., Regi J, A., Andrews, K.G., Ajantha, P. A. and Augusti, K. T. 2010. Garlic oil and vitamine prevent the adverse effects of lead acetate and ethanol

separately as well as in combination in the drinking water of rats. *Indian J Clin Biochem* 25 (3): 280–288.

Salah EL-Deen, M., and Rogers, W. A., 1993. Changes in total protein and transaminase activities of grass carp exposed to diquat. *J. Aquat. Anim. Health* 5: 280-6.

Sallam, K. H., el-Sebaey, E. S. and Morshdy, A. M. 1999. Mercury, cadmium and lead levels in bagrus bayad fish from the river nile, delta region, egypt. *Journal of Egyptian Public Health Association* 74 (1-2): 17-26.

Sandhir, R., Julka D. and Gill, K. D. 1994. Lipoperoxidative damage on lead treatment in rat brain and its implications on membrane bound enzymes. *Pharmacol Toxicol* 74: 66–71.

Santschi, P. H. 1988. Factors controlling the biogeochemical cycle of trace elements in fresh and coastal waters as revealed by artificial radioisotopes. *Limnol oceanogr* 33 (4, part 2): 848 – 866.

Sastry, K. V. and Gupta P. K. 1979. The effect of Cadmium on the digestive system of freshwater fish, Channa Punctatus. *Arch. Environ. Contam. Toxicol* 8: 397 – 407

Satchel, G. H. 1991. Physiology and form of fish circulation. Cambridge University Press, Cambridge, Great Britain pp. 235.

Sawhney, A. K., and Johal, M. S. 2000. Erythrocyte alterations induced by Malathion in *Channa Punctatus* (Bloch). *Bull. Environ. Contam. Toxicol* 64: 398 - 405.

Schlenk, D. and Benson, W. H., (Eds). 2001 Target Organ Toxicity in Marine and Freshwater Teleosts, Volume 1 Organs, Taylor and Franchis, London and New York.

Schmitt, C. J., Hinck, J. E., Blazer, V. S., Denslow, N. D., Dethloff, G. M., Bartish, T. M. and Coyle, J. J. 2005. Environmental contaminants and biomarker responses in fish from the rio grande and its u.S. Tributaries: Spatial and temporal trends. *Science Total Environment* 350 (1-3): 161-193.

Schreck, B., Carl W. C. and Martin S. F. 2001. Effects of stress on fish reproduction, gamete quality and progeny. *aquaculture* 197: 3 24

Schreck, C. K., Whaley, R.A., Bass, M. L., Maughan, O. E. and Solazzi, M. 1976. Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock. *Journal of the Fisheries Research Board of Canada* 33: 76 – 84.

Secombes, C. 1996. The nonspecific immune system: cellular defenses. In: The Fish Immune System: Organism, Pathogen, and Environment. (Iwama, G. & Nakanishi, T., eds.), Academic Press, Sand Diego, California, pp. 84.

Shalaby, A. M., Khattaby, Y. A. and Abdel Rahman, A. M. 2006. Effect of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia *Oreochromis niloticus. J. Venom. Anim. Toxins Incl. Trop. Dis.* 12 (2): 172 – 201

Shawat, A B., Oamar, B. and Baharul, H. 2010 Histopathology of the intestine of *Clarias batrachus* L. Under Heavy metal stress. *The Chittagoag Univ. J. B. Sci* 5 (1&2): 45 – 53

Silvestre, F., Dierick, J., Dumont, V., Dieu, M., Raes, M. and Devos. P. 2006. Differential protein expression profiles in anterior gills of Eriocheir sinensis during acclimation to cadmium. *Aquatic Toxicology* 76: 46–58.

Simir, M., Saeed, I. and Shaker, M. 2008. Assessment of heavy metals pollution in water and sediments and their effects on *Oreochromis niloticus* in northern Delta lakes, Egypt. 8th international symposium on Tilapia in aquaculture.

Singh, A. P., Singh, A. K. and Singh, J. P. N. 2007. Cadmium induced changes on the secretion of branchial mucous cells of peppered loach, *Lepdocephalichthys guntea. J. Exp. Zool* 10 (1): 65-68.

Singh, R. K., Chavan, S. L. and Sapkale, P. H. 2006. Heavy metal concentrations in water, sediments and body tissues of red worm (tubifex spp.) collected from natural treatments in mumbai, india. *Environmental Monitoring Assessment,* 129 (1-3): 471-481.

Sivam G. P. 2001. Recent advances on the nutritional effects associated with the use of garlic as supplement. *Am. Soc. Nutr. Sci*. 1106 -8.

Smith S. 2000. Introduction, Respiration, Circulation and Hematology. Physiological effects of metals. http://www.kcl.ac.uk/ip/christerhogstrand/ courses/he0323/intro,%20 respiration, %20 circulation, %20 and %20 hematology.doc. Retrived 2/3/2012.

Snedecor, O. W. and Cochran, W. O. 1982. Statistical Methods. 7th Ed, Iowa University Press, Ames, Iowa.

Song, Y., Xu, H., Ren, L., Gong, P. and Zhou, Q. 2002. Eco-toxicological effects of heavy metals on the inhibition of seed germination and root elongation of chinese cabbages in soils. *Huan Jing Ke Xue,* 23 (1): 103-107.

Sovenyl, J. and Szakolczal, J. 1993. Studies on the toxic and immunosuppressive effects of cadmium on the common carp. *Acta Vet. Hung* 41 (3-4): 415- 426.

Sprocati, A. R., Alisi, C., Segre, L., Tasso, F., Galletti, M. and Cremisini, C. 2006. Investigating heavy metal resistance, bioaccumulation and metabolic profile of a metallophile microbial consortium native to an abandoned mine. *Science Total Environment,* 366 (2-3): 649-658.

SPSS, 1998 SPSS-Inc for Windows release. Chicago, 10.

Srivastava, A. K. and Agrawal, S. J. 1983. Changes induced by manganese in fish testis. *Experientia* 39: 1309-1310.

Srivastava, K. C. 1988. Aqueous extraction of onion, garlic and ginger inhibit platelet aggregation and alter arachidonic acid metabolism. *Biomedical Biochemistry Acta* 43: 335-336.

Stohs, S. J. and Bagchi, D. 1990. Oxidative mechanisms in the toxicity of metal ions. *Free Radical and Biological Medicine* 18: 321–336.

Stoskopf, M. K. 1993. In: Fish Medicine. pp. 113-131. Edited by M. K. Stoskopf. Saunders, Philadelphia.

Strunjak – Perovic, 1., Coz – Rakoval R., Topic Popovic N. and Jadan M. 2009. Seasonality of nuclear abnormalities in gilthead sea bream sparus aurata (L) erythrocytes. *Fish physiol. Biochem* 35: 287.

Svodova, Z., Vykusova, B. and Machova, J. 1994. The effects of pollutants on selected haematological and biochemical parameters in fish. In Muller, R & R Lloyd (eds) sublethal and chronic effects of pollutants on freshwater fish. FAO fishing News books, Great Britain: 39-52.

Swingle, H. S. 1961. Relationships of pH of pond waters to their suitability for fish culture. Proceedings Pacific Scientific Congress 9 (1907), Vol. 10. *Fisheries* 72-75.

Tahmina, H., Rakibul, H., Nazmul, H., and Md, A., 2015. The Effect of Chromium on Glucose Content of Freshwater Fish, *Heteropneustes fossilis*. *American Journal of Zoological Research* 3 (1): 1-3. doi: 10.12691/ajzr-3-1-1.

Taiwo, V. O. 2005. Lecture note on fish diseases and pathology, University of Ibadan, Ibadan. pp25.

Talas, Z. S., Orun, I., Ozdemir, I., Erdogan, K., Alkan, A. and Yılmaz, I. 2008. Antioxidative role of selenium against the toxic effect of heavy metals (Cd+2, Cr+3) on liver of rainbow trout (*Oncorhynchus mykiss* Walbaum 1792). *Fish Physiology and Biochemistry* 34: 217–222. doi: 10.1007/s10695-007-9179-9

Taylor, D. 1983. The significance of the accumulation of cadmium by aquatic organisms. *Ecotoxicol. and Environ. Safety.* 7 (1): 33-42.

Teh, S. J., Adams S. M. and Hinton D. E. 1997. Histopatological biomarkersnin freshwater fish populations exposed to different types of contaminant stress. *Aquat Toxicol* 33: 75 – 85.

Teugels, G. G. 1982. Preliminary results of a morphological study of five African species of the subgenus Clarias (Pisces: Clariidae) Journal of Natural History 16 (3): 439-464.

Tewe, O. O. 1997. Sustainability and Development: Paradigms from Nigeria Livestock Industry. Inaugural Lecture Faculty of Agriculture and forestry, University of Ibadan 72pp.

The toxic farms of China www.thefishsite.com/fishnews/7404/the-toxic-fish-farms-of-china/print 7/20/2008.

Thomaston, W. W. and Zeller, H. D. 1961. Results of a six-year investigation of chemical soil and water analysis and lime treatment in Georgia fish ponds. Proceedings Annual Conference South Eastern Association Game and Fisheries Commission 15: 236-245.

Tjalkens, R. B., Valerio L. G, Jr., Awasthi Y. C., Petersen D. R., 1998. Association of glutathione S-transferase isozyme-specific induction and lipid peroxidation in two inbred strains of mice subjected to chronic dietary iron overload. *Toxicol Appl Pharmacol* 151: 174–81.

Ugwumba, A. O. and Ugwumba A. A. 2003. Aquaculture options and the future of fish supply in Nigeria. *The Zoologist* 2 (2): 96 – 122.

Valerino, D. M. and McCormark, J. J. 1971. *Biochem. Pharmacol* 20: 47.

Vallachira, A. 1998. Veterinary Materia Medica. First edition. Jaypee Brothers Medical Publishers (P) Ltd. B-3 EMCA House, 23/23 B Ansari Road, Daryagonj, Post Box 7193, New Delhi 110002, India. 229 pp.

Van der Oost, R., Beyer, J. and Vermeulen, N. P. E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *environmental toxicology and pharmacology* 13: 57-149.

van Vuren, J. H. J. 1980. The Plasma proteins of some freshwater fish in their natural environment. *J. Lim Soc of Southern African*, 6 (2): 96-100.

van Vuren, J. H. J. 1986. The effects of toxicants on the haematology of *Labeo umbratus* (Teleostei; cyprinidae). *Comparative Biochemistry and Physiology* 83C: 155-159.

Vanderberg, G.W., Leatherland, J. F. and Moccia, R. D. 1998. The effects of beta-agonist ractopamine on growth hormone and intermediary metabolite concentrations in rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* 29: 79-89.

Varshney, R. and Kale, R. K. 1990. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *Int. J. Radiat. Biol* 58: 733-743.

Veith, G. D., Defoe, D. L. and Bergstedt, B. V. 1979. Measuring and Estimating the Bioaccumulative Factor of Chemical in Fish. *J. Fish Res Board Can* 36: 1040-1047.

Velma, V., Vutukuru S., Tchounwou P. B. 2009. Ecotoxicology of hexavalent chromium in freshwater fish: a critical review. *Reviews on environmental health* 24 (2): 129.

Verma, S. K., Jain V. and Verma D. 2008. Garlic - "The spice of life": composition, cooking chemistry and preparations. *Journal of Herbal Medicine and Toxicology* 2 (2): 21-28.

Verma, S. R., Rani, S. and Delela, R. C. 1981. Isolated and combined effects of pesticides on serum transaminases in *Mystus vittatus* (African catfish). *Toxicol. Lett*. 8: 67- 71.

Vesey, D. A. 2010. Transport pathways for cadmium in the intestine and kidney proximal tubule: Focuson the interaction with essential metals. *Toxicol. Letters.* 198(1): 13-19.

Warner, C., Mark., and Whitney W. R. (2006). Comparison between serum values of pond and intensive race way culture, channel catfish *Ictalurus punctatus* (Rafinesque). *Journal of fish biology* II (5): 385-391.

Watanabe, T., Viswanath, K. and Shuichi, S. 1997. Trace minerals in fish nutrition. *Aquaculture* 151: 85-207.

Water management in fish farming http://fisheryfarming.blogspot.com.ng/search?q=TDS, Posted Monday, 9 February 2015.

Watson, R. M. 2001. Evaluation of fish health assessment index as Biomonitoring tool for heavy metal contamination in the Olifants river catchment area. Doctoral Thesis. Johannesbury Rand Afrikaans University.

Weatherley, A. H. and Gill, H. S. 1983. Relative growth of tissues at different somatic growth rates in rainbow trout *Salmo gairdneri* Richardson. *J. Fish Biol.* 22: 43–60.

Webb, M. A., Feist, G. W., Fitzpatrick, M. S., Foster, E. P., Schreck, C. B., Plumlee, M., Wong, C. 2006. Mercury concentrations in gonad, liver, and muscle of white sturgeon acipenser transmontanus in the lower columbia river. *Archives of environmental contamination and toxicology* 50 (3): 443-451.

Wedemeyer, G. A., McLeay, D. J. and Goodyear, C. P. 1984. Assessing the tolerance of fish and fish population to environmental stress. The problems and methods of monitoring. Pages 163-190 in V.W. Cairns, P. V. Hodson, and J. O. Nriagu, editors. Contaminants effects on fisheries. Wiley, New York.

Weis, J. S. and Weis P. 1989. Effects of Environmental Pollutant on Early fish development. *Rev. Aquat Sci*. 45-73

Wendelaar-Bonga, S. E. 1997. The Stress response in fish. *Physiological reviews* 77: 591-625.

Wepener, W. 1990. The effects of heavy metals at different pH on the blood physiology and metabolic enzymes in *Tilapia sparmanii* (Cichlidae). M.Sc. Thesis, Rand Afrikaans University, Johannesburg, South Africa.

West, W. Q. B., and Biney, C.A., 1991. African Fisheries and Environment RAFR Publication, FI/91/1. 26pp.

Wester, P. W., Vethaak, A. D. and Van Muiswinkel, W. B. 1994. Fish as biomarkers in immunotoxicology. *Toxicology* 86 (3): 213-232.

WHO, (World Health Organisation), 2003. Malathion in drinking water. Background document for preparation of WHO guidelines for drinking water quality, Geneva, World Health Organistaion (WHO/SEME/WSH/03.04/103).

WHO, 1992. Environmental Health Criteria, No. 134, Environmental aspects, Geneva WHO.

WHO, (World Health Organization) (1989). Guide lines for drinking water quality. Vol. 1, Recommendations WHO, Geneva, p. 19.

Wicklund, A. and Runn, P. 1988. Calcium effects on cadmium uptake, redistribution and elimination in Minnows, *Phoxinus phoxinus*, acclimated to different calcium concentrations. *Aquatic Toxicology*, 13: 109-122.

Wiegertjes, G. F., Stet, R. J. M., Parmentier, H. K. and Van Muiswinkel, W. B. 1996. Immunogenetics of diseases resistance in fish: A comparative approach. *Dev. Comp Immunol* 20: 365-381.

Witeska, M., Malgorzata, E. K. and Katarzyna, S. 2011. The effects of cadmium on common carp erythrocyte morphology polish J. of environmental stud. 20 (3): 783-788.

Witeska, M. 2005. Stress in fish-hematological and immunological effects of heavy metals *Electronic Journal of Ichthyology* 1: 35-42.

Wolf, J. C. and Wolfe M. J. 2005. A brief overview of nonneoplastic heptic toxicity in fish. *Toxicol pathol* 33: 75 – 85

Wong, C. K. and Wong, M. H. 2000. Morphological and biochemical changes in the gills of Tilapia (*Oreochromis mossambicus*) to ambient cadmium exposure. *Aquatic Toxicol.* 48 (4): 517-527

Wu, R. S. S. 2002. Hypoxia: from molecular responses to ecosystem responses. *Marine pollution bulletin* 45: 35-45.

Xue, Y. G., Gu, X. Y., Wang, X. R., Sun, C., Xu, X. H., Sun, J. and Zhang, B. G., 2009. The Hydroxyl Radical Generation and Oxidative Stress for the Earthworm Eisenia Fetida Exposed to Tetrabromobisphenol A, *Ecotoxicology* 18 (6): 693–699.

Yakhnenko, V. M. and Klimenlove, I. V. 2009. Specific features of blood cells composition and structure in fishes from pelegial and coastal zones of Lake Baikal. *Biology bulletin* 36 (1): 37-44.

Yaoling, L., Jiunrong, C., Mengsyh, S., Mingler, S., Li Y. L., Chen, J. R., Shien, M. S. and Shien, M. J. 1998. The effects of garlic powder on the hypolipidemic function and antioxidative status in hamsters. *Nutr. Sci. J*. 23: 171-87.

Younis, E. M., Abdel – wraith, A. A. and Al. Asgah, N. A. 2012. Hematological and enzymatic responses of Nile tilapia *Oreochromis niloticus* during short and long term sublethal exposure to zinc. *African J. Biotechnol* 11 (19): 4442 – 4446.

Yunxia, Q., Jianzhong S. and Guoliang W. 2001**.** A review of principal bacterial diseases of mariculture fish. *Transactions of Oceanology and Limnology* 2: 78-87.

Zeynab, A., Mohammadkazem K., Sohrab K. E. and Hossein R. 2012. Comparison of Lethal Concentrations (LC50-96 H) of Cdcl2, Crcl3 and Pb (NO3)2 in Common Carp (*Cyprinus carpio*) and Sutchi Catfish (*Pangasius Hypophthalmus*) *Iranian Journal of Toxicology* 6 (18): Autumn.

Zinkl, J. G. 1986. Avian Hematology. In: Schalms Veterinary Hematology, Jain, N.C. (Ed.). 4th Edn. Lea and Febiger, Philadelphia, PA., USA., pp: 256-273.

Zyadah, M. A. and Abdel-Baky, T. E. 2000. Toxicity and bioaccumulation of copper, zinc, and cadmium in some aquatic organisms. *Bulletin of Environmental Contamination and Toxicology*, 64: 740–747.

APPENDIX A

PREPARATION OF STOCK

1.0mg/l standard of lead and cadmium were prepared using 0.1599g of lead nitrate and 0.2282g of cadmium sulphate respectively made up to 1000ml with distilled water for sub lethal study.

Cadmium Stock Solution
Cadmium stock of 10,000mg/L standard solution of cadmium was prepared by weighing and dissolving 166.0g of analar grade cadmium sulphate in 9L of deionizd water.
1ml = 1mg cadmium.

Lead Stock Solution
Lead stock of 10,000mg/L standard solution of lead was prepared by weighing and dissolving of 144.0g analar grade lead nitrate in 9L of deionized water (standard volume)
1ml = 1mg lead.

APPENDIX B

Group water and ambient temperature (^{0}C) mean ± SEM during sublethal experimental study.

Month	Time of day	n.	Water temp.	Ambient temp.
November	9.00am	60	25.50 ± 0.50^a	26.60 ± 0.50^a
	12.00moon	60	27.50 ± 0.5^b	32.57 ± 0.50^b
	4.00pm	60	28.60 ± 0.49^c	37.37 ± 0.90^c

Means with the same letter in a column are not significantly different according to DMRT at ($P \geq 0.05$.). There were higher significant ($p \leq 0.05$) value in water temperature in order 4.00>12.00noon>9.00am.

APPENDIX B CONT'D

Group water and ambient temperature (^{0}C) mean ± SEM during sublethal experimental study.

Month	Time of day	n.	Water temp.	Ambient temp.
December	9.00am	60	24.47 ± 0.50^a	27.23 ± 0.81a
	12.00moon	60	28.60 ± 0.49^b	34.85 ± 2.32b
	4.00pm	60	28.50 ± 0.50^c	35.07 ± 2.55^c

Means with the same letter in a column are not significantly different according to DMRT at (P≥0.05.). There were a higher significant (p≤0.05) value in water temperature in order 4.00pm, 12.00noon>9.00am. Also there were higher significant (p≤0.05) value in ambient temperature in order 4.00pm, 12.00noon>9.00am.

Month	Time of day	N	Water temp.	Ambient temp.
January	9.00am	62	23.00 ± 1.34^a	25.00 ± 1.92a
	12.00moon	62	24.06 ± 1.67^b	33.10 ± 1.94b
	4.00pm	62	26.55 ± 1.07^c	37.07 ± 1.40c

Means with the same letter in a column are not significantly different according to DMRT at (P≥0.05.). There were higher significant (p≤0.05) value in water temperature in order 4.00pm > 12.00pm > 9.00am. Also there was high significant (p≤0.05) value in ambient temperature in order 4.00pm > 12.00noon > 9.00am.

APPENDIX B CONT'D

Group water and ambient temperature (^{0}C) mean ± SEM during sublethal experimental study.

Month	Time of day	N	Water temp.	Ambient temp.
February	9.00am	24	25.87 ± 0.48^a	27.50 ± 0.78a
	12.00moon	24	27.58 ± 0.50^b	32.92 ± 1.21b
	4.00pm	24	27.67 ± 1.20^b	36.58 ± 3.69c

Means with the same letter in a column are not significantly different according to DMRT at (P≥0.05.). There was a higher significant (p≤0.05) value in water temperature in order 4.00pm and 12noon > 9.00am. there was no significant difference in 4.00pm and 12.00noon water temperature. Also there were higher significant (p≤0.05) value in ambient temperature in order 4.00pm > 12.00noon > 9.00am.

APPENDIX C

Protocol for protein estimation according to the method of Gornall *et al*., 1949

Test tube no.	**1**	**2**	**3**	**4**	**5**
Stock BSA (ml)	0.1	0.2	0.3	0.4	0.5
Distilled water (ml)	0.9	0.8	0.7	0.6	0.5
Biuret reagent (ml)	4	4	4	4	4
BSA Concentration (mg/ml)	1	2	3	4	5
Absorbance (540 nm)	0.160	0.310	0.462	0.651	0.790

APPENDIX D

Protocol for the estimation of hydrogen Peroxide

Test tube	1	2	3	4	5	6	7
H_2O_2 (ml)	0.05	0.10	0.15	0.20	0.30	0.40	0.50
Dichromate/acetic acid (ml)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Distilled water (ml)	0	0	0	0	0	0	0.50
H_2O_2 concentration (µ mole)	95	90	85	80	70	60	100
Absorbance (570nm)	0.104	0.200	0.300	0.400	0.600	0.800	0.484
	0.049	0.094	0.145	0.199	0.295	0.395	

APPENDIX E

Preparation of GSH Standard Curve

Stock (ml)	PO4 buffer (ml)	Ellman Reagent conc. (ml)	Absorbance (412nm)	GSH (µg/ml)
0.02	0.48	4.5	0.04	8
0.05	0.45	4.5	0.101	20
0.10	0.40	4.5	0.194	40
0.20	0.30	4.5	0.380	80
0.30	0.20	4.5	0.572	120
0.40	0.10	4.5	0.749	160

APPENDIX F

Protocol of hydrogen peroxide generation

Reagents	Volume
Buffer	2.5mls
AFS	250Ml
Sorbitol	100Ml
XO	100µL
H_2SO_4	25Ml
Sample	50µL

APPENDIX G

GPx Assay protocol

Phosphate buffer	500
NaN3	100
GSH	200
H_2O_2	100
Sample	500
Distilled water	600