Chemicals such as industrial effluents induces some level of alterations in the naturally occurring chemical composition of aquatic phase which in turn alters the behavioral, biochemistry, and general physiology of aquatic fauna. Chemical additives effluent was analyzed to determine its physicochemical parameters. The fish, Clarias gariepinus was exposed to lethal concentrations of the effluent for 96 hours and the (96-h LC50) value for the acute toxicity was found to be 0.30mg/L. Mortality of exposed fishes increased as the concentrations of the effluent increases. The impact of long term exposure to the effluent was also evaluated through changes of biochemical and haematological parameters as well as the assessment of heavy metal bioaccumulation in selected biomarkers. It was concluded that fish can bioaccumulate heavy metals from polluted environments. The chemical additives effluent had some degree of negative impact on the biochemistry, haematology and metal bioaccumulation pattern of Clarias gariepinus. This finding should be useful to ecotoxicologists, aquatic resources managers, laboratory scientists, researchers and students at various levels of higher learning.

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Dahunsi Olatunde studied at Ladoke Akintola University of Technology, University of Maiduguri and Covenant University, all in Nigeria. His research interests are Environmental Biomonitoring, Aquatic Toxicology and Ecotoxicology. He is the recipient of international grants, He has attended several international conferences and is a prolific writer.

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Dahunsi Olatunde
Oranusi Solomon

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## An aquatic toxicological research study in tropical Africa

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# DEDICATION

To the one who brought me joy, happiness, fulfillment and makes me feel being a real man, **Beulah Oluwagbemisola**.

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I sincerely appreciate God the giver of life, by whose power and will I have achieved this feat. May all glory and honour be to His name forever. I want to use this medium to appreciate my biological father; Pastor Stephen Olaolu Olawuyi Dahunsi for his tremendous contribution to my life and academic pursuit up till this extent. He has and is still labouring so incensantly both financially and morally to see me become an erudite scholar in life. May your efforts never be in vain in Jesus name. My mother, Lady Evangelist F.B. Dahunsi has done a great job in bringing me up to this starture, she and my siblings (Olawale, Olakunle, Olajide, Olaleke, Olabode and Bolanle) are all appreciated. My treasure, Beulah Oluwagbemisola has been a wonderful blessing to my life, I commend your courage and determination for a successful life and I know together, we shall achieve great feats.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Biomonitoring is a scientific technique for assessing environment degradation including exposures to natural and synthetic chemicals, based on sampling and analysis of an individual organism's tissues, organs and fluids. This technique takes advantage of the knowledge that chemical that has entered the organisms leave behind markers to reflect the exposure. The marker may be the chemical itself or may also be a breakdown product of the chemical or some biological changes in the organisms that is a result of the action of the chemical/toxicant on the individual. The results of these assessments provide qualitative informations about the quantity of both natural and artificial chemicals that have entered and remained in the organisms (via bioaccumulation and biomagnification) and the corresponding effects caused. Due to the consistency between the selected organisms and the corresponding living space, biomonitoring can directly offer the data on the potential effects and actual integrated toxicities of pollutants, reflecting the corresponding level of environmental damage. Precaution may be drawn based on the sensitive biomonitoring of chronic effects induced at low concentration/dose of pollutants for long-term exposure. These characters endowed biomonitoring with attractive advantages of wide practicability, high sensitivity and integration, which are qualities that the conventional chemical analysis lacks (Zhang, 2004). For the biomonitoring of aquatic pollution, the organisms in the given aquatic systems are sampled for the analysis of various biological responses to pollutants exposures. An organism can be termed a perfect bioindicator when it has the following qualties: (1) it can accumulate high levels of pollutants without death, (2) it lives in a sessile style, thus definitely representing the local pollution, (3) it has enough abundance and wide distribution for the repetitious sampling and

comparison, (4) its life is long enough for the comparison between various ages, (5) it can afford suitable target tissue or cell for the further research at microcosmic level, (6) easy sampling and easy raising in the laboratory, (7) it keeps alive in water, (8) it occupy the important position in food chain and well dose-effect relationship can be observed in it (Yan and Lv, 1989). Finding such bioindicator for biomonitoring is a hectic task because the candidate bioindicator with several characters is practicable according to the specific monitoring purpose. There are abundant organisms living in water system such as plankton, sedentary benthos, fish and bacteria which promise the feasibility of biomonitoring. Due to the fact that water quality directly affects their population, species, fecundity, physiology, abundance and living behavior, they may act as the bioindicators for the evaluation of water pollution. The common biomonitoring methods for aquatic metal pollution include biota population, bacteria test, acute and chronic toxicity assays and residue analysis among others. The method of biota population is usually performed by counting the species and amounts of various organisms in the tested water system. Many bacteria live in surface water, ground water, and other natural environmental water, which offers the possibility for water quality assessment especially for hygiene using bacteria test most probable of which is the Coliform test. Fish and algae are usually used for the acute toxicity assay of pollutants such as industrial effluents, pesticides and heavy metals. The data on half lethal or effect concentration ($LC_{50}$ or $EC_{50}$) obtained from these assays can serve as the powerful evidence for the enactment of water quality regulations for industrial effluent discharge regarding various pollutants. It can also be used for the risk assessment of the pollution levels of the water bodiesas well estimation of water treatment performance. Researches on chronic toxicity of pollutants at low levels may range from molecular reaction to individual alterations, including genotoxicity, embryo toxicity, histopathological alterations, physiological changes and behavioral

abnormality etc. Biomonitoring using chronic toxicity assay may sensitively indicate the pollution stress posed by the pollutants at sublethal levels. Residue analysis can afford the information on the accumulation, distribution and transfer properties of the pollutants in the target organisms by the chemical analysis due to the occurrence of bioaccumulation and biomaginification for many xenobiotics in aquatic organisms. Other methods like productivity determination can also reflect aquatic pollution by measuring other parameters like the chlorophyll contents, photosynthesis and nitrogen fixation in aquatic plants (Yang, 2006). When compared with the conventional chemical analysis of aquatic environmental matrix, i.e. water and sedimentanalysis, biomonitoring exhibits obvious predominance as follows: (1) biomonitoring reveals the subtle biological changes of organisms affected by exogenous chemicals, which is usually missed by the conventional chemical analysis; (2) reveals the integrated effects of the complex pollutants on the organisms in the environment; (3) has high sensitivity due to the rapid responses induced in the organisms exposed to pollutants, which helps to the declare of the precaution; (4) realizes the monitoring of the pollutants at low levels which were below the detection limits of the instrumental analytical techniques due to the occurrence of the chronic toxicities of the pollutants in the organisms under long-term exposure; (5) allows widely sampling even at remote areas; (6) avoids the limits of the convention chemical analysis such as continuous sampling, needs of expensive instruments. As an appealing tool, biomonitoring exerts unparalleled functions in the evaluation of environmental pollution, especially for the metal pollution in aquatic ecosystem.

### 1.1. Bioindicators for aquatic pollution

The typical method for biomonitoring is based on bioindicators. As shown in a review concerned with the used of bioindicators by Burger (2006), over 40% of the bioindicator papers were about metal pollution, wherein plants, invertebrates, fish,

mammals were the dominant used bioindicator species. For aquatic metal pollution, the common used bioindicators includes plankton, insects, mollusks, fish, plant, bird etc. Each bioindicator show the special merits for the biomonitoring of metal pollution in aquatic ecosystem when compared to the others.

**1.2. Biochemical and Haematological alterations**

With the development of biological techniques, researches on the interaction between the pollutants and biological macromolecules such as protein, enzyme and nucleic acid may indicate the action mechanism of the pollutants. Precaution can thus be sensitively made at various levels. Many biomarkers have currently been developed such as metallothionein (MT), oxidative stress, cytotoxicological responses such as genotoxicity, lysosomal alterations, immunocompetence and gencholinesterase activities etc. Some special proteins can be purified to serve as biomarker for metal exposure as well. Suitable selection of biochemical and haematological biomarkers should be made based on specific conditions such as target pollutants, tested organisms, investigated areas etc. MTs are a kind of soluble metal-binding proteins with low molecular weight. It exists in most eukaryotes where its primary role is the regulation of homeostasis of the essential metals especially copper and zinc. They have an additional protective role through their binding of toxic metals such as cadmium and mercury. Depending on the full understanding of its function and on the possibility of measuring its concentration in tissues, MT may be regarded as an early sign of alarm in the early stage of heavy metals contamination. Increased expression of MT in response to harmful levels of these metals has been demonstrated for several aquatic species such as gastropods, insects, crustaceans, mussels and fishes. A high responsiveness was found in MT induction by cadmium in two crustaceans and a clear relationship between cadmium concentration in water and MT levels in tissues existed (Ramo

*et al*., 1995). In another finding, MT levels in the whelk *T. clavigera* from 11 sites in the coastal waters of Hong Kong were significantly correlated with Cadmium body concentrations and a clear relationship between the dissolved Cadmium exposure and the resultant MT levels in the animals was established, which is an indication that MTs are credibility biomarkers for the evaluation of some related metal exposure (Graham and Wang, 2004). Simultaneous study of MT contents and lysosomal membrane stability (LMS) in *Mytilus galloprovincialis* (L.) showed that MT contents were significantly less and LMS values were significantly greater in mussels collected from the reference station compared to those from heavy metal (Cd, Pb, Cu and Zn) polluted sites in the Gulf of Thermaikos (Domouhtsidou *et al*., 2004), showing the feasibility of actual use of MT in the assessment of environmental pollution. Metallothionein mRNA was also reported to be implicated for biomonitoring. Quantitation of MT mRNA from the New Zealand common bully (*Gobiomorphus cotidianus*) could indicate the expression of MT in the liver tissue due to copper exposure for 48 hours. In addition, the heptic MT mRNA levels do not correlate with fish age, sex or sampling location, which avoids other factors' disturbance during heavy metal biomonitoring process. A comparison of two populations of common bullies from a polluted and a control site showed a 2-fold higher mean MT mRNA levels in fish from the polluted site, proving the efficiency of using MT mRNA as biomonitoring tool (Laurie, 2004). Chlorophyll a fluorescence, as a potential valuable ecotoxicological endpoint, could be used with a range of aquatic phototrophs. Chlorophyll a fluorescence-based ecotoxicological bioassays have been applied in the assessment of aquatic pollution including heavy metals. The main advantages are that it is rapid, non-invasive and non-destructive, while the major weakness is the lack of clear ecological relevance. Future research focusing on aquatic chlorophyll a fluorescence ecotoxicology may be on the standardization of test protocols and

statistical techniques (Ralph *et al*., 2007). Biomarkers indicating oxidative stress in the various organisms are proposed for the biomonitoring of aquatic metal pollution. In a research by Farombi *et al*., (2007), the activities of superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST), glutathione (GSH) concentration and malondialdehyde (MDA) formation were reported to investigate the oxidative stress in African cat fish (*C. gariepinus*) from Nigeria Ogun River with metal pollution (Zn, Cu, Cd, As and Pb). He demonstrated that alterations in antioxidant enzymes, glutathione system and lipid peroxidation reflected the presence of heavy metal in the corresponding tissues, therefore confirming a rational use of biomarker of oxidative stress in biomonitoring of aquatic metal pollution. Antioxidant and biotransformation enzymes in *Myriophyllum quitense* were reported as biomarkers of heavy metal exposure in Auquia River basin. *M. quitense* reacted to the pollution stress increasing the activity of GST, glutathione reductase and peroxidase. Elevated enzyme activities agreed to different pollution levels, especially inorganic nitrogen loads combined with elevated lead and aluminum concentrations, thus presenting *M. quitense* as a good biomonitor for assessment of water quality in the polluted aquatic ecosystem (Nimptsch *et al*., 2005). Biochemical stress of glutathione (GSH) levels in aquatic moss *Fontinalis species* showed alterations due to $Cd^{2+}$ over 10-day exposure period (Bleuel *et al*., 2005). Test of GST activity in two marine gastropods (*Monodonta lineate* and *N. lapillus*) exposed to copper and cadmium showed a significant reduction of *N. lapillus* GST after copper exposure but had no effect on *M. lineate* GST, indicating that the selection of suitable biochemical biomarkers should consider the experimental species as well as the pollutant in question (Cunha *et al*., 2007). Cellular biomarker including genotoxicity, lysosomal alterations, immunological responses and other protein or enzyme index, can also be used for monitoring estuarine environments. Genotoxic effects are evaluated as

DNA strands breaks by single cell gel electrophoresis (or Comet assay) and as chromosomal alterations occurs by the micronucleus test in gill cells. Lysosomal alterations are assessed by the neutral red retention time (in haemocytes), lipofuscin accumulation, ultrastructure, size and number (in digestive cells). The phagocytic activity is used to evaluate the immunocompetence. Bolognesi *et al*., (2005) found Micronucleus frequency to correlate with Mercury concentration in *M. galloprovincialis*. Likewise, a general genotoxicity and lysosomal alterations were found in the Mediterranean mussel (*M. galloprovincialis*) from the River Cecina, accompanied by an elevation of tissue metal levels. Those based on DNA and lysosomal membrane integrity exhibited early biomarkers as they were induced at similar degree in native and transplanted mussels, while the alterations of micronuclei frequency, lipofuscin accumulation and mean lysosomal diameter resulted from cumulative pollution events (Nigro *et al*., 2006). Based on genetic variation, the correlation between a particular metal and the bands resulting from the use of a specific RAPD primer on *P. viridis* was reported as an efficient biomonitoring tool of heavy metal contamination (Yap *et al*., 2007). A dose and time response both in phagocytic activity of haemocytes and lysosomal structure in the Asian clam (*Corbicula fluminea*) exposed to 10_$g^{L-1}$ of cadmium demonstrated their use as biomarkers in freshwater biomonitoring (Champeau, 2007). The enzymes cholinesterases (ChEs) showing properties of both typical acetylcholinesterase and pseudocholinesterase in foot muscle of *M. lineate* and *N. lapillus* were found to increase by cadmium in vivo exposure and inhibited by copper in vitro exposure (Cunha *et al*., 2007). Some new biomarkers are now being explored for feasibility of its practical use. A polypeptide of 22 kDa of molecular weight (LF22) was induced in *Limnoperna fortunei* exposed to sublethal levels of Cd II, Cu II and Hg II. The concentration of LF22 was tripled in the presence of Cd II, indicating it as a useful biomarker of heavy metal exposure (Belaich *et al*.,

2006). High sensitivity and high speciality of these biomarkers responding to the aquatic metal pollution can exhibit the deleterious effects as well as the potential toxic mechanisms.

**1.3. Morphological and behavioral observation**

Morphological and behavioral observations provide the most direct effects of toxicants on the organisms, which can be easily noticed, thus being an interesting topic of the related fields. In the biomonitoring of metal pollution in aquatic ecosystem, many techniques based on morphological observation have been developed using various model organisms. Some of them are standardized as the criteria for the evaluation of individual or combined toxicities and for the risk assessment of environmental pollution. Acute lethal assay is the most common method for the toxicity evaluation of chemicalsand other xenobiotics including metal compounds. Many organisms are involved ranging from zooplankton to fish while many of them has been standardized as the routine tests. The index of 12, 24, 96-h $LC_{50}$ and others have been used for the toxicity classification of the chemicals. The results of lethality can contribute to the perfect references for the accurate selection of the sublethal levels of pollutants for simulative exposure experiments and also provide the useful information on the right comparative assessment of environmental pollution. Other sublethal toxic effects have been proposed as the biomonitoring approaches for the more sensitive evaluation of chemical exposure compared to lethality. The toxicity endpoints include avoidance, feeding depression, valve closure behavior etc. The parameters such as $EC_{50}$, LOEC etc. are used in this aspect. If avoidance (sensu evasion, displacement) of contaminants occurs in real situations, then bioassays involving forced exposure severely underestimate pernicious effects of contamination. The study on the avoidance of copper contamination by field populations of *Daphnia longispina* showed significant avoidance to copper when exposed to a gradient from 3 to

87_g$^{L-1}$. An intense association was observed between other endpoints and avoidance, furthermore, avoidance was much more sensitive than lethality (Lopes, 2004). As a complementary tool, avoidance assays is recommended for ecological risk assessments and effluent biomonitoring because such assays can provide cost-effective and ecologically relevant information. The chronic feeding assays appear to be a rapid, cheap and effective tool to be used in biomonitoring studies. An increase in cadmium and zinc at sublethal levels resulted in significant reductions of the feeding rate of two freshwater crustaceans: *Atyaephyra desmarestii* (Decapoda) and *Echinogammarus meridionalis* (Amphipoda). The LOECs of zinc were 1.29mg$^{L-1}$ for *A. desmarestii* and 0.4mg$^{L-1}$ for *E. meridionalis*. The LOEC of cadmium was 6.53_g$^{L-1}$ for both species (Pestana *et al*., 2007). Based on the closure daily rhythm and the corresponding dose-response indices, the valve closure behavior in the clam such as *C. fluminea* can be also used as a toxicity endpoint for the biomonitoring of the aquatic heavy metals (Liao *et al*., 2007). For aquatic plants, parameters such as foliar injury, chlorophyll content and phytomass may indicate the harmful effects after exposure to heavy metal. Perceptible effects with increasing exposure to the metal was obtained based on these parameters when three aquatic plants (*Hydrilla verticillata* Presl, *Pistia stratiotes* L. and *Salvinia molesta* D.S. Mitchell) were treated with different concentrations of mercury. A positive relationship between leaf injury index (LII) and doses of the metal was obtained in the case of floating plants (Mhatre and Chaphekar, 1985). Simple bioassay based on these parameters is equally feasible in biomonitoring and toxicity studies.

**1.4. Bioaccumulation**

Bioaccumulation is an important process through which chemicals can affect living organisms. An increase in the concentration of a chemical in a biological organism over time may occur compared to the chemical's concentration in the environment.

Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost. Several processes including uptake, storage and elimination are involved during bioaccumulation.

More and more attention has been drawn due to the wide occurrence of metal pollution in aquatic system. Some heavy metals may transform into the persistent metallic compounds with high toxicity, which can be bioaccumulated in the organisms, magnified in the food chain, thus threatening human health and existence (Jin, 1992). Various harmful effects including abnormal development of fetus, procreation failure, mutation, cancers and immunodeficiency has been exhibited in humans due to aquatic metal exposure (Chang *et al*., 2000). Monitoring and prevention of heavy metal pollution is one of the hot topics in environmental researches. Heavy metals in aquatic system can be naturally produced by the slow leaching from soil/rock to water, which are usually at low levels, and as such causing no serious deleterious effects on human health (Chang *et al*., 2000). However, the development of industry and agriculture over the decades has promoted the rapid increase of environmental metal pollution and the resulting degradation. Aquatic heavy metal pollution usually represents high levels of Hg, Cr, Pb, Cd, Cu, Zn, Ni etc. in water bodies (Huang and Li, 2002; Liang *et al*., 2004). The anthropogenic activities such as discharge of heavy metal wastewater contribute to the predominant causation. The wastewater in most cases originates from mining, mill run, metallurgy, plating, chemical and pesticides plants, curry and paper making industries. Although some metallic compounds can be strongly absorbed onto the suspended particles and sediments, they are able to be released into the water under the suitable conditions such as pH values and Eh, leading to further contamination of aquatic milleu (Xu and Yang, 1996). Some heavy metal including Hg, Cr, Cd, Ni, Cu, Pb etc. introduced into environmental water system may pose high toxicities on the aquatic organisms (Wu and Zhao,

2006). As an example, cadmium is a priority environmental contaminant with consequences for human health and the maintenance of bio-diversity in affected ecosystems. Ecosystem-based approach to cadmium research is highlighted based on the overview of recent developments in the field by Campbell, (2006). Wide occurrence of metal pollution exists worldwide now, including China. For example, investigations on Yangtze River showed the occurrence of the various levels of heavy metal in alongshore-aquatic areas with the predominant elements of Zn, Pb, Cd, Cu, Cr. Some elements with high affinity to sulfur atoms such as Cd, Pb, Hg and Cu detected in Yangtze River are liable to produce the potential toxicity (SEPA, 2001). Survey on the water quality in Shanghai City showed that Cd was the main pollutant, while Hg was at the second highest level. Determination of Cu, Pb, Zn and Cd in the surface sediments in Huangpu River indicated that the level of Pb in the mainstream was twice the national water quality standard. Serious pollution of Cu, Zn, Cd and Pb was found in nine branch rivers, wherein 100, 75, and 62.5% of samples contained the high levels of Pb, Cd and Hg, respectively, which all exceeded the corresponding national water quality standard limits in Suzhou River (Chang and Qu, 2005). Different levels of various metal pollutants are reported in many other inland and marine water systems in China (Chen, 2002).

Considering the use of some rivers and lakes as water supplies, threats are thus posed on human health via drinking water, polluted vegetable and foodstuff etc. besides the disruption of the natural environment. Chemical and physical analyses of the environment matrix such as water and sediment are the most direct approach to reveal the heavy metal pollution status in the environment, while it cannot afford the powerful evidence on the integrated influence and possible toxicity of such pollution on the organisms and ecosystem in general. Bioaccumulation usually results from a dynamic equilibrium between exposure from the outside

environment and uptake, excretion, storage, and degradation within an organism. Understanding of the dynamic process of bioaccumulation is a critical consideration in the regulation of chemicals such as aquatic metals. As exemplary, a simulative exposure experiment was carried out to test the suitability of *Mya arenaria* as a new sensitive biomonitor of butyltin (TBT) pollution in the marine system based on its special high bioaccumulation ability for butyltin compounds (Zhou *et al*., 2003). According to the first-order kinetic model developed by Yamada and Takayanagi, (1992), the kinetic parameters of the accumulation rate constant (Ku) and bioconcentration factor (BCF) were calculated. The parameter of Ku ranged between 0.54 and 2.97 for *M. arenaria* and from 0.062 to 0.30 for *Mytilus edulis*, respectively. The BCF ranged from 15538 to 91800 for *M. arenaria* and from 1813 to 9000 for *M. edulis* (control species), respectively, after 28 days exposure. During the subsequent depuration test, it was found that the depuration rate constant was in the range of 0.0074 to 0.0098 $day^{-1}$ for *M. arenaria* and 0.019 to 0.0328 $day^{-1}$ for *M. edulis* based on first-order kinetics (Gomez-Ariza *et al*., 1999). The biological half-life of TBT elimination ($t1/2$) ranged from 71 to 94 days for *M. arenaria* and from 21 to 36 for *M. edulis*. The extremely high level of TBT in *M. arenaria* showed that it has stronger ability to accumulate or lower rate to metabolize this kind of environmental pollutants than other sampled species, which promised *M. arenaria* as a potential new biomonitor to indicate butyltin pollution in oceanic environment (Yang *et al*., 2006).

**1.5. Fish as Indicator Organism**

Fish has attracted much attention in the biomonitoring of water pollution due to its special biological characters such as relatively big body size, long life cycle, easy to culturing etc. More importantly, fish species are at the top position in the aquatic food chain and may directly affect the health of humans, which makes it much of significance for the biomonitoring process. In the beginning of 1990s, lethal test of

fish was proposed to evaluate marine pollution and was widely used as the main biomonitoring method. Behavioral response of fish was also suggested to evaluate the toxicity of the pollutants (Carns, 1981). The acute lethal rate, growth, reproduction, metabolism and fecundity of the fish are all veritable biomarkers for biomonitoring of aquatic pollution. Various fish species have been reported in this respect such as zebrafish, catfish, tilapia, medaka, the Chinese rare minnow, loach etc. As an example, acute and chronic toxicities of cadmium on the juvenile loach showed good dose-related effects could be induced. The 24 and 48-h $LC_{50}$ were 1.22 and 0.85mg$L^{-1}$ respectively. The lowest observable effect concentration was 0.08mg$L^{-1}$ and the highest unobservable effect concentration was 0.04mg$L^{-1}$. The results indicated the Chinese loach could serve as the suitable bioindicator for heavy metal pollution (Jia, 2001). A comprehensive review article described fish bioaccumulation and biomarkers in environmental risk assessment and a suite of fish biomarkers such as metallothioneins (MTs), hematological and biochemical parameters, immunological parameters, reproductive and endocrine parameters, histological and morphological parameters were employed to evaluate exposure to or effects of environmental metal pollution on aquatic ecosystems (van der Oost *et al*., 2003). Relative long experimental period and high cost, however, sometimes limit the use of fish species in biomonitoring procedures.

### 1.6. Catfishes

Catfishes form a well diversified group of ray-finned fish which are named for their prominent barbels. They range in size and behavior from the heaviest and longest, the Mekong giant catfish from Southeast Asia and the second longest, the wels catfish of Eurasia, to detritivores and even to a tiny parasitic species commonly called the candiru, *Vandellia cirrhosa* (Wikipedia, 2012). There are armour-plated types and also naked types, which are scaleless. Not all catfishes

have prominent barbels as some are defined by features of their skull and swimbladder. Catfishes are of considerable commercial importance; many of the larger species are reared for foods while many of the smaller species are important in the aquarium hobby. Catfish are nocturnal (Animal world, 2012; Scientific American, 2012).

### 1.6.1. Distribution and habitat

Catfish species are commonly found inhabiting inland or coastal waters of every continent except in the Antarctica. They are most diverse in tropical South America, Africa, and Asia (Lundberg and Friel, 2003) and more than half of all species live in the Americas. They are the only ostariophysans that have entered freshwater habitats in Madagascar, Australia, and New Guinea (Bruton, 1996).

They are found in freshwater environments, though most inhabit shallow and running water (Bruton, 1996). Members of not less than eight families lives underground (hypogean) with three families that also inhabit caves i.e. troglobitic (Langecker and Longley, 1993). One such species *Phreatobius cisternarum*, is known to live underground in phreatic habitats (Froese and Daniel, 2007). Numerous species from the families Ariidae and Plotosidae, and a few species from among the Aspredinidae and Bagridae, are found in salt water (Schafer, 2005; Monks, 2006).

### 1.6.2. Morphology

Most catfishes are bottom feeders, negatively buoyant, meaning that they will usually sink rather than float due to a reduced gas bladder and a heavy, bony head. They have a variety of body shapes, though most have a cylindrical body with a flattened ventrum to allow for benthic feeding (Bruton, 1996). The flattened head

allows for digging through the substrate as well as perhaps serving as a hydrofoil. Most have a mouth that can expand to a large size and contains no incisiform teeth; catfishes generally feed through suction or gulping rather than biting and cutting prey (Bruton, 1996). However, some families, notably Loricariidae and Astroblepidae, have a suckermouth that allows them to fasten themselves to objects in fast-moving water. Catfishes also have a maxilla reduced to a support for barbels; this means that they are unable to protrude their mouths as other fish such as carp. Catfishes are scaleless; their bodies are often naked. In some species, the mucus-covered skin is used in cutaneous respiration, where the fish breathes through its skin (Bruton, 1996).

Juvenile catfishes, like most fish have relatively large heads, eyes and posterior median fins in comparison to larger, more mature individuals. These juveniles can be readily placed in their families, particularly those with highly derived fin or body shapes; in some cases identification of the genus is possible. As far as known for most catfishes, features that are often characteristic of species such as mouth and fin positions, fin shapes, and barbel lengths show little difference between juveniles and adults. For many species, pigmentation pattern is also similar in juveniles and adults. Thus, juvenile catfishes generally resemble and develop smoothly into their adult form without distinct juvenile specializations. Exceptions to this are the ariid catfishes, where the young retain yolk sacs late into juvenile stages, and many pimelodids, which may have elongated barbels and fin filaments or coloration patterns (Lundberg *et al*., 2004).

### 1.6.3. Taxonomy

The catfishes are a monophyletic group. This is supported by molecular evidence (Sullivan *et al*., 2006). Catfish belong to a superorder called the Ostariophysi,

which also includes the Cypriniformes, Characiformes, Gonorynchiformes and Gymnotiformes, a superorder characterized by the Weberian apparatus. Some place Gymnotiformes as a sub-order of Siluriformes, however this is not widely accepted. Currently, the Siluriformes are said to be the sister group to the Gymnotiformes, though this has been debated due to more recent molecular evidence (Nelson, 2006). As of 2007 there are about 36 extant catfish families, and about 3,093 extant species have been described (Ferraris *et al*., 2007a). This makes the catfish order the second or third most diverse vertebrate order; in fact, 1 out of every 20 vertebrate species is a catfish (Lundberd and Friel, 2003).

The taxonomy of catfishes is quickly changing. In some 2007 and 2008 papers, *Horabagrus*, *Phreatobius*, and *Conorhynchos* were not classified under any current catfish families (Ferraris *et al*., 2007b). There is disagreement on the family status of certain groups; for example, Nelson (2006) lists Auchenoglanididae and Heteropneustidae as separate families, while the All Catfish Species Inventory (ACSI) includes them under other families. Also, FishBase and the Integrated Taxonomic Information System lists Parakysidae as a separate family, while this group is included under Akysidae (Nelson, 2006; Catfish families, 2007; Froese and Daniel, 2007; ITIS, 2007). Many sources do not list the recently revised family Anchariidae (Heok Hee and Sparks, 2005). The family Horabagridae, including *Horabagrus*, *Pseudeutropius*, and *Platytropius*, is also not shown by some authors but presented by others as a true group (Sullivan et al., 2006). Thus, the actual number of families differs between authors. The species count is in constant flux due to taxonomic work as well as description of new species. On the other hand, our understanding of catfishes should increase in the next few years due to work by the ACSI (Nelson, 2006).

The rate of description of new catfishes is at an all-time high. Between 2003 and 2005, over 100 species have been named, a rate three times faster than that of the past century (Ferraris *et al*., 2005). In June, 2005, researchers named the newest family of catfish, Lacantuniidae, only the third new family of fish distinguished in the last 70 years (others being the coelacanth in 1938 and the megamouth shark in 1983). The new species, Lacantuniidae, *Lacantunia enigmatica*, was found in the Lacantun river in the Mexican State of Chiapas (Rodile-Hernandez *et al*., 2005).

According to morphological data, Diplomystidae is usually considered to be the most primitive of catfishes and the sister group to the remaining catfishes, grouped in a clade called Siluroidei. Recent molecular evidence contrasts the prevailing hypothesis, where the suborder Loricarioidei are the sister group to all catfishes, including Diplomystidae (Diplomystoidei) and Siluroidei; though they were not able to reject the past hypothesis, the new hypothesis is not unsupported. Siluroidei was found to be monophyletic without Loricarioid families or Diplomystidae with molecular evidence; morphological evidence is unknown that supports Siluroidei without Loricarioidea (Sullivan *et al*., 2006).

**1.6.4. Aquaculture**

Catfishes are easy to farm in warm climates, leading to inexpensive and safe food at local grocers. About 60% of U.S. farm-raised catfishes are grown within a 65-mile (100-km) radius of Belzoni, Mississippi (Morris, 1993). Channel catfish (*Ictalurus punctatus*) supports a $450 million/yr aquaculture industry (Lundberg and Friel, 2003). Catfishes cultured in inland tanks or channels are considered safe for the environment, since their waste and disease are contained and not spread to the wild (Rogers, 2006). In Asia, many catfish species are important as food. Several walking catfish (Clariidae) and shark catfish (Pangasiidae) species are heavily cultured in Africa and Asia (Growfish).

Moreover, catfishes have widely been caught and farmed for food for hundreds of years in Africa, Asia, Europe, and North America. Judgments as to the quality and flavor vary, with some food critics considering catfish as being excellent food, while others dismiss them as watery and lacking in flavor (Baker, 1988). In Central Europe, catfishes were often viewed as a delicacy to be enjoyed on feast days and holidays. Migrants from Europe and Africa to the United States brought along this tradition, and in the Southern United States, catfish is an extremely popular food. The most commonly eaten species in the United States are the channel catfish and the blue catfish, both of which are common in the wild and increasingly widely farmed.

### 1.7. Clarias gariepinus

*Clarias gariepinus* or African sharptooth catfish is a species of catfish of the family *Clariidae,* the airbreathing catfishes.

#### 1.7.1. Natural Distribution

They are found throughout Africa and the Middle East and live in freshwater lakes, rivers, swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems. The African sharptooth catfish was introduced all over the world in the early 1980s for aquaculture purposes and is therefore found in countries far outside its natural habitat like Brazil, Vietnam and India.

#### 1.7.2. Description

The African sharptooth catfish is a large, eel-like fish, usually of dark gray or black coloration on the back, fading to a whitish belly. In Africa, this catfish is second in size only to the Vundu of the Zambesian waters (Ecotravel, South Africa). *Clarias gariepinus* has an average adult length of 1 to 1.5 meters reaching a maximum total length of 170 cm (67.0 inches), (Froese and Daniel, 2011). These fish have slender

bodies, a flat bony head, notably flatter than in the genus *Siluris*, and a broad, terminal mouth with four pairs of barbels. They also have a large, accessory breathing organ composed of modified gill arches. Also, only the pectoral fins have spines. They can weigh up to 29 kg (Ecotravel, South Africa).

### 1.7.3. Habits

It is a nocturnal fish like many other catfishies; it feeds on living as well as dead animal matter. Because of its wide mouth, it is able to swallow relatively large prey whole. It has been known to take large waterbirds such as the Common Moorhen (Anoop *et al*., 2009). It is also able to crawl on dry ground to escape drying pools. Furthermore, it is able to survive in shallow mud for long periods of time, between rainy seasons thereby earning the name, Mud fish. African catfish sometimes produce loud croaking sounds, similar to the voice of the crow.

### 1.7.4. Rearing of *Clarias gariepinus*

The rearing of the African sharptooth catfish in Africa started in the early 1970s in Central and Western Africa as it was realized that it was a very suitable species for aquaculture as:

- It grows fast and feeds on a large variety of agriculture by-products.
- It is hardy and can tolerate adverse water quality conditions.
- It can be raised in high densities resulting in high net yields (6–16 t/ha/year).
- In most countries, it fetch a higher price than tilapia as it can be sold live at the market.
- It matures and is relatively easy to reproduce in captivity.
- It has tolerance for low oxygen and imparts good flavours to food.

The sharptooth catfish, *Clarias gariepinus* is of ecologic importance and commercially valued fish in the Nigerian fishing industry. These sharp-tooth catfish are frequently and widely cultured in ponds, and occur freely in African natural fresh waters.

**Figure 1:** African Catfish (*Clarias Gariepinus*). Source: Wikipedia, 2012.

**1.7.5. Scientific Classification**

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Clariidae

Genus: *Clarias*

Species: *Clarias Gariepinus* (Burchell, 1822)

**1.8. Background of the study**

Excessive deposition of industrial effluents and other xenobiotics into the environment and the resulting pollution is a well known problem in Nigeria. Monitoring this pollution and assessing its effect on organisms is therefore an important issue. This is why it has become important to look for organisms which can be used as biological indicators or monitors of pollutants in the aquatic system. Finding such bioindicator with several characters for biomonitoring is usually an hectic task as abundant organisms living in water system such as plankton, sedentary benthos, fish and bacteria promise the feasibility of the biomonitoring methods. As water quality directly affects their population, species, abundance and living behavior, they may act as the bioindicators for the evaluation of water pollution. In Nigeria, several studies have been carried using fishes and other bioindicator organisms. However, there is need for more studies especially those that has to do with toxicity, bioaccumulation and biomagnification as it affects man rigorously in the food chain.

Also, fish has attracted much attention in the biomonitoring of water pollution due to its special biological characters such as relatively big body size, long life cycle, easy to raise etc. More importantly, fish species are at the top position in the aquatic food chain and may directly affect the health of humans, which makes it much of significance for the biomonitoring using fish. Lethal test of fish as well as

behavioral response are good in evaluating pollutants toxicity, marine and freshwater pollution and are widely used as the main biomonitoring method. The acute lethal rate, growth, reproduction, metabolism and fecundity of the fish can all be used for biomonitoring of aquatic pollution.

**1.9. Statement of the problem**

The Chemical additives effluent used in this study is the waste product of a company producing adhesives, emulsions, resins and other chemicals in an industrialised region of Nigeria. The wastewater is usually partially treated and then chanelled into the nearby river which in turn serves several purposes to the residents of the community by providing them with water for washing, drinking, and also provide them with aquatic resources as many fish species and other Benthic Macroinvertebrates (BMIs) are found in the river which as become a source of income to the local fishermen in the area. Moreover, recent studies in the area have all suggested a high level of metal pollutants in the area. Lead among other metals have recently been found to be bioaccumulated in large quantities in both aquatic organisms and harvested crops (maize) grown within this community hence the evaluation of the toxicity of this Chemical additives effluent as one of the major possible and veritable source of metal pollution in this environment. There is therefore need for qualitative research in aquatic toxicology, aquatic biomonitoring and aquatic pollution using a renewal bioassay procedure in other to evaluate the extent and impacts of pollution activities on aquatic resources as well as on the humans who are the end users of the water and other resources. A research of this nature foccussing on the biochemical and haematological disorderliness as well as monitoring of heavy metals and their subsequent effects on life is essential and will go a long way to contribute to national and state efforts currently being made to sanitise Nigeria and rid her off all sorts of pollution in her numerous and vast water bodies.

### 1.10. Objectives of the study

The objective of this research therefore was to use different biomonitoring tools in the assessment of *Clarias gariepinus* exposed to lethal and sublethal concentrations of chemical additives effluent as a prelude to advice on the need for effective hazard analysis critical point control application in aquaculture, waste and pollution management. This will be achieved by doing the following:

- Evaluating the physical and chemical characteristics of the Chemical additives effluent
- Carrying out the acute toxicity test and evaluating the $LC_{50}$ from the i.e. the concentration of the effluent in the aquatic environment at which half or 50% of the biota will die
- Carrying out chronic toxicity tests using *Clarias gariepinus* as test organism
- Biochemical profile analysis to evaluate the effluent's toxic effect to the fish
- Haematological indices analysis to evaluate the effluent's toxic effect to the fish
- Digestion of fish specimen for metal bioaccumulation analysis
- Statistical analysis of data for conclusions and inferences to be drawn

### 1.11. Scope of the study

This research was limited to the evaluation of the toxicity of Chemical additives effluent using a renewal laboratory bioassay procedure and African Catfish, *Clarias gariepinus* as the model bioindicator organism.

### 1.12. Hypotheses

This study will investigate the following hypotheses:

**Hypothesis 1**: The physicochemical parameters of the chemical additives effluent do not conform to FEPA standard for effluent discharge into the aquatic environment;

**Hypothesis 2**: The chemical additives effluent does not impart significant behavioral response on the treated fishes during the exposure periods;

**Hypothesis 3**: There is no significant correlation between biochemical parameters of treated fishes exposed to different concentrations of the effluent;

**Hypothesis 4**: There is no significant correlation between haematological parameters of treated fishes exposed to different concentrations of the effluent;

**Hypothesis 5**: There is no biomagnification of heavy metals in different biomarkers of the treated fishes after exposure.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

Water pollution may be defined as any impairment in its native characteristics by addition of anthropogenic contaminants to the extent that it either cannot serve to humans for drinking purposes and/or to support the biotic communities, such as fish (Agrawal *et al*., 2010). A change in the quality of water by the presence of toxins/contaminants, makes it potentially harmful to life forms, instead of sustaining them (Agrawal *et al*., 2010). Organic pollution of inland water systems in Africa is alarming, in contrast to the situation in developed countries of the world and it is often the result of extreme poverty, economic and social under-development (Kanu and Achi, 2011). Water resources pollution and contamination in Nigeria and other developing nations has become a serious problem recently. Apparently, human and ecological disorder experienced in industrial settlements as a result of improper disposal of chemicals such as effluent calls for careful surveillance on the state of the environment. Only few chemicals have been ecologically tested in Nigeria for safety in spite of their environmental and ecological impact. The Federal Government of Nigeria has since been emphasizing the need for adequate environmental protection in any technological and socio-economic development by strictly asking industrial operators to sustainably manage the disposal of chemical into natural environment (DPR, 2002).

Exposure of organisms to xenobiotics such pesticides, insecticides, herbicides and other synthetic materials is a serious matter in environmental and toxicological chemistry. In an ecosystem, intricate relationships exist between the organisms and their surroundings, exposure of an ecosystem to such toxicant may result in loss of species diversity, which is an important characteristic of healthy ecosystems

(Olufayo and Alade, 2012). Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. Biological monitoring techniques like haematological and biochemical variables have become attractive and useful for monitoring environmental quality, water pollution, and the health conditions of aquatic organisms (Kohler *et al*., 2007; Kori-Siakpere and Ubogu, 2008; Olufayo and Jatto, 2011). Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety (Ward and Parrish, 1982). Toxic substances may be introduced deliberately or accidentally into the aquatic ecosystem, impairing the quality of water and making it unsuitable for aquatic life. When the concentration of the toxic substance is higher than what the homeoistasis of the aquatic organisms can control, it results in death/ organ or cause damages. For an organism like fish, organs such as opercula, and may also cause physical damages to fish particularly on the skin, liver and gill could be impaired (Oyedapo and Akinduyite, 2011). A toxic substance is a chemical pollutant that is not a naturally occuring substance in aquatic ecosystems. The greatest contributors to toxic pollution are herbicides, pesticides and industrial compounds (Agrawal, *et al*., 2010). The effects of waste waters discharged into water bodies can be acute which occurs rapidly and are clearly defined as fatal and rarely reversible or may be chronic which normally have lingering effects after long period of exposure and may ultimately cause death (Adewoye *et al*., 2005). The entry of toxicants into aquatic media may affect the water quality parameter which in turn leads to changes in the haematological variables of fish, due to its close association with the external environment (Carvalho and Fernandes, 2006; Kavitha *et al*., 2010). The acute toxicity of a chemical can easily be evaluated in a short term test and death determines the end point. From an ecological point of view, survival, growth, reproduction, spawning and hatching success provide reactions and adoption to environmental parameters

regardless of whether they are natural or man-made. Macroscopically, overt signals of toxicity are almost always preceded by changes at the organs, tissues, cellular and molecular levels (Dutta, 1996; Pathak *et al*., 2000, Easley *et al*., 2001; Lohiya *et al*., 2002). Although the aquatic environment is not the ultimate sink and the aquatic invertebrates and fish are not the target organisms, as a toxicant the agent can produce an adverse effect in any biological system, seriously damaging its structure or function or causing death (Easley *et al*., 2001). Adverse response may be defined in terms of a measurement that is outside the "normal" range for healthy organisms, such as abnormal mortality, reproduction or growth. It has been reported that biological monitoring techniques like haematological and biochemical variables are attractive and useful for monitoring environmental quality, water pollution, and the health conditions of aquatic organisms (Celik, 2004; Kohler *et al*., 2007; Kori-Siakpere and Ubogu, 2008; Olufayo, 2009; Kavitha *et al*., 2011). Biochemical biomarkers like glucose, protein, and enzymes are frequently used as an indicator of the general state of health and early warning of stress in fish under stressful conditions (Barnhorn and van-Vureu, 2004; Abou El-Naga *et al*., 2005; Osman *et al*., 2010). Heavy metals have been reported to have negative impact on all relevant parameters and caused histo-pathological changes in fish (Maity *et al.,* 2008).

Fishes exposed to toxicants undergo stress, which is a state of re-established homeostasis, a complex suite of mal-adaptive responses (Chrouses, 1998). Under stress, physiological and biochemical responses may be compromised, becoming detrimental to the fish's health and well being at which point the fish is termed distressed (Barton and Iwama, 1991). Fishes in a contaminated environment show some altered behavioral patterns which may include avoidance, locomotive activity and aggression and these may be attempts by the fish to escape or adjust to the stress condition (Gormley and Teather, 2003; Morgan *et al*., 1991, Olufayo and

Alade, 2012). Metals are transported in the blood by binding to specific plasma protein (Joseph and Raj, 2010). The fishes serves as bio-indicators of water quality and the impact of the chemicals can be well understood by analysing either blood or serum of the fish, because blood is a pathophysiological reflector of whole body (Sharma and Singh, 2004).

A chemical that is toxic to one animal also may be toxic to other forms of life (Munkittrick *et al*., 2005). Oil pollution is one of the environmental constraint that produces aqua-toxicological effects which are deleterious to aquatic life (Agbogidi *et al.,* 2005). Martin-Skilton *et al.,* (2008) demonstrated that acute exposure of juvenile turbot, *Scophthalmus maximus* to the prestige fuel oil elicits alterations in some hepatic biotransformation enzymes with different sensitivities, and leads to decreased levels of testosterone in plasma of juvenile turbot which might threaten reproductive capability of exposed individuals. Saffa and Mohsen, (2011) observed that commercial petroleum fuel had a negative impacy on the growth performance and survival of Nile Tilapia. The eco-physiological effects of crude oil on *Macharium lunatus* has also been reported (Bamidele and Agbogidi, 2006). Abdel-Hadi *et al*., (2011) observed that oxytetracycline induced significant mortality in experimental Tilapia. Many laboratory studies have shown the toxicity of plant extract to fish and changes in haematological and biochemical profiles leading to death of fish (Ayotunde and Ofem, 2008; Olufayo and Jatto, 2011). Botanical products when used extensively may enter aquatic systems such as streams, rivers, and lakes, which may have an effect on non-target organisms in due course of time (Singh and Singh, 2005; Dongmeza *et al.,* 2006; Tiwari and Singh, 2006; Winkaler *et al.,* 2007; Gabriel *et al.,* 2009; Kavitha *et al*., 2011). Adequate management of our environment requires the correct tools which allows us to accurately predict the

fate and effects of contaminants within the environment (Borhan and Rahimah, 2011).

Fish haematology is known to be an essential tool to the fisheries biologist, as it acts as a frontline sensitive indicator of vital physiological and biochemical functions as well as status of nutrition, health, diseases and stress responses of the organism subjected to changes in environmental conditions. Therefore, the striking alterations in the blood parameters and associated pathological changes in fishes under influence of various toxic agents have attracted the attention of workers in the field (Nair, 2002). Bhatia *et al.,* (2004) reported that fish are highly sensitive to very low concentrations of endosulfan and that blood is the primary target of pesticides action. Blood being the medium of intercellular and intracellular transport, which comes in direct contact with various organs and tissues of the blood, the physiological state of an animal at a particular time is reflected in its blood (Sharmila and Marithanayayagam, 2004). Changes in haematology can act as sensitive, early warning signals of the possible toxic effects of metals on health and physiological status (Weeks *et al*., 1992). Haematological parameters are therefore widely used as indicators of condition (Bowerman *et al*., 2000; Dauwe *et al*., 2006; Rogival *et al*., 2006). The amount of red blood cells, haemoglobin concentration, haematocrit and red blood cell indices are indicative for the oxygen transport capacity of the blood (Ots *et al*., 1998). Metals such as cadmium and lead are also known to cause anemia, which might be reflected as lowered haematocrit values, lower haemoglobin concentrations and smaller mean corpuscular volume (Nyholm, 1998; Iolascon *et al*., 2009). Moreover, lead directly influences haemoglobin production through inhibition of the enzyme δ-aminolevuline acid dehydratase, ALAD (Bergdahl, 1998; Papanikolaou *et al*., 2005), which catalyzes the second step in the porphyrin and haem biosynthetic pathway. Zinc, which plays

an essential role in the functioning of this enzyme, is replaced by lead, which causes malfunction of the enzyme. ALAD has been used successfully as a biomarker for metal pollution (Vanparys *et al*., 2008). Haematological characteristics, such as amount of red blood cells, haemoglobin concentration, haematocrit and red blood cell characteristics (mean corpuscular volume and mean corpuscular haemoglobin), were measured to evaluate the impact of metal pollution on haematological status. The haematological status is important for the performance and survival of the bird (Kilgas *et al*., 2006; Nadolski *et al*., 2006). Haematological parameters have already been used as biomarkers for biologically active metal concentrations in organisms. Because of their fast response to metal pollution, these markers can be used to scan for toxic effects in an early stage, for example in humans (Papanikolaou *et al*., 2005) and mice (Rogival *et al*., 2006). The entry of toxicants into aquatic media therefore may affect the water quality parameters which in turn leads to changes in the haematological variables of fish and other aquatic lives due to close association with the external environment (Carvalho and Fernandes, 2006; Kavitha *et al*., 2010).

Heavy metal contamination of aquatic ecosystems has long been recognised as a serious pollution problem (Ayandiran *et al*., 2009). They are particularly severe in their action due to persistence in biological amplification through the food chain (Adami *et al*., 2002; Waqar, 2006; Vutukuru, 2005; Olojo *et al*., 2005; Erdogrul and Erbilir, 2007; Senthil *et al*., 2008; Honggang *et al*., 2010). Heavy metals have long been recognized as serious pollutants of the aquatic system because contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosylene and Jankaite, 2006; Farombi *et al*., 2007). The heavy metals that are toxic to many organisms at very low concentrations and are never beneficial to

living beings are Hg, Cd and Pb (Dural *et al*., 2006). Mercury is classified as one of the most toxic metals, which are introduced into the natural environment by human interference (Ishikawa *et al*., 2007). Lead is a potentially toxic chemical that may be directly ingested by man or indirectly through aquatic animals like fish and shellfish .The effects of lead on man include mental retardation, learning dysfuntion, and loss of coordination (Goodman and Gilman, 1992). The main sources of heavy metal pollution are the agriculture, industry and mining activities (Kumar *et al*., 2007). Organisms develop a protective defense against the deleterious effects of essential and unessential heavy metals and other xenobiotics that produces degenerative changes like oxidative stress in the body (Filipovic and Raspor, 2003; Abou El-Naga *et al*., 2005). As a result of metal absorption, regulation, storage and excretion mechanisms, the tissue differ in bioaccumulation rates and their roles in these processes (Storelli *et al*., 2006). Due to the presence of metal-binding proteins in some tissues, such as metallothioneins in the liver, they can bioaccumulate significantly higher metal concentrations than other organs (Ploetz *et al*., 2007; Uysal *et al*., 2009). High metal concentrations in the gills can point out the water as the main source of contamination (Bervoets and Blust, 2003). Total metal level in gills have been observed to be influenced by absorption of metals onto the gill surface, and also through complexion with the mucous (Rashed, 2001; Storelli *et al.*, 2006; Dural, 2006; Erdogrul and Erbilir, 2007).

Excessive deposition of metals in the environment and the resulting pollution is a well-known problem in several areas around the world. Monitoring this pollution and assessing its effect on organisms is therefore an important issue. It has become important to look for organisms which can be used as biological indicators or monitors of those pollutants. Xenobiotics compounds usually concentrate in the tissues of aquatic biotas and are known to produce cumulative deleterious effects (Abbas, 1998; Abbas and Mahmood, 2003, 2004). Therefore, the application of

environmental toxicology studies on non-mammalian vertebrates is rapidly expanding for the evaluation of the effects of noxious compounds (Ayoola, 2008a, b). Indiscriminate discharge of such compounds that contains mixtures of heavy metals such as herbicide, pesticides, detergents, chemical effluents etc, their careless handling, accidental spillage or discharge of treated effluents into natural waterways have harmful effects on the fish population and other forms of aquatic life and may contribute long term effects in the environment (Akhtar, 1986; Olojo *et al*., 2005; Ayoola, 2008a, b). Toxic chemicals cause tissues damage and histophathological degradations as the fish show haematological responses to toxicants; and generally, such degradation of histological origin occurs in the gills, livers, heart, kidney and epidermis of animals. Van Dyk *et al*., (2005) reported sublethal levels of metal mixtures of cadmium and zinc to have influence on the histological responses in exposed specimens. Because the liver of fish can be considered a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health (Myers *et al*., 1998), and exhibit the effects of a variety of environmental pollutants (Hinton *et al.,* 1992). Moreover, the liver has play a major role in complex enzymatic processes of tetraiodothyronine (thyroxine)-tri-iodothyronine (T4 - T3) conversion. The metabolic rate of hepatocytes is certainly modulated by thyroid hormones. Thyroid dysfunction may perturb liver function, and liver disease affects thyroid hormone metabolism (Malik and Hodgson, 2002). Therefore, more attention has to be paid on the functions of liver when affected by chemicals. The liver plays primary roles in the metabolism and excretion of xenobiotics compounds with morphological alterations occurring in some toxic conditions (Rocha and Monteiro, 1999). Metals in effluents can either increase or decrease histopathological changes, depending on the additive effects of the reacting metals in such an effluent, concentration, fish species type, physiological status of the fish species, length of exposure and other

factors (Paris-Palacious *et al*., 2000). The monitoring of histological alteration in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds both in field and in the laboratory. Contamination of aquatic phase by detergent has been reported in aquatic organisms such as fishes (Adewoye and Fawole, 2002; Adham *et al*., 2002; Adewoye *et al*., 2005; Ogundiran *et al.,* 2007, 2009 and 2010). These pollutants build up in the food chain and are responsible for the adverse effects and death in aquatic organisms (Farkas *et al*., 2002). Fishes are widely used to evaluate the health of aquatic ecosystem and physiological changes serves as biomarkers of environmental pollution (Kock *et al*., 1996). Production of wholesome aquatic foods demands adequate management of the aquatic environment through effective screening for toxicants for corrective actions.

Fish are commonly situated at the top of the food chain and therefore, they can accumulate large amount of toxicants (Yilmaz *et al*., 2007). Fish are also considered as one of the most susceptible aquatic organisms to toxic substances present in water (Alibabic *et al*., 2007). Since the fish meat represents a major components of human diet, the presence of heavy metals in the aquatic environment and their accumulation in fish call for concern (Erdogrul and Erbilir, 2007; Alibabic *et al*., 2007; Keskin *et al*., 2007). *Clarias gariepinus* is most widely used because it is hardy since it is able to tolerate both well and poorly oxygenated waters. It is widely cultivated in Nigeria water bodies, hence used as biological indicators of ecotoxicological studies. Fish constitutes an important aspect of human food due to the high level of quality protein and essential amino acids for the proper growth and functioning of body muscles and tissues. *Clarias gariepinus* inhabit freshwater, it's suitable species for aquaculture because it grows fast and feeds on a large variety of agricultural by-products and can tolerate adverse water quality conditions.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1. Toxic Sample Collection

The effluent used for the toxicity test was collected from the discharge point of a company that produces chemical additives and emulsions. The collections were made bi-monthly between June 2010 to July 2011, and between the hours of 8.00am to 9.00am on the days of sample collection. The samples (about 5000ml each) were collected into clean plastic gallons and kept in the refrigerator to avoid further activities of microorganisms before the experiment commenced. The waste waters were then pooled together to avoid variability in concentration.

### 3.2. Test Organism

The test organism; *Clarias gariepinus* of standard length between 22-25cm and 171- 180g body weight were purchased from a commercial Agricultural farm and transported in a big bowl to the Laboratory. The test organisms were almost of the same size and weight since variability in size may lead to different responses to the effluent of the same concentration.

The test organisms were kept in a large plastic container of about 60 litres that has already been washed and rinsed with 5% potassium trioxonitrate to remove any adhered metals and thereafter acclimatized for a period of two weeks. During this period of acclimatization, renewal bioassay was employed and fishes were fed twice daily (12 hourly) with a formulated fish feed containing 40% crude protein content.

### 3.3. The Physico-Chemical Analysis

The physico-chemical analysis of the effluent was carried out prior to the laboratory experiment and it is to quantify the concentrations of the metals and other parameters in the effluent of study using the APHA/AWWA/WEF, (1995) standard method for examination of water and waste waters.

### 3.4. Toxicity Test

The test organisms were acclamatized for two weeks during which the water was renewed daily using a renewal bioassay procedure while the fishes were fed twice daily. The toxicity test was then carried out in two phases i.e the acute and the chronic evaluations.

#### 3.4.1. Acute Evaluation

After acclamatization, range finding test using the ASTM, (2007) method was conducted to determine the definitive concentrations to be used for the acute test. Five different concentrations were set up in replicates, these are 0.00 (control), 0.30 mg/L, 0.40 mg/L, 0.50 mg/L, and 0.60 mg/L. A total of ten fishes were introduced into each concentration including the control. Mortalities were recorded at intervals and at the end of 96 hours, the following were determined:

a. Total number of death (mortality) after 96 hours

b. The percentage mortality at 96 hours

c. The $LC_{50}$ which is the concentration at which half or 50% of the test organism died on exposure

Since the organisms were exposed for 96 hours, the 96-h $LC_{50}$ was determined from the graph of percentage mortality against concentration. Arithmetic Graphic method was used to determine the 96-h $LC_{50}$.

### 3.4.2. Chronic Evaluation

After the 96hr$LC_{50}$ was evaluated, four different concentrations were set up on the basis of the $LC_{50}$ value obtained. They are the 20%, 10%, 5% and the 2.5% of the $LC_{50}$ value respectively and these are 0.06mg/L, 0.03 mg/L, 0.015 mg/L and 0.0075 mg/L. A control experiment was also set up. The solutions were renewed every 48 hours and the entire exposure period was 42 days. This was to allow the RBC of the test organisms undergo a complete cycle of maturation during the exposure period.

### 3.5. Biochemical Analysis

At the 42nd day, three organisms per concentration were randomly selected for biochemical analysis. Blood used was collected from the fish heart through cardiac puncturing with a needle and syringe, spin in a centrifuge at 5000rpm and biochemical indices like Total Cholesterol, Total serum protein, Serum Albumin, Globulin and Total Glucose level were analysed.

### 3.5.1. Total protein determination

The total Plasma protein was determined by the method of Cannon *et al*., (1974) thus: At alkaline pH value, protein forms a stable complex with Copper II ion, whic is photometrically measured with calorimeter. Three test tubes were set up (Blank, standard and test). 2.5ml of Copper II ion solution was added to the blank tube, 2.5ml of Copper II ion solution and 0.05ml of commercially prepared standard was added to the standard tube and 2.5ml of Copper II ion solution and 0.05ml of plasma sample was added to the test tube. The content of the tube were well mixed and incubated for for 15 minutes at 37°C. The wavelenght was adjusted to 550nm and the cuvette blank was used to zero the calorimeter. The content of the sample tube and standard tubes were filled separately into the cuvette and

inserted in the compartment of the calorimeter. The reading was taken as absorbance of the test and standard.

TOTAL PROTEIN = Absorbance of test/Absorbance of standard x Concentration of standard (4.5g)

### 3.5.2. Plasma glucose determination

Plasma Glucose was determined by the method of Cooper and McDaniel, (1970) thus: Glucose and oxygen react in the presence of glucose oxidase yielding gluconic acid and hydrogen peroxide. Hydrogen peroxide subsequently oxidizes the dyes in a reaction mediated by peroxidase producing a blue colour from the dyes. The intensity of this blue colour is proportional to the glucose cocentration in the sample. Three test tubes (Blank, standard and test) were set up in a rack. 0.5ml of glucose oxidase solution was added to the test tubes, then 3.0ml of buffered peroxidase to the test tube. 0.03ml of serum was added to the sample tube and 0.03ml of the standard solution to the standard tube. They were mixed and incubated for 20 minutes at 37°C. After incubation, 3ml of 30% Sulphuric acid was added to the tubes contents. The wavelenght was adjusted to 520nm, the cuvette blank was used to zero the calorimeter. The content of the sample and standard tubes were filled separately into the cuvette and inserted in the compartment of the calorimeter. The reading was taken as absorbance of the test and standard.

GLUCOSE = Absorbance of test/Absorbance of standard x Concentration of standard (4.5g/d)

### 3.5.3. Serun albumin determination

Serum albumin was determined using the method of Gustafsson, (1976) thus: The addition of albumin to a buffer solution of a dye (indicator) binds some of the dye,

causing a change in the colour of the solution. Over a certain range, the amount of colour change will be proportional to the amount of albumin present. Three test tubes (Blank, standard and test) were set up in a rack. 3.0ml of Bromocresol (BCG) was added to the test tubes, 0.01ml of the serum to the sample tube, 0.01ml of the standard solution to the standard tube while 0.01ml of distilled water was added to blank tube. The content of each test tube was mixed thoroughly and allowed to react for 30 minutes. After incubation, 3ml of 30% Sulphuric acid was added to the content of each tube. The wavelenght was adjusted to 630nm (red filter) and the cuvette blank was used to zero the calorimeter. The content of the sample and standard tubes were filled separately into the cuvette and inserted in the compartment of the calorimeter. The reading was taken as absorbance of the test and standard.

ALBUMIN = Absorbance of test/Absorbance of standard x Concentration of standard (4.5g/d)

### 3.5.4. Total cholesterol determination

Total Cholesterol level was determined by the method of Warnick, (1991) as follow: Cholesterol reacts with acetic acid in the presence of concentrated Sulphuric acid to give a green colour. The intensity of the colour produced is measured calorimetrically at 570nm and is proportional to amount of cholesterol in the sample. Three test tubes were set up in a rack (Test, standard and blank) containing acetic anhydride mixture. 2.5ml of this mixture was put in the three test tubes, 0.025ml of the serum was added to the sample tube, then 0.025ml of prepared standard cholesterol solution (200mg) was added to the standard tube and 0.025ml of distilled water to the blank test tube. The content of each tube was thoroughly mixed and allowed to cool for 10 minutes or incubate for 5 minutes at

$37^0$ C. Then 0.5ml of concentrated Sulphuric acid was added. Immeditely after each addition, the test tubes were allowed to cool in water for 10 minutes. The wavelenght was adjusted to 570nm (red filter) and the cuvette blank was used to zero the calorimeter. The content of the sample and standard tubes were filled separately into the cuvette and inserted in the compartment of the calorimeter. The reading was noted as optical density of the test and standard. The standard was prepared by dissolving 200mg (0.2g) of pure cholesterol in 100ml ethanol. The standard thus contains 200mg of cholesterol.

CHOLETEROL = Absorbance of test/Absorbance of standard x Concentration of standard (200mg/d).

### 3.5.5. Globulin determination

The Globulin content was measured by subtracting the value of Albumin from that of Total protein.

GLOBULIN = TOTAL PROTEIN - ALBUMIN

### 3.6. Haematological analysis

0.5ml of blood was sampled from 3 fishes in each container by cardiac puncture using lithium heparin as anticoagulant at the beginning and the end of the experiment. The routine method of fish haematology designed by Blaxhall, (1973) was employed. The RBC count (RBCc, $x10^6$ µl) was determined by counting the erythrocyte from 5 small squares of Neubaner hemocytometer using Vulpian dillution solution. The hematocrit (PCV, %) was determined by duplicate using heparised cappilary tubes centrifuged for 4 minutes at 13000 rpm in a micro hematocrit centrifuge. The photometrical cyanohemoglobin method was used for determining the hemoglobin concentration (Hb, g/dl) using standard formular

(Svobodova, 2001). The White blood cell count (WBC) was evaluated according to the routine clinical methods (Wintrobe,1978).

Mean Corpuscular Volume (MCV):- This is the average volume of a single RBC count which is calculated from the data obtained for RBCc and PCV using standard formular by Torts *et al*., (1988)

MCV = PCV×10/RBC ($10^6$) fl (fentrolitres)

Mean Corpuscular Haemoglobin (MCH):- This is the quantity or amount of haemoglobin present in one RBC. It is the amount of Hb expressed in relation to the volume of one RBC and is calculated from the data obtained for Hb and RBCc using standard formular by Torts *et al*., (1988).

MCH = Hb×10/RBC ($10^6$) pg (pictograms)

Mean Corpuscular Haemoglobin Concentration (MCHC):- This is the concentration of Hb in one RBC. It is the amount of Hb expressed in relation to the volume of one RBC and also calculated using the formular propounded by (Torts *et al*., 1988) .

MCHC = Hb×100/PCV g/dl (decilitres).

**3.7. Digestion of specimen for metal analysis**

The specimens were dissected to remove the various organs, which were then kept in the freezer prior to analysis. The dissected parts were oven dried at 70 to 73°C until constant weight was obtained. The specimens were then grounded to fine powder and stored in desiccators in order to avoid moisture accumulation before digestion. The digestion procedure was carried out as described by Kotze *et al*., (2006). Twenty ml of concentrated nitric acid (55%) and 10ml of perchloric acid

(70%) were added to approximately 1g tissue (dry mass) in a 100ml Erlenmeyer flask. The digestion was done on a hotplate (200 to 250°C) until the solutions were clear (Van Loon, 1980). The solutions were then filtered through an acid-resistant 0.45mm filter paper and made up to 50ml each with distilled water. The samples were stored in clean glass bottles prior to the determination of the metal concentration using a PYE UNICAM Atomic Absorption Spectrophotometer (AAS). A standard sample, consisting of tuna homogenate (sample IAEA-350) from the International Atomic Energy Agency Marine Environment Laboratory, was prepared and used as a control in accordance with the above-mentioned procedures with every set of samples, to ensure accuracy of data through comparison. Analytical standards were prepared from Holpro stock solutions. Prior to use all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24h, rinsed in distilled water, acid-washed in 1M HCl for another 24h and rinsed again in distilled water (Giesy and Wiener, 1977).

### 3.8. Statistical analysis

The statistical analysis of the haematological parameters was done using the SPSS 10 package. The values obtained were confirmed using one-way ANOVA at 0.05 level of significance. Further test on those found to be significant was done using Duncan Multiple Range tests (DMRT).

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1.Physicochemical Characteristics of chemical additives Effluent

This is shown in table 1. The data obtained have some of its values conforming to FEPA, (1991) specifications for maximum limits allowed for effluent discharge into water bodies while the values for lead, cyanide, total hardness, calcium, oil and grease and alkalinity do not conform to the standard.

### 4.2. Behavioural Responses

During the toxicity test as shown in table 2, *Clarias gariepinus* exhibited distress behavioural responses due to the effects of the chemical additives effluent. These were noticed by the sudden change in the organism's response to the environment such as erratic swimming , gasping for breath and frequent surfacing which increases as the concentration increases. As the experiment progressed, the test organisms were seen to get weaker and those that couldn't tolerate the concentrations any longer went into comatose. Normal behaviour were however observed in the control.

### 4.3. Mortality

The result of the acute toxicity shows the absence of mortality in the lower concentrations while maximum mortality was observed in the highest concentration. Figure 1 shows the arithmetic graph of percentage mortality against concentration for the acute evaluation. The 96-h $LC_{50}$ was calculated to be 0.30mg/L

### 4.4. Effects of effluent on the biochemical profile of *Clarias gariepinus*

Table 3 reveals the biochemical parameters of C. Gariepinus after 42 days exposure to sub-lethal concentrations of the chemical additive effluent. The glucose and total protein increases with concentration, while the cholesterol decreases with increase in concentration.

### 4.5. Effects of effluent on the haematological indices of *Clarias gariepinus*

Table 4 shows the result of the haematological parameters of the test organisms after exposure to sub-lethal concentrations of the chemical additives effluent for 42 days. These are the WBC, RBC, Hb, MCV, PCV, MCH and MCHC. In comparison with the control, all the parameters showed an increase in values obtained as the concentration increased for the same parameter except for WBC and MCH which showed decrease in values as concentration increases. The PCV, Hb and RBC values for the treated organisms all showed a level of significant difference ($P<0.05$) from the control at the highest concentrations while all the values obtained for MCV, MCH, MCHC and WBC showed no significant difference from the control stocks. The RBC's were also discovered to have undergone lysis during the exposure period.

### 4.6. Concentration of metals in the various organs

The highest concentrations of most of the analyzed metals were recorded in the liver (Table 5), while the lowest ones were in the muscle (Table 6). A significantly higher level of Cu was found in the liver than in other fish organs. This study revealed high levels of Fe in liver while Zinc and Nickel had the highest concentration in the gill (Table 7) than in liver. Manganese, Magnesium, Lead and Cadmium were found to reach their maximum level of bioaccumulation in the liver. Accumulation of metals in the gut, kidney and head capsule were also

observed to be concentration dependent as in other organs (Tables 8, 9 and 10).

**Table 1: Physicochemical composition of Chemical Additives Effluent**

| Parameters | Chemical Additives Effluent (mg/L) | F.E.P.A. 1991 Specification (mg/L) |
|---|---|---|
| Ph | 6.7 | 6.9 |
| DO | 2.6 | 5.0 |
| BOD | 0.4 | 5.0 |
| Total suspended solid | 72 | 30 |
| Oil & Grease | 12.5 | 10.0 |
| Alkalinity | 65.0 | 45.0 |
| Iron | 0.6 | 1.0 |
| Cadmium | 5.5 | <1.0 |
| Chromium | 0.05 | <1.0 |
| Sulphide | 0.25 | 0.2 |
| Nitrate | 3.3 | 20 |
| Cyanide | ND | 20 |
| Lead | 9.6 | <1.0 |
| Total hardness | 52.0 | - |
| Total solid | 396 | - |
| Magnesium | 0.59 | - |
| Nickel | 1.01 | - |
| Copper | 0.08 | <1.0 |
| TDS | 324 | - |

**KEY: ND=** Not detected

**Table 2: Behavioral responses of C. *gariepinus* during exposure to sublethal concentrations of chemical additives effluent.**

| **Behaviour** | **Concentrations** | | | | |
|---|---|---|---|---|---|
| | **0.00** | **0.06** | **0.03** | **0.015** | **0.0075** |
| Erratic swimming | - | + | + | + | + |
| Gasping for breath | - | + | + | + | + |
| Loss of reflex | - | + | + | + | + |
| Frequent surfacing | - | - | + | + | + |
| Motionlessness | - | + | + | + | + |
| Hyperventilation | - | + | + | + | + |
| Discolouration | - | - | + | + | + |

Absent (-), Present (+)

**Table 3: Mean and Standard Deviations for biochemical parameters of *Clarias gariepinus* exposed to different concentrations of chemical additives effluent for 42 days.**

| Concentrations (mg/L) | Parameters | | | | |
|---|---|---|---|---|---|
| | **Glucose** | **Total protein** | **Albumin** | **Globulin** | **Cholesterol** |
| 0.00 | 43.333 ±8.819$^{b}$ | 2.4333 ±0.088$^{c}$ | 1.5667 ±0.088$^{b}$ | 0.8667 ±0.088$^{b}$ | 150.000 ±15.275$^{a}$ |
| 0.06 | 40.000 ±11.547$^{a}$ | 3.6000 ±0.264$^{a}$ | 2.2333 ±0.176$^{a}$ | 1.0667 ±0.088$^{a}$ | 147.000 ±13.316$^{a}$ |
| 0.03 | 40.00 0±5.773$^{b}$ | 3.3667 ±0.033$^{bc}$ | 1.5333 ±0.176$^{b}$ | 1.1333 ±0.145$^{ab}$ | 139.333 ±41.462$^{a}$ |
| 0.015 | 66.666 ±13.333$^{b}$ | 3.1000 ±0.057$^{ab}$ | 2.0000 ±0.057$^{ab}$ | 1.1000 ±0.000$^{ab}$ | 140.666 ±21.827$^{a}$ |
| 0.0075 | 80.000 ±23.094$^{b}$ | 3.0000 ±0.152$^{ab}$ | 3.0000 ±0.152$^{ab}$ | 1.2000 ±0.100$^{a}$ | 134.667 ±20.827$^{a}$ |

Note: Mean or values with the same alphabet are not significantly different ($P<0.05$)

**Table 4: Mean and Standard Deviations for haematological parameters of *Clarias gariepinus* exposed to different concentrations of chemical additives effluent for 42 days.**

| Concentrations (mg/L) | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | PCV (%) | Hb (g/dl) | RBC ($10^6$µl) | MCV (fl) | MCH (pg) | MCHC (g/dl) | WBC (µl) |
| 0.00 | 25.56[b] | 10.3667[b] | 3.1667[b] | 90.983[b] | 33.846[b] | 36.113[b] | 736033.33[b] |
| | ±3.17 | ±1.2197 | ±0.4177 | ±1.74 | ±0.42 | ±0.23 | ±151219.05 |
| 0.06 | 41.66$_a$ | 15.1000[a] | 4.0667[a] | 90.046[b] | 30.270[b] | 34.066[b] | 1066666.6[b] |
| | ±3.48 | ±0.6000 | ±0.2728 | ±2.68 | ±0.55 | ±1.36 | ±112885.38 |
| 0.03 | 30.64[b] | 12.5667[b] | 3.4333[b] | 98.386[b] | 33.056[b] | 35.370[b] | 800000.0[b] |
| | ±1.76 | ±0.7446 | ±0.3930 | ±5.43 | ±1.58 | ±0.37 | ±80531.56 |
| 0.015 | 30.13[b] | 09.5333[b] | 2.9667[b] | 99.816[b] | 36.493[b] | 35.993[b] | 920800.0[b] |
| | ±2.40 | ±0.4842 | ±0.8192 | ±4.78 | ±0.99 | ±0.75 | ±129326.46 |
| 0.0075 | 32.00[b] | 10.4667[b] | 3.1667[b] | 101.076[b] | 33.816[b] | 32.820[b] | 1062400.0[b] |
| | ±1.73 | ±0.2906 | ±0.1764 | ±1.07 | ±1.23 | ±0.89 | ±90735.66 |

Note: Mean values with the same alphabet for same parameter are not significantly different ($P<0.05$)

(g/dl) = gramme/decilitre

fl = fentrolitre

pg = pictogram

(µl) = microlitre

mg/l = milligramme/litre

**Table 5: Bioaccumulation of metals in the Liver of *Clarias gariepinus* at sub-lethal concentrations (±se)**

| Concentration (%) | Metals (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Nickel** | **Copper** | **Zinc** | **Magnesium** | **Manganese** | **Iron** | **Lead** | **Cadmium** |
| 0.00 | ND | ND | ND | ND | ND | ND | ND | ND |
| 0.06 | 0.3002 ±0.300[a] | 0.1072 ±0.107[a] | 5.1460 ±0.610[a] | 16.1208 ±0.161[a] | 1.3075 ±0.130[a] | 8.1812 ±3.818[a] | 0.3136 ±0.1815[a] | 0.8917 ±0.0215[a] |
| 0.03 | 0.1909 ±0.190[a] | 0.4003 ±0.200[a] | 4.2311 ±0.423[a] | 16.0112 ±0.421[a] | 1.2982 ±0.178[a] | 8.6214 ±0.046[a] | 0.1677± 0.150[a] | 1.0158 ±0.015[a] |
| 0.015 | 0.1801 ±0.180[ab] | 0.1865 ±0.186[a] | 4.2142 ±0.421[a] | 16.0064 ±0.421[a] | 1.3201 ±0.291[a] | 10.0859 ±1.712[a] | 0.3918 ±0.168[a] | 2.1067 ±0.016[a] |
| 0.0075 | 0.1782 ±0.178[ab] | 0.1132 ±0.112[a] | 3.2492 ±1.521[a] | 14.2141 ±1.182[a] | 0.9921 ±0.429[a] | 9.1200 ±2.114[a] | 0.2253 ±0.105[a] | 2.2249 ±0.011[a] |

Means within column having the same alphabet(s) are not significantly different (P > 0.05). Se = Standard error, ND= not detected

**Table 6: Bioaccumulation of metals in the Muscle of *Clarias gariepinus* at sub-lethal concentrations (±se)**

| Concentration (%) | Metals (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Nickel | Copper | Zinc | Magnesium | Manganese | Iron | Lead | Cadmium |
| 0.00 | ND | ND | ND | ND | ND | ND | ND | ND |
| 0.06 | 0.5362 ±0.536[a] | 0.0010 ±0.001[a] | 1.4417 ±0.144[ab] | 9.6585 ±6.150[a] | 1.1321 ±0.113[b] | 6.8713 ±2.684[a] | 0.1671 ±0.167[a] | 0.0173 ±0.0173[b] |
| 0.03 | 0.5993 ±0.599[a] | 0.0022 ±0.001[a] | 4.8559 ±1.855[a] | 7.5444 ±3.352[a] | 1.3101 ±0.132[b] | 8.0015 ±0.084[a] | 0.1272 ±0.010[a] | 0.0101 ±0.0097[b] |
| 0.015 | 1.0819 ±0.108[a] | 0.3958 ±0.039[a] | 2.0496 ±0.204[ab] | 9.8567 ±0.953[a] | 0.6429 ±0.121[b] | 4.8337 ±1.421[ab] | 0.1364 ±0.278[a] | 1.0152 ±0.0152[b] |
| 0.0075 | 0.8883 ±0.882[a] | 0.2900 ±0.100[a] | 4.6256 ±2.311[a] | 10.7345 ±1.767[a] | 0.9071 ±0.096[a] | 9.4083 ±1.124[ab] | 0.2480 ±0.396[a] | 2.0424 ±0.0039[a] |

Means within column having the same alphabet(s) are not significantly different (P > 0.05). Se = Standard error, ND= not detected

**Table 7: Bioaccumulation of metals in the Gills of *Clarias gariepinus* at sub-lethal concentrations (±se)**

| Concentration (%) | Metals (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Nickel** | **Copper** | **Zinc** | **Magnesium** | **Manganese** | **Iron** | **Lead** | **Cadmium** |
| 0.00 | ND | ND | ND | ND | ND | ND | ND | ND |
| 0.06 | 1.9016 ±0.101[a] | 0.0010 ±0.000[a] | 9.4218 ±0.042[ab] | 10.3485 ±3.151[a] | 0.0021 ±0.066[ab] | 6.6713 ±2.079[a] | 0.1711 ±0.157[a] | 0.0273 ±0.0273[b] |
| 0.03 | 2.1193 ±0.193[a] | 0.0010 ±0.100[a] | 12.0552 ±1.055[a] | 10.5424 ±3.350[a] | 0.0100 ±0.012[b] | 7.9015 ±0.278[a] | 0.1372 ±0.010[a] | 0.0134 ±0.0077[b] |
| 0.015 | 2.4814 ±0.048[a] | 0.3955 ±0.039[a] | 12.1826 ±0.560[ab] | 11.1570 ±0.814[a] | 0.0426 ±0.021[b] | 9.0137 ±1.625[a] | 0.4314 ±0.268[a] | 1.0251 ±0.0147[b] |
| 0.0075 | 3.7883 ±0.037[a] | 0.3308 ±0.330[a] | 11.1252 ±1.101[a] | 12.6797 ±1.508[ab] | 0.1011 ±0.100[a] | 9.7013 ±3.021[a] | 0.4250 ±0.296[a] | 2.0224 ±0.0038[a] |

Means within column having the same alphabet(s) are not significantly different (P > 0.05). Se = Standard error, ND= not detected

**Table 8: Bioaccumulation of heavy metals in the Gut of *Clarias gariepinus* at sub-lethal concentrations (±se)**

| Concentration (%) | Metals (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Nickel** | **Copper** | **Zinc** | **Magnesium** | **Manganese** | **Iron** | **Lead** | **Cadmium** |
| 0.00 | ND | ND | ND | ND | ND | ND | ND | ND |
| 0.06 | 0.0864 ±0.106[a] | 0.2300 ±0.023[a] | 1.9403 ±0.019[ab] | 11.1235 ±2.120[a] | 0.0921 ±0.016[b] | 4.6713 ±2.019[a] | 0.1691 ±0.167[a] | 0.0263 ±0.0263[b] |
| 0.03 | 0.1230 ±0.193[a] | 0.3100 ±0.139[a] | 2.0439 ±1.20[ab] | 10.7444 ±2.352[a] | 0.2001 ±0.102[b] | 5.9015 ±0.218[a] | 0.1652 ±0.010[a] | 0.0142 ±0.0075[b] |
| 0.015 | 0.1281 ±0.128[a] | 0.1215 ±0.069[a] | 1.0886 ±0.583[ab] | 13.7417 ±0.743[a] | 0.0401 ±0.701[b] | 5.8337 ±1.622[a] | 0.3314 ±0.268[a] | 1.0351 ±0.0157[b] |
| 0.0075 | 0.1001 ±0.280[a] | 0.1708 ±0.170[a] | 4.0201 ±2.431[a] | 14.5001 ±1.508[a] | 0.1014 ±0.964[ab] | 4.4083 ±3.121[a] | 0.4049 ±0.276[a] | 2.0124 ±0.0098[a] |

Means within column having the same alphabet(s) are not significantly different (P > 0.05). Se = Standard error, ND= not detected

**Table 9: Bioaccumulation of metals in the kidney of *Clarias gariepinus* at sub-lethal concentrations (±se)**

| Concentration (%) | Metals (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Nickel** | **Copper** | **Zinc** | **Magnesium** | **Manganese** | **Iron** | **Lead** | **Cadmium** |
| 0.00 | ND | ND | ND | ND | ND | ND | ND | ND |
| 0.06 | 0.3362 ±0.526[a] | 0.0010 ±0.001[a] | 1.4417 ±0.144[ab] | 8.6555 ±6.150[a] | 0.1321 ±0.113[b] | 6.8713 ±2.684[a] | 0.1981 ±0.167[a] | 0.0113 ±0.0163[b] |
| 0.03 | 0.6233 ±0.519[a] | 0.0022 ±0.001[a] | 4.8559 ±1.855[a] | 6.5414 ±3.352[a] | 0.3101 ±0.132[b] | 8.0015 ±0.084[a] | 0.2272 ±0.010[a] | 0.0101 ±0.0097[b] |
| 0.015 | 1.0619 ±0.100[a] | 0.3958 ±0.039[a] | 2.0496 ±0.204[ab] | 9.6557 ±0.953[a] | 0.5429 ±0.121[b] | 4.8337 ±1.421[ab] | 0.3164 ±0.268[a] | 0.1032 ±0.0142[b] |
| 0.0075 | 1.4183 ±0.282[a] | 1.9900 ±0.100[a] | 4.6256 ±2.311[a] | 12.7345 ±1.767[a] | 0.7071 ±0.096[a] | 9.4083 ±1.124[ab] | 0.3380 ±0.296[a] | 0.1243 ±0.0039[a] |

Means within column having the same alphabet(s) are not significantly different (P > 0.05). Se = Standard error, ND= not detected

**Table 10: Bioaccumulation of metals in the head capsule of *Clarias gariepinus* at sub-lethal concentrations (±se)**

| Concentration (%) | Metals (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Nickel | Copper | Zinc | Magnesium | Manganese | Iron | Lead | Cadmium |
| 0.00 | ND | ND | ND | ND | ND | ND | ND | ND |
| 0.06 | 0.6362 ±0.533[a] | 0.0110 ±0.101[a] | 1.3417 ±0.104[ab] | 11.3585 ±6.130[a] | 1.1321 ±0.113[ab] | 6.8713 ±2.684[a] | 0.0571 ±0.112[a] | 0.0173 ±0.0173[b] |
| 0.03 | 0.5993 ±0.539[a] | 0.0021 ±0.001[a] | 3.5559 ±1.355[a] | 10.5240 ±2.152[a] | 1.3101 ±0.132[b] | 6.0015 ±0.084[a] | 0.0752 ±0.010[a] | 1.0101 ±0.0097[b] |
| 0.015 | 1.0819 ±0.108[a] | 0.1958 ±0.019[a] | 2.1496 ±0.234[b] | 11.8560 ±0.653[a] | 0.6429 ±0.121[b] | 4.8337 ±1.421[ab] | 0.0064 ±0.231[a] | 2.0152 ±0.0152[b] |
| 0.0075 | 1.0883 ±0.482[a] | 0.2100 ±0.100[a] | 4.5236 ±2.310[a] | 12.7215 ±1.710[a] | 0.9071 ±0.096[a] | 7.4033 ±1.124[ab] | 0.0630 ±0.346[a] | 2.0424 ±0.0039[a] |

Means within column having the same alphabet(s) are not significantly different (P > 0.05). Se = Standard error, ND= not detected

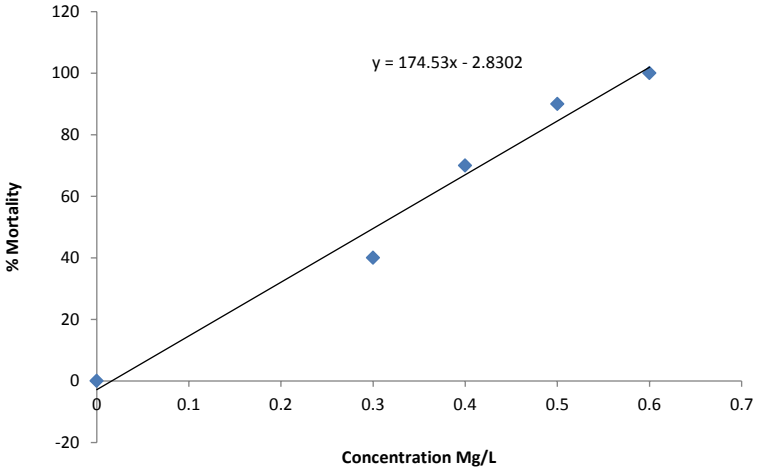


**Figure 2. $LC_{50}$ Determination for *Clarias gariepinus* exposed to lethal concentrations of chemical additives effluent**

# CHAPTER FIVE

## 5.0 DISCUSSION

**Hypothesis 1**: The physicochemical parameters of the Chemical additives effluent do not conform to FEPA standard for effluent discharge into the aquatic environment:-

The study shows the chemical additives effluent to be higher in total suspended solid (TSS), Oil and grease, cadmium, sulphide, lead, alkalinity, total hardness, total solid, magnesium, nickel and total dissolved solid than the standard specification used which shows the effluent to be toxic for discharge into our immediate environment while other parameters are in conformity with the standard. This corresponds to the findings of (Adewoye *et al.,* 2005) that the observed characteristics features may have resulted from the organic loads in the wastewater. From this result therefore, the study supports hypothesis 1 (Table 1).

**Hypothesis 2**: The Chemical additives effluent does not impart significant behavioral response on the treated fishes during the exposure periods:-

The abnormalities observed prior to mortality is an indication of depleted oxygen content due to higher demand for oxygen. Consequently, it was observed in this study that the abnormal behaviour and mortality rate of the test organisms increaded with increase in the concentrations of pollutant. This correspond to the findings of Shobha et al., (2007) that the behaviour and mortality rate of *C. catla* during experimentation was found to depend on both duration of exposure and concentration of the toxicant.

The introduction of the effluent at different concentrations impaired the swimming pattern, skin colouration, feeding rate and general behaviour of test fish. Also, the variation in the behavioural responses and mortality in the sub-lethal test in comparison with the acute test can be attributed to the low level of accumulation of

the effluent. The mucus covering the entire body of the test organisms might have resulted from the excretion of some accumulated metals in their tissues and organs. The investigation further show that fishes can tolerate low concentrations of pollutants with reduced mortality and this agrees with Oyedapo and Akinduyite, (2011) that the abnormal behaviour observed in fish subjected to *Morinda lucida* increased with increasing concentration of the pollutant used. The 96-h $LC_{50}$ value for the acute test was 0.30mg/L which mean that at this concentration of the effluent in the aquatic environment, half of the entire natural population will become dead and this corresponds to the discovery of Adewoye *et al*., (2005) that at this concentration, the fitness of the natural population of an aquatic environment would be relatively impaired and as the concentration increases, the mortality rate also increase. Since the effluent induced behavioural responses in the test organisms, the hypothesis 2 is not supported by this study (Table 2).

**Hypothesis 3**: There is no significant correlation between biochemical parameters of treated fishes exposed to different concentrations of the effluent:-

The significant ($P < 0.05$) increase in glucose which was concentration and time dependent may be considered to be manifestations of stress induced by the chemical additives effluent. Glucose increase is a general response of fish to acute and sub-lethal pollutant effects (Ceron, 1997). Increase in serum glucose levels in fish under stress was reported by Almeida *et al,*. (2001), Chowdhury *et al*., (2004), Bedii and Kenan, (2005). This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis (Almeida *et al.,* 2001).

There is an increase in serum protein recorded in this work which is in agreement with Oruc and Uner, (1999) who reported increase in liver protein following

exposure to 2,4 Diamine for 30 days. There is a significant decrease in serum protein observed in the 0.015mg/L and 0.0075mg/L concentrations and this may be due to the toxic stress which may reduce protein content in tissues. This is supported by Singh and Khare, (1999); Desai, (2002) that proteins are mainly involved in the architecture of the cell. During chronic period of stress, they are also a source of energy. During stress condition, fish need more energy to detoxify the toxicant and to overcome stress. Since fish have fewer amount of carbohydrate, the next alternative source of energy is protein to meet the increased energy demand. The depletion of protein may have been due to their degradation and possible utilization of degraded products for metabolic purposes. Shobha *et al.,* (2007) also observed that decrease in the protein content as observed in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or due to directing the free aminoacids for the synthesis of proteins, or for the maintenance of osmo and ionic regulations. It could also be due to the production of heat shock proteins or destructive free radicals or could be a part of heavy metal induced apoptosis. Nassr-Allah, (2007) also recorded serum protein decrease in fish exposed to phenol.

Cholesterol was found to decrease considerably in this work which may be due to utilization of stored and circulatory cholesterol and other lipid fractions in the treated fish to counteract toxic effects produced. This result conforms closely with Singh *et al*., (2010) who observed a decrease level of cholesterol in *Channa punctatus* exposed to phorate. Rani, (2001), Shankar and Kulkamir, (2007) also observed the same trend in *Notopterus notopterus* during stress. There is also time dependent significant ($P < 0.05$) serum albumin and globulin elevation due to the effluent exposure. From the study, values for all the biochemical parameters except cholesterol are significantly different, therefore, hypothesis 3 is rejected (Table 3).

**Hypothesis 4**: There is no significant correlation between haematological parameters of treated fishes exposed to different concentrations of the effluent:- The high WBC count recorded could be due to attempt by the fishes to fight against the antigens (pollutants) and this led to the production of more antibodies (WBC) to improve the health status of the organisms. This agrees with Ates *et al*., (2008) that the increase in WBC during acute and sub-lethal treatment may be due to stimulated lymphomyeloid tissue as a defence mechanism of the fish to tolerate the toxicity. The increase in leucocyte count indicates the stimulatory effects of the toxicant on immune system and also depend on the toxicant stress. The gradual depression in the values of WBC as the concentrations increased may be due to the breakdown of vital metabolic activities as a result of possible blockage in the metabolic pathway which led to the gradual reduction in the toxiproduction of WBC.

The observed depression in haematocrit (PCV) percentage and haemoglobin concentration of the organism on exposure to the effluent could be a result of the bioaccumulation of the toxicant in the body. This depression in the two indices was as a result of uncontrolled lysis of the RBC due to the toxicity level of the effluent while the decrease in haematocrit compared to the haemoglobin standards was attributed to shrinkage of the erythrocytes. These are in agreement with Atamanalp and Yanik, (2003); Martinez and Souza, (2002); Kavitha *et al*., (2011) that the decrease in haemoglobin content during stress condition may indicate a decrease in the rate of haemoglobin synthesis which lead to impaired oxygen supply to various tissues resulting in decrease in the number of RBC through hemolysis. The lysis of erythrocyte leads to a reduction in haematocrit value. The mean cell volume (MCV) showed an elevated trend in values in comparison with the control, but a depression was recorded at higher concentrations. The values obtained for the

erythrocyte constant is in agreement with Devi *et al.*, (2004), and Ogundiran *et al*., (2007). The decreasing trend in mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were evident in the organisms kept in different concentrations and this correlates with the findings of Kavitha *et al*., (2011) that MCHC is an indicator of RBC swelling and the lowered MCHC during treatment might have resulted from release of young erythrocytes containing less haemoglobin into circulation. The values obtained for MCV, MCH, MCHC and WBC in all the concentrations are not significantly different and as such supports hypothesis 4 while RBC, PCV and Hb values does not support the hypothesis (Table 4).

**Hypothesis 5**: There is no biomagnification of heavy metals in different biomarkers of the treated fishes after exposure:-

In present study, the highest concentrations of most of the analyzed metals was recorded in the liver, while the lowest ones were in the muscle. Such pattern has been observed in a number of other studies, covering several fish species (Rashed, 2001; Dural *et al*., 2006; Storelli *et al*., 2006; Ploetz *et al*., 2007; Pyle *et al*., 2006; Agah *et al*., 2009). Muscle is generally considered to have a weak accumulating potential (Bervoets and Blust, 2003; Erdogrul and Erbilir, 2007; Uysal *et al.*, 2009). High accumulating ability of the liver is a result of the activity of metallothioneins, the proteins that can be binded to some metals, such as Cu, Cd and Zn, thus reducing their toxicity and allowing the liver to accumulate high concentrations (Wu *et al*., 2006; Ploetz *et al*., 2007; Uysal *et al*., 2009). Due to the above discussed reasons, liver has been recommended by many authors as the best environmental indicator of both the water pollution and chronic exposure to heavy metals (Dural *et al.*, 2006; Agah *et al*., 2009; Messaoudi *et al*., 2009).

A significantly higher level of Cu was found in the liver than in other fish tissues which has also been observed by other authors (Rashed, 2001; Wu, *et al*., 2006; Storelli *et al*., 2006; Farag *et al*., 2007; Yilmaz *et al*., 2007; Uysal *et al*., 2009). According to Pyle *et al*. (2006), the liver Cu concentrations are usually regulated by a homeostatic control below 50 μg $g^{-1}$ dw, and can exceed this threshold only if the control mechanisms are overloaded. High Cu levels found in the present study might imply loss of regulatory control of liver Cu (Pyle *et al*., 2006). The present study revealed high levels of Fe in liver. Fe has been found to reach maximum concentrations in liver (Dural *et al*., 2006; Yilmaz *et al*., 2007; Uysal *et al*., 2009). Zinc reached higher levels in the gill than in liver, although Rashed, (2001) presented opposite finding. Several studies have determined the highest Zn concentrations in gills (Dural *et al*., 2006; Yilmaz *et al*., 2007). Nickel had the highest concentration in the gill, which agrees with findings of other studies, suggesting the gills as the centre of their accumulation (Rashed, 2001; Storelli *et al*., 2006). Gills could be important as a site of direct metal uptake from water (Storelli *et al*., 2006). High metal concentrations in gills can point out the water as the main source of contamination (Bervoets and Blust, 2003). According to Dural *et al.,* (2006); Erdoğrul and Erbilir, (2007), total metal levels in gills can be influenced by absorption of metals onto the gill surface, but also through the element complexion with the mucous, that is very difficult to remove from lamellae prior to the analysis. Manganese and Magnesium were found to reach their maximum level of bioaccumulation in the liver suggesting the liver as the major site for their bioaccumulation. Most of the metals were found in this study to have the least bioaccumulation in the muscle. This is in contrast to the findings of Kotze *et al*., (2006) and Senthil *et al.,* 2008 who reported significant bioaccumulation of metals in fish muscle. Lead was found to have its highest level in the gills suggesting the gill as the site of its bioaccumulation. This result agrees

with authors (Bols *et al*., 2001; Vindohini and Narayanam, 2008) that the gill is the centre of lead accumulation where it causes lesions and other gills damages. Cadmium revealed the highest concentration in liver. This is similar to the results obtained by other researchers (Dural *et al.,* 2006; Alibabic *et al*., 2007; Fianko *et al*., 2007; Yilmaz *et al*., 2007) who stated that increased Cd levels is worrying, especially considering the fact that it could be one of the most toxic heavy metals, even at relatively low concentrations and very hazardous for fish genetic material.

It was observed in this study that accumulation of heavy metals in the liver followed the order of Mg > Fe > Zn > Mn> Cd > Pb > Cu > Ni. In the case of the muscle, the order was Mg > Fe > Zn >Mn > Ni > Cd > Cu > Pb. In the gill, the order was Mg > Zn > Fe > Ni > Cd > Pb > Cu > Mn. In the gut, the order was found to be Mg > Fe > Zn > Cd > Pb > Cu > Mn > Ni. In the kidney, the order was Mg > Fe > Zn > Cu > Ni > Mn > Pb > Cd while in the head capsule, the order of metal bioaccumulation was found to be Mg > Fe > Zn > Cd > Mn > Ni > Cu > Pb. In all the metals analysed, the bioaccumulation of magnesium, iron and zinc proportion was significantly increased in the liver, gill and gut of *Clarias gariepinus*. The result conformed closely with the study done by Vinodhini and Narayanam, (2008) where they carefully observed the trend of bioaccumulation of heavy metals in various organs of the fresh water fish *Cyprinus carpio* (common carp) exposed to heavy metal contaminated water system. The present study reveals bioaccumulation of heavy metals in all biomarkers at all the concentrations except control; therefore, hypothesis 5 is not supported (Tables 5 to 10).

# CHAPTER SIX

## 6.0. CONCLUSION AND SUMMARY

### 6.1. Conclusion

The recorded significant differences in the biochemical and haematological parameters as well as the bioconcentration of metals in the fish under study may be attributed to the observed differences in the behavioural and metabolic responses of the fish to the effluent; these differences can also be attributed to the differences in the physiological role of each tissue. It is then clear from the study that fish has the tendency to bioaccumulate metals in a polluted environment.

In conclusion, its evident from this study that increasing concentration of the chemical additives effluent when present in any water body could lead to abnormal bahavioural responses and haematological dysfunction in fish health and general condition. Man is the final reciepient of toxic bioaccumulated chemicals via the food chain and environment, effective application of hazard analysis critical control point (HACCP) monitor is stressed. There is therefore a need for preventive measures to be taken in order to prevent the indiscriminate discharge of this effluent into nearby streams and ponds. Its hence recommended that the application of appropriate effluent technology be adopted by the concerned industries and individuals.

### 6.2. Contributions to knowledge

As at the time of this research, the Chemical additives effluent had not receive any prior attention and so, its toxicological effects in the aquatic environment as well as on humans have not been quantified in scientific research. This finding have successfully overcome this hurdle by bringing to limelight, the implications of

discharging this effluent either in a partially treated or untreated form into the environment and have also established its many deleterious effects on living organisms exposed to it at various levels in the food chain. This will definitely go a long way to help policy makers in formulating laws that militate against the effects of such effluents and other xenobiotics.

Moreover, this research explored a good number of biomarkers to reveal the levels of metal bioaccumulation in various sites of the organism's body as a prelude for researchers and individuals to understand the efficacy environmental phenomena like bioaccumulation and biomagnification and to guide against such effects.

### 6.3. Recommendations

The methods used in this research were adequate to indicate that heavy metals are present in the Chemical additives effluent and are bioavailable. However, the data provided in this study are not definitive in asserting the level of biochemical, haematological as well as heavy metal toxicity and bioaccumulation. Consequently, additional investigations are needed. Such studies should, first explore other bioindicators like Benthic Macroinvertebrates (BMIs), algae and others. Also, future research should consider depurating the BMIs to eliminate adsorbed contaminants and gut contents. Furthermore, analysis of BMI body parts separately is an effective way of determining these organism's centres for heavy metals bioaccumulate. Significant information on whether these metals have synergistic or antagonistic behaviors would be provided and would help in management and protection of the aquatic biota.

In addition to this, histopathological studies should be carried out, the liver which is usually the target organ should be prepared for probable histopathological degradations. Lesions such as congestion of central vein, vacoulation of

hepatocyte, oedema, cellular infiltration and cellular necrosis should be checked for.

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## GLOSSARY

**Abnormalities**: Or dysfunctional behavior refers to something deviating from the normal or differing from the typical (such as an aberration).

**Acclamatization**: Or acclimation in British English, is the process in an individual organism adjusting to a gradual change in its environment.

**Accumulation**: The gathering or amassing of objects of value.

**Acute toxicity**: This is the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short space of time.

**Agriculture**: Also called farming or husbandry, is the cultivation of animals, plants, fungi, and other life forms for food, fiber, biofuel and other products used to sustain life.

**Agrochemicals**: Or agrichemical a is a generic term for the various chemical products used in agriculture.

**Albumin**: Egg white; dried egg white refers generally to any protein that is water soluble, moderately soluble in concentrated salt solutions and experiences heat denaturation.

**Antagonistic behavior**: Aggressive behavior between conspecifics usually involves fighting over a limiting resource such as food, water, space or mates.

**Anthropogenic activities**: An effect or object resulting from human activities.

**Anthropogenic contamination**: The term is sometimes used in the context of pollution emissions that are produced as a result of human activities but applies broadly to all major human impacts on the environment.

**Antibody**: An antibody (Ab), also known as an immunoglobulin (Ig), is a large Y-shaped protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses.

**Antigen**: A substance that evokes the production of one or more antibodies.

**Aquaculture**: Also known as aquafarming, is the farming of aquatic organisms such as fish, crustaceans, molluscs and aquatic plants.

**Assay**: An investigative (analytic) procedure in laboratory medicine, pharmacology, environmental biology, and molecular biology for qualitatively assessing or quantitatively measuring the presence or amount or the functional activity of a target entity (the analyte) which can be a drug or biochemical substance or a cell in an organism or organic sample.

**Benthic**: The collection of organisms living on or in sea or lake bottoms.

**Bioaccumulation**: The accumulation of substances, such as pesticides or other organic chemicals in an organism.

**Biochemistry**: Sometimes called biological chemistry, is the study of chemical processes in living organisms, including but not limited to living matter.

**Bioindicator**: These are species used to monitor the health of an environment or ecosystem. They are any biological species or group of species whose function, population, or status can be used to determine ecosystem or environmental integrity.

**Biological half-life**: The biological half-life or elimination half-life of a substance is the time it takes for a substance (for example a metabolite, drug, signalling

molecule, radioactive nuclide, or other substance) to lose half of its pharmacologic, physiologic or radiologic activity.

**Biomagnification**: Also known as bioamplification or biological magnification, is the increase in concentration of a substance that occurs in a food chain as a consequence of persistence, food chain energetics or low (or nonexistent) rate of internal degradation/excretion of the substance (often due to water-insolubility).

**Biomarker**: Or biological marker, is an indicator of a biological state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

**Biomonitoring**: The measurement of the body burden of toxic chemical compounds, elements, or their metabolites in biological substances.

**Biota**: The total collection of organisms of a geographic region or a time period, from local geographic scales and instantaneous temporal scales all the way up to whole-planet and whole-timescale.

**Biotic community**: A group of interdependent organisms inhabiting the same region and interacting with each other.

**Bottom feeder**: An aquatic animal that feeds on or near the bottom of a body of water which could be the ocean, a lake, a river or an aquarium.

**Carbohydrate:** An organic compound that consists only of carbon, hydrogen, and oxygen, usually with hydrogen: oxygen atom ratio of 2:1.

**Calorimeter**: An object used for calorimetry, or the process of measuring the heat of chemical reactions or physical changes as well as heat capacity.

***Clarias gariepinus*:** Or African sharptooth catfish is a species of catfish of the family Clariidae, the airbreathing catfishes.

**Centrifuge**: A piece of equipment generally driven by an electric motor that puts an object in rotation around a fixed axis applying a force perpendicular to the axis.

**Cellular infiltration** : Migration of cells from their sources of origin, or direct extension of cells as a result of unusual growth and multiplication, thereby resulting in fairly well-defined foci, irregular accumulations, or diffusely distributed individual cells in the connective tissue and interstices of various organs and tissues; used especially with reference to such changes associated with inflammations and certain types of malignant neoplasms.

**Cholesterol**: This is a waxy, fat-like substance that occurs naturally in all parts of the body.

**Chordata:** Members of the phylum Chordata are deuterostome animals possessing a notochord, a hollow dorsal nerve cord, pharyngeal slits, an endostyle, and a post-anal tail for at least some period of their life cycles.

**Chronic toxicity**: Property of a substance that has toxic effects on a living organism, when that organism is exposed to the substance continuously or repeatedly.

**Clariidae**: This is the family commonly known as the "Walking Catfishes" owing to their ability to move overland after their previous home has 'dried up'.

**Classification**: Biological classification, or scientific classification in biology, is a method of scientific taxonomy used to group and categorize organisms into groups.

**Coastal water**: The interface between terrestrial environments and the open ocean encompasing many unique habitats, such as estuaries, coastal wetlands, seagrass meadows, coral reefs, mangrove and kelp forests, and upwelling areas.

**Concentration**: The abundance of a constituent divided by the total volume of a mixture.

**Contamination**: The presence of a minor and unwanted constituent (contaminant) in a material, in a physical body, in the natural environment, at a workplace, etc.

**Crustaceans**: A very large group of arthropods, usually treated as a subphylum, which includes such familiar animals as crabs, lobsters, crayfish etc.

**Cytology**: The branch of life science, which deals with the study of cells in terms of structure, function and chemistry.

**Degradation**: The process of deterioration of characteristics of an object with time; moving back; gradual decline; decline in quality; breakdown of matter due to the impact of external forces in conformity with the laws of nature and time.

**Deleterious**: Harmful, often in a subtle or unexpected way.

**Depuration**: To cleanse or purify or become cleansed or purified.

**Detergent:** A surfactant or a mixture of surfactants with "cleaning properties in dilute solutions.

**Detritivores**: Known as detritophages or detritus feeders or detritus eaters or saprophages, are heterotrophs that obtain nutrients by consuming detritus (decomposing plant and animal parts as well as organic fecal matter.

**Dibromochloropropane:** Also known as DBCP, is a nematicide Pesticide that was widely used agriculturally from 1955-1977 in the United States and was at one time the most heavily used pesticide in the US.

**Discolouration:** A soiled or discolored appearance.

**DNA**: Deoxyribonucleic acid molecules are informational molecules encoding the genetic instructions used in the development and functioning of all known living organisms and many viruses.

**Dose-effect:** The dose–response relationship, or exposure–response relationship, describes the change in effect on an organism caused by differing levels of exposure (or doses) to a stressor (usually a chemical) after a certain exposure time.

**Ecological balance:** A state of dynamic equilibrium within a community of organisms in which genetic, species and ecosystem diversity remain relatively stable, subject to gradual changes through natural succession.

**$EC_{50}$:** The term half maximal effective concentration ($EC_{50}$) refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after some specified exposure time.

**Effluent:** An outflowing of water or gas from a natural body of water, or from a human-made structure.

**Embryo toxicity:** Toxic effects on the embryo of a substance that crosses the placental membrane.

**Endosulfan:** Is an off-patent organochlorine insecticide and acaricide that is being phased out globally. There are two isomers, endo and exo, known popularly as I and II.

**Emulsion:** A mixture of two or more liquids that are normally immiscible (nonmixable or unblendable).

**Environment:** The physical and biological factors along with their chemical interactions that affect an organism.

**Enzyme:** Large biological molecules responsible for the thousands of chemical interconversions that sustain life.

**Eukaryotes:** Organism whose cells contain complex structures enclosed within membranes.

**Evaluation:** A systematic determination of a subject's merit, worth and significance using criteria governed by a set of standards.

**Exposure:** An act of subjecting or an instance of being subjected to an action or an influence

**Fauna:** All of the animal life of any particular region or time.

**Fecundity:** The quality or power of producing abundantly; fruitfulness or fertility.

**Free radicals:** Atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules.

**Freshwater:** Fresh water is naturally occurring water on the Earth's surface in ice sheets, ice caps, glaciers, bogs, ponds, lakes, rivers and streams and underground as groundwater in aquifers and underground streams.

**Gastropods:** The Gastropoda or gastropods more commonly known as snails and slugs, are a large taxonomic class within the phylum Mollusca.

**Gel electrophoresis:** A method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments based on their size and charge.

**Genotoxicity:** Describes a deleterious action on a cell's genetic material affecting its integrity.

**Globulin:** A family of globular proteins that have higher molecular weights and water solubility values than the albumins.

**Gluconeogenesis:** (Abbreviated GNG) is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as pyruvate, lactate, glycerol, and glucogenic amino acids.

**Glucose:** Also known as D-*glucose*, dextrose, or grape sugar) is a simple monosaccharide found in plants.

**Groundwater:** Water located beneath the earth's surface in soil pore spaces and in the fractures of rock formations.

**Glycolysis:** The metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvate, $CH_3COCOO^- + H^+$.

**Habitat:** An ecological or environmental area that is inhabited by a particular species of animal, plant or other type of organism.

**HACCP:** Hazard analysis and critical control points is a systematic preventive approach to food safety and pharmaceutical safety that identifies physical, allergenic, chemical and biological hazards in production processes that can cause the finished product to be unsafe, and designs measurements to reduce these risks to a safe level.

**Haematology:** The study of blood, the blood-forming organs and blood diseases.

**Haemoglobin:** Abbreviated Hb or Hgb) is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (with the exception of the fish family Channichthyidae) as well as the tissues of some invertebrates.

**Heavy metal:** A member of a loosely defined subset of elements that exhibit metallic properties.

**Herbicides:** Also commonly known as weedkillers, are pesticides used to kill unwanted plants.

**Hepatopancrease:** The hepatopancreas, digestive gland or midgut gland is an organ of the digestive tract of arthropods, molluscs and fish.

**Homeostasis:** Tendency of a system, especially the physiological system of higher animals to maintain internal stability owing to the coordinated response of its parts to any situation or stimulus that would tend to disturb its normal condition or function.

**Hygiene:** Refers to the set of practices perceived by a community to be associated with the preservation of health and healthy living.

**Hyperventilation:** Or overbreathing is the state of breathing faster or deeper than normal,(hyperpnoea) causing excessive expulsion of circulating carbon dioxide.

**Hypogean:** Located under the earth's surface; underground.

**Ichthyology:** The branch of zoology devoted to the study of fish.

**Immune system:** A system of biological structures and processes within an organism that protects against disease.

**Immunodeficiency:** (Or immune deficiency) is a state in which the immune system's ability to fight infectious disease is compromised or entirely absent.

**Immunological response:** The integrated body system of organs, tissues, cells, and cell products such as antibodies that differentiates self from nonself and neutralizes potentially pathogenic organisms or substances.

**Impairment:** A medical condition that leads to disability.

**Incubation:** The time elapsed between exposure to a pathogenic organism, a chemical or radiation, and when symptoms and signs are first apparent.

**Insect:** A class of invertebrates within the arthropod phylum that have a chitinous exoskeleton, a three-part body (head, thorax and abdomen), three pairs of jointed legs, compound eyes and one pair of antennae.

**Insecticide:** A pesticide used against insects. They include ovicides and larvicides used against the eggs and larvae of insects respectively.

**Invertebrates:** Animal species that do not develop a vertebral column.

**Juvenile:** The *Juvenile* may refer to: *Juvenile* status, or minor (law), prior to adulthood.

**Lipid peroxidation:** Refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage.

**Macroinvertebrates:** Animals that have no backbone and are visible without magnification.

**Mammals:** Members of class Mammalia, air-breathing vertebrate animals characterized by the possession of endothermy, hair, three middle ear bones, and mammary glands functional in mothers with young.

**Maturation:** The process of becoming mature; the emergence of personal and behavioral characteristics through growth processes.

**MCH:** The mean corpuscular hemoglobin*, or "*mean cell hemoglobin" (*MCH*), is the average mass of hemoglobin per red blood cell in a sample of blood.

**MCV:** The mean corpuscular volume, or "mean cell volume", is a measure of the average red blood cell size that is reported as part of a standard complete blood count.

**Metabolism:** The set of life-sustaining chemical transformations within the cells of living organisms.

**Metallic compounds:** A metallic compound is a compound that contains one or more metal elements.

**Metallothiones (MT):** A family of cysteine-rich, low molecular weight (MW ranging from 500 to 14000 Da) proteins.

**Microorganisms:** Tiny one-celled organisms, viruses, fungi, and bacteria, and are found everywhere in the world.

**Mollusks:** Common name molluscs or mollusks, are a large phylum of invertebrate animals.

**Monophyletic group:** A taxon (group of organisms) which forms a clade, meaning that it consists of a species and all its descendants.

**Mussels:** The common name used for members of several families of clams or bivalvia mollusca, from saltwater and freshwater habitats.

**Nocturnal:** Active at night ( opposed to diurnal ).

**Oedema:** Also known as dropsy, is where there is an excessive build-up of fluid in the body's tissues.

**Ontogeny:** The development or course of development especially of an individual organism.

**Organic pollution:** Occurs when an excess of organic matter, such as manure or sewage, enters the environment.

**Osmoregulation:** The active regulation of the osmotic pressure of an organism's fluids to maintain the homeostasis of the organism's water content; that is, it keeps the organism's fluids from becoming too diluted or too concentrated.

**Oxidation pond:** A pond that contains partially treated wastewater which is then left to allow the growth of algae and bacteria which decompose therest of the waste.

**Oxidative stress:** Reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.

**Photometry:** The science of the measurement of light, in terms of its perceived brightness to the human eye.

**Photosynthesis:** A process used by plants and other organisms to convert the light energy captured from the sun into chemical energy that can be used to fuel the organism's activities. Physiology: is the scientific study of function in living systems.

**Piscidal:** Fatal to fish.

**Plankton:** (Singular plankter) are any organisms that live in the water column and are incapable of swimming against a current.

**Pollutants:** Substance or energy introduced into the environment that has undesired effects, or adversely affects the usefulness of a resource.

**Population:** A *population* is all the organisms that both belong to the same group or species and live in the same geographical area.

**Porphyrin:** Any of a class of water-soluble, nitrogenous biological pigments (biochromes), derivatives of which include the hemoproteins.

**Renewal bioassay:** A test used to evaluate the relative potency of a chemical without continuous flow of solution.

**Reproduction:** (Or procreation) is the biological process by which new "offspring" individual organisms are produced from their "parents".

**Risk assessment:** The determination of quantitative or qualitative value of risk related to a concrete situation and a recognized threat (also called hazard).

**Sedentary**: A type of lifestyle without physical exercise.

**Spectrophotometer:** An instrument which measures the amount of light of a specificed wavelength which passes through a medium.

**Toxicants:** Man-made (synthetic) substance that presents a risk of death, disease, injury, or birth defects in living organisms through absorption, ingestion, inhalation, or by altering the organism's environment.

**Troglobitic organisms:** Small cave-dwelling animals that have adapted to their dark surroundings.

**Vertebrates:** Animals that are members of the subphylum Vertebrata (chordates with backbones and spinal columns).

**Wastewater:** Also written as waste water, is any water that has been adversely affected in quality by anthropogenic influence.

**Xenobiotics:** A chemical which is found in an organism but which is not normally produced or expected to be present in it.

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