EFFECT OF BATTERY WASTES ON AFRICAN CATFISH *Clarias gariepinus* FINGERLINGS

BY

AKIWOWO, USMAN ABIODUN

FAQ/13/0988

MARCH, 2019

EFFECT OF BATTERY WASTES ON AFRICAN CATFISH (*Clarias gariepinus*)

BY

AKIWOWO,USMAN ABIODUN

FAQ/13/0988

A PROJECT REPORT SUBMITTED TO

THE DEPARTMENT OF FISHERIES AND AQUACULTURE

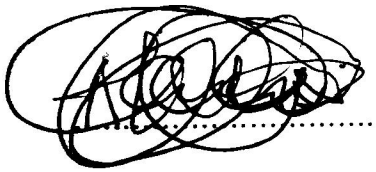
IN PARTIAL FUFILMENT OF REQUIREMENT FOR THE AWARD OF BACHELOR OF FISHERIES AND AQUACULTURE IN THE FACULTY OF AGRICULTURE

FEDERAL UNIVERSITY OYE-EKITI,EKITI STATE, NIGERIA

MARCH 2019

DECLARATION

I, AKIWOWO, USMAN ABIODUN hereby declare that this project titled "EFFECT OF BATTERY WASTE ON AFRICAN CATFISH (Clarias gariepinus) FINGERLINGS'' was carried out by me in the department of Fisheries and Aquaculture under the supervision of DR. A.M AKINSOROTAN. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree at any University.



22/03/2019

Akiwowo, Usman Abiodun

DATE

CERTIFICATION

This is to certify that this project work was carried out by MR AKIWOWO, USMAN ABIODUN with MATRIC NUMBER FAQ/13/0988 in the department of Fisheries and Aquaculture of Federal University, Oye-Ekiti.

_____ _____

DR. A.M AKINSOROTAN DATE

PROJECT SUPERVISOR

_____ _____

DR. J. B. OLASUNKANMI DATE

HEAD OF DEPARTMENT

DEDICATION

This research project is dedicated to Almighty Allah. The All-compassionate, All-beneficent and All-merciful. Who through His rare and unending magnanimities saw me through the course of this research project.

Also to my late mother Alhaja Maryam Olatoke Ayandunmade Adeniji, My stepfather Alhaji Abdulrafiu Alani Adeniji and my fiancée Ayinla Nofisat Omolara.

To my uncles Engr. Abdul Rahman Yusuf and Engr. Abdul Razaq Yusuf.

ACKNOWLEGDEMENTS

My unalloyed, unadulterated and profound thankfulness are to Almighty Allah, the Lord of the worlds; for the priceless and invaluable gift of life and enablement He freely granted me for the successful completion of this research project.

I am immeasurably indebted to my late mother Alhaja Maryam Olatoke Ayandunmade Adeniji for her guidance, tutelage, moral, social, psychological, financial support and encouragement. If not for her, Tertiary education could have proved elusive, unattainable and inaccessible for me. May Allah overlook your shortcomings and inadequacies and make you an inmate of Aljanat Firdaos Amin.

I will be an absolute ingrate, if I fail to lucidly and succinctly acknowledge the enormous and uncounted contributions of my stepfather Alhaji AbdulRafiu Alani Adeniji who took over the baton of care and provisions from where my mother ended the race, you are indeed an epitome of kindness and generosity. May Allah in His unrestricted magnanimity place blessings and happiness without measures at your beck and call, and to my siblings; Zaynu Abideen Olamide Adeniji, Giyath Ayomide Adeniji and mother of the house Marizuqah Mofolorunto Abike Adeniji you all contributed immensely to this project work.

I bountifully express special thanks to my uncles Engr. AbdulRahman Yusuf and Engr. AbdulRazzaq Yusuf, for their constant financial assistance, prayers, support, care, tutelage and guidance. May Allah continue to strengthen the family ties. To their wives sister Sherifat and Hafsah Yusuf your motherly role, especially after the demise of my mother cannot be over-emphasized.

Special thanks to my supervisor Dr. Akinsorotan Ademola Michael for his tutelage, mentorship and up-to- date intellectual guidance. You are truly endowed with sound knowledge in your area of specialization; you are an astute man who is very simple but frank, sincere, caring, hardworking, dynamic, active, strong-willed and decisive, independent, determined, discipline, self-sufficient, humble and not easily discouraged. As your mentee, am proud to say you are a worthy mentor, who combined humility with academic brightness and ingenuity. May God grant you sound health, enormous wealth, and divine wisdom and preserve you to mentor scores more.

My appreciation to all the lecturers in my department: Dr. Olasunkanmi J.B, Dr. Babalola T.O, Prof. Araoye P.A, Prof. Nwana, Dr. Ariyomo T.O, Dr. Okeke O.S, Dr. Omobepade B.P, Mr Jimoh, Dr. Jegede T., Mr Oyawoye T.O. who have at one point in time impacted knowledge in me. Thanks so much.

Unique thanks to my mother in the department in person of Dr Ariyomo T.O, for her huge, and unflinching contributions and sacrifices during the course of this project work. May God in His supreme majesty place prosperity beyond measure within your grasp.

Thanks to Mr Jimoh for his outstanding intellectual contributions to this project work

My Course mate, Adekunle Dele Sumbo , Olatoye Dolapo Salim, Salami Michael , Mustapha Qazeem, Oyinloye Emmanuel, Olanikanju Olajumoke, Falayi Christianah, Fatoyinbo Oluwabunmi, Olaniyan Blessing, Ojo Oluwanifemi, Alabi Bisola. I won't forget all the memories we have made together.

ABSTRACT.

The study examined the effect of battery waste on African catfish, Clarias gariepinus. Massive spent batteries when disposed indiscriminately constitute nuisance and cause resource waste as well as environmental pollution to the ecosystems. The fish were exposed to five different concentrations as T_1 5g,T_2 10g, T_3 15g, T_4 20g, T_5 25g,and the control as T_0 for four days and later left for four weeks as possible bio-remediation. Physicochemical parameters and heavy metals were analyzed in accordance with standard methods. Clarias gariepinus fingerlings exposed to lethal and sub lethal concentrations of battery waste were investigated in a static bioassay with particular reference to behavioral changes, survival, and histopathological changes. Early symptoms of battery waste lethal poisoning were respiratory distress, hyperactivity, erratic swimming, loss of equilibrium and increased breathing activity. Behavioral response was dose dependent and decreased with decrease concentration. The histopathological changes of the gills, liver, and intestinal tissues of fish exposed to battery waste with sub lethal concentration for four weeks showed gill distortion, hyperplasia and excessive mucus accumulation. Histology of the liver of *Clarias gariepinus* exposed to 5g/l battery waste showing normal morphology of hepatocyte with no inflammation. : Histology of Liver of *Clarias gariepinus* exposed to 25g/l battery waste showing diffuse lobular hepatocytes necrosis with severe inflammation but no fibrosis.

TABLE OF CONTENT

LIST OF TABLES

TABLES PAGE

LIST OF PLATES

PLATES PAGE

CHAPTER ONE

1.0 INTRODUCTION

Waste disposal had always been a subject of concern to municipalities, industries, agricultural farms, government and non-governmental agencies, and designated environmental authorities. However, the concern is not limited to cost (finance, space and time), but includes the environmental impacts, especially as it borders on health related issues (Emenike et al., 2012).

A battery is a simple assembly of different parts made of different materials including metal, ceramics, polymers and chemicals etc. with relatively small to large amount depending on the capacity. Among these substances few may contains hazardous and toxic properties on the health and environment with local or even global impacts beyond the local scale of releasing (Swarnakar et al., 2008).Batteries are found in many of the electronic devices we have in our homes, businesses and public agencies,

Batteries currently contain acidic or alkaline chemicals and heavy metals such as mercury, cadmium, lead, zinc, manganese, nickel, silver and lithium (Palacin, 2009). When a battery is disposed of in a solid waste landfill or incinerator, the battery can leach its toxic constituents and contaminate air, soil, surface water and groundwater (Yang, 2007).

The disposal of batteries after the end-of-life into our environments prone a great danger to our entire ecosystem including land, water, plants, animals,and humans. It is not essentially each of the components are harmful but nevertheless the toxic properties of different chemicals and metals containing hazards properties attributes to environmental pollution (Swarnakar et al., 2008). A review article published by Grandjean et al., 2006 stated that the few industrial

chemicals (lead, methylmercury, polychlorinated biphenyls [PCBs], arsenic, and toluene) can possibility lead to neurodevelopmental disorders in the humans for example, attention deficit disorder, mental retardation and autism. Other types of batteries waste metals such as cadmium and lead, which has adverse effects such as damage kidneys and child's growth, cause brain damage etc. needs to reduce.

Fish should be a major test organism in ecotoxicological studies because of their link to man in the food chain. They are particularly useful for the assessment of water-borne and sediment-deposited toxins where they may provide advanced warning of the potential danger of new chemicals and the possibility of environmental pollution (Powers 1989, Oshode et al., 2008). In addition, fish health is essential to the success of aquaculture industry, an industry of growing importance in protein production for humans (Oshode et al., 2008). In this study, *Clarias gariepinus* will be exposed to battery waste of different concentrations and the effects on hematological parameters and histopathology of the gills, kidney and liver will be investigated. This study will be designed to assess the risk posed by battery waste to the aquatic environment as it relates bioaccumulation of toxicants in fish. Therefore analyzing the impact of battery waste on aquatic organism will help to understand the potential of waste component to disrupt the ecological flow.

1.1 STATEMENT OF RESEARCH PROBLEM

Batteries are very important to the modern man because of the need to power electronic appliances but the fact remains that these batteries are made from combinations of different chemicals which can be termed to be hazardous. Batteries, after use becomes a waste which has to be disposed either in incinerators or landfills. The chemical contents are non-degradable

materials, so when rainfalls and erosion occurs, the constituents in batteries can find their way into the aquatic ecosystem. Moreso, most of these landfills and incinerators are created on bear soil which means that the waste can infiltrate the soil and get to groundwater bodies. The method of disposing waste in Nigeria is not regulatedhence people discharge this product indiscriminately and they end up in landfills and invariably find their way into the aquatic ecosystem and leach its components into the water.

1.2 JUSTIFICATION

Fish is a very important source of protein to the human race so it is paramount that anthropogenic activities that can threaten the continuous existence of fish should be studied and curtailed in other to further protect the human race which is at the top of the food chain. Batteries are manufactured to serve different beneficial purposes and so it is necessary tool to humans but when it comes to disposal of battery waste, it is also important to investigate the adverse effect of battery waste on the aquatic ecosystem because it is always at the receiving end of the pollution that comes with waste disposal and since this batteries contain heavy metals, therefore there is need for a bioassay in order to ascertain the level of prospective damage that can be caused by batteries on aquatic life. Currently in Nigeria, there is paucity of information on the amount of battery waste generated in the country and their effects on the environment, be it water, soil or air. Little or no information is currently available about the toxicity of battery waste on fish and such information will be useful in deciding battery waste disposal methods and treatment options. *Clarias gariepinus* was chosen for this assay because of its hardy nature of not being easily susceptible to diseases and also because it is widely accepted fish for culture in the country.

1.3 RESEARCH OBJECTIVES

The main purpose of this study was to examine the effect of battery waste on African Catfish, *Clarias gariepinus* while the specific objectives were as follows;

- ✓ To assess the behavioral changes of *Clarias gariepinus* exposed to battery waste
- ✓ To evaluate the hematological properties of *Clarias gariepinus* exposed to battery waste
- ✓ To evaluate some histological properties of *Clarias gariepinus* exposed to battery waste
- ✓ To evaluate the heavy metal concentration in *Clarias gariepinus* exposed to battery waste
- ✓ To calculate the differential toxicity $_{LC50}$ of different concentrations of battery waste on *Clarias gariepinus*

1.4 RESEARCH HYPOTHESIS

The hypothesis that will be tested in this study will be as follows;

Ho_1: there are no significant changes in the mortality rate of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ha_1: there are significant changes in the mortality rate of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ho_2: there are no significant changes in the behavior of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ha_2: there are significant changes in the behavior of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ho_3: there are no significant changes in the morphology of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ha_3: there are significant changes in the morphology of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ho_4: there are no significant changes in the hematological properties of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ha_4: there are significant changes in the hematological properties of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ho_5: there are no significant changes in the histological properties of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ha_5: there are significant changes in the histological properties of *Clarias gariepinus* exposed to varying concentrations of battery waste;

Ho_6: there is no significant difference in the heavy metal concentration of *Clarias gariepinus* exposed to varying concentration of battery waste;

Ha_6: there is significant difference in the heavy metal concentration of *Clarias gariepinus* exposed to varying concentration of battery waste;

Ho_7: the differential toxicity (Lc_{50}) of varying concentrations of battery waste on *Clarias gariepinus* does not differ significantly,

Ha_7: the differential toxicity (Lc_{50}) of varying concentrations of battery waste on *Clarias gariepinus* differs significantly,

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TAXONOMY AND IDENTIFICATION OF *Clarias gariepinus*

The African catfish Clarias gariepinus belongs to the family of *Clariidae* and it is the widest culture species in this family. *Clarias* is a genus of catfishes (order Siluriformes) of the family Clariidae, the air-breathing catfishes. The name is derived from the Greek*chlaros*, which means lively, in reference to the ability of the fish to live for a long time out of water. It is characterized by the ability to grow on a wide range of artificial and tolerance to low dissolved oxygen adverse aquatic condition (Mwita and Nkwengulila, 2008).

Clarias has been found to be paraphyletic. A species of Heterobranchus (*H. longifilis*) clusters deeply inside the *Clarias* group (Mwita and Nkwengulila, 2008). *Clarias gariepinus* is circum-tropical (Teugels, 1984). *Clarias* species are recognized by their long-based dorsal and anal fins, which give them a rather eel-like appearance. These fish have slender bodies, a flat, bony head, 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 (*Sudarto et al., 2004).* The head is long and the body is 3.0-3.5 times as long as the head. The dorsal fin has 62-82 rays and the anal fin has 50-65 rays. The various teeth are all or mostly granular, forming a band, which is 1.3-2.5 times the width of the band of teeth on the pre-maxillary. There are 31-35 long and fine gill rakers on the whole of the first gill arch. The color varies considerably, but is usually blackish; on the back and white or silvery grey on the belly and the flank are grayish olive. The fins are black except for the ventral and pectorals, which are almost grey or transparent.

Table 2.1 Taxonomic classification of *C.gariepinus*

Kingdom	*Animalia*
Phylum	*Chordata*
Subphylum	*Vertebrata*
Class	*Actinopterygii*
Order	*Siluriformes*
Family	*Clariidae*
Genus	*Clarias*
Species	*gariepinus*

(Source: Gunter and Fink; 2004)

2.2 DESCRIPTION

Clarias gariepinus are readily recognized by their cylindrical body with scaleless skin, flattened bony head, small eyes, elongated spineless dorsal fin and four pairs of barbels around a broad mouth. The upper surface of the head is coarsely granulated in adult fishes but smooth in young fish (Van Oijen, 1995). The anal, caudal and dorsal fins are not united. The males can be easily recognized by a distinct sexual papilla located immediately behind the anal opening. This sexual papilla is not present in female fish. The body is greyish-black with the underside of the head and body a creamy-white colour (Van Oijen, 1995), with a distinct black longitudinal band

on each side of the ventral surface of the head (which is absent in young fish of less than 9 cm long). Larger fish (more than 9 cm) are mottled with an overall grey-khaki colour. Skin coloration is known to change slightly according to substrate and light intensity in culture systems.

2.3 DISTRIBUTION

Clarias gariepinus is indigenous to the inland waters of much of Africa and they are also endemic in Asia Minor in countries such as Israel, Syria and the south of Turkey. *C. gariepinus* has been widely introduced to other parts of the world including the Netherlands, Hungary, much of South-East Asia and East Asia. This species can be cultivated in areas with a tropical climate, areas with access to geothermal waters or with the use of heated recirculating water systems. It is a hardy fish that can be densely stocked in low oxygen waters making it ideal for culture in areas with a limited water supply. Its air-breathing ability, high fecundity, fast growth rate, resistance to disease and high feed conversion efficiency makes *C. gariepinus* the freshwater species with the widest latitudinal range in the world.

2.4 MORPHOLOGY

African catfish are elongate with fairly long dorsal and anal fins. The dorsal fin has 61-80 soft rays and the anal fin has 45-65 soft rays. They have strong pectoral fins with spines that are serrated on the outer side (Teugels 1986). This species can attain sizes of up to 1.7 meters including the tail and can weigh up to 59 kg when fully grown. They possess nasal and maxillary barbels and somewhat smallish eyes. Their coloring is dark grey or black dorsally and cream colored ventrally. Adults possess a dark longitudinal lines on either side of the head; however, this is absent in young fish. Adult's heads are coarsely granulated, while the head is smooth in

the young. The head is large, depressed, and heavily boned. The mouth is quite large and sub terminal (Skelton 1993).

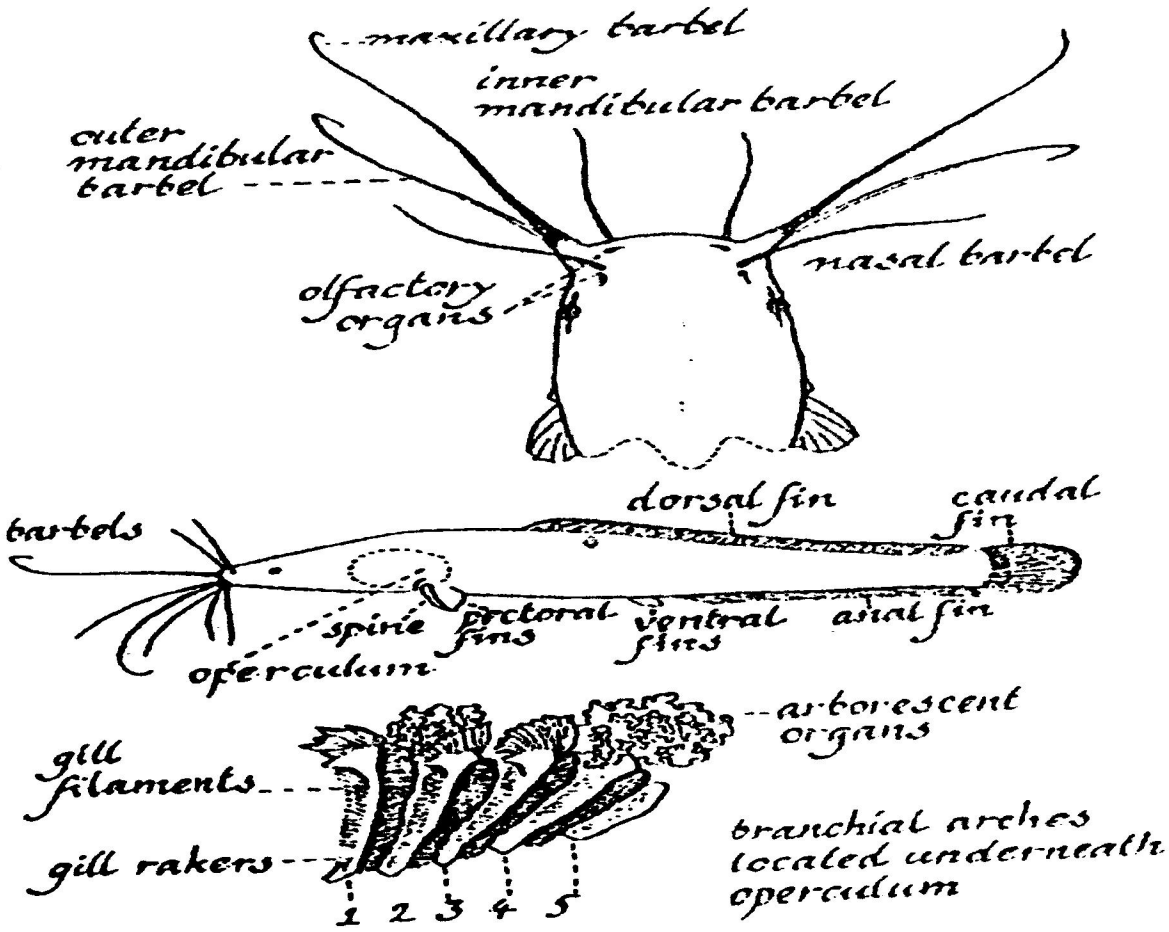


Fig.1. Morphological characteristics of Catfish, *Clarias gariepinus*

Source: Viveen et al. (1986).

2.5 HABITAT AND BIOLOGY

This species is found in lakes, streams, rivers, swamps and floodplains, many of which are subject to seasonal drying. The most common habitats are floodplain swamps and pools where they can survive during the dry season(s) due to their accessory air breathing organs (Froese *et al.*, 2014).

Clarias gariepinus undertake lateral migrations from the larger water bodies, in which they feed and mature at about the age of 12 months, to temporarily floody marginal areas in order to breed. These reproductive migrations typically take place shortly after the onset of the rainy season(s). The final gonadal maturation is associated with rising water levels. Under stable environmental conditions, adult *C. gariepinus* have mature gonads year-round. Under ideal conditions, a ripe female may lay about 60 000 eggs/kg. Prior to mating, males compete aggressively for females with which they mate in single pairs, the female swishing her tail vigorously to mix the eggs and sperm and distribute the fertilized eggs. The adhesive eggs stick to submerged vegetation and hatch in 20–60 hours, depending on temperature. The yolk sac is absorbed within 3–4 days and the stomach is fully functional within 5–6 days after onset of exogenous feeding. Sexual differentiation begins between 10 and 15 days after hatching. Larvae feed and grow rapidly in the warm (usually >24 °C) nutrient rich floodplains, reaching 3-7 g within 30 days. As flooded marginal areas dry up with the end of the rains, juveniles and adults make their way back to deeper water. In areas with two rainy seasons, there are usually two reproductive peaks during the year, corresponding in intensity to the magnitude of the rains (Anoop *et al.*, 2009).

2.6 BATTERIES

A battery is a device consisting of one or more electrochemical cells with external connections provided to power electronic devices such as flashlights, smartphones, and electric cars (*Crompton, 2000*). When a battery is supplying electric power, its positive terminal is the cathode and its negative terminal is the anode (Pauline, 1988.)The terminal marked negative is the source of electrons that when connected to an external circuit will flow and deliver energy to an external device. When a battery is connected to an external circuit, electrolytes are able to move as ions within, allowing the chemical reactions to be completed at the separate terminals

and so deliver energy to the external circuit. It is the movement of those ions within the battery which allows current to flow out of the battery to perform work. Historically the term "battery" specifically referred to a device composed of multiple cells, however the usage has evolved additionally to include devices composed of a single cell (*Pistoia et al., 2005).*

The batteries can be classified into two categories including primary and secondary battery. The primary batteries are disposable batteries and requirements of chemical depend on voltage, whereas secondary batteries are rechargeable. Batteries come in many shapes and sizes, from miniature cells used to power hearing aids and wristwatches to small, thin cells used in smartphones, to large lead acid batteries used in cars and trucks, and at the largest extreme, huge battery banks the size of rooms that provide standby or emergency power for telephone exchanges and computer data centers. According to a 2005 estimate, the worldwide battery industry generates US$48 billion in sales each year, with 6% annual growth. Batteries have much lower specific energy (energy per unit mass) than common fuels such as gasoline. In automobiles, this is somewhat offset by the higher efficiency of electric motors in producing mechanical work, compared to combustion engines.

2.6.1 CLASSIFICATION OF BATTERIES

A flow sheet is given below with another classification of batteries, which divides the batteries in two categories including physical and chemical.

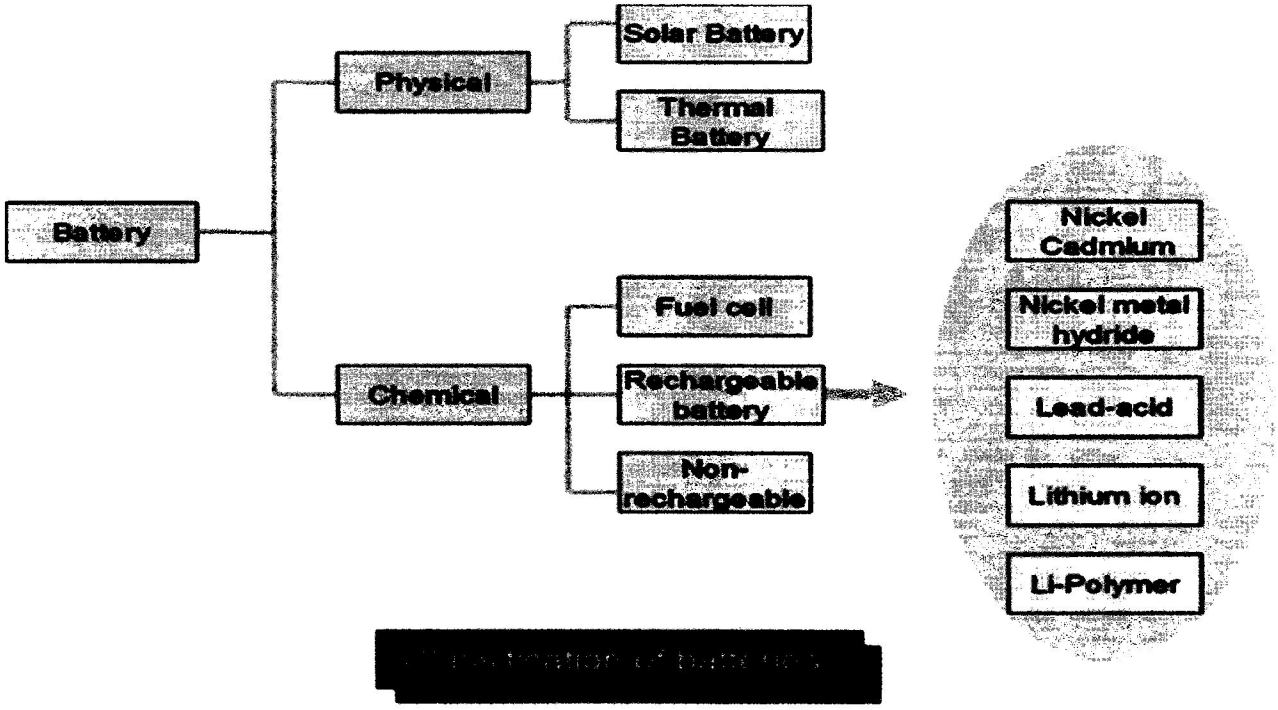


Figure 1: Classification of batteries

2.6.2 TYPES OF BATTERIES

2.6.2.1 Primary batteries

These are the most common household battery. These batteries automatically convert chemical energy into electrical energy. This kind of battery cannot be recharged and are thrown away straight after use. They are commonly known as alkaline batteries, contain zinc and manganese chemistry, and are often used in torches, toys, smoke detectors, watches, calculators, hearing aids, radios and remote controls.

2.6.2.2 Secondary batteries

These are rechargeable and can be used repeatedly. These batteries are usually nickel cadmium, nickel metal hydride or lithium ion. Secondary batteries are commonly found in

cordless phones, cordless drills, mobile phones, laptops and computers, shavers, digital cameras, video cameras and house alarms. Although rechargeable, secondary batteries may need to be recycled when they no longer hold a charge. Secondary batteries, also known as *secondary cells*, or *rechargeable batteries*, must be charged before first use; they are usually assembled with active materials in the discharged state. Rechargeable batteries are (re)charged by applying electric current, which reverses the chemical reactions that occur during discharge/use. Devices to supply the appropriate current are called chargers.

The oldest form of rechargeable battery is the lead–acid battery, which are widely used in automotive and boating applications. This technology contains liquid electrolyte in an unsealed container, requiring that the battery be kept upright and the area be well ventilated to ensure safe dispersal of the hydrogen gas it produces during overcharging. The lead–acid battery is relatively heavy for the amount of electrical energy it can supply. Its low manufacturing cost and its high surge current levels make it common where its capacity (over approximately 10 Ah) is more important than weight and handling issues. A common application is the modern car battery, which can, in general, deliver a peak current of 450amp. The sealed valve regulated lead–acid battery (VRLA battery) is popular in the automotive industry as a replacement for the lead–acid wet cell. The VRLA battery uses an immobilized sulfuric acid electrolyte, reducing the chance of leakage and extending shelf life. VRLA batteries immobilize the electrolyte. The two types are:

- Gel batteries (or "gel cell") use a semi-solid electrolyte.
- Absorbed Glass Mat (AGM) batteries absorb the electrolyte in a special fiberglass matting.

Other portable rechargeable batteries include several sealed "dry cell" types that are useful in applications such as mobile phones and laptop computers. Cells of this type (in order of increasing power density and cost) include nickel–cadmium (NiCd), nickel–zinc (NiZn), nickel metal hydride (NiMH), and lithium-ion (Li-ion) cells. Li-ion has by far the highest share of the dry cell rechargeable market. NiMH has replaced NiCd in most applications due to its higher capacity, but NiCd remains in use in power tools, two-way radios, and medical equipment.

In the 2000s, developments include batteries with embedded electronics such as USBCELL, which allows charging an AA battery through a USB connector ,nano ball batteries that allow for a discharge rate about 100x greater than current batteries, and smart battery packs with state-of-charge monitors and battery protection circuits that prevent damage on over-discharge. Low self-discharge(LSD) allows secondary cells to be charged prior to shipping.

2.6.3 CELL TYPES

Many types of electrochemical cells have been produced, with varying chemical processes and designs, including galvanic cells, electrolytic cells, fuel cells, flow cells and voltaic piles.

2.6.3.1 Wet cell

A *wet cell* battery has a liquid electrolyte. Other names are *flooded cell*, since the liquid covers all internal parts, or *vented cell*, since gases produced during operation can escape to the air. Wet cells were a precursor to dry cells and are commonly used as a learning tool for electrochemistry. They can be built with common laboratory supplies, such as beakers, for demonstrations of how electrochemical cells work. A particular type of wet cell known as a concentration cell is important in understanding corrosion. Wet cells may be primary cells (non-

rechargeable) or secondary cells (rechargeable). Originally, all practical primary batteries such as the Daniell cell were built as open-top glass jar wet cells. Other primary wet cells are the Leclanche cell, Grove cell, Bunsen cell, Chromic acid cell, Clark cell, and Weston cell. The Leclanche cell chemistry was adapted to the first dry cells. Wet cells are still used in automobile batteries and in industry for standby power for switchgear, telecommunication or large uninterruptible power supplies, but in many places batteries with gel cells have been used instead. These applications commonly use lead–acid or nickel–cadmium cells.

2.6.3.2 Dry cell

A *dry cell* uses a paste electrolyte, with only enough moisture to allow current to flow. Unlike a wet cell, a dry cell can operate in any orientation without spilling, as it contains no free liquid, making it suitable for portable equipment. By comparison, the first wet cells were typically fragile glass containers with lead rods hanging from the open top and needed careful handling to avoid spillage. Lead–acid batteries did not achieve the safety and portability of the dry cell until the development of the gel battery. A common dry cell is the zinc–carbon battery, sometimes called the dry Leclanché cell, with a nominal voltage of 1.5 volts, the same as the alkaline battery (since both use the same zinc–manganese dioxide combination). A standard dry cell comprises a zinc anode, usually in the form of a cylindrical pot, with a carbon cathode in the form of a central rod. The electrolyte is ammonium chloride in the form of a paste next to the zinc anode. The remaining space between the electrolyte and carbon cathode is taken up by a second paste consisting of ammonium chloride and manganese dioxide, the latter acting as a depolarizer. In some designs, the ammonium chloride is replaced by zinc chloride.

2.6.3.3 Molten salt

Molten salt batteries are primary or secondary batteries that use a molten salt as electrolyte. They operate at high temperatures and must be well insulated to retain heat.

Reserve

A reserve battery can be stored unassembled (un-activated and supplying no power) for a long period (perhaps years). When the battery is needed, then it is assembled (e.g., by adding electrolyte); once assembled, the battery is charged and ready to work. For example, a battery for an electronic artillery fuze might be activated by the impact of firing a gun. The acceleration breaks a capsule of electrolyte that activates the battery and powers the fuze's circuits. Reserve batteries are usually designed for a short service life (seconds or minutes) after long storage (years). A water-activated battery for oceanographic instruments or military applications becomes activated on immersion in water.

2.6.4 TYPES OF CHEMICALS USED IN BATTERIES

The primary batteries (non -chargeable) uses various types of chemicals such as zinc, carbon, zinc chloride, alkaline, lithium with Cu O, FeS_2, MnO_2, CrO_2, mercury oxide, silver oxide, and magnesium etc. The secondary Batteries (Re-Chargeable) contains chemical like Ni-Cd, lead acid, Ni-Mh, Ni-Zn, Ag-Zn, lithium ion etc. Lithium has many industrial applications (reviewed in Birch, 1988). Lithium does not have a known biological use and does not appear to be an essential element for life (Lenntech, 2007). The amount of lithium in the human body is approximately 7 mg. Lithium is absorbed from the gastrointestinal tract (Linakis, 2007) and excreted primarily through the kidneys after approximately 24 h (Freeman and Freeman, 2006). Non-supervised or indiscriminate therapeutic use of lithium carbonate can produce certain toxic

symptoms in the neuromuscular, cardiovascular and gastrointestinal system as well as more serious renal damage and can even cause death. The serum (blood) level under therapeutic lithium treatment should not exceed 11.1 mg/L Li and must be carefully monitored. Nevertheless, patients on long-term lithium treatment may suffer from severe neurotoxic effects while serum lithium concentrations are normal (Stern, 1995). Lithium has numerous effects in humans and in other organisms (Moore et al., 1995). Phiel and Klein (2001) reviewed the cause and effect of lithium and showed that therapeutic levels of lithium are clearly able to inhibit functioning of multiple enzymes in the body. Lithium, as a medicine, has multiple effects on embryonic development, glycogen synthesis, hematopoiesis (the formation and development of blood cells involving both proliferation and differentiation from stem cells), and other processes. Lithium is generally found naturally in the aquatic and terrestrial environment but in small concentrations.

Copper Oxide

Copper is an essential metal. However, although it is an effective biocide, it may also affect non target organisms and cause environmental concerns (Kiaune *et al.*,2011). The toxicity of copper in water is greatly affected by the chemical form or speciation of the copper and to what degree it is bound to various ligands that may be in the water, making the copper unavailable to organisms (Burridge *et al.*, *l*2010). The speciation is essential for understanding the copper's bioavailability and subsequent toxicity to aquatic organisms (Thomas *et al.*, 2010). Copper oxide when used in antifouling paint leaches from the boat surfaces and enters the water as a free copper ion (Cu^{+}), which is immediately oxidised to Cu^{2+} and forms complexes with inorganic and organic ligands (Thomas *et al.*, 2010). Copper is a trace element needed at miniscule levels for the proper functioning of all organisms (Kiaune *et al.*, 2011). However, it can be toxic at higher concentrations

(Burridge *et al.*, 2010). Copper is generally toxic to aquatic organisms, with a lethal concentration 50 (LC_{50}) value varying from 5 to 100,000 μg L−1 (USEPA 1995). However, organisms have different mechanisms by which they cope with and process copper (Thomas *et al.*, 2010). Generally copper is actively regulated in fish, decapod crustaceans and algae. It is stored in bivalves, barnacles and aquatic insects (Brix *et al.*, 2000). The bioavailability, bio-distribution to various parts of the organism and bioaccumulation of copper are dramatically influenced by water chemistry. Therefore, water pH, hardness, organic content and salinity play important roles in copper-induced toxicity (Burridge *et al.*, 2010).Thus, increased pH accentuates copper toxicity because of the reduced competition between copper and hydrogen ions at the cell surface (Wilde *et al.*, 2006). In a similar manner, cations that are involved in water hardness also compete with Cu2+ for biological binding sites (Boulanger *et al.*, 2003). Copper bound to organic matter is widely thought to be non-bioavailable and, therefore, non-toxic (Brooks *et al.*, 2007). Dissolved organic carbon (DOC) content is among the most important factors in reducing copper toxicity in both fresh- and salt-water species (Kiaune *et al.*,2011). DOC forms organic complexes with copper, thereby reducing copper's bioavailability (Kiaune *et al.*,2011). The effects of DOC on reducing the toxicity of copper have been reported in fish (Playle *et al.*, 1993), bivalves, (Brooks *et al.*, 2007), echinoderms (Lorenzo *et al.*, 2006), macroalgae (Brooks *et al.*,2008), unicellular algae (Florence *et al.*, 1986), estuarine copepod (Hall *et al.*, 2008) and planktonic crustaceans (Kramer *et al.*, 2004). Some authors confirm that water salinity influences the bio-distribution and bioaccumulation of copper, affecting its toxicity (Polo *et al.*, 2011).

Cadmium (Cd)

Cadmium is a naturally occurring nonessential trace element and its' tendency to bio accumulate in living organisms often in hazardous levels, raises environmental concern (Sfakianakis

et al., 2015). Cadmium production, consumption and emissions to the environment have increased dramatically during the 20th century, due to its industrial use (batteries, electroplating, plastic stabilizers, pigment), and consequently lead to contamination of aquatic habitats (Järup, 2003). This heavy metal has been shown to accumulate mainly (about 75 %) in kidney, liver and gills of freshwater fish (Chowdhury *et al.*, 2006), but it can also be deposited in the hearts (Cattani *et al.*, 1996) and other tissues (Melgar *et al.*, 1997) and cause pathological changes of varying severity in above mentioned organs (Thophon *et al.*, 2004). Morphological and histological alterations in liver of fishes exposed to cadmium have been documented (Thophon *et al.*, 2003). Higher doses of cadmium caused visible external lesions such as discoloration and necrosis on livers of *Cyprinus carpio*, *Carassius auratus* and *Corydoraspaleatus* (Cavas *et al.*, 2005). Omer *et al.,* (2012) reported histopathological alterations in liver, intestine and kidneys of tilapia fish (*Oreochromis niloticus*) exposed to cadmium.

Copper (Cu)

Copper (Cu) is an essential trace metal and micronutrient for cellular metabolism in living organisms on account of being a key constituent of metabolic enzymes (Monteiro *et al.*, 2009). However it can be extremely toxic to intracellular mechanisms in aquatic animals at high concentrations which exceed normal levels (Abdel-Tawwab *et al.*, 2007). Fish can accumulate copper via diet or ambient exposure (Sfakianakis *et al.,* 2015). Even at low environmental concentrations, copper shows distinct affinity to accumulate in the fish liver (Chowdhury *et al.*, 2004). Copper-induced histological alterations are found in the gill, kidney hematopoietic tissue, mechanoreceptors, chemoreceptors, and other tissues (Sorensen, 1991). Morphological and histological alterations in liver of fishes exposed to copper have been documented (Varanka *et al.*, 2001). Higher doses of copper caused visible external lesions such as discoloration and necrosis on livers of *Cyprinus carpio*,

Carassiusauratus and *Corydoras paleatus* (Cavas *et al.*, 2005). (Arellano *et al.*, 1999) reported vacuolization of endothelial cells in fish liver by after copper exposure. Hepatocyte vacuolization, necrosis, shrinkage, nuclear pyknosis and increase of sinusoidal spaces were the distinct changes observed in the liver of copper-exposed fish (Figueiredo-Fernandes *et al.*, 2007). Exposure of Nile tilapia (*Oreochromis niloticus*) to sublethal levels of Cu has been shown to cause histopathological alterations in gills (edema; vasodilation of the lamellar vascular axis) and livers (vacuolation and necrosis) (Figueiredo-Fernandes *et al.*, 2007).

Lead (Pb)

Lead (Pb) is a persistent heavy metal which has been characterized as a priority hazardous substance (Sfakianakis *et al.*, 2015). Although Pb is a naturally occurring substance, its environmental concentrations are significantly increased by anthropogenic sources which include base metal mining, battery manufacturing, Pb-based paints and leaded gasoline (Monteiro *et al.*, 2011). Lead in water may come from industrial and smelter discharges; from the dissolution of old lead plumbing, lead containing pesticides, through precipitation, fallout of lead dust, street runoff, and municipal wastewater (Sepe *et al.*, 2003). Aquatic organisms bio accumulate Pb from water and diet, although there is evidence that Pb accumulation in fish, is most probably originated from contaminated water rather than diet (Cretì *et al.*, 2010). Lead deposits in various fish organs: liver, kidneys and spleen, but also digestive tract and gills (Jezierska B, Witeska M, 2006). Accumulation of lead in different fish species has been determined in several works (Castro-González *et al.*, 2008), leading to disorders in fish body. When *C. batrachus* exposed to 5 ppm of lead nitrate for 150 days, it exhibited marked inhibition of gonadal growth and showed decrease in cholesterol and lipid levels in brain, testis and ovary whereas the liver showed an elevation of both (Katti SR, Sathyanesan AG, 1983).

Zinc (Zn)

Zinc (Zn) is the second most abundant trace element after Fe and is an essential trace element and micronutrient in living organisms, found almost in every cell and being involved in nucleic acid synthesis and occurs in many enzymes (Sfakianakis *et al.*, 2015). Additionally, Zn is involved in more complicated functions, such as the immune system, neurotransmission and cell signaling (Hogstrand C, 2011). It may occur in water as a free cation as soluble zinc complexes, or can be adsorbed on suspended matter. Zinc and its compounds are extensively used in commerce and in medicine. The common sources of it are galvanized ironwork, zinc chloride used in plumbing and paints containing zinc (Clarke *et al.*, 1981). Zinc wastes can have a direct toxicity to fish at increased waterborne levels (Niyogi S, Wood CM, 2006), and fisheries can be affected by either zinc alone or more often together with copper and other metals (Alabaster JS & Lloyd R, 1982).

2.6.5 TOXICITY OF BATTERIES ON ENVIRONMENT

Disposal of batteries after the end-of-life into our environment possess a great danger to our entire ecosystem including land, water, plants, animals, and humans. It is not essentially each of the components are harmful but nevertheless the toxic properties of different chemicals and metals containing hazards properties attributes to environmental pollution. Batteries contain acidic or alkaline chemicals, heavy metals, and the lithium (button) batteries may even pass an electric current to damage or kill tissue. A review article published by Grandjean et al., stated that the few industrial chemicals (lead, methylmercury, polychlorinated biphenyls [PCBs], arsenic, and toluene) can possibility lead to neurodevelopmental disorders in the humans for example, attention deficit disorder, mental retardation and autism. Other types of batteries waste metals such as *cadmium and lead,* which has adverse effects such as damage kidneys and child's

growth, cause brain damage etc. needs to reduce. Literature reports that batteries use cathodes with nickel, cobalt and solvent-based electrode processing showed severe health and environmental hazards. In addition, potassium leakage from the batteries can cause severe chemical burns thereby affecting the eyes and skin. Furthermore, the landfills also generate methane gas leading to the 'greenhouse effect' and global climatic changes. Lithium with CuO, FeS_2, MnO_2, CrO_2 affects in plants and animals interacts with sodium and potassium as well as with enzymes requiring magnesium.

Environmental problems

Batteries are identified as a problem material in the waste stream. Batteries are made from a variety of chemicals to power their reactions. Some of these chemicals, such as nickel and cadmium, are extremely toxic and can cause damage to humans and the environment. In particular, they can cause soil and water pollution and endanger wildlife. For example, cadmium can cause damage to soil micro-organisms and affect the breakdown of organic matter. It can also bio accumulate in fish, which reduces their numbers and makes them unfit for human consumption.

2.6.6 TOXICITY OF BATTERIES TO HUMANS

Batteries are safe, but precaution applies when touching damaged cells and when handling lead acid systems that have access to lead and sulfuric acid. Several countries label lead acid as hazardous material, and rightly so. Let's look at the hazards if not properly handled. Lead is a toxic metal that can enter the body by inhalation of lead dust or ingestion when touching the mouth with lead-contaminated hands. If leaked onto the ground, the acid and lead particulates contaminate the soil and become airborne when dry. Children and fetuses of pregnant women are

most vulnerable to lead exposure because their bodies are developing. Excessive levels of lead can affect a child's growth, cause brain damage, harm kidneys, impair hearing and induce behavioral problems. In adults, lead can cause memory loss and lower the ability to concentrate, as well as harm the reproductive system. Lead is also known to cause high blood pressure, nerve disorders, and muscle and joint pain. Researchers believe that Ludwig van Beethoven became ill and died from lead poisoning. (Lenntech, 2007)

The sulfuric acid in a lead acid battery is highly corrosive and is potentially more harmful than acids used in other battery systems cool the affected tissues and to prevent secondary damage. Immediately remove contaminated clothing and thoroughly wash the underlying skin. Always wear protective equipment when handling the sulfuric acid. *Cadmium,* which is used in nickel-cadmium batteries, is considered more harmful than lead if ingested. Workers at Ni-Cd manufacturing plants in Japan have been experiencing health problems from prolonged exposure to the metal, and governments have banned the disposal of nickel-cadmium batteries in landfills. The soft, whitish metal that occurs naturally in the soil can damage kidneys. Cadmium can be absorbed through the skin by touching a spilled battery. Since most Ni-Cd batteries are sealed, there are no health risks in handling them. The caution applies when working with an open battery.

Nickel-metal-hydride is considered non-toxic and the only concern is the electrolyte. Although toxic to plants, nickel is not harmful to humans. Lithium-ion is similarly benign — the battery contains little toxic material. Nevertheless, caution is required when working with a damaged battery. When handling a spilled battery, do not touch your mouth, nose and eyes, and wash your hands thoroughly.

2.6.7 Effect on Human

Keep small batteries out of children's reach. Children younger than four are most likely to swallow batteries, and the most common types ingested are button cells. The battery often gets stuck in the esophagus (the tube that passes food) and the electrical current burns the surrounding tissue. Doctors often misdiagnose the symptoms, which can show as fever, vomiting, poor appetite and weariness. Batteries that make it through the esophagus often move through the digestive tract with little or no lasting damage. The concern of a parent is not only to choose safe toys, but also to keep small batteries away from young children. Charging batteries in living quarters should be safe. This also applies to lead acid. Ventilate the dwellings regularly as you would a kitchen when cooking. Lead acid produces some hydrogen gas but the amount is minimal when charged correctly. Hydrogen gas is explosive and one would need a concentration of 4% to create an explosion. This level would only be achieved if large lead acid batteries were charged in a sealed room. Over-charging *a lead acid battery* can produce *hydrogen-sulfide*. The gas is colorless, very poisonous, inflammable and has the odor of rotten eggs. Hydrogen sulfate also occurs naturally during the breakdown of organic matter in swamps and sewers; it is also present in volcanic gases, natural gas, and some well waters. Being heavier than air, the gas accumulates at the bottom of poorly ventilated spaces. Although noticeable at first, the sense of smell deadens and potential victims may be unaware of its presence. As a simple guideline, hydrogen sulfide becomes harmful to human life if the odor is noticeable. Turn off the charger, vent the house and stay outside until the odor disappears. [Jürgen Garche 2001]

2.7 Mechanism of pollutants absorption in fish

Over the past few decades, mechanism of pollutant absorption in fish has been studied. For example, a study by (Farombi et al., 2007) has determined an accumulation rate of heavy metals in aquatic organisms. Heavy metals accumulated in the tissues of aquatic living can be a public health concern to both animals and humans (Ashraf, 2005). The permeability of the gills and body surface of the fish varies with temperature and other physical variables (Eddy, 2005). The absorption of metals by aquatic organism involves the transfer of metals to the circulatory system across the epithelial barrier of gills, digestive systems or integument (Deb et al., 1999). This transfer across the epithelial cells consists of three ways. The first one is the uptake by the apical membrane, the interface with the external environment. Secondly, is the movement through the cell and interaction with intracellular ligands and lastly is the efflux across the baso-lateral membrane, the interface with the circulatory system. Organs that serve as the sites for uptake (e.g., gills, intestine and digestive gland) also tend to become concentrated with metals and therefore, exhibit relatively high potentials for bio-accumulation. The higher the metal concentration in the environment, the more it may be taken up and accumulated in the fish (Jezierska et al., 2006). Water hardness (mainly related to calcium concentration) affects the uptake of metals across the gill epithelium (Maceda-Veiga et al., 2012). The excessive uptake of nutritive metals such as Fe, Cu and Zn can be toxic to the fish (Akan et al., 2012).

Fish exposed to organo-chlorine pollutants will be directly absorbed via gill membranes, and the gastrointestinal membrane via the food uptake (Sharma et al., 2009). All organo-chlorine pollutants have the ability to bio concentrate and bio-accumulate. It was reported that the concentration of metals in fish tissues were not directly related to the corresponding concentrations in water (Maceda-Veiga et al., 2012), which means not all the metals taken up are

accumulated in fish body because fish can regulate metal concentration in tissues to a certain degree. Accumulation of metals can be detected in different parts of the fish for example, the accumulation of Cu have been commonly detected in the liver of the fish while Hg have been detected in both liver and muscle tissues of the fish (Chen et al., 2004). There are three possible routes for a substance to enter in a fish: through gills, food and skin (Chen et al., 2004). Gill is the first targets of waterborne metals since it is exposed to the aquatic environment. Several studies also claimed that the major route of bio uptake for metals that are concentrated in fish is across the gill epithelium. The accumulated rate is depends on the balance between the uptake rate, metabolism of the chemical and excretion rate. The accumulation of metals in the fish body is via gills and the food consumption by the fish (Akan et al., 2012). There are some metals that are essential for the fish. However, these metals can be a toxic to the fish when it is exceed the permissible level. Toxic effects of metals become worst when various metabolic activities inside the organism's body fail to detoxify.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION OF THE STUDY AREA

The bioassay experiments was carried out using the Fisheries laboratory of the Faculty of Agriculture, Federal University Oye-Ekiti, Ekiti State while hematological and histological analysis was done at Central Research Laboratory of the Federal University of Technology Akure, Ondo state. The bioassay experiments was conducted under standard static bioassay procedure (Reish and Oshida, 1987; American Public Health Association,1987). This involves carefully controlled environmental conditions as to define the response of the test organism to battery waste.

3.2 PREPARATION OF TEST MATERIAL

Used and discarded batteries were collected and the content of the batteries was removed and grinded with pestle and mortal to a powdery form. The battery content was then weighed out and 1g of the powdery form was mixed with 1litre of distilled water.

3.3 COLLECTION AND ACCLIMATIZATION OF EXPERIMENTAL FISH

Four hundred and fifty (450) live, and apparently healthy fingerlings *C. gariepinus* fingerlings of a measured length and weight were purchased from Afe Babalola Fish Farms (ABUAD FARM) situated in Ekiti-State. The fish was transported in plastic bowl at 0070H and acclimatized for 1 week in the laboratory, inside transparent rectangular plastic tanks (40cm X 29cm X 28cm) container of 60 liters capacity, filled with 20 litre distilled water at Fisheries Research Laboratory of the Federal University Oye-Ekiti, Ekiti State. The fish was fed diet containing 45% crude protein during the acclimatization period. Feeding was discontinued 48

hours before the commencement of the experiments, to minimize the production of waste in the test container.

3.4 TOXICITY TEST

3.4.1 RANGE FINDING TESTS

A preliminary range finding test was conducted on the fingerlings fish to determine the toxicity level of battery waste using standard methods/procedure (American Public Health Association, 1987). In the range finding tests, one control and five tests in triplicates will be set up for each experiment. Grinded battery waste of 1g, 10g, 20g, 30g and 40g mixed with distilled water was introduced randomly and tested for 24hours. The behavior and mortality of the test fish in each tank was monitored and recorded every 15minutes for the first hour, once every hour for the next three hours and every four hours for the next 24hours period on the experiments. In the experiment, 18 (40cm X 29cm X 28cm) plastic tanks of 60litres capacity each was filled with 20litres aerated, distilled water. Twelve *C. gariepinus* fingerlings was batch-weighed with a top-loading mettle balance and distributed randomly in triplicate per treatment. The fish was held in aquarium containing distilled water. The fish was allowed to acclimatize for more than one week under laboratory conditions to allow it adapted to experimental conditions. The period of acclimatization was extended beyond one week to ascertain the condition of the fish. The fish was inspected for disease conditions and general fitness. Fish was individually examined and the healthy ones was used in the study. The average body weight and total body length was determined to the nearest mg and 0.1 cm respectively. The fish was fed during the period of

acclimatization and the water was changed every four days in order to remove fecal and unconsumed feeds. Feeding was discontinued during the 96-hour test period.

3.4.2 DEFINITIVE TEST

The definitive test was conducted using concentration of battery waste of earlier determined from the range finding test. A total of 18 tanks were used for this experiments which included the control and five other treatments, a total number of 12 fish was put in each tank. Concentrations used where 0g/l for control, 5g/l, 10g/l, 15g/l, 20g/l and 25g/l. The plastic tanks were covered with nets during the 96h; there was no water change or feeding throughout the 96hours test period.

3.5 BIOLOGICAL DATA

Fish mortality was monitored and recorded hourly for the first four hours, 4hours for the next 24hours and subsequently every 24 hours for the next 96hours.The inability of the fish to respond to external stimuli was used as an index of death. Dead fish was removed immediately with a scoop net to avoid contamination due to rotting. Apart from monitoring and recording fish mortality, the fish behavior such as erratic swimming, air gulping, loss of reflex, discoloration and molting were monitored.

3.6 DETERMINATION OF LETHAL CONCENTRATION

LC_{50} which is the concentration of battery waste estimated to be lethal to 50% of the test organism after exposure time of 96-hour, was determined graphically using Probit and Logit transformation (USEPA,2000).

3.7 HAEMATOLOGICAL EXAMINATION OF FISH

One test fish was removed from each tank for blood analysis. 2-10ml blood per fish was collected from the severed caudal peduncle of the fingerlings using 2ml disposable plastic heparinized syringes treated with EDTA as anti-coagulant. The blood was stored at -4^{0}C in deep freezer prior to analysis. The blood analysis followed the method describe by Svobodova *et al.* (1991).

3.7.1 BLOOD CELL COUNTS

Haemocytometer was used in blood cell counts. The apparatus consist of a counting chamber, a cover slip, white and red pipette for the blood and a plastic mouth piece for drawing the fluid into pipette. The blood diluting fluid was prepared as describe by Svobodova *et al.* (1991). The blood cell was counted on the counting chamber of haemocytometer with the aid of compound microscope.

RBC = No of cells counted X 5 X10 X 200 ($10^{12}mm^3$)

WBC = No of cells counted X 0.25 X 10 X 20 (10^9mm^3)

3.7.2 PACKED CELLS VOLUME (PVC)

Non clotted blood was drawn by capillary action into microhaematocrit tubes; one end of the tube was sealed with synthetic sealant. The sealed tube was centrifuged in a microhaematocrit centrifuge. Centrifugation lasted for five minutes at 10500 rpm. The packed cell volume was measured using microhaematocrit reader and expressed as percentage.

3.7.3 RED BLOOD CELL

The erythrocyte count was determined in heparinized blood diluted by the Hayem solution at a ratio of 1:200.The flask (bublet) method after Bürker was used for the dilution of the blood. The blood was diluted in special glass bublets. First, a special pipette was used to put an accurate amount of 4975 µl of Hayem solution (filtered before use) into the bublet; then 25 µl of heparinized blood was added, using a flushing micropipette. The micropipette was rinsed several times by repeatedly sucking the solution, the bublet was closed with a rubber stopper and its content was stirred by circling motion for 2 to 3 min. A dropper was used to fill Bürker's counting cell with the diluted blood. The red blood cells were counted in 20 rectangles, regularly distributed over the whole lattice of the counting cell. The counting was done at a 200-fold magnification. The resultant counted amount of erythrocytes was then reduced 100 times and the resultant value is the number of erythrocytes in $T.1^{-1}$ (Tera = 10^{12}) (Z. Svobodová, B. Vykusová 1991).

3.7.4 WHITE BLOOD CELL

The leucocyte count was determined in heparinized blood, diluted with a solution after Procházka and Škrobák at a ratio of 1:200. The Procházka-Škrobák solution had the following composition; sodium chloride NaCl 3.88g, sodium sulphate Na_2SO4 2.50g, sodium monohydrophosphate dodecahydrate Na2HPO4.12H2O 2.91g, potassium dihydrophosphate KH_2PO_4 0.25 ml, formaldehyde 37% 7.50 ml, brilliant cresil blue 0.10g, distilled water at 1000 ml. Fresh (newly prepared) solution was not used for the determination itself, the solution was left to stand for about 2 weeks before it was filtered and used. The flask method after Bürker was used for diluting the blood. Special glass flasks (bublets) or penicillin phials (volume about 15 to

25 ml) was used for the dilution, first, 4975 µl of the Procházka-Škrobák solution was put in the flasks or phial by means of a special pipette and then 25 µl of heparinized blood was added by means of a rinsing micropipette. The solution was then sucked repeatedly several times to rinse the micropipette, the flask was closed by means of a rubber stopper and its content was stirred by a cycling motion for 2–3 minutes. A dropper or a Pasteur pipette was then used to fill Bürker's counting cells with the diluted blood. The leucocytes were counted in 100 large squares. The counting was done at a 200-fold magnification. The total number of leucocytes, counted in the 100 large squares, was multiplied by 0.5 to give the leucocyte count, expressed in $G.1^{-1}$ (G-giga = 10^9)Svobodová Z., Pravda D., Paláčková J. (1991).

3.8 HISTOLOGICAL EXAMINATION OF TEST ORGAN

At the end of the experiments, one fish per treatment was sampled after 96hour of exposure to battery waste for histological analysis. The fish was anesthetized in aerated buffered tricaine methanesulfonate (MSS222) to depress the CNS so as to reduce the impact of pain. The fish was sacrificed with a blow on the head, using a mallet and was dissected to remove the liver and the gills. The organs was fixed in 10 % formalin for 3 days after which the tissue was dehydrated in periodic acid Schiff's reagent (PAS) following the method of Hughes and Perry (1976) in graded levels of 50%,70%,90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding.

The organs were then embedded in molten wax. Tissue was sectioned into a thin section (5-7µm) by means of a rotator microtome and was dehydrated and stained with Harris haematoxyllin-eosin (H&E) stain.Bancroff and Cook,(1994) using a microtone and each section was cleared by placing in warm water (38^0C) where it was picked with clean slide and oven

dried at 58^0C for 30 minutes to melt the wax. Slides containing sectioned materials/tissue was cleared using xylene and graded levels of 50%, 70%, 90% and 100% alcohol for 2 minutes each.

The section was stained in haematoxyline eosin for ten minutes. The stained slides was observed under a light microscope. At varying X100 magnification, sections was examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus PM C35 AD4) a camera (OlympusC40 AB -4) and an automatic light exposure unit (Olympus PM CS5P).The film was removed carefully from the camera and developed into negatives. This was done by placing the film in 400ml fixer(hydroquine as reducing agent, bromide as restrainers and sodium sulfite as preservatives to prevent premature oxidation) fix at 20^0C for 4 minutes with several agitations to prevent excess gram on the film. The finished negative then was washed and rinsed. The reel was then removed from the tank and the fresh negatives were hung up to dry. Printing was done using special paper, which was coated with light-sensitive materials. The enlarger was used to increase the size of the image. Toning solution was used to control its intensity and colour. The negative was finally printed out.

3.9 WATER QUALITY

Water quality monitoring was done prior to the experiment, during the experiment and after the experiment. Water quality parameters to be determined include pH, Dissolved Oxygen Concentration and Temperature.

3.9.1 pH

This was determined using a digital pH meter (Mettle Toledo 320). The electrode was inserted into the bottle containing the water sample after standardization in different buffer, after which the reading was taken.

3.9.2 DISSOLVED OXYGEN

This was measured using a digital dissolved oxygen meter(Jenway-9071) once in a day at 0800hr.

3.9.3 TEMPERATURE

This was taken using a mercury-in-glass thermometer which was placed in the medium inside the test container until the reading was stable. The reading was taken at 10.00hr on each day of the experiment.

3.9.4 HEAVY METAL ANALYSIS

Heavy metals concentration was determined using AOAC (1990) method. Two grams (2 g) of each of the samples was heated in a muffle furnace at 600°C until it changed to ash. Thirty milliliters (30ml) of 0.1M H_2SO_4 was used to digests the ash, and the solution will be made up to 100ml with deionized water and then filtered. The concentrations of these heavy metals in the battery, gill, liver of *Clarias gariepinus* was measured by Atomic Absorption Spectrophotometer AAS; model SSI UV 2101 at the Central Research Laboratory of the Federal University of Technology Akure, Ondo State, Nigeria. The instrument setting and operational conditions was done in accordance with the manufacturers' specifications. Results were expressed in mg/kg dry weight for the body parts and mg/litre for the water samples.

3.10 STATISTICAL ANALYSIS

The data resulting from the experiments was subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package Science Software). Duncan's multiple comparisonwas used to evaluate the comparison between means at $P \leq 0.05$ (Duncan,1955). Also

the descriptive statistics such as percentages and histogram was used to analyze parameters such as water quality and survival. T-test was used for determining the significant differences between different treatments, using SPSS version 17 for windows.

CHAPTER FOUR

4.0 RESULTS

4.1 Water quality parameters

The water quality parameters that was tested were temperature, pH and dissolved oxygen concentration. It was measured at 24hours interval during the 96hours period of the experiment and the results are shown in the table1 below.

Table 1: Physiochemical parameters of test medium with treatment and hours as variation

variation	parameters	Ph	Temperature(^{0}C)	Dissolved oxygen	Conductivity
Hours	12 hours	9.33± 0.26^{c}	25.89± 0.34^{c}	5.50± 0.12^{bc}	243.72± 0.52^{e}
	24 hours	8.17± 0.22^{b}	25.17± 0.12^{c}	4.89± 0.24^{a}	267.11± 0.28^{f}
	36 hours	8.06± 0.19^{b}	25.72± 0.30^{c}	5.17± 0.90^{b}	236.89± 0.22^{a}
	48 hours	8.00± 0.17^{b}	25.78± 0.13^{c}	5.17± 0.15^{b}	242.50± 0.15^{c}
	60 hours	7.67± 0.11^{a}	24.59± 0.12^{b}	5.11± 0.76^{b}	240.11±1.03^{c}
	72 hours	7.94± 0.56^{ab}	25.56± 0.17^{c}	5.50± 0.12^{bc}	241.56± 0.98^{d}
	84 hours	7.67± 0.11^{a}	22.89± 0.17^{a}	5.17± 0.90^{b}	239.50± 1.18^{b}
	96 hours	7.61± 0.12^{a}	26.06± 0.17^{d}	5.44± 0.12^{b}	241.67± 0.75^{d}
Treatment	T01	7.25± 0.13^{a}	25.34± 0.38^{b}	5.25± 0.16^{b}	238.50±1.71^{a}
	T02	7.25± 0.16^{a}	25.38± 0.26^{b}	5.25±0.16^{b}	241.00± 1.72^{ab}
	T03	7.37± 0.18^{a}	25.88± 0.35^{bc}	5.25± 0.16^{b}	238.00± 2.92^{a}
	T11	8.25± 0.25^{b}	25.50± 0.42^{b}	5.63± 0.18^{c}	240.50± 3.94^{ab}
	T12	8.25± 0.25^{b}	25.63± 0.46^{b}	5.38± 0.18^{b}	241.00± 4.18^{ab}
	T13	8.25± 0.25^{b}	25.75± 0.31^{bc}	5.50± 0.19^{bc}	242.88± 2.87^{b}
	T21	8.75± 0.41^{bc}	25.75± 0.53^{bc}	5.38± 0.18^{b}	245.63± 3.77^{d}
	T22	8.37± 0.32^{b}	25.63± 0.32^{b}	5.50± 0.19^{bc}	246.25± 3.69^{d}
	T23	8.75± 0.37^{bc}	25.50± 0.42^{b}	5.38± 0.18^{b}	244.88± 4.49^{cd}
	T31	8.13± 0.30^{b}	25.25± 0.41^{b}	5.38± 0.18^{b}	244.88± 4.30^{cd}

T32	8.25± 0.25^{b}	25.00± 0.27^{b}	5.13± 0.13^{b}	246.38± 4.03^{d}
T33	8.13± 0.30^{b}	25.63± 0.32^{b}	5.25± 0.16^{b}	246.50± 4.27^{d}
T41	8.25± 0.25^{b}	23.00± 2.31^{a}	5.25± 0.25^{b}	246.50± 4.00^{d}
T42	8.00± 0.33^{b}	23.38± 2.23^{a}	5.13± 0.23^{b}	250.38± 3.96^{f}
T43	7.75± 0.25^{ab}	23.25± 2.19^{a}	5.13± 0.23^{b}	248.50± 4.38^{e}
T51	8.25± 0.25^{b}	25.63± 0.18^{b}	5.00± 0.27^{b}	243.75± 4.77^{bc}
T52	7.75± 0.25^{ab}	25.63± 0.26^{b}	4.88± 030^{a}	244.50± 5.27^{c}
T53	7.88± 0.23^{ab}	26.38± 0.38^{c}	4.75± 0.31^{a}	244.38± 4.80^{c}

Mean ± S.E with different superscript across row is significantly different at $p< 0.05$

4.2 Behavioral observation of African catfish exposed to battery waste

The tables below shows the observations of different parameters in seconds based on the different concentration of battery waste. On addition of battery waste, there was an increased in tail fin movement, operculum ventilation, air gulping count.

Table 2: Observations on tail fin movement in seconds of the fishes when exposed to varying concentration of battery waste.

	Treatment					
Observations in seconds	**Treatment 0**	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
First	24.67± 0.89^{a}	64.00±1.53^{d}	54.67± 2.85^{b}	55.67±4.84^{b}	65.67±2.3a3^{e}	60.67±2.03^{c}
second	21.00±	54.00±	63.00±3.51^{d}	57.33±	66.67±2.3	57.33±1.7

	0.58[a]	7.57[b]		2.40[c]	3[e]	8[c]
Third	23.00± 1.15[a]	60.67± 2.60[c]	56.67±2.60[b]	61.67±3.17[c]	67.67±6.23[d]	56.33±0.88[b]

Mean ± S.E with different superscript across row is significantly different at $p < 0.05$

Table 3: Observations on operculum ventilation count (in seconds) of the fishes when exposed to varying concentration of battery waste.

	Treatment					
Observations in seconds	**Treatment 0**	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
First	31.67± 0.88[a]	71.00±0.58[d]	69.67±1.20[c]	66.33±1.45[a b]	64.00± 0.58[b]	65.33±0.67[a b]
second	30.67± 0.88[a]	73.00±1.52[d]	70.00±5.20[c]	66.00±1.52[b c]	65.33±0.33[b]	66.67±0.33[b c]
Third	31.67± 0.88[a]	72.67±1.20[c]	78.67±0.33[d]	67.00±1.16[b c]	66.67±0.33[b c]	65.67±0.33[b]

Mean ± S.E with different superscript across row is significantly different at $p < 0.05$

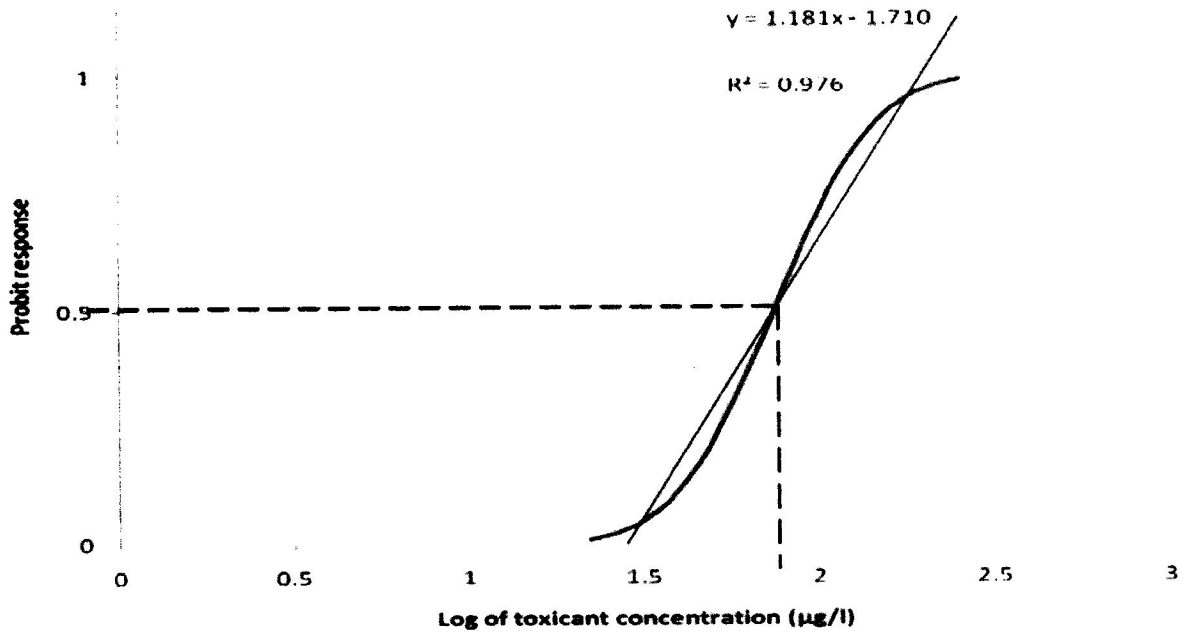
Table 4: Observations on Air gulping index of the fishes when exposed to varying concentration of battery waste

	Treatment

Observations in seconds	Treatment 0	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
First	25.67± 0.33[a]	71.00±1.52 [c]	67.00±6.66[b]	74.00±0.58[d]	84.00±1.15 [e]	68.67±2.85 [b]
Second	24.00± 0.58[a]	74.00±2.51 [c]	72.67±1.86[b]	76.00±2.08[d]	85.00±1.53 [e]	72.67±0.33 [b]
Third	24.00± 0.58[a]	72.00±3.05 [b]	75.33±1.20[c] [d]	75.67±1.45[c] [d]	89.00±1.00 [d]	74.00±0.58 [c]

Mean ± S.E with different superscript across row is significantly different at $p < 0.05$

4.3 ESTIMATION OF LC_{50}



LC_{50} for *Clarias gariepinus* exposed to varying concentration of battery waste were 2.22 µg/l, 2.73µg/l, 2.82 µg/l, 3.44 µg/l, and 3.93µg/l after 24, 48, 72 and 96hours.

4.4 Hematological examination

Table 4 shows the result obtained from the analysis of hematological examination of the fishes. The concentrations of battery waste used were 0grams, 5grams, 10grams, 15grams, 20grams and 25grams. Red blood cell (Rbc) count was highest at 5grams concentration of battery waste and lowest at 25grams concentration of battery waste. White blood cell (Wbc) count was highest at 5grams concentration of battery waste and lowest at no concentration of battery waste. Lymphocyte was highest at 5grams concentration of battery waste and lowest at 25 grams concentration of battery waste. Neutrophil was highest at 20grams concentration of battery waste and lowest at 5grams concentration of battery waste. Packed cell volume was highest at 20grams concentration of battery waste and lowest at 25 grams concentration of battery waste. The result below also shows the Analysis of variance of the hematological parameter at a significant level of 1%.

Table 6: Hematological Parameter of African Catfish Exposed to Varying Concentration of Battery waste

	Treatment					
Parameters	**Treatment 0**	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
RBC	15.00± 2.89^{b}	45.00± 1.15^{c}	29.00± 2.65^{b}	18.33± 3.84^{b}	25.00± 2.89^{b}	8.33± 2.19^{a}
WBC	2.00± 0.77^{a}	25.00± 1.53^{a}	4.33± 1.45^{a}	2.33± 0.88a	3.33± 0.33^{a}	4.00± 1.15^{a}
Lymp	30.00± 1.15^{c}	80.00± 1.73^{d}	35.00± 3.10^{c}	20.00± 5.00^{b}	75.00± 2.89^{d}	15.00± 5.00^{b}
Neutro	70.00± 1.15^{d}	20.00± 1.73^{a}	65.00± 3.10^{e}	80.00± 5.00^{d}	25.00± 2.89^{b}	78.33± 6.01^{d}
PCV	37.00± 3.05^{c}	37.00± 1.52^{b}	42.00 ± 1.15^{d}	40.00± 2.89^{c}	60.00± 1.15^{c}	29.00± 2.08^{c}

Mean ± S.E with different superscripts within column are significantly different at $p< 0.05$

4.5 Heavy metal analysis

The result below show the concentration of these various heavy metals Arsenic, Cadium, Lead (Pb),Nickel() and Lithium() at different levels of concentration of battery waste. Consequently, the concentration of heavy metals increased after the addition of more battery waste. It was observed that lithium had the highest concentration at every added concentration of battery waste.

Table 5: Heavy Metal in Water Sample exposed to varying concentration of Battery waste

TREATMENTS		ARSENIC MG/L	CADMIUM MG/L	LEAD MG/L	NICKEL MG/L	LITHIUM MG/L
T0	Mean	.000000	.009000	.089000	.012000	8.825000
	Std. Error of Mean	.	.	.	.	.
T1	Mean	.001000	.012000	.089000	.026000	10.045000
	Std. Error of Mean	.	.	.	.	.
T2	Mean	.002000	.015000	.109000	.057000	10.494000
	Std. Error of Mean	.	.	.	.	.
T5	Mean	.005000	.025000	.195000	.121000	15.173000
	Std. Error of Mean	.	.	.	.	.
T3	Mean	.002000	.001700	.117000	.058000	11.363000
	Std. Error of Mean	.	.	.	.	.
T4	Mean	.003000	.021000	.152000	.083000	12.120000
	Std. Error of Mean	.	.	.	.	.
Total	Mean	.002167	.013950	.125167	.059500	11.336667
	Std. Error of Mean	.0007032	.0034219	.0168729	.0160432	.8947759

Mean ± S.E with different superscripts within column are significantly different at $p< 0.0$

4.6 HISTOLOGY OF LIVER AND GILLS OF THE EXPOSED FISH

Histology of liver:

PLATE 1: Histology of the liver of the 5g/l, showing normal morphology of hepatocyte with no inflammation. (X400)

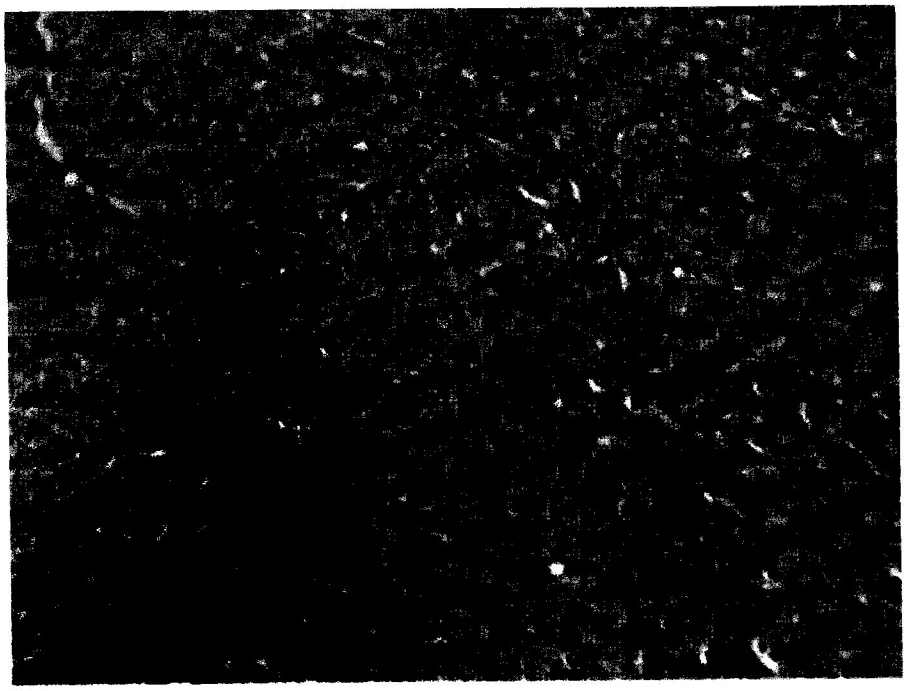


PLATE 2: Histology of Liver of *Clarias gariepinus* exposed to the 10g/l of battery waste showing normal hepatocyte with mild inflammation. (X400)

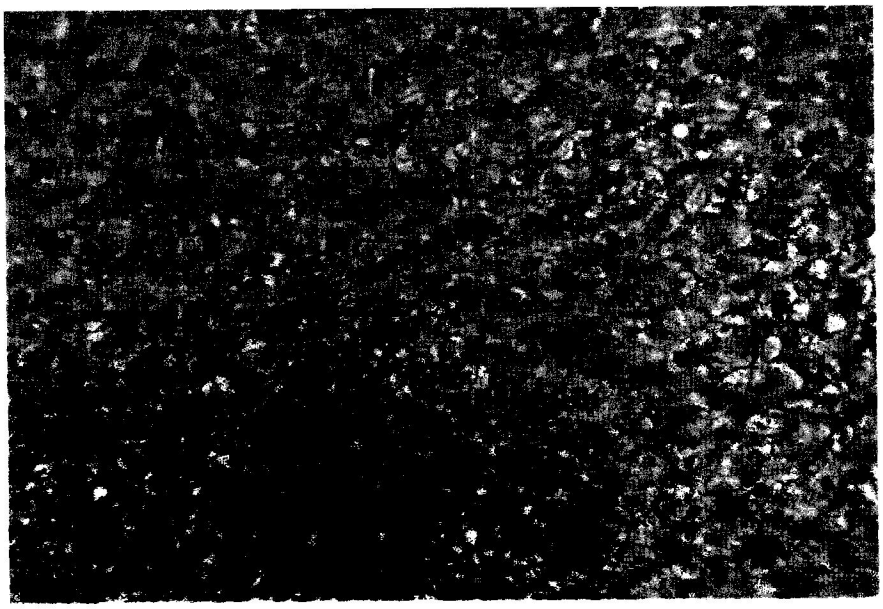


PLATE 3: Histology of Liver of *Clarias gariepinus* exposed to 15g/l of battery waste showing mild steatosis, patchy lobular and hepatocyte necrosis. (X400)

PLATE 4: Histology of Liver of *Clarias gariepinus* exposed to 20g/l of battery waste showing moderate steatosis with multifocal hepatocyte necrosis and inflammation but no fibrosis. (X400)

PLATE 5: Histology of Liver of *Clarias gariepinus* exposed to 25g/l battery waste showing diffuse lobular hepatocytes necrosis with severe inflammation but no fibrosis. (X400)

Histology of gills of *Clarias gariepinus* exposed to varying degree of battery waste

PLATE 6: Gills of *Clarias gariepinus* exposed to 5g/l showing remarkable morphology with no sign of necrosis or inflammation. (X400)

PLATE 7: Histology of Gill of *Clarias gariepinus* exposed to 10g/l of battery waste showing unremarkable morphology with no necrosis and inflammatory infiltrate is unremarkable compared to the control. (X400)

PLATE 8: Histology of Gill of *Clarias gariepinus* exposed to 15g/l of battery waste showing no sign of necrosis but with significant lymphocytic inflammatory infiltrates.(X400)

PLATE 9: Histology of Gill of *Clarias gariepinus* exposed to 20g/l of battery waste showing multifocal area of necrosis with moderate lymphocytic inflammatory infiltrates. (X400)

PLATE 10: Histology of *Clarias gariepinus* exposed to 25g/l of battery waste showing severe necrosis with severe inflammation. (X400)

CHAPTER FIVE

5.0 DISCUSSION

Scholars described fish growth as the end product and an integrator of the reactions involving the intrinsic and extrinsic factors (including the aquatic medium) in which the fish finds itself. According to Ivoke et al, (2007) specific features of the catfish environment are of primary importance in determining the growth and survival of the species in varying degrees. Naturally, most organisms possess well defined range of physiochemical tolerance. The result obtained from the analysis of the physiochemical parameters of both the control and the other concentrations used during the experiment are shown in Table 1 above. The result obtained revealed that there is significant difference between the control and varying concentrations. No distinct changes were recorded in the temperature, dissolved oxygen concentration and conductivity throughout the course of the experiment. The implication of the pH result obtained from this study is that there reduction in pH with time as pH recorded at first 24hour was higher than at the final 96hours. Though the pH recorded for this study still falls within the Federal Environmental Protection Agency limit. For best fish production, Ayodele and Ajani (1999) recommended a pH of range of 6.5 - 9 while Kunle (2000) recommended a pH range of 6.5 and 8.5.

In the current study, abnormal fish behavior occurred more at higher concentrations and shows that fish behavior is dose-dependent. Abnormalities observed in fish behavior are an indication that fish undergone stress when exposed to toxicants (Adesina, 2008). From the result above it was showed that behavioral changes such as tail movement, operculum ventilation count and air gulping index increased per second after exposure of *Clarias gariepinus* to battery waste compared to the control test organism. This shows that fish behavior changes with changes with

the introduction of toxicant to the water body and this conforms with the findings of Olufayo Akinpelu, (2012) who worked on exposure of *O. niloticus* fingerlings to fresh root-bark hot ethanolic extract(FRBHEE) of *Moringa oleifera*. Death could have occurred either by direct poisoning or indirectly by making the medium unconducive or even by both, whichever is the case, the source of death was batter waste. Several abnormal behavior such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increase opercula activities, surface to bottom movement, sudden quick movement, resting at the bottom were similar to the observations of (Rahman et al., 2002, Aguigwo, 2002 and Omoniyi et al., 2002). The stressful and erratic behavior of *Clarias gariepinus* juvenile in the experiment indicates respiratory impairment; probably due to the effect of the toxicant battery waste on the gills. The fishes became inactive at higher concentrations with increased time of exposure to toxicant.

The changes in the hematological parameters of fish are a helpful biomarker for evaluating their health status Řehulka et al.(2004). According to Van Vuren, (1986) Studies have shown that when the water quality is affected by toxicants, any physiological change will be reflected in the values of one or more of the hematologic parameters. Thus water quality is one of the major factors responsible for individual variations in fish hematology since they are sensitive to slight fluctuations that may occur within their internal milieu (Fernandez and Mazon, 2003). In this study, it was observed that White Blood Cell increased with increase in concentration of battery waste, this could be due to the attempt of the fish to fight against the pollutants which may lead to production of more WBC to improve the health status of the fish thus, this is similar to those reported in *C. gariepinus* exposed to petroleum oil and *Parkia bioghobossa* pods extracts (Adewoye 2010). The increase in WBC with increase in concentration observed in this study is similar with findings of (Nath and Banerjee, 1995; Mazon *et al.*, 2002)

who reported significantly higher WBC in fish exposed to increased copper concentration. Mishra and Srivastava (1980) also reported an increase in leucocytes count when they exposed fishes to heavy metals. From the hematological study it was also observed that red blood cell decreased with increase in concentration of battery waste as shown in the table above. It showed that red blood cell was highest in 5g/l concentration which was of battery waste compared to 25g/l concentration which was the lowest. This conforms with the result of the study conducted by Holcombe *et al.*(1976) who found that there was decline in RBC values of brook trout *(Salvalinus fontinalis)* on exposure to long term effects of lead. It was suggested that the reduction in the RBCs counts during treatment, may be due to the development of hypoxia that led to either increase in the destruction of RBS or decrease in the genesis of RBCs due to non-availability of Hb content in cellular medium (Akinrotimi *et al.*, 2012; Vasantharaja *et al.*, 2012). The lymphocyte was observed to decrease with increase of battery waste concentration; this is corroborated by Srivastava and Narain (1982) who also reported an decrease in the number of lymphocytes in *Heteropneustes fossillis* treated with endrin and nuvacron. Similarly, lymphocytosis was reported in *Ictalurus punctatus* subjected to hypoxia (Grizzle and Rogers, 1976; Scoot and Rogers, 1981). In fish, lymphocytes participate in inflammatory processes (Martins, 2000), although the function of these cells remains unclear (Tavares-Dias; Moraes, 2004). It is possible that they are recruited to the focus on the lysate by the defense mechanisms, which might explain the high numbers of these cells in the blood circulation of the infected *C. gariepinus.* According to Ayandiran *et al.* (2010) and Gabriel *et al.* (2011), normal PCV values usually fall within the range of 20-35% and are rarely greater than 50% for fish. However, from the result of this study which showed PCV to be normal at control concentration with 37% while that of the highest concentration of battery waste was recorded to be decreased at 29%. This has

showed that with increase in the concentration of battery waste in a water body there is decrease in the packed cell volume in blood of the fish, this could be because transport of metals in fish occurs through the blood where the ions are usually bound to proteins (Ayandiran *et al.*, 2009); and pollutants generally produce relatively rapid changes in blood characteristics of fish (Johansen *et al.*,1994; Moussa *et al.*,1994; Ezzat *et al.*, 1998; Rizkalla *et al.*, 1999).

In ecotoxicological studies, histopathology is gaining importance for rapid evaluation of the toxic effect of pollutant and considered as an important tool for examining the effect in different organs and even tissue of the body (Latif et al., 2013). Many investigators reported lesions in different organs of fish in response to various chemical contaminants like heavy metals and pesticide (Omitoyin et al.,2006; Ayoola and Ajani, 2008; Velmurugan et al., 2009a,).In the present study, the histopathological examination of the gill and liver tissues of juvenile *Clarias gariepinus* exposed to varying concentrations of battery waste revealed that the liver and gills were affected.

The result of this study demonstrates that the liver of fish in the 5g/l concentration exhibits a normal hepatocyte and there were no pathological abnormalities compared to the concentration of 10g/l, 15g/l, 20g/l and 25g/l (Plate 1-5) where steatosis (fatty liver disease), patchy lobular, hepatocyte necrosis and inflammation were observed. This agrees with the result of Loganathan *et al.*(2006) who reported severe necrosis, inflammation and degeneration of hepatocytes in the liver tissue of *Labeo rohita* exposed to zinc. It showed that with increase in concentration of battery waste the resultant effects become more severe on the liver. Hepatocyte necrosis of the liver tissues observed in this study was because of the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis (El-Naggar *et al.*,

2009). Alterations in liver hepatocytes which includes the formation of vacuoles in the hepatocytes and patchy lobular associated with stress have been well studied and reported, this is as a result of histopathological lesion on liver tissue. (Metelev *et al.*, 1971).

The examination of the gills showed that the fish in the concentration of 5g/l of battery waste did not undergo any significant changes in morphology due to the mild nature of the toxicant, and no signs of necrosis or inflammation compared to the fish exposed to higher doses of battery waste which showed degeneration of the morphology of the gill, significant lymphocytic inflammatory infiltrates, necrosis and inflammation, this conforms with the findings of Winkaler *et al.* (2001) and Tkatcheva *et al.* (2004) who reported that gills may change morphologically in order to maintain physiological functions under effects of pollution. When the gills are damaged, hypoxic condition occurs due to alteration in the gas exchange mechanisms (Das and Mukherjee, 2003). The heavy metal observed in this study showed that arsenic, cadmium, lead, nickel and lithium were present in the battery waste in varying proportions and they all increased in the fish with increase in the concentration of battery waste added to the fish tank. Lithium ion was identified to be highest metal in the tissue of the fish. This shows that fish takes up and bio-accumulates heavy metals in various quantities depending on their concentrations in the water. This agrees with the results of Oluyemi *et al* (2008) and Kemdrin (1979) who reported on the level of heavy metals in aquatic organism from different water bodies.

5.1 CONCLUSION AND RECOMMEDATIONS

This study has proved that battery waste is toxic to aquatic life and the aquatic ecosystem at large. This study has been able to prove that it is important for battery waste not to be dumped indiscriminately in water bodies because they have resultant effects on health and quality of aquatic organism and this is important because humans are at the top of the food chain and can be affected adversely by consuming fishes in battery waste waters as they contain heavy metals that are toxic. The changes in haematological and histological properties of the test organism have shown that are they reliable indices in toxicity assay. Hence, good knowledge of fish response to various stressors will be immensely helpful in improving production of fish and in providing information on ways of effectively controlling and monitoring stress in aquaculture.

Due to the findings of this study, it is recommended that the relevant agencies in charge of waste disposal should take cognizance of battery waste and map out methods to intercept the waste before it comes in contact with water bodies. People living in rural and urban areas alike needs to be sensitized on the harmful effects of dumping waste that include battery waste indiscriminately in water bodies or sewage systems that ultimately leads to water bodies. Furthermore, the government and relevant agencies should diverse means of proper waste disposal in communities and they should favor rechargeable batteries to non-rechargeable once as it decreases the amount of battery waste in circulation.

5.2 REFERENCES

Akan J., S. Mohmoud, B. Yikala and V. Ogugbuaja, 'Bioaccumulation of Some Heavy Metals in Fish Samples from River Benue in Vinikilang, Adamawa State, Nigeria', *American Journal of Analytical Chemistry*, vol. 3, p. 727, 2012

Akinrotimi, O.A. and U.U. Gabriel, 2012.Haematological profile of Clarias gariepinus broodfish raised in water recirculating system. Adv. Agric. Sci. Eng.Res., 2: 97–103

American Public Health Association (1987). Standard methods for the examination of water and wastewater, American Public Health Association (APHA, AWWA, WPCF), 20th ed., Washington, DC.

Ashraf W., 'Accumulation of heavy metals in kidney and heart tissues of Epinephelus microdon fish from the Arabian Gulf', *Environmental Monitoring and Assessment*, vol. 101, no. 1-3, pp. 311--316, 2005.

Ayandiran t.a., fawole o.o., adewoye s.o. And ogundiran m.a., (2010): Bio-concentration of metals in the head capsule and skeleton of *clariasgariepinus* exposed to sublethal concentrations of soap and detergent effluents. *Agri. & biol. J. North america.* Issn online: 2151-7525.

Ayoola, S.O. and E.K. Ajani, 2008. Histopathological effects of Cypermethrin on Juvenile African Catfish (Clarias gariepinus). J. Biol., Res., 1: 1–14

Chen B. and C. Liao, 'Farmed tilapia *Oreochromis mossambicus* involved in transport and biouptake of arsenic in aquacultural ecosystems', *Aquaculture*, vol. 242, no. 1, pp. 365-380, 2004.

Crompton, T. R. *Battery Reference Book* (third ed.). Newnes. p. Glossary 3. *ISBN 0080499953*. *Retrieved 2016-03-18,* 2000

Das, P., Ayyappan, S., Jena, J. and Das, B. (2004) Acute toxicity of ammonia and its sub-lethal effects on selected haematological and enzymatic parameters of mrigal, Cirrhinus mrigala (Hamilton). Aquaculture Research, 35, 134-143.

Deb S. and T. Fukushima, 'Metals in aquatic ecosystems: mechanisms of uptake, accumulation and release-Ecotoxicological perspectives', *International Journal of Environmental Studies*, vol. 56, no. 3, pp. 385-417, 1999.

Eddy F., 'Ammonia in estuaries and effects on fish', *Journal of Fish Biology*, vol. 67, no. 6, pp. 1495--1513, 2005.

Emenike, C.U, Fauziah, S.H and Agamuthu, P 2012. Leachate risk: Bioaccumulation of heavy metals in fish. Proceeding of the 7th Asian Pacific Landfill Symposium, Bali, Indonesia, October 8th-10th, 2012

Ezzat a.a., abdel aziz s.h., el nady f.e. And abdel–barr m. (1998): Impacts of sublethal concentrations of lead on haematology of *siganus rivulatus*. Proceeding of the 8th int. *Conf. Environ.* Protection is a must.

Farombi E., O. Adelowo and Y. Ajimoko, 'Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River', *International Journal of Environmental Research and Public Health*, vol. 4, no. 2, pp. 158-165, 2007.

Froese, Rainer, and Daniel Pauly, eds. Species of *Clarias* in FishBase. 2011

Gabriel U.U., Akinrotimi O.A., Eseimokumo F. 2011 –Haematological responses of wild Nile tilapia Oreochromis niloticus after acclimation to captivity – J.Biol. Sci. 4: 225-230.

Grandjean P, Landrigan PJ, 2006. Developmental neurotoxicity of industrial chemicals, Lancet; 368: 2167–78*ISSN: 1735-6865*

Haylor GS, 1993. Aspects of the biology and culture of the African catfish Clarias gariepinus (Burchell 1822) with particular reference to developing African countries. Recent Advances in Aquaculture, 4:233-294.

Huisman EA and Richter CJJ, 1987. Reproduction, growth, health control and aquacultural potential of the African catfish, Clarias gariepinus (Burchell 1822). Aquaculture, 63(1-4):1-14.

Latif, A., M. Ali, A.H. Sayyed, F. Iqbal, K. Usman, M. Rauf and R. Kaoser, 2013. Effect of Copper Sulphate and Lead Nitrate, Administered Alone or in Combination, on the Histology of Liver and Kidney of Labeo rohita. Pak. J. Zool., 45: 913–920

Maceda-Veiga A., M. Monroy and A. de Sostoa, 'Metal bioaccumulation in the Mediterranean barbel (*Barbus meridionalis*) in a Mediterranean River receiving effluents from urban and industrial wastewater treatment plants', *Ecotoxicology and Environmental Safety*, vol. 76, pp. 93-101, 2012.

Maheswaran, R., A. Devapanl, S. Muralidharan, B. Velmurugan and S. Ignaeimuthu, 2008. Hematological studies of fresh water fish, Clarias batradrus (L) exposed to mercuric chloride. IJIB., 2: 49–54

Medja n. And golemi s.,(2011): Histopathology of larve *carassius gebelio* biomarker of pollution with cyanobacteria of shkodra lake water, albania, *j. Environ. Res.Develop.*, 6(2), 281-285,

Moussa fi, abou shabana mb and el-toweissy my (1994): Short and long term effects of acid stress on survival, behavioral and some cellular blood constituent in the catfish *clarias lazera. Bull. Nat.inst.Oceanogr. & fish. A.r.e.* 20 (2), 229-248.

Mwita, CJ; Nkwengulila, G. (2008). "Molecular phylogeny of the clariid fishes of Lake Victoria, Tanzania, inferred from cytochrome b DNA sequences". Journal of Fish Biology. 73 (5): 1139–1148. doi:10.1111/j.1095-8649.2008.01935.

Omitoyin, B.O., E.K. Ajani and A.O. Fajimi, 2006. Toxicity Gramoxone (paraquat) to juvenile **African catfish, Clarias gariepinus** (Burchell, 1822). Amer. Eur.J. Agric. Environ. Sci., **1: 26–30**

Oshode, O. A., **Bakare, A. A., Adeogun,** A. O., Efuntoye M. O. and Sowunmi, A. A. 2008. Ecotoxicological Assessment Using *Clarias Gariepinus* and Microbial Characterization of Leachate from Municipal Solid Waste Landfill. *Int. J. Environ. Res., 2(4): 391-400, Autumn 2008*

Palacin 2009. Recent advances in rechargeable battery materials: a chemist's perspective, Chem. Soc. Rev., , 38, 2565–2575

Pauling, Linus (1988). "15: Oxidation-Reduction Reactions; Electrolysis.". General Chemistry. New York: Dover Publications, Inc. p. 539. *ISBN 978-0-486-65622-9*.

Pistoia, Gianfranco (2005-01-25). Batteries for Portable Devices. Elsevier. p. 1. ISBN 0080455565. Retrieved 2016-03-18.

Powers, D. A. 1989. Fish as model systems. Science **246**, 352 – 358.

Reed, W., J. Burchard, A.J. Hopson, J. Jennes, I. Yaro (1967). *Fish and Fisheries ofNorthern Nigeria.* 1st ed. Ministry of Agriculture, Northern Nigeria. 226p

Reish, D. J. and Oshida, P. S. (1987). Manual of methods in aquatic environment research. Part 10. Short term static bioassays. FAO Fish Tech. Pap. (247), 62.

Rizkalla eh, abd el -haleem me and shalaby ame (1999): Hematological changes in *cyprinus carpio l.* As a result of short and long term exposure to different combinations of copper, cadmium and zinc. *Egypt j. Aquat. Biol. & fish.* 175-193.

Sharma C., B. Rosseland, M. Almvik and O. Eklo, 'Bioaccumulation of organochlorine pollutants in the fish community in Lake Arungen, Norway', *Environmental Pollution*, vol. 157, no. 8-9, pp. 2452-2458, 2009.

Sudarto; Teugels, Guy G.; Pouyaud, Laurent (2004). "Description of a New Clariid Catfish, Clarias pseudonieuhofii from West Borneo (Siluriformes: Clariidae)" (PDF). Zoological Studies. 43 (1): 8–19. Retrieved 2009-06-24.

Svobodova z, Pravda d and j Palackova., (1991): Unified methods of haematological examination of fish. Research inst. of fish culture and hydrobiology Vodnany, Czecholovakia, , *p 31.*

Teugels GG, 1986. A systematic revision of the African species of the genus Clarias (Pisces; Clariidae). Ann. Mus. R. Afr. Centr. Sci. Zool., 247:199 pp.

Teugels GG; Guyomard R; Legendre M, 1992. Enzymatic variation in African clariid catfishes. Journal of Fish Biology, 40(1):87-96.

Usepa (united states environmental protection agency) (2000) draft aquatic life water quality criteria for selenium. Epa-822-d-04-001. Washington, dc: usa.

Van Oijen MJP, 1995. Key to Lake Victoria fishes other than haplochromine cichlids, Appendix I. In: Witte F, Van Densen WLT, eds. Fish Stocks and Fisheries of Lake Victoria. A Handbook for Field Observations. UK: Samara Publishing Limited, 209-300

Velmurugan, B., M. Selvanayagam, E.I. Cengiz andE. Unlu, 2009b. Histopathological Changes in the Gill and Liver Tissues of Freshwater Fish, Cirrhinus mrigala Exposed to Dichlorvos. Int.J. Braz. Arch. Biol. Technol.,52: 1291–1296

Velmurugan, B., T. Mathews and E.I. Cengiz, 2009a. Histopathological effects of Cypermethrin on gill, liver and kidney of fresh water fish Clarias gariepinus (Burchell, 1822), and recovery after exposure. Environ. Technol., 30: 1453–1460

Yang, W., 2007. Problems exposed in the domain of recycling and using of battery in China. Environment 1, 68–71 (in Chinese).