Effects of Some Heavy Metal Pollutants On Glutathione Production In *Clarias Gariepinus* (Burchell, 1822) In An *In Situ* Bio-Assay In River Galma, Kaduna State, Nigeria

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Abstract

Pollution of the aquatic media poses a lot of problems to the living biota within them and other organisms dependent on them for survival. Pollution from heavy metals are particularly deleterious as they are persistent within the ecosystem and have the tendency to bio-accumulate especially in higher vertebrates that depend directly or indirectly on them. Fishes possess different defensive mechanisms to counteract the impact of toxicants.

This research focuses on the effects of some heavy metals on production of glutathione in *Clarias gariepinus* in an *in situ* bioassay in River Galma.120 samples (40 per exposure) of 20-45g size range of juveniles were exposed to the river environment for fourteen days in a cage system at five different locations along the river course for three different periods of the year. Glutathione were assayed for after the 7th and 14th day of exposure. Heavy metal (Pb, Cr, Cd, Zn and Mn) contents were also tested for in digested pooled samples of water, livers and gills of the fish.

The results indicate significant difference in the levels of glutathione production in the gills in September at $P<0.05$ level of significance but not in the livers and kidneys. Glutathione production levels were highly significant in the gills and kidneys, and significant in the livers amongst the sites. Glutathione production levels were highly significant in the kidneys in all the months of exposures and locations. Highest mean values were obtained in all the organs of fish and in all the exposures from Kakeyi site. There were significant differences in the Chromium concentration in the gills and livers of the fish in all the exposures. There were strong positive correlation in glutathione production levels and Pb, Cr and Zn concentrations. Also, there was significant difference ($P < 0.05$) amongst the fish organs, water samples and Lead (Pb) concentrations. This research also established the presence of heavy metals examined in varying degrees of concentrations in water samples as well as in fish organs with the exception of Cadmium which was below detectable limit in fish organs.

Glutathione can therefore, be used as biomarkers of pollution in River Galma to give early warning on environmental pollution to the community and policy makers.

Keywords: Oxidative stress, Glutathione, Reactive Oxygen Species, Heavy Metals, Biomarkers

1. Introduction

The presence of toxic metals in environmental matrices is one of the major concerns of pollution control and environmental agencies in most parts of the world (Tay *et al*. 2009). Any metal or metalloid may be considered a "contaminant" (or "pollutant") if it occurs in places where it is unwanted and in unwanted concentrations, or in a

form or concentration that is detrimental to the environment, biota or to humans (Reena *et al*. 2011). The harmful effects on the aquatic ecosystem become evident especially when such discharges are in large quantities such that they become deleterious to both aquatic organisms and other organisms dependent on them for their survival. The contamination of water, soils sediments and biota by heavy metals is of major concern because of their toxicity, persistence and bioaccumulative nature (Ikem *et al*. 2003).

Fish accumulate toxic chemicals such as heavy metals directly from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediments and food (Labonne *et al*. 2001; Goodwin *et al*. 2003; Osman *et al*. 2007).

Heavy metals are a group of elements with a mass density greater than 4.5g/cm^3 which tend to release electrons in chemical reactions and form simple cations. Heavy metals in the solid and liquid states are characterized by good heat and electrical conductivity, and are glossy and opaque. They have high melting and boiling points. They are malleable with usually monoatomic pairs and these include copper, cobalt, chromium, cadmium, iron, zinc, lead, tin, mercury, manganese, nickel, molybedmium, vanadium. Metalloids are antimony, arsenic, astatine, boron, germanium, silicon, tellurium and selenium. Heavy metals of note in environmental science literature include lead, mercury, cadmium, chromium, copper, manganese, nickel, zinc and silver. Some heavy metals are essential elements while others are non-essential (Maity *et al*. 2008). Heavy metals such as copper, iron, chromium and nickel are essential metals since they play an important role in biological systems, where as cadmium and lead are non-essential metals, as they are toxic, even in trace amount (Fernandes *et al*. 2008). Arsenic, cadmium and lead have no known bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic (Duruibe *et al*. 2007). Heavy metals can be bioaccumulated and biomagnified via the food chain and finally assimilated by human consumers resulting in health risks (Agah *et al*. 2009). Due to these, in the last 50 years, environmental conditions have changed at an unprecedented rate, impacting heavily on ecological processes (Diffenbaugh & Field, 2013).

Alkallak (2013) demonstrated a significant difference in the accumulation concentration of cadmium and lead in the liver, kidneys, intestine, gills and muscles of the infected and un-infected fishes (*Silurus glanis*). The metals are brought into contact with the organs and tissues of the fish and consequently, accumulated to a different extent in different organs or tissues of the fish. Once heavy metals are accumulated by aquatic organisms, they can be transported through the upper class of the food chain (Ayandiran *et al*. 2009). As heavy metals cannot be degraded, they are continuously being deposited and incorporated in water sediments and aquatic organisms (Linnik & Zubenko 2000). Similarly, Dahunsi *et al*. (2012) in their work on bioaccumulation pattern of cadmium and lead in the head capsule and body muscle of *Clarias gariepinus* exposed to paint emulsion effluents, demonstrated that fish can bioaccumulate heavy metals from a polluted environment which often result in reduction or impairment of natural population size and could be variable sources of these metals to man.

Exposure and ingestion of heavy metals can cause a myriad of physiological and neurological problems in both plants and animals and, ultimately deleterious effects in man and other higher consumers. Exposure to lead has been associated with reduced IQ, learning dissabilities, slow growth, hyper-activity, anti-social behaviours and impaired hearing (Dahiya *et al*. 2005).

Among various causes of fresh water and riverine pollution, heavy metals are of considerable importance and consideration (Sthanadar *et al*. 2013). Edward *et al*. (2013) observed that though, heavy metals were below detectable level in the water samples, the levels of bioaccumulation in fish parts examined were beyond tolerable levels (WHO and FEPA recommendations) making the fishes unfit for human consumption. Organisms in aquatic environments are usually exposed to a complex mixture of chemicals including parent compound and their transformation products causing multiple damages at the organisms, population and ecosystem level, in organ function, reproductive stages and biological diversity (Ginebreda *et al*. 2014; Vorosmarty *et al*. 2010). High levels of heavy metals in soil, water and atmosphere vis-à-vis the biota are often related to industrial activities, burning of fossil fuels, chemical dumping, application of agro-allied chemicals such as fertilizer and certain pesticides (Oyekunle *et al*. 2012). The eight most common pollutant heavy metals listed by the Environment Protection Agency (EPA) are: As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn (Athar & Vohola 2001). Zinc deficiency causes anaemia and retardation of growth and development**.** Its deficiency also results in poor immunity since it is known to have a role in the immune system. Even Cr (VI) is taken up in amount of 150 μg/day that are required for collagen, but it leads

to irritation of the stomach, leading to ulcers and kidney and liver damage at higher concentration (Dayan & Paine 2001). In humans, higher concentration of lead and mercury is associated with autoimmune disease such as rheumatoid arthritis as well as interfere with the proper functioning of the kidney and circulatory system that leads to injury of central nervous system (Patrick 2006). Effects of cadmium on aquatic organisms are analogous to those in humans, and include skeletal deformities and impaired functioning of kidneys in fish. Skeletal deformities in fish can result in an impaired ability of the fish to find food and avoid predators; hence, this sub lethal effect becomes a lethal effect (Landis & Yu 2003). Cadmium is a very toxic metal, and also an environmental and industrial pollutant which is present in soil, water, air and food (Kaplan *et al.* 2011). Roopha & Latha (2013) reported that cadmium exposure induced oxidative stress; delay in sexual maturation and impaired hormones in developing rat ovary. Chromium can make fish more susceptible to infection; high concentrations can damage and/or accumulate in various fish tissues and in invertebrates such as snails and worms (WHO 1995).   The main concern about the absorption of chromium depends on its speciation. Chromium (VI) penetrates cell membranes, where as Chromium (III) does not; thus, chromium (VI) may cause genotoxic effects and cancer where as chromium (III) does not (WHO 1995; Tarley *et al.* 2001).

Zinc (Zn) is a cofactor to more than 300 enzymes involved in important functions such as RNA and DNA metabolism and plays a major role in the stabilization of the structure of a large number of proteins, including signaling enzymes at all levels of cellular signal transduction (Chasapis *et al.* 2012). The most wide spread human deficiency is noted in zinc, for which over two billion humans, mostly in developing countries, suffer from inadequate amounts of zinc in their diet. The resulting effects include growth retardation and increased susceptibility to infection, with a net outcome of a contributory cause of death in more than over 800,000 children per year (Ananda 2003). The process of biomagnification, which involves the increase of metals concentration in organisms on the upper level of the trophic chain, is most visible in the aquatic environment (Dobrowolski & Skowronska 2001). Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms (Storelli *et al.* 2005). For the normal metabolism of the fish, the essential metals must be taken up from water, food or sediments (Canli & Atli 2003). These essential metals can also produce toxic effects when the metal intake is excessively elevated (Tuzen 2003). Heavy metal contaminated food can seriously deplete some essential nutrients in the body that are further responsible for decreasing immunological defenses, intra-uterine growth retardation, impaired psycho-social faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Iyenger & Nair, 2000; Turkdogan *et al.* 2003; Arora *et al.* 2008). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj 2005; Vosyliene & Jankaite 2006; Farombi *et al.* 2007). Various harmful effects including abnormal development of fetus, procreation failure, and immuno-deficiency has exhibited due to aquatic metal exposure. Studies have also indicated that fish are able to accumulate and retain heavy metals from their environment and that accumulation of metals in tissues of fish is dependent upon exposure concentration and duration as well as other factors such as salinity, temperature, hardness and metabolism of the animals (Karthikeyan *et al.* 2007)

The consumption of heavy metal contaminated food can seriously deplete some essential nutrients in the body that are further responsible for decreasing immunological defenses, intra-uterine growth retardation, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Khan *et al.* 2008). Heavy metals are persistent inorganic pollutants of the environment. Some heavy metals may transform into the persistent metallic compounds with high toxicity, which can be bioaccumulated in the organism, magnified in the food chain, thus threatening human health (Jin 1992).

The most anthropogenic sources of metals are industrial, petroleum contamination and sewage disposal (Santos *et al.* 2005). Examples of non-point source pollution include agricultural runoff (pesticides, pathogens, and fertilizers), storm-water and urban runoff, and atmospheric deposition _wet and dry deposition of persistent organic pollutants such as polychlorinated biphenyls (PCBs) and mercury (Ritter *et al.* 2002). The ultimate sink for many of these contaminants is the aquatic environment, either due to direct discharges or to hydrologic and atmospheric processes (Stegeman & Hahn 1994).

 Non-enzymatic antioxidants are represented by ROS (Reactive Oxygen Species) scavengers (both hydrophilic such as low-molecular mass thiols, glutathione (GSH), metallothioneins (MTs), ascorbic and uric acids, as well as

lipophilic ones such as vitamin E and carotenoids (Viarengo *et al*. 2007). Also, Non enzymatic antioxidants are biological molecules that can act as antioxidants by either quenching a free radical directly or indirectly by promoting a process responsible for radical scavenging indirectly (Irshad & Chaudhary 2002).

Glutathione is the major non-enzymatic antioxidant in all cells. Glutathione is the most copious non-protein thiol found at milli-molar concentrations in most cells (Ueno *et al*. 2002). Glutathione (GSH) is tripeptide, which has many biological roles including protection against reactive oxygen and nitrogen species. As a carrier of an active thiol group in the form of a cysteine residue, it acts as an antioxidant either directly by interacting with reactive oxygen/nitrogen species (ROS and RNS, respectively) and electrophiles or by operating as a co-factor for various enzymes. The chemical structure of GSH determines its potential functions and its broad distribution among all living organisms reflects its important biological role. GSH has been found in all mammalian cells. Probably most importantly, GSH is responsible for protection against ROS and RNS, and detoxification of endogenous and exogenous toxins of an electrophilic nature. Other functions include (i) maintaining the essential thiol status of proteins and other molecules; (ii) storage of cysteine reserves both in the cell and for inter-organ transfer; (iii) involvement in the metabolism of estrogens, leukotrienes, and prostaglandins; (iv) participation in the reduction of ribonucleotides to deoxyribonucleotides; (v) participation in the maturation of iron-sulfur clusters in proteins; (vi) copper and iron transfer; (vii) signal transduction from the environment to cellular transcription machinery. Glutathione exists in reduced (GSH) and oxidized (GSSG) forms. In the reduced state, the thiol group of cysteine is able to donate a reducing electron directly to unstable molecules such as ROS. In donating an electron, GSH itself becomes reactive, but readily reacts with another reactive GSH to form GSSG. GSH is a low molecular weight thiol. It can react directly with ROS species, thereby detoxifying them. In addition, GSH is used as a conjugating molecule by GST (glutathione S- transferase) to ease excretion of xenobiotics. GSH is also used as a reducing equivalent in the metabolism of reactive intermediates, several studies have shown that antioxidants that are affected by reactive oxygen species show adaptive responses to xenobiotics that produce oxyradicals (Di Giulio *et al*. 1995) and are potential biomarkers for oxidative stress in fish (Van der Oost *et al*. 2003). Glutathione sulfhydryl is tripeptide protein made of three amino acids namely cystein, glutamate or glutamic acid and glycine. It was first discovered by J. deRey-Pailhade from human eyeball in 1888. The enzymes derived from GSH are glutathione peroxidase and glutathione S-transferase. When the GSH level rises the enzymes levels also rise. The reduced and oxidized forms of glutathione (GSH and GSSG) act in concert with other redox-active compounds (e.g., NAD (P) H) to regulate and maintain cellular redox status (Jones *et al*. 2011). Glutathione and other antioxidants can also be produced from the oxidative stress generated from their activities. For instance, Eissa *et al*. (2014) observed that enzyme activities act as biomarkers for oxidative stress induced by metacercarial affections in cultured *Oreochromis niloticus* and *O. aureus*. Glutathione modulates cell proliferation and plays a key role in protecting cells against oxidants (Kumaraguruparan *et al*. 2005).

Reactive oxygen species are induced by substances such as transitional metal ions, pesticides, and petroleum pollutants (Slaninova *et al*. 2009; Lushchak 2011). Hypoxic conditions, temperature and acidifications generally render the fish more sensitive to toxication where as increase in mineral contents decrease metal toxicity (Abdullahi & Javed 2006). In general, almost all metals strongly bind to thiol groups of cysteine amino acids, making free cysteine an effective chelator of metal ions. However, not only free metals but also potentially dangerous xenobiotics like herbicides, and metabolites such as anthocyanins, are bound to GSH.

A disturbance in the balance between the pro-oxidants and antioxidants leading to detrimental biochemical and physiological effects is known as oxidative stress. Oxidative stress is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses in living organisms (Nishida 2011). This is a harmful condition in which increase in free radical production, and/or decreases in antioxidant levels can lead to potential damage of lipids, proteins and DNA (Padmini *et al*. 2004). Oxidative and nitrative stress results from increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) mediated by pollutants (Scoullos *et al*. 2007). For example, a recent meta-analysis showed that oxidative stress increased with an increase in the duration of physiological stress, while acute exposure mostly resulted in up-regulation of the antioxidant response (Costantini *et al*. 2011a). Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans (El-Shehawi *et al*. 2013). Metals are well known inducers of oxidative stress, and assessment of oxidative damage and antioxidant defenses in fish can reflect metal contamination of the aquatic environment (Livingstone 2003). Fish livers have comparatively more chemical affinity to bioaccumulate cadmium as compared to heavy metals like Zn, Cr and Ni

(Sthanadar *et al*. 2013). Rajagopalan *et al*. (2010) demonstrated how constant production of free radical resulted in increased exploitation of the antioxidants leading to their depletion in which the levels of non-enzymatic antioxidants such as reduced glutathione, vitamins C and E were significantly reduced in the alcohol and PUFA (poly unsaturated fatty acid) treated livers of rats because of their complete utilization due to the oxidative stress.

Environmental stress has been demonstrated to cause an increase in the oxidative stress, an imbalance in the antioxidant status (Yildirim *et al*. 2010). There is substantial evidence that environmental pollution increases oxidative stress (Olivia *et al*. 2012). Environmental stress as well as variety of physical conditions may lead to the production of certain protein in fish. Some of these proteins are capable of protecting the cells against damages that may result from such environmental perturbations; while others are involved in the regulation of various genes. Cellular antioxidant defense systems in biological systems are impaired when exposed to environmental pollutants, but the levels of antioxidants in living organisms can increase in order to restore the imbalance caused by oxidative damage. A number of pollutants including heavy metals have been linked with the presence of free radicals which may induce oxidative stress in biological systems (Osuala 2012). Also, Saliu *et al*. (2012) reported that the levels of GST-GSH, SOD, CAT and MDA were all reduced in fishes (*Clarias gariepinus*) exposed to $Pb(NO_3)_2$ in comparison with the control indicating the effect of the pollutant on the fish while the reverse was the case in those fish exposed to $ZnCl_2$ with increase in antioxidants except MDA. Also, numerous low molecular weight antioxidants such as glutathione, β-carotene (vitamin A), ascorbate (vitamin C), and tocopherol (vitamin E) can participate in the process of eliminating oxy-radicals (Van Der Oost *et al*. 2003). Similarly, Paul & Sengupta (2013) demonstrated how sub-lethal concentration of lead acetate has the capacity to bio-accumulate, thereby altering the normal functional activities of freshwater fish *C. punctata*. Furthermore, the association of oxidative stress including variation in its anti-oxidant profile suggests that the defense system of *C. punctata* is significantly compromised upon metal exposure at low concentrations.

Peakall (1994) defined biomarkers as changes in biological response (ranging from molecular through cellular and physiological responses to behavioural changes) which can be related to exposure to, or toxic effects of environmental chemicals. Fish biomarkers are promising for ERA (Environmental Risk Assessment) as supplements to existing chemical measures (Vander Oost *et al*. 2003). In addition, systematic use of multiple biomarkers has been found as most useful in the assessment of pollutants' effects (Tsangaris *et al*. 2010). For instance, oxidative stress markers (GSH and SOD_ Glutathione and Super oxide dismutase) correlated with heavy metal accumulation in some location- which shows that they could be used in monitoring environmental pollution (Taiwo *et al*. 2014). Biomarkers are measurements in body fluids, cells or tissues indicating biochemical or cellular modifications due to the presence and magnitude of toxicant, or of host response (NRC 1987).

 The River Galma is currently loaded with run-offs from agricultural and municipal activities of the surrounding communities along its course at various adjoining tributaries (most of which are seasonal); and contain myriads of toxic pollutants (heavy metals inclusive). There may also be contribution of toxic pollutants from the few industrial activities in Chikaji and Dakace areas.

Although many research have been conducted to assess the toxicity of heavy metals in algae, however, the number of studies dealing with the toxic effect of heavy metal on aquatic animals including fish are limited (Harmon *et al*. 2005). Similarly, unlike with molluscs (Viarengo *et al*. 2007), for fish, the studies on the response of caged specimens are scarce. Thus, this investigation attempted to bridge the gap and bring to fore the biochemical responses of fish to heavy metal loads in River Galma.

Viarengo *et al*. (2007) suggested that bio-monitoring programmes should use caging studies to obtain highly sensitive early warning signs of the effects of exposure to environmental pollution. Caging allows the exposure of individual fish to conditions at a certain site, for known time (Almroth 2008). Caged forage fish can provide results useful for guiding and defining priorities for more thorough effects-driven assessments (Munkittrick & McMaster 2000; Munkittrick *et al*. 2000) for determining cumulative impacts of multiple contaminant stressors. Also, Yousafzai *et al*. (2010) showed that omnivorous fish may bioaccumulate more heavy metals than carnivorous fish in natural habitats. In this regard, the test sample, *Clarias gariepinus* is an omnivorous species. River Galma receives variable levels of pollution from different sources of anthropogenic activities along its banks (Butu & Bichi, 2013). Thus, the use of *Clarias gariepinus* in *in-situ* investigation on the possible effects of xenobiotics in River Galma would possibly provide early warning to the users of the river, farmers and policy makers.

Falfushynska *et al*. (2011); Hansen *et al*. (2006a; 2006b) used 14 days in their separate research. Thus, the current investigation also used 14 days to determine the effects of pollutants on glutathione production in *Clarias gariepinus* in River Galma.

Common practices of fish farming, such as capture, confinement, transportation and water quality, varying temperature and climatic conditions, etc are stressful to fish and can lead to increase in the incidence of diseases, immunological imbalances, mortality and impairment of growth and survival. For instance, Adeyemo *et al*. (2009) demonstrated significant difference in the values of neutrophil and lymphocytes of the stressed fish relative to the baseline data obtained from the control.

The knowledge of the levels of heavy elements in our environment is necessary for the purposes of setting background values of these elements, monitoring their accumulation in the biota regularly and estimating the amount of the metals that may possibly get trans-located across the compartments in the entire ecosystem (Oyekunle *et al*. 2012). In this regard, fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bio-indicators of environmental pollution (Dautremepuits *et al*. 2004). In this investigation, heavy metals such as lead, cadmium, zinc, chromium and manganese were tested for in water and fish organs such as liver and gill so as to determine their concentrations in setting background values of these elements.

2. Materials and Methods

2.1 *Description of Study Area*

Galma River is one of the main tributaries of River Kaduna. It has its headwaters near the north western edge of the Jos Plateau and falls near the Magami village into Kaduna plains. The main tributaries of Galma River are Shika River in the middle course and the Rivers Kinkiba and Likarbu in its lower course. The Galma reservoir which is popularly called Zaria dam was constructed across the Galma River in 1975. The major land use in the catchment areas is farming and animal rearing. There are also some industrial and municipal activities (in the surrounding towns and villages such as Chikaji, Dakache and Sabon Gari areas) that produce myriads of wastes that ultimately get to the river either in the short- or long-run through run-offs and seepages. The few industries are located in Chikaji and Dakace. The main tributaries of the river in the sampling areas are all located in the Sabon Gari Local Government Area of Kaduna State (Figure 1).
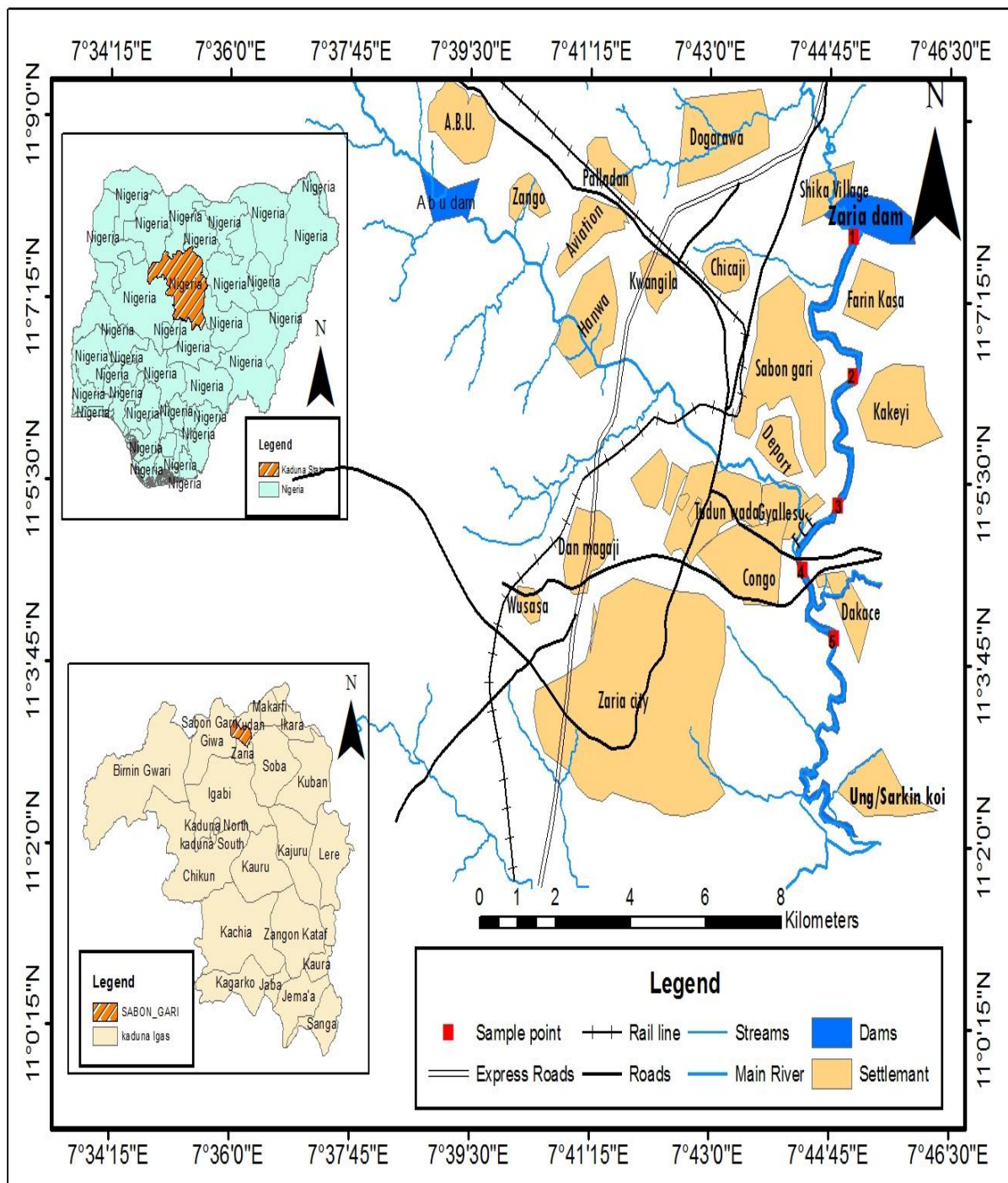
Figure 1 Map of Zaria and its Environment Showing Study Areas.

Source: Satellite Image (2013).

2.2 *Cage Construction*

Five cages were constructed manually using PVC (polyvinyl chloride) pipes of various sizes. One inch pipes were used to form the upper and lower frames of the cage. These were measured and cut to size (50cm). PVC of half inch were used as the side and basal bars. Each bar was 50cm long. Holes were made in each frame with the aid of red-hot iron rod wide enough to accommodate the bar conviniently. Holes were also made along the central and side axes of the two lower frames to accommodate the vertical and horizontal bars while the other PVC frames were punctured only on the central axis. A minimum of sixteen holes at regular interval per frame were created. The PVC frames were connected with the aid of L-shaped elbow joints. For proper and firm fitting of the elbow joints, an adhessive was applied at each joint ready to be fitted into the frame. Shredded foams were placed in the PVC frame to ensure lightness and floatation. The adhesive was also applied on the bars for proper fitting into the holes. The length of each frame was 50cm in-to-in. Holes were also created at the joints to accommodate more bars to fill up the spaces. A lid was constructed at the upper end of the cage. An inch PVC pipes were used with the four sides connected to the frame at one side via T-shaped PVC connector joint after two holes were made at the side. A net of 2cm mesh size was attached to the inside of the cage with the aid of an adhesive and the free ends of the nets were tack on the side bars through the use of fish twine thread and needle. An iron mesh was used to cover the inner layer of the cage to avoid attack on the content of the cage from predators such as crabs. Floaters in form of foams were attached to the cage on the outside through the use of thread at either sides of the cage. Another foam was attached on the lid to increase buoyancy and floatation capacity of the cage. And another PVC of two inch was used to cover the four edges of the upper frame of the cage in order to hold the nets firmly at the upper base.

2.3 *Acclimatization of Clarias gariepinus*

A total of one-hundred and twenty samples (40 per exposure) of *Clarias gariepinus* of 20-45g size range were purchased from the commercial fish farmer (Bagauda, Kano) and kept in concrete tank for a period of two weeks in which they were fed with Cupens, 2mm size feed morning and afternoon five days a week. The holding facility was aerated with Blagdon aerator (KOI Air, KA25). The same number and size range of fish were acclimatized as samples during the October and December exposures when the first set of samples have been taken to the river. This helped the fish to be relieved of stress as a result of transportation and change of environment; as well as to shed off toxicity of its former environment.

2.4 *Sample Location and Collection*

Five (5) sites were selected along the river. The first site was located at Shika Reservoir (Zaria Dam). Shika reservoir was used as reference site because it serves as the upper course of the river and relatively located far from the industrial areas and the municipal waste load was also relatively low compared with other sites. Other sites were located around Kakeyi village (which receives municipal wastes and effluents from the neighbouring villages and towns such as Sabon Gari), two sites around FCE, Congo (FCEI and FCEII at about 500 metres apart and FCEI receives municipal wastes and effluents, agricultural run-offs from some parts of Sabon Gari, Chikaji, Kakeyi and Farin Kasa villages. FCEII site receives municipal wastes and effluents, agricultural run-offs from various parts of the town including Tudun Wada, PZ and Congo via the Kubani stream which adjoins the main stream at this point. Waters from Kakeyi along the stream also joins here; Dakace village at about 1000 metres apart from FCEII and receives municipal and agricultural wastes and effluents from Dakace village and the few industries located in the area (Figure 1). Farming and other agricultural activities take place in both wet and dry season. The choice of these sites were made base on security reasons and ease of accessibility

Water samples from each site were collected in 2L plastic containers from each site for physico-chemical parameters, nutrients and heavy metals (Cadmium, lead, zinc, chromium and manganese) analyses. Samples meant for Dissolved Oxygen were collected in 300ml BOD bottles and treated with 2ml manganous sulphate at the point of collection. These parameters were measured to ensure that the fish were in good condition during the period of exposure.

Forty samples of *Clarias gariepinus* (eight per site) were transported to the river in a special mesh using cage system for each sampling site and then suspended into the water in which the fish were fed once at dawn after 24 hours of exposure within the natural ecosystem for fourteen (14) days. The fish samples were harvested from each site after the first 7 days of exposure and then, 14 days after exposure and kept in a large plastic container and

transported to the laboratory in each case. Each sample was dissected and gill, liver and kidney removed and homogenized in 0.9% physiological saline solution the same day and kept in the test tube meant for each organ from each fish and for each site and then refrigerated prior to analyses for non- enzymatic antioxidants (glutathione) and heavy metals. For best results the samples were harvested from each sampling site the same day and in the early hours of the day. Prior to exposure, glutathione levels in the gill, liver and kidney were tested for, serving as control from which comparison were made with the various sites.

The whole process (experiment) was carried-out or repeated in October and in contrasting dry season (December) for comparison.

2.5 *Metal Analysis*

Each of the fresh organs (gill and liver) of samples of *Clarias gariepinus* from the various sites were homogenized in 0.9% physiological saline with a ceramic mortar and pestle and then digested in containers containing nitric acid. The digestion was done by heating 1ml of the homogenized organ of the sample of the various site in each case, with 7.5ml of concentrated nitric acid, 2.5ml of concentrated hydrochloric acid in 50ml graduated beaker at 150^0C until near dryness in a fume chamber. After which the beaker was brought down and allowed to cool. The content in each beaker was made up to 50ml with distilled water. Samples drawn and digested after 7 days period of exposure and those drawn after 14 days period were mixed together and a pooled sample was obtained. Same was done for samples from October and December, 2014 exposures. The pooled samples of digested tissues were tested for lead, zinc, cadmium, chromium and manganese concentrations via Graphite Furnace Atomic Absorption Spectrophotometry (AA WIN PRO) in Soil Science Department of Ahmadu Bello University, Zaria.

Digested water samples were pooled for three months (August to October, 2014 and November, 2014 to January, 2015) and analysed for lead, zinc, cadmium, chromium and manganese by Graphite Furnace Atomic Absorption Spectrophotometry (AA WIN PRO) in Soil Science Department of Ahmadu Bello University, Zaria. Prior to this, the water sample from each site were digested on monthly basis and then pooled by the same process described above.

2.6 *Glutathione Analysis*

Each liver pieces, kidneys and gills used for measurements of reduced glutathione (GSH) concentrations in *Clarias gariepinus* from each site were homogenized in 0.9% physiological saline using ceramic mortar and pestle.

The following reagents were used for the analysis: 0.2M phosphate buffer (8.40g of NaH_2PO_4 and 9.94g of Na_2HPO_4 was dissolved in distilled water and made up to 1000ml mark in a volumetric flask. The buffer was adjusted to pH8.0); 10% Trichloroacetic acid (10g of TCA was dissolved in distilled water and made up to 100ml in the volumetric flask); and Ellman' reagent (19.8mg of 5,5'-dithiobis nitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate).

To 150μL of the tissue homogenate (in phosphate-saline pH 7.4), 1.5ml of 10% TCA was added, and centrifuge at 1500g for 5min. 1.0ml of the supernatant was treated with 0.5ml of Ellman's reagent and 3.0ml of phosphate buffer (2.0m pH 8.0). The absorbance was read at 412nm. Estimation of Reduced Glutathione was determined by the method of Ellman (1959) as described by Rajagopalan *et al*. (2004). The amount of glutathione was calculated using a GSH standard curve and expressed as microngrams of GSH formed/mg protein in each case.

2.7 *Data Analysis*

Graphical representation of the various heavy metal concentrations were indicated using error bar charts. Same was also done for the glutathione concentrations in gills, livers and kidneys of the various sites. Concentrations of the metals (mg/ml) were plotted against (vertical) sampling locations (horizontal).

One way Analysis of Variance (ANOVA) followed by Duncan Multiple Range test was used to determine the differences between the reference site samples and the samples from other sites at the different periods of exposure using IBM SPSS Inc. (version 20.0 for Windows) statistical package at $P<0.05$ level of significance.

The relationship between heavy metals concentration and glutathione production levels were determined by carrying-out Spearman's Correlation Coefficient.

3. Results

3.1 *Glutathione Production levels in Clarias gariepinus*

From the ANOVA and DMRT results there was significant difference in the gills assayed for in the month of September while there were no significant differences in the kidneys and livers in September as well as in all the organs of the fish in all the months of exposures. The mean values obtained ranged from 55.55 ± 14.03 (Day 7 gill) to 148.22 ± 34.15 (Day 14 liver) in September. The mean values obtained ranged from 88.18 ± 29.77 (Day 7 gill) to 163.59 ± 63.29 (Day 14 liver) in October. The mean values obtained ranged from 53.14 ± 19.17 (Day 7 liver) to 170.87 ± 62.93 (Day 14 gill) in December. (Table 1).

The results of the bio-assay amongst the locations indicate that glutathione production levels were highly significant in gills and kidneys of the fish while there was significance in the glutathione production levels in the liver of the fish. Highest mean values were obtained in all the organs of fish from Kakeyi in all the exposure periods followed by samples from FCEII sites in all the organs. Lowest mean values were obtained in Shika dam gill and FCEII kidney. (Table 2). There were no significant differences amongst the months of exposures (Table 3).

When the means of values of glutathione production levels were compared amongst the sites (locations) and the months of exposures there were high significance differences in the kidneys of the fish in all the months of exposures. There were also significance differences in the gill and livers of those fish exposed in the month of October; and significant in the gill of the fish in December exposure. (Table 4).

There were no significant differences amongst the organs of the fish. (Table 5).

From the results of Spearman's Correlation Coefficient analysis there were correlation between glutathione production levels and all the heavy metals under study, with strong positive correlation with Pb, Cr, and Zn. (Table 6).

3.2 *Heavy Metals*

From the ANOVA and DMRT of the results the comparison of heavy metals in the gill and liver samples and that of water samples indicate: there were significant differences $(P < 0.05)$ in Chromium concentrations from the gills and livers of the fish from the various sites of exposure. (Table 7).

There were no significant differences in the heavy metals amongst the sites (locations).

Pb concentration had its lowest in Kakeyi with 0.091 ± 0.06 while the highest was 0.536 ± 0.02 from Shika Dam.

Cr concentration ranged from 0.379 ± 0.29 to 0.834 ± 0.17 (Shika Dam).

Cd was not detected in any of the sites except in water sample from Kakeyi.

Zn concentration ranged from 0.095 ± 0.05 (Shika Dam) to 0.339 ± 0.30 (FCEII).

Mn concentration ranged from 0.119 ± 0.09 (Kakeyi) to 0.178 ± 0.15 (Shika Dam). (Table 8)

There was significant difference $(P < 0.05)$ amongst the fish organs, water samples and Lead (Pb) concentrations. While there were no significant differences amongst the fish organs, water samples and the remaining heavy metals (Cr, Cd, Zn and Mn). (Table 9).

Table 1 Monthly Glutathione production levels from the 7th and 14th day Bio-assay in Organs of *Clarias gariepinus* from an *in situ* Exposure in River Galma

Months	Body Part	Day 7	Day 14	Total	P value
September	Gill	55.55±14.03	130.96±28.48	**93.26±18.93**	0.039*
	Liver	87.70±21.05	148.22±34.15	**117.96±21.19**	0.162ns
	Kidney	77.77±18.67	84.63±22.59	**81.20±14.01**	0.820ns
October	Gill	88.18±29.77	95.51±34.84	**91.84±21.87**	0.876ns
	Liver	101.65±34.27	163.59±63.29	**132.62±35.56**	0.410ns
	Kidney	107.09±40.08	104.96±33.68	**106.03±24.96**	0.968ns
December	Gill	97.61±32.71	170.87±62.93	**134.24±35.57**	0.326ns
	Liver	53.14±19.17	93.82±31.59	**73.49±18.65**	0.297ns
	Kidney	67.80±22.32	76.78±25.34	**72.29±16.16**	0.796ns

Table 2 Glutathione Bio-assay in Fish Organs of *Clarias gariepinus* Amongst the Locations of the Sites of Exposure in River Galma

Location	Gill	Liver	Kidney
Control	104.96±0.00a	117.73±0.00a	130.50±0.00a
Shika Dam	83.92±32.34ab	129.55±47.79a	100.00±34.46ab
Kakeyi	169.93±34.97a	164.98±45.92a	131.67±23.98a
FCE I	162.64±37.80a	143.45±30.04a	98.77±18.36ab
FCE II	117.23±46.77a	92.43±33.77ab	58.11±19.48bc
Dakace	0.00±0.00b	0.00±0.00b	0.00±0.00c
Total	**106.45±15.21**	**108.02±15.30**	**86.51±10.91**
P value	**0.008****	**0.021***	**0.001****

Table 3 Glutathione Bio-assay in Fish Organs of *Clarias gariepinus* Amongst the Months of Exposures in River Galma

Months	Gill	Liver	Kidney
September	93.26±18.93a	117.96±21.19a	81.20±14.01a
October	91.84±21.87a	132.62±35.56a	106.03±24.96a
December	134.24±35.57a	73.49±18.65a	72.29±16.16a
Total	**106.45±15.21**	**108.02±15.30**	**86.51±10.91**
P value	**0.446ns**	**0.266ns**	**0.437ns**

Table 4 Comparison of Glutathione Production levels in Fish Organs Amongst Sites and Months of Exposures of *Clarias gariepinus* in an *In Situ* Bio-assay in River Galma

Location	September			October			December		
	Gill	Liver	Kidney	Gill	Liver	Kidney	Gill	Liver	Kidney
Control	104.96±0.00a	117.73±0.00ab	130.50±0.00a	104.96±0.00a	117.73±0.00ab	130.50±0.00a	104.96±0.00ab	117.73±0.00a	130.50±0.00a
Shika Dam	113.47±55.32a	149.64±63.12ab	117.73±24.12b	138.30±41.85a	239.01±33.34a	182.27±12.06a	0.00±0.00b	0.00±0.00a	0.00±0.00c
Kakeyi	118.44±63.12a	130.49±7.09ab	103.54±17.02ab	169.50±46.10a	264.54±125.54a	200.00±35.47a	221.85±82.96a	99.93±22.06a	91.49±0.71b
FCE I	109.22±65.25a	136.88±77.31ab	54.61±2.13b	138.29±17.73a	174.47±26.96ab	123.40±41.14a	240.43±85.82a	119.01±72.34a	118.30±20.57ab
FCE II	113.47±42.55a	173.05±34.05a	80.85±22.70ab	0.00±0.00b	0.00±0.00b	0.00±0.00b	238.22±50.99a	104.26±27.67a	93.48±5.68b
Dakace	0.00±0.00a	0.00±0.00b	0.00±0.00c	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00a	0.00±0.00c
Total	**93.26±18.93**	**117.96±21.19**	**81.20±14.01**	**91.84±21.87**	**132.62±35.56**	**106.03±24.96**	**134.24±35.57**	**73.49±18.65**	**72.29±36.73**
P value	**0.505ns**	**0.214ns**	**0.007****	**0.013***	**0.048***	**0.003****	**0.042***	**0.104ns**	**0.000****

Mean values with the same alphabet are not significantly different

Table 5 Glutathione Bio-assay in the Fish Organs of *Clarias gariepinus* from the 7th and 14th day *in situ* Exposure in River Galma

Fish Organ	Day 7	Day 14	Total	P value
Gill	80.48±15.17	132.45±25.35	**106.45±15.21**	**0.087ns**
Liver	80.83±14.80	135.22±25.64	**108.02±15.30**	**0.075ns**
Kidney	84.22±16.03	88.79±15.25	**86.51±10.91**	**0.838ns**

Table 6 Spearman's Correlation of Glutathione Bioassay with the Heavy Metals

	Glutathione	*Pb*	*Cr*	*Cd*	*Zn*	*Mn*
Glutathione	1.00					
Pb	0.95	1.00				
Cr	0.96	0.90	1.00			
Cd	0.55	0.30	0.62	1.00		
Zn	0.87	0.85	0.71	0.33	1.00	
Mn	0.53	0.72	0.56	-0.13	0.31	1.00

Table 7 Heavy Metals From pooled samples of Gill and Liver from the *in situ* exposures of *Clarias gariepinus* in River Galma

Location	Gill					Liver				
	Pb	Cr	Cd	Zn	Mn	Pb	Cr	Cd	Zn	Mn
Shika Dam	0.02±0.01b	0.44±0.22ab	0.00±0.00a	0.20±0.11a	0.03±0.01a	0.08±0.06a	0.00±0.00b	0.00±0.00a	0.19±0.10a	0.03±0.02a
Kakeyi	0.31±0.14a	0.78±0.11a	0.00±0.00a	0.26±0.08a	0.11±0.04a	0.31±0.10a	0.78±0.11a	0.00±0.00a	0.16±0.06a	0.09±0.05a
FCE I	0.31±0.10a	0.78±0.11a	0.00±0.00a	0.18±0.07a	0.41±0.38a	0.20±0.16a	0.56±0.29ab	0.00±0.00a	0.17±0.07a	0.42±0.37a
FCE II	0.12±0.06ab	0.25±0.21b	0.00±0.00a	0.10±0.08a	0.08±0.07a	0.06±0.04a	0.25±0.21ab	0.00±0.00a	0.11±0.08a	0.09±0.07a
Dakace	0.00±0.00b	0.00±0.00b	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00b	0.00±0.00a	0.00±0.00a	0.00±0.00a
Total	**0.15±0.05**	**0.45±0.10**	**0.00±0.00**	**0.15±0.04**	**0.13±0.08**	**0.13±0.05**	**0.32±0.10**	**0.00±0.00**	**0.13±0.03**	**0.13±0.08**
P value	**0.061ns**	**0.020***	**0.580ns**	**0.225ns**	**0.489ns**	**0.210ns**	**0.031***		**0.371ns**	**0.451ns**

Mean values with the same alphabet are not significantly different from each other.

Table 8 Heavy Metals in Pooled Water Sample from the Sites of Exposure in River Galma

Location	Pb	Cr	Cd	Zn	Mn
Shika Dam	0.536±0.02a	0.834±0.17a	0.00±0.00a	0.095±0.05a	0.178±0.15a
Kakeyi	0.091±0.06a	0.379±0.29a	0.005±0.01a	0.11±0.07a	0.119±0.09a
FCE I	0.277±0.243a	0.379±0.29a	0.00±0.00a	0.11±0.09a	0.132±0.10a
FCE II	0.449±0.07a	0.379±0.29a	0.00±0.00a	0.339±0.30a	0.149±0.11a
Dakace	0.363±0.16a	0.379±0.29a	0.00±0.00a	0.14±0.08a	0.14±0.11a
Total	**0.343±0.07**	**0.47±0.11**	**0.001±0.00**	**0.159±0.06**	**0.143±0.04**
P value	**0.308ns**	**0.694ns**	**0.486ns**	**0.761ns**	**0.996ns**

Table 9 Heavy Metals Comparison in Gill, Liver from the Fish Samples of the *in situ* Bio-assay and Water Samples from the Sites of Exposure in River Galma

Source	Pb	Cr	Cd	Zn	Mn
Gill	0.15±0.05b	0.45±0.10a	0±0.00a	0.15±0.04a	0.13±0.08a
Liver	0.13±0.05b	0.32±0.10a	0±0.00a	0.12±0.03a	0.13±0.08a
Water Sample	0.34±0.07a	0.47±0.11a	0±0.00a	0.16±0.06a	0.14±0.04a
Total	**0.19±0.03**	**0.41±0.06**	**0±0.00**	**0.14±0.02**	**0.13±0.04**
P value	**0.022***	**0.530ns**	**0.379ns**	**0.837ns**	**0.984ns**
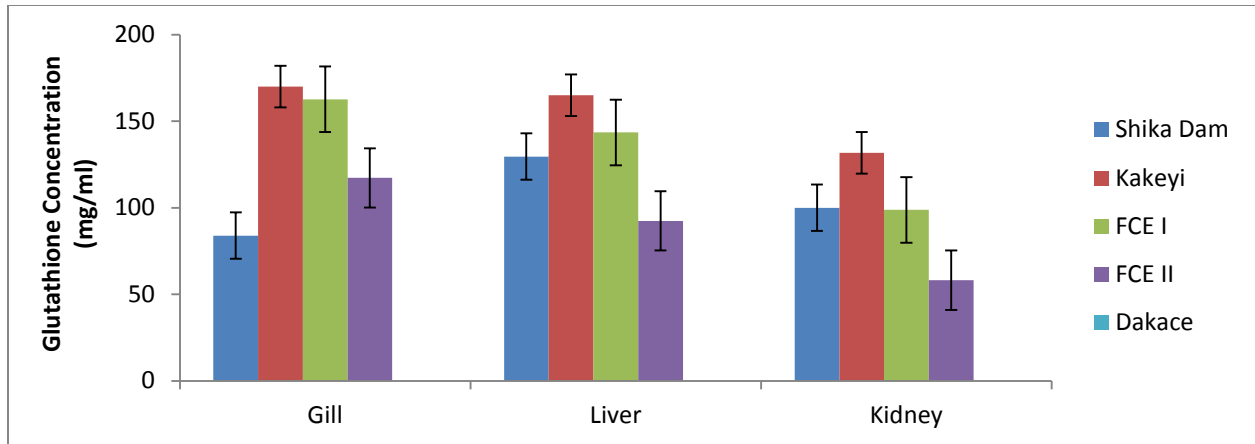
Figure 2 An Error bar indicating the various Glutathione concentrations in each site from all the 7th and 14th day *insitu* bio-assay of *Clarias gariepinus* in River Galma
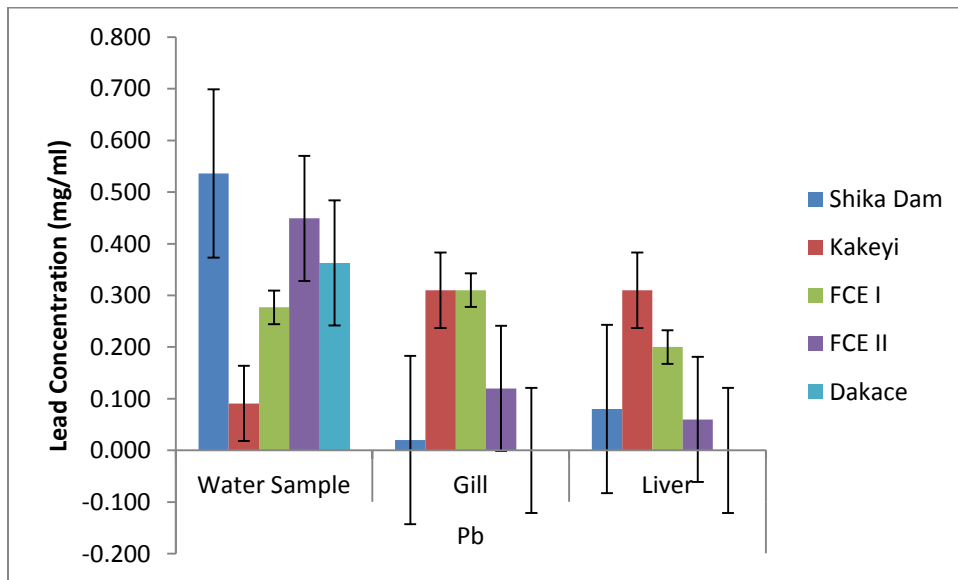

Figure 3 An Error bar indicating Lead Concentrations (mg/ml) in Water samples and Fish organs in all the sites.
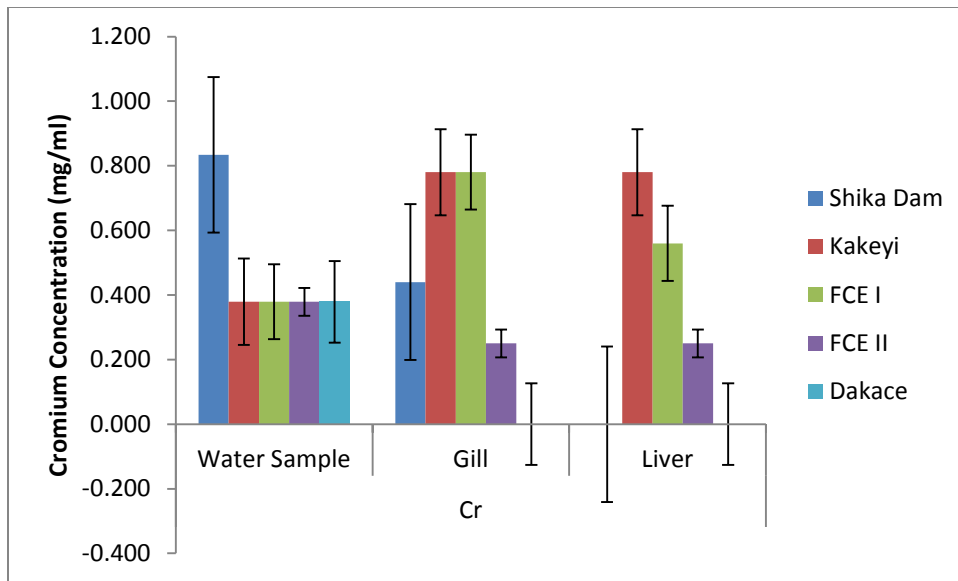
Figure 4 An Error bar indicating Chromium Concentrations (mg/ml) in Water samples and Fish organs in all the sites.
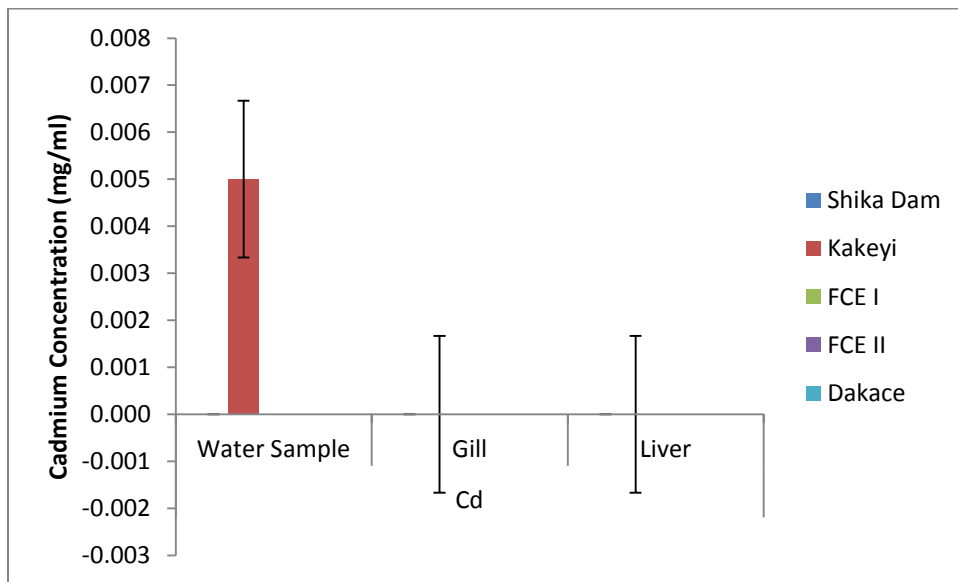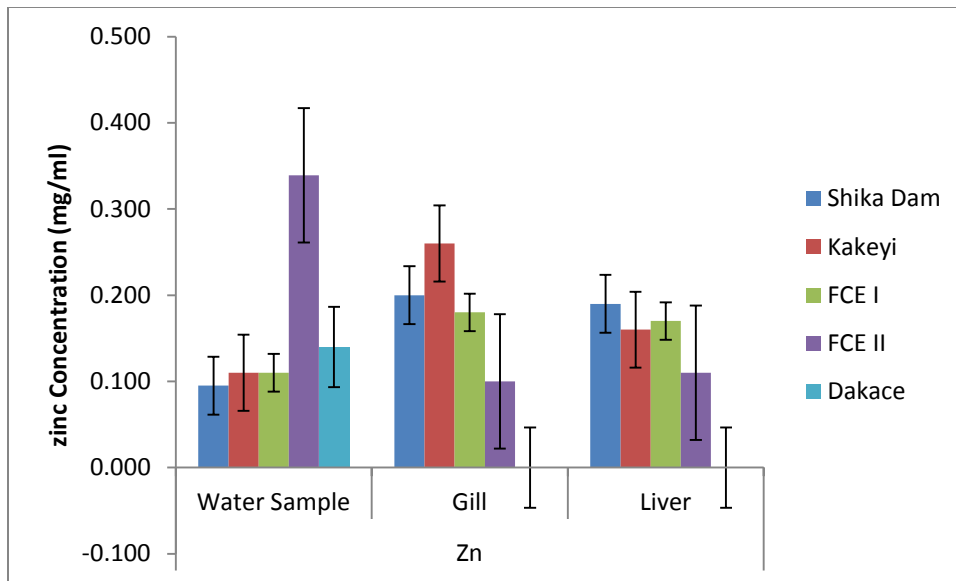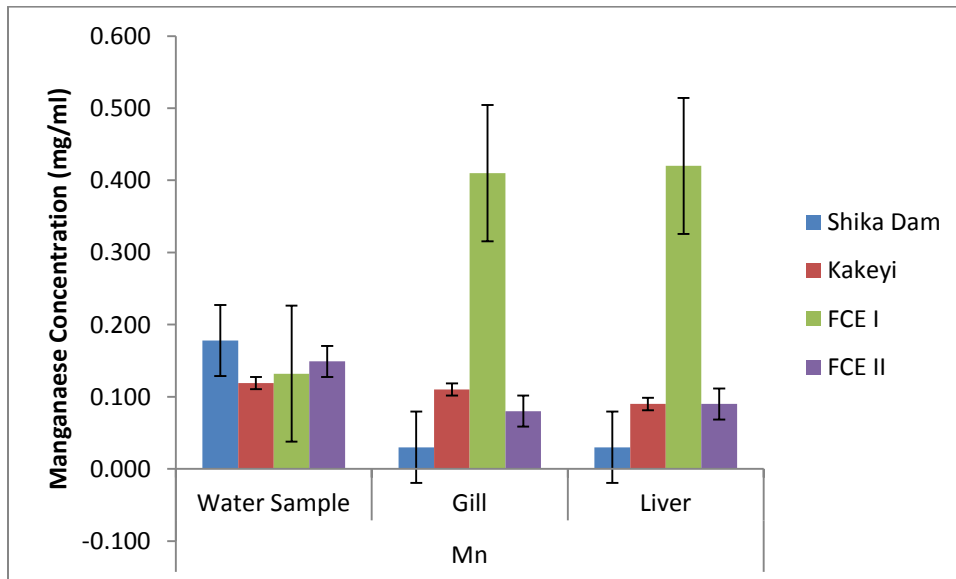

Figure 5 An Error bar indicating Cadmium Concentrations (mg/ml) in Water samples and Fish organs in all the sites.

Figure 6 An Error bar indicating Zinc Concentrations (mg/ml) in Water samples and Fish organs in all the sites.

Figure 7 An Error bar indicating Manganese Concentrations (mg/ml) in Water samples and Fish organs in all the sites.

4. Discussions

Fish is one of the chief protein sources for humans that play a role in lowering the blood cholesterol level and offers omega-3 fatty acids that minimize the danger of stroke and heart related disorders (Al- Busaidi *et al*. 2011). Of all aquatic species, fish are particularly sensitive to waterborne contamination and are recognized as bio-indicators for water quality monitoring. To attenuate the negative effects of Reactive Oxygen Species (ROS), fish possess an antioxidant defence system like other vertebrates that utilizes enzymatic and non-enzymatic mechanisms. The use of fish in environmental monitoring has become increasingly important in recent years in the investigation of natural variability, as well as anthropogenic substances, many of which function as prooxidants, accumulating in aquatic environments (Almroth 2008).

The kidney being an organ of excretion (amongst other functions) was actively engaged in dealing with the xenobiotics in the environment of the fish. The pollutants especially heavy metals need to be eliminated from the body as soon as possible that is most likely the reason why there were high significant differences in all the months of exposures because there is need for increased glutathione production to counteract the effects of the pollutants. This is in agreement with the works of Moniuszko-Jakoniuk *et al*. (2005); Michalak (2006); Jurczuk *et al*. (2006) when they indicated that the body fights the stress caused by excessive cadmium content by increasing the production of antioxidants such as glutathione, metallothionein, flavonoids and other chemical compounds.

The livers of the fish produced more of the antioxidant during the months of September and October. This may be due to the fact that the liver is the organ of detoxification and since, there was high volume of water the gills were spared of the effects (at least to some extent) of xenobiotics during respiration and other biological functions of the body necessary for survival. When the pollutants get into the internal organs of the fish they must be detoxified if the immune system of the fish at the moment is not overwhelmed. The up-regulation of the production levels of glutathione as the days increase was in response to the oxidative stress in their environment. These results are in conformity with those found by Lattuca *et al*. (2009) who showed that the liver of *Odontesthes nigricans* exhibited a better control of the oxidative damage than the gills, allowing minimization of intracellular damage when it is exposed to environmental stressful conditions. Furthermore, Liver possesses high potential for ROS generation, which seems to be efficiently counterbalanced by powerful protective mechanisms to detoxify and repair damaged lipid and proteins (Oliveira *et al*. 2008; Lattuca *et al*. 2009; Nahrgang *et al*. 2010). Also, measuring the same biomarker in different locations simultaneously gives us information about the pollution status of the region and provides a better comprehension of the mechanisms of response of the organisms to pollutants (Giarratano *et al*. 2010). There were however, high levels of production of glutathione in the gills of the fish during the December exposure in which the water levels were down and the dilution factor having been removed led to the concentration of the heavy metals vis a vis pollutants in the aquatic matrices. This concentration may have led to the increase observed in the glutathione production in the gill since it is the first point of contact with the environment. For most of the cellular reaction, glutathione must be available in reduced form (GSH) to prevent the harmful effects of ROS induced oxidative stress (Meyer 2008).

Glutathione production levels were highly significant in the gills and kidneys of the fish and and significant in the livers of the fish. This signifies the importance of these organs in the survival and adaptability of the fish to the polluted environment. The strong positive correlation of glutathione production levels with Pb, Cr, and Zn, and correlations with Cd and Mn further buttresses the fact that the polluted environment led to the generation of reactive oxygen species and creating oxidative stress on the fish with consequent increase in the glutathione production levels in order to counteract the effects of the xenobiotics. Similar results were obtained by Taiwo *et al*. (2014) when they demonstrated that oxidative stress markers (GSH and SOD_ Glutathione and Super oxide dismutase) correlated with heavy metal accumulation in some location- which shows that they could be used in monitoring environmental pollution. Glutathione is a key player in this antioxidative system, with a significant function in ROS scavenging and as a redox buffer to keep the cellular redox state in balance (Meyer & Hell 2005). Also, fish tissues are endowed with an antioxidant defence system to protect them from oxidative stress caused by metals (Atli *et al*. 2006). The antioxidant defence system in this regard is the glutathione in the liver, gill and kidney of the fish.

Highest mean values of glutathione production were obtained from Kakeyi site in all the organs of the fish in all the exposure. This may be as a result of high levels of municipal discharge from the neighbouring towns and villages especially from Sabon Gari area into the river body. This high content of run-off is also witnessed in FCEI where the glutathione production levels were equally high in all the organs. Lowest mean values were obtained in the gills of fish samples from Shika dam. Shika dam served as the reference point because it is relatively located at the upper course of the river and is relatively far from municipal discharge. This is in conformity with the work of Mahboob *et al.* (2014) when they observed that levels of GSH concentrations were increased by 44.8%, 35.3% and 32.7% in the kidney, heart and liver but were decreased by 33.6% in the gill in the fish samples collected from Wadi Hanefah Reservoir.

The significant difference observed in the fish organs, water samples and Lead (Pb) concentrations; and the significant differences in Chromium (Cr) concentrations observed in the gills and livers of the fish may be as result of urban and agricultural run-offs into the river system. Lead is well known to be a very toxic heavy metal even in its lowest concentration. Its detection and other heavy metals in the fish organ can serve as biomarkers used in bio-monitoring to give biological information that is, the effects of pollutants on living organisms. A link between DNA damage and concentration of Cr and Mn in soft tissues was observed in mussels in the most polluted site (Dallas *et al.* 2013). The harmful effect of toxic chemicals on natural ecosystems has led to an increasing demand for early – warning systems to detect those toxicants at very low concentration levels (Durrieu *et al.* 2006).

Lead, Chromium and Manganese had their highest values in Shika Dam. These heavy metals may have been gotten from River Kaduna since River Galma is one of its main tributaries or they may have been retained within the embankments of the reservoir over time and being washed off from time to time. Zinc however, had its highest in Kakeyi which is located downstream below Shika Dam where the river body is beginning to have its impact from the surrounding towns and villages.

It is important to note that whether ROS will act as damaging, protective or signaling factors depends on the delicate equilibrium between ROS production and scavenging at the proper site and the time (Gratao *et al*. 2005). Despite the considerable understanding of their links with contaminant exposure, the use of biomarkers is often limited by their strong variability due to natural biological and environmental cycles (Nahrgang *et al*. 2010a).

5. Conclusions

Glutathione production levels were highly significant (P<0.05) in the Kidneys and gills of *Clarias gariepinus* and significant in the livers of the fish in all the *in situ* bio-assays indicating that River Galma is polluted and that these organs were essential for the survival of the fish in the medium. Kidney is a very suitable organ for determination of antioxidants as biomarkers of oxidative stress in the environment since there were high significant differences in glutathione production levels in this organ.

This research establishes the presence of heavy metals in River Galma with varying degree of concentrations with significant differences in Chromium concentrations in the gills and livers of the fish; significant difference in the concentration of Lead amongst water samples and fish organs.

There were correlations between the heavy metals and glutathione production levels. Therefore, glutathione can be used as biomarkers of heavy metal pollutant in River Galma.

6. Recommendations

The out-come of this research can serve as an invaluable information to the members of the immediate community and the policy makers on environmental issues. It is also an eye-opener to the scientific community.

Gills, livers and Kidneys of fish are good organs in determination of glutathione levels in response to oxidative stress in the aquatic environment.

Better caging system and security measures should be adopted to ensure greater success in future *in situ* exposure. Further research should be carried out to test the outcome of this research (especially established research institutes) on the use of glutathione as biomarkers especially in polluted tropical freshwaters.

7. Acknowledgements

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