ASSESSMENT OF FLUORIDE BIOACCUMULATION IN CATFISH GROWN IN FLUORIDE RICH WATERS

Jophillene Bejumula

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Environmental Sciences and Engineering of the Nelson Mandela African Institution of Science and Technology, Tanzania

Arusha, Tanzania

July, 2021

ABSTRACT

Fluoride bioaccumulation in fish poses a significant impact to their growth performance, survival and consequently to the upper trophic levels. Few studies have reported the impact of high fluoride concentration and their accumulation to catfish in African settings. A 60-days African catfish (*Claris gariepinus*) cultivation was conducted at Ngarenanyuki ward and at the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratory in Arusha, Tanzania, to determine fluoride bioaccumulation and its effect on growth and survival of the catfish. The juvenile catfishes were exposed to water containing 36 mg F^{-}/L in a fishpond and synthetic water containing fluoride concentrations (NaF) of 5, 15, 36 and 45 mg/L in the aquaria set at NM-AIST laboratory. Fish growth and survival was determined and the fluoride concentrations in fish tissues were analysed by ion selective electrode. At the end of the experiment, fish survival rate was greater than 90% in fluoride levels of 5, 15, 36 mg/L compared to 65.8% in 45 mg/L aquaria treatment. Also, significant fluoride bioaccumulation was observed in fish bones (222.00 mg/kg, dry weight) followed by gills (177.4 mg/kg), skin (9 mg/kg) and low amount in fillets (1.467 mg/kg). In all these tissues, fluoride bioaccumulation significantly rose with increase in fluoride levels in the water and exposure time ($p < 0.001$). From this experiment, it is concluded that high fluoride occurrence in surface water led to increased bioaccumulation in the African catfish and correlates with exposure time. Therefore, to grow catfish in water containing more than 45 mg/L requires a deflouridation process to take place.

AUTHOR'S DECLARATION

I, Jophillene Bejumula, declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Jophillene Bejumula

30/07/2021

Name and Signature of the Candidate Date

The above declaration is confirmed by:

Prof. Revocatus Machunda

30/07/2021

Name and Signature of the Main Supervisor Date

Prof. Kelvin Mtei

30/07/2021

Name and Signature of the Co-Supervisor Date

Dr. Liliane Pasape

30/07/2021

Name and Signature of the Co-Supervisor Date

COPYRIGHT

CERTIFICATION

The undersigned certify that has read and found the dissertation acceptable by the Nelson Mandela African Institution of Science and Technology, Tanzania

Prof. Revocatus Machunda

30/07/2021

Name and Signature of the Main Supervisor — Date

Prof. Kelvin Mtei

30/07/2021

Name and Signature of the Co-Supervisor — Date

Dr. Liliane Pasape

30/07/2021

Name and Signature of the Co-Supervisor — Date

ACKNOWLEDGEMENTS

I would like to give my sincere thanks to the School of Materials Energy Water and Environmental Sciences of the Nelson Mandela African Institution of Science and Technology, Tanzania for admission to the program and financial support through the ACE II Program at WISE - Futures Centre.

I would like to express my deep appreciation to my supervisors Prof. Revocatus Machunda, Prof. Kelvin Mtei and Dr. Liliane Pasape of the Nelson Mandela African Institution of Science and Technology for their support, advice and encouragement throughout my study. I also thank them heartily for patiently reading the work and giving valuable suggestions.

Special thanks to Mr. David Mtweve and Ms. Theresia Shirima of Nelson Mandela African Institution of Science and Technology for their friendly help throughout laboratory work and data analysis. I also wish to sincerely thank Mr. Humphrey Msongo, the Laboratory technician of Ngurudoto deflouridation centre in USA River – Arusha as well as Mr. Makemia Mabula, a PhD student at the university of Dar es salaam for their assistance on statistical analysis.

DEDICATION

I would like to dedicate this work to my beloved late brother Mr. Phinillene Bejumula.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

ADG	Average Dairy Gain
ANOVA	Analysis Of Variance
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization
H+	Hydrogen ions
ITPS	Intergovernmental Technical Panel on Soils
K	Condition Factor
MANOVA	Multivariate Analysis of Variance
NBS	National Bureau of Statistics
NH^{+}_{4}	Ammonium
NH_3	Ammonia
$NH_3^{-}N$	Ammonia-Nitrogen
NM-AIST	Nelson Mandela African Institution of Science and Technology
SE	Standard error
SGR	Specific Growth Rate
SR	Survival Rate
TBS	Tanzania Bureau of Standards
TDS	Total Dissolved Solids
TSS	Total Suspended Solid
USA	United States of America
WG	Weight Gain
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

The aquaculture industry is now becoming an important subsector for fisheries production, and thus it bridging the gap between supply and demand of fisheries products, following the decline in global capture of fisheries production (Subasinghe *et al.*, 2009). In addition, it has been recently viewed as an alternative strategy to combat poverty and food insecurity in developing countries in particular, where food security is still a challenge.

Globally, aquaculture is mainly conducted in freshwater (Singh & Tripathi, 2015). In most of sub-Saharan African countries, pond culture systems form an important production section. The carp, tilapia, rainbow trout and catfish are the species that dominate the world aquaculture production (Fabrice, 2019). In Africa, catfish is the second most farmed freshwater fish species, just after the tilapia. The catfish popularity is due to several characteristics ranging from high market acceptability, rapid growth rate and tasty flavour (Hossain *et al.*, 2006) to high disease resistance (Fitzsimmons, 2010). Despite having many good characteristics, catfish production is yet to take off in most of African countries, including Tanzania. Besides, economic, technological and water quality issues are some among other serious constraints that limit catfish production within the region. In most cases, retarded growth and mortalities in fish have been associated with water quality issues.

Fluoride toxicity is one of the water quality concerns for many countries in the world (Kaur *et al.*, 2017). While most of water sources are said to have naturally ideal parameters such as: Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), pH, Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), Conductivity and Ammonia-Nitrogen (NH_3-N) for Aquaculture, there exist areas with exceptionally high fluoride concentrations. Moreover, higher levels of fluorides have been reported to pose many health and socio-economic problems to humans (Mbabaye *et al.*, 2018). Not only in humans that high levels of fluoride have been associated with poor health outcomes, but also in fish growth in particular. For instance, Shi *et al.* (2009) reported significant inhibited growth of sturgeon fish that was reared in freshwater with fluoride concentrations greater than 10 mg F^-/L. Gupta and Poddar (2014), and Shingadia (2014), reported abnormal changes in behaviours, chromatophores functions, reproductive system, haematology, serum, genotoxicity, Cytotoxicity, histology and tissue biomolecules to fishes exposed on high fluoride concentrations.

Tanzania is one of the top ten countries in the world with high fluoride groundwater concentrations. Others include: Australia, China, Ghana, India, Kenya, Sri Lanka, and the USA (Jagtap *et al.*, 2012; Kimambo *et al.*, 2019; Rizzu *et al.*, 2020; Addison *et al.*, 2020). Central and northeast regions of Tanzania, especially Singida, Arusha, Shinyanga, Manyara, Mara and Kilimanjaro, are reported to have their water sources highly affected by higher fluoride contents (Malago *et al.*, 2017; Thole *et al.*, 2013).

The Meru district (located in Arusha region) is one amongst areas within the country, whose both surface and underground water are reported to contain high levels of fluoride up to 1103.70 mg/L (Mbabaye *et al.*, 2018). Implications of this higher level content of fluoride within the area have widely been studied in humans and plants. A study by Ghiglieri *et al.* (2010) reported 26 mg/L of fluoride bioaccumulation in some crops grown under irrigation of Ngarenanyuki river during the dry. Masawe *et al.* (2019) revealed that maize plants grown in 116.93 mg F^-/L soil accumulated mean fluoride (mg F^-/L) of 38.560, 24.251, 10.629, 7.7756 and 2.100 in the roots, leaves, stem, grain and cob, respectively. In addition, the bean plants grown in the 129.2 mg F^-/L soil accumulated mean fluoride in roots, leaves and stem up to 16.27, 11.328 and 8.459 mg F^-/L, respectively. Rizzu *et al.* (2020) reported maize and bean grains collected from Ngarenanyuki area contains higher amounts of fluoride than other fluoride endemic areas such as parts of Burundi, India and Ethiopia.

However, limited information exists about fluoride bioaccumulation, and associated effects on growth and survival of African catfish which are mainly reared in different Tanzania regions. Catfish farmed in different ponds of Ngarenanyuki ward of Meru district in Arusha region, Tanzania are exposed to similar and, or more fluoride levels reported to cause growth and survival effects to catfish grown elsewhere (Gupta & Poddar, 2014; Shingadia, 2014; Shi *et al.*, 2009). Most of these ponds abstracted water from surrounding rivers reported to contain high levels of fluoride. This practice may accelerate catfishes to accumulate a significant amount of fluoride in their body tissues particularly in skeletal tissues that may reduce their growth rate and affect their population.

Due to livelihood diversification initiatives in this area, ponds for culturing catfish have been increasingly dug and filled with waters from the nearby flowing rivers, which contain elevated levels of fluoride (Mbabaye *et al.*, 2018). Since fluoride is already known to have adverse effects on human health, it therefore, of paramount importance to extend research interest in this area so as to understand the growth performance and survival of commercially important fish species farmed in there. Furthermore, it is critical importance to foster research that will contribute to knowledge and address challenges that might be associated with such higher fluoride occurrences in the area. Therefore, these initiatives will boost commercially grown fish species production in the area as well as avoiding other related fluoride toxicity effects along the food chain.

Most studies conducted in various parts of Africa, including Tanzania, have established the baseline information on fluoride occurrences, removal technologies for domestic use, and its impacts to human health (Malago *et al.,* 2017). However, no information is available with respect to the effect of fluoride on growth and survival of other organisms such as fish, especially in areas where fluoride concentration is extremely high. It is based on this background that the present study was tailored to understanding the growth performance and survival of one of the highly valuable fish species (the catfish) farmed in in fluoride rich areas in Tanzania.

1.2 Statement of the Problem

Most studies conducted in various parts of Africa, including Tanzania, have established the baseline information on fluoride occurrences, removal technologies for domestic use, and its impacts to human health (Malago *et al.,* 2017). However, limited information exists about fluoride bioaccumulation, and associated effects on growth and survival of African catfish, which are mainly reared in different regions of Tanzania. In Meru district; the Ngarenanyuki ward in particular, located in Arusha region is one amongst other areas within the country, whose both surface and underground water have been reported to contain high levels of fluoride up to 1103.70 mg/L (Mbabaye *et al.,* 2018). However, by the time the present study was conducted, there was no any study reported to have been done for investigating the fluoride bioaccumulation and their effects on growth performance and survival of the catfish reared within the area. Therefore, this is the gap (the missing link) the present study was undertaken to fill in.

1.3 Rationale of the Study

Tanzania is one of the top ten countries in the world with high fluoride groundwater concentrations. Central and northeast regions of Tanzania including Arusha regions are reported to have their water sources highly affected by higher fluoride levels. Moreover, not only that high level of fluoride has been associated with poor health outcomes in humans, but also in growth performance and survival of fish in particular. For instance, Shi *et al.* (2009) reported significant inhibited growth of sturgeon fish that was reared in freshwater with fluoride concentrations greater than 10 mg F^{-}/L). Meru district (Ngarenanyuki ward in particular) located in Arusha region is one amongst areas within the country, whose both surface and underground water contain high levels of fluoride up to 1103.70 mg/L (Mbabaye *et al.,* 2018). Catfish farmed in different ponds of Ngarenanyuki ward of Meru district in Arusha region, Tanzania are exposed to similar and, or more fluoride levels reported to cause growth and survival effects to catfish grown elsewhere (Gupta & Poddar, 2014; Shingadia, 2014; Shi *et al.,* 2009). Concurrently, this region is as well considered as the most potential for catfish culture in Tanzania. It is based on this background, that the present study was tailored to understanding the growth performance and survival of catfish farmed at Ngarenanyuki ward. Furthermore, it is critical importance to foster research that will contribute to knowledge and address challenges that might be associated with such higher fluoride occurrences in the area. This is because, a serious concern on problems related to catfish production as a result of impaired growth rate, survival and reproduction shall extend socio-economic problems as catfish are largely grown for commercial purpose in the area. In addition, these initiatives will boost commercially grown fish species production in the area as well as avoiding other related fluoride toxicity effects along the food chain.

1.4 Research Objectives

1.4.1 General Objective

To investigate fluoride bioaccumulation in catfish reared in fluoride rich waters.

1.4.2 Specific Objectives

(i) To determine fluoride bioaccumulation in catfish tissues of different fish age groups.

(ii) To examine the interference of high fluoride levels in water to fish growth.

1.5 Research Hypotheses

(i) Is there any significant difference in mean fluoride bioaccumulation in Clarias gariepinus tissues reared at different fluoride levels?

(ii) Are there any catfish growth interferences caused by high fluoride levels in waters?

1.6 Significance of the Study

The findings of this study are going to contribute to existing knowledge on the extent fluoride can impact growth performance and survival of the catfish. This is urgent as catfish growth performance and their survival ship have major impact on their production, and thus the aquaculture industry in general. Further, the study results will aid fish farmers and other stakeholders to understand and address the effects of high fluoride concentrations on the growth performance and survival of the *Clarias gariepinus*. Knowledge on the effect of fluoride on growth performance and survival of African catfish will help fish farmers, aquaculture experts and fishery managers to make informed decision regarding fluoride control such as water treatment and/or development of African catfish traits which are more tolerant to high fluoride concentrations. The results of the present study also have practical relevance to catfish consumers. Consumers of catfish farmed from areas of fluoride-contaminated water can benefit from the findings by paying attention to their fish food intake. These results of the present study also have scientific relevance. Scientific literature is scarce on the bioaccumulation and effect of fluoride in *Clarias gariepinus*; this report tries to fill that gap.

1.7 Delineation of the Study

The present study focused on investigating bioaccumulation and effect of fluoride on the growth performance and survival rate of *Clarias gariepinus*. The Scope of study in the dissertation contains the explanation of what information or subject is being analysed. Research is usually limited in scope by sample size, time and geographic area.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fluoride Occurrence and Chemistry

Fluoride is among the elements found in the natural environments and human surroundings such as rocks, soil, surface and ground water. These named surroundings are said to be major sources of high fluoride concentrations in drinking waters sourced from springs, rivers, lakes, wells and dams (Mbabaye *et al.,* 2018). The major sources of fluoride ions in rocks and soils are fluorapatite, fluorites (fluorspar), phlogopite, lepidolite, topaz ($Al_2(FOH)_2SiO_4$) and its associated mineral compounds resulted by volcanic eruptions (Kitalika *et al.,* 2018; Roy & Dass, 2013). Several places in the world have been reported to contain fluoride in its ground waters (Kimambo *et al*., 2019). According to different studies conducted globally, more than 25 countries in the world have high fluoride levels in water sources and that, more than 200 million people depend on that high fluoridated waters for drinking and aquaculture which pose serious health effects (Maity *et al.,* 2018). Fluoride toxicity have brought many health, social and psychological problems to most countries in the word, to such an extent that some countries declared endemic for fluoride in the world (Kaur *et al,* 2017).

Groundwaters in most African countries have been reported to contain high fluoride concentrations above 1.5 mg/L a maximum limit recommended by WHO for drinking water. The East African Rift valley as depicted in Figure 1 have been reported as a high fluoride zone whereby 80 million people exhibit fluorosis symptoms (Mohan *et al.,* 2012). Several places in Tanzania such as Singida, Arusha, Arumeru and Shinyanga as depicted in Figure 2 have been reported to contain high concentrations of fluoride in ground water sources (Thole *et al.*, 2013 & Malago *et al.,* 2017).

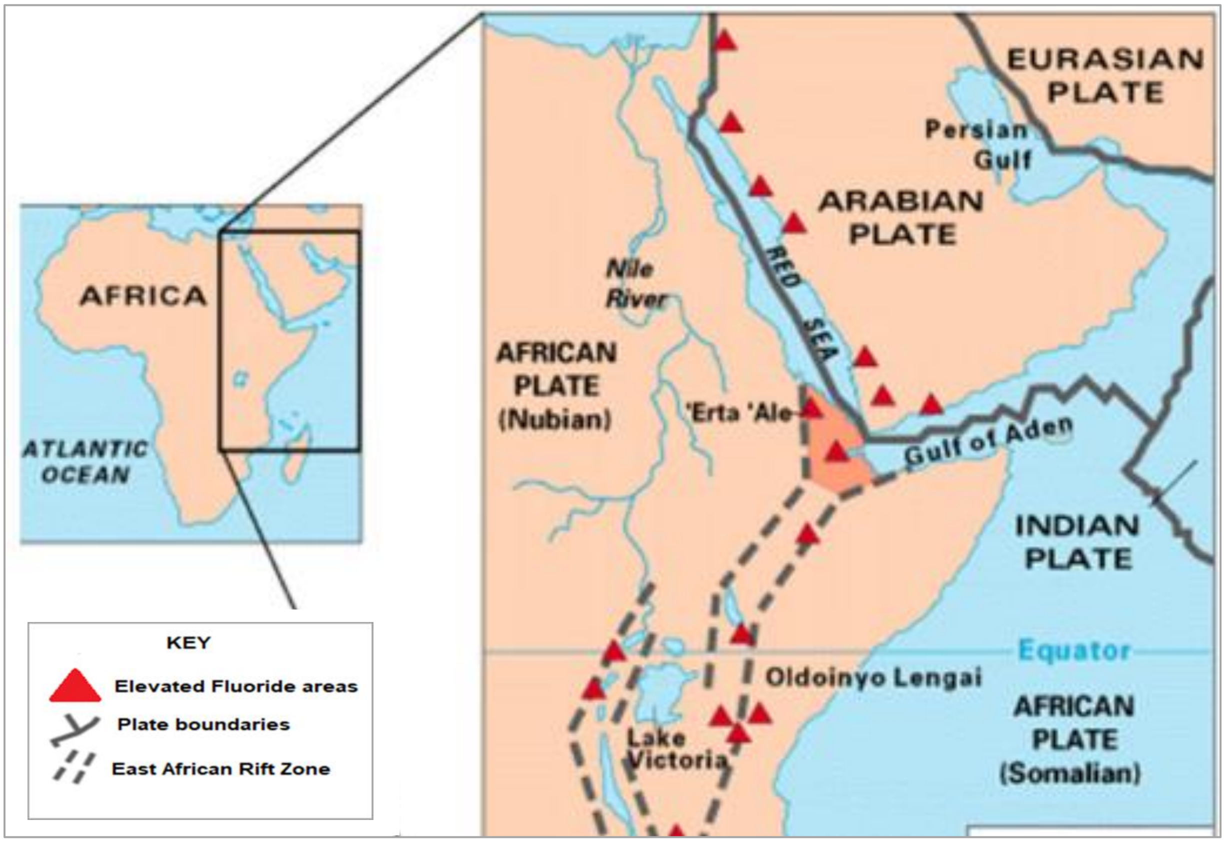


Figure 1: Distribution of fluoride occurrences along the East African Rift Valley (Johansen, 2013)

Kitalika *et al.* (2018) reported that, about 30% of the rivers in Tanzania contain more than 1.5 mg/L of fluoride levels which is a maximum concentration levels recommended by World Health Organization (WHO) and also above 4 mg/L a maximum concentration allowed by Tanzania Bureau of standards (TBS) for drinking water. Mbabaye *et al.* (2018) pointed out that fluoride distribution and occurrences in surface and ground waters along the slopes of Mount Kilimanjaro, Meru and along the rift valley as depicted in Figure 1 had been documented in Tanzania since 1950. In addition, the ground water quality report of Tanzania by the British Geological survey depicts the severity and widespread of fluoride resulted by volcanic activities in the rift valley zone in the south west and northern part of Tanzania.

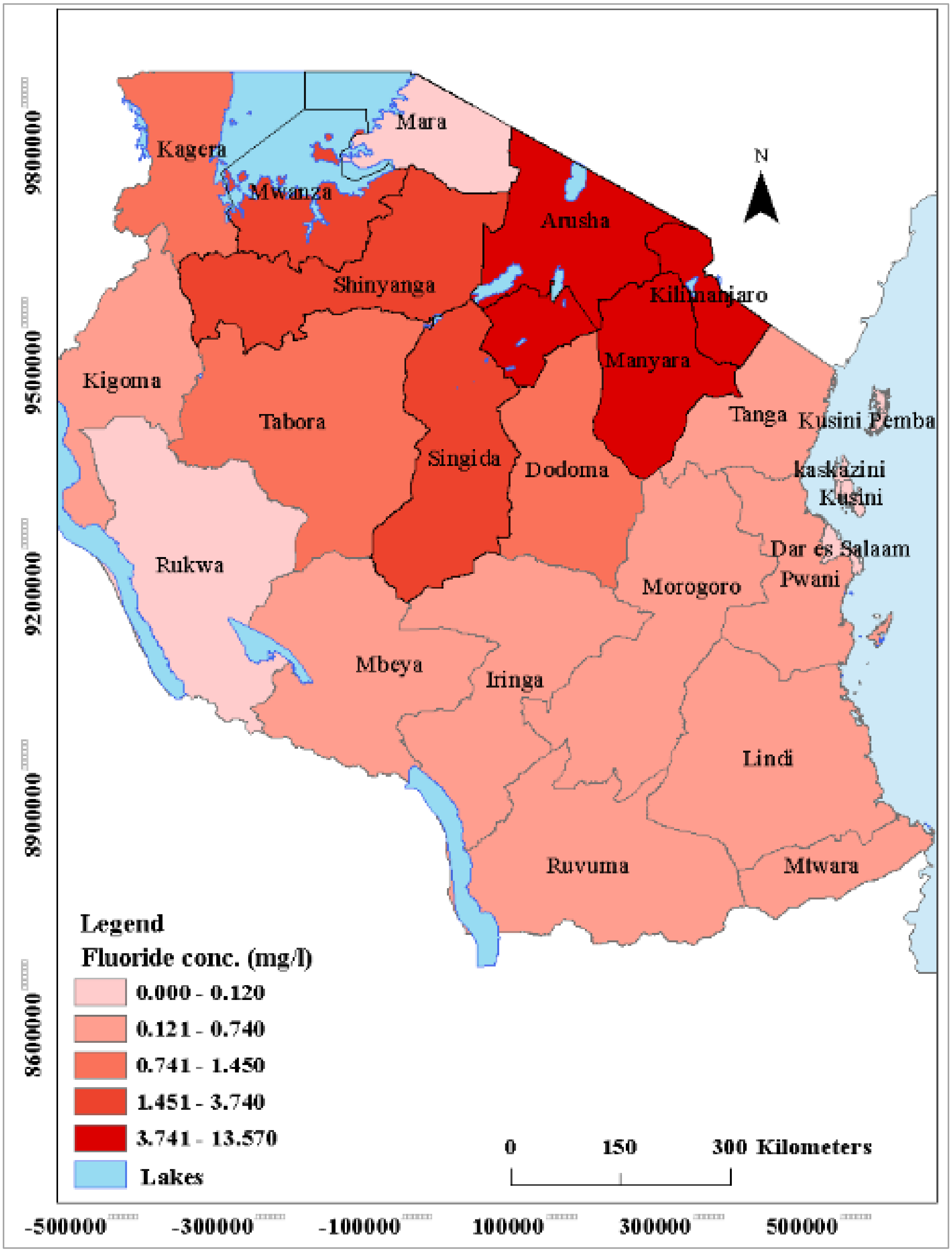


Figure 2: Tanzania regions documented with high fluoride levels (Malago *et al.*, 2017)

It has been reported that, most of the water sources in Meru district, Tanzania such as Ngarenanyuki Olkung'wado Spring, Ngarenanyuki Olkung'wado River, Leguruki Shishtoni dam, Lake Tulisia Arusha in leguluki, Big Momella Lake in Leguruki have picked with fluoride concentrations of 31 mg/L, 30 mg/L, 38 mg/L, 974.90 mg/L and1103.70 mg/L respectively (Mbabaye *et al.*, 2018).

2.2 Aquaculture of Catfish Species

Catfish (Order Siluriformes) are widely distributed fish species primarily inhabiting in many of inland or coastal waters tropics and temperate regions (Armbruster, 2011; Jin *et al.*, 2016). High ability for artificial spawning, adaptability to earthen ponds, high tolerance to low dissolved oxygen, relatively high resistance against infectious diseases, and relatively high feed conversion efficiency, make catfish best candidates for aquaculture (Jin *et al.*, 2016).

The global importance cannot be overstated. Many countries around the world such as China and Vietnam, are now heavily engaging in catfish aquaculture (Liu *et al.*, 2008). In the United States, catfish accounts for over 60 % of all USA aquaculture production (Liu, 2011). The production of catfish is also growing in Africa. In 2017, the catfish production reached USD 644 million, from USD 79 325 in 2005 (Food and Agriculture Organization [FAO] & Intergovernmental Technical Panel on Soils [ITPS], 2018).

Catfish farming in the Africa faces unprecedented challenges including economic and technological constraints, quality feeds and water quality issues (Wanja *et al.*, 2020). Singh *et al.*, (2017), argues that aquatic systems are final sink of most environmental contaminants including fluoride toxicant compounds and thus contributing to increased environmental surface area for most of aquatic organism fluoride exposure. In many occasions, retarded growth and mortalities in fish have been associated with water quality issues (Agano *et al.*, 2017). Water quality parameters affect the growth and fecundity of cultured fish (Agano *et al.*, 2017). Retarded growth and fecundity of cultured fish may in one way or another reduce increased fish production and fish industry profitability. Therefore, continued investigation of water quality parameters that affect fish survival and growth performance are much needed to support the catfish industry.

2.3 Water Quality

Water quality refers to as the totality of all physical, chemical, biological, and aesthetic characteristics of water that influence its beneficial use (Boyd, 1998). Boyd (1998) once opined that any characteristic of water that in production systems that effects survival, reproduction, growth, and production of aquaculture species, influences management decisions, causes environmental impacts, or reduces product quality and safety can be considered a water quality variable. In freshwater aquaculture, some of the important parameters which normally receive high attention include, dissolved oxygen, ammonia, nitrites, pH, temperature and chemical pollutants (Boyd, 1998; Olurin & Aderibigbe, 2006; Bajpai & Tripath, 2010).

Dissolved oxygen (DO) in water plays an important role in the biology of cultured organisms (Boyd, 2001). The DO is essential for growth and survival of fish because it affects fish respiration as well as nitrite and ammonia toxicity (Mallya *et al.*, 2007). Low DO concentration may result into poor fish growth, mortality, and diseases (Tucker & Robinson, 1991). Many studies report on minimum DO requirement for catfish species. Tucker and Robinson (1991) reported 4-5 ppm as the minimum DO requirements in Channel Catfish hatcheries. According to Santhosh (2017), Catfish can survive in low oxygen concentration of 4 mg/L. Also, Setiadi *et al.* (2019) reported that *Clarias* specie prefer DO concentration above 3 mg/L.

In aquaculture systems, ammonia come from fish excretion and/or from bacteria decomposition of organic matter in the fish feeds (El-Sayed, 2004). Ammonia exist either as un-ionized NH_3 which is highly toxic (Tomasso, 1994) or ionized NH_4^+ which is less toxic (El-Sayed, 2006). Un-ionized NH_3 is closely correlated with water pH, temperature and DO (Lim & Webster, 2007). The concentration of un-ionized NH_3 increases with increase in water pH and temperature (Durborow *et al.*, 1997). Nevertheless, low DO elevates ammonia toxicity (Lim & Webster, 2007). Schram *et al.* (2010) advised that water NH_3 concentration for African catfish should not exceed 0.34 mg NH_3-N/L to reduce the risk of reduced growth and feed intake. In addition to ammonia, high levels of nitrites in culturing media also cause “Brown blood” disease, a situation whereby fish blood fails to carry sufficient amounts of oxygen as of hemoglobin being permanently bound to nitrite to form methemoglobin. Roques *et al.* (2013) opined that in order to prevent the risk of reduced growth and feed intake in African catfish aquaculture, water nitrite concentration need to be kept as low as below 0.6 mg/ L.

pH is defined as a negative logarithm of the hydrogen ions (H+) in the water (Boyd, 1998). The acid and alkaline lethal points are approximately pH 4 and 11, respectively (Boyd, 1998). The optimum pH range for growth and health of most freshwater organisms is 6.5-9.0 (Boyd, 1998). At high pH (>9) most ammonium in water get converted to toxic ammonia (NH_3) which can kill fish (Boyd, 2001). According to Lang *et al*. (2007), pH range of 6.5-8 is desirable for growth of *Clarias gariepinus.*

Water temperature is an important variable that influences all other biological and chemical processes in aquaculture operation. El-Sayed (2006) reported that high water temperatures reduce the amount of DO while increasing the metabolic rate and oxygen demand. Each species has its optimum temperature where it grows best. *Clarias gariepinus* perform well when reared at mean temperature of 26 °C (Ogunji & Awoke, 2017). *Clarias gariepinus* fingerlings are not able to survive when water temperature reaches to 40 ^{o}C (Ogunji & Awoke, 2017). Britz and Hecht (1987) observed high growth rates between 25 and 33 °C.

Aquaculture systems can also be polluted by a variety of pollutants that originate from natural and anthropogenic sources which are toxic to the fishes (Singh & Tripathi , 2015). Fluoride is one of them. Different minerals such as fluorapatite, fluorites, phlogopite, lepidolite, topaz and other compounds resulted by volcanic eruptions are reported to be the major sources of fluoride in rocks, soils and aquatic environment (Roy & Dass, 2013). In most circumstances, Fluorite (CaF_2) solubility is reported to control the highly dissolved fluoride concentrations in aquatic environment and this is attributed by soft, alkaline and calcium - deficient waters (Ozsvath, 2009).

2.4 Fluoride Bioaccumulation and its Effects in Fish

Fluoride is a cumulative poison into body tissues which its bioaccumulation values increases with exposure time to different organisms including African catfish (Ozsvath, 2009). Studies have indicated the possibilities of different species of fish to accumulate a significant amount of fluoride in their bones (Dalgleish *et al*., 2007). For instance, a study conducted by Chowdhury *et al.* (2018) found that *Rohu* (*Labeo rohita*) fish species contained fluoride concentrations of about 3.16 mg/kg in the bones and 2.28 mg/kg in flesh. Also, Arctic char (*Salvinu salpinus*) fish found in temperate areas was found to accumulate 16.6 mg/kg in muscles and 1150 mg/kg in bones when living in water with 2 – 20 mg/L of fluoride (Christensen, 1987).

Aquatic organisms experience behavioural and physiological changes when exposed to high levels of fluoride in the water. A fluoride level exposure of about 5.75 mg/L to fish leads to changes in the shape, size and quality of fish chromatophores pigment thus impairing fish coloration and camouflage (Shingadia, 2014). Recent studies that explored the detrimental features of fluoride revealed that it affects soft tissues such as brain, liver, Kidney and pancreas as equal as it does to hard tissues like bones (Singh *et al.*, 2017). Furthermore, Barbier *et al.*, (2010) argues that, fluoride deleterious effects to organisms precisely depend on fluoride dosage, exposure time and type of cell being exposed. In addition, other studies revealed that fluoride bioaccumulation and deleterious effects will depend on water temperature and intraspecific fish size (Camargo, 2003). Uo and Ie (2013), revealed limited growth in freshwater teleost fish exposed to fluoride concentrations of 77.7 mg/L by 24 % as compared to control fish in 60 exposure days. Also, Jha (2004) reported on the serious impacts to fish growth and cell proliferation when exposed to high fluoride levels. Fish exposure to varied fluoride concentrations impair their weight gain, increase in size and survival rates (Uo & Ie, 2013). Other fish species such as rainbow rearing trout recorded threshold of 50 % mortality under 8.5 mg/L fluoride exposure (Wright, 1977). A coastal Shrimp (*Palaemon elegans*) decreased by 118.19 % of its growth after being exposed to extra 0.5 mg/L fluoride concentrations in a fluoridated sea water (El-said & Sallam, 2008). The acuteness of toxicity increases with an increase of fluoride concentrations and thus immediate effects are being felt by various fish species (Camargo, 2003). High fluoride concentrations in water bodies have impacted aquatic organisms such as fishes in different aspects such as change of behaviours, chromatophores, reproductive system, haematology, serum, tissue biomolecules, genotoxicity, Cytotoxicity, histology, genobiotics (Kaur *et al.*, 2017). Fresh water catfish (*Clarias batrachus*) is reported to be affected in blood cell morphology and antioxidant, superoxide dismutase and catalase enzymes the condition which resulting into a persisting condition referred as oxidative stress under high fluoride exposure (Gupta & Poddar, 2014).

In humans, several studies have revealed the high intake of fluoride from drinking water has caused a great danger to health problems in the world (Latham & Grech, 1967). About 35 % - 48 % of ingested fluorides by humans are retained in their bodies especially in skeletal and calcified tissues leading to fluorosis (Samal *et al.*, 2016). In Tanzania and elsewhere in the world as depicted in Fig. 2, dental fluorosis (teeth mottling), skeletal fluorosis and crippling fluorosis have been reported in many parts of the country including, Arusha, Kilimanjaro, Shinyanga, Singida and Mwanza (Mbabaye *et al.*, 2018).

Dental Fluorosis (Kaseva, 2005) Skeletal Fluorosis (Kaseva, 2005) Crippling Fluorosis (Eliuza, 2004)

Figure 3: Documented fluorosis problems in Tanzania and elsewhere in the world (Mbabaye *et al.*, 2018)

Most of the studies conducted in Tanzania have focused on addressing the fluoride distribution, occurrences, removal technologies and associated impacts to human health. However, little is known about the effect of fluoride in other organisms, especially to catfish which are highly farmed and consumed by humans. Fluoride concentration in the Ngarenanyuki River is said to be 26 mg/L (Malago *et al.*, 2017), although Mbabaye *et al.* (2018) reported a fluoride level of up to 30 mg/L. Fluoride bioaccumulation and effects on fish growth and survival rate had not been evaluated, thus becoming of high research interest as the fluoride level far exceeds permissible limit set by the World Health Organisation (WHO) standard as well as that of Tanzania Bureau of Standards (TBS) (Kitalika *et al.*, 2018). There is the need to assess the effect of fluoride on fish's welfare taking into consideration the levels of fluoride and its bioaccumulation in catfish, resulted impacts as well as its potentiality to be transferred to humans through dietary intake.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Clarias gariepinus fingerlings used in the study were procured from catfish farmer in Moshi municipality (Being reared at less than 1 mg/L Fluoride waters), a municipal located 86 kilometres from Arusha City. The wide coverage of the area is dominated by alluvial deposits and volcanic rocks (Ghiglieri *et al.*, 2010).

3.2 Study Site

The present study took place at Ngarenanyuki ward which is located at Meru district in Arusha region, Tanzania (Figure 4). The aquifer of the study area and its associated underground rocks are magmatic and metamorphic in nature with high fluoride contents. These rocks have been reported to influence the high fluoride levels in most of surface water bodies within the area..Surface waters in the area are utilized by local communities for domestic uses, and agricultural activities including fish farming. Fish farming that operates in Ngarenanyuki ward is mainly done in ponds. In this type of fish farming, fish farmers set their equipment and draw fluoride rich water from surface water structures to the ponds.

The experiment was carried out for 60 days at Ngarenanyuki ward and the Nelson Mandela African Institution of Science and Technology (NM AIST), located in Arusha -Tanzania (Fig. 4).

.

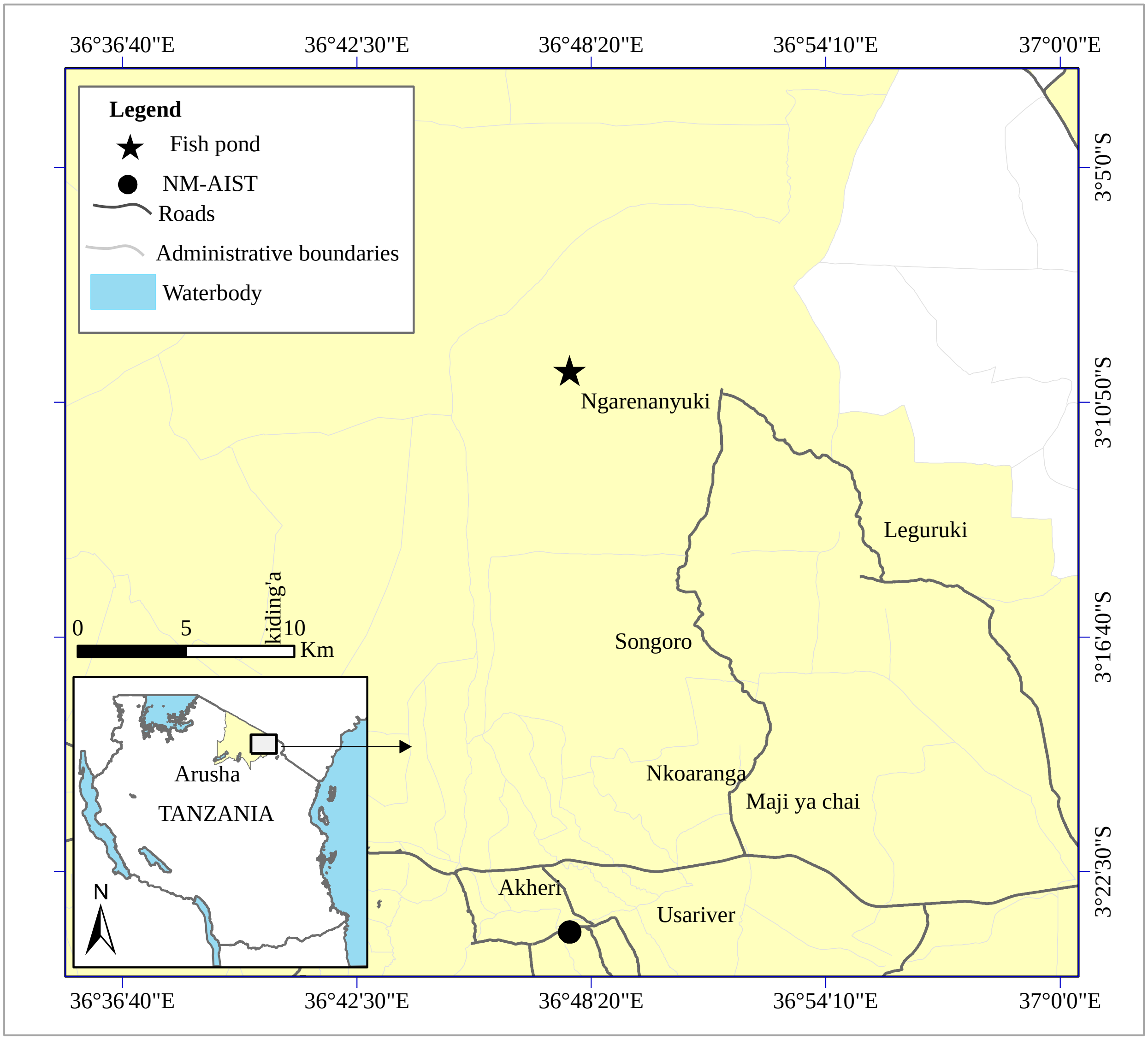


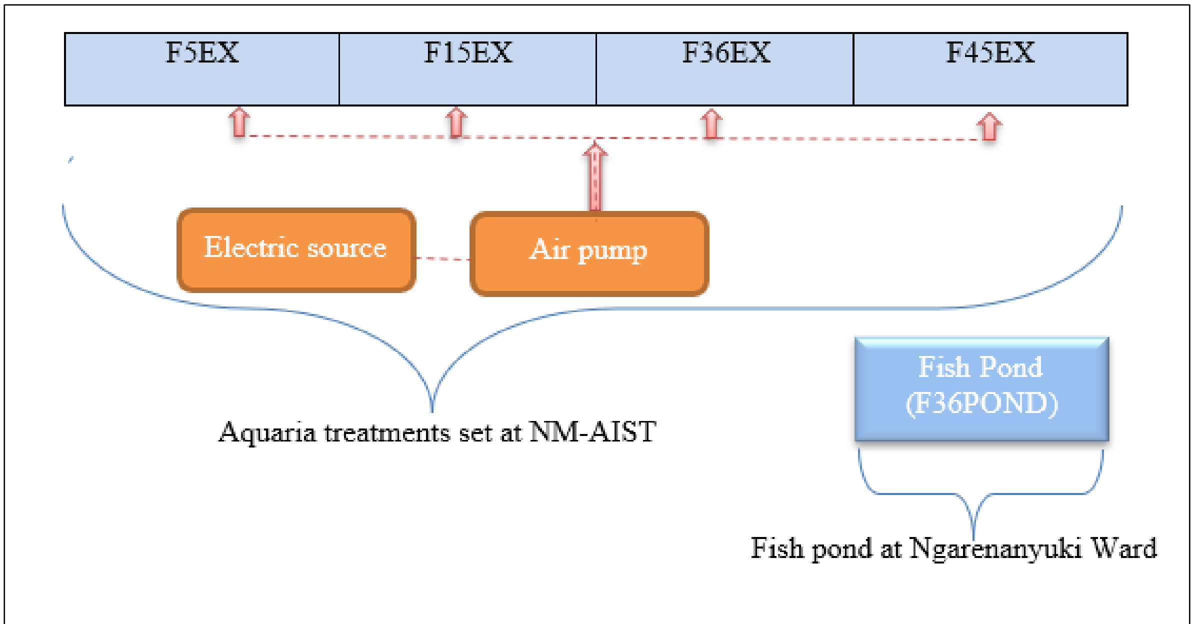
Figure 4: Map of Tanzania showing the location of the Ngarenanyuki ward in Arusha region where the experiment was conducted (NBS)

3.3 Collection of Samples

The 150, one-month old fishes weighing between 19 g and 21 g were procured from the fish hatchery in Moshi municipality. The 150 fingerlings were split into two unequal group of 120 and 30 individuals. The 120 *Clarias gariepinus* fingerlings were then transported to the experimental aquaria at the NM-AIST laboratory while the 30 were sent to the Ngarenanyuki secondary school pond. In both cases, fingering transportation was achieved using oxygenated plastic bags. No mortality occurred during transportation. On the other hand, the water samples were collected and sent to the NM AIST Laboratory for fluoride concentration determination. Fluoride concentration determination was conducted using fluoride ion selective electrode method. The Fluoride concentration of the water from hatchery pond ranged between 0.25 mg/L to 0.76 mg/L.

3.4 Experimental Design and Facilities

The experimental design comprised of 3.375 m^3 aquaria. One was used as control and other three were used treatment aquaria. To ensure similar fluoride bioaccumulation conditions between the treatments and the control, bioaccumulation limiting factors namely Mg, Ca and Cl were determined from pond waters prior experiment. The treatment aquaria were filled with a multi element standard solutions prepared following the dilution theory (Casellato *et al.*, 2012). The resultant solutions contained in the aquaria had similar concentrations of Mg (12.4 mg/L), Ca (13.4 mg/L) and Chloride (191.43 mg/L)) as that of the ponds but with varied fluoride concentrations in the order of (5, 15, 36 and 45 mg/L) (Figure 5). In Figure 5 and Figure 6, the 36 mg/L fluoride concentration compartment was considered as a control aquarium as it was more or less the same with fish pond fluoride concentration at Ngarenanyuki secondary school at Ngarenanyuki Ward.



Key: Treatment abbreviated F5EX, F15EX, F36EX, F45EX, F36POND stands for 5,15,36, 45 F^-mg/L aquaria and 36 F^-mg/L fish pond respectively.

Figure 5: Hypothetical experimental set up at NM-AIST and Ngarenanyuki ward in Arusha region

Figure 6: Field photographs taken during experimental set up at NM -AIST (a) and (b) and Ngarenanyuki Secondary School (c) and (d) in Arusha region

Before stocking the fish fingerlings into respective aquarium and fishpond compartment, 150 two-month catfish fingerlings reared below 1 mg/L fluoride waters from Moshi Municipality in Kilimanjaro were acclimatized to suite experimental conditions for 14 days. The confirmation of the varied fluoride levels was done by the use of fluoride ion selective electrode method. Then, 120 fish fingerlings were stocked in the aquaria treatments, each with 30 fingerlings and the remaining 30 fingerlings were stocked in the fishpond compartment.

3.5 Water Quality Assessment

3.5.1 Water Quality Parameters

In each Aquarium, water quality parameters (pH, dissolved oxygen, and temperature) were recorded twice a day at 09:00 am and 4:00 pm using a pH meter (HANNA model No. HI 98128) and oxy-guard meter (HANNA model No. HI 98186) (for oxygen and temperature). During the experimental period, all treatment Aquaria sections were supplied with aeration by using RS Electrical Pump (Model: RS 390) to ensure sufficient DO levels for well-being. The multi standard solution exchange was conducted once a week to control the water quality while maintaining the required fluoride level throughout the experimental period. Water samples were taken from Ngarenanyuki fishpond and aquarium sections set at NM-AIST Laboratory on weekly basis, and then, frozen at $\leq$ -18 ^{o}C and transported to the NM-AIST laboratory where analysis for ammonia and nitrite concentration using phenol method was done (Parsons, 1972).

3.5.2 Laboratory Analysis of Water Hardness, Chloride and Fluoride Concentrations

Hardness and chloride of water from the fish ponds (Ngarenanyuki secondary school pond) were determined by using EDTA Titrimetric Method and Argentometric method, respectively. Fluoride concentrations were determined by the use of fluoride ion selective electrode (Carey & Coleman, 2014). The electrode was calibrated using standard solution NaF (1000 mg/L, 100 mg/L, and 10 mg/L). The TISAB II were added to both standard solutions of NaF and prepared samples in a ratio of 1:1 to ensure adjustment of the ionic strength of the samples.

3.6 Fish Feed and Feeding Schedule

All fishes were fed on industrial manufactured feed pellets until the end of the experiment. The feeds were bought from a commercial shop known as TAZAMA Company Limited based in Arusha, Tanzania. The nutritional composition of the feed included: Cereals (corn wheat), Fish meal, Soybean meal, Fish oil, Amino acid, Mono, Calcium phosphate, vitamins and minerals, anti-oxidant and anti-mould. Generally, the feed contained 40 % of protein and with no fluoride associated ingredients, thus assumed to contribute similar fluoride bioaccumulation trends as all catfish under experiment were fed the same fish feed (Table 1). During the first month of the experiment, Fish juveniles required more protein to boost their growth and thus, were fed three times a day at 8.00 am, 12.00 pm and 4.00 pm at the rate of 10 % of their body weight and thereafter, reduced to 5 % of their body weight in the subsequent month (El-Sayed *et al.,* 2003).

Table 1:Nutritional composition of the feed

Feed type	Description	Size	Fish age (Months)
Starter (1) 44 %	Powder	150-200 µm	1 - 2
Starter (2) 40 %	Crumbles & pellets	0.1 – 1 mm	
Grower (1) 36 %	Floating & pellets	2 - 3 mm	2-3
Grower (2) 32 %	Floating & pellets	3 – 4 mm	
Storage	Fish feed were stored in the room temperature and not exposed to direct sunlight or rain		
Ingredients	Cereals (corn wheat), Fish meal, Soybean meal, Fish oil, Amino acid, Mono, Calcium phosphate, vitamins and minerals, anti-oxidant and anti-mould. (Total protein =40 %)		

TAZAMA Company Ltd of Arusha Tanzania (http://www.tazamatanzania.com/)

3.7 Growth Performance and Survival Rate

Before placing the fish into their respective aquarium sections, their initial weights and length were measured using a weighing balance (OHAUS – Explorer 124, Version 2.01/2.01) and a 30 cm-Fish Data Ruler respectively (Figure 7).

Figure 7: Photographs taken during the experiment while measuring weight (right) and length (left) of catfish

Subsequent weight and length were taken after every 20 days. Measurement of weight and lengths was done up to the 60th day of the experimental period. On the other hand, fish mortality was observed on a daily basis. The dead fishes were scooped out of the treatment sections. Fish growth performance parameters such as weight gain (WG), average daily gain (ADG), specific growth rate (SGR) and survival rate (SR) were calculated by using the formulae as applied in Umey *et al.* (2019).

$$WG = Final\ weight - Initial\ weight \tag{1}$$

$$ADG = \frac{Weightgain}{Culturing\ days} \tag{2}$$

$$SGR = \frac{\ln(Final\ weight) - In(Initial\ weight)}{Culturing\ days} \times 100\ \% \tag{3}$$

$$SR = \frac{Number\ of\ fish\ at\ the\ end\ of\ the\ experiment}{Number\ of\ fish\ at\ the\ start\ of\ the\ experiment} \times 100\ \% \tag{4}$$

3.8 Analysis Fluoride Bioaccumulation

At the end of experiment (under this study referring to after 20th, 40th and 60 days), one Cat fish, each from treatment types, thus making a total of three samples was randomly selected and dissected to obtain tissues for bones, skin, gills and flesh or fillets. The samples were placed in the cooler to be transported to the NM–AIST Laboratory for experimental analysis. All sample tissues were stored at -80 °C until Fluoride analyses were done. Prior experimental analysis, samples were then thawed, dried for 6 hours at 105 °C and ground into powders for fluoride concentration determination (Shi *et al.,* 2009). Fluoride content from each fish species and in respective treatment was determined by using fluoride specific electrode method (Gonzalo & Camargo, 2012).

3.9 Data Analysis

Statistical analysis for the differences in growth performance and survival rates of *Clarias gariepinus* reared at different fluoride levels were done using R statistical Package (version 3.6.1) program. The data were presented as mean ± SE (Standard error of the mean) in tabular and graphical formats. Before any statistical analysis was carried out, a Shapiro test was used to understand the distribution normality of the data at a significance of 0.05. Differences in water quality parameters were checked using the Kruskal Wallis test. Similarly, Kruskal Wallis test was used to test whether there were any interferences of growth in catfish caused by high fluoride concentrations in waters. Three-way Analysis of Variance (ANOVA) was used to detect whether there was any significant difference in mean fluoride concentration between tissue, time and treatments. All results were considered statistically significant at $p<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Water Quality Conditions

The water quality parameters recorded from both experimental aquaria and fishpond are presented in Table 2 using means and standard deviations. Also, Figure 8 and Figure 9 graphically portrays the status of water quality parameters. Generally, the waters quality parameters recorded during the experiment were found to be within the recommended range for the *Clarias gariepinus* rearing. In all aquaria treatments and fish pond, water temperatures varied from 17.59 ^{o}C to 27.28 ^{o}C. No significant difference in mean temperature was observed between treatments and fish pond (F=0.051, p-value=0.995). Dissolved oxygen varied from 6.893 to 7.759 mg/L. No significant difference in DO was observed between aquaria treatments. There was significant lower DO in fish pond as compared to aquaria treatments (Kruskal-Wallis chi-squared = 36.1, df = 4, (p = 2.76 x 10^{-07}). Lower dissolved Oxygen (DO) in the fish pond is likely to be a result of bio-decomposition of increased debris together with feed remnants due to less frequency of water exchange in the pond as compared to aquaria treatments. Similarly, pH in aquaria treatments did not differ significantly (p >0.05) but all were lower as compared to that in fishpond water (Kruskal-Wallis chi-squared = 15.656, df = 4, p-value = 0.003517). Nitrite and ammonia in all treatments varied from 0.1756 to 0.3928 mg/L with median value of 0.2206 and 0.0008 to 0.0056 mg/L with median value of 0.0046 respectively. However, their values were relatively higher in ponds as compared to that in experimental aquaria (*p* >0.05). This might have been caused by increased accumulation of fish feed and debris resulted from less water exchange frequency in the fish pond.

Table 2: Determined water quality parameters at different fluoride levels in aquaria treatments and fish pond during the entire period of the experiment

Parameters	Fluoride levels in Treatments (mg/L)				
	F5EX	**F15EX**	**F36EX**	**F36POND**	**F45EX**
Temperature	24.65 ±0.202	24.74 ±0.129	24.75±0.024	24.68± 2.108	24.67±0.034
DO	7.020 ±0.044	7.550±0.183	7.200±0.041	5.66± 0.042	7.6607±0.051
pH	8.02±0.082	7.92±0.08	8.01±0.09	8.03± 0.031	8.03±0.079
Ammonia	0.004±0.001	0.003±0.004	0.003±0.0114	0.005±0.0114	0.002±0.002
Nitrite	0.215±0.049	0.351±0.052	0.343±0.05	0.349±0.05	0.222±0.01

Treatment abbreviated F5EX, F15EX, F36EX, F45EX, F36POND stands for 5,15,36, 45 mg F^-/L aquaria and 36 mg F^-/L fish pond respectively, whereas DO: Dissolved Oxygen, pH: Potential of Hydrogen (measure of hydrogen ions concentration in the substance)

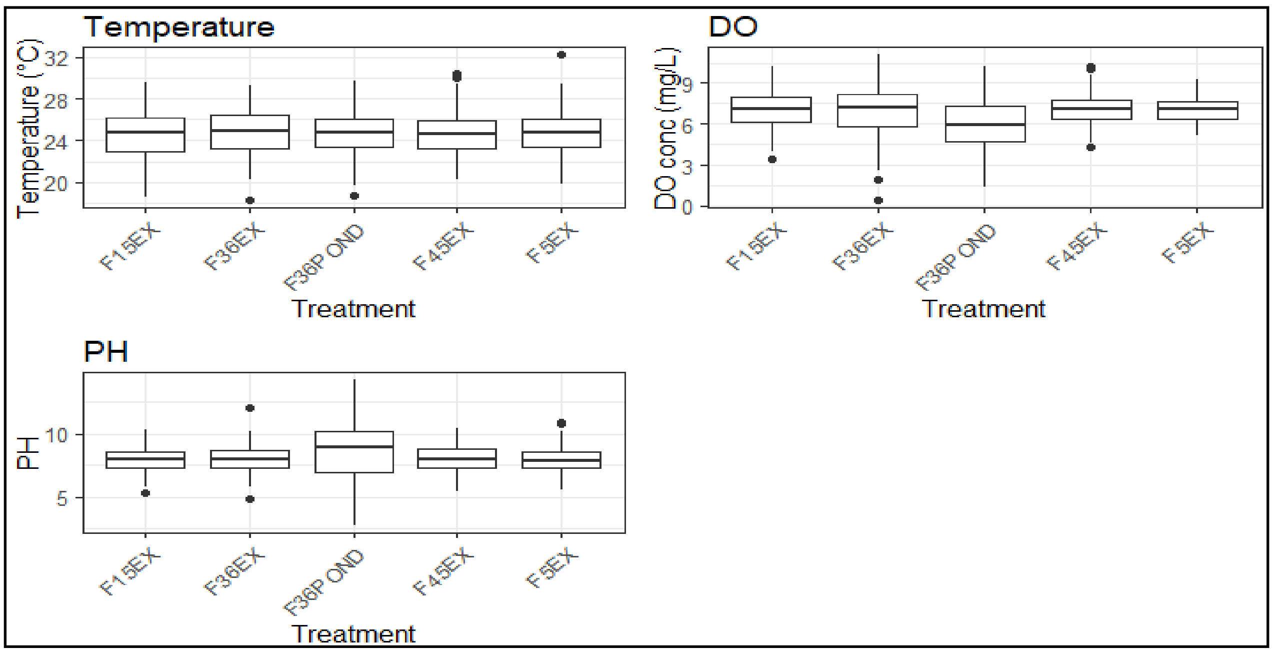


Figure 8: Mean Temperature, Dissolved Oxygen and pH values recorded in the experimental aquaria and fish pond

The abbreviations "F5EX","F15EX","F36EX", and "F45EX" represent aquaria with 5 mg F^-/L, 15 mg F^-/L, 36 mg F^-/L and 45 mg F^-/L respectively while "F36POND" represents 36 mg F^-/L in the fishpond.

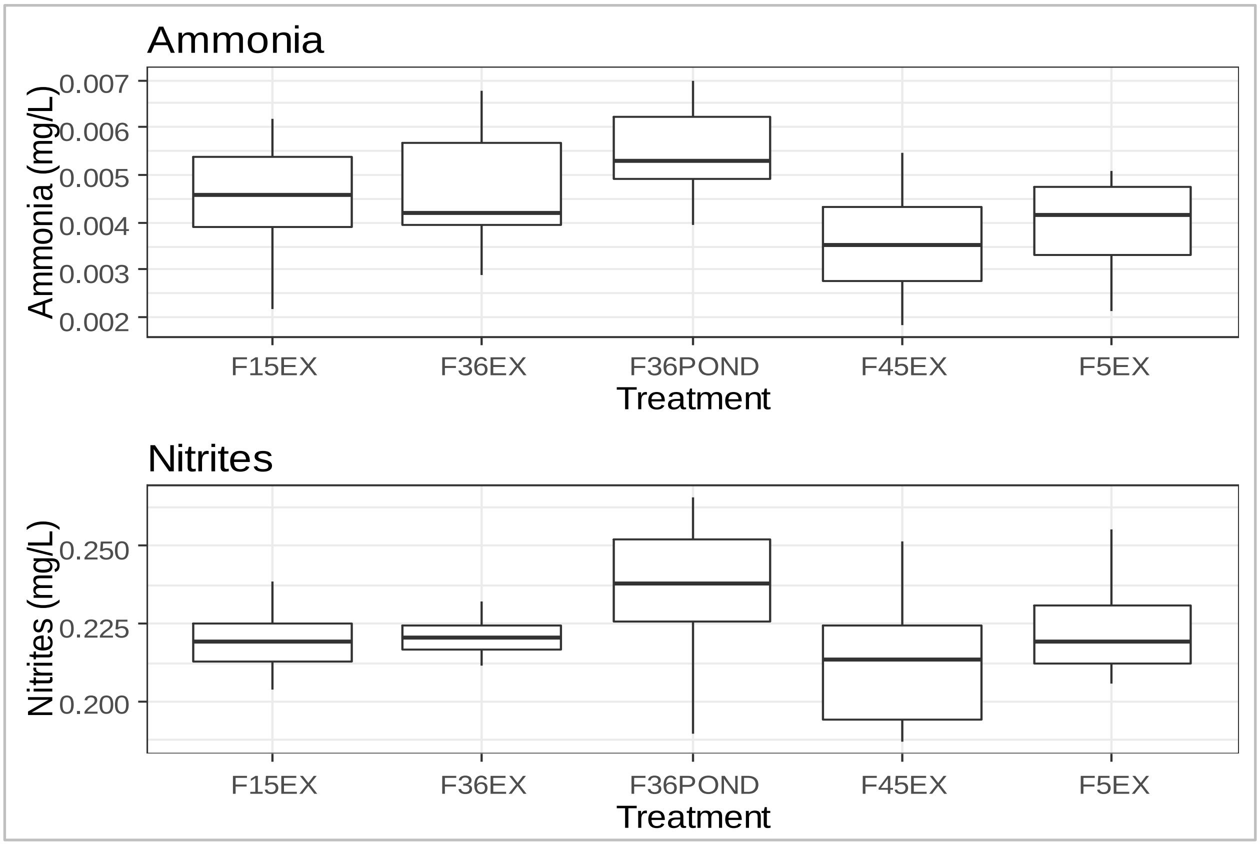


Figure 9: Ammonia and Nitrites concentrations recorded in the experimental aquaria and fish pond

The abbreviations "F5EX","F15EX","F36EX", and "F45EX" represent aquaria with 5 mg F^-/L, 15 mg F^-/L, 36 mg F^-/L and 45 mg F^-/L respectively while "F36POND" represents 36 mg F^-/L in the fishpond

A similar observation of the water temperature recorded during this study was reported by Umaru *et al.* (2016) that recorded water temperature of 20.0 to 33.7 °C, pH of 6.88 to 8.51 and the DO of 4.2 to 7.76 mg/L. Previous researchers opined that in order to prevent the risk of reduced growth and feed intake in African catfish culture, the water ammonia should not exceed 0.34 mg/L (Schram *et al.*, 2010) while that of nitrite should be less than 0.6 mg/L (Roques *et al.*, 2013). In the present study, Ammonia and nitrite concentrations were observed to be very low, despite their differences among treatments.

4.2 Growth Performance of Catfish Reared at Different Fluoride Levels

Growth performance parameters of catfish at different fluoride levels are shown in Table 3. At the beginning of 60 culturing days of the experiment, catfish weight and length were not significantly different from each other in all treatments (p>0.05). Albeit a significant difference was observed among treatments at the end of the experiment (p<0.05) (Table 3).

Table 3: The growth performance of catfish at different fluoride levels

Growth Parameters	Treatments				
	F5EX	**F15EX**	**F36EX**	**F36POND**	**F45EX**
Initial mean body weight (g)	19.92	19.89	19.88	19.87	19.9
Final mean body weight @20 days (g)	53.98	44.06	43.59	43.52	38.06
Final mean body weight @ 40 days(g)	79.99	64.03	59.05	59	50.77
Final mean body weight @ 60 days(g)	93.6	76.8	69.89	70	57.23
WG @ 20 days (g)	34.06	24.17	23.71	23.65	18.16
WG @ 40 days (g)	26.01	19.97	15.46	15.48	12.71
WG @ 60 days (g)	13.61	12.77	10.84	11	6.46
ADG @ 20 days (g)	1.703	1.2085	1.1855	1.1825	0.908
ADG @ 40 days (g)	1.3005	0.9985	0.773	0.774	0.6355
ADG @ 60 days (g)	0.6805	0.6385	0.542	0.55	0.323
SGR @ 20 days (%/day)	0.0498	0.0398	0.0393	0.0392	0.0324
SGR @ 40 days (%/day)	0.0197	0.0187	0.0152	0.0152	0.0144
SGR @ 60 days (%/day)	0.0079	0.0091	0.0084	0.0085	0.006
Mean initial length (cm)	16.05	16.06	16.05	16.05	16.05
Mean final length/fish (cm)	34.06	36.04	37.03	37.03	40.05
Length gain (cm)	18.01	19.98	20.98	20.98	24
Mean SR (%)	97.78	96.26	91.21	93.61	65.79

Treatment abbreviated F5EX, F15EX, F36EX, F45EX, F36POND stands for 5,15,36, 45 mg F^-/L aquaria and 36 mg F^-/L fish pond respectively, whereas WG: Weight gain, ADG: Average daily weight gain, SGR: Specific growth rate, SR: Survival rate. (Data source: Recorded data from study experiment)

The findings in the present study indicate that; WG, ADG and SGR decreas with increase in fluoride media concentrations (Fig. 10 a, b & c). These findings are supported by Agniwanshi *et al.* (2014) who also reported loss of body weight in freshwater catfish *Heteropneustes fossilis* with increase in water fluoride concentrations. Pairwise comparisons using Wilcoxon rank sum test indicated high ADG for catfish reared at fluoride level of 5 mg/L as compared to those reared at higher fluoride levels in aquaria and fish pond ($p \geq 0.0011$). In Figure 10 (b), While the 36 mg F^-/L fish pond and 36 mg F^-/L experimental aquarium showed no significant difference in terms of ADG, lower ADG values were recorded in catfish reared at 45 mg F^-/L aquarium. Specific growth rate (SGR) determined was found to be high in catfish reared in 5 mg F^-/L and 15 mg F^-/L aquaria as compared to those reared at 36 mg F^-/L and 45 mg F^-/L waters as depicted in Figure 10 (c). However, SGR was found to be lower in catfish grown at 45 mg F^-/L aquarium than 36 mg F^-/L aquarium and fish pond. These findings align with those reported by Shi *et al.* (2009) on Siberian sturgeon (*Acipenser baerii)* which showed better fish SGR at lower fluoride concentrations, such as 3.1 mg/L and 0.26 mg/L, than those in higher fluoride levels such as 7.8, 18.7, and 51.8 mg/L. Uo and Ie (2013) also observed that weight gain and specific growth rate decreased significantly as a function of both fluoride concentration and exposure time in freshwater common carp (*Cyprinus carpio*).

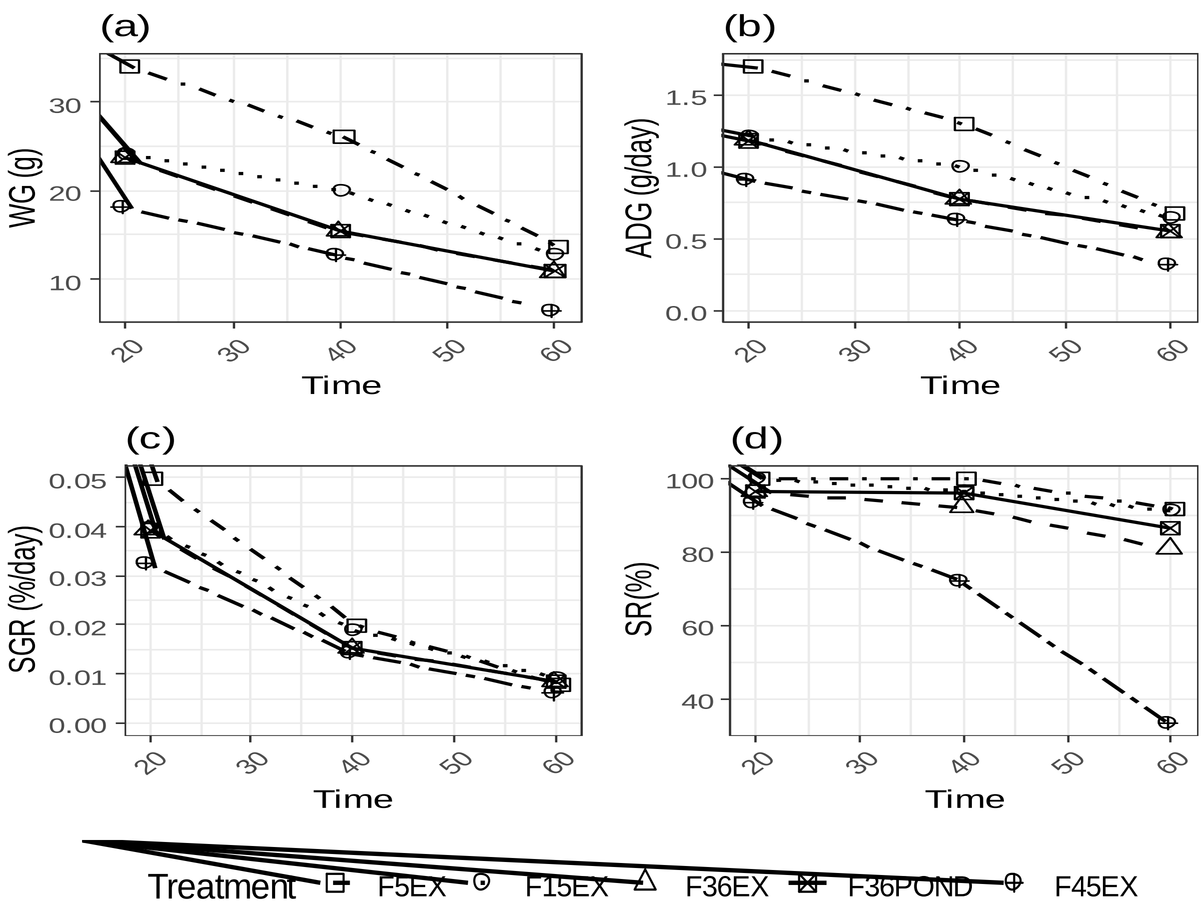


Figure 10: Growth performance of catfishes reared at different fluoride concentrations

Treatment abbreviated F5EX, F15EX, F36EX, F45EX, F36POND stands for 5,15,36, 45 mg F^-/L aquarium and 36 mg F^-/L fishpond respectively, whereas WG: Weight gain, ADG: Average daily weight gain, SGR: Specific growth rate, SR: Survival rate

The findings in the present study indicate that length and weight decrease as fluoride concentrations rise (Table 3 and Appendix 3). Other previous studies including Azmat *et al.* (2007), and Vishal and Gaur (2015) found negative relationship for length and weight against fluoride concentration in *Johnius belangerii* and *Puntius ticto fish*, respectively. Since all catfish in the present study were reared in optimum rearing conditions and given sufficient feed to support the survival, the observed differences in the overall ADG and SGR of the fish might have been influenced by increase in water fluoride concentrations.

While working on the effects of fluoride water on the growth performance of catfish (*Heteropneustes fossilis* and *Clarias batrachus*) Bajpai and Tripath (2010) observed that fish growth decreased as fluoride increased to 3.5 mg/L NaF. A review by Singh and Tripathi (2015) indicates that high fluoride concentrations affect the level of glucose, lipid, protein, cholesterol and glycogen, which play a great role in growth, reproduction and fish survival. Given the high fluoride concentrations in the study area, these findings may be of great economic implications as far as aquaculture practices are highly increasing in the study area. The experienced changes in fluoride concentrations due to geological changes resulted from climate stressors and other human activities particularly agriculture, houses and road construction may potentially affect catfish production, not only in the study area but also in other fluoride rich regions in the country.

4.3 Survival Rates of Catfish Reared at Different Fluoride Levels

At the end of 60 culturing days of experiment, treatments with 5 mg F^-/L and 15 mg F^-/L were found with high mean survival rate of 97.8 % and 96.3 % respectively. While in the 36 mg F^-/L aquarium treatment and fish pond there was a slight drop to 91.2 % and 93.6 % respectively (Figure 10 (d)). The results indicated a drop of fish mean survival rate (65.8 %) in the 45 mg F^-/L treatment. The average mean survival rate was found to be 97.3 % in the first 20 culturing days, significantly dropped to 91.4 % and 76.7 % at the end of 40^{th} and 60^{th} culturing days respectively. Similar findings were reported by Parker (2020) on inch rainbow trout (*Oncorhynchus mykiss*) fish that increased mortality rate within first 72 hours while exposed to 22 mg F^-/L. Since the environmental and dietary conditions were adequate throughout the experiment, the reduced fish mean survival rate might have been caused by the increased fluoride accumulation in catfish (Figure 11 (a) & (b)). Additionally, 53 % of fish mortalities that occurred during the last 20 culturing days of the experiment was recorded in the 45 mg F^-/L aquarium followed by 36 mg F^-/L aquarium (19.4 %), 36 mg F^-/L fish pond (13.8 %), 15 mg F^-/L (8.3 %) and very low at 5 mg/L (5.5 %) aquarium as seen in Figure 10 (d), and these fish mortalities might have been contributed by a significant increase of bioaccumulation trends with an increase of exposure time. Sharmin *et al.* (2013) reported on the similar findings where by catfish (*Clarias batrachus)* reared in 0, 15 and 30 mg F^-/L survived throughout the 120 culturing days while those being exposed to 50 mg F^-/L resulting into 50% mortality rate, and extremely serious with 100% mortality rate at 60 mg F^-/L. Of more important, Sharmin *et al.* (2013) revealed that catfish begun becoming weak, settling on the ground of the rearing containers and died as fluoride concentrations increased beyond 40 mg F^-/L. In addition, Wright, (1977) observed similar findings while rearing rainbow trout fish which recorded threshold of 50 % mortality under 8.5 mg/L fluoride exposure.

4.4 Fluoride Concentration in Different Fish Tissues

A Three-way ANOVA test indicated significant difference in fluoride concentration between tissue, time and treatment (Table 4 and 5). There was also an interaction between time and tissues. The highest concentration levels were found during the last 20 culturing days of the experiment in catfish reared at 45 mg F^-/L. There was a great fluoride bioaccumulation tendency in fish bones followed by gills, skin and very rare in fish flesh (Figure 11 (a)). Generally, the level of fluoride concentration in both catfish reared in aquaria treatments and fish ponds in different tissues increased with increase in fluoride media concentration and exposure time (Figure 11 (a) & (b) and Appendix 2). A multiple comparison of means indicated that the fish reared in pond with 36 mg F^-/L had significant higher fluoride bioaccumulation in all tissues compared to experimental aquaria in 5 mg F^-/L, 15 mg F^-/L and 36 mg F^-/L, except for 45 mg F^-/L aquarium (p-value<0.005). The multiple comparison test for difference in mean fluoride concentration between treatments, time and tissues are detailed in Appendix 1.

Table 4: Three-way ANOVA test for statistical differences in fluoride concentration levels among tissues of the catfish

Predictor	Sum of Squares	*df*	Mean Square	*F*	*p*	partial η^2	partial η^2 90% CI [LL, UL]
Time	83986.54	1	83986.54	39.24	.000	.66	
Treatment	71499.86	4	17874.97	8.35	.000	.63	[.28, .71]
Tissues	530007.19	3	176669.06	82.54	.000	.93	[.84, .94]
Time x Treatment	30251.50	4	7562.88	3.53	.025	.41	[.04, .53]
Time x Tissues	59686.70	3	19895.57	9.30	.000	.58	[.25, .68]
Treatment x Tissues	55949.24	12	4662.44	2.18	.060	.57	[.00, .54]
Time x Treatment x Tissues	20691.84	12	1724.32	0.81	.642	.33	[.00, .24]
Error	42807.04	20	2140.35				

Note. df, F, LL and UL represent the, degree of freedom, F-Statistics, lower-limit and upper-limit of the partial η^2 confidence interval, respectively

Table 5: Mean concentration (mg F/kg dry weight) in *Clarias gariepinus* exposed to waterborne fluoride for 60 culturing days

Treatments	Tissues			
	Bone	Gills	Skin	Fillet
F5EX	119	112	7.95	0.282
F15EX	156	115	9.62	0.411
F36EX	233	207	39.1	1.64
F36POND	234	208	40.6	2.14
F45EX	368	245	50.3	2.86
Mean (mg/L)	222.26	177.13	29.49	1.47

The abbreviations "F5EX","F15EX","F36EX", and "F45EX", respectively represent aquaria with 5 mg F^-/L, 15 mg F^-/L, 36 mg F^-/L and 45 mg F^-/L while "F36POND" represents 36 mg F^-/L in fish pond

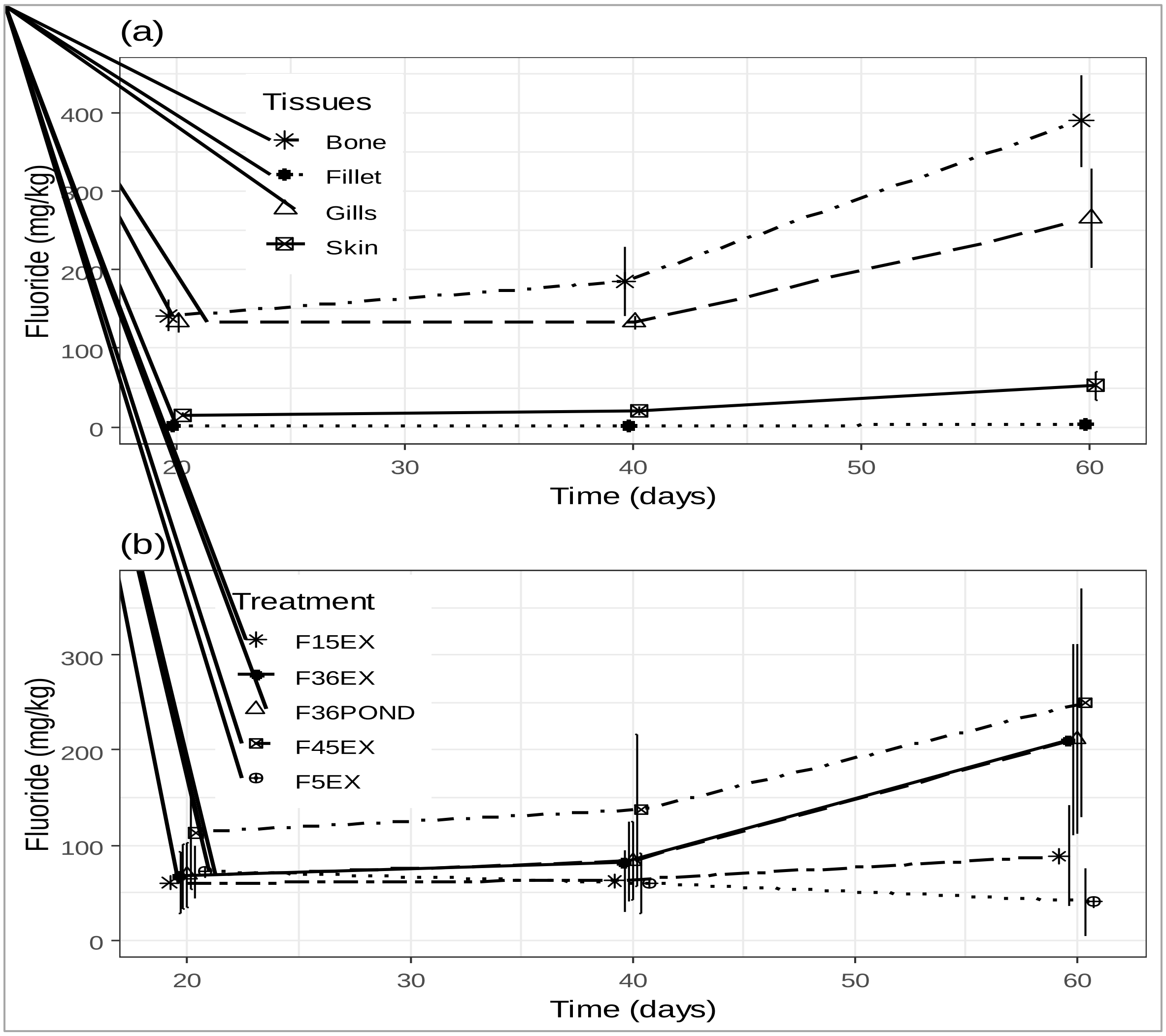


Figure 11: Fluoride bioaccumulation trends recorded during 60 culturing days of catfish exposed to different fluoride levels

The abbreviations "F5EX","F15EX","F36EX", and "F45EX", respectively represent aquaria with 5 mg F^-/L, 15 mg F^-/L, 36 mg F^-/L and 45 mg F^-/L while "F36POND" represents 36 mg F^-/L in pond

Other studies reported fluoride bioaccumulation in body tissues in other fish after exposure to waterborne Fluoride (Gikunju *et al*., 1991; Camargo, 2003; Shi *et al.*, 2009; Aguirre-Sierra *et al.*, 2013; James *et al.*, 2014). While studying the bioaccumulation of fluoride in 4 tilapia fishes (*Tilapia nilotica, T. zilii*, *T. leucost* and *Micropterus salmoides*) from lakes of great Rift Valley in Kenya, Gikunju *et al*. (1991) found high fluoride concentration ranging from 117 to 211 mg F/kg and from 1.3 to 3 mg F/kg in the fish bones and fillets respectively. Shi *et al.* (2009) found a maximum fluoride concentration of 3204.4 $mgkg^{-1}$ in bones, 1401.2 $mgkg^{-1}$ in cartilage, 389.4 $mgkg^{-1}$, 100.1 $mgkg^{-1}$ in skin, 389.4 $mgkg^{-1}$ in skin and 5.2 $mgkg^{-1}$ in muscles of the Siberian sturgeon (*Acipenser baerii*). El-said and Sadaawy (2013) also reported high fluoride contents in bones but less in flesh tissues of the Egyptian *Tilapia nilotica.* These findings also support results of the present study suggesting fluoride bioaccumulation resulted from aquatic medium. Previous studies reported high fluoride concentration up to 1103 mg/L (Mbabaye *et al.*, 2018), especially in the surface water sources which are also used for aquaculture. Therefore, the bioaccumulation of fluoride in the catfish found extreme levels of fluoride in the wildness is an important subject for future research.

In the present study, the fluoride bioaccumulation in fish fillets reared in 36 mg/L was found to be 2.14 $mgkg^{-1}$. Since the fillets are parts of the fish often consumed by humans, intake of 200 g fillet with 2.14 mg F/kg would provide 0.428 mg of fluoride, which is well below the maximum (1.5 mg) daily fluoride intake considered as safe by the WHO and/or 4.0 mg by the Tanzania Bureau of Standards (Mbabaye *et al.*, 2018). In order to exceed 4.0 mg dietary limit, ones need to consume over 800 g of fish fillet containing 5.54 mg $F^{-}kg^{-1}$ as in 45 mg/L reared fishes.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study found a significant fluoride bioaccumulation in catfishes reared in fluoride rich waters. The fluoride bioaccumulation trends increased with water media fluoride concentration and exposure time. Significant difference in mean fluoride bioaccumulation was revealed in *Clarias gariepinus* tissues, the bones being the portion of the fish with elevated amount of fluoride concentrations followed by gills and very minute in the skin and to the fish flesh. High fluoride bioaccumulation trends occurred at the last 20 culturing days as compared to first 20 culturing days of the experiment. The results revealed that catfish growth and survival rate reared in 5 up to 36 mg F^-/L waters were not highly affected as compared to catfishes grown above 45 mg F^-/L aquarium treatment waters. Noticeable growth effects such as reduced weight gain, reduced specific growth rate and survival rates that occurred to catfishes under the experiment increased with increase of fluoride concentrations. Based on the findings of the present study it was concluded that varying water fluoride levels in Ngarenanyuki area could potentially accelerate high fluoride bioaccumulation in African catfish and contribute to reduced growth and survival rate of catfish reared in the area.

These findings will certainly benefit aquaculture industry in the area by providing guiding information on optimal growth rates of catfish that could bring maximum profit. Again, this study will contribute to more information towards several interventions that is ongoing to address the challenges associated with high water fluoride occurrences in the area.

5.2 Recommendations

The present study suggests the following recommendations that would increase knowledge relevant for increasing catfish production in fluoride rich regions in Tanzania and/or elsewhere in the world:

(i) The present study consisted catfish rearing at the fish pond with +/-36 mg/L throughout the experiment. The baseline water quality data for the present study as referred from previous studies indicated lower fluoride concentration of 28.6 mg/L of the river stream feeding the fish pond. Thus, it is important to conduct a long-term water quality monitoring of at least 3 to 5 years to explore on the temporal fluoride level changes. This will contribute to increased understanding and knowledge of fluoride level concentration which might be used for agricultural and fishing activities adaption and thus result into high yield.

(ii) Fluoride occurrences highly documented in the region suggests geological rocks to be the source of high fluoride levels in waters. However, the observed farming activities with the use of pesticides might have contributed to the increased trends of fluoride in the area. It is thus very important to conduct a study and assess the fluoride ratio in waters contributed by the use of pesticides in the region.

(iii) The current study assessed the effect of fluorides on growth performance and survival rate of the catfish (*Clarias gariepinus*) for only 60 days. Further studies should be carried out to prolong the culture period until fishes reach their marketable size to confirm whether there is any depression or improvement on growth performance and survival rate of the fish.

(iv) The present study assessed fluoride bioaccumulation in fish reared up to maximum of fluoride water level concentration of to 45 mg/L only. Given the high variability in fluoride concentration among the water bodies in the study area, further efforts should be made to identify the maximum fluoride level for survival and growth of the catfish. It is recommended that deflouridation of fish pond should be conducted to keep fluoride level quite below 5mg/L for aquaculture profitability.

REFERENCES

Lang, R. P. A., & Tiersch, T. R. (2007). Early out-of-season induced spawning of channel catfish Ictalurus punctatus (Rafinesque) conditioned in heated earthen ponds. *Acquaculture Research*, 38(5), 498–507.

Addison, M. J., Rivett, M. O., Robinson, H., Fraser, A., Miller, A. M., Phiri, P., Mleta, P., & Kalin, R. M. (2020). Fluoride occurrence in the lower East African Rift System, Southern Malawi. *Science of the Total Environment*, 712(December 2019), 1-16.

Makori, J. A., Abuom, P. O., Kapiyo, R., Anyona, D. N., & Dida, G. O. (2017). Effects of water physico-chemical parameters on tilapia *(Oreochromis niloticus)* growth in earthen ponds in Teso. *Fisheries and Aquatic Sciences*, 20(30), 1–10.

Agniwanshi, S., Shedpure, M., & Jain, N. (2014). Effect of sodium fluoride on body weight gain and gonadosomatic index in freshwater catfishes. *Journal of Industrial Pollution Control*, 30(2), 339–343.

Aguirre-Sierra, A., Alonso, Á., & Camargo, J. A. (2013). Fluoride bioaccumulation and toxic effects on the survival and behavior of the endangered white-clawed crayfish Austropotamobius pallipes (Lereboullet). *Archives of Environmental Contamination and Toxicology*, *65*(2), 244–250.

Barbier, O., Arreola-Mendoza, L., & Del Razo, L. M. (2010). Molecular mechanisms of fluoride toxicity. *Chemico-Biological Interactions*, 188(2), 319–333.

Boyd, C. E. (1998). *Water quality and aquaculture: Preliminary considerations.* Kluwer Academic Publishers. https://link.springer.com

Boyd, C. E., & Thunjai, T. (2001). Pond soil pH measurement. *Journal of the World Aquaculture Society*, 32(2), 141–152.

Camargo, J. A. (2003). Fluoride toxicity to aquatic organisms: A review. *Chemosphere*, *50*(3), 251–264.

Carey, C. M., & Coleman, S. S. (2014). Method for the analysis of total fluoride in fluoride-releasing dental varnishes. *Caries Research*, *48*(4), 306–311.

Casellato, S., Masiero, L., & Ballarin, L. (2012). Toxicity of fluoride to the freshwater mollusc.

The International Society for Fluoride Research, 45(March), 35–46.

Chowdhury, C., Khijmatgar, S., Kumari, D., Chowdhury, A., Grootveld, M., Hegde, C., & Lynch, E. (2018). Fluoride in fish flesh, fish bone and regular diet in south-coastal area of Karnataka state of India. *Indian Journal of Dental Research*, 29(4), 414–417.

Christensen, B. (1987). Uptake and release of fluoride in arctic Char. *Combustion Science and Technology*, 25(5–6), 241–242.

Dalgleish, T., Williams, J. M. G. ., Golden, A. M. J., Perkins, N., Barrett, L. F., Barnard, P. J., Yeung, C., Murphy, V., Elward, R., Tchanturia, K., & Watkins, E. (2007). Fluoride assessment in Tilapia fish species. *Journal of Experimental Psychology*, 136(1), 23–42.

Demelash, H., Beyene, A., Abebe, Z., & Melese, A. (2019). Fluoride concentration in ground water and prevalence of dental fluorosis in Ethiopian Rift Valley: Systematic review and meta-analysis. *Public Health*, 19(1), 1–9.

Durborow, R. M., Crosby, D. M., & Brunson, M. W. (1997). Nitrite in fishponds. *Southern Regional Aquaculture Center*, 462, 1-4.

Johansen, E. S. (2013). *The effects of fluoride on human health in Eastern Rift Valley, Northern Tanzania.* 35. http://www.duo.uio.no/

El-said, G. F., & Sallam, N. A. (2008). The uptake of fluoride concentration and its effects on the growth rate of shrimps (*Palaemon elegans*, Rathke). *Chemistry and Ecology*, 24(3), 191–205.

El-said, G. F., & El-sadaawy, M. M. (2013). Seasonal variation of boron and fluoride in tilapia nilotica from an Egyptian fish farm in relation to human health hazard assessment. *Human and Ecological Risk Assessment: An International Journal*, 12(46), 37–41.

El-Sayed, A. F. M., Mansour, C. R., & Ezzat, A. A. (2003). Effects of dietary protein level on spawning performance of Nile tilapia (Oreochromis niloticus) broodstock reared at different water salinities. *Aquaculture*, 220(1–4), 619–632.

El-Sayed, A. F. M. (2004). Protein nutrition of farmed tilapia: Searching for unconditional sources. *International Symposium of Tilapia in Aquaculture*, 6, 364–378.

El-Sayed, A. F. M. (2006). *Tilapia culture.* Publishing, Oxford, UK. https://www.google.com

Fabrice, T. (2019). F*ish domestication: An overview: Animal Domestication*. https://www.google.com

FAO, & ITPS. (2018). Status of the world's soil resources (SWSR) – Main report. *Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils, Rome, Italy*. https://www.google.com

Fitzsimmons, K. M. (2010). *Tilapia: The most important aquaculture species of the 21st century*. https://www.google.com

Vishal, R., & Gaur, R. (2015). Impact of high sodium fluoride concentration on length- weight relationship and condition factor in *Puntius ticto* of Lake Nainital, India. *Journal of Global Biosciences*, 4(1), 1180–1185.

Ghiglieri, G., Balia, R., Oggiano, G., & Pittalis, D. (2010). Prospecting for safe (low fluoride) groundwater in the Eastern African Rift: The Arumeru District (Northern Tanzania). *Hydrology and Earth System Sciences*, 14(6), 1081–1091.

Gikunju, J. K., Maitho, T. E., Birkeland, J. M., & Lökken, P. (1991). Fluoride in fish from lakes of great Rift Valley, Kenya. *Ecology of Food and Nutrition*, *0244*(June), 85–90.

Gonzalo, C., & Camargo, J. A. (2012). Fluoride bioaccumulation in the signal crayfish *Pacifastacus leniusculus* (Dana) as suitable bioindicator of fluoride pollution in freshwater ecosystems. *Ecological Indicators*, 20, 244–251.

Gupta, S., & Poddar, A. (2014). Sodium fluoride toxicity in the fresh water cat fish *Clarias batrachus* (Linn.): Effects on the erythrocyte morphology and antioxidant enzymes. *Research Journal of Environmental Toxicology*, (8), 68-76.

Hossain, M. Y., Ahmed, Z. F., Leunda, P. M., Jasmine, S., Oscoz, J., Miranda, R., & Ohtomi, J. (2006). Condition, length-weight and length-length relationships of the Asian striped catfish *Mystus vittatus* (Bloch, 1794) (Siluriformes: Bagridae) in the Mathabhanga River, southwestern Bangladesh. *Journal of Applied Ichthyology*, 22(4), 304–307.

Jagtap, S., Yenkie, M. K., Labhsetwar, N., & Rayalu, S. (2012). Fluoride in drinking water and defluoridation of water. *Chemical Reviews*, 112(4), 2454–2466.

James, R., & Thomas, A. (2014). Accumulation of fluoride from natural water sources in *Oreochromis mossambicus* (Peters, 1852). *Scholars Academic Journal of Biosciences,* 2(9), 659–662.

Jha, A. N. (2004). Genotoxicological studies in aquatic organisms: An overview. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, 552(1–2), 1–17.

Jin, Y., Liu, S., Yuan, Z., Yang, Y., Tan, S., & Liu, Z. (2016). *Catfish genomic studies: Progress and perspectives*. Elsevier Inc. https://www.google.com

Armbruster, J. W. (2011). Global catfish biodiversity. *American Fisheries Society Symposium*, 77, 15–37.

Kaur, R., Saxena, A., & Batra, M. (2017). A review study on fluoride toxicity in water and fishes: Current status, toxicology and remedial measures. *International Journal of Environment, Agriculture and Biotechnology*, 2(1), 456–466.

Kimambo, V., Bhattacharya, P., Mtalo, F., Mtamba, J., & Ahmad, A. (2019). Fluoride occurrence in groundwater systems at global scale and status of defluoridation – State of the art. *Groundwater for Sustainable Development*, *9*(August 2018), 1-55.

Kitalika, A. J., Machunda, R. L., Komakech, H. C., & Njau, K. N. (2018). Fluoride variations in rivers on the slopes of mount meru in Tanzania. *Journal of Chemistry*, 2018, 1-19.

Kut, K. M. K., Sarswat, A., Srivastava, A., Pittman, C. U., & Mohan, D. (2016). A review of fluoride in african groundwater and local remediation methods. *Groundwater for Sustainable Development*, 2–3, 190–212.

Latham, M. C., & Grech, P. (1967). The effects of excessive fluoride intake. *American Journal of Public Health and the Nation's Health*, 57(4), 651–660.

Liu, Z., Li, R. W., & Waldbieser, G. C. (2008). Utilization of microarray technology for functional genomics in ictalurid catfish. *Journal of Fish Biology*, 72(9), 2377–2390.

Liu, Zhanjiang. (2011). Development of genomic resources in support of sequencing, assembly, and annotation of the catfish genome. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, 6(1), 11–17.

Maity, J. P., Hsu, C. M., Lin, T. J., Lee, W. C., Bhattacharya, P., Bundschuh, J., & Chen, C. Y. (2018). Removal of fluoride from water through bacterial-surfactin mediated novel hydroxyapatite nanoparticle and its efficiency assessment: Adsorption isotherm, adsorption kinetic and adsorption Thermodynamics. *Environmental Nanotechnology, Monitoring and Management*, 9(November 2017), 18–28.

Malago, J., Makoba, E., & Muzuka, A. N. N. (2017). Fluoride Levels in Surface and Groundwater in Africa: A Review. *American Journal of Water Science and Engineering*, 3(1), 1-17.

Mallya, Y. J., & Thorarensen, H. (2007). The effects of dissolved oxygen on fish growth in aquaculture: Final Project Report. *The United Nations University Fisheries Training Programme,* 2007 (December), 1-30.

Mbabaye, G. K., Minja, R. J. A., Mtalo, F., Legonda, I., & Mkongo, G. (2018). Fluoride occurrence in domestic water supply sources in Tanzania: A case of meru district Arusha region. *Tanzania Journal of Science*, *44*(3), 72–92.

Mohan, D., Sharma, R., Singh, V. K., Steele, P., & Pittman, C. U. (2012). Fluoride removal from water using bio-char, a green waste, low-cost adsorbent: Equilibrium uptake and sorption dynamics modeling. *Industrial and Engineering Chemistry Research*, 51(2), 900–914.

Mondal, K., & Nath, S. (2015). Fluoride contamination on aquatic organisms and human body at Purulia and Bankura District of West Bengal, India. *Bulletin of Environment, Pharmacology and Life Sciences*, 4(June), 112–114.

Ogunji, J. O., & Awoke, J. (2017). Effect of environmental regulated water temperature variations on survival, growth performance and haematology of African catfish, *Clarias gariepinus*. *Nature*, 15(1-2), 26–33.

Olurin, K. B., & Aderibigbe, O. A. (2006). Length-weight relationship and condition factor of pond reared juvenile *Oreochromis niloticus*. *World Journal of Zoology*, 1(2), 82–85.

Masawe, K., Mtei, K. M., & Machunda, R. (2019). Distribution of fluoride in beans and maize grown along the slope of Mount Meru, Tanzania. *Journal of Biodiversity and Environmental Sciences*, 15(5), 29–36.

Ozsvath, D. L. (2009). Fluoride and environmental health: A review. *Reviews in Environmental Science and Bio/Technology, 8*, 59–79.

Parker. (2020). *Fluoride regulatory framework and aquatic toxicity: Review Phase I.* https://www.google.com

Parsons, T. R., & Strickland, J. D. H. (1972). A practical handbook of seawater analysis. Second Edition. https://www.google.com

Lim, C., & Webster, C. D. (2007). Tilapia—Biology, Culture and nutrition. A*quaculture International, 123*, 169–170.

Azmat, R., Talat, R., & Ahmed, K. (2007). The length-weight relationship, condition factor and impact of fluoride concentration in *Johnius belangerii* of Arabian Sea. *Research Journal of Environmental Technology*, 1 (3), 138–143.

Rizzu, M., Tanda, A., Canu, L., Masawe, K., Mtei, K., Deroma, M. A., Roggero, P. P., & Seddaiu, G. (2020). Fluoride uptake and translocation in food crops grown in fluoride-rich soils. *Journal of the Science of Food and Agriculture*, *100*(15), 5498–5509.

Roques, J. A. C., Schram, E., Spanings, T., Schaik, T. Van, & Abbink, W. (2013). The impact of elevated water nitrite concentration on physiology, growth and feed intake of African catfish *Clarias gariepinus* (Burchell 1822). *Aquaculture Research*, *2*, 1–12.

Roy, S., & Dass, G. (2013a). Fluoride contamination in drinking water-A review. *Resources and Environment*, *3*(3), 53–58.

Samal, P., Jena, D., Sahoo, D., & Behera, S. (2016). Fluorosis: An epidemic hazard and its consequences on livestock population. *Indian Farmer*, 3(3), 180-184.

Bajpai, S., & Tripathi, M. (2010). Effect of fluoride on growth bioindicators in stinging. *Research Report*, 43(December), 232–236.

Santhosh, S. B. (2017). Guidelines for water quality management for fish culture in Tripura. *ICAR Research Complex*, 27 (October), 210-990.

Schram, E., Roques, J. A. C., Abbink, W., Spanings, T., Vries, P. De, Bierman, S., Vis, H. Van De, & Flik, G. (2010). The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (*Clarias gariepinus*). *Aquaculture*, 306(1–4), 108–115.

Setiadi, E., Taufik, I., Widyastuti, Y. R., Ardi, I., & Puspaningsih, D. (2019). Improving productivity and water quality of catfish, *Clarias* sp. cultured in an aquaponic ebb-tide system using different filtration. *Fisheries and Marine Science*, 236(2019), 1-12.

Sharmin, S. S., Billah, B., Ahmed, K., & Salam, A. (2013). Toxicity of Sodium Fluoride (NaF) to Juvenile Catfish, *Clarias Batrachus*. *Dhaka Univerisity Jounal of Biological Sciences*, 22(1), 1–7.

Shi, X., Zhuang, P., Zhang, L., Feng, G., Chen, L., Liu, J., Qu, L., & Wang, R. (2009). The bioaccumulation of fluoride ion (F^-) in Siberian sturgeon (*Acipenser baerii*) under laboratory conditions. *Chemosphere*, 75(3), 376–380.

Shingadia, H. U. (2014). Chronic revelation to sodium fluoride induces pathohistological alterations in intestine of *Poecilia reticulata*. *Bionano Frontier*, 7(2), 293–296.

Singh, N., & Tripathi, M. (2015). Fluoride toxicity in freshwater fishes and aquaculture: A review. *Indian Journal of Life Science*, 4(2), 115–124.

Singh, R., Hussain, M. A., Kumar, J., Kumar, M., Kumari, U., & Mazumder, S. (2017). Chronic fluoride exposure exacerbates headkidney pathology and causes immune commotion in *Clarias gariepinus*. *Aquatic Toxicology*, 192, 30–39.

Subasinghe, R., Soto, D., & Jia, J. (2009). Global aquaculture and its role in sustainable development. *Reviews in Aquaculture*, 1(1), 2–9.

Thole, B., Masamba, W. R. L., & Mtalo, F. W. (2013). Water defluoridation by bauxite-gypsum magnesite (B-G-Mc) based filters calcined at 350 500C. *International Journal of Physical Sciences*, 8(19), 956–962.

Tomasso, J. R. (1994). Toxicity of Nitrogenous Wastes to Aquaculture Animals. *Reviews in Fisheries Science*, 2(4), 291-314.

Tucker, C. S., & Robinson, E. H. (1991). Channel catfish farming handbook. *Springer Science & Business Media*, 1st Edition, New York, USA. https://www.google.com/

Umaru, J., Auta, J., Oniye, S. J., & Bolorunduro, P. I. (2016). Growth and economic performance of African catfish, *Clarias gariepinus* (Burchell, 1822) Juveniles to imported and local feeds in floating bamboo cages. *International Journal of Fisheries and Aquatic Studies*, 4(2), 221–226.

Umey, S., Mat, S., Fui, N., & Ching, F. (2019). Comparison on growth performance, body coloration changes and stress response of juvenile river catfish, *Pangasius hypophthalmus* reared in different tank background colour. *Aquaculture Research, May*, 2591–2599.

Uo, Y. O. L., & Ie, L. I. X. (2013). Effects of fluoride on growth, body composition, and serum biochemical profile in a freshwater teleost , *Cyprinus carpio*. *Environmental Toxicology and Chemistry*, 32(10), 2315–2321.

Britz, P. J., & Hecht, T. (1987). Temperature preferences and optimum temperature for growth of African sharptooth catfish (*Clarias gariepinus*) larvae and post- larvae. *Aquaculture,* 63, 205–214.

Wanja, D. W., Mbuthia, P. G., Waruiru, R. M., Mwadime, J. M., Bebora, L. C., Nyaga, P. N., & Ngowi, H. A. (2020). Fish husbandry practices and water quality in central Kenya: Potential risk factors for fish mortality and infectious diseases. *Veterinary Medicine International*, 2020, (1-10).

Wright, D. A. (1977). Toxicity of fluoride to brown trout (*Salmo trutta*). *Eradication Iron Pollution*, 1972, 57–62.

APPENDICES

Appendix 1: Multiple comparison test for difference in mean fluoride concentrations between treatments, time and tissues

These table show detailed information of multiple comparison of fluoride accumulated values (mg/kg) between tissues, treatments and exposure time. Of more important differences between tissues, treatments and exposure time were recorded. Lower and upper fluoride accumulated values between the compared tissues, treatments and parameters were also recorded as seen in the table. Along every comparison, p values were recorded. Generally, the multiple comparison was conducted for five categories including exposure time against treatment interactions, exposure time against tissues interactions, tissues, exposure times and treatments.

Between treatments

	diff	lwr	upr	p.adj
F36EX-F15EX	49.908723	-23.42017369	123.2376197	0.317329
F36POND-F15EX	50.76492684	-22.56396986	124.0938235	0.300774
F45EX-F15EX	96.29295462	22.96405793	169.6218513	0.004458
F5EX-F15EX	-10.45154186	-83.78043855	62.87735483	0.994252
F36POND-F36EX	0.856203831	-72.47269286	74.18510052	1
F45EX-F36EX	46.38423161	-26.94466508	119.7131283	0.390692
F5EX-F36EX	-60.36026487	-133.6891616	12.96863183	0.152741
F45EX-F36POND	45.52802778	-27.80086891	118.8569245	0.409659
F5EX-F36POND	-61.2164687	-134.5453654	12.11242799	0.142843
F5EX-F45EX	-106.7444965	-180.0733932	-33.41559979	0.001292

Between Time

Days	diff	lwr	upr	p.adj
40-20	6.635705866	-41.26633028	54.53774202	0.940227
60-20	85.52575589	36.98235057	134.0691612	0.000266
60-40	78.89005002	29.7735744	128.0065256	0.000881

Between tissues

Tissues	diff	lwr	upr	p.adj
Fillet-Bone	-226.5429273	-288.1386711	-164.9471836	0
Gills-Bone	-50.8715761	-112.4673199	10.72416766	0.138686
Skin-Bone	-198.5113789	-260.1071226	-136.9156351	1.30E-10
Gills-Fillet	175.6713512	114.0756075	237.267095	4.45E-09
Skin-Fillet	28.03154847	-33.56419529	89.62729224	0.623841
Skin-Gills	-147.6398028	-209.2355465	-86.044059	3.44E-07

Time x tissues interaction

	diff	lwr	upr	p.adj

40 Days:Bone-20 Days:Bone	43.76315	-84.3161	171.8424	0.98866859
60 Days:Bone-20 Days:Bone	247.076	110.5431	383.6089	7.27E-06
20 Days:Fillet-20 Days:Bone	-141.16	-269.24	-13.0811	0.019505078
40 Days:Fillet-20 Days:Bone	-140.572	-268.652	-12.4931	0.020383687
60 Days:Fillet-20 Days:Bone	-139.252	-267.331	-11.1727	0.022490945
20 Days:Gills-20 Days:Bone	-8.89175	-136.971	119.1875	0.999999999
40 Days:Gills-20 Days:Bone	-9.05261	-137.132	119.0266	0.999999998
60 Days:Gills-20 Days:Bone	123.9738	-4.10537	252.053	0.065958432
20 Days:Skin-20 Days:Bone	-126.185	-254.265	1.893762	0.056882544
40 Days:Skin-20 Days:Bone	-121.583	-249.662	6.496562	0.077157387
60 Days:Skin-20 Days:Bone	-89.1218	-217.201	38.95736	0.432421915
60 Days:Bone-40 Days:Bone	203.3129	61.42373	345.202	0.000595208
20 Days:Fillet-40 Days:Bone	-184.923	-318.698	-51.1491	0.001047483
40 Days:Fillet-40 Days:Bone	-184.335	-318.11	-50.5611	0.001099788
60 Days:Fillet-40 Days:Bone	-183.015	-316.789	-49.2407	0.001226636
20 Days:Gills-40 Days:Bone	-52.6549	-186.429	81.11947	0.967117965
40 Days:Gills-40 Days:Bone	-52.8158	-186.59	80.95861	0.966397589
60 Days:Gills-40 Days:Bone	80.21069	-53.5637	213.9851	0.652581673
20 Days:Skin-40 Days:Bone	-169.949	-303.723	-36.1742	0.003535595
40 Days:Skin-40 Days:Bone	-165.346	-299.12	-31.5714	0.00507977
60 Days:Skin-40 Days:Bone	-132.885	-266.659	0.889377	0.052998222
20 Days:Fillet-60 Days:Bone	-388.236	-530.126	-246.347	1.19E-10
40 Days:Fillet-60 Days:Bone	-387.648	-529.537	-245.759	1.25E-10
60 Days:Fillet-60 Days:Bone	-386.328	-528.217	-244.439	1.39E-10
20 Days:Gills-60 Days:Bone	-255.968	-397.857	-114.079	7.78E-06
40 Days:Gills-60 Days:Bone	-256.129	-398.018	-114.239	7.67E-06
60 Days:Gills-60 Days:Bone	-123.102	-264.991	18.78696	0.146801214
20 Days:Skin-60 Days:Bone	-373.261	-515.151	-231.372	4.08E-10
40 Days:Skin-60 Days:Bone	-368.659	-510.548	-226.77	5.96E-10
60 Days:Skin-60 Days:Bone	-336.198	-478.087	-194.309	8.82E-09
40 Days:Fillet-20 Days:Fillet	0.588012	-133.186	134.3624	1
60 Days:Fillet-20 Days:Fillet	1.908415	-131.866	135.6828	1
20 Days:Gills-20 Days:Fillet	132.2686	-1.50578	266.043	0.055168025
40 Days:Gills-20 Days:Fillet	132.1077	-1.66664	265.8821	0.055746956
60 Days:Gills-20 Days:Fillet	265.1342	131.3598	398.9085	9.17E-07
20 Days:Skin-20 Days:Fillet	14.97489	-118.799	148.7493	0.999999769
40 Days:Skin-20 Days:Fillet	19.57769	-114.197	153.3521	0.999996146
60 Days:Skin-20 Days:Fillet	52.03849	-81.7359	185.8129	0.969773351
60 Days:Fillet-40 Days:Fillet	1.320403	-132.454	135.0948	1
20 Days:Gills-40 Days:Fillet	131.6806	-2.09379	265.4549	0.057310169
40 Days:Gills-40 Days:Fillet	131.5197	-2.25466	265.2941	0.057908742
60 Days:Gills-40 Days:Fillet	264.5462	130.7718	398.3205	9.67E-07
20 Days:Skin-40 Days:Fillet	14.38688	-119.387	148.1612	0.999999849
40 Days:Skin-40 Days:Fillet	18.98968	-114.785	152.764	0.999997192
60 Days:Skin-40 Days:Fillet	51.45048	-82.3239	185.2248	0.97215493

20 Days:Gills-60 Days:Fillet	130.3602	-3.4142	264.1345	0.062387394
40 Days:Gills-60 Days:Fillet	130.1993	-3.57506	263.9737	0.063031953
60 Days:Gills-60 Days:Fillet	263.2258	129.4514	397.0001	1.09E-06
20 Days:Skin-60 Days:Fillet	13.06648	-120.708	146.8408	0.999999946
40 Days:Skin-60 Days:Fillet	17.66928	-116.105	151.4436	0.999998676
60 Days:Skin-60 Days:Fillet	50.13008	-83.6443	183.9044	0.976989127
40 Days:Gills-20 Days:Gills	-0.16086	-133.935	133.6135	1
60 Days:Gills-20 Days:Gills	132.8656	-0.90878	266.64	0.053065365
20 Days:Skin-20 Days:Gills	-117.294	-251.068	16.48067	0.137040826
40 Days:Skin-20 Days:Gills	-112.691	-246.465	21.08347	0.176298951
60 Days:Skin-20 Days:Gills	-80.2301	-214.004	53.54427	0.652251726
60 Days:Gills-40 Days:Gills	133.0264	-0.74792	266.8008	0.052511001
20 Days:Skin-40 Days:Gills	-117.133	-250.907	16.64154	0.138285381
40 Days:Skin-40 Days:Gills	-112.53	-246.304	21.24434	0.177811686
60 Days:Skin-40 Days:Gills	-80.0692	-213.844	53.70514	0.654985136
20 Days:Skin-60 Days:Gills	-250.159	-383.934	-116.385	3.52E-06
40 Days:Skin-60 Days:Gills	-245.556	-379.331	-111.782	5.33E-06
60 Days:Skin-60 Days:Gills	-213.096	-346.87	-79.3213	9.46E-05
40 Days:Skin-20 Days:Skin	4.6028	-129.172	138.3772	1
60 Days:Skin-20 Days:Skin	37.0636	-96.7108	170.838	0.998060593
60 Days:Skin-40 Days:Skin	32.4608	-101.314	166.2352	0.999419948

Time x treatment interaction

	diff	lwr	upr	p.adj
40 Days:F15EX-20 Days:F15EX	1.716632	-313.745	317.1783	1
60 Days:F15EX-20 Days:F15EX	28.02058	-287.441	343.4822	1
20 Days:F36EX-20 Days:F15EX	7.17522	-308.286	322.6369	1
40 Days:F36EX-20 Days:F15EX	22.11745	-293.344	337.5791	1
60 Days:F36EX-20 Days:F15EX	150.1707	-165.291	465.6324	0.91994318
20 Days:F36POND-20 Days:F15EX	7.990315	-307.471	323.452	1
40 Days:F36POND-20 Days:F15EX	22.88557	-292.576	338.3472	1
60 Days:F36POND-20 Days:F15EX	151.1561	-164.306	466.6177	0.916240198
20 Days:F45EX-20 Days:F15EX	52.77441	-262.687	368.236	0.99999872
40 Days:F45EX-20 Days:F15EX	76.5681	-238.894	392.0297	0.999876066
60 Days:F45EX-20 Days:F15EX	189.2736	-126.188	504.7352	0.695225541
20 Days:F5EX-20 Days:F15EX	11.36265	-287.911	310.6358	1
40 Days:F5EX-20 Days:F15EX	-0.50743	-315.969	314.9542	1
60 Days:F5EX-20 Days:F15EX	-20.4177	-361.155	320.3198	1
60 Days:F15EX-40 Days:F15EX	26.30395	-289.158	341.7656	1
20 Days:F36EX-40 Days:F15EX	5.458588	-310.003	320.9202	1
40 Days:F36EX-40 Days:F15EX	20.40082	-295.061	335.8625	1
60 Days:F36EX-40 Days:F15EX	148.4541	-167.008	463.9157	0.9261344
20 Days:F36POND-40 Days:F15EX	6.273683	-309.188	321.7353	1
40 Days:F36POND-40 Days:F15EX	21.16894	-294.293	336.6306	1
60 Days:F36POND-40 Days:F15EX	149.4395	-166.022	464.9011	0.922620687
20 Days:F45EX-40 Days:F15EX	51.05777	-264.404	366.5194	0.999999163

40 Days:F45EX-40 Days:F15EX	74.85147	-240.61	390.3131	0.999904907
60 Days:F45EX-40 Days:F15EX	187.5569	-127.905	503.0186	0.707801468
20 Days:F5EX-40 Days:F15EX	9.646014	-289.627	308.9192	1
40 Days:F5EX-40 Days:F15EX	-2.22406	-317.686	313.2376	1
60 Days:F5EX-40 Days:F15EX	-22.1343	-362.872	318.6032	1
20 Days:F36EX-60 Days:F15EX	-20.8454	-336.307	294.6163	1
40 Days:F36EX-60 Days:F15EX	-5.90313	-321.365	309.5585	1
60 Days:F36EX-60 Days:F15EX	122.1501	-193.312	437.6118	0.984324375
20 Days:F36POND-60 Days:F15EX	-20.0303	-335.492	295.4314	1
40 Days:F36POND-60 Days:F15EX	-5.13501	-320.597	310.3266	1
60 Days:F36POND-60 Days:F15EX	123.1355	-192.326	438.5972	0.983181665
20 Days:F45EX-60 Days:F15EX	24.75382	-290.708	340.2155	1
40 Days:F45EX-60 Days:F15EX	48.54752	-266.914	364.0092	0.999999564
60 Days:F45EX-60 Days:F15EX	161.253	-154.209	476.7146	0.871968017
20 Days:F5EX-60 Days:F15EX	-16.6579	-315.931	282.6153	1
40 Days:F5EX-60 Days:F15EX	-28.528	-343.99	286.9336	1
60 Days:F5EX-60 Days:F15EX	-48.4383	-389.176	292.2992	0.999999845
40 Days:F36EX-20 Days:F36EX	14.94223	-300.519	330.4039	1
60 Days:F36EX-20 Days:F36EX	142.9955	-172.466	458.4571	0.943671549
20 Days:F36POND-20 Days:F36EX	0.815096	-314.647	316.2767	1
40 Days:F36POND-20 Days:F36EX	15.71036	-299.751	331.172	1
60 Days:F36POND-20 Days:F36EX	143.9809	-171.481	459.4425	0.940743122
20 Days:F45EX-20 Days:F36EX	45.59919	-269.862	361.0608	0.999999807
40 Days:F45EX-20 Days:F36EX	69.39288	-246.069	384.8545	0.999961318
60 Days:F45EX-20 Days:F36EX	182.0984	-133.363	497.56	0.746631165
20 Days:F5EX-20 Days:F36EX	4.187426	-295.086	303.4606	1
40 Days:F5EX-20 Days:F36EX	-7.68265	-323.144	307.779	1
60 Days:F5EX-20 Days:F36EX	-27.5929	-368.33	313.1446	1
60 Days:F36EX-40 Days:F36EX	128.0533	-187.408	443.5149	0.976481276
20 Days:F36POND-40 Days:F36EX	-14.1271	-329.589	301.3345	1
40 Days:F36POND-40 Days:F36EX	0.768124	-314.694	316.2298	1
60 Days:F36POND-40 Days:F36EX	129.0387	-186.423	444.5003	0.974923537
20 Days:F45EX-40 Days:F36EX	30.65695	-284.805	346.1186	0.999999999
40 Days:F45EX-40 Days:F36EX	54.45065	-261.011	369.9123	0.999998091
60 Days:F45EX-40 Days:F36EX	167.1561	-148.306	482.6178	0.840821798
20 Days:F5EX-40 Days:F36EX	-10.7548	-310.028	288.5184	1
40 Days:F5EX-40 Days:F36EX	-22.6249	-338.087	292.8368	1
60 Days:F5EX-40 Days:F36EX	-42.5352	-383.273	298.2024	0.999999972
20 Days:F36POND-60 Days:F36EX	-142.18	-457.642	173.2812	0.94601669
40 Days:F36POND-60 Days:F36EX	-127.285	-442.747	188.1765	0.977643617
60 Days:F36POND-60 Days:F36EX	0.985392	-314.476	316.447	1
20 Days:F45EX-60 Days:F36EX	-97.3963	-412.858	218.0653	0.99822954
40 Days:F45EX-60 Days:F36EX	-73.6026	-389.064	241.859	0.999921983
60 Days:F45EX-60 Days:F36EX	39.10286	-276.359	354.5645	0.999999975
20 Days:F5EX-60 Days:F36EX	-138.808	-438.081	160.4651	0.933387163

40 Days:F5EX-60 Days:F36EX	-150.678	-466.14	164.7835	0.918049945
60 Days:F5EX-60 Days:F36EX	-170.588	-511.326	170.1491	0.887792039
40 Days:F36POND-20 Days:F36POND	14.89526	-300.566	330.3569	1
60 Days:F36POND-20 Days:F36POND	143.1658	-172.296	458.6274	0.943172787
20 Days:F45EX-20 Days:F36POND	44.78409	-270.678	360.2457	0.999999848
40 Days:F45EX-20 Days:F36POND	68.57778	-246.884	384.0394	0.999966449
60 Days:F45EX-20 Days:F36POND	181.2833	-134.178	496.7449	0.752257816
20 Days:F5EX-20 Days:F36POND	3.372331	-295.901	302.6455	1
40 Days:F5EX-20 Days:F36POND	-8.49775	-323.959	306.9639	1
60 Days:F5EX-20 Days:F36POND	-28.408	-369.146	312.3295	1
60 Days:F36POND-40 Days:F36POND	128.2705	-187.191	443.7322	0.976144307
20 Days:F45EX-40 Days:F36POND	29.88883	-285.573	345.3505	0.999999999
40 Days:F45EX-40 Days:F36POND	53.68252	-261.779	369.1442	0.999998407
60 Days:F45EX-40 Days:F36POND	166.388	-149.074	481.8496	0.84508638
20 Days:F5EX-40 Days:F36POND	-11.5229	-310.796	287.7503	1
40 Days:F5EX-40 Days:F36POND	-23.393	-338.855	292.0686	1
60 Days:F5EX-40 Days:F36POND	-43.3033	-384.041	297.4342	0.999999965
20 Days:F45EX-60 Days:F36POND	-98.3817	-413.843	217.0799	0.998034838
40 Days:F45EX-60 Days:F36POND	-74.588	-390.05	240.8736	0.999908762
60 Days:F45EX-60 Days:F36POND	38.11747	-277.344	353.5791	0.999999982
20 Days:F5EX-60 Days:F36POND	-139.793	-439.067	159.4797	0.929925617
40 Days:F5EX-60 Days:F36POND	-151.664	-467.125	163.7981	0.914290699
60 Days:F5EX-60 Days:F36POND	-171.574	-512.311	169.1637	0.883565558
40 Days:F45EX-20 Days:F45EX	23.79369	-291.668	339.2553	1
60 Days:F45EX-20 Days:F45EX	136.4992	-178.962	451.9608	0.960489395
20 Days:F5EX-20 Days:F45EX	-41.4118	-340.685	257.8614	0.999999892
40 Days:F5EX-20 Days:F45EX	-53.2818	-368.743	262.1798	0.999998553
60 Days:F5EX-20 Days:F45EX	-73.1921	-413.93	267.5454	0.999970963
60 Days:F45EX-40 Days:F45EX	112.7055	-202.756	428.1671	0.992466627
20 Days:F5EX-40 Days:F45EX	-65.2055	-364.479	234.0677	0.999965524
40 Days:F5EX-40 Days:F45EX	-77.0755	-392.537	238.3861	0.999866178
60 Days:F5EX-40 Days:F45EX	-96.9858	-437.723	243.7517	0.999254231
20 Days:F5EX-60 Days:F45EX	-177.911	-477.184	121.3623	0.707964213
40 Days:F5EX-60 Days:F45EX	-189.781	-505.243	125.6806	0.691479134
60 Days:F5EX-60 Days:F45EX	-209.691	-550.429	131.0462	0.658851514
40 Days:F5EX-20 Days:F5EX	-11.8701	-311.143	287.4031	1
60 Days:F5EX-20 Days:F5EX	-31.7804	-357.588	294.027	0.999999999
60 Days:F5EX-40 Days:F5EX	-19.9103	-360.648	320.8272	1

Appendix 2: Raw data of fluoride bioaccumulation values recorded during three different analysis periods undertaken during of 60 culturing days

Recorded data under appendix 2 were taken during data analysis for fluoride bioaccumulation values (mg/kg) during the present study. In detail, the table shows all recoded data during the first 20th culturing days recorded as 1st analysis phase followed by data analysis conducted at 40th culturing day recorded as 2nd analysis phase and lastly were the analysis conducted at 60th culturing day recorded as 3rd analysis phase. The recorded values were rooting from catfish samples taken from 5, 15, 36 and 45 mg F^{-}/L aquaria treatments as well as 36 mg F^{-}/L fishpond.

Fish Sample ID	Aquarium & pond Fluoride concentrations	Aquarium & pond ID	Bioaccumulation in different tissues (mg/kg)				Culturing day	Date	Analysis phase
			Bone	Gills	Skin	Fillet			
1	**Experimental Aquarium (5mg/L)**	F5EX	118.6077	112.1272	8.0767	0.3077	20th_day	30th Oct, 2019	1st analysis
2		F5EX	118.6094	111.9897	8.2685	0.126	20th_day	30th Oct, 2019	
3		F5EX	118.6521	112.02	7.9305	0.2797	20th_day	30th Oct, 2019	
			118.623	112.0456	8.0919	0.2378			
4		F5EX	118.9149	112.0408	8.2686	0.3097	40th_day	30th Oct, 2019	2nd analysis
5		F5EX	118.901	112.0353	8.2423	0.3385	40th_day	30th Oct, 2019	
6		F5EX	118.9968	111.8786	8.1147	0.3853	40th_day	30th Oct, 2019	
			118.9376	111.9849	8.2085	0.3445			
7		F5EX	119.7816	112.1452	7.4372	0.3105	60th_day	30th Oct, 2019	3rd analysis
8		F5EX	119.6898	111.9313	7.5376	0.1436	60th_day	30th Oct, 2019	
9		F5EX	119.6177	112.1322	7.6554	0.3343	60th_day	30th Oct, 2019	
			119.6964	112.0696	7.5434	0.2628			
10	**Experimental Aquarium (15mg/L)**	F15EX	118.2578	113.77	8.9271	0.3263	20th_day	30th Oct, 2019	1st analysis
11		F15EX	118.7132	114.0179	8.4016	0.4613	20th_day	30th Oct, 2019	
12		F15EX	118.4802	113.9252	8.8932	0.3416	20th_day	30th Oct, 2019	
			118.4838	113.9044	8.7406	0.3764			
13		F15EX	123.0689	114.9235	9.3159	0.3844	40th_day	30th Oct, 2019	2nd analysis
14		F15EX	123.8581	114.9393	9.6684	0.4533	40th_day	30th Oct, 2019	
15		F15EX	123.4686	115.2218	9.3972	0.4158	40th_day	30th Oct, 2019	
			123.4652	115.0282	9.4605	0.4178			
16		F15EX	227.5671	115.127	10.76	0.276	60th_day	30th Oct, 2019	3rd analysis
17		F15EX	227.3446	114.8193	10.316	0.4893	60th_day	30th Oct, 2019	
18		F15EX	227.4202	115.2341	10.858	0.5512	60th_day	30th Oct, 2019	
			227.4439	115.0601	10.645	0.4388			
19	**Experimental Aquarium (36mg/L)**	F36EX	124.4534	128.0239	17.775	0.5354	20th_day	30th Oct, 2019	1st analysis
20		F36EX	124.4414	127.9163	16.989	0.8548	20th_day	30th Oct, 2019	
21		F36EX	124.4461	127.6755	16.729	0.7791	20th_day	30th Oct, 2019	
			124.447	127.8719	17.164	0.7231			
22		F36EX	164.3003	142.2024	22.441	1.0147	40th_day	30th Oct, 2019	2nd analysis

23		F36EX	164.2354	141.9694	22.365	0.9929	40th_day	30th Oct, 2019	
24		F36EX	164.2661	142.2864	22.836	1.0157	40th_day	30th Oct, 2019	
			164.2673	142.1527	22.547	1.0078			
25		F36EX	411.4058	350.2464	77.666	3.2334	60th_day	30th Oct, 2019	3rd analysis
26		F36EX	411.4761	349.8791	77.389	3.2223	60th_day	30th Oct, 2019	
27		F36EX	411.4284	349.7722	77.709	3.1362	60th_day	30th Oct, 2019	
			411.4368	349.9659	77.588	3.1973			
28	**Experimental Aquarium (45mg/L)**	F45EX	244.3795	181.9193	25.174	1.0807	20th_day	30th Oct, 2019	1st analysis
29		F45EX	244.4973	182.2964	24.901	0.9142	20th_day	30th Oct, 2019	
30		F45EX	244.5483	182.039	25.095	0.9637	20th_day	30th Oct, 2019	
			244.475	182.0849	25.057	0.9862			
31		F45EX	356.3841	152.282	36.584	2.6012	40th_day	30th Oct, 2019	2nd analysis
32		F45EX	356.643	152.2079	36.251	2.4293	40th_day	30th Oct, 2019	
33		F45EX	356.5459	152.2321	36.616	2.5567	40th_day	30th Oct, 2019	
			356.5243	152.2407	36.484	2.529			
34		F45EX	503.962	400.1917	89.014	5.056	60th_day	30th Oct, 2019	3rd analysis
35		F45EX	504.2888	400.1499	89.204	4.9956	60th_day	30th Oct, 2019	
36		F45EX	504.3135	400.0216	89.503	5.0986	60th_day	30th Oct, 2019	
			504.1881	400.1211	89.24	5.0501			
37	**Ngarenanyuki pond (36mg/L)**	F36POND	125.1124	128.7889	19.005	0.6653	20th_day	30th Oct, 2019	1st analysis
38		F36POND	124.9871	128.0077	18.966	0.8644	20th_day	30th Oct, 2019	
39		F36POND	125.0076	129.0088	18.99	0.9968	20th_day	30th Oct, 2019	
			125.0357	128.6018	18.987	0.8422			
40		F36POND	164.8797	142.9868	24.988	1.6655	40th_day	30th Oct, 2019	2nd analysis
41		F36POND	163.9989	141.8976	23.977	1.8779	40th_day	30th Oct, 2019	
42		F36POND	164.8877	142.009	24.099	1.8766	40th_day	30th Oct, 2019	
			164.5888	142.2978	24.354	1.8067			
43		F36POND	411.9687	351.9988	77.999	3.6775	60th_day	30th Oct, 2019	3rd analysis
44		F36POND	412.4898	350.9873	78.019	3.8443	60th_day	30th Oct, 2019	
45		F36POND	412.769	351.8735	79.008	3.7545	60th_day	30th Oct, 2019	

Appendix 3: Growth parameters recorded during three different analysis periods of catfish 60 culturing days

Data under appendix 3 shows recorded raw values for catfish growth parameter taken after every 20th culturing days. It therefore, presents data recoded on 20th, 40th and 60th culturing days revealing the differences in catfish length, weight gain (WG), average daily gain (ADG) and Specific Growth Rate (SGR).

Parameters	Treatment				
	F5EX	**F15EX**	**F36EX**	**F36POND**	**F45EX**
Initial mean body weight (g)	19.92	19.89	19.88	19.87	19.9
Final mean body weight @20 days (g)	53.98	44.06	43.59	43.52	38.06
Final mean body weight @ 40 days(g)	79.99	64.03	59.05	59	50.77
Final mean body weight @ 60 days(g)	93.6	76.8	69.89	70	57.23
WG @ 20 days (g)	34.06	24.17	23.71	23.65	18.16
WG @ 40 days (g)	26.01	19.97	15.46	15.48	12.71
WG @ 60 days (g)	13.61	12.77	10.84	11	6.46
ADG @ 20 days (g)	1.703	1.2085	1.1855	1.1825	0.908
ADG @ 40 days (g)	1.3005	0.9985	0.773	0.774	0.6355
ADG @ 60 days (g)	0.6805	0.6385	0.542	0.55	0.323
SGR @ 20 days (%/day)	0.0498	0.0398	0.0393	0.0392	0.0324
SGR @ 40 days (%/day)	0.0197	0.0187	0.0152	0.0152	0.0144
SGR @ 60 days (%/day)	0.0079	0.0091	0.0084	0.0085	0.0060
Mean initial length (cm)	16.05	16.06	16.05	16.05	16.05
Mean final length/fish (cm)	34.06	36.04	37.03	37.03	40.05
Length gain (cm)	18.01	19.98	20.98	20.98	24

RESEARCH OUTPUTS

(i) Publication

Bejumula, J., Machunda, L. R., Pasape, J. L., & Mtei, M. K. (2021). Assessment of Fluoride Biocumulation Potential in African Catfish (Clarias gariepinus) Reared in Fluoride Rich Water. *Tanzania Journal of Science*, 47 (2), 472-484.

(ii) Poster presentation