Sudan University of Science & Technology

College of Graduate Studies

Bioaccumulation of Heavy Metals in Tilapia Fish (Oreochromis niloticus)

التراكم الحيوي للمعادن الثقيلة في أسماك البلطي (Oreochromis niloticus)

A thesis Submitted in partial Fulfilment for the Requirements of MSc
degree In Chemistry

By

Michael Mathiang Ngong Kuach

BSc. Education (Chemistry), 2012

Supervisor

Dr. Mohammed El Bushra Hassan Ahmmed

Oct, 2020

DEDICATION

This piece of work is dedicated to my lovely family, most particularly my lovely wife ***Tereza Agel Achien*** and my dad for their unlimited support to accomplish this master program.

ACKNOWLEDGEMENTS

Firstly, thanks go to God almighty for giving me the power, strength and health to complete this work.

I would also like to acknowledge and appreciate the support and guidance given to me by the supervisor of my thesis **Dr. Mohammed El-Bushra Hassan** (department of Chemistry, Sudan university of Science & Technology).

Special thanks also to Alawia Imam Pharmaceutical Research Center at the University of Medical Sciences (UMST) and the Central Forensic Laboratory for having given me an opportunity to use their facilities for my sample preparation and analysis. I should also pay a very special thanks to my colleagues for their solidarity and most particularly **Afra Mohmmed** and **LaduD.Morris**. Last but not the least, I am very much grateful to the academic staffs of the Sudan university of Science & Technology and to **prof. Moukaila Noma** of UMST. A very special thank goes to **Dr. Samuel Kartukila** who was very instrumental in the data analysis for this research.

Best Regards.

ABSTRACT

Fish are widely used to evaluate the health of any aquatic ecosystem because their biological responses serve as biomarkers of an environmental contaminant. Besides their ecological value, Nile tilapia contributes immensely to a healthy diet. However, consumption of fish is contaminated by heavy metals lead to various diseases. The aim of this study was to determine the bioaccumulation levels of seven heavy metals (Cr, Zn, Cu, Fe, Pb, Hg, and Cd) in Nile Tilapia fish (*Oreochromis Niloticus*) collected from Almorada fish market (Omdurman locality).

The samples of fresh fish were randomly collected from the local fishermen during the month of October 2019 and packed in an ice box before being transported to the laboratory. The fish samples were tagged into A, B, C and D for easier identification, with length of 8, 9.5,11, and 13 cm and weight of 0.195, 0.206, 0.472 and 0.551 kilograms/wet, respectively. Aqua regia solution (prepared by HNO_3: HCl at the ratio of 1:3, respectively) was used for the samples digestion. The samples were subsequently assayed using inductive couple plasma atomic emission spectroscopy.

Different organs of the fishes accumulated varying quantities of heavy metals. In *Oreochromis Niloticus*, the level of heavy metals varied significantly among fish size and organs. The highest level of heavy metals was found in gill followed by the head and finally the muscle. However, the accumulation of potassium and sodium followed the sequence Head > Gill > Muscle and Head > Muscle > Gill, respectively. There were statistically significant for the level of Lead (p= 0.03), Chromium (p= 0.041) and potassium (p= 0.044) in the tilapia fish tissues rendering them above the threshold limit set by WHO/FAO 2003 as a result become toxic to human consumption. This suggests that the fish samples could be used to monitor Lead and Chromium pollution levels in the River Nile.

المستخلص

تستخدم الأسماك على نطاق واسع لتقييم صحة أي نظام بيئي مائي وذلك لاستجابتها البيولوجية والتي تعمل كمؤشرات حيوية للملوثات البيئية. ألي جانب قيمتها البيئية، فأن أسماك البلطي تسهم بشكل كبير في النظام الغذائي الصحي. وبالرغم من ذلك، يؤدي استهلاك الأسماك الملوثة بالمعادن الثقيلة إلى أمراض مختلفة. تهدف هذه الدراسة الي تحديد مستويات التراكم الحيوي لسبعة معادن ثقيلة وهي Cr و Zn و Cu و Fe و Pb, Hg , Cd في أسماك البلطي (*Oreochromis Niloticus*) والتي جمعت من سوق الموردة للاسماك محلية ام درمان.

تم جمع عينات من الأسماك الطازجة بشكل عشوائي من الصيادين المحليين خلال شهر أكتوبر 2019 وعبئت في صندوق ثلج قبل نقلها إلى المختبر. تم تسمية عينات الأسماك بالأحرف أ، ب، ج، د وه حتى يسهل التعرف عليها ,وبأطوال 11،9.5،8 و13 سم ووزن رطب 0.195 ،206. 0 ،0.472 و0.551 كجم على التوالي. تم استخدام محلول الماء الملكي (المحضر من HNO_3: HCl وبنسبة 3:1 علي التوالي) لعملية تهضيم العينات. تم تحليل العينات لاحقا باستخدام التحليل الطيفي للانبعاث الذري للبلازما.

وجد أن هنالك تباين في تراكم كميات المعادن الثقيلة مع أختلاف أعضاء الأسماك. ففي أسماك البلطي، اختلف مستوى المعادن الثقيلة بشكل جوهري بناءا على أحجمها وأعضاءها. تم العثور على أعلى مستوى من المعادن الثقيلة في الخياشيم تليها الرأس وأخيرا العضلات. ولكن ، معدل تراكم البوتاسيوم والصوديوم يتبع التسلسل: الرأس > الكبد > العضلات و الرأس > العضلات > الكبد، على التوالي. كانت هناك دلالة إحصائية لمستوى الرصاص ($p=0.03$) والكروم ($p=0.041$) والبوتاسيوم ($p=0.044$) في أنسجة أسماك البلطي مما يجعلها أعلى من القيمة المسموح بها وفقا لمنظمة الصحة العالمية/منظمة الأغذية والزراعة 2003 مما يجعلها سامة للاستهلاك البشري. ومن هنا يمكن أن يقترح امكانية استخدام عينات الأسماك لرصد مستويات تلوث الرصاص والكروم في نهر النيل.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

1. INTRODUCTION AND LITERTURE REVIEW

1.1 GENERAL BACKGROUND

The Nile is Africa's longest river which covers a distance of 6,650 Km. It is a major south-north flowing river in the north eastern Africa, with confluences of White and Blue Nile converging in Khartoum-Sudan and its tributaries including Atbara among others are believed to be the principal natural sources of fresh water for human, agriculture, livestock and wildlife (**Magboul and Suleiman, 2015)**.

Due to recent increase in urbanization and industrialization in Khartoum, the level of municipal wastewater has rapidly increased (sewage water and industrial effluents) and as a result of no proper waste disposal mechanism led to serious environmental pollution. The effluent discharged into the Nile river may have drastic effect on the aquatic organism and fish, either directly or indirectly (**Abdalrahaman, 2015**).

The river Nile can be considered as a model polluted aquatic ecosystem for assessment of biomarker responses in fish because it receives untreated wastes from various drain outlets (**Osman and Kloas, 2010; Osman, et al., 2012**). The use of biomarkers has become an important tool for modern environmental assessment as they can help to predict pollutants involved in the monitoring program. Currently, the use of biomarkers for monitoring environmental quality has gained considerable interest in the assessment of river condition in many places around the world (**Tejeda-Vera et al., 2007; Falfushynska et al., 2009**).

There are many different biomarkers that occur at many different levels of organization from sub-cellular to whole-organisms. The biomarker may be the chemical itself (bioaccumulation). It may also be molecular biomarkers, biochemical biomarkers and tissues biomarkers. Metal bioaccumulation is a major route, through which increased levels of the pollutants are transferred across the food chain and finally assimilated by human consumers resulting in health risks (**Ambedkar and Muniyan, 2011**). Therefore, it is important to always determine the bioaccumulation capacity for heavy metals by fishes in order to assess potential risk to human health and take appropriate action to protect public health and the environment (**Otitoloju , 2002; Ambedkar and Muniyan, 2011**). Contaminant residues in fish tissues may ultimately reach concentrations hundreds or thousands of times above those measured in the water. For this reason, monitoring fish tissue contamination serves an important function as an early warning biomarker of aquatic pollution (**Barak and Mason, 1990**).

Fish are widely used to evaluate the health of aquatic systems and their biological responses serve as biomarkers of environmental contaminants. Besides its ecological value, Nile tilapia, (*Oreochromis niloticus*) also economically important as a food source by low income families along the river Nile. Nile tilapia is one of the most common freshwater fish used in toxicological studies, because it presents a number of characteristics that may make it an appropriate model that can be used as indicator species in biomonitoring programs (**Figueiredo-Fernandes et al., 2007; Osman and Kloas , 2010**).

Nile tilapia (Oreochromis niloticus), is one of the most important fish species that is consumed and is very important in world fisheries (FAO, 2011). Nile tilapia is a fresh water fish species that is commonly farm and consume among the African and Asian communities. This fish has amazing characteristics of being hardy, prolific, fast growing and tropical in nature as well, making it an important bio-indicator for metal concentration. Tilapia species are beneficial to human health as they make up a major part of balance diet. Fish muscle contains 19.5g/100g protein, 2g/100g fat, 0.70g/100g vitamin B12, 6.3g/100g vitamin D3 and 0.40g/100g vitamin E, in the raw edible parts. Apart from proximate composition, Nile tilapia contains iron, zinc, calcium, iodine, selenium, phosphorus, magnesium, sodium, potassium, manganese, sculpture, copper (Cu) and at the levels of 1.1mg, 1.2mg, 95mg, 11g, 26g, 190mg, 26mg, 81mg, 280mg, 0.052mg, 240mg and 0.031mg per 100g raw edible part, respectively (Bogard et al., 2015).

Given their significance in the study of carcinogenic potential of contaminants present in water samples, Fish in general are being used as bio indicators since they can metabolize, concentrate and store water borne pollutants (El-sapped, et al., 2012).

The purpose of this study was to determine the level of some heavy metals (Cr, Zn, Fe, Cd, Hg, Cu and Pb) in river Nile and tributaries in using Nile tilapia (Oreochromis Niloticus) collected from Almorada fish market (Omdurman locality) as a proxy. Hence, our research would like to contribute to the on-going debate on the association between the environment changes and their impact on the (human) fauna and flora.

1.2 LITERATURE REVIEW

1.2.1 Heavy metals

Heavy metals are a group of metals with atomic density greater than 4g/cm3 or 5 times greater than water. They are also known as trace elements because they occur in minute concentrations in the biological systems. Depending on their concentration, they may exert beneficial or harmful effects on plants, animals and human life. Some of these metals are toxic to the living organisms even at low concentration, whereas others are biologically essential and become toxic at relatively high concentrations (Bruno Streit, 1998). Varying considerable attentions have been given to heavy metals so far due to their long-term persistence, toxicity, and bioaccumulation and bio magnification at various tropic levels (El-Batrawy et al., 2018).

Heavy metals enter the aquatic food chain either by direct consumption of water and food or through non-dietary routes across permeable membranes such as the muscle and gills. Therefore, levels of heavy metals in fish always reflect their concentration in sediment and water of that particular aquatic ecosystem in which they were collected from and time of exposure. Fish can accumulate heavy metals in their tissues by absorption along gill surface and kidney, liver and gut tract wall to higher levels than environmental concentration (Sivakumar and Xiaoyu, 2019).

1.2.2 Heavy Metal Bioaccumulation:

Metals have the tendency to accumulate in various organs of the aquatic organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards. Bioaccumulation of metals can only take place if the rate of uptake by the organism exceeds the rate of elimination. Metals are non-biodegradable, and once they enter the aquatic environment, bio-concentration may occur in fish tissue by means of metabolic and bioasorption processes (Carpene et al., 1990; Wicklund-Glynn, 1991). From an environmental point of view, bio-concentration is important because metal ions usually occur in low concentrations in the aquatic environment and subtle physiological effects go unnoticed until gross chronic reactions (e.g. changes in population's structure, altered reproduction, etc.) become apparent. Protein binding of metals has been the subject of a recent major review on the molecular biology of metal toxicology. Many different classes of proteins are known to play a role in the disposition of metals in the body nonspecific binding to proteins such as serum albumin or hemoglobin plays a role in metal transport in the blood stream and in the distribution of metal between red cell and plasma (Zalups and koropatmick, 2000). Environmental toxicity is concerned with the adverse effect that chemicals can include, when released into the natural environment of living organism. Chemicals reach people directly through water and air and indirectly through the food chain. Most of the world environmental pollutants are the result of human intervention and hence human activities (Mance, 1990)

Industrial wastes and mining can create a potential source of heavy metal pollution in the aquatic environment. Under certain environmental conditions, heavy metals might accumulate up to toxic concentrations and cause ecological damage (Guven et al., 1999). Thus, heavy metals, acquired through the food chain as a result of pollution, are potential chemical hazards, threatening consumers. Metal contamination of aquatic ecosystems has long been recognized as a serious pollution problem. When fish are exposed to elevated levels of metals in a polluted aquatic ecosystem, they tend to take these metals up from their direct environment (Seymore, 1994).

Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi et al., 2007). Pollutants are able to accumulate along the aquatic food chain with severe risk for animal and human health. Toxic heavy metal contamination mostly occurred in aquaculture farms and frequently occurs in Groundwater Rivers, estuaries, wetland and coastal areas. Of particular concern are the highly toxic non-nutrient elements such as mercury (Hg), lead (Pb), and cadmium (Cd) (Nevo et al., 1986).

The rate of bioaccumulation of heavy metals in aquatic ecosystem relies mostly on the ability of fish species and any other aquatic organism to digest metals and the concentration of such pollutants in the river. Bioaccumulation rate of heavy metals could also be increase by feeding habits of organism and the concentration of heavy metals in the surrounding soil sediments (Eneji et al.,2011).Rate of heavy metals accumulation also in fish meat depends particularly on the age, gender and the location of fish cultivation, the higher the accumulation of heavy metals in the water body, the higher are the bioaccumulation of heavy metals contained in the network aquatic organisms (Junianto et al. 2017)

The presence of pollutants has been associated with decreased fertility and other reproductive abnormalities in birds, fish, shellfish and mammals and also altered immune function. Heavy metals like cadmium are known to accumulate in marine organisms and cause rapid genetic changes (Nimmo et al., 1978; Nevo et al., 1986). The toxicity of these elements is due to their ability to cause, oxidative damage to living tissues. Damage includes enhanced lipid per oxidation, DNA damage, enzyme inactivation and the oxidation of protein sulfhydryl groups (Taiz and Zeiger, 1998).

Toxic heavy metal can cause dermatological diseases, skin cancer and internal cancers (liver, kidney, lung and bladder), cardiovascular disease, diabetes, and anemia, as well as reproductive, developmental, immunological and neurological affects in the human body. Metal contamination sources are typically derived from natural sources: mining, industrial Waste discharges, sewage effluent, harbor activities and agrochemicals. It is also possible that environmental toxicants may increase the susceptibility of aquatic animals to various diseases by interfering with the normal functioning of their immune, reproductive and developmental processes. Prolonged exposure to water pollutants even in very low concentrations have been reported to induce morphological, histological and biochemical alterations in the tissues which may critically influence fish quality (Kalay and Canli, 2000).

Transport of metals in fish occurs through the blood where the ions are usually bound to proteins. The metals are brought into contact with the organs and tissues of the fish and consequently accumulated to a different extent in different organs and tissues of the fish. Most heavy metals released into the environment find their way into the aquatic environment as a result of direct input, atmospheric deposition and erosion due to rainwater, therefore aquatic animals may be exposed to elevated levels of heavy metals due to their wide use for anthropogenic purposes (Kalay and Canli, 2000).

Toxicity of heavy metals is very important in environmental pollution and play an important role in marine ecosystems as pollutants or essential elements. One of the important features that distinguished metals from other pollutants is that they are not biodegradable and become more toxic even at sub trace level when present in soluble ionic or complexes from toxic metal ions. The exception includes metals contained in certain pesticide formulation, mostly dithiocabamte fungicides compounds. Heavy metal ions can be entrapped in the cellular structure and subsequently biosorbed onto the binding sites present in the cellular structure. This method of uptake is independent of the biological metabolic cycle and is known as biosorption or passive uptake. The heavy metal can also pass into cell across the cell membrane through the cell metabolic cycle. This mode of metal uptake is referred as active uptake. The metal uptake by both active and passive modes can be termed as bioaccumulation (lyer et al., 2004).

Some of the toxic effects are related to the promotion of cellular oxidative stress by such metals, both by increasing the cellular levels of reactive oxygen species and by damage to the antioxidant systems in animals and algae (Okamoto et al., 1999).

1.2.3 Copper

Copper is an essential trace metal in small concentrations for several fish metabolic functions. Copper forms an essential part of variety of enzymes (free radical defense) and liver proteins homocuprien and heptacuprien It is also used as fungicide, algaecide and herbicide and in municipal water treatment systems Copper is widely used for plumbing purposes, can be added as a salt to water supply reservoirs to suppress algal growth, is a component in agricultural pesticides, and can be a byproduct of acid mine drainage (Hem, 1985).

Copper is a natural element which is widely distributed in soils, rocks and in rivers and the sea. The Cu is mostly used by communities but is sometimes quite toxic to life in rivers. It can be introduced into the environment as a result of anthropogenic activities, agricultural runoffs, sludge from public own treatment work and the municipal and industrial solid waste dumped into the river water. It may also be attributed to domestic sewage and runoff from extensive farmed areas. Other factors that may contribute to the high concentration of cu in the environment is copper compounds used in electroplating industries such as cupric suphthenate and cupric acetate and in fertilizers such as copper naphthenate and cuprous oxide from the paint industries. The dissolved form of copper compound is quite toxic to aquatic animals and plants. The toxicity of copper can be reduced by binding it to a particulate matter in the river water and when the water is hard (Hem, 1985).

Copper is found in living organisms, enzymes to carrying oxygen. However, accumulation of copper in liver leads to cirrhosis; in brain leads to death of neurons and resulting neurological symptoms; and in kidney leads to renal tubular damage. Aquatic organisms require Cu in trace quantities in order to grow. In unpolluted conditions, this metal is accumulated from 103 to 104 times the level present in seawater according to the United State Environmental Protection Agency (USEPA).

1.2.4 Iron

Iron is a major element in various primary minerals, notably the, Ferro magnesium, group of silicates together with other compounds including oxides, which form workable ores. Although less prominent in the secondary (clay) minerals it readily forms, hydrous oxides in soil along with aluminum. Hence, most soils contain significant amounts of iron. Although ferric compounds are relatively insoluble, iron, like aluminum, can form a number of soluble hydroxyliron species depending on the pH, and small fraction can be mobilized by organic linkages. Ferrous ions are taken up by plants. They are most prominent under reducing conditions and mobile unless precipitated as sulphide. Iron is an essential minor nutrient. Iron is present in respiratory pigments such as the cytochrome catalyses, and peroxides in aerobic cells. Fishes may be harmed by iron compounds in poorly oxygenated waters with low pH where the iron is present mainly in form of soluble compounds (Karar, 1997).

As the gill surface of the fish tends to be alkaline, soluble ferrous iron can be oxidized to insoluble ferric compounds, which then cover the gill lamellae and Inhibit respiration. At low water temperature and in the presence of iron, iron – depositing bacteria will multiply rapidly on gills and further contribute to the oxidation of ferrous iron compounds (Karar, 1997).

Iron deficiency in some instances may impair the bactericidal activity of neutrophils and depresses immunoglobulin synthesis, migratory inhibition factor and T lymphocyte transformation. However, data are often conflicting; several reports state that iron deficiency does not alter these specific immune Parameters. Nevertheless, the concentration of iron in body tissues do appear to affect the pathogenesis of infectious disease (Karar, 1997).

1.2.5 Lead

Lead occurs naturally in the environment. However, most lead concentrations that are found in the environment are a result of human activities. Due to the application of lead in gasoline, an Unnatural lead-cycle has consisted. In car engines lead is burned, so that lead salts (chlorines, bromines, and oxides) will originate. These lead salts enter the environment through the exhausts of cars. The larger particles will drop to the ground immediately and pollute soils or surface waters, the smaller particles will travel long distances through air and remain in the atmosphere. Part of this lead will fall back on earth when it is raining. This leadcycle caused by human production is much more extended than the natural leadcycle, and has led pollution to be a worldwide issue (Dowidar et al., 2001)

Lead was used in plumbing pipes and as an aid for combustion of gasoline (tetraethyl lead) (Hem, 1985). It could be found in solder used to joint copper fittings, in pipes in water-distribution service lines, and in brass and bronze fixtures. (Hem, 1985) stresses the importance of dry and wet deposition of particulate lead as a major source of lead into the environment. Lead, bound to particulates, can be washed into streams after storms and adheres to soil particles.

The high level of lead could be also due to the industrial discharges from super phosphate factories, traffics of high way or motor vehicles as well as the extensive use of agrochemicals such as fertilizers, pesticides and growth promoters (Tolba et al., 1994). Lead can enter the human body through uptake of food (65%), Water (20%) and air (15%) and cause several unwanted effects, such as: Disruption the biosynthesis of hemoglobin and anemia, a rise in blood pressure, kidney damage and Miscarriages and subtle abortions, disruption of nervous systems, brain damage, declined fertility of men through sperm damage and diminished learning abilities of children and behavioral disruptions of children, such as aggression, impulsive behavior and hyperactivity. Lead can enter a fetus through the placenta of the mother. Because of this, it can cause serious damage to the nervous system and the brains of unborn children (Jarup, 2003)

The concentrations normally encountered are soil 2-20ug g-1; animal tissue 0.1-3ug g-1 and freshwater1-20ug g-1. Lead exerts adverse effects on the resistance of the body to disease. It causes suppresses the immune system, particularly the hormonal response in animals. This suppression often occurs at very low subclinical dosages and, therefore, may be detrimental to the health of animals and perhaps of man by mechanisms other than the typical well documented toxicity which occurs at larger dosages. Severe contamination with Pb leads to brain damage, anemia, liver and kidney diseases. The human health action level for lead contaminant is 1.7ug g-1 wet weight (USFDA, 1993).

1.2.6 Zinc

Zinc is a common element found in the earth's crust, as well as in the air, soil, and water. It is also found in all foods. Zinc is widely used as a constituent of brass and bronze and for galvanizing steel, as well as a white pigment in paint and rubber (Hem, 1985). Zinc also is used some fertilizers that may leach into the groundwater. This metal may be important for human body growth and development; however, drinking or eating food contaminated with high levels of zinc can lead to many diseases such as stomach cramps, nausea and vomiting. Zinc can be introduced into the environment by either natural or man-made activities through erosion from rocks and soil or urban runoff, mine drainage and municipal sewages which are always highly concentrated with zinc (Damodharan, 2013).

Zinc is an essential metal for maximum activity of many enzymes and contributes to the development and maintenance of the thymus. Much of the toxicological studies have, therefore, dealt with the effect of zinc deficiency on the immune response (El-Sikaily et al., 2003) Concentration of up to 0.4mg/l has been reported in some estuarine waters. Concentrations at this level are lethal to mollusk' s larvae. Toxic levels for adult fish and shellfish are about 10ppm (ElSikaily et al., 2003). Chromium (Cr) is an essential trace metal for living organisms, but, its high toxicity, mutagenicity and carcinogenicity render it hazardous at very low concentration (Cheung et al., 2002).

1.2.7 Chromium

Chromium, in various forms, is used in many industrial applications, such as chrome plating operations (Hem, 1985). As the application of chromium is extensive in various industries like chrome planting, wood preservation and alloy formation, chromium –associated pollution is of increasing concern. Apart from traditional physicochemical treatments, a number of biological assays using microorganisms have been studied and developed to remedy chromium contaminated water. The two major processes being investigated are adsorption of metals on biological materials (i.e. biosorption) including cells of microorganisms and plants, and dissimulator reduction of metal ions from higher violent state to lower one (i.e. biotransformation) through enzymatic reaction or indirectly with metabolite produced (Lyer et al., 2004).

Chromium is widely distributed in soils and vegetation although the concentrations generally very low. The levels in some basic igneous soils such as serpentine are relatively high. It is toxic to animals, particularly in the hexavalent state although less so to plants. As with other heavy metals, pollution problems can arise from the discharge of waste products from electro-planting manufacture of alloys and other industrial processes. The concentrations normally encountered are soils 10-200ug g-1; plants materials 0.05-0.5ug g-1; animal tissue 0.01- 0.3ug g1- and freshwater 0.1-0.5 ug g-1. The USEPA considers chromium concentrations in the water, 0.1mg/l, as threats to the marine environment while levels lower than 0.05mg/l present minimal risks. However, smaller aquatic organisms are very sensitive to Cr, and it can eventually be bioaccumulated to the higher levels of the trophic web (PesoAguiar et al., 2002) to as being from 1-10mg/l, in a large variety of marine species (GESAMP, 1982).

1.2.8 Cadmium

Cadmium is non-essential and toxic metal which is distributed and released into the aquatic environment by industrial sources such as mining, refining of ores. Other sources of cadmium are plating process, phosphate fertilizers and gasoline containing of this toxic metal. Heavy metals also enter the aquatic environment from the rocks and soil directly exposed to the water surface (Rashed, 1999).

Cadmium is an element with atomic number of 48, atomic weight of 112.4 and the density of 8.65gms/cm3.Some of the most common sources is coal burning plus agricultural inputs like fertilizers, pesticides, refined petroleum products and detergent etc. Exposure to any form of cadmium has drastic health effects such as; lung diseases with clear link to lung cancer, kidney dysfunction and in very high levels may possess serious health problems related to bone defects in humans and animals and sometime can cause death (Rajeswari et al.,1994).

The purpose of this study was to determine the levels of bioaccumulation of some heavy metals (Cr, Zn, Cu, Fe, Pb, Hg, Cd) in river Nile and tributaries in using Nile Tilapia fish (Oreochromis Niloticus) collected from Almorada fish market (Omdurman locality) as a proxy. Hence our research would like to contribute to the ongoing debate in the literature on the association between the environmental changes and their impact on (human) fauna and flora.

1.3 STATEMENT OF RESEARCH PROBLEM

Health and environmental problems arising from heavy metals present in aquatic ecosystem and their bioaccumulation in fish species are well known because of anthropogenic activities such agricultural activities, disposal of waste material from industries and chemicals. Over hundreds of thousand people could be affected due to unsustainable environmental management and lack of clear and strong environmental protection measures being taken which has consequently resulted into pollution of rivers that sustained the livelihoods of the local populations. Heavy metals are very dangerous to human if taken or consume in high concentration through food chain of tilapia fish which bio accumulate some heavy metals as a result of chemicals containing wastewater being released into the rivers. The purpose of this study was to determine the levels of bioaccumulation of some heavy metals (Cr, Zn, Cu, Fe, Pb, Hg, Cd) in river Nile and tributaries in using Nile Tilapia fish (Oreochromis Niloticus) collected from Almorada fish market (Omdurman locality) as a proxy. Hence, our research would like to contribute to the ongoing debate in the literature on the association between the environmental changes and their impact on (human) fauna and flora.

1.4 RESEARCH QUESTION

What are the levels of heavy metals (Cr, Zn, Cu, Fe, Pb, Cd and Hg) concentration in tissues of Nile Tilapia (*Oreochromis Niloticus*) Fish in Almorada fish market of Omdurman locality?

1.5 OBJECTIVES OF THE STUDY

1.5.1 General Objective

-To determine the levels of heavy metals (Cr, Zn, Cu, Fe, Pb, Cd, and Hg) concentration in gill, muscle and head of Nile Tilapia Fish (Oreochromis Niloticus) collected from Almorada fish market of Omdurman along the Nile.

1.5.2 Specific Objectives

A-To determine the types of heavy metals in Nile tilapia fish of Almorada fish market in Omdurman.

B- To determine the levels concentration of heavy metals in the Nile tilapia fish collected from Omdurman fish market.

C-To compare the concentration of heavy metals in Nile tilapia of Omdurman market to standard thresholds of human safety as per the WHO regulations.

CHAPTER TWO

MATERIALS AND METHODS

2.1 INTRODUCTION

This chapter gives an account of the chemicals used for preparing of the fish samples. Furthermore, instrumentation used to analyze the elements in the sample solution is also presented.

2.2 STUDY DURATION

The was study implemented from the 1st – 6th Oct 2019.

2.3 STUDY AREA AND POPULATION

The study was conducted at Almorada fish market situated along the White Nile bank of Omdurman at Khartoum state. Almorada is an ancient and prestigious district in Omdurman city bordered from the east by Nile Street. It is border on the south by Khour Abuanjah and from the north Alhashmab and Alomara district. Almorada features a hot desert climate, with only the months of July and August seeing significant precipitation.

This locality is one of the most densely populated and has always brought so many visitors from different parts of the Sudan as earlier as 4:00 am in the morning to get the best part of Nile rich fish of different kind caught by the local fishermen [http/www.sudan.gov.sd]. Poor waste disposal, agricultural activities and other anthropogenic activities along the Nile and its tributaries led to the need to undertake this study and comprehend the levels of heavy metals in the tissues of the fish sold and consume by the local communities of Omdurman city.

Figure (1): Locations of Almorada fish market

https://www.google.com/maps/place/Omdurman,+Sudan

2.4 SAMPLING OR COLLECTION OF FISH SAMPLES

Four fresh fish samples tagged according to identification number and of different sizes, length and weight were randomly purchased directly from the local fishermen at the Almorada fish market of Omdurman (Almorada) during the month of October and were packed in an ice box and then transported to Alawia Imam research centre for pharmaceutical and development at the University of medical science and Technology –UMST for samples preparation. The samples were collected considering their differences in size and length in order to determine the possible bioaccumulation of heavy metals due to this existing variation which may add considerable value (Akan et al., 2012).

2.5 REAGENTS AND INSTRUMENTS

Chemical reagents used were Aqua regia solutions (HCl&HNO_3) (AstaChem, India) for the digestion of the sample. Analytical instrument that was used to analyze the samples was inductive coupled plasma atomic emission spectroscopy (ICPE-9000, Shimadzu, Japan). Glassware, beakers, measuring cylinder, volumetric flask, crucible, hot plate & Magnetic stirrers (Jenway, UK), sensitive balance (Ohaus - pioneer Tm, China), Furnace Carbolite (Carbolite Gero, Malaysia) mortar and pestle washed and rinsed with deionized water.

2.6 SAMPLE PREPARATION AND DRY DIGESTION METHOD

The fish samples were kept and transported in an icebox in order to preserve and avoid any possible spoilage. The samples were categorized into A, B, C and D, measurements were taken to determine the weight (0.2061 kg,0.195 kg, 0.551 kg, & 0.472 kg) and the size such as length in cm (23, 20, 32, and29) and width (9.5, 8, 13, and 11cm) of the samples before digestion respectively. The fish samples were dissected to remove the part of head, muscle and gills and their different weight taken before and after digestion. The samples were ash overnight in furnace of carbolite. The desired materials were grounded using mortar and pestle to homogenize the sample.

2.7 PREPARATION OF AQUA REGIA SOLUTIONS

Extraction of the sample was done using the aqua regia solutions of HNO3: HCl in the ratio of 1:3. After digestion the residue was diluted to 25ml.

2.8 DATA MANAGEMENT AND ANALYSIS

Standardized data sheet developed in Ms-excel was used to computerize data related to fish code, weight, size, type of heavy metals identified, and concentration of heavy metals. The data was analyzed using SPSS version 16. Inferential statistics of ANOVA was performed to assess association between the type of heavy metals and the level of bioaccumulation.

CHAPTER THREE

RESULTS AND DISSCUSSION

Fish are often at the top of the aquatic food chain and tend to concentrate large amounts of some heavy metals from the water (Mansour and Sidky, 2002). Bioaccumulation of heavy metals is toxic to fish (Chattopadhyay et al., 2002; Ayotunde & Offen, 2012 (Mansour and Sidky, 2002). Bioaccumulation of heavy metals is toxic to fish (Chattopadhyay et al., 2002; Ayotunde & Offen, 2012).

Some heavy metals, such as copper, can combine with other contaminants to produce an additive toxic effect on fish (Yacoub, 2007). Chromium bioaccumulation in fish has been reported to cause impaired respiratory and osmoregulatory functions through structural damage to gill epithelium while Cadmium and lead are highly toxic non-essential heavy metals and they do not have a role in biological processes in living organisms (El-Naggar et al., 2009). Thus, even in low concentration, Cd and Pb could be harmful to fishes. Lead was found to cause pathological changes in tissue and organs (El-Naggar et al., 2009).

Nile tilapia (Oreochromis niloticus), is one of the most important fish species that is consumed and is very important in world fisheries (FAO, 2011). Nile tilapia contains iron, zinc, calcium, iodine, selenium, phosphorus, magnesium, sodium, potassium, manganese, sulphur, copper (Cu) and at the levels of 1.1mg, 1.2mg, 95mg, 11mg, 26mg, 190mg, 26mg, 81mg, 280mg, 0.052mg, 240mg and 0.031mg per 100g raw edible part, respectively (Bogard et al., 2015).

Heavy metals are high-density nonbiodegradable metals and metalloids with prolonged toxic effects, which, upon accumulation in the aquatic environment, are transferred to the aquatic biota through various pathways (Khalifa et al., 2010). These substances can, in turn, have carcinogenic, cytotoxic and mutagenic effects on humans who consume these organisms. Although fish is highly nutritious, its high consumption rate can have significant deleterious effects on human health because of accumulated toxic metals beyond permissible safety limits (Rauf et al., 2009). The accumulation of heavy metals in fish occurs in many of its organs. Among the different organs, gills were found to have the highest concentrations of heavy metals (Golovanova, 2008). Heavy metals concentrations in different tissues of the studied fish (Tilapia nilotica) are provided in Table 3.1.

Table 3.1 The level of metal concentration (mg) in each Fish Samples (A, B, C & D) in their different tissues (Gill, Head & Muscle) collected from Almorada Fish Market in Omdurman- Sudan as the data was analyzed using ICP – spectrometry.

Sample	Part	Cr (mg/kg)	Zn (mg/kg)	Na (mg/kg)	Mg (mg/kg)	K (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Pb (mg/kg)
A	Gill	8.4	31.3	218.6	718.3	2.2	54.7	281.1	843.3	14.5
	Muscle	1.3	0.4	8.4	41.2	6.2	4.8	1.6	15.5	3.7
	Head	6.5	0.9	15	39.1	7.4	28.3	6.1	15.2	17.8
B	Gill	4.5	2.2	53.1	40.2	40.2	15.8	9.05	17.2	12.7
	Muscle	1.4	0.7	16.3	140.9	7.8	5.2	8.9	48.2	3.5
	Head	3.6	4.7	133.3	41.9	52.4	34.5	44.9	194.6	19.5
C	Gill	0.7	0.3	2.6	2.9	0.5	2.6	2	22.9	2.0
	Muscle	0.5	0.3	2.9	5.7	0.6	1.9	2.7	35.3	1.3
	Head	1.4	1.8	21.2	53.0	3.9	4.85	21.7	181.0	3.9
D	Gill	0.7	0.5	23.4	7.7	8.5	2.8	3.0	93.5	1.9
	Muscle	0.5	0.2	4.8	4.4	1.6	1.8	0.9	1.3	1.4
	Head	1.0	0.7	4.1	4.1	1.2	3.4	5.6	108.8	2.8

As can be seen, *Tilapia nilotica* fish from Omdurman demonstrated the presence of high level of heavy metals.

The above results for accumulation of heavy metals (Cr, Zn, Na, Mg, K, Cu, Fe, Ca and Pb) according to factors (Gill, Muscle & Head) in samples (A, B, C and D) were statically treated by ANOVA and the obtained data are presented in Table 3.2 below.

Table 3.2 The ANOVA analysis for accumulation of heavy metals (Cr, Zn, Na, Mg, K, Cu, Fe, Ca and Pb) according to factors (Gill, Muscle & Head) in fish samples (A, B, C and D)

Metals	permissible level FAO/WHO 1999 (mg/kg)	factors	N	Mean (mg/kg)	Std. Deviation	P-value of F-test	
						Samples	Factors
Cr	0.15-1.0	Gill	4	3.55	3.66	0.041*	0.131
		Muscle	4	0.9	0.46		
		Head	4	3.11	2.54		
Zn	-	Gill	4	8.53	15.17	0.498	0.447
		Muscle	4	0.36	0.22		
		Head	4	2	1.82		
Na	-	Gill	4	74.4	98.35	0.484	0.433
		Muscle	4	8.09	5.94		
		Head	4	43.38	60.33		
Mg	0.5-1.0	Gill	4	192.25	351.09	0.446	0.523
		Muscle	4	48.01	64.19		
		Head	4	34.51	21.18		
K	-	Gill	4	12.85	18.56	0.044 *	0.368
		Muscle	4	4.04	3.51		
		Head	4	16.23	24.25		

Cu	30	Gill	4	18.94	24.6	0.136	0.27
		Muscle	4	3.41	1.84		
		Head	4	17.74	15.93		
Fe	43	Gill	4	73.76	138.23	0.532	0.499
		Muscle	4	3.53	3.66		
		Head	4	19.56	18.46		
Ca	-	Gill	4	244.2	400.87	0.688	0.524
		Muscle	4	25.08	20.79		
		Head	4	124.89	82.26		
Pb	0.214	Gill	4	7.75	6.77	0.030 *	0.058
		Muscle	4	2.44	1.32		
		Head	4	10.96	8.88		

The present study revealed significant differences in Cr concentrations in Gill (3.55) than the Permissible level (0.15-1.0) in all tissues (p<0.041*). Pb concentrations were higher (7.75) than the Permissible level (0.214). In Gill it was high than other parts. The different organs in the body accumulate a specific metal to a high level though others do not accumulate the metal though present in the medium (Al-Kahtani, 2009). In the present study, Pb accumulated in the order gill > Head > muscle. The gill is the one of sites that creates a path for the heavy metal to enter into the fish body (Bols et al., 2001). Our results show that the gill accumulates the highest level of metals followed by the head and finally the muscle except for potassium and sodium in the sequence of Head > Gill > Muscle and Head > Muscle > Gill respectively. Bioaccumulation of heavy metal in muscles is lower. Similar results were also described by (Jabeen et al., 2012).

In the study done by Ammar et al. (2019) on the samples of Nile Tilapia fish collected from Dongola and Morowe of the Sudan showed that the fish collected from the River Nile of the two localities indicated the presence of high levels of Pb; more than the permissible limits (0.214 and 0.5-0.6 ppm) according to FAO/WHO (1999) and US EPA (2003). As well as, Afroza et al. (2019) evaluate the bioaccumulation of chromium (Cr), lead (Pb), nickel (Ni) and copper (Cu) in cultured Nile tilapia. The bioaccumulation of Pb, Cr and Ni in Nile tilapia in this study exceeds the permissible limits set for heavy metals; this is potentially risky for consumers. Comparison of the study conducted in the Almorada Fish market and that of other studies has been described in Table 3.3 below.

Table 3.3 The Comparison of heavy metal content in various tissues of fish sample (mg/kg) from my study and that of other studies as reported in the literature

Sample Location	**Tissue**	**Heavy Metals**						**Reference**
		Cr **(mg/kg)**	Zn **(mg/kg)**	Cu **(mg/kg)**	Fe **(mg/kg)**	Ca **(mg/kg)**	Pb **(mg/kg)**	
Merowe, Sudan	Muscle	6.03	4.30	2.81	8.52	-	9.96	Ammar et al., 2019
	Liver	7.72	1.39	1.20	1.76	-	0.21	
Kolleru Lake, Kerala, India	Muscle	0.46	0.22	1.84	3.60	20.79	1.32	Sekhar et al., 2014
	Gill	1.26	-	1.50	-	-	2.59	
	Liver	0.77	-	2.96	-	-	4.63	
Almorada fish Market, Omdurman-Sudan	Muscle	3.10	1.20	11.68	12.23	95.68	8.30	This study
	Gill	3.60	15.17	24.60	138.6	0.90	6.77	
	Head	2.54	1.82	15.90	18.40	82.26	8.00	

In the present study, Heavy metals concentrations (ppm) were increased in gills. Muiruri et al. (2013) reported that the concentration of Pb, Mn, Cd and Cr in water, and Pb, Ni and Mn in Tilapia fish Gills were found to be higher than the

WHO recommended limit. The function of uptake and excretion in fish determine the accumulation of metal in fish. The gills are likely sites of metal uptake from water, due to their large surface area and the close proximity of the internal constituent of the body and external environment (Wepener, 1997). The high accumulation of heavy metals in gills may be attributed to the metallothionein proteins which are synthesized in gills when fishes are exposed to heavy metals and detoxify them. These proteins are thought to play an important role in protecting them from damage by heavy metal toxicants. Also, gills are the site directly exposed to the ambient conditions and also are known for their excretory function even for some metals like zinc (Jobling, 1995; Matthiessen and Brafield, 1977). Goel (2006) had observed that fish and other organisms that respire through the gills can absorb metals through their respiratory surfaces.

The results revealed the presence of Pb and Cr in higher concentrations than threshold limits. The higher level of Pb and Cr was related to the highest levels of Pb and Cr in the aquatic plant, sediment and Nile water. Tilapia nilotica is herbivorous and eat aquatic plants and clams that play a role as filters for heavy metals (Kallaf et al., 1994). It was concluded that the study area contains higher levels of Pb and Cr. This is because there were human activities that cause pollution to the water. High levels of Pb in the fish tissues may as a result of the industrial wastes which introduce to the river body and contains high levels of Pb, but the edible tissues of the fish, as well as the Nile water, were all in the safety baseline levels for human consumption (Mohamed et al., 1990; Issa, 1994). The head concentrates higher levels than the muscles. Ahmed et al., (2010) also observed more concentration of minerals and heavy metals in the head of fish species.

In the present study, heavy metals concentrations were decreased in Muscles. The lowest concentrations of metals were found in muscle tissues, this may due to the little blood supply to the muscular tissues and related to lower metabolic activities of muscle (Kalkan, et al., 2015).

There are two reasons for lower metals accumulation in muscle:

1- The presence of a mucous layer coating the fish skin surface creates a barrier that protects fish muscle tissue by forming complexes with the heavy metals that are present in the surrounding environment (Uysal, 2008).

2- Jagakumar and Paul (2006) have stated that other tissues do not transport heavy metals to muscle.

CHAPTER FOUR

GENERAL CONCLUSIONS, LIMITATIONS OF THE RESEARCH AND RECOMMENDATIONS

4.1 GENERAL CONCLUSION

It can be concluded that, *Tilapia Nilotica* fish was found to be a good bio-assay indicator for water pollution with heavy metals. *Tilapia Nilotica* fish from Omdurman demonstrated the presence of high amounts of heavy metals. Gill exhibits high levels of heavy metals. Muscle (as edible part) of fish Tilapia nilotica under study showed lower metals accumulation and safety levels for man consumption.

The purpose of this study was to determine the levels of bioaccumulation of some heavy metals (Cr, Zn, Cu, Fe, Pb, Hg, Cd) in river Nile and tributaries in using Nile Tilapia fish (Oreochromis niloticus) collected from Almorada fish market (Omdurman locality). Four fresh fish samples tagged according to identification number and of different size, length and weight were randomly purchased directly from the local fishermen at the Almorada fish market of Omdurman locality in Sudan during the month of October and were packed in an ice box and then transported to Alawia Imam research centre for pharmaceutical and development at the University of medical science and Technology –UMST for samples preparation. In The present study, Heavy metals concentrations (ppm) in different tissues of Tilapia nilotica there was statistically significant difference in lead. Chromium and Potassium but increased in gills and lessen only in muscle tissue which make it safe for human consumption

This study successfully investigated all the set objectives. A summary of conclusions that support the main objectives of each investigation is drawn below:

- Tilapia nilotica fish was found to be a good bio-assay indicator for water pollution with heavy metals (HMs).
- *Tilapia Nilotica* fish from Omdurman demonstrated the presence of high amounts of HMs in Gill.
- Muscle (as edible part) of *Tilapia Nilotica* fish under study showed lower metals accumulation and safety levels for human consumption.

4.2 LIMITATIONS OF THE RESEARCH

- The sample size was too small which may affect the reliability and accuracy of the results of the study.
- The period of data collection was too short, as this was done once in the month of oct, 2019 which may not actually represent the bioaccumulation of heavy metals throughout the year in the Almorada Fish market of Omdurman
- The age of the fish samples was not put into consideration which is an important factor in determining the period of exposure to water contamination.
- The research was short on the correlation of the bioaccumulation of heavy metals and the fish size (length) and weight, although these measurements were taken the statistical analysis performed did not put them into consideration.

4.3 RECOMMENDATIONS

- The local authorities should keep an eye on random disposal of waste materials at the shore of the river, as these contribute to the pollution of the water bodies.
- Environmental conservation awareness should be carried out among the inhabitants along the Nile to preserve the aquatic organisms.
- Authorities should ensure that, the international guide lines of water safety are adhered to strictly by the Factories operating along the Nile.
- Presence of the heavy metals on the collected fish species according to this study is dangerous and further investigation should be done by expertise to ascertain the magnitude of this contamination.
- Use of fertilizers on the farms along the river Nile should be within the acceptable limits for the aquatic organisms' safety in order to avoid washing of heavy metals into the water bodies.
- There shall be need to increase the sample size in any of the future research for reliability and accuracy of the study.
- Scope of the study shall be extensive to get the actual representation throughout the year on the bioaccumulation of the heavy metals, because it takes time for the heavy metals to find their ways into the aquatic organism

REFERENCES

Agriculture Organization of the United Nations. Fisheries Department, 2000. The State of World Fisheries and Aquaculture, 2000 (Vol. 3). Food & Agriculture Org.

Akan, J.C., Mohmoud, S., Yikala, B.S. and Ogugbuaja, V.O., 2012. Bioaccumulation of some heavy metals in fish samples from River Benue in Vinikilang, Adamawa State, Nigeria.

Ali, S., Elfaki, J., Adam, M., Dafalla, M., Ahmed, H. and Ali, M., 2017. Assessment of heavy metals contamination in the Nile River water and adjacent sediments: a case study from Khartoum City and Nile River State, Sudan. Eurasian Journal of Soil Science, 6(3), pp.285-294.

Al-Kahtani, M.A., 2009. Accumulation of heavy metals in Tilapia fish (*Oreochromis niloticus*) from AL-Khadoud spring, AL-Hassa, Saudi Arabia. American Journal of Applied Sciences, 6(12), p.2024.

Ambedkar, G. and Muniyan, M., 2011. Bioaccumulation of some heavy metals in the selected five freshwater fish from Kollidam River, Tamilnadu, India. Advances in Applied Science Research, 2(5), pp.221-225.

Barak, N.E. and Mason, C.F., 1990. Mercury, cadmium and lead in eels and roach: the effects of size, season and locality on metal concentrations in flesh and liver. Science of the Total Environment, 92, pp.249-256.

Belton, B., van Asseldonk, I.J.M. and Thilsted, S.H., 2014. Faltering fisheries and ascendant aquaculture: Implications for food and nutrition security in Bangladesh. Food Policy, 44, pp.77-87.

Bogard, J.R., Thilsted, S.H., Marks, G.C., Wahab, M.A., Hossain, M.A., Jakobsen, J. and Stangoulis, J., 2015. Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes. Journal of Food Composition and Analysis, 42, pp.120133.

Bols, N.C., Brubacher, J.L., Ganassin, R.C. and Lee, L.E., 2001. Ecotoxicology and innate immunity in fish. Developmental & Comparative Immunology, 25(8-9), pp.853-873.

Braunbeck, T., Hinton, D.E. and Streit, B. eds., 1998. Fish ecotoxicology (Vol. 86). Springer Science & Business Media.

Carpene, E., Cattani, O., Serrazanetti, G.P., Fedrizzi, G. and Cortesi, P., 1990. Zinc and copper in fish from natural waters and rearing ponds in Northern Italy. Journal of fish biology, 37(2), pp.293-299.

Cheung, S.G., Tai, K.K., Leung, C.K. and Siu, Y.M., 2002. Effects of heavy metals on the survival and feeding behaviour of the sandy shore scavenging gastropod Nassarius festivus (Powys). Marine pollution bulletin, 45(1-12), pp.107-113.

Damodharan, U., 2013. Bioaccumulation of heavy metals in contaminated river water-Uppanar, Cuddalore, South East coast of India. Perspectives in Water Pollution, pp.23-34.

Dowidar, M., Abdel-Magid, S. and Salem, S., 2001. Biochemical effect of lead and cadmium on glutathion peroxidase superoxide dismutase activity, copper and selenium level in rat. 2nd Int. sc. In Conf., Fac. vet. Med., Mansura univ.

El-Batrawy, O.A., El-Gammal, M.I., Mohamadein, L.I., Darwish, D.H. and ElMoselhy, K.M., 2018. Impact assessment of some heavy metals on tilapia fish, Oreochromis niloticus, in Burullus Lake, Egypt. The Journal of Basic and Applied Zoology, 79(1), p.13.

EL-sappah, A., shawky, A., sayed-ahmad, m.a.h.a.s.s.e.n. and youssef, m., 2012. Nile tilapia as bio indicator to estimate the contamination of water using SDS-page and RAPDPCR techniques. egyptian journal of genetics and cytology, 41(2), pp.209-227.

El-Sikaily, A., Khaled, A. and El Nemr, A., 2004. Heavy metals monitoring using bivalves from Mediterranean Sea and Red Sea. Environmental Monitoring and Assessment, 98(1-3), pp.41-58.

Falfushynska, H.I. and Stolyar, O.B., 2009. Responses of biochemical markers in carp Cyprinus carpio from two field sites in Western

Ukraine. Ecotoxicology and Environmental Safety, 72(3), pp.729-736.

Farombi, E.O., Adelowo, O.A. and Ajimoko, Y.R., 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River. International journal of environmental research and public health, 4(2), pp.158-165.

Figueiredo-Fernandes, A., Ferreira-Cardoso, J.V., Garcia-Santos, S., Monteiro, S.M., Carrola, J., Matos, P. and Fontaínhas-Fernandes, A., 2007. Histopathological changes in liver and gill epithelium of Nile tilapia, Oreochromis niloticus, exposed to waterborne copper. Pesquisa Veterinária Brasileira, 27(3), pp.103-109.

IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution, 1982. The Review of the Health of the Oceans (Vol. 15). Unesco.

Glynn, A.W., 1991. Cadmium and Zinc Kinetics in Fish: Studies on Water-Borne ^{109}Cd and ^{65}Zn Turnover and Intracellular Distribution in Minnows, Phoxinus phoxinus. Pharmacology & toxicology, 68(6), pp.485-491.

Goel, P.K., 2006. Water pollution: causes, effects and control. New Age International.

Golovanova, I.L., 2008. Effects of heavy metals on the physiological and biochemical status of fishes and aquatic invertebrates. Inland Water Biology, 1(1), p.93.

GÜVEN, K., ÖZBAY, C., ÜNLÜ, E. and SATAR, A., 1999. Acute lethal toxicity and accumulation of copper in Gammarus pulex (L)(Amphipoda). Turkish Journal of Biology, 23(4), pp.513-522.

Hem, J.D., 1985. Study and interpretation of the chemical characteristics of natural water (Vol. 2254). Department of the Interior, US Geological Survey.

Humphries, P., King, A.J. and Koehn, J.D., 1999. Fish, flows and flood plains: links between freshwater fishes and their environment in the MurrayDarling River system, Australia. Environmental biology of fishes, 56(1-2), pp.129-151.

Jabeen, G., Javed, M. and Azmat, H., 2012. Assessment of heavy metals in the fish collected from the river Ravi, Pakistan. Pakistan Veterinary Journal, 32(1), pp.107-111.

Järup, L., 2003. Hazards of heavy metal contamination. British medical bulletin, 68(1), pp.167-182.

Kalay, M. and CanI, M., 2000. Elimination of essential (Cu, Zn) and nonessential (Cd, Pb) metals from tissues of a freshwater fish Tilapia zilli. Turkish Journal of Zoology, 24(4), pp.429-436.

Kalkan, H., Şişman, T. and Kılıç, D., 2015. Assessment of heavy metal bioaccumulation in some tissues of Leuciscus cephalus from Karasu River, Erzurum-Turkey. Austin J Environ Toxicol, 1(1), p.1004.

Khalifa, K.M., Hamil, A.M., Al-Houni, A.Q.A. and Ackacha, M.A., 2010. Determination of heavy metals in fish species of the Mediterranean Sea (Libyan coastline) using atomic absorption spectrometry. International Journal of PharmTech Research, CODEN (USA): IJPRIF ISSN, pp.09744304.

Karar, O.O.A (1997). Indication of the effect of proximity to Atlantis II Deep on selected fauna of the Red Sea, institute of Environmental Studies, University of Khartoum, pp89 [Thesis].

Iyer, A., Mody, K. and Jha, B., 2004. Accumulation of hexavalent chromium by an exopolysaccharide producing marine Enterobacter cloaceae. Marine pollution bulletin, 49(11-12), pp.974-977.

Mance, G., 2012. Pollution threat of heavy metals in aquatic environments. Springer Science & Business Media.

Matthiessen, P. and Brafield, A.E., 1977. Uptake and loss of dissolved zinc by the stickleback Gasterosteus aculeatus L. Journal of Fish Biology, 10(4), pp.399-410.

Muiruri, J.M., Nyambaka, H.N. and Nawiri, M.P., 2013. Heavy metals in water and tilapia fish from Athi-Galana-Sabaki tributaries, Kenya. International Food Research Journal, 20(2).

Nevo, E., Noy, R., Lavie, B., Beiles, A. and Muchtar, S., 1986. Genetic diversity and resistance to marine pollution. Biological Journal of the Linnean Society, 29(2), pp.139-144.

Nimmo, D.R., Rigby, R.A., Bahner, L.H. and Sheppard, J.M., 1978. The acute and chronic effects of cadmium on the estuarine mysid, Mysidopsis bahia. Bulletin of environmental Contamination and Toxicology, 19(1), pp.80-85.

Okamoto, O.K., Shao, L., Hastings, J.W. and Colepicolo, P., 1999. Acute and chronic effects of toxic metals on viability, encystment and bioluminescence in the dinoflagellate Gonyaulax polyedra. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 123(1), pp.75-83.

Osman, A.G. and Kloas, W., 2010. Water quality and heavy metal monitoring in water, sediments, and tissues of the African Catfish Clarias gariepinus (Burchell, 1822) from the River Nile, Egypt. Journal of Environmental Protection, 1(04), p.389.

Osman, A.G., Abuel-Fadl, K.Y. and Kloas, W., 2012. In situ evaluation of the genotoxic potential of the river Nile: II. Detection of DNA strand-breakage and apoptosis in Oreochromis niloticus niloticus (Linnaeus, 1758) and

Clarias gariepinus (Burchell, 1822). Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 747(1), pp.14-21.

Otitoloju, A.A., 2002. Evaluation of the joint-action toxicity of binary mixtures of heavy metals against the mangrove periwinkle Tympanotonus fuscatus var radula (L.). Ecotoxicology and Environmental safety, 53(3), pp.404415.

Panchanathan, J. and Vattapparumbil, I.P., 2006. Patterns of cadmium accumulation in selected tissues of the catfish Clarias batrachus (Linn.) exposed to sublethal concentration of cadmium chloride. Veterinarski Arhiv, 76, pp.167-177.

Parvin, A., Hossain, M.K., Islam, S., Das, S.S., Munshi, J.L., Suchi, P.D., Moniruzzaman, M., Saha, B. and Mustafa, M.G., 2019. Bioaccumulation of heavy metals in different tissues of Nile tilapia (Oreochromis niloticus) in Bangladesh. Peer-reviewed Journal of the Nutrition Society of Malaysia, 25(2), p.237.

Peso-Aguiar, M.C., Smith, D.H., Assis, R.D.C.F., Santa-Isabel, L.M.D., Peixinho, S., Gouveia, E.P., Almeida, T.C.A., Andrade, W.D.S., Carqueija, C.R.G., Kelmo, F. and Carrozzo, G., 2000. Effects of petroleum and its derivatives in benthic communities at Baía de Todos os Santos/Todos os Santos Bay, Bahia, Brazil. Aquatic Ecosystem Health & Management, 3(4), pp.459-470.

Rajeshkumar, S. and Li, X., 2018. Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Toxicology reports, 5, pp.288-295.

Rauf, A., Javed, M. and Ubaidullah, M., 2009. HEAVY METAL LEVELS IN THREE MAJOR CARPS (CATLA CATLA, LABEO ROHITA AND CIRRHINA MRIGALA) FROM THE RIVER RAVI, PAKISTAN. Pakistan Veterinary Journal, 29(1).

Seymore, T., 1994. Bioaccumulation of metals in Barbus marequensis from the Olifants River, Kruger National Park and lethal levels of manganese to juvenile Oreochromis mossambicus (Doctoral dissertation, University of Johannesburg).

Taiz, L., 1998. Plant defenses: surface protection and secondary metabolites In: TAIZ L; ZEIGER E. Plant Physiology, pp.350-353.

Tejeda-Vera, R., López-López, E. and Sedeño-Díaz, J.E., 2007. Biomarkers and bioindicators of the health condition of Ameca splendens and Goodea atripinnis (Pisces: Goodeaidae) in the Ameca River, Mexico. Environment international, 33(4), pp.521-531.

Tolba, K., El-Neklawy, G. and Niazi, Z., 1994. Lead residue in tissue of slaughtered cattle kept at agricultureal and industrial areas. J. Egypt. Vet. Med. Ass, 54(2), p.199.

USFDA, U. and Food and Drug Administration, 1993. Guidance documents for cadmium, chromium, lead and nickel. Center for Food Safety and Applied Nutrition US Food and Drug Administration, Washington, DC.

Uysal, K., Emre, Y. and Köse, E., 2008. The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICPOES) in Beymelek Lagoon (Antalya/Turkey). Microchemical journal, 90(1), pp.67-70.

Vinodhini, R. and Narayanan, M., 2008. Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). International Journal of Environmental Science & Technology, 5(2), pp.179-182.

Wepener, V., 1997. Metal ecotoxicology of the Olifants River in the Kruger National Park and the effect thereof on fish haematology (Doctoral dissertation, University of Johannesburg).

Yacoub, A.M., 2007. Study on some heavy metals accumulated in some organs of three River Nile fishes from Cairo and Kalubia governorates. African J. Biol. Sci, 3(3), pp.9-21.

Zalups, R.K. and Koropatnick, D.J. eds., 2000. Molecular biology and toxicology of metals. CRC Press.

Zhao, F., Zhuang, P., Song, C., Shi, Z.H. and Zhang, L.Z., 2010. Amino acid and fatty acid compositions and nutritional quality of muscle in the pomfret, Pampus punctatissimus. Food Chemistry, 118(2), pp.224-227.