بسم الله الرحمن الرحيم

Bioaccumulation of heavy metals in Nile tilapia (*Oreochromis niloticus)* and African Cat Fish (*Clarias gariepinus)Collected* from White and Blue Nile Rivers-Sudan

تراكم المعادن الثقيلة فى أسماك البلطي النيلى(*Oreochromis niloticus*) وأسماك القرموط *(Clarias gariepinus)* المجمعة من النيل الأبيض والنيل الأزرق بالسودان

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بسم الله الرحمن الرحيم

وَهُوَ الَّذِي سَخَّرَ الْبَحْرَ لِتَأْكُلُواْ مِنْهُ لَحْمًا طَرِيًّا وَتَسْتَخْرِجُواْ مِنْهُ حِلْيَةً تَلْبَسُونَهَا وَتَرَى الْفُلْكَ مَوَاخِرَ فِيهِ وَلِتَبْتَغُواْ مِن فَضْلِهِ وَلَعَلَّكُمْ تَشْكُرُونَ (14)

صدق الله العظيم

سورة النحل

الاية (14)

Dedication

This effort is dedicated…

To my mother

Who always prays for me

To my father

Who always encouraging me

To my husband

Who always help me

To our son and daughters

To my brothers and sisters

And to all those I love

haram

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First, I thank ALLA who gave me the aptitude and patience to conduct and finish this study.

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My thanks extend to my daughters and my son for making my life full of joy.

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ABSTRACT

This study was conducted to investigate the occurrence and bioaccumulation of some heavy metals, Copper, chromium, cadmium, Lead, Zinc and Iron in water, sediment and two fishes , *Oreochromis niloticus* and *Clarias gariepinus.* And evaluate water parameters there in Three different stations (Jebal Awlia,Adobaseen and Alamab) in White Nile and three different stations (Soba,Algreef and Bori) in the Blue Nile where chosen as study areas. These were carried for three seasons using the apparatus adopted for heavy metals determination the Atomic Absorption Spectrometer (AAS) A Analyst 700, Perkin –Elmer also the different tissues from fish, muscles, liver, kidney and gills were prepared by standard histological techniques to investigate pathological changes.

Values of water criteria in the tested areas were suitable for rearing Nile tilapia fish.

Water and sediments Cu, Cr, Cd, Pb, Zn and Fe level were significantly ($P \leq 0.05$) higher at locations (Adobaseen, Alamab)in White Nile and (Soba , Bori) in the Blue Nile than the other locations, This may be attributed to the pollution source, industrials zone and sewage effluents and thermal station. Summer season reflected the highest ($P \leq 0.05$) levels of Cr, Cd Pb and Zn whereas Cu level was significantly ($P\leq0.05$) higher in winter. And Fe higher in autumn. Also for sediments all studied metals the highest ($P \leq 0.05$) levels were found in summer season.

In fish muscles, the levels of all metals were significantly ($P \leq 0.05$) affected by sampling locations and sampling seasons, whereas Cd and Fe were not affected by sampling seasons

Oreochromis niloticus accumulate Cr, Zn and Fe higher significantly ($P \leq 0.05$) than *Claria*s *gariepinus*.but its have Cu high and there were no significant difference in accumulation of Cd and Pb between the two species.

It was clear that all heavy metals were significantly higher in fish Gills and, liver tissue and kidney than in the edible muscle tissue. This may depend on the target organ for each element where it might be deposited.

The muscles of *Oreochromis niloticus& , Clarias gariepinus* from White and Blue Nile during summer, winter and autumn. Shows Edema between muscle bundles and focal areas of necrosis. Also splitting of muscle fibers .The most common lesions in the liver of both studied fish were focal areas of necrosis vacuolation and dilation and thrombosis formation in central vein, rupture in the hepatocytes, Lymphocytic infiltration and dead nuclei. Moreover, cell damage was seen. Gills show proliferation in the epithelium of gill filaments and secondary lamellae, congestion in blood vessels of gill filaments and atrophy of secondary lamellae, Edematous changes and sloughing of secondary lamellae. The kidney of both fish shows some changes included, Blood congestion, Shrinking of glumeruli Vacuolation in glomerulus, less cellular and Edema in Bowman's capsule.

This suggests that the fish samples could be used to monitor heavy metals pollution levels in the White and Blue Nile river.

Heavy metals in the edible parts of the fish indicated safe levels for human consumption and concentrations in the muscles are generally accepted by the international legislation limits.

Therefore, it is a must to manage such factories to be environmentally consonant by treating its wastes before exposure to the environment.

ملـخص الـبـحث

اجريت هذه الدراسه للتحقق من وجود و تراكم بعض المعادن الثقيله وهي(النحاس، الكروم ، الكادميوم، الزنك، الرصاص و الحديد) في الماء و التربه بالاضافه للعضلات و الكبد و الخياشيم والكلي لسمكتي البلطي النيلي و القرموط كما تم تقييم لقياسات الماء و ذلك في ثلاث محطات علي النيل الابيض و هي (جبل اولياء و الدباسيين و اللاماب) و ثلاث محطات علي النيل الازرق و هي (سوبا و الجريف شرق و بري) تم اختيارها مناطق للدراسه لثلاثه مواسم متتاليه باستخدام جهاز الامتصاص الذري كما تم تحضير ذات الاعضاء للتحقق من التغيرات النسيجيه فيها

قياسات الماء في المناطق المحدده اظهرت انها ملائمه لتربيه البلطي النيلي. كل المعادن الثقيله المدروسه في عينات التربه و الماء اظهرت ارتفاعا معنوي في محطات اللاماب و الدباسيين علي النيل الابيض و في سوبا و بري علي النيل الازرق. وذلك قد يرجع لبعض مصادر التلوث قرب المناطق الصناعيه و مخرج الصرف الصحي و المحطات الحراريه

فصل الصيف اظهر مستوي عالي من الكروم و الكادميوم و الرصاص و الذنك بينما كان النحاس عالي في فصل الشتاء و الحديد عالي في فصل الخريف في الماء. كل القراءات و القيم كانت اقل من الحد المسموح به حسب منظمه الصحه العالميه 2011. اما التربه فقد كانت كل المعادن فيها عاليه في فصل الصيف

في عضلات الاسماك نجد ان مستوي كل المعادن المدروسه قد تاثر معنويا بالموسم و مكان الصيد بينما الكادميوم و الحديد لم تتاثر قيمهم بالموسم.

اظهرت اسماك البلطي النيلي تراكم علي معنويا في الكروم و الذنك و الحديد من اسماك القرموط بينما احتوي القرموط علي النسبه الاعلي معنويا من النحاس كما لايوجد فرق معنوي بين نوعي الاسماك في تراكم الكادميوم و الرصاص.

كل المعادن الثقيله في عضلات الاسماك كانت اقل من الحد المسموح به حسب منظمه الصحه العالميه 2011.

وقد اظهرت الدراسه ان كل المعادن الثقيله كانت اعلي في خياشيم و كبد وكلي الاسماك معنويا عنه في عضلاتها.

التغيرات النسيجيه التي تمت ملاحظتها في العضلات شملت استسقاء بين الاربطه وموت بعض الخلايا مما ادي حدوث فجوات اضافه لتشقق في الياف العضلات. وقد ظهرت بعض التغيرات في نسيج الكبد شملت موت بعض الخلايا مما ادي حدوث فجوات بالاضافه لتكون بعض التخثر في الوريد المركزي و تمزق و انفجار في خلايا الكبد كما ظهرت بعض الخلايا الميته و الانويه الميته كذلك كما اظهرت الخياشيم تضخم في النسيج الطلائ للخيوط الثانويه و احتقان دموي في الاوعيه الدمويه علي الخيوط الغلصميه و ضمور في الخيوط الثانويه كم لوحظ انسلاخ في غشاء الخيوط الثانويه. كما اظهرت الكلي بعض التغيرات شملت الاحتقان الدموي و انكماش الكبيبه الكلويه وجود فراغات فيها كما لوحظ تجمع السائل الدموي حول محفظه بومان.

لهذا يقترح استخدام عينات الاسماك لمتابعه و جود و تراكم المعادن الثقيله في النيل الابيض و النيل الازرق كما يجب ان تدير المصانع مخلفاته قبل ان تلقي بها في البيئه الخارجيه.

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List of Abbreviations

PH .... The degree of acidity or alkalinity

DO2………………………. Dissolved Oxygen

NO3………………………..Nitrate

PO4……………………….**.** Phosphate

Cu…………………………Copper

Cr…………………………..Chromium

Cd…………………………..Cadmium

Pb…………………………...Lead

Zn…………………………...Zinc

Fe…………………………....Iron

WN…………………………White Nile

BN…………………………...Blue Nile

W……………………………Winter

S……………………………..Summer

Au……………………………Autumn

Se……………………………Season

ATSDR ……………………...Agency for Toxic Substances and Disease Registry

Chapter one

CHAPTER ONE

INTRODUCTION

The Blue Nile, which is known as Aabbay in Ethiopia, has its source at Tana Lake (3.100 km2). The lake is located in north western Ethiopia lying nearly 1.800 meter above sea level and 1.500 kilometers upstream from Khartoum. The Blue Nile drops about 410 meters and picks up the flow of two seasonal tributaries, the Dinder and Rahad Rivers **(Collins1900- 1988).** During flooding it carries large quantities of silt from the highlands of Ethiopia **(El-Khodari, 2003).**

From its major source, Victoria Lake, a body of fresh water of 69.485 km2 (27.000 square miles) in surface and one of the largest freshwater lakes of the world White Nile flows northwards through Uganda into Sudan **(Collins1900-1988).** The annual flow for the White Nile varies between 20 and 22 billion cubic meters (**El-Khodari, 2003).**

The White Nile has a much lower gradient than the Blue Nile and consequently its terraces rise far more gently **(El-Khodari, 2003).**

Possible sources of contamination of the White Nile water are numerous. These include industrial and domestic sewage effluents and surface run off from urbanization and agricultural land. Within Sudan, the White Nile is exposed to pollution and cultural eutrophication.

During the last century, some factories were built along the White Nile, and many more will be built in the future. Existing factories include cement, sugar, and tanneries. Waste waters from these factories, as well as from a sewage treatment plant located south of Khartoum; find their way directly into the White Nile.

At Khartoum, the Blue Nile and the White Nile merge into the single river. From downstream Khartoum, the river is called River Nile. About, 320 km north of

Khartoum, they joined by the seasonal Atbara river that rises in the Ethiopian highland.

The Blue Nile and the Atbara are subjected to heavy seasonal fluctuations in flow as a result of the seasonal rains of the Ethiopian highlands. Between the months of July and September, flow increases dramatically due to heavy rains, but the Blue Nile may run empty during dry seasons or droughts.

Khartoum state has an area of 22,122 km2 and an estimated population of approximately 7,152,102 (2008).it is divided to 7 lower government level called Al mahalyat they translate it as localities, it is similar to districts, The three rivers White Nile, Blue Nile and the River Nile formed the geographical map of Khartoum state divided into three cities (Khartoum, Omdurman and Khartoum Bahri) .The city Khartoum is chosen for this study not only because it is the capital of Sudan and therefore most probably expecting a impacts on the river water quality, but also located in the point of meeting for the main two Rivers Blue and White Nile.

Khartoum is located at the confluence of the White and Blue Niles at 370 meters above sea level **(Collins1900-1988)**. It is situated between latitudes 15º26'and 15º45'N and longitudes 32º25' and 32º40'E. The terrain in this region is generally flat or gently sloping; only interrupted by occasional hills of rocky outcrops while sand dunes provide a gently undulating topography. This flat landscape is also broken by the floors and terraces of the Nile valleys.

The population density in Sudan was 14.6 people/km2 in1955 and 22.7 in 1970 but declined to 14.8 in 1980 rising slightly to 16.0 in 1998 **(Ali, 1999).** This declining trend is Indicative of the large areas progressively occupied, legally as a result of

planning, replanting and resettlemint programmers, and in an unauthorized way by new migrants and land speculators.
According to 2004 census there was about six million people living in the city Khartoum and Sudan's current total population was around thirty five million people. **(Ali, 1999).**

From ancient times, fishing is a major source of food for humanity and provides employment and economic benefits to those engaged in this activity **(FAO, 1998**).

Khartoum state has limited natural fisheries scattered on the Blue and White Nile River: Fiteihab, Azozab, Al- Kalakla and Jabel Awlia Dam in the White Nile and Al- Gereif and Soba in the Blue Nile and Al-Mourada in the Main Nile **(Mohammed, 2004).**

The White Nile River Fisheries contribute about more than half of total fish marketed in the state, which was estimated to be at around 12.5% **(FD, 2003).** Thus, increasing tendency in exploitation of its fish products has led to negative effect on their fish stocks and continuous yield as reduction in fish composition of each fishery **(Kawai, 1994)**

Sudan fresh water Fisheries is small-scale of intensive labor conducted by artisanal craftsmen. The artisanal nature defined by that the level of income; the mechanical sophistication; the quantity of production; fishing range; the political influence; the market outlets; the employment the social mobility and financial dependence keep them subservient to the economic decisions and operating constraints put upon them by those who buy their production. **(Saeed, 2004).**

Urbanization is taking place in many urban centers in the Sudan. The most important centers adjacent to the Nile system is Khartoum at the Blue and White Nile confluence. It now hosts about one fifth of the Sudanese population "about 6 millions". Emigration of citizen to Khartoum is continuously increasing. And many industrial projects are continuously implemented. This will certainly impose more pressure on the Nile water around Khartoum. Remarkable effects on both its quality and quantity will be taking place. The former is affected by inputs from industrial, agricultural and domestic sewage sources, and the latter is affected by Pumping more water to satisfy the needs of the growing population and various developmental needs. **(Ali, 1999).**

Aquatic pollution is "any effect of human activity which alters the natural conditions of the aquatic environment" **(Pandy *et al.*, 2005).**

For thousands of years, the people of Sudan have been dependant on the Nile's water for survival since it is the main drinking water source in Sudan. One of the main environmental problems in Sudan is the problem with water pollution. This is particularly truth for Khartoum more than in any other city along the river, because Khartoum is the biggest city in Sudan and the increasing number of citizens has certainly increased the sanitation problem **(Ali, 1999).**

Due to population growth in Khartoum and the parallel increasing anthropogenic activities, Water pollution is increasing over time and facing an increasing problem **(Ali, 1999)**.

Polluted effluents discharged to the water system derive from several sources. One of the major sources is the disposal of untreated or semi-treated domestic effluents into water bodies.

Excessive use of fertilizers and pesticides is another major source of water pollution despite the success in considerable reduction of the use of agro-chemicals during the past decade. Additionally, many of the industrial establishments do not comply with the law, by dumping their wastewater untreated into surface water bodies as well as inject it into groundwater.

The Nile systems in Sudan host many towns, many agricultural schemes and industrial projects (e.g. Sugar factories) are situated between or at its banks. This system therefore is vulnerable to pollution risks. Because it is sensitive to any change and even a mild degree of eutrophication could have a serious effect on it, due to its high temperature and radiation inputs **(Hammerton, 1972**).

There are many factories in the city of Khartoum that have impacts directly or indirectly on the two Niles, such as clay pits, i.e. industries for manufacture of building blocks, spread along the bank of Blue Nile for more than 50 kilometers. The clay pits produce a lot of carbon dioxide generated from combustion of mud and outputs.

In Khartoum the public sewage system covers only a small part of the city. After treatment, the effluent goes to the groundwater, with an expected serious effect to its quality, **(UNESCO, National commission)**. A part of the population still use traditional earth pits. For the public sewage system, there are two plants for municipal waste water treatment in Khartoum. Those treatments plants are located at Soba (south of Khartoum) and Elhaj Yousif- (east of Khartoum North).

The treated sewage effluent is used in irrigation of trees and other fodder cultivations. This is the situation as planned for but in certain situations like rainy seasons the sewage effluents may find an access to the river water.

The human contact also may threaten by the water quality; people are using the Nile system for swimming, bathing, car and clothes washing which may cause bacteriological pollution.

Few industries are using water for industrial purposes either as an ingredient of products or in cooling systems. The most serious source of industrial pollution to the Nile in Sudan is the sugar factories. Which dispose highly enriched organic waste to both the White and Blue Nile

The level of treatment achieved at those factories, the ecological consequences of waste disposal need a scientific assessment to assess the effects on the water oxygen content, light penetration and Biological Oxygen Demand (B.O.D) and their consequences on primary productivity, and bottom flora and fauna.

Khartoum state is located between the White Nile & the Blue Nile marked by its long shores where man activities such as industries, construction, reclamation, tourism, etc. can affect the water biota and habitat. The effect can be through the direct discharge of harmful substances into the water environment, or indirectly such as fume resulting from combustion of petroleum or any industrial substances which are discharged into atmosphere then transferred to the aquatic environment (the electric power generating station and traffic).

Sudan's industrial sector is currently undergoing rapid change and expansion. Industry in Khartoum varies from medium to small industries and there are about (8) categories of industries.

Fertilizers, pesticides, hormones and other chemicals used in agricultural production are Agrochemicals' usage. Fertilizers in run off rise the nutrients level in running water leading to algal blooms and multiplication of macrophysics and alter the phytoplankton species composition and hence all the ecosystem components. Urban runoff, solutions of much solid water which may be of domestic or industrial origin and automobiles gaseous exhausts are dissolved and carried to the Nile. Some recently established buildings e.g. student hostiles, Hotels are disposing their sewage effluents directly in the Nile system. Precise assessment of their danger is very necessary.

Recreational sites created at the banks of the Blue Nile, White Nile and conference areas are also threatening the quality of the Nile water. Very recent analyses have recorded high bacteriological counts **(Elhassan, 2002).**

Power stations located near the Nile are using its water for cooling and disposing their exhaust oil in the Nile. Former survey recorded high values of oil and grease near Burri power station on the Blue Nile. **(Elhassan ,2002)**.

Intensification of agricultural production in other parts of Sudan “in the west and the east", maximizes the hazard of run-off containing agricultural chemicals. Unknown amounts of these Chemicals reach the Nile system either by drift through wind action during spraying or run-off through the fields or irrigation canals during the rainy season.   Although concentrations of pesticide residues in the open water body are considerably lowered by dilution, adverse effects were recorded.

Residues of organ chlorine compounds have been recorded in Fish from Lake Nubia more than 200km downstream. The Fish "kass". *Hydrocyon Forkalii* fat tissue was found to have the highest residue levels ranging from 0.4 - 3.3 ppm, followed by muscles with concentrations 0.01- 0.25 ppm (**ElZorgani *et al,* 1998**).

Objectives:

The main objectives of the study are geared towards determining the current heavy metals pollution status of the White and Blue Nile River around Khartoum state with the aim of:

1) Evaluating the relevance of Nile tilapia *Oreochromis niloticus* and African Cat Fish**,** *Clarias gariepinus* as a bio-indicator of pollution.

2) To investigate the bioaccumulation of heavy metals concentrations (Copper, Cadmium, Chromium, Lead, Zinc and Iron) in the water, sediments of the rivers and tissues of the selected fish species and their impact on fish tissues

3) To identify any variation in the occurrences and levels of these metals with different location of fish couth.

4) To help in developing of monitoring programmed that could predict the effects pertinent to both environments on the one hand and fauna on the other

5) To establish basic information about the residues of some heavy metals in consumable commercial fishes for human safety

Chapter two

CHAPTER TWO

LTERATURE REVIEW

In aquatic systems, the natural concentrations of metal ions are principally dependent on the ambient distribution, weathering and leaching of these elements from the soil in the catchments area. Human activity, such as industrial and traffic emission and various land use practices may increase heavy metal loading in to aquatic ecosystems (**Tarvainen *et al*, 1997)**

2.1 Aquatic pollution:

Pollution of fresh water environment as a consequence of mans activities is an ever growing problem, and its risk is particularly acute in semi-enclosed bodies of water.

GESAMP (1993) defined marine pollution as "The introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of sea water and reduction of amenities" A measure of pollution is inevitable, and acceptable, provided that its impact can be shown to be minimal. There are three basic approaches to the measurements of pollution. One is to measure the level of pollutants in the field, and evaluate these in the light of laboratory determinations of toxicity: this poses various problems. Thus, it assumes that all the pollutants are known, and largely ignores possible synergistic effects between them. The pollutant may occur only intermittently in the environment, and may be concentrated by organisms or in the sediment. Toxicity is determined by short-term laboratory experiments, whilst the field effects may result from long-term exposure. This method is suitable only for certain situations involving the effect of specified pollutant on the particular species. A second approach is to use natural water or substrate sample, and to test these for pollutant activity by field or laboratory bioassay. The problems here are that the pollution effects may be long-term, may affect only certain life –cycle stages of particular species, or may operate via food chain or other complex biological interactions. These are very difficult to cover in any experiments format, and make the extrapolation of bioassay results to the field situation an extremely dubious procedure. The third approach is to assess the impact of pollution by measuring changes in specific species. This is tempting in its simplicity and apparent objectivity, and has been the basic of variety of studies, which are usually referred to as monitoring or surveillance programmes.

The effect of pollution on fisheries may range from the immediate, such as the sudden death of a substantial number of fish, to the more prolonged effects such as defective development or faulty reproduction. Effects may be directly on individual fish eggs, larvae, or adults or indirectly, through the food chain **(GESAMP, 1982; Abd-Elmoneim *et al*. 1994, and Ahmed *et. al*. 1994).**

Heavy metals are commonly found in natural waters and some are essential to living organisms, yet they may become highly toxic when present in high concentrations. **(Ibok,. *et.al* 1989)**These metals also gain access into ecosystem through anthropogenic source and get distributed in the water body, suspended solids and sediments during the course of their mobility.

The rate of bioaccumulation of heavy metals in aquatic organisms depends on the ability of the organisms to digest the metals and the concentration of such metal in the river. (**Olaijire. *et al*, 2000)**

Aquatic animals' bioaccumulate trace metals in considerable amounts and stayed over a long period. Fishes have been recognized as a good accumulator of organic and inorganic pollutants .Age of fish, lipid content in the tissue and mode of feeding are significant factors that affect the accumulation of heavy metals in fishes. They are finally transferred to other animals including humans through the food chain. **(King *ET، al.2003*).**

Pollution affects the environment in several ways. Pollutants are, however, toxic to all species. Some pollutants are toxic having a direct effect on the metabolism of aquatic organisms. More common are of indirect effects. In addition, pollutants may detriment organisms through altering the physical or chemical conditions of their environment. Very severe pollution eliminates life comprehensively and therefore destroys ecological communities. However, most types and concentrations of pollutants do not have such an extreme harm to organisms simply by their presence. They are toxic as having direct physiological effects on organisms **(Frid and Dobson ,2002**)

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According to **(Huss *et al,* 2003)** problems related to chemical contamination of the environment are nearly all man-made. Hundreds of millions tons of disposable materials and sludge from sewage treatment plants that drained into the sea consist of chemicals used in agriculture and untreated sewage drained from large urban populations. All these participate and contaminate the coastal marine environments or freshwater environments. From there the chemicals find their way into fish and other aquatic organisms. Increasing amounts of chemicals may be found in predatory species because of biomagnifications, which is the concentration of the chemicals in the higher levels of the food chain. These chemicals may be there as a result of bioaccumulation, when increasing concentrations of chemicals in the body tissues accumulate over the life span of the individual. In this case, a large (i.e. an older) fish will have a higher content of the chemical concerned than a small (younger) fish of the same species. The presence of chemical contaminants in seafood is therefore highly dependent on geographic location, species, fish size, feeding patterns, solubility of chemicals and their persistence in the environment

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).**

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 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. **(Kalay and Canli, 2000).**

2.2 Monitoring fresh water environment:

The basic principle of monitoring is to commence before the onset of any pollution and measure selected organisms changes, and so define the natural or baseline situation. These measures are subsequently repeated at regular intervals. In a continuing unpolluted state, the expectation is for organism organs to show only specific and expected patterns of change. The appearance of changes other than these can then be attributed to pollution, and their extent taken as a measure of severity of the pollution. The need for monitoring the fate and effects of these chemicals has been recognized for many years due to the presence and potential impact of pollutants in humans and wildlife. The choice of environmental matrix to be monitored depends on the chemical of concern, potential target, and specific questions being asked. For monitoring of actual human exposure, it is possible to analyze several human tissues such as blood, milk, urine, even hair or placenta. Marine organisms including those used as seafood as well as other organisms not usually consumed by human can also be examined. Method based on biological effects and their underlying mechanisms can complement, and for some applications could replace, the use of analytical chemistry in monitoring the aquatic environment **(Hahn, 2002).** The major advantages of such biological, mechanism-based method are their toxicological specificity, rapidity, and low cost. Here, toxicological specificity refers to the relationship between the assay response and the toxic potential rather than simply the contaminant concentrations of the sample being analyzed and it is called functional toxicology **(Hahn, 2002).**

2.3 Heavy metals pollution:

Heavy metals are natural elements that occur in the earth crust. Some of these are necessary for human health and metabolic function in minute amount **(Abdulla, 1990; Haward, 2002 and Smith, 2007)**

Trace elements are natural components of rocks and soil that enter the water environment as a consequence of weathering and erosion. The term heavy metal is used synonymously with trace metal and includes both essential and non-essential trace metals and essentially imply those elements which in the metal form show specific weight above 5 **(FAO,1975)**

Metals are redistributed naturally in the environment by both geologic and biologic cycles rainwater dissolves rocks and ores and physically transports material to streams and rivers, adding and deleting from adjacent soil, and eventually to the ocean. Three measures of levels of heavy metals in fresh water habitats are usually available namely concentrations in waters, sediments and biota. The measurement of dissolved metal concentrations presents analytical problems. These concentrations are typically low, often at the limits of analytical detection, may therefore require pre concentration and liable to inadvertent contamination during collection and analysis.

Dissolved concentrations, moreover, vary greatly over time. For example with tidal cycle, freshwater run-off, season, etc. monitoring programmes need to be intensive over extended periods. In aquatic systems, metals are present as dissolved ions and complexes, colloids, suspended solids and solids in sediments. Concentrations of these metal ions are strongly dependent on biological processes and pH, activities of organic and scavenging processes. The ecological risk produced by metals largely depends on their form in water, the capacity of metals for complexion, sedimentation, and bioaccumulation. Most important, however, measurements of dissolved heavy metal concentrations provide an assessment of total metal, not of that portion which is bioavailability that is available for uptake and accumulation by aquatic organisms **(Bryan and Langston, 1992).**

Bremner (2005) stated that many elements, which are present in seafood are essential for human life at low concentration, however, they can be toxic at high concentrations. Other element like mercury, cadmium and lead show no known essential function in life and are toxic even at low Concentration when ingested over a long period, therefore many consumers regard any presence of these elements in fish as a hazard to health

Heavy metals accumulate in sediments, particularly organically rich sediments, and sediment metal concentrations are therefore high, easily measured and much less susceptible to accidental contamination. Furthermore, sediments offer a degree of time integration, overcoming the worst effects of temporal variability of heavy metal availability. **(Bryan and Langston, 1992)**

Heavy metals are commonly found in natural waters and some are essential to living organisms, yet they may become highly toxic when present in high concentrations **(Ibok. *etal*, 1989).** These metals also gain access into ecosystem through anthropogenic source and get distributed in the water body, suspended solids and sediments during the course of their mobility **(Olaijire and Imeokparia 2000)**

The rate of bioaccumulation of heavy metals in aquatic organisms depends on the ability of the organisms to digest the metals and the concentration of such metal in the river. Also it has to do with the concentration of the heavy metal in the surrounding soil sediments as well as the feeding habits of the organism. Aquatic animals (including fish) bioaccumulate trace metals in considerable amounts and stay over a long period. Fishes have been recognized as a good accumulator of organic and inorganic pollutants **(King and Jonathan, 2003).**

Odoemelam *et al.* (1999) revealed high concentrations of heavy metals such as Cd, Pb, Cu, Ni, Zn, Mn, Mg and Co in some rivers within the proximity of some industrial cities in Nigeria. The discharge of industrial wastes containing toxic heavy metals into water bodies may have significant effects on fish and other aquatic organisms, which may endanger public health through consumption of contaminated seafood and irrigated food crops.

Nwaedozie *et al,* (1998) reported that zinc contamination affects the hepatic distribution of other trace metals in fish. Zinc, copper and manganese, which are essential elements, compete for the same site in animals. This, no doubt, would affect tissue metal concentrations as well as certain physiological processes.

The aquatic environment is continuously being contaminated with chemicals from agriculture and urban activities. In many aquatic systems, metal concentrations are elevated over natural background levels due to a continuous release of metals from industrial and agricultural sources. Frequently, metals are present as mixtures in the environment because of their concomitant release from mining activities or industrial uses. Copper and zinc play an important role in cellular metabolism acting as co- factors in a number of important enzymes. However, they can become toxic when elevated concentrations are introduced into the environment **(Marr *et al.*, 1996; Karan *et al.*, 1998).**

Metals differ from other toxic substances in that they are neither created nor destroyed by humans. Nevertheless, utilization by humans influences the potential for health effects in at least two major ways: First, by environmental transport, that is by human or anthropogenic contributions or air, water, soil and food, second by altering the speciation or biochemical form of the element **(Beijer and Jeernelov, 1986)**

Metals in raw surface water reflect erosion from natural sources, fallout from the atmosphere, and additions from industrial activities **(Goyer, 1985)**

2.4 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 **(Puel *et al*., 1987; USEPA, 1991)**. Bioaccumulation of metals can only take place if the rate of uptake by the organism exceeds the rate of elimination **(Specie and Hamelink, 1985)**. Metals are non-biodegradable, and once they enter the aquatic environment, bioconcentration 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, bioconcentration 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 (**Gumg *et al* 1994; Lee and Stuebing, 1990)**. 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) **(Nimmo *et al.* 1978, Nevo *et al*. 1986).**

The presence of pollutants have 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 sulfydryl 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**).

Mineral and trace metals are essential to life, as they constitute the basic structural components of the skeletal system, blood protein, enzymes and certain hormones and vitamins. The body uses minerals to produce energy and carry out important biochemical processes such as enzyme reactions, hormones production, and nerve transmissions **(Bennet and Dooly, 1982)**

Minerals toxicity is attributable to an acute dose or gradual build up of toxicant **(Mahmoud and Jarrar, 2002)**. Mineral toxicants are either macro minerals or micro trace minerals (heavy metal). Heavy metals have atomic weight between 63.549 and 200.59 and are usually stored in bones, muscle tissues and some organs All heavy metals exist in surface water in colloidal, particulate and dissolved phases although dissolved concentrations are generally low **(Kies,1989)**.

Aquatic organisms may be adversely affected by heavy metals in the aquatic environment. The toxicity is largely a function of the water chemistry, sediment composition and heavy metals concentration in the aquatic system. According to (**Jarup, 2003)** slightly elevated levels of metal in natural waters may cause one or more of the following sub lethal effects in the aquatic organisms:

1- Histological or morphological changes

2- Physiology such as suppression of growth and development and changes in circulation.

3- Enzymes activity and blood chemistry.

4- Behavior, especially swimming performance.

5- Reproductive activity.

Many organisms are able to regulate the metal concentrations in their tissues. Fish and crustacean can excrete essential metals, such as Cu, Zn and Fe that are present in excess. But excretion of non essential metals, such as Cd, is usually met with less success **(Connel, 1975)**.

Mullet, *Mugil cephalus*, collected from the polluted southern basin of Lake Macquarie (Australia) showed higher copper concentrations in their tissues as compared to those collected from the Clyde River estuary, comparatively pristine. Zinc concentrations in muscle tissues of fish from Lake Macquarie were higher than that from the Clyde River estuary, but were same in the other tissues. Metals concentrations in tissues were at levels that may reduce or effect fish growth, reproduction and survival **(Kirby *et al*., 2001).**

Heavy metals are stable and persistent environmental contaminants of both fresh and marine waters and their sediments. Interest in metals like Zn, Cu, Fe and Mn, which are required for metabolic activities in organisms, lies in the narrow "window" between their essentiality and toxicity. Other heavy metals like Cd, Hg, Cr and Pb may exhibit extreme toxicity even at low levels under certain conditions, thus necessitating regular monitoring of sensitive Aquatic environments **(Fatoki and Mathabatha, 2001)**

Sediments represent an important sink for trace metals in aquatic systems, and metal concentrations in sediment can be several orders of magnitude greater than in the overlying water. Sediment-associated metals pose a direct risk to decrial and deposit-feeding benthic organisms, and may also represent long-term sources of contamination to higher tropic levels **(Eimers, *et al,* 2001; Luoma, 1983, 1989).**

2.5 Toxicity of heavy metals:

Heavy and toxic metals are 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)**.Once they have entered the human body through food chain, they go none accumulating in different parts of the human body producing adverse effects. Presence of these metals in water even at very low levels must be monitored because in water these are present in soluble form and directly go to human metabolism.

Human destructive influence on the aquatic environment is in the form of sub lethal pollution which results in chronic stress conditions that have negative effect on aquatic life. The main source of freshwater pollution can be attributed to discharge of untreated waste, dumping of industrial effluent and run-off from agricultural fields. Stress response is characterized by physiological changes and effect of pollutants on fish is assessed by acute and chronic toxicity tests.

2.6 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, 1992**). Copper is naturally occurring in rocks, soil, water, and air, as well as in plants and animals **(ATSDR, 1999c)**. It can be introduced into the environment after being dissolved from plumbing pipes and fixtures, in storm water runoff in agricultural settings, and in copper smelting. Upon entering the water, copper dissolves rapidly and binds to suspended particles; some can be taken up by plants and animals **(ATSDR, 1999c)**.

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 **(Forstner and Wittmann, 1979)**.

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), Cu is accumulated by aquatic organisms with concentration factor of $5x10^3$ in the soft tissues of mollusks, and of $1x10^3$ in the muscles of fish. Crustaceans appear to be the most adept at regulating Cu concentrations in their bodies and detoxification occurs by formation of granules **(GESAMP, 1982)**

2.7 Cadmium:

Cadmium can be a byproduct of the smelting and refining of copper ores and is used in electroplating, paints, printing ink, and plastics. It is used as a stabilizer for polyvinyl chloride, in Electrical batteries (nickel-cadmium batteries), and in fluorescent and video tubes. It can enter the environment as a leach ate from buried wastes, through vaporization and atmospheric deposition, from metallurgical processes, and from the combustion of fossil fuels **(Hem, 1992)**. Once in the environment, cadmium can be bioaccumulated and biomagnified by animals and fish **(ATSDR, 1999b)**.

Measurable amount of cadmium occur in many soils and plant materials and some attention has been paid to its concentration in these materials for geochemical prospecting, in view of its association with zinc and other metals. The main anthropogenic pathway through which cadmium enters the environment is via wastes from industrial processes such as electroplating, plastics manufacturing, mining and metallurgical process. It is also used in the chemical and paint industries. Its often discharged as an effluent in to drainage course or reaches the atmosphere through stack emissions. Some naturally occurring concentrations are soils 0.03-0.3ug g^{-1}; plants materials 0.01-0.03ug g^{-1}; animal tissue 0.05-0.5 ug g^{-1} and freshwater 1-10ug g^{-1}. The exact mechanism for Tran epithelial uptake of cadmium in aquatic organisms is still not understood. However, evidence exists for an interaction between cadmium and the calcium uptake pathway: the ionic radii of cadmium (0.97^{o} A) and calcium (0.99^{o} A) are almost identical and it has been suggested that Cd^{2+} ions transverse the apical gill epithelium via Ca^{2+} -channels in aquatic animals. Cadmium uptake was shown to be inhibited by external lanthanum, unspecific calcium –channel. **(Sunda *et al.*, 1978; Engel and Fowler, 1979)**

There is much concern about the levels present in the environment, because it is an accumulative poison to mammals. It becomes concentrated in some organs where it can exceed 100ug g^{-1}. Cadmium accumulated in liver, kidney rather than in muscle and may be replacing the zinc in some enzymes and has a long half –life time (10-30yr) **(Kotsonis and Klaassen, 1977 and 1978)**.

The ingestion of small amount of contaminated fish over long periods may be leads to some form of cadmium intoxication.Cadmium intoxication can lead to oxidative damage in tissues by enhancing per oxidation of cell membrane lipids and altering the antioxidant defense armory of the cells. Cadmium induced Hepatic and renal injury in chronically exposed rats, likely role of hepatic cadmium-metallothionein in nephrotoxicity. Severe contamination with cadmium leads to itai-itai disease. The human health action level for cadmium contaminant is 3.7ug g^{-1} wet weight **(USFDA, 1993; El-Sikaily *et al*., 2003)**

2.8 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 hydroxyl-iron 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 does appear to affect the pathogenesis of infectious disease.

2.9 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 lead-cycle caused by human production is much more extended than the natural lead-cycle, and has caused lead 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, 1992).** It could be found in solder used to joint copper fittings, in pipes in water-distribution service lines, and in brass and bronze fixtures (Shelton, undated). **(Hem ,1992)** 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. Health concerns have caused a decrease in the amount of lead used in gasoline; paints, ceramic products, caulking, and pipe solder **(ATSDR, 1999d)**

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 $^{-1}$; plants materials 0.05-3ug 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 sub-clinical 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).**

2.10 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, 1992).** Zinc also is used to make ointments, dyes, and wood preservatives **(ATSDR, 1995).** Many forms of zinc, including zinc chloride, zinc oxide, zinc sulfate, and zinc sulfide, are found in hazardous waste sites (**ATSDR, 1995).** Zinc enters the environment as a leach ate. Although most zinc binds tightly to soil particles, small amounts can be taken up by fish **(ATSDR, 1995).**

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 **(koller, 1980; 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 **(koller, 1980; El-Sikaily *et al,* 2003).**

2.11 Chromium:

Chromium (Cr) is an essential trace metal for living organisms , but, its high toxicity, mutagencity and carcinogenicity render it hazardous at very low concentration **(Cheung *et al,* 2002)** .

Chromium, in various forms, is used in many industrial applications, such as chrome plating operations **(Hem, 1992).** It also is used in leather tanning processes and wood preserving **(ATSDR, 2001b).** It can enter the environment through fossil-fuel combustion, waste incineration, and cement-plant emissions (Shelton, undated). Once in the environment, chromium Adheres strongly to soil particles; little is dissolved in water and taken up by fish **(ATSDR, 2001b**).

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 be eventually bioaccumulated to the higher levels of the trophic web **(Peso-Aguiar *et al*.,2002)** to as being from 1-10mg/l, in a large variety of marine species **(GESAMP, 1982)**

Chapter three

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Sites:

For purposes of the fulfillment of the requirement of execution of the study programme that covers the periods January 2014 to March 2015 the following subsections were duly adopted in order to lead subsequently to the anticipated results. Selection of the study sites undergone procedure and after preliminary surveys. It is considered that study sites should represent, as much as possible, a wide range of White and Blue Nile around Khartoum and different human activities. The study sites were:

3.1.1 The White Nile three main sites were selected and they were:

1/ Jebel Awlia dam located across the White Nile some 50 kilometers south of Khartoum at the open water of White Nile.

2/ Eldobaseen bridge area near the exit of the sewage effluence treatment ponds in southern Khartoum town.

3/Ellamab site near the Khartoum industrial zone

3.1.2 The Blue Nile the main sites selected were as follows:

1/Soba west around the industrial zone

2/ Algreef shareg as Small landing site considered as clean site

3/Bori area before meeting White Nile at Almogran near Bori thermal Station

The study sites location were shown in (fig.1 and table.1)

Table 1. Location of the study sites:

River name	*Site No*	*Location name*	*GPS Location*
White Nile	**1**	Jebel Awlia dam	15°14'19"N 32°28'42"E
	2	Eldobaseen bridge area	15°30'45"N 32°28'18"E
	3	Ellamab area	15°44'59"N 32°28'10"E
Blue Nile	**4**	Bori area	15°36'45"N 32°33'24"E
	5	Algreef shareg	15 34 57.N 32 35 20E
	6	Soba west	15.44 59 N 32.46 E

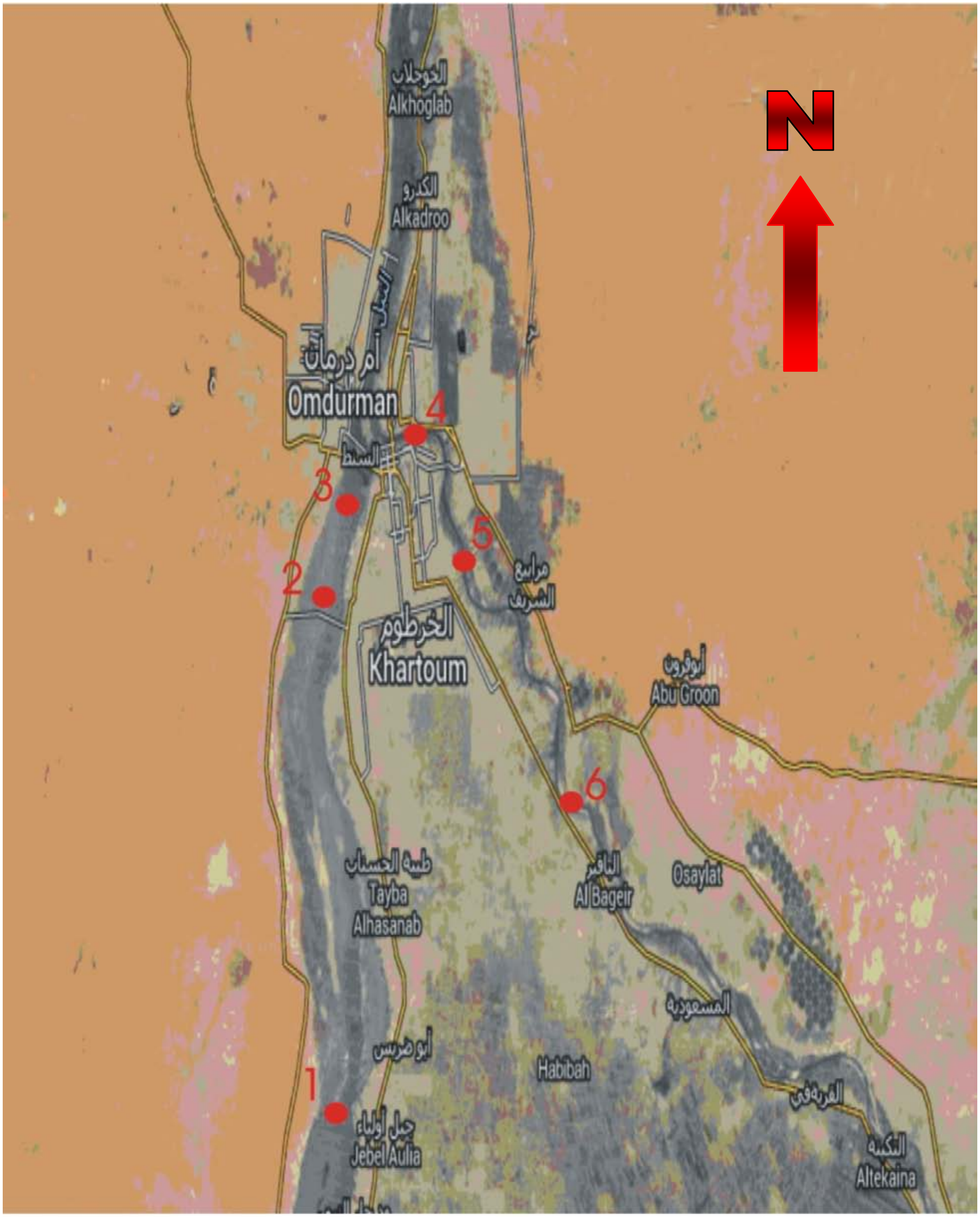

Fig.1 Location of the study sites along White and Blue Nile Rivers in Khartoum town.

3.2 Physiochemical parameters:

3.2.1 Physical measurements:

Temperature (C^o): surface water temperature was measured using an ordinary centigrade measuring thermometer.

PH: Water pH was determined by taking water sample and measuring its pH by portable digital Ph meter

3.2.2 Chemical measurements:

Dissolved oxygen: surface water sample were taken in 250 ml clean glass bottles. Samples were analyzed according to the standard Winkler method as modified by **(Grasshoff 1983)**

Nutrients: surface water sample were taken in 1000ml clean plastic bottles and analyzed according to **(ROMPE. 1999)** for nitrate and phosphate concentrations

3.3 Samples collection and pretreatment

Generally, when studying water pollution some clearly contaminated stations are singled out and compared with a clean monitoring site. In this study were similar sites were examined in order to identify useful background levels as a reference for intra specific comparisons within White and Blue Nile around Khartoum State on which information has been very scarce. Comparisons with control sites were also done to see if there is any impact. For a broader picture of both the environmental conditions of the area under investigation and possible bioaccumulation patterns, sediment samples were collected to assess total concentrations.

A total of 180 sample of *tilapia* fish and 180sample of *claries* fish from the two rivers at the six stations. Also 90 sample of water and 90 samples of sediments were collected from the two rivers 10 samples of each were collected seasonally from each river. Details of sampling location are presented in figure (1) and the GPS reading of the sampling sites were given in table (1)

3.3.1 Sediments samples:

Surface Sediment samples were taken using a P V C cylindrical tube sampler, placed in plastic bags and transported to the laboratory for further investigation.

3.3.2 Water samples

Thirty replicates of water samples were taken from a depth of 60cm or less from each site at morning hours. Direct measurements were taken in the field except dissolved oxygen and heavy metal that were measured at the laboratory. Water samples were preserved by the addition of one ml of concentrated nitric acid per liter until the time of analysis.

3.3.3 Fish samples:

Two different commercial fish species were chosen according to their trophic level. Nile tilapia, Oreochromis *niloticus* and African Cat Fish, *Clarias gariepinus*, sampled from the White and the Blue Nile rivers at the three stations on each river at three seasons.

Each sample collected was dissected and samples from muscle, gill, kidney and liver tissues were taken for determining the heavy metals and histopathological changes.

3.4 Determination of heavy metals

3.4.1 Sediments samples:

For determining the heavy metals of sediments samples: weighing out (0.5-1g) of powder were taken in a beaker, moisted with distilled water , 10 ml concentrated of hydrofluoric acid + 5 ml concentrated of perchloric acid (HCIO4) +2.3ml concentrated nitric acid were added . Samples were heated using a hot sand bath to dryness. The residues were dissolved in 10 ml (50%) HCL then heated on a hot plate for 4 minutes and transfered to polyethylene flask. Then the target elements were measured using Flame Atomic Absorption Spectrophotometer. According to **(Price *et al* 1997)**

3.4.2 Water samples:

Water samples were filtered through 0.45µl membrane filter. The required volume (100 ml) of the filtrate was collected and (Zinc, Copper, Cadmium, Lead, Iron and Chromium) were measured by `Flame Atomic Absorption Spectrophotometer **(Price *et al,* 1997)**

3.4.3 Fish samples:

The fish flesh samples were dried using an electric oven at 105c for at least 9 hours till a constant weight is achieved. Dry samples were grinded using manual porcelain mortar. All tools were made of stainless steel to avoid contamination with metal residues. Powder samples were kept in air tight plastic bags till they were used for analysis.

Weight 2.0g of powder sample placed in a silica evaporating crucible of known weigh, the crucible was placed on a hot plate and allow smoking until completely charred the transferred to a muffle furnace at of 470 c and ashed at this temperature for three hours. When ashing is complete, cooled, and extracted, with minimum amount of hydrochloric acid. Then Evaporated to dryness, then extracted again with 10ml of 25% HCL, boiled and filtered into 100ml calibrated flask, the filter washed with warm 1%HCL, solution and make up to 100ml with water and mixed. This solution is used for the determination of heavy metals. The procedure used was followed by **(Price *et al,* 1997)**.

3.5 HIistopathological Examination:

Tissue specimens from fresh fish were taken from (gills, muscles, kidney and livers) and fixed in 10 % buffered neutral formalin. Then processed using the normal histological techniques to obtain five micron thick paraffin sections then stained with Hematoxylin and Eosin and examined under light microscope.

3.6 Statistical Analysis:

Statistical analysis of data was carried out using SPSS statistical package program Factorial arrangement 3X2X2X2 CRBD complete randomized design followed by LSD

Chapter four

CHAPTER FOUR

RESULTS

4.1 Physiochemical parameters:

Physic-chemical characteristics of the sampling sites are summarized in table (2 and 3). The results showed that the highest water temperature from Blue Nile in summer while lower was found at White Nile in winter. The pH, NO3, PO4 was significantly higher at White Nile.

Table (2). Physic-chemical characteristics of the water at White and Blue Nile at different season.

Factors		water.temp (c)	pH	Do2 (ml/l)	NO3 (ml/l)	PO4 (ug/l)
Season	**Water Source**					
Winter	White Nile	25.1	7.2	7.8	0.93	2.14
	Blue Nile	29.2	6.5	8.4	0.26	1.33
Summer	White Nile	26.1	6.6	6.7	0.19	1.00
	Blue Nile	31.2	6.5	6.6	0.78	0. 81
Autumn	White Nile	26.7	7.0	7.0	0.94	1.13
	Blue Nile	31.2	6.5	7.0	0.62	0.89
White Nile		26.2	6.9*	7.1	0.68*	1.42*
Blue Nile		31.8*	6.7	7.3*	0.55	1.01
Winter		27.5^{b}	6.3^{c}	8.1^{a}	0.54^{b}	1.73^{c}
Summer		30.6^{a}	6.9^{a}	6.6^{b}	0.48^{c}	0.90^{b}
Autumn		28.5^{b}	6.6^{b}	7.0^{b}	0.78^{a}	1.01^{a}

*: significant at (P<0.05).

a,b,c, means within the same column followed by different superscripts are significantly different.

Table (3) The Physic-chemical characteristics of water from different stations in White and Blue Nile.

stations	Parameters				
	water.temp (c)	pH	Do2 (ml/l)	NO3 (ml/l)	PO4 (ug/l)
Jebal awli	25.4	6,6[b]	7.7[a]	0.23[c]	1.80[c]
Adubaseen	26.0	7.2[a]	6.8[c]	0.83[b]	2.00[b]
Alamab	26.7	7.1[a]	7.0[b]	0.96[a]	3.11[a]
Soba	29.0	6.9[b]	6.9[c]	0.74[a]	1.80[a]
Algreef	30.7	6.5[c]	7.5[a]	0.30[b]	1.73[b]
Bori	30.8	7.0[a]	7.4[b]	0.63[c]	1.59[c]

*: significant at ($P<0.05$).

a,b,c, means within the same column followed by different superscripts are significantly different.

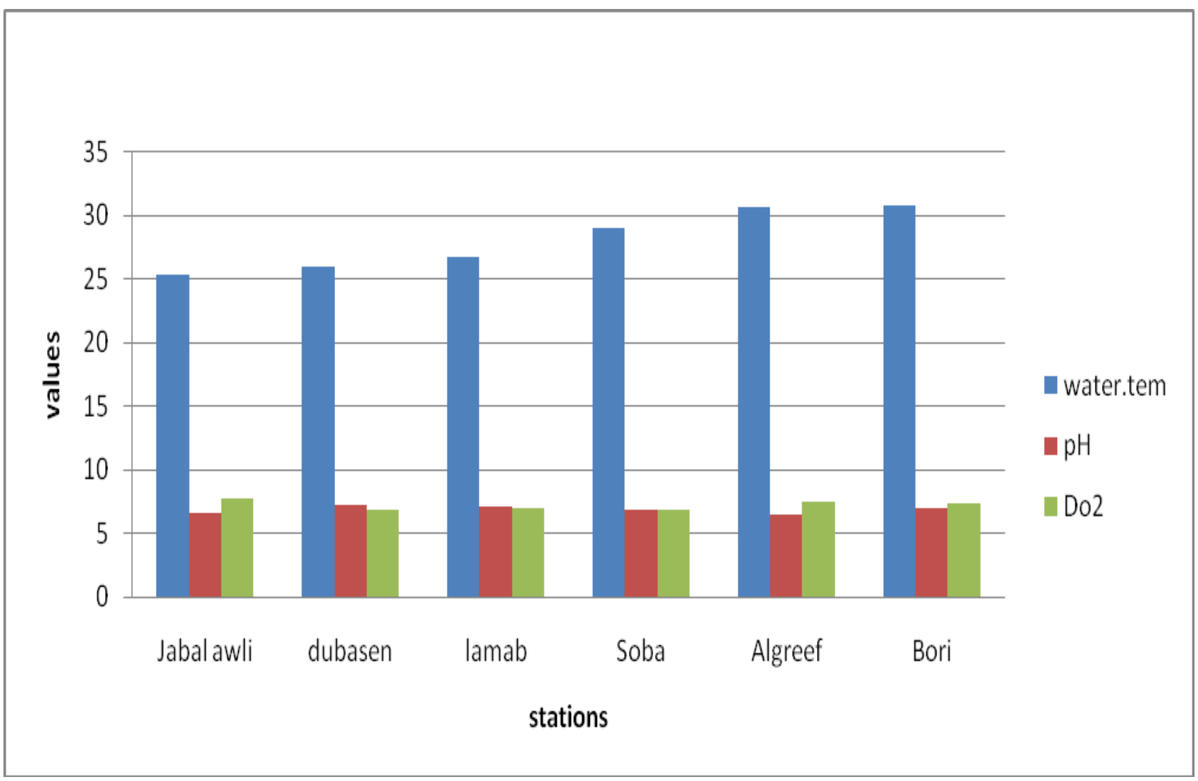

Fig.(2) Distribution of water temperature, pH and dissolved oxygen in water from different stations in White and Blue Nile.

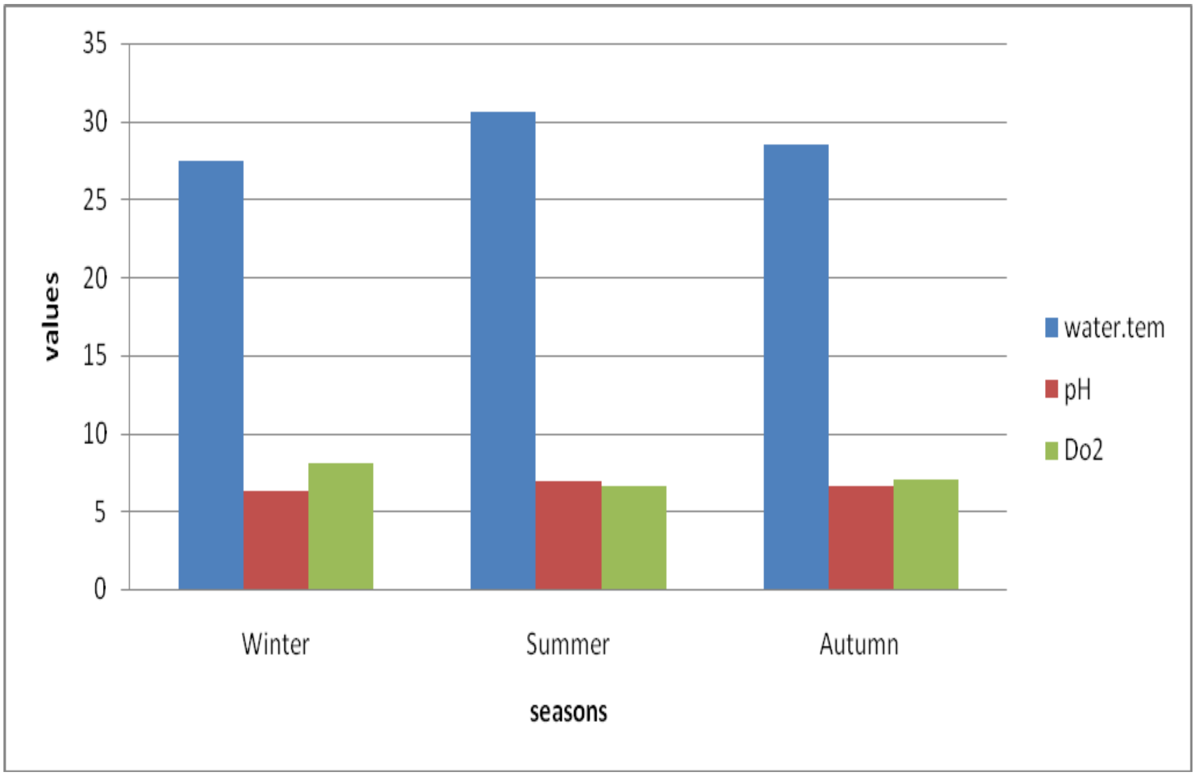

Fig.(3) Distribution of water temperature, pH and dissolved oxygen in the water of White and Blue Nile in different seasons

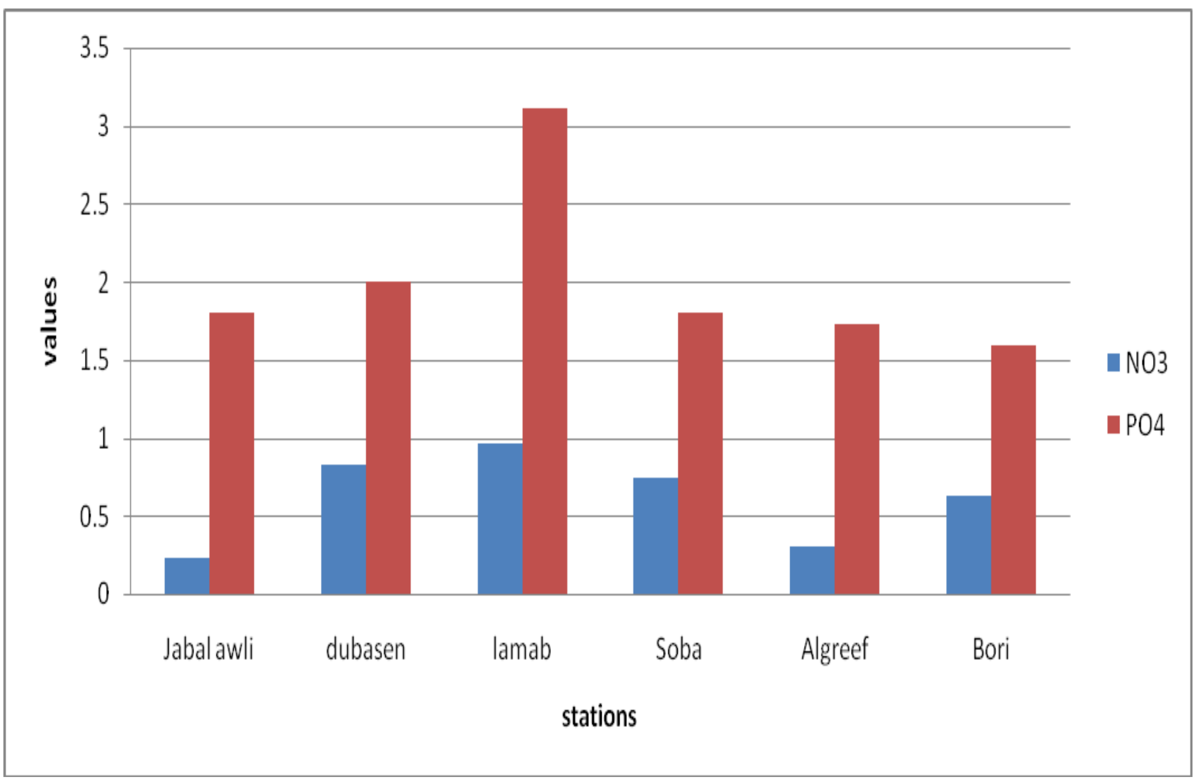

Fig.(4) Distribution of nitrate and phosphate in the water from different stations in White and Blue Nile

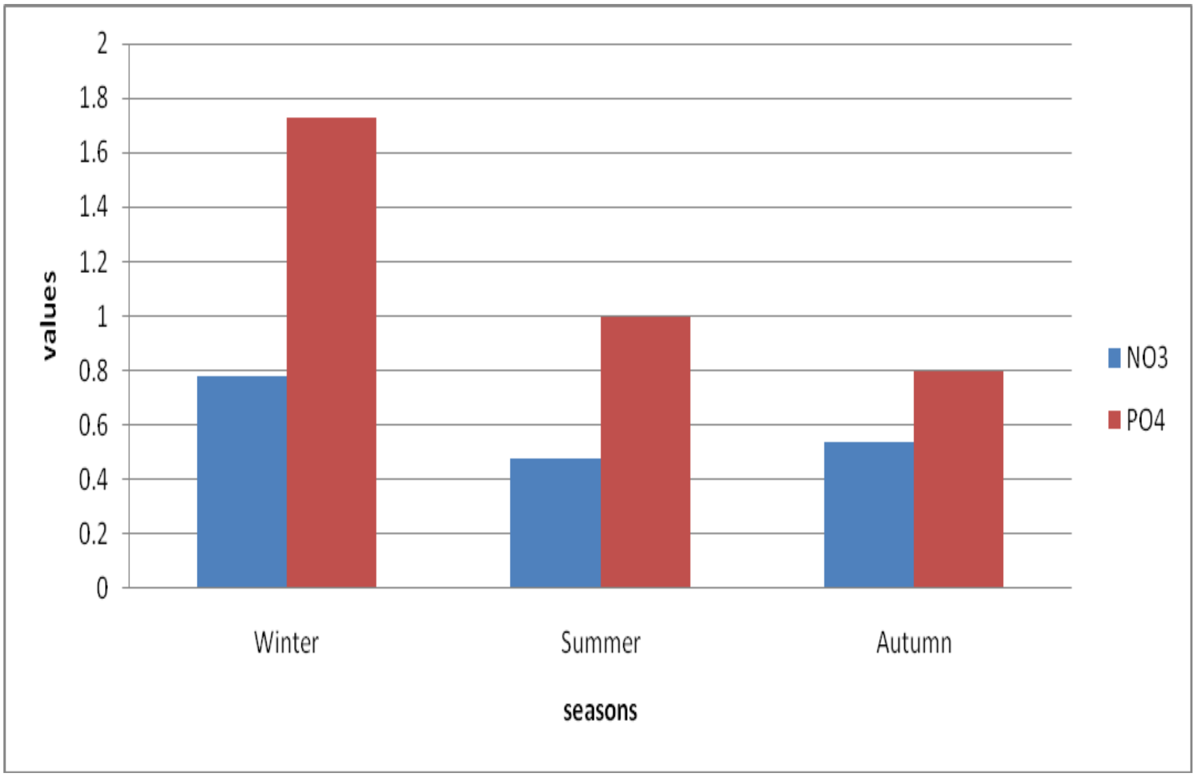

Fig.(5) Distribution of nitrate and phosphate in the water of White and Blue Nile in different seasons

4.2 Heavy metals in water samples:

The results of the levels of concentration of heavy metals in water samples are presented in Table (4, 5). Figures (6, 7)

The results showed that the highest heavy metal concentration in water. The mean concentration of Cu in water from Blue Nile was 0.80±.05 ppm which was significantly higher than that from White Nile 0.61±01 ppm.

Cr in water sample from the Blue Nile was 0.05±.08 ppm was significantly lower than that from White Nile 0.06±.002 ppm.

Cd and Pb were higher in the Blue Nile and they were 0.20±.05 and 0.20±.06 respectively. While in the White Nile were 0.06±.06 and 0.04±0.02 ppm respectively.

Fe concentration from Blue Nile was0.05±.08 ppm it was significantly lower than White Nile 0.08±.28 ppm.

There was no significant difference in Zn concentration between White and Blue Nile

The order of heavy metals concentration in water samples in the two rivers was

Cu > Zn > Cd > Pb >Fe > Cr.

Table (4) Shows Heavy metals concentration in waters from White and Blue Nile in different seasons:

Factors		Cu	Cr	Cd	pb	Zn	Fe
Se	WaterSource						
W	WN	0.98	0.04	0.04	0.01	0. 04	0.04
	BN	0.93	0.03	0.09	0.12	0.09	0.02
S	WN	0. 25	0.12	0.12	0.08	0.61	0.07
	BN	0.78	0.09	0.44	0.42	0.33	0.04
Au	WN	0.61	0.03	0.03	0.03	0.26	0.15
	BN	0.70	0.03	0.07	0.06	0.41	0.10
WN		0.61±01	0.06±.002*	0.06±.06	0.04±0.02	0.30±.08	0.08±.28*
BN		0.80±.05*	0.05±.08	0.20±.05*	0.20±.06*	0.27±.02	0.05±.08
Winter		0.95±.02[a]	0.03±.04[b]	0.06±.08[b]	0.06±.07[b]	0.06±.13[c]	0.03±.03[b]
Summer		0.51±.47[b]	0.10±.06[a]	0.28±.09[a]	0.25±.02[a]	0.47±.06[a]	0.05±04[b]
Autumn		0.65±.08[b]	0.03±.05[b]	0.05±.03[b]	0.01±.21[c]	0.33±.03[b]	0.12±.08[a]
Permissible limits(WHO,2011)		1.0	0.3	0.5	0.5	0.5	0.3

*: significant at (P<0.05). a,b,c, means within the same column followed by different superscripts are significantly different (P<0.05).

The interactions between subjects are significant 0.000 except Cu and Cr.

Table (5) Shows Heavy metals concentration in water in different stations from White Nile and Blue Nile

stations	Heavy metals											
	Cu	**(%)**	**Cr**	**(%)**	**Cd**	**(%)**	**Pb**	**(%)**	**Zn**	**(%)**	**Fe**	**(%)**
Jabal awli	0.01[c]	67%	0.01[c]	7%	0.01[c]	57%	0.03[c]	60%	0.10[c]	16%	0.10 [a]	43%
dubasen	0.04[b]	80%	0.02[b]	22%	0.04b	80%	0.14[a]	84%	0.42[a]	51%	0.11 [a]	31%
lamab	0.51[a]	29%	0.20[a]	59%	0.06 [a]	16%	0.03[b]	9%	0.39 [b]	58%	0.05[b]	51%
Soba	0.04[b]	77%	0.12 [a]	77%	0.16 [a]	64%	0.18[a]	80%	0.44[a]	44%	0.07 [a]	87%
Algreef	0.70[a]	11%	0.01 [b]	3%	0.07[b]	10%	0.02 [c]	3%	0.08[c]	9%	0.06[a]	2%
Bori	0.79 [a]	19%	0.02 [b]	5%	0.16[a]	4%	0.10 [b]	17%	0.31[b]	2%	0.03[b]	19%

%: the percentage of metal occurrence in all samples.

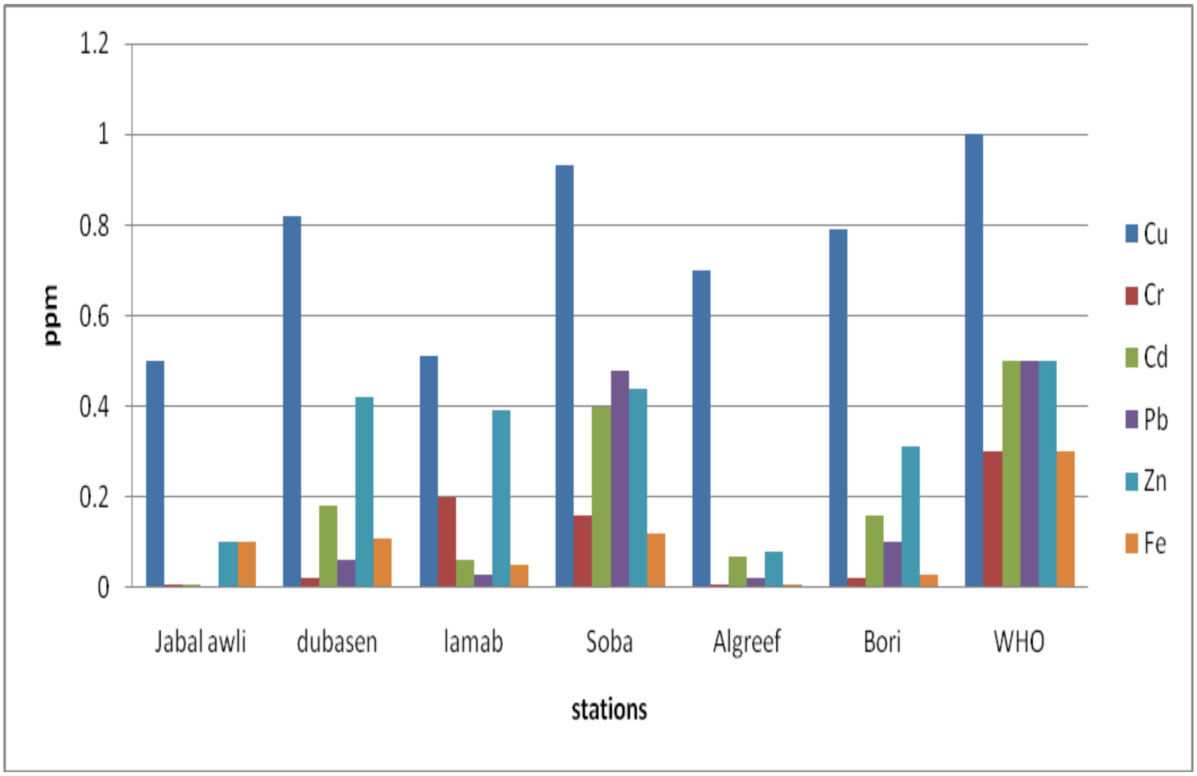

Fig (6). Heavy metals in water from different stations in White and Blue Nile visa WHO guidelines.

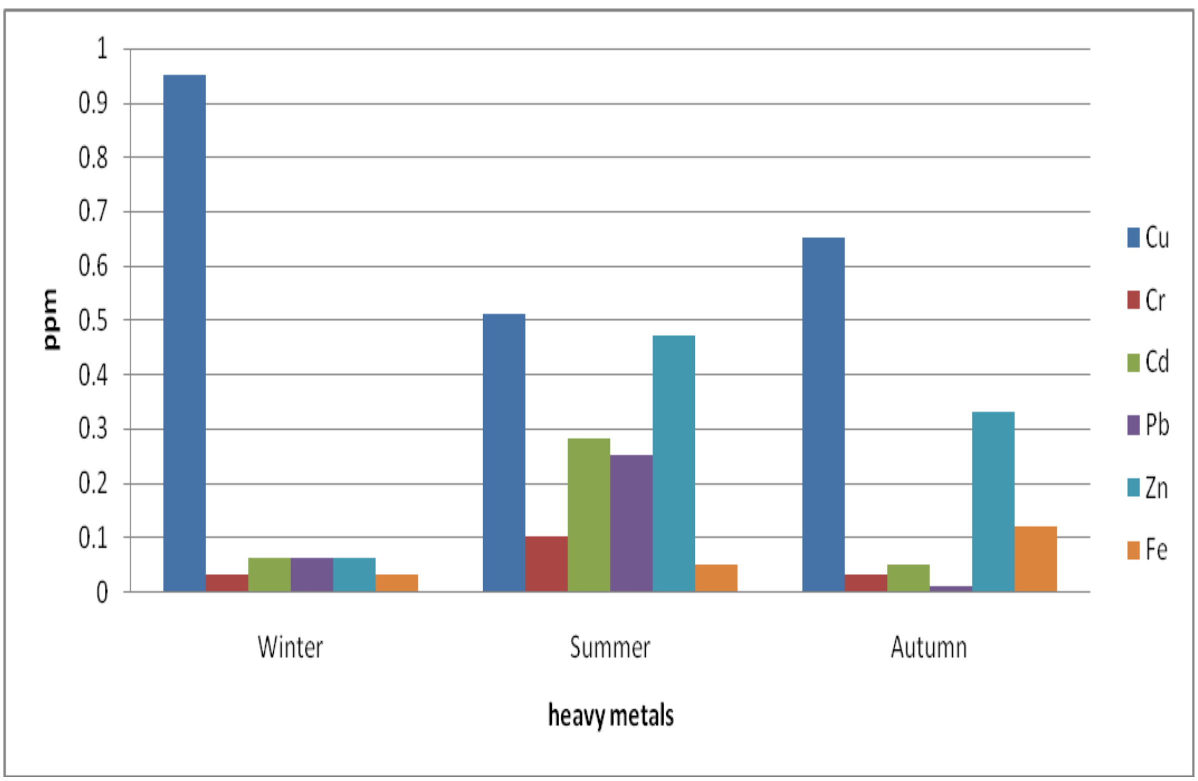

Fig (7). Heavy metals in water of White and Blue Nile in different seasons.

4.3 Heavy metals in sediment samples:

The results of the levels of concentration of heavy metals in sediments are presented in Table (6, 7). Figures (8.9).

The results showed that Cu was the highest concentrated heavy metals in sediments from Blue Nile 2.76±.31; while it was 2.30±.06 from White Nile

The mean concentration of Cd and Pb in the sediment from Blue Nile was 1.55±.21 and 2.12±.08 respectively and it were significantly higher than that from White Nile 1.38±.05 and 1.20±.06 respectively

The mean concentration of Cr and Fe in the sediment from White Nile was 1.70±.10and 1.99±.07 respectively and it were significantly higher than that from Blue Nile 1.09±.09 and 1.43±.04respectively

There was no significant difference between White and Blue Nile in accumulation of Zn in sediments.

The order of Cu, Cd, Pb and Fe concentration in sediments from all stations in different season was summer > winter > autumn. And summer, winter > autumn for Cr and Zn.

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Table (6) Shows Heavy metals concentration in sediments from White and Blue Nile in different seasons:

Factors		Cu	Cr	Cd	pb	Zn	Fe
Se	WS						
W	WN	2.40	1.95	1.31	1.02	1.73	2.01
	BN	2.50	1.01	1.49	2.22	1.63	1.32
S	WN	3.32	2.26	1.93	1.74	1.82	2.73
	BN	4.47	1.30	2.17	2.97	1.92	1.99
Au	WN	1.19	0.90	0.91	0.85	0.96	1.23
	BN	1.31	0.71	1.01	1.18	0.82	0.97
WN		2.30±.06	1.70±.10*	1.38±.05	1.20±.08	1.50±.05	1.99±.07*
BN		2.76±.31*	1.09±.09	1.55±.21*	2.12±.06*	1.46±.06	1.43±.04
Winter		2.45±.07[b]	1.48±.16[a]	1.40±.12[b]	1.62±.01[b]	1.68±.15[a]	1.66±.05[b]
Summer		3.89±.09[a]	1.78±.08[a]	2.05±.08[a]	2.35±.05[a]	1.87±.27[a]	2.36±.09[a]
Autumn		1.25±.07[c]	0.80±.06[b]	0.96±.01[c]	1.01±.19[c]	0.89±.29[b]	1.10±.08[c]

*: significant at (P<0.05). a,b,c, means within the same column followed by different superscripts are significantly different (P<0.05).

Table (7) Shows Heavy metals concentration in sediments in different stations From White and Blue Nile

stations	Heavy metals											
	Cu	(%)	Cr	(%)	Cd	(%)	Pb	(%)	Zn	(%)	Fe	(%)
Jabal awli	1.22b	15%	0.51[c]	12%	0.56[c]	16%	0.35 [b]	12%	0.76[b]	6%	1.40 [c]	53%
dubasen	2.93 [a]	55%	1.66[b]	37%	1.98 [a]	39%	1.72 [a]	38%	1.90[a]	35%	2.71 [a]	31%
lamab	2.75 [a]	41%	2.94[a]	78%	1.54 [b]	33%	1.54[a]	30%	1.84 [a]	27%	1.86[b]	58%
Soba	3.75[a]	53%	1.78 [a]	26%	1.91[a]	42%	2.90[a]	36%	1.95[a]	54%	1.98 [a]	40%
Algreef	1.83[c]	18%	0.69 [b]	8%	1.23[c]	17%	1.05 [c]	5%	0.89[c]	12%	0.56[c]	6%
Bori	2.72 [b]	29%	0.80 [b]	10%	1.52[b]	19%	2.41 [b]	32%	1.56[b]	8%	1.77[b]	21%

%: the percentage of metal occurrence in all samples.

The interactions between subjects are not significant of all factors except the cadmium in sediments

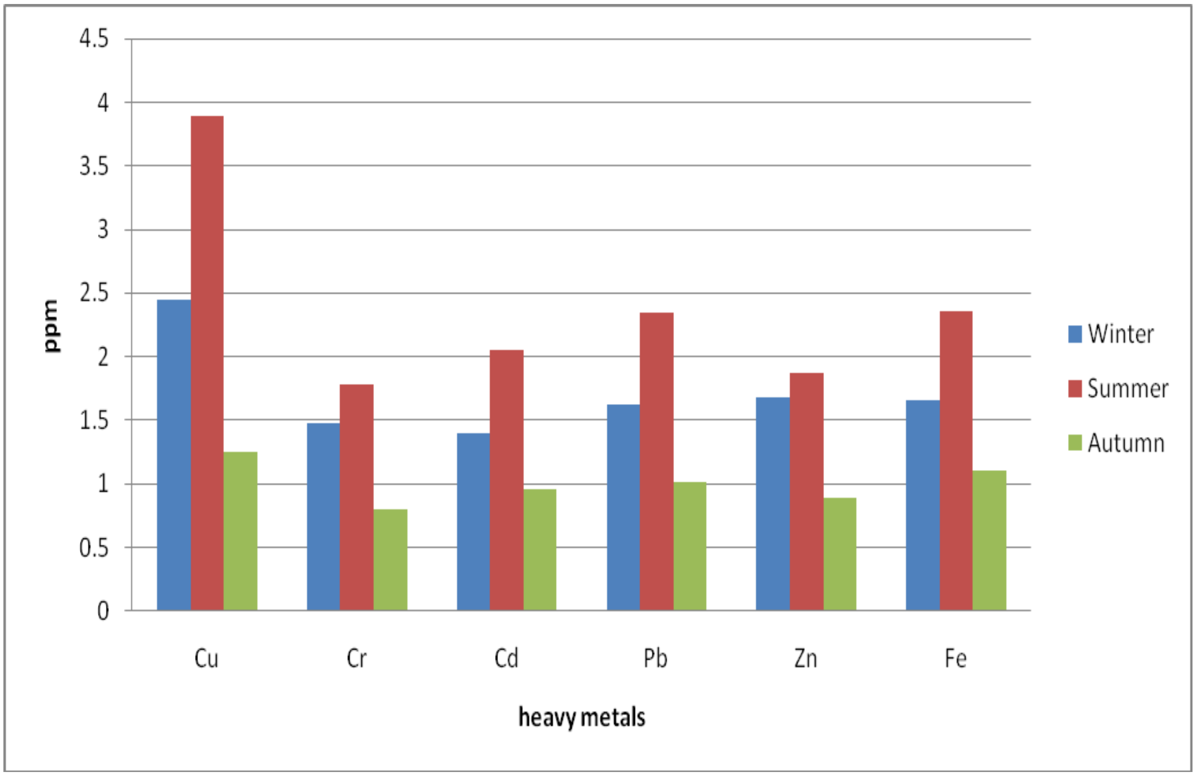

Fig :(8). Heavy metals in sediments of White and Blue Nile in different seasons.

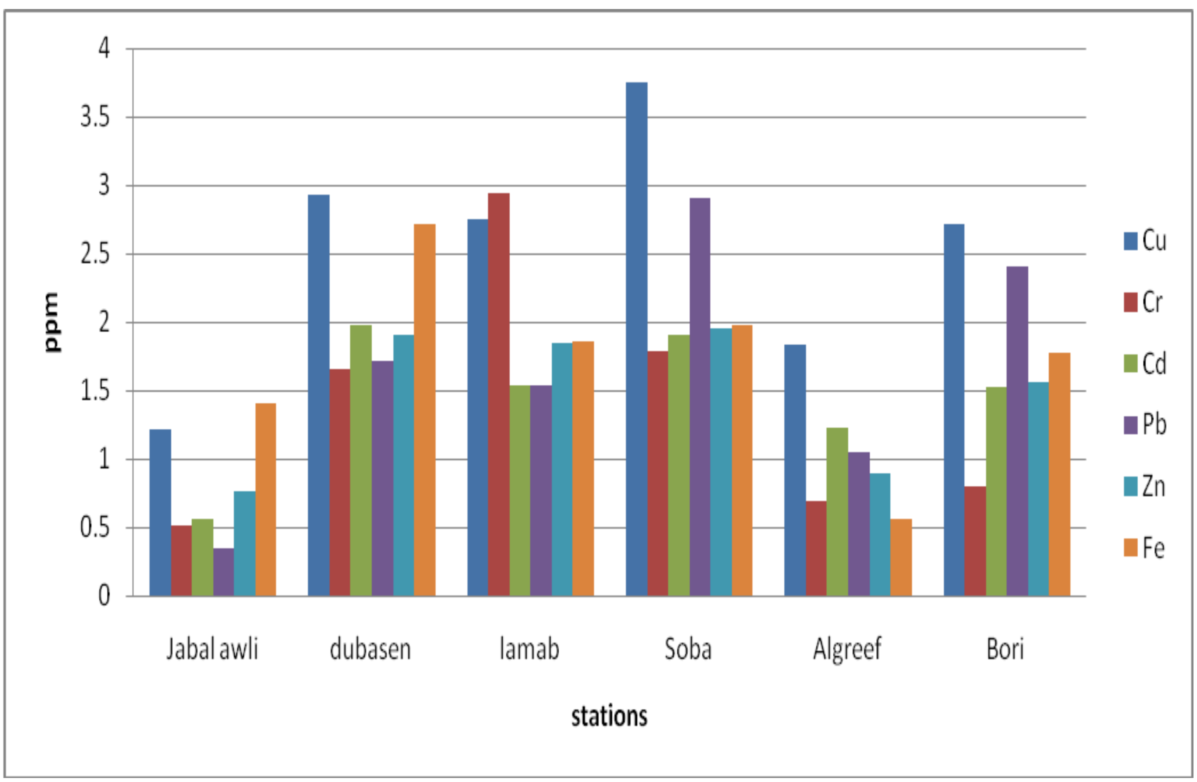

Fig :(9). Heavy metals in sediments of White and Blue Nile from different stations.

4.4 Heavy metals in fish muscles samples:

The results of the levels of concentration of heavy metals in fish muscle samples from different stations are presented in Table (8, 9, 10 and 11) figure (10, 11 and 12).

The percentage of occurrence of Cu in muscle of fish samples from different stations Jabal Awlia, Dobasen, Alamab. Soba, Algreef, and Bori were rage between 3% to 19% and occurrence of Cr in fish muscles were rage between 2% to 18% and Cd were rage between 2% to 19% and Pb were rage between 5% to 52% and Zn were rage between 6% to 61 and Fe rage between 4% to 21%.

The highest mean concentration of Cu was 0.59 ±0.02 ppm in *Clarias gariepinus* fishes in the Soba station while *Oreochromis niloticus* had the lower 0.34±.03 ppm. But *Oreochromis niloticus* had the highest levels of Cr (0.21±0.02 ppm), Zn (0.54 ± .05 ppm) and Fe (0.21 ± .02 ppm) than *Clarias gariepinus* ($p < 0.05$). This had Cr (0.14±.05 ppm), Zn (0.36 ±.02 ppm) and Fe (0.07 ±.01 ppm). There is no significant difference between *tilapia* and *Clarias* in accumulation of Cd and Pb in muscle.

The lowest value of Cu, Cd and Pb was found in the fish muscles collected from the White Nile they were 0.28±.01and 0.09± .02 and 0.23± .04 respectively, while the highest values of Cu, Cd and Pb was found in the fish muscles from Blue Nile 0.43± .03 and 0.27±.05 and 0.41±.04 respectively. The mean concentration of Cr in the fishes muscle from the Blue Nile was 0.07±.02ppm. It was significantly lower than that from the White Nile 0.29± .02 ($p < 0.05$).

There was no significant difference between White Nile and Blue Nile in accumulation of Zn and Fe in fish muscle. Also there were no significant difference between the female and male in accumulation of all hevay metals in fish muscles ($p < 0.05$). All heavy metals in fish muscle were below the Permissible limits **(WHO, 2011)** .

The order of Cu, Cr concentration in fishes muscles from all stations in different season was summer > winter > autumn. And for Pb and Zn were summer, winter > autumn But there is no significant difference in the accumulation of Cd and Fe in all seasons.

Table (8) Heavy metals concentration in fish muscles from White and Blue Nile in different seasons

Factors		**Cu**				**Cr**				Cd			
		Tilapia		*Clarias*		*Tilapia*		*Clarias*		*Tilapia*		*Clarias*	
Season	**Water S**	female	male	female	male	female	male	female	male	female	Male	female	male
Winter	WN	0.32	0.21	0.42	0.40	0.38	0.58	0.16	0.22	0.16	0.15	0.04	0.05
	BN	0.23	0.12	0.37	0.43	0.02	0.05	0.03	0.05	0.21	0.40	0.11	0.37
Summer	WN	0.56	0.92	0.43	0.33	0.42	0.38	0.19	0.29	0.09	0.08	0.06	0.03
	BN	0.24	0.45	0.39	0.20	0.06	0.14	0.09	0.06	0.28	0.32	0.27	0.36
Autumn	WN	0.26	0.31	0.06	0.14	0.19	0.18	0.38	0.15	0.13	0.03	0.11	0.13
	BN	0.32	0.26	0.19	0.19	0.08	0.09	0.09	0.07	0.20	0.19	0.20	0.20
White Nile		0.28±.01				0.29± .02*				0.09± .02			
Blue Nile		0.43± .03 *				0.07±.02				0.27±.05*			
Tilapia		0.34± .03				0.21± .02*				0.19± .02			
Clarias		0.59±.02*				0.14±.05				0.17±.01			
Winter		0.33±.04[b]				0.18±.025[b]				0.19±.02			
Summer		0.42±.02[a]				0.27±.039[a]				0.20±.07			
Autumn		0.23±.02[c]				0.15±.062[b]				0.15±.07			
Female		0.31±.03				0.17±.02				0.16±.02			
Male		0.33±.03				0.19±.02				0.19±.02			
Permissible limits(WHO,2011)		1.0				0.5				0.3			

*: s Significant (P<0.05). a,b,c, means within the same column followed by different superscripts are significantly different (P<0.05).

Table (9) Heavy metals concentration in fish muscle in different stations from White and Blue Nile.

stations	**Heavy metals**												
	Cu	(%)	Cr	(%)	Cd	(%)	**stations**	Cu	(%)	Cr	(%)	Cd	(%)
Jabal awli	0.41[a]	60%	0.03[c]	3%	0.11[a]	54%	Soba	0.58 [a]	17%	0.15[a]	6%	0.53[a]	19%
dubasen	0.36[b]	70%	0.31[b]	10%	0.12[a]	70%	Algreef	0.52[a]	6%	0.01[c]	2%	0.18[b]	2%
lamab	0.31[b]	10%	0.53 [a]	18%	0.06[b]	11%	Bori	0.19[b]	3%	0.05[b]	4%	0.10 [b]	2%

%: the percentage of metal occurrence in all samples.

Table (10) Heavy metals concentration in fish muscle from White and Blue Nile in different seasons

Factors		**Pb**				**Zn**				Fe			
		Tilapia		*Clarias*		*Tilapia*		*Clarias*		*Tilapia*		*Clarias*	
Season	**Water S**	female	male	Female	male	female	male	female	male	female	male	female	Male
Winter	WN	0.37	0.28	0.09	0.14	0.67	0.95	0.29	0.63	0.17	0.20	0.02	0.11
	BN	0.40	0.62	0.46	0.27	0.29	0.81	0.24	0.28	0.49	0.30	0.04	0.08
Summer	WN	0.35	0.32	0.15	0.17	0.68	0.60	0.14	0.19	0.17	0.14	0.03	0.04
	BN	0.09	0.41	0.20	0.49	0.23	0.68	0.82	0.43	0.12	0.28	0.09	0.07
Autumn	WN	0.34	0.14	0.07	0.18	0.48	0.53	0.09	0.32	0.10	0.07	0.10	0.08
	BN	0.25	0.11	0.40	0.18	0.30	0.16	0.29	0.28	0.11	0.22	0.17	0.09
White Nile		0.23± .04				0.47± .05				0.11± .02			
Blue Nile		0.41±.04*				0.41±.03				0.17±.07			
Tilapia		0.23 ± .04				0.54 ± .05*				0.21 ± .02*			
Clarias		0.24 ±.06				0.36 ±.02				0.07 ±.01			
Winter		0.32 ±.05[a]				0.49 ±.07[a]				0.14±.03			
Summer		0.37 ±.04[a]				0.51 ±.06[a]				0.14 ±.04			
Autumn		0.23 ±.06[b]				0.32 ±.05[b]				0.13 ±.06			
Female		0.35 ±.04				0.40 ±.06				0.14 ±.02			
Male		0.29 ±.04				0.49 ±.04				0.13 ±.02			
Permissible limits(WHO,2011)		0.5				1.0				0.5			

*: significant at ($P<0.05$). a,b,c,d means within the same column followed by different superscripts are significantly different ($P<0.05$).

Table (11) Heavy metals concentration in fish muscle in different stations From White and Blue Nile

stations	**Heavy metals**						
	Pb %	**Zn%**	Fe%	**stations**	**Pb%**	**Zn%**	Fe%
Jabal awli	0.30 [a] 67%	0.65 [a] 6%	0.13 [a] 12%	Soba	0.85[a] 52%	0.66[a] 22%	0.20[a] 21%
dubasen	0.24[b] 67%	0.43[b] 61%	0.16 [a] 16%	Algreef	0.11 [c] 5%	0.09[b] 10%	0.16 [b] 21%
lamab	0.19[b] 29%	0.37[b] 15%	0.04[c] 4%	Bori	0.27 [b] 16%	0.48 [a] 7%	0.15[c] 5%

%: the percentage of metal occurrence in all samples.

The interaction between subject were not significant for all factors except its significant ($P<0.05$ for Water Source X Fish In Cr and Zn. And for Water Source X seasons in Cu. and for Fish species X seasons in Cu and Cr

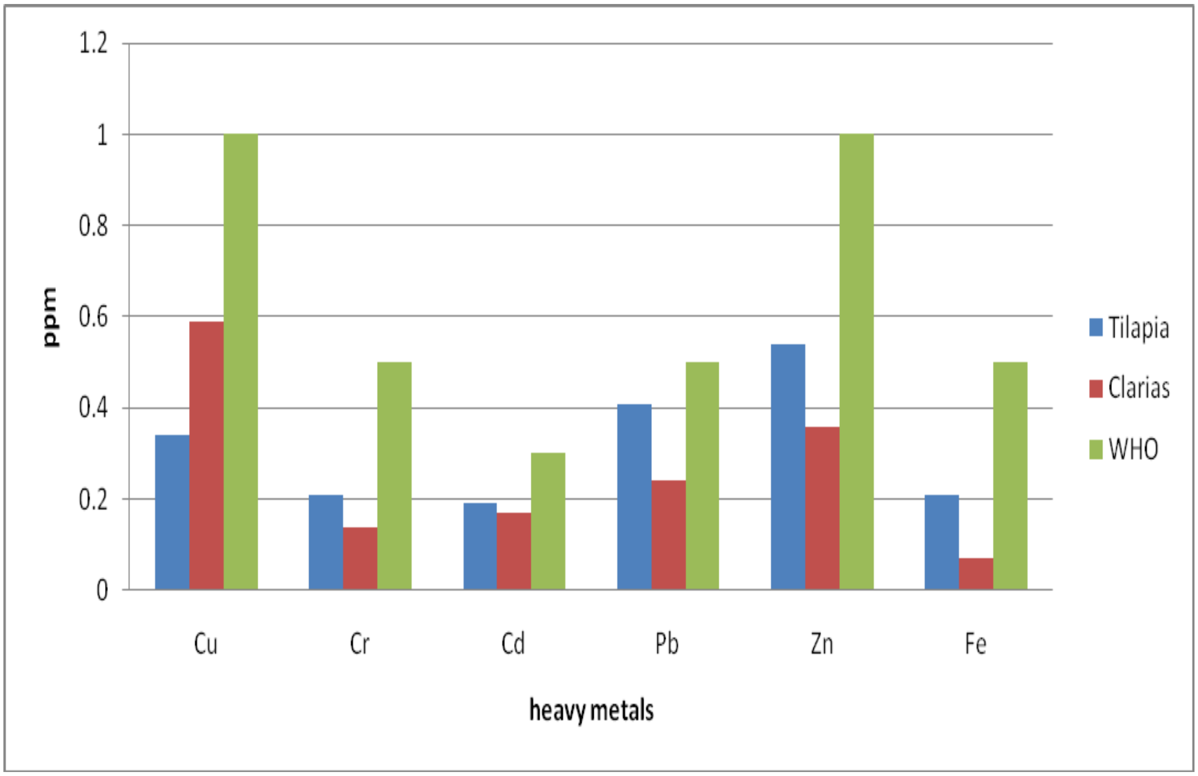

Fig: (10). The concentration of heavy metals in fish muscles of the Studied Fish species

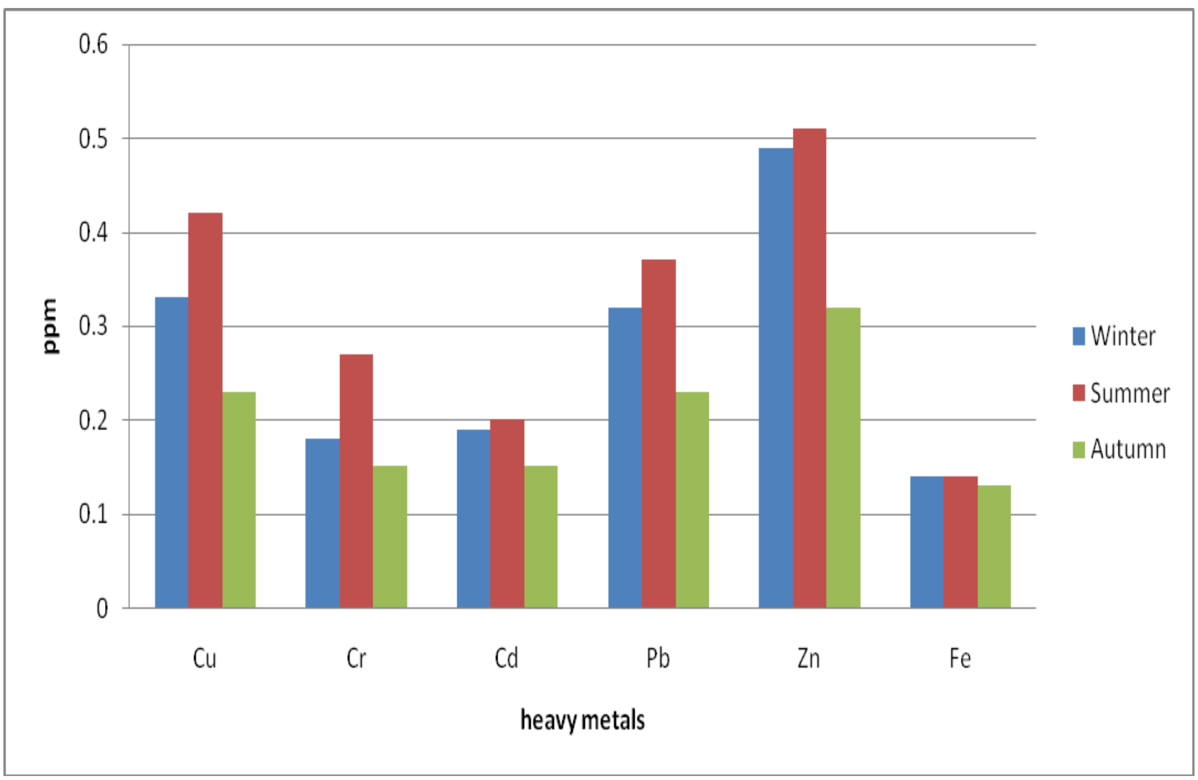

Fig: (11). The concentration of heavy metals in fish muscles in different seasons.

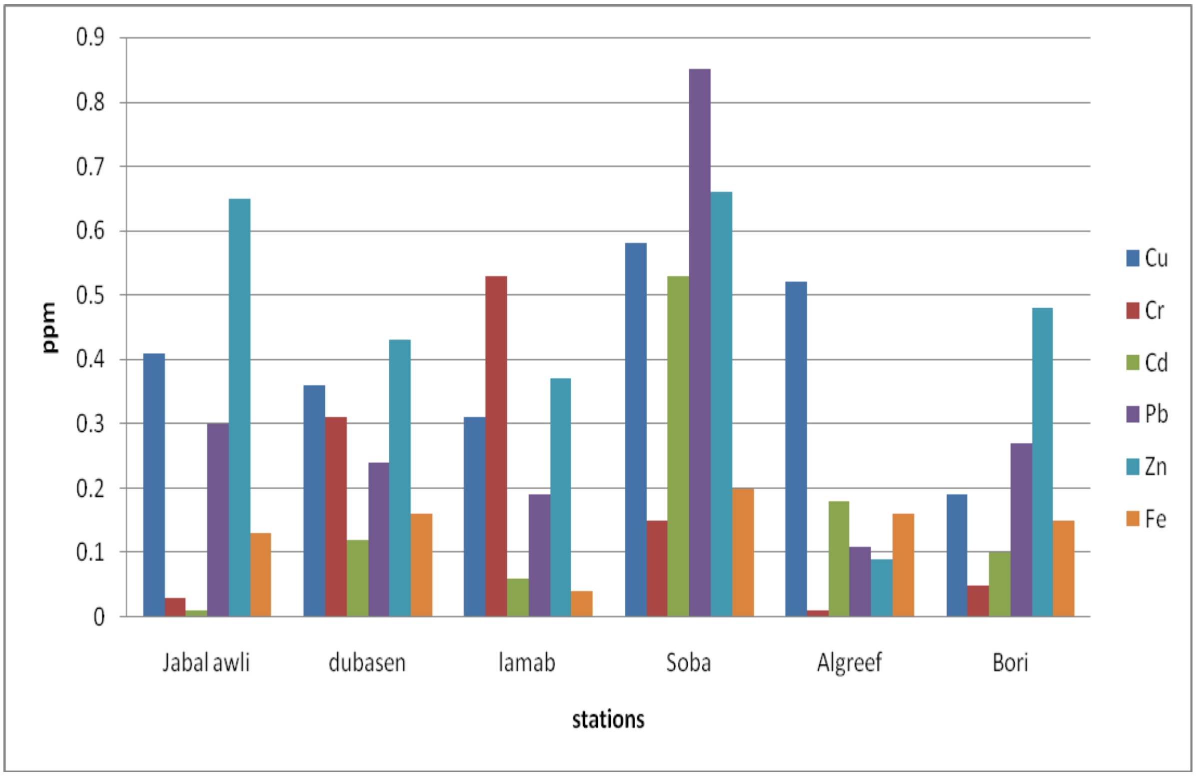

Fig: (12). The concentration of heavy metals in fish muscles in different station in White and Blue Nile.

4.5 Heavy metals in fish liver samples:

The results of the levels of concentration of heavy metals in fish liver samples from different stations are presented in Table (12, 13, 14 and 15) and figures (13, 14 and15).

The percent of occurrence of Cu in liver of fish from different stations Jebal awlia, Dobaseen, Alamab. Soba,Algreef, and Bori were range between 9% to 84% and Cr were range between 8% to 50% and Cd were range between 10% to 80% and Pb were were range between 10% to 81% and Zn were range between 5% to 72% and Fe were were range between 9% to 52%

The highest concentrations of Cu were 1.21 ±.14 in Oreochromis *niloticus*.and the lower was 0.91 ±.01 in *Clarias gariepinus*. And Cd is 0.43 ±.09 in *Clarias gariepinus* while it was 0.30 ± .04 in *Oreochromis niloticus* in different fishing stations. Also *Oreochromis niloticus* had the highest levels of Pb (1.85 ± .32) and Fe (0.29 ± .02) than *Clarias gariepinus* which had Pb (0.54 ±.07) and Fe (0.19 ±.09) ($p < 0.05$).

There is no significant difference between *Oreochromis niloticus* and Clarias *gariepinus* in accumulation of Cr and Zn in fish liver.

The lowest value of Cr and Zn was found in the fish liver collected from Blue Nile 0.25±.03 and 0.75 ±.05 respectively, while the highest value was found in the fish liver from white Nile 0.78± .02 , 1.08 ± .11 respectively.

The mean concentration of Cu and Cd in the fish liver from Blue Nile was 2.90 ±.27, 0.59±.06. Which was significantly higher than that from White Nile 0.26 ±.07, 0.14± .01 ($p < 0.05$) respectively.

There is no significant difference between White and Blue Nile in accumulation of Fe in fish liver.

There were no significant difference($p < 0.05$) between the female and male in accumulation of all studied metals except the Cu and Pb in which the female had accumulate 1.74 ±.07, 1.90 ±.09 and male has accumulate(0.41 ±.02, 0.48 ±.05) respectively.

The order of Cu and Pb concentration in fishes from the all stations in different season was summer > winter > autumn. And summer, winter > autumn for, Cr and Cd.Zn and Fe. Accumulation in fish liver.

Table (12) Heavy metals concentration in fish liver from White and Blue Nile In different seasons

Factors		**Cu**				**Cr**				Cd			
		Tilapia		*Clarias*		*Tilapia*		*Clarias*		*Tilapia*		*Clarias*	
Season	**Water S**	female	male	Femal e	male	female	male	female	male	female	male	female	Male
Winter	WN	0.49	0.71	0.48	0.29	0.48	0.53	0.23	0.28	0.41	0.13	0.10	0.07
	BN	0.30	0.12	0.35	0.44	0.10	0.19	0.26	0.44	0.35	0.65	0.92	1.08
Summer	WN	3.25	0.71	0.33	0.68	0.46	0.54	0.21	0.27	0.12	0.16	0.08	0.12
	BN	0.12	0.30	0.31	0.36	0.21	0.19	0.36	0.51	0.43	0.79	0.56	0.93
Autumn	WN	0.32	0.44	0.63	0.44	0.27	0.15	0.25	0.17	0.11	0.04	0.23	0.10
	BN	0.13	0.15	0.22	0.26	0.18	0.10	0.15	0.24	0.22	0.22	0.46	0.49
White Nile		0.26 ±.07				0.78± .02*				0.14± .01			
Blue Nile		2.90 ±.27 *				0.25±.03				0.59±.06*			
Tilapia		1.21 ±.14*				0.28± .02				0.30 ± .04			
Clarias		0.91 ±.01				0.28±.07				0.43 ±.09*			
Winter		0.40 ±.07^{b}				0.31 ±.03^{a}				0.46 ±.05^{a}			
Summer		2.01 ±.05^{a}				0.35 ±.07^{a}				0.40 ±.03^{a}			
Autumn		0.32 ±.04^{c}				0.19 ±.08^{b}				0.23 ±.04^{b}			
Female		1.74 ±.07*				0.30 ±.04				0.40 ±.01			
Male		0.41 ±.02				0.26 ±.02				0.33 ±.02			

*: significant at ($P<0.05$). a,b,c, means within the same column followed by different superscripts are significantly different ($P<0.05$).

Table (13) Heavy metals concentration in fish liver in different stations from White and Blue Nile.

stations	**Heavy metals**						
	Cu (%)	Cr (%)	Cd (%)	**stations**	Cu (%)	Cr (%)	Cd (%)
Jabal awli	0.60^{a} 74%	0.09^{c} 10%	0.29^{a} 64%	Soba	0.052^{c} 59%	0. 21^{a} 17%	0.137^{c} 35%
dubasen	0.45^{b} 84%	0.50^{b} 31%	0.14^{c} 80%	Algreef	2.20^{a} 9%	0.20^{a} 9%	0.30^{a} 10%
lamab	0.23^{c} 28%	1.75^{a} 50%	0.21^{b} 27%	Bori	1.91^{b} 11%	0.19^{b} 8%	0.21^{b} 10%

%: the percentage of metal occurrence in all samples.

Table (14) Heavy metals concentration in fish liver from White and Blue Nile In different seasons

Factors		Pb				Zn				Fe			
		Tilapia		*Clarias*		*Tilapia*		*Clarias*		*Tilapia*		*Clarias*	
Season	**Water S**	female	male	female	male	female	male	female	male	female	male	female	Male
Winter	WN	0.36	0.36	0.17	0.25	1.21	1.39	1.31	2.01	0.34	0.28	0.23	0.13
	BN	0.37	0.39	0.99	0.81	0.62	0.91	0.82	1.24	0.38	0.44	0.24	0.29
Summer	WN	0.45	0.38	0.12	0.33	0.98	1.49	0.41	1.89	0.29	0.48	0.18	0.07
	BN	0.23	0.55	0.66	0.37	0.35	1.05	1.44	0.75	0.23	0.18	0.19	0.27
Autumn	WN	0.23	0.24	0.38	0.29	0.69	0.51	0.42	0.35	0.35	0.10	0.21	0.10
	BN	0.25	0.21	0.53	0.59	0.37	0.73	0.35	0.42	0.16	0.18	0.16	0.21
White Nile		0.58 ±.021				1.08 ± .11*				0.23 ± .02			
Blue Nile		1.80 ± .02*				0.75 ±.04				0.24 ±0.3			
Tilapia		1.85 ± .32*				0.88 ± .11				0.29 ± .02*			
Clarias		0.54 ±.07				0.95 ±.07				0.19 ±.09			
Winter		0.47 ±.03[b]				1.19 ±.13[a]				0.29 ±.03[a]			
Summer		2.77 ±.14[a]				1.08 ±.18[a]				0.24 ±.08[a]			
Autumn		0.34 ±.07[c]				0.48 ±.05[b]				0.19 ±.05[b]			
Female		1.90 ±.09*				0.77 ±.10				0.25 ±.02			
Male		0.48 ±.05				1.06 ±.09				0.23 ±.01			

*: significant at (P<0.05). a, b, c, means within the same column followed by different superscripts are significantly different (P<0.05).

Table (15) Heavy metals concentration in fish liver in different stations from White and the Blue Nile

stations	Heavy metals												
	Pb	%	Zn	%	Fe	%	stations	Pb	%	Zn	%	Fe	%
Jabal awli	0.51 [b]	77%	0.62 [b]	5%	0.13 [b]	9%	Soba	0.23[c]	81%	0.87[b]	29%	0.32[a]	30%
dubasen	0.20[c]	74%	1.78[a]	72%	0.44 [a]	52%	Algreef	0.91[a]	10%	0.46[c]	18%	0.10 [b]	22%
lamab	0.58[a]	30%	0.84[c]	29%	0.12[b]	31%	Bori	0.70 [b]	22%	0.92 [a]	%19	0.10[b]	9%

%: the percentage of metal occurrence in all samples.

The interaction between subject effect is not significant for all factors except the Water S Xfish in Cu, Cr and Cd.and for Water S X season in Cd and Fe. And for Water S X sex in Cu and Cd.and for Water S X Fish X sex X seas in Zn.

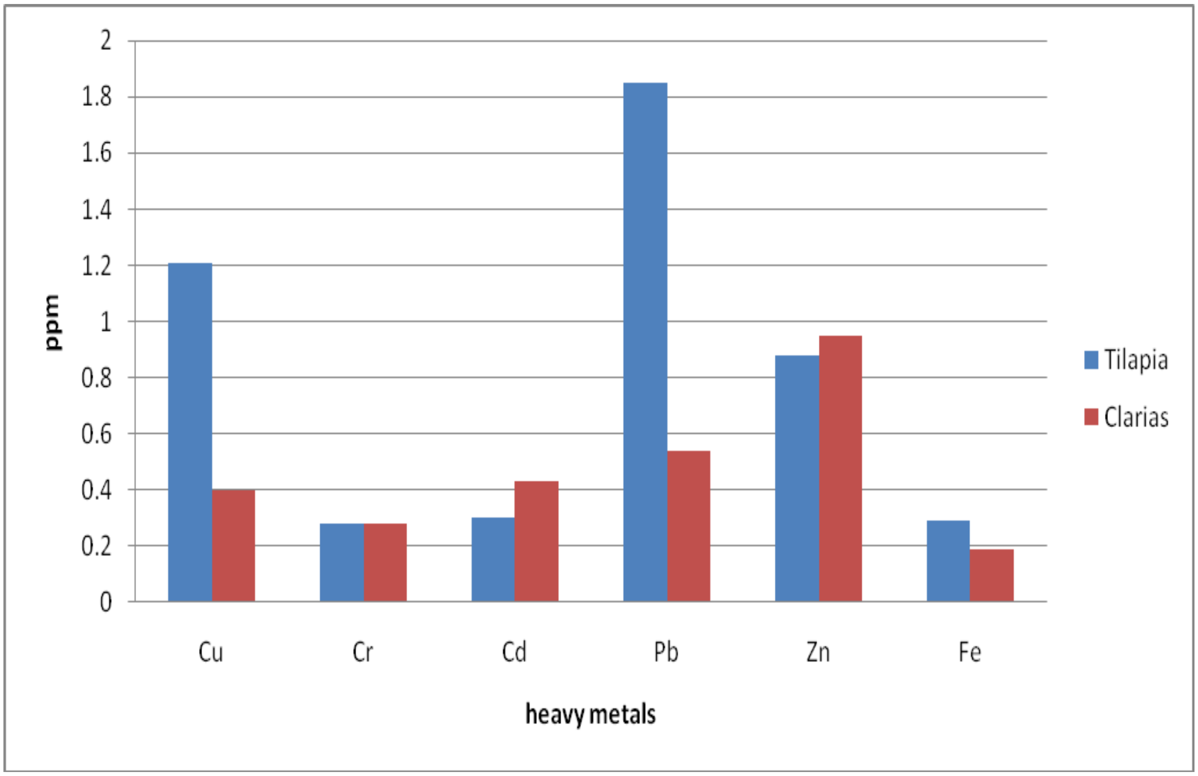

Fig:(13). Shows heavy metals in fish liver from White and Blue Nile.

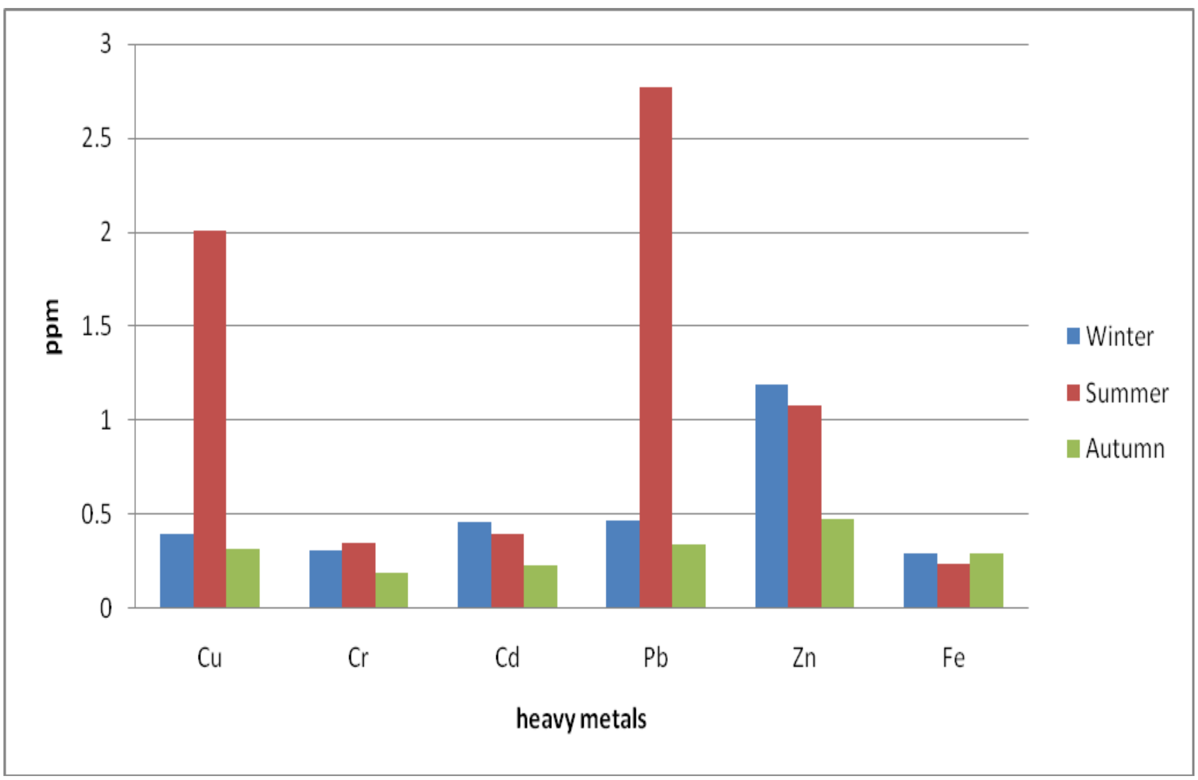

Fig:(14). Heavy metals in fish liver in different season.

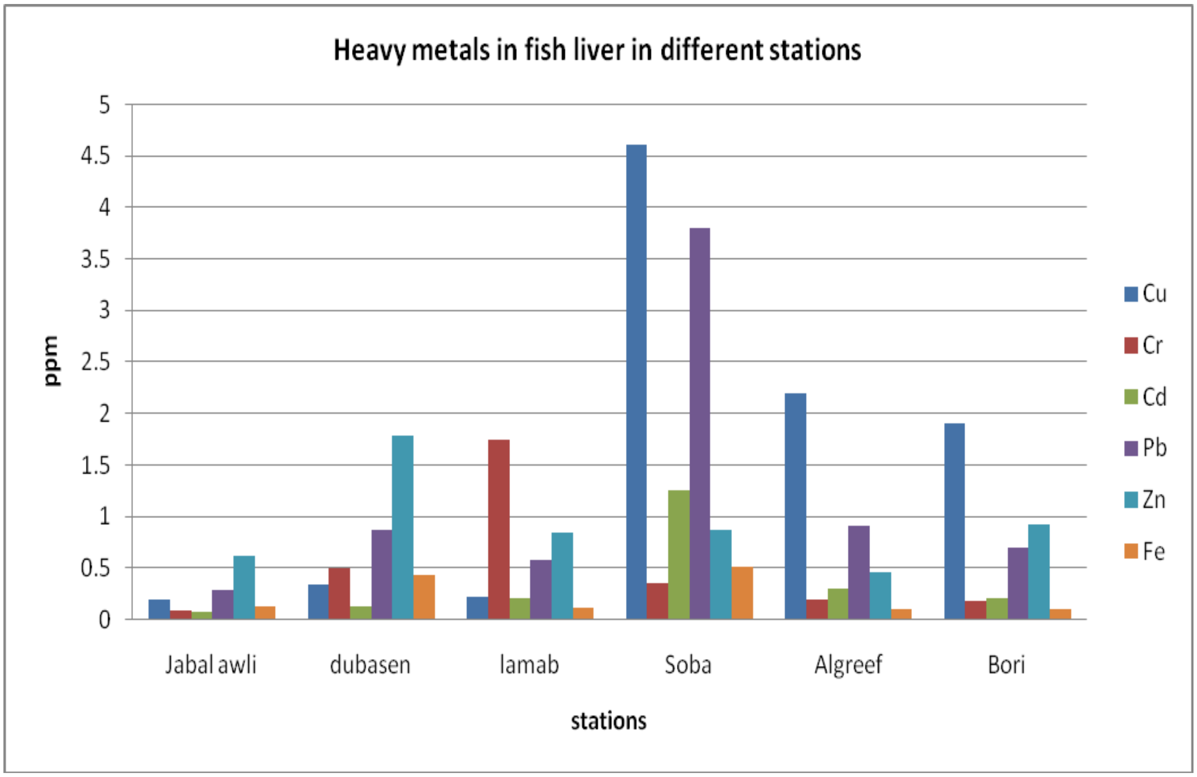

Fig :(15). Heavy metals in fish liver from different stations.

4.6 Heavy metals in fish gill samples:

The results of the levels of concentration of the heavy metals concentration in fish gills samples from different stations are presented in Table (16, 17, 18 , 19) and figures(16, 17 , 18) The percentage of occurrence of Cu in gills of fish samples from different stations Jebal awlia Dobasen,A lamab. Soba,Algreef, and Bori were range between 4% to 64%, Cr were 6% to 62% and Cd were between 5% to 39% and Pb were between 5% to 65% and Zn were 8% to 60% and Fe were between 9% to 60%.

The highest concentrations of Cu and Fe are 2.16 ±.03ppm and 0.29 ± .02and was related to *Oreochromis niloticu* fishes in the fishing stations. But *Clarias gariepinus* had 0.72 ±.06, and 0.19 ±.09 respectively.

There was no significant difference ($p < 0.05$) between *Oreochromis niloticus* and Clarias *gariepinus* in accumulation of Cr, Cd, Pb and Zn in fish gills.

The lowest value of Cr, Zn was found in the fish gills collected from the Blue Nile they were 0.23 ±.06, 0.50 ±.05 respectively, while the highest value was found in the fish gills from White Nile 0.68 ± .04, 0. 0.85 ± .07 respectively. The mean concentration of.Cu, Cd and Pb in the fishes gills from the Blue Nile was 2.35 ± .05.and 0.63 ± .06 and 0.90 ±.08 respectively. They were significantly ($p < 0.05$) higher than that from the White Nile 0.53 ±.07, 0.23 ± .05 and 0.37 ± .06 respectively.

And there was no significant difference between the White and Blue Nile in accumulation of Fe in gills. And there were no significant difference($p < 0.05$) between the female and male in accumulation of all studied metals except the Cu which the female had accumulate 2.30 ±.01and male has accumulate 0.59 ±.07.

The order of Cu concentration in fishes gills from the all stations in different season was summer > winter > autumn. And summer, winter > autumn for Cr, Cd, Pb, Zn and Fe for accumulation in fish gills.

Table (16) Heavy metals concentration in fish gills from White and Blue Nile In different seasons

Factors		**Cu**				**Cr**				Cd			
		Tilapia		*Clarias*		*Tilapia*		*Clarias*		*Tilapia*		*Clarias*	
Season	**Water S**	female	male	female	male	female	male	female	male	female	male	female	Male
Winter	WN	0.74	0.73	0.73	0.47	0.94	0.68	0.62	0.75	0.33	0.26	0.22	0.18
	BN	0.66	0.40	0.49	0.65	0.05	0.24	0.21	0.27	0.34	0.76	0.82	0.16
Summer	WN	0.75	2.96	0.57	0.31	0.68	0.82	0.62	0.88	0.21	0.28	0.37	0.10
	BN	0.53	0.37	0.63	0.75	0.28	0.42	0.25	0.32	0.54	0.54	0.25	0.86
Autumn	WN	0.57	0.46	0.48	0.62	0.55	0.57	0.47	0.54	0.19	0.31	0.11	0.17
	BN	0.25	0.42	0.64	0.61	0.09	0.17	0.15	0.30	0.32	0.28	0.36	0.53
White Nile		0.53 ±.07				0.68 ± .04*				0.23 ± .05			
Blue Nile		2.35 ± .05*				0.23 ±.06				0.63 ± .06*			
Tilapia		2.16 ±.03*				0.46 ± .04				0.36 ± .05			
Clarias		0.72 ±.06				0.45±.018				0.49 ± .07			
Winter		$0.73 \pm .02^{b}$				$0.47 \pm .05^{a}$				$0.51 \pm .06^{a}$			
Summer		$1.58 \pm .21^{a}$				$0.53 \pm .09^{a}$				$0.49 \pm .12^{a}$			
Autumn		$0.51 \pm .06^{c}$				$0.35 \pm .08^{b}$				$0.28 \pm .08^{b}$			
Female		2.30 ±.01*				0.41±.04				0.41 ±.05			
Male		0.59 ±.07				0.50 ±.04				0.45 ±.05			

*: significant at ($P<0.05$). a,b,c,d means within the same column followed by different superscripts are significantly Different ($P<0.05$).

Table (17) Heavy metals concentration in fish Gills in different stations from White and Blue Nile

stations	**Heavy metals**											
	Cu	(%)	Cr	(%)	Cd	(%)	**stations**	Cu	(%)	Cr	(%)	Cd	(%)
Jabal awli	0.87^{a}	80%	0.15^{c}	6%	0.45^{a}	67%	Soba	3.72^{a}	17%	0.34^{a}	28%	1.24^{a}	39%
dubasen	0.70^{b}	64%	0.61^{b}	39%	0.18^{b}	84%	Algreef	1.78^{b}	4%	0.17^{b}	10%	0.31^{b}	5 %
Lamab	0.42^{c}	33%	1.3^{a}	62%	0.10^{b}	26%	Bori	1.55^{b}	9%	0.18^{b}	11%	0.34^{c}	5%

%: the percentage of metal occurrence in all samples.

Table (18) Heavy metals concentration in fish gills from White and Blue Nile in different seasons

Factors		Pb				Zn				Fe			
		Tilapia		*Clarias*		*Tilapia*		*Clarias*		*Tilapia*		*Clarias*	
Season	**Water S**	female	male	female	male	female	male	female	male	female	male	female	Male
Winter	WN	0.59	0.52	0.16	0.33	1.11	1.17	0.59	1.58	0.36	0.31	0.05	0.03
	BN	0.68	0.71	1.62	1.35	0.18	0.66	0.96	0.43	0.20	0.34	0.14	0.17
Summer	WN	0.48	0.66	0.37	0.20	1.09	1.39	0.51	0.37	0.30	0.47	0.29	0.22
	BN	0.56	0.83	1.22	1.79	0.34	0.82	0.66	0.86	0.22	0.25	0.14	0.31
Autumn	WN	0.47	0.29	0.25	0.12	0.51	0.48	0.90	0.49	0.14	0.15	0.06	0.09
	BN	0.40	0.38	0.50	0.70	0.33	0.22	0.18	0.40	0.16	0.15	0.13	0.22
White Nile		0.37 ± .06				0.85 ± .07*				0.20± .02			
Blue Nile		0.90 ±.08*				0.50 ±.05				0.19±.03			
Tilapia		0.55 ± .06				0.69 ± .07				0.29 ± .02*			
Clarias		0.72 ±.03				0.66 ±.08				0.14 ±.05			
Winter		0.74 ±.07[a]				0.84 ±.08[a]				0.20 ±.02[b]			
Summer		0.76 ±.05[a]				0.75 ±.08[a]				0.25 ±.08[a]			
Autumn		0.39 ±.17[b]				0.44 ±.04[c]				0.14 ±.02[c]			
Female		0.61 ±.06				0.61 ±.07				0.18 ±.02			
Male		0.66 ±.06				0.74 ±.07				0.21 ±.02			

*: significant at ($P<0.05$). a,b,c, means within the same column followed by different superscripts are significantly different ($P<0.05$).

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Table (19) Heavy metals concentration in fish Gills in different stations from White and Blue Nile

stations	Heavy metals												
	Pb %		**Zn %**		Fe %		**stations**	**Pb %**		**Zn %**		Fe %	
Jabal awli	0.60[c]	70%	0.34 [c]	8%	0.11[c]	10%	Soba	1.36[a]	65%	0.87[a]	60%	0.31[a]	42%
dubasen	0.57[a]	84%	1.31[a]	19%	0.30 [a]	60%	Algreef	0.97 [b]	5%	0.12[c]	8%	0.09 [b]	15%
lamab	0.19[b]	20%	0.90[b]	21%	0.19[b]	5%	Bori	0.37 [c]	1%	0.51 [b]	13%	0.18[c]	9%

%: the percentage of metal occurrence in all samples.

The interaction between subjects has not significant for all factors except the Water S X Fish in pb and Cd And for Water S X season in pb and for Fish X season sig in Cr and Zn

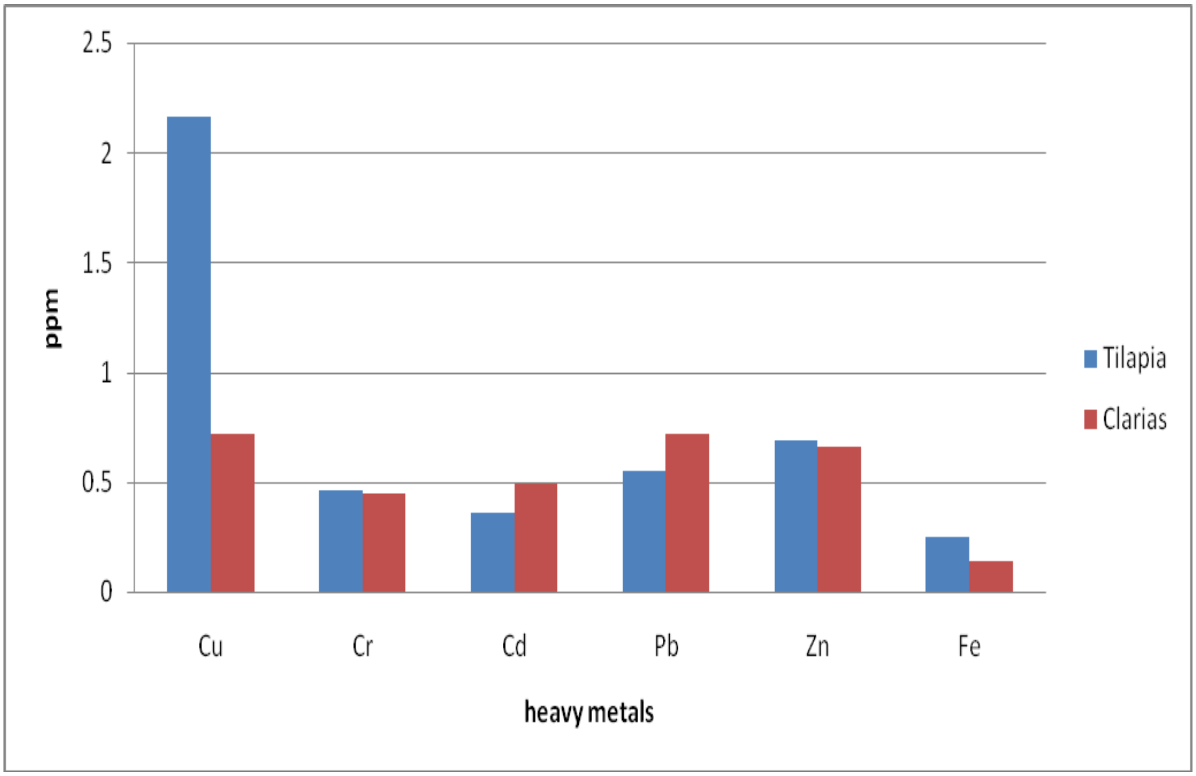


Fig :(16). Heavy metals in *Oreochromis niloticus& Clarias gariepinus* gills

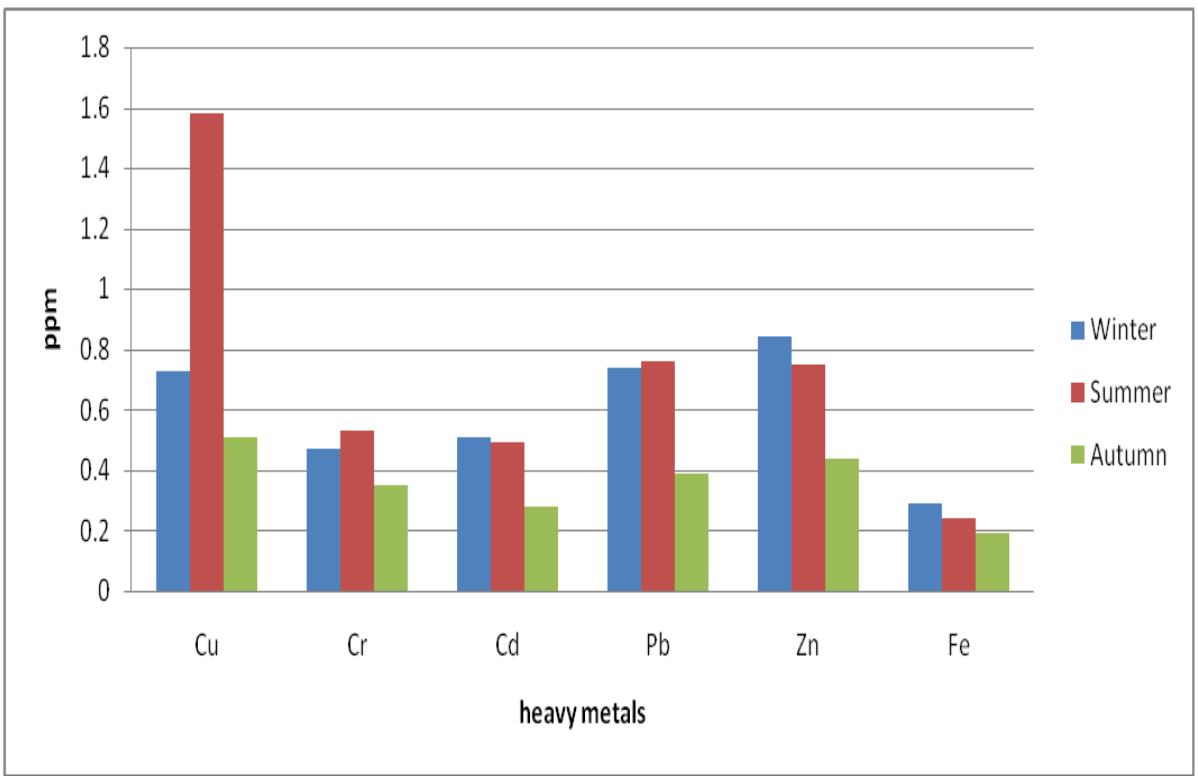


Fig :(17). Heavy metals in *Oreochromis niloticus* and *Clarias gariepinus* gills in different seasons.

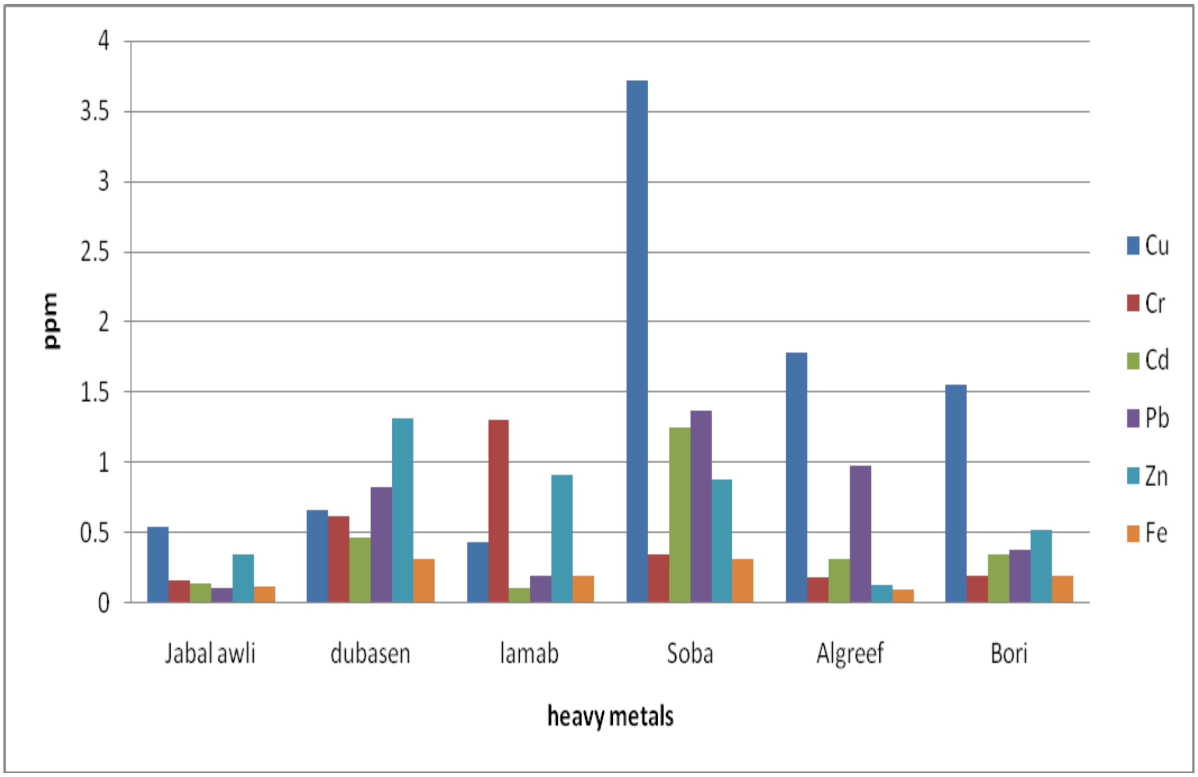


Fig :(18). Heavy metals in *Oreochromis niloticus* and *Clarias gariepinus* gills from different stations.

4.7 Heavy metals in fish kidney samples:

The results of the levels of concentration of the heavy metals in fish kidney samples from different stations are presented in Table (20, 21, 22, and 23).and figures (19, 20, 21, and 22).

The percentage of occurrence of Cu in kidney of fish samples from different stations Jebal awli, Dobasen, Alamab. Soba, Algreef, and Bori were range between 3% to 65% and Cr were between 7% to 33%and Cd were between 5% to 26% and Pb were between 4% to60% and Zn were between 8% to 67% and Fe were between 6%to 53%.

The highest concentrations of Cr were 0.30 ± .02 ppm in *Oreochromis niloticus* fishes in the fishing stations. But Clarias *gariepinus* had 0.16 ±.04. And Cu in *Clarias gariepinus* was 0.69±.08 significantly higher than in *Oreochromis niloticus* 0.47 ± .04.

There is no significant difference between: *Oreochromis niloticus* and *Clarias gariepinus* in accumulation of Cd, Pb, Zn and Fe in fish kidney.

The lowest value of, Cr, Zn was found in the fish kidney collected from Blue Nile they were 0.14 ±.01, 0.44 ±.03 respectively, while the highest value was found in the fish kidney of fish from White Nile 0.32 ± .02, 0.83 ± .07 respectively. The mean concentration of Cd in the fish's kidney from the Blue Nile was 0.35±.05. And It was significantly ($p < 0.05$) higher than that from the White Nile 0.17± .02.

There was no significant difference between White and Blue Nile in accumulation of Cu, Pb and Fe in fish kidney.

There were no significant difference ($p < 0.05$) between the female and male in accumulation of all studied metals in fish kidney.

The order of Pb concentration in fishes from the all stations in different season was summer > winter > autumn. And summer, winter > autumn for Cu and Cr and there were no significant difference in accumulation of Cd, Zn and Fe in fish kidney in different seasons.

Table (20) Heavy metals concentration in fish kidney from White and Blue Nile in different seasons

Factors		**Cu**				**Cr**				Cd			
		Tilapia		*Clarias*		*Tilapia*		*Clarias*		*Tilapia*		*Clarias*	
Season	**Water S**	female	male	female	male	female	male	female	male	female	male	female	male
Winter	WN	0.39	0.54	0.38	0.85	0.55	0.31	0.22	0.23	0.31	0.25	0.05	0.06
	BN	0.54	0.33	1.03	0.69	0.06	0.14	0.14	0.17	0.38	0.40	0.44	0.47
Summer	WN	0.67	0.98	0.46	0.74	0.47	0.77	0.19	0.26	0.13	0.42	0.06	0.06
	BN	0.39	0.40	0.54	0.69	0.10	0.19	0.10	0.16	0.17	0.23	0.37	0.62
Autumn	WN	0.38	0.36	0.52	0.30	0.37	0.17	0.18	0.16	0.19	0.19	0.07	0.14
	BN	0.32	0.30	0.50	0.38	0.23	0.17	0.08	0.07	0.22	0.22	0.25	0.36
White Nile		0.55± .04				0.32 ± .02*				0.17± .02			
Blue Nile		0.51±.05				0.14 ±.01				0.35±.05*			
tilapia		0.47 ± .04				0.30 ± .02*				0.26 ± .02			
Clarias		0.69±.08*				0.16 ±.04				0.25 ±.08			
Winter		0.59 ±.05^{a}				0.23 ±.02^{a}				0.29 ±.03			
Summer		0.61 ±.02^{a}				0.28 ±.05^{a}				0.26 ±.08			
Autumn		0.39 ±.04^{b}				0.18 ±.05^{b}				0.21 ±.05			
female		0.55 ±.04				0.22±.01				0.22 ±.02			
male		0.51 ±.04				0.23 ±.05				0.28 ±.03			

*: significant at (P<0.05). a,b,c, means within the same column followed by different superscripts are significantly different (P<0.05).

Table (21) Heavy metals concentration in fish kidney in different stations from White and Blue Nile

stations	**Heavy metals**												
	Cu	(%)	Cr	(%)	Cd	(%)	**stations**	Cu	(%)	Cr	(%)	Cd	(%)
Jabal awli	0.67^{b}	70%	0.08^{c}	8%	0.27^{c}	64%	Soba	0.05 ^{c}	11%	0. 13^{a}	10%	0.14^{a}	20%
dubasen	0.35^{c}	77%	0.16^{b}	10%	0.37^{a}	67%	Algreef	0.26^{b}	8%	0.09^{b}	7%	0.19^{a}	2 %
lamab	0. 66^{a}	27%	0.72^{a}	33%	0.23^{b}	26%	Bori	0.57^{a}	3%	0.06^{b}	7%	0.10 ^{c}	7%

%: the percentage of metal occurrence in all samples.

Table (22) Heavy metals concentration in fish kidney from White and Blue Nile in different seasons

Factors		Pb				Zn				Fe			
		Tilapia		*Clarias*		*Tilapia*		*Clarias*		*Tilapia*		*Clarias*	
Season	**Water S**	female	male	female	male	female	male	female	male	female	male	female	Male
Winter	WN	0.38	0.47	0.12	0.19	0.83	1.45	1.14	1.21	0.26	0.27	0.03	0.03
	BN	0.20	0.25	0.51	0.53	0.30	0.47	0.28	0.40	0.14	0.23	0.10	0.12
Summer	WN	0.33	0.45	0.24	0.25	0.98	1.22	0.37	0.66	0.59	0.13	0.14	0.04
	BN	0.22	0.30	0.25	1.18	0.13	0.33	0.36	1.02	0.16	0.36	0.16	0.14
Autumn	WN	0.39	0.19	0.21	0.15	0.45	0.67	0.63	0.31	0.11	0.11	0.22	0.19
	BN	0.14	0.16	0.33	0.36	0.12	0.13	0.84	0.84	0.13	0.27	0.24	0.29
White Nile		0.28 ±.07				0.83 ± .07*				0.18 ± .03			
Blue Nile		0.37 ± .03				0.44 ±.03				0.19 ±.01			
Tilapia		0.29± .03				0.59 ± .07				0.23 ± .02			
Clarias		0.36±.08				0.67±.06				0.14 ±.01			
Winter		0.33±.04[b]				0.76 ±.09				0.15 ±.04			
Summer		0.40 ±.03[a]*				0.63 ±.12				0.21 ±.03			
Autumn		0.24 ±.02[c]				0.50±.06				0.20±.04			
Female		0.28 ±.03				0.54 ±.07				0.19 ±.03			
Male		0.37 ±.03				0.73 ±.08				0.18 ±.03			

*: significant at (P<0.05). a,b,c, means within the same column followed by different superscripts are significantly different (P<0.05).

Table (23) Heavy metals concentration in fish kidney in different stations from White and Blue Nile

stations	Heavy metals						
	Pb %	**Zn %**	Fe %	**stations**	**Pb %**	**Zn %**	Fe %
Jabal awli	0.40 [a] 70%	0.51 [c] 8%	0.14[c] 7%	Soba	0.10[c] 30%	0.60[a] 67%	0.27[a] 45%
Dubasen	0.30[b] 80%	1.02[a] 20%	0.21 [b] 53%	Algreef	0.18 [b] 8%	0.47[b] 28%	0.18[c] 6%
Lamab	0.33[c] 22%	0.97[b] 25%	0.21[a] 21%	Bori	0.19 [a] 10%	0.25 [c] 19%	0.20[b] 10%

%: the percentage of metal occurrence in all samples.

The interaction between subject effects has to be not significant for all factors except the Water S X fish for Cr Cd. Pb and Zn and for Water S X season in Cr and Zn and for Fish X season in Fe. and for sex X season in Cr and Pb.

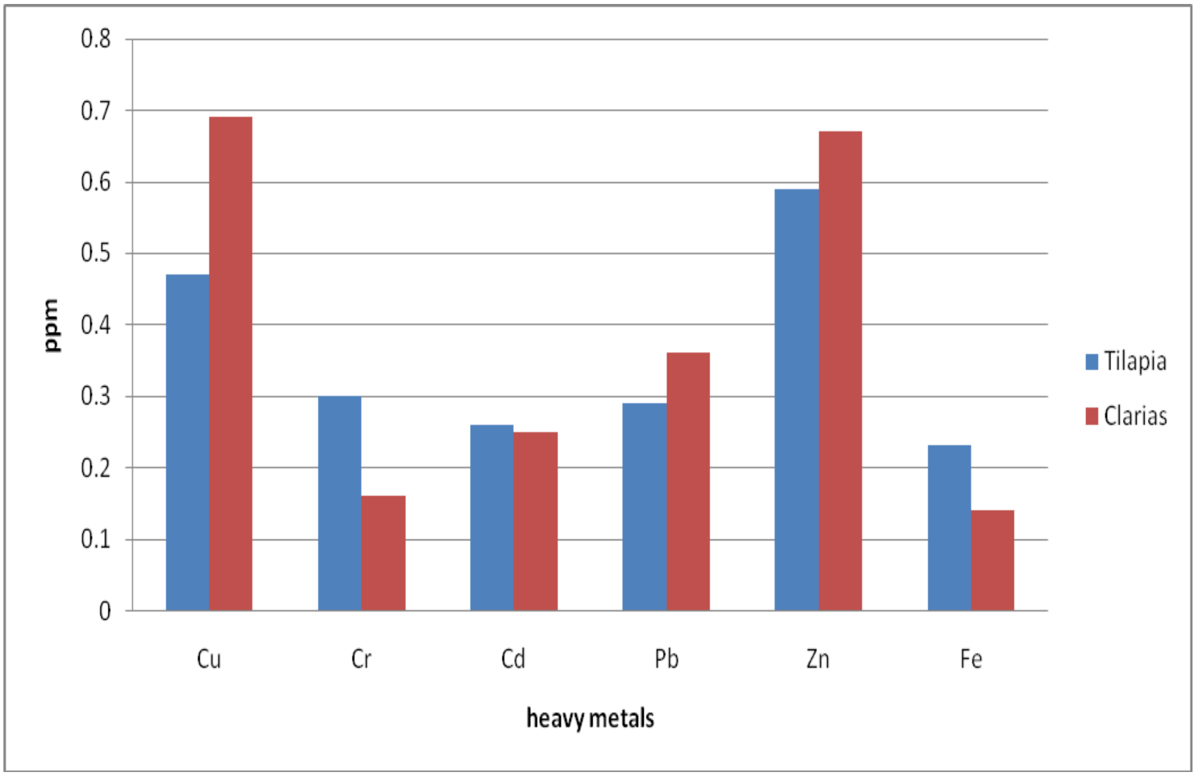


Fig :(19). Heavy metals in *Oreochromis niloticus& Clarias gariepinus* kidney from White and Blue Nile.

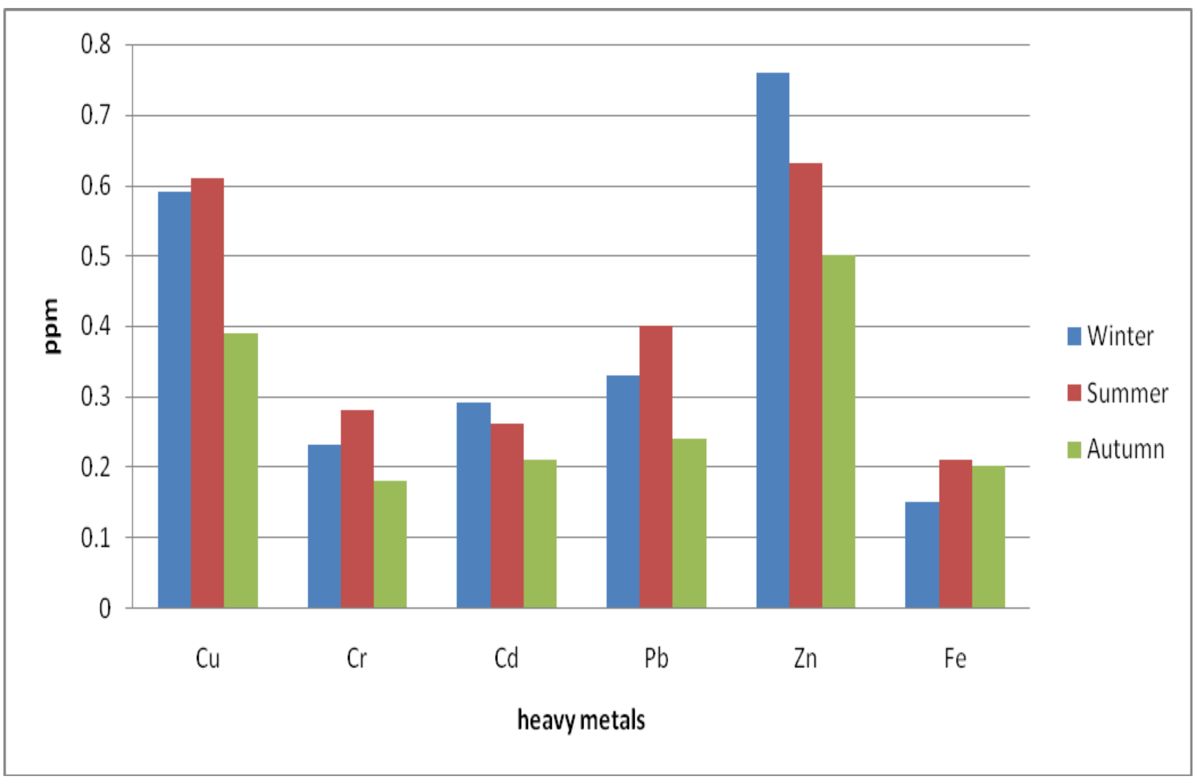


Fig :(20). Heavy metals in *Oreochromis niloticus& Clarias Gariepinus* kidney in different seasons.

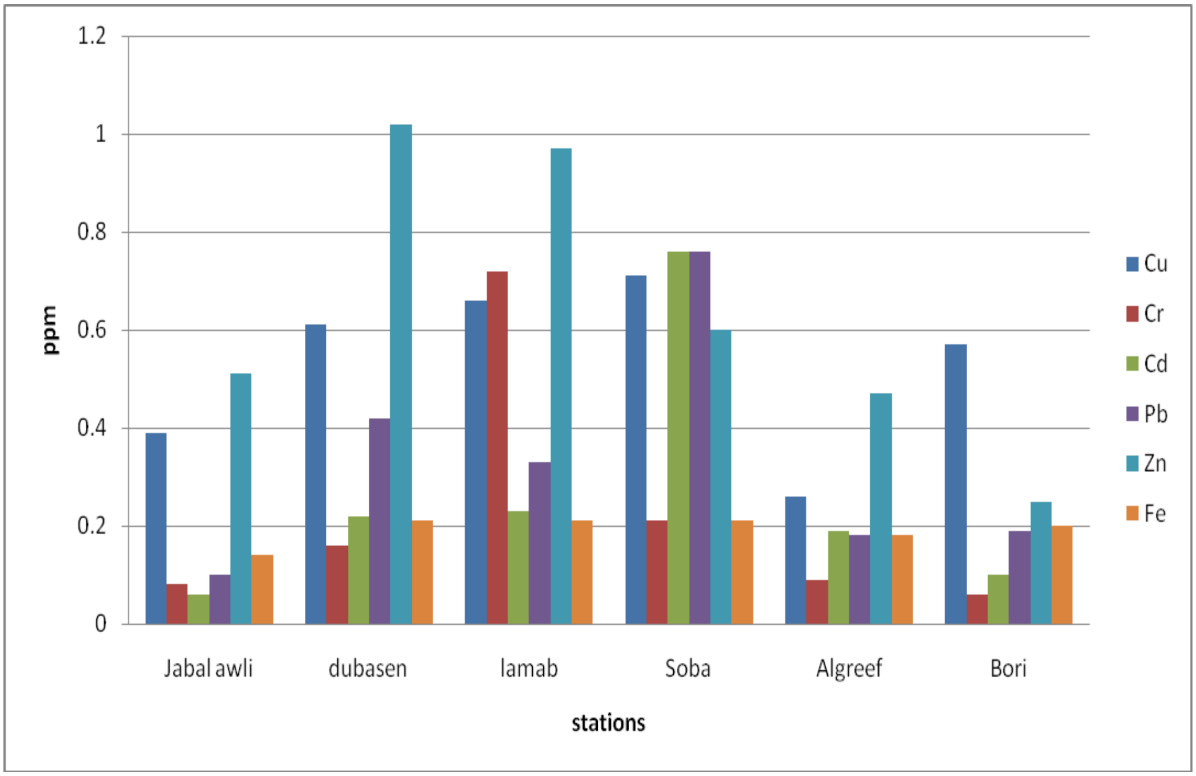


Fig :(21). Heavy metals in *Oreochromis niloticus& Clarias Gariepinus* kidney in different stations.

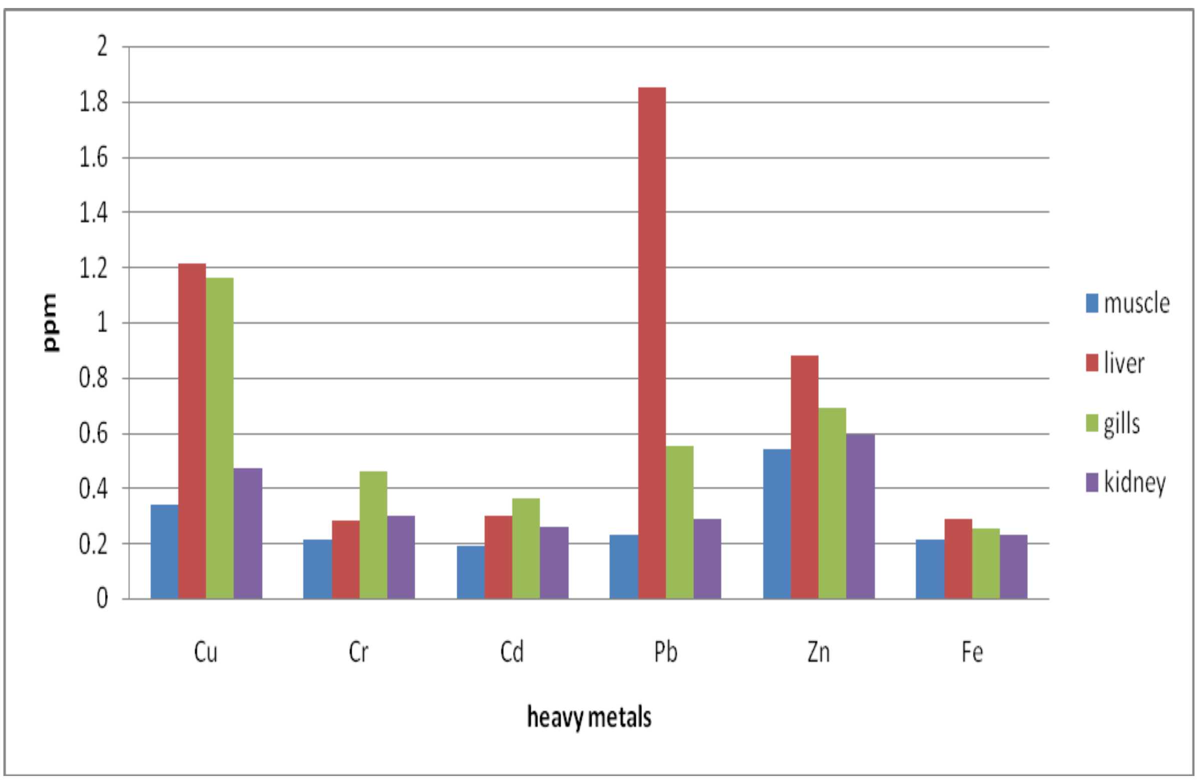


Fig :(22). Heavy metals in *Oreochromis niloticus* organs from White and Blue Nile.

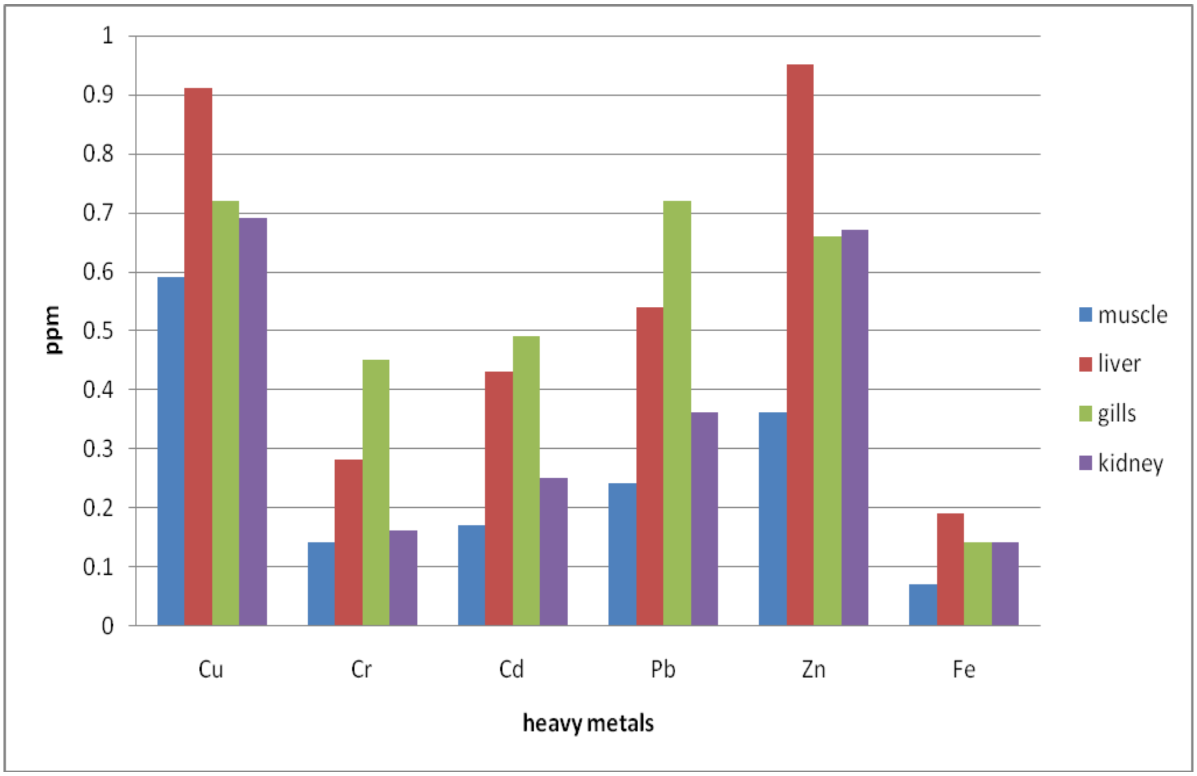


Fig :(23). Heavy metals in *Clarias gariepinus* organs from White and Blue Nile.

4.8 Histopathological results:

Sections from the muscles, liver, gills and kidney were prepared from both fish species from different location in White and Blue Nile. They were examined under the light microscope. The microscopic observation were reported for each organ and the intensity of histopathological changes were tabulated in table (24) and are presented in the plates (1_27) Sections from muscles of Oreochromis *niloticus&, Clarias gariepinus*fish from White and Blue Nile during summer, winter and autumn .shows the normal structures of the muscles. And several histopathological alterations were seen. The pathological findings included Edema between muscle bundles and focal areas of necrosis. Also splitting of muscle fibers was seen Plates (1-6).

The most common lesions in the liver of both studied fish were focal areas of necrosis vacullation and dilation and thrombosis formation in central vein, rupture in the hepatocytes Lymphocytic infiltration and pyknosis. Also cell damage was seen. Plates (7-15). Sections from gills of both fish species shows the normal histological structures of the gills. The histopathological alterations in the gills of *Oreochromis niloticus&, Clarias gariepinus* from white and Blue Nile during winter, summer and autumn were more or less similar. They included proliferation in the epithelium of gill filaments and secondary lamellae, congestion in blood vessels of gill filaments and atrophy of secondary lamellae, Edematous changes, sloughing of secondary lamellae were also seen. Plates (16-21). Sections of the Kidney show the normal histological structures of the kidney. The histopathological alterations in the kidney of both fish included, Blood congestion, shrinking of glumeruli, Vacillation in glomeruli. Edema in Bowman's capsule was also observed Plates (22-27).

Plate 1. Section through the muscle of *Oreochromis niloticus* at Jebal Awllia station in White Nile Notice the normal tissues of muscles fibers. H&E X 200.

Plate 2. Section through the muscle of Clarias gariepinus at Algreef station in Blue Nile Notice the normal tissues of muscles fibers. H&E X 200.

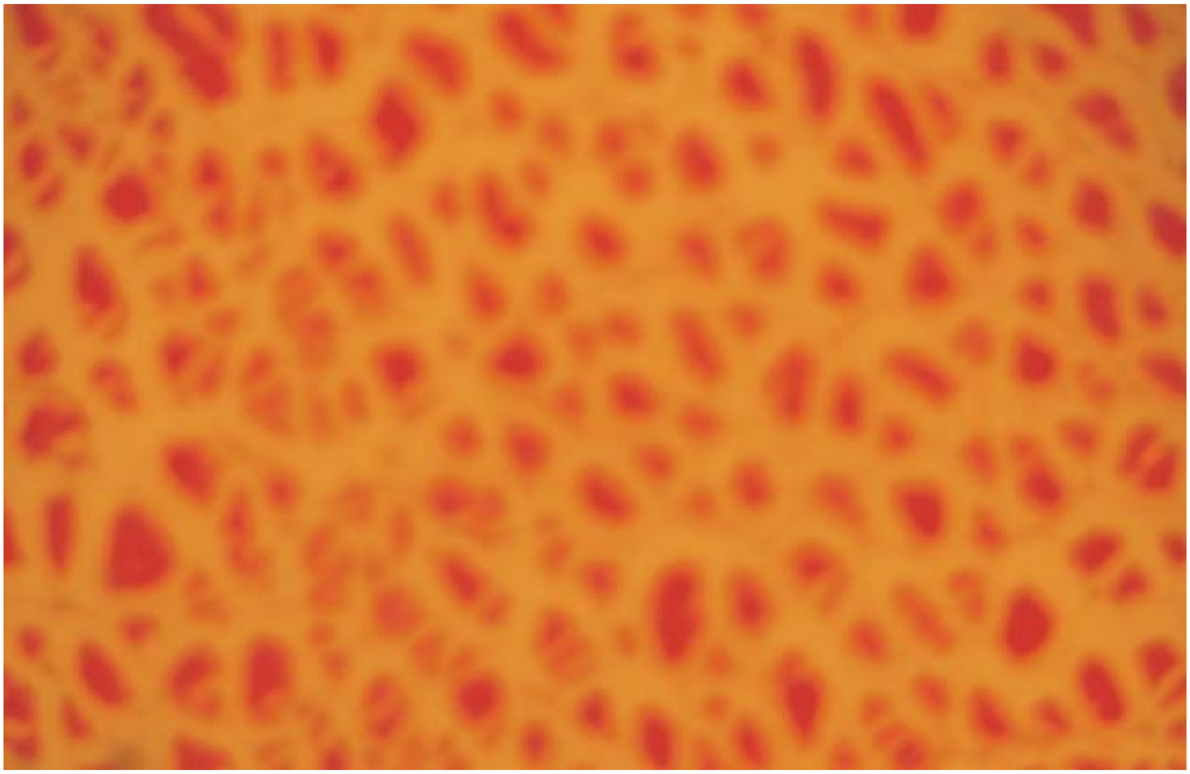


Plate 1.

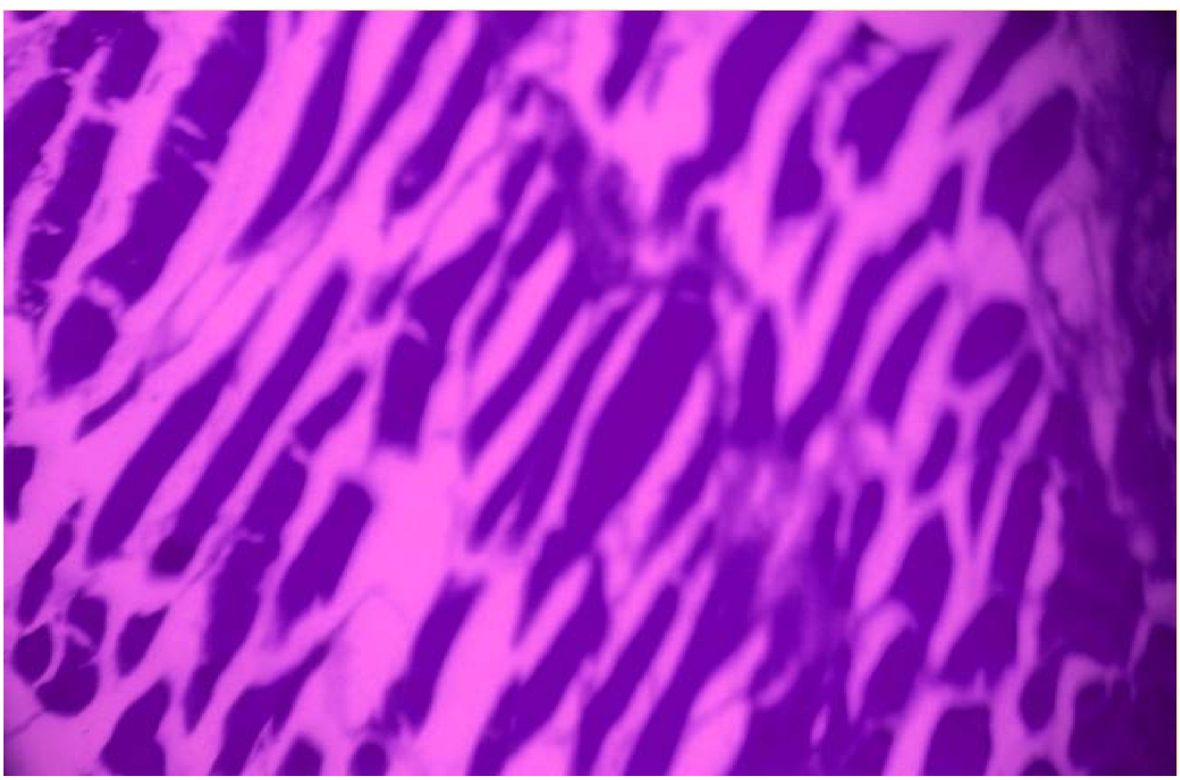


Plate 2.

Plate 3. Section through the muscles of *Oreochromis niloticus* at Bori station in Blue Nile Notice edema between muscle bundles (E). H&E X 200.

Plate 4. Section through the muscle of *Clarias Oreochromis s* at Adobaseen station in White Nile Notice the fatty changes) F.CH).H&E X 400.

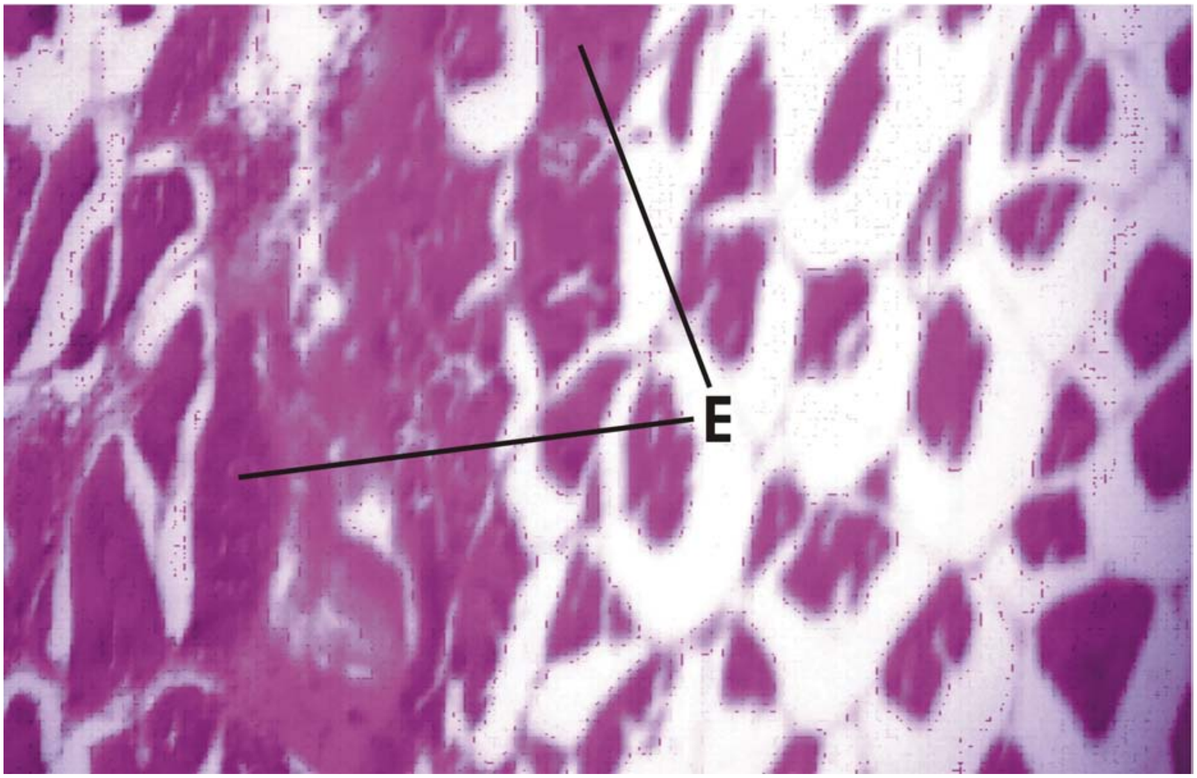


Plate 3.

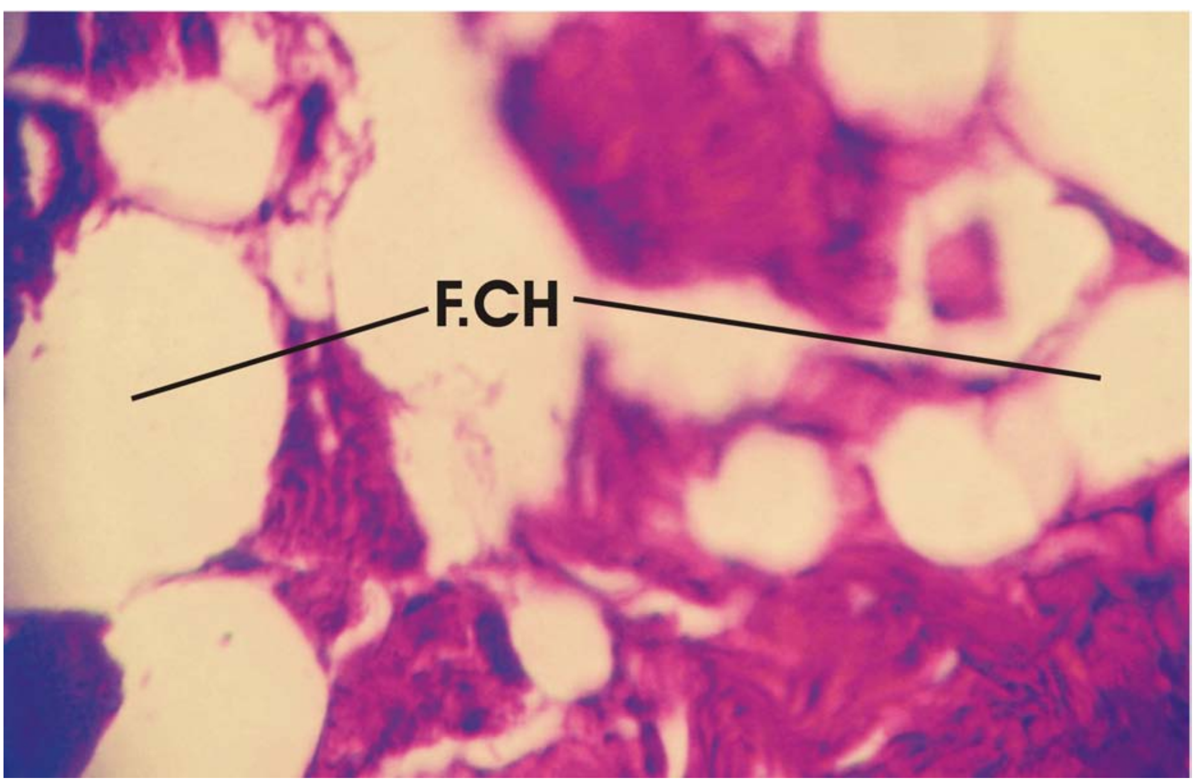


Plate 4.

Plate 5. Section through the muscle of *Oreochromis niloticus* at Adobaseen station in White Nile Notice focal areas of necrosis (FN). H&E X200

Plate 6. Section through the muscle of *Oreochromis niloticus* at Soba station in Blue Nile Notice splitting of muscle fibers (S). H&E X200

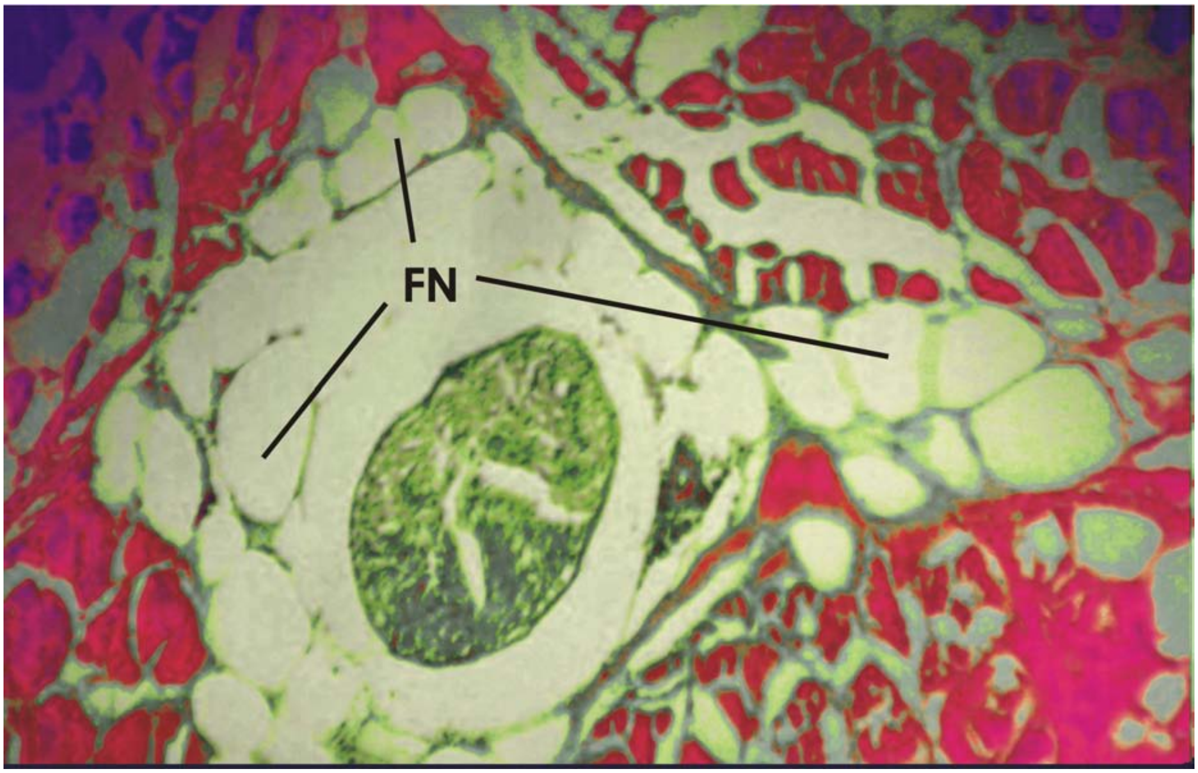


Plate 5.

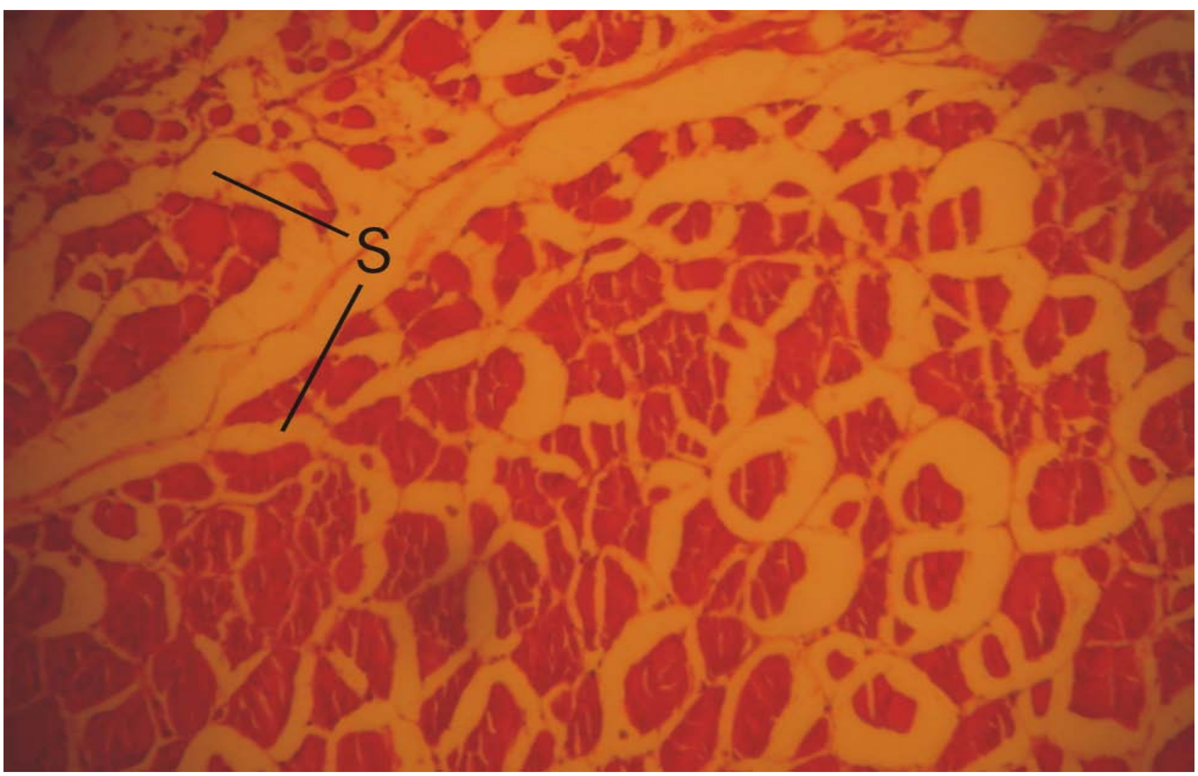


Plate 6.

Plate 7. Section through the liver of *Oreochromis niloticus* at Algreef station in Blue Nile Notice hepatocyes (H).H&E X200

Plate 8. Section through the liver of *Oreochromis niloticus* at Algreef station in Blue Nile Notice hepatocytes (H), the central vein (CV) .H&E X200

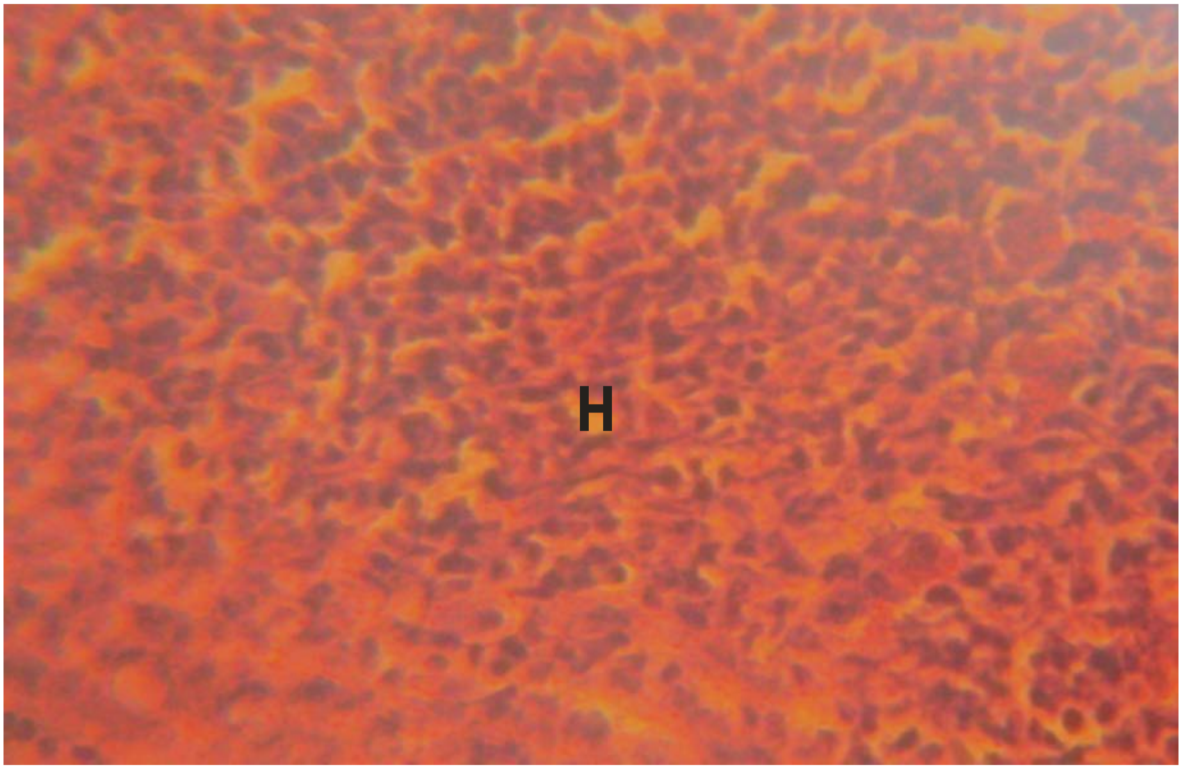


Plate 7.

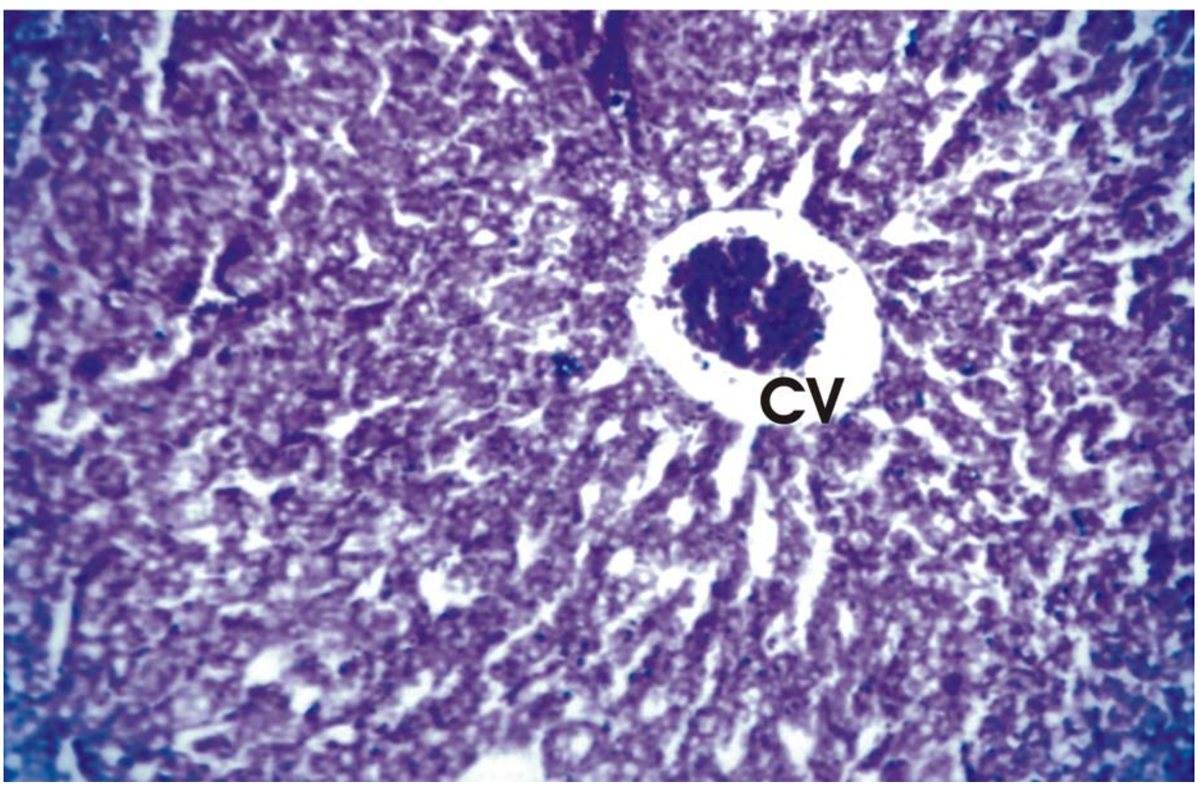


Plate 8.

Plate 9. Section through the liver of *Oreochromis niloticus* at Soba station in Blue Nile Notice lymphocytic infiltration (LI) pyknosis (PY) .H&E X400

Plate 10. Section through the liver of *Oreochromis niloticus* at Alamab station in White Nile Notice Dilation and thrombosis formation in central vain (CV). H&E X200

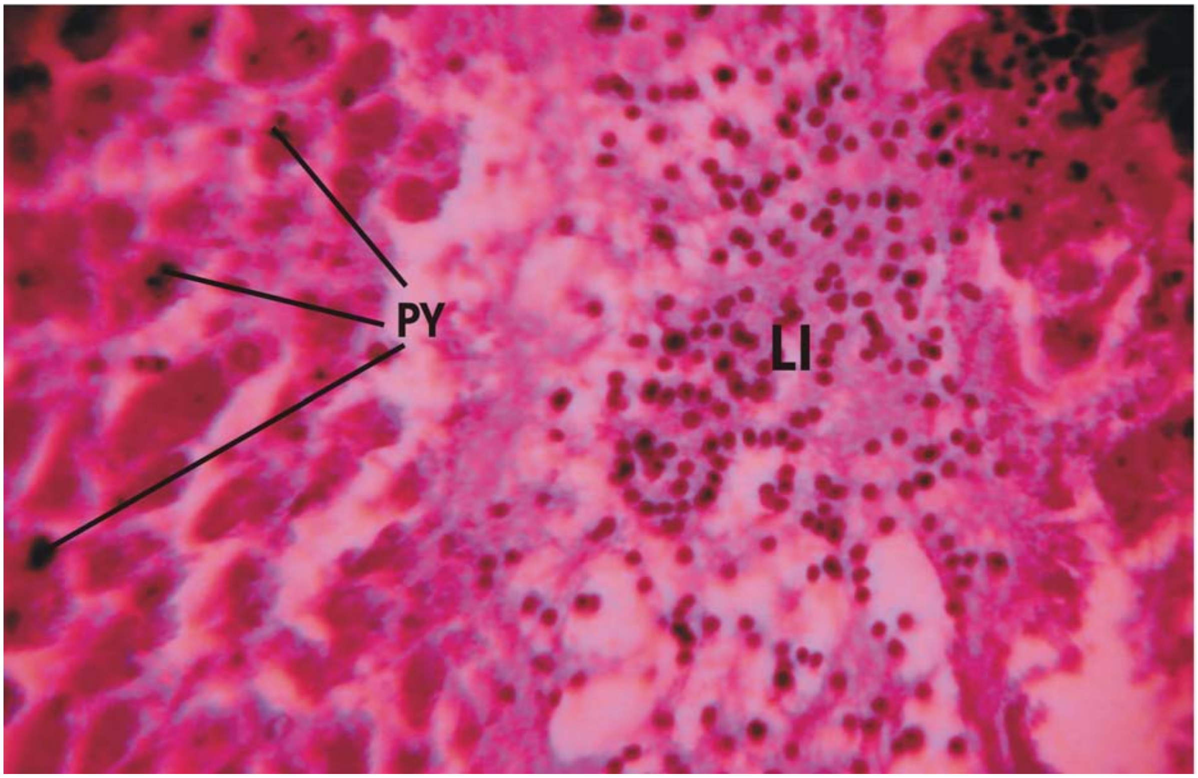
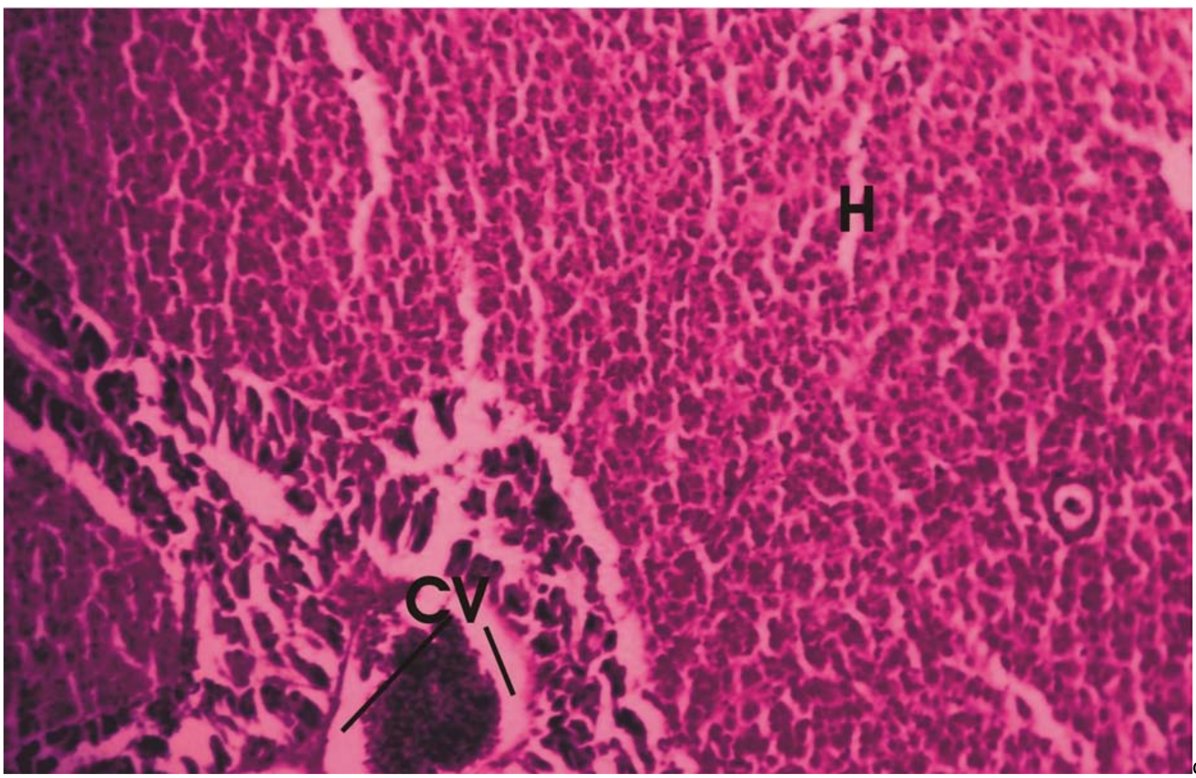


Plate 9.



c

Plate 10.

Plate 11. Section through the liver of *Oreochromis niloticus* at Alamab station in White Nile Notice Dilation and thrombosis formation in central vain (CV). H&E X400

Plate 12. Section through the liver of *Oreochromis niloticus* at Alamab station in White Nile Notice Focal areas of necrosis (FN).H&E X200

Plate 11.

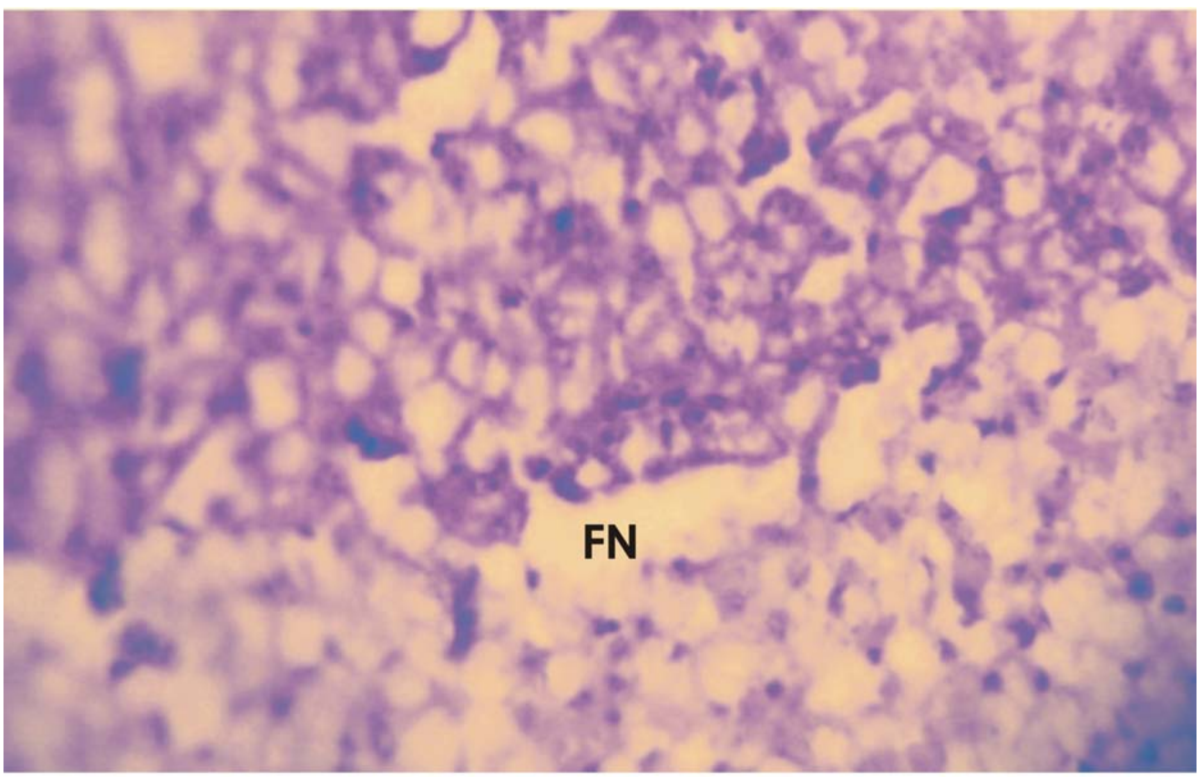


Plate 12.

Plate 13. Section through the liver of *Clarias gariepinus* at Adobaseen station in White Nile Notice ruptured hepatocytes (RH) and vacuolation (V) H&E X200

Plate 14. Section through the liver of *Oreochromis niloticus* at Adobaseen station in White Nile Notice ruptured hepatocytes (RH) and vaculation (V) H&E X200

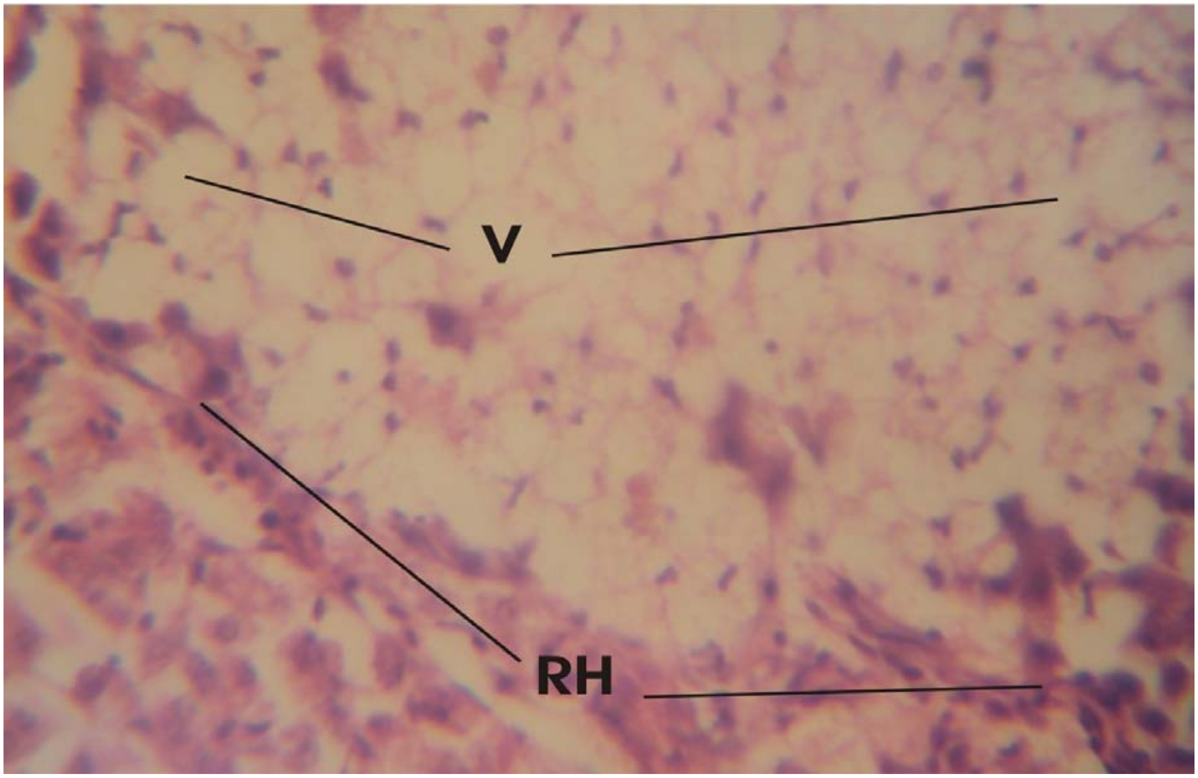


Plate 13.

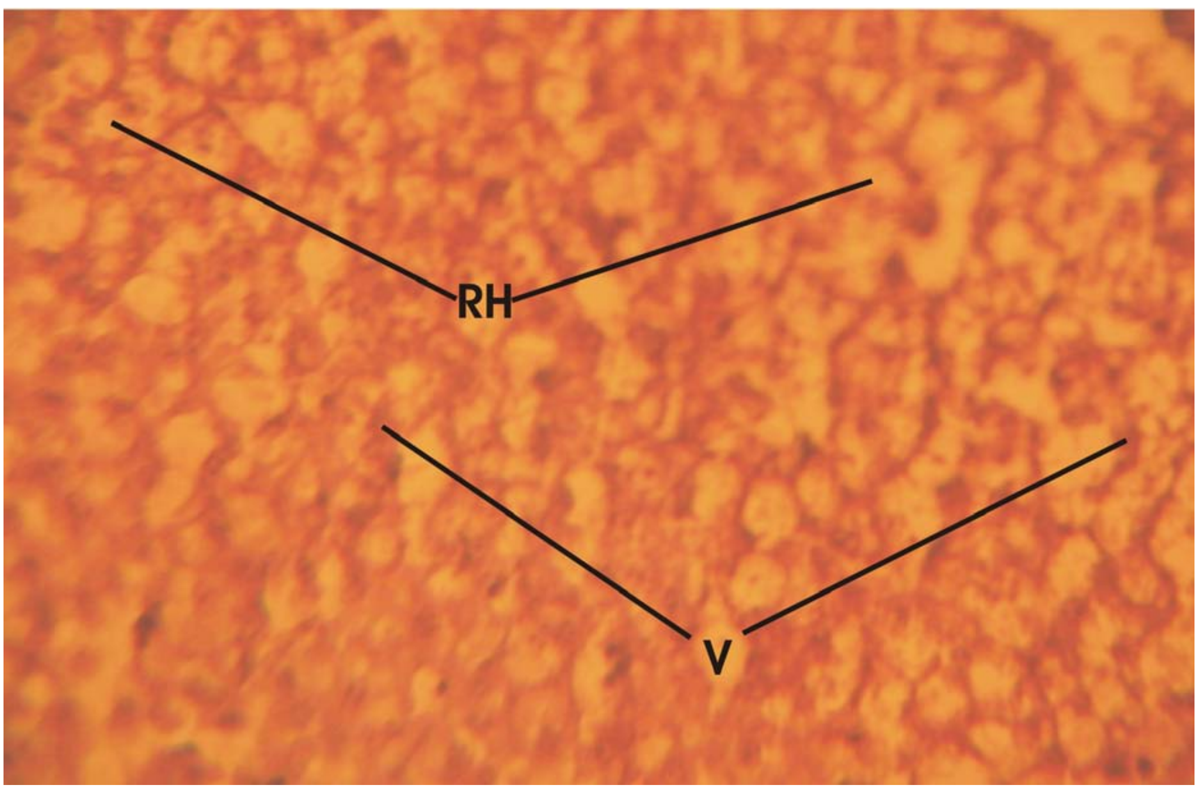


Plate 14.

Plate 15. Section through the liver of *Clarias gariepinus* at Adobaseen station in White Nile Notice cell damage (CD).H&E X200

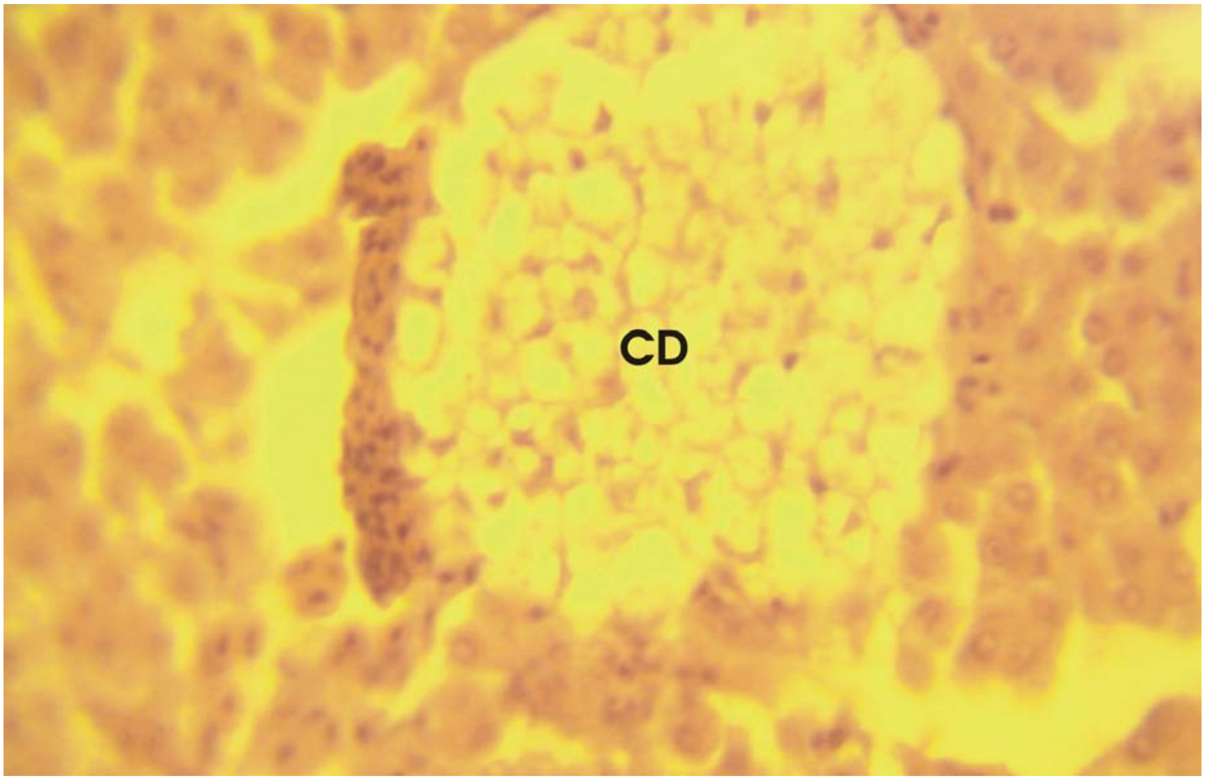


Plate 15.

Plate 16. Section through the control gills of *Clarias gariepinus* at Jebal Awlia station in White Nile Notice primary lamellae (PL), secondary lamellae (SL), mucous cell (MC) .H&E X200

Plate 17. Section through the control gills of *Oreochromis niloticus* at Jebal Awlia station in White Nile Notice secondary lamellae (SL).H&E X100

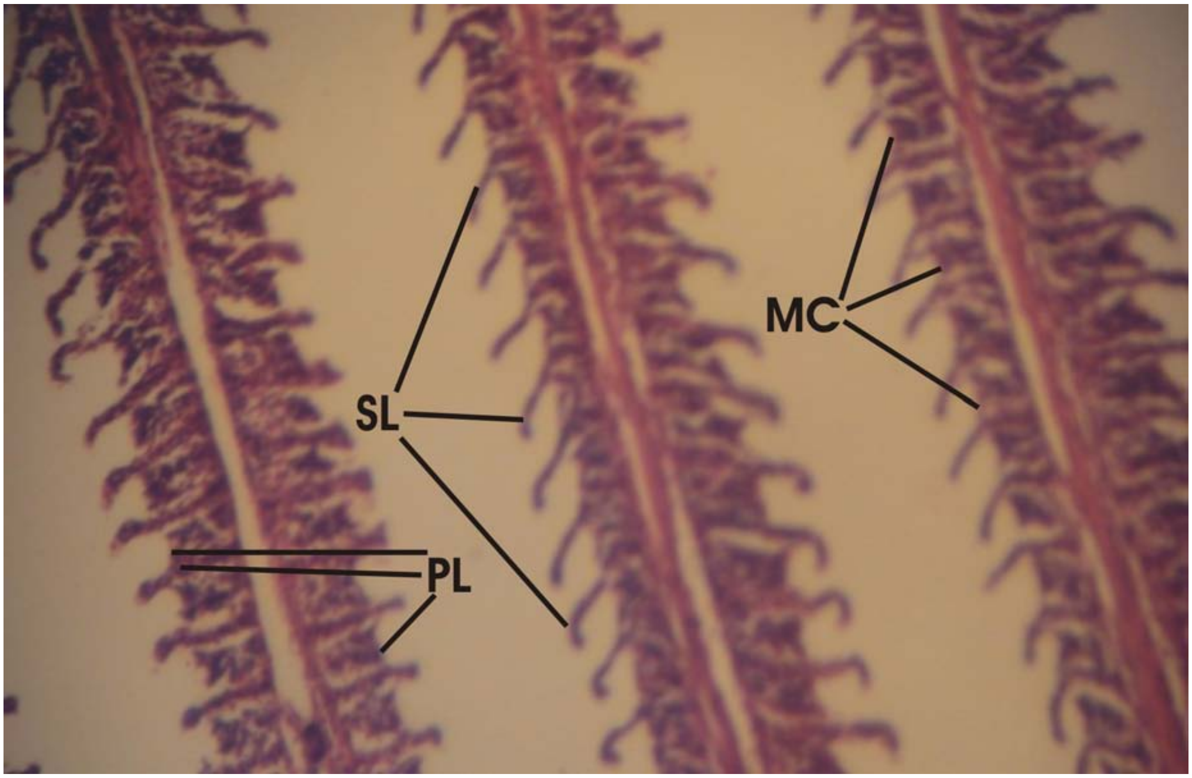


Plate 16.

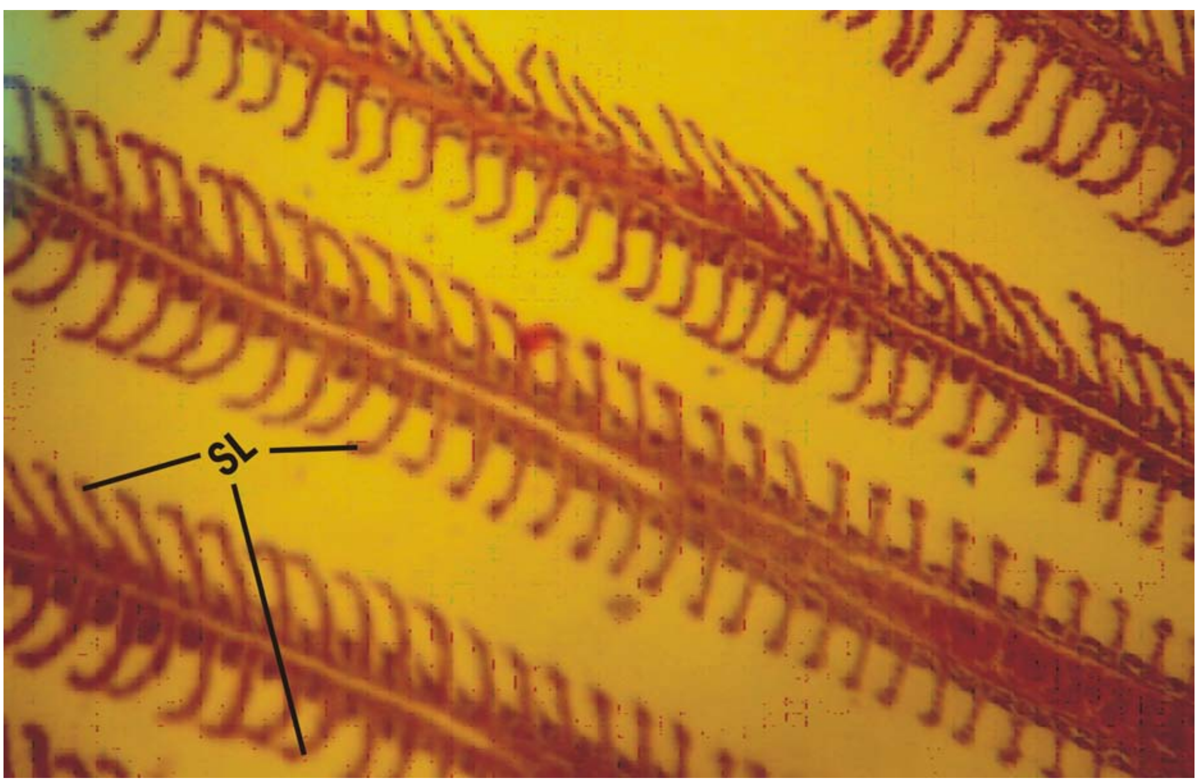


Plate 17.

Plate 18. Section through the gills of *Oreochromis niloticus* at Bori station in Blue Nile Notice proliferation in the epithelium of gill filaments (EPC) H&E X400

Plate 19. Section through the gills of *Clarias gariepinus* at Adobaseen station in White Nile Notice congestion in blood vessels of gill filaments and atrophy of secondary lamella .H&E X400

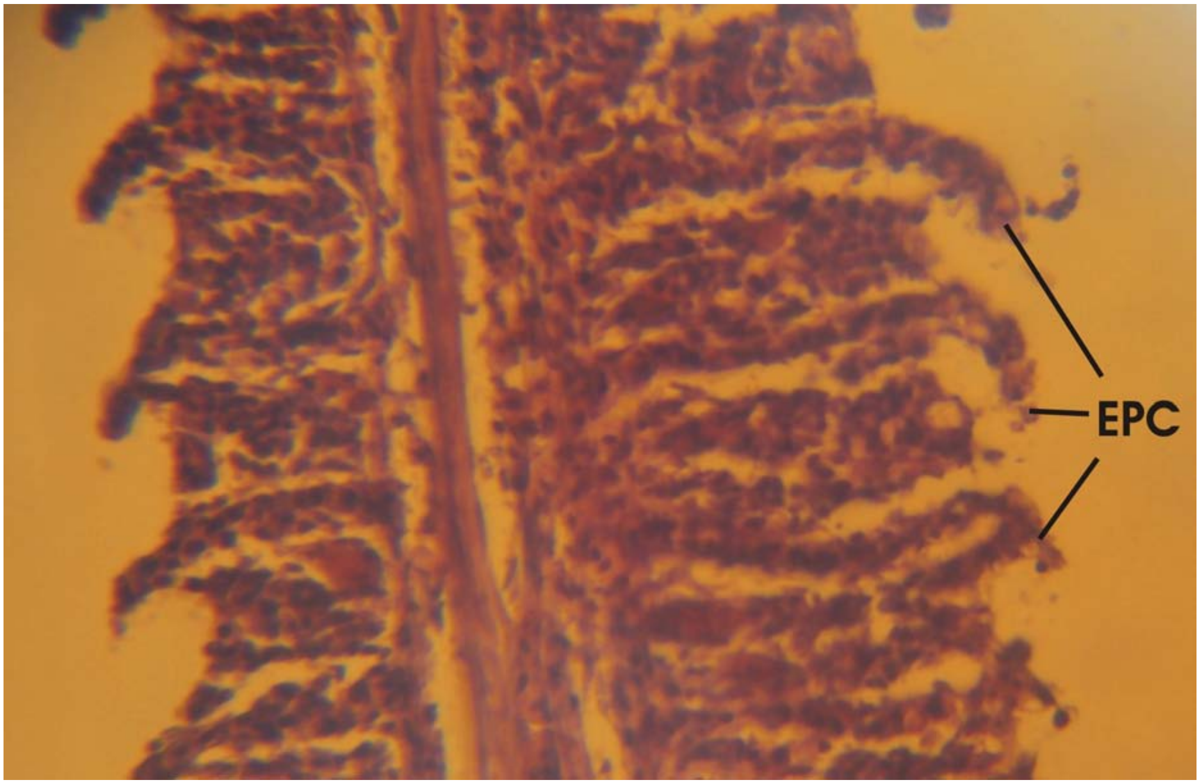


Plate 18.

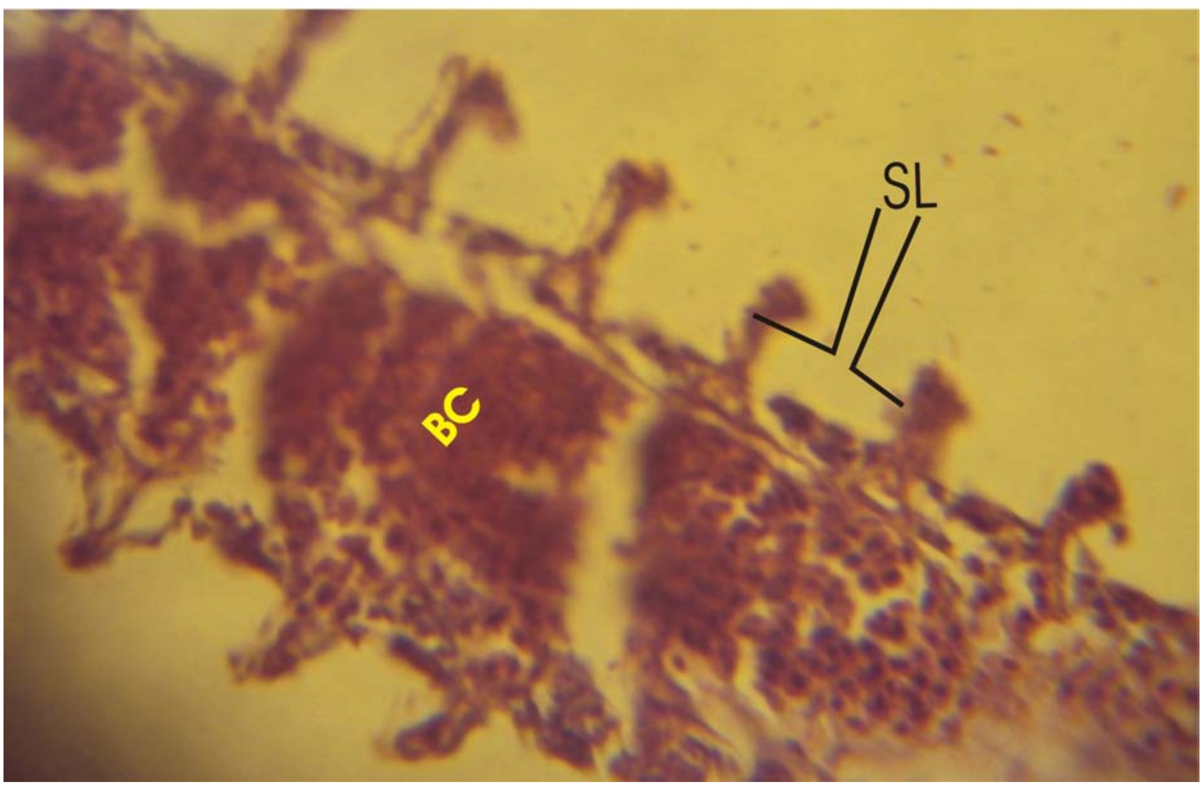


Plate 19.

Plate 20. Section through the gills of *Clarias gariepinus* at Adobaseen station in White Nile Notice edema in secondary lamellae and gill filaments.H&E X400.

Plate 21. Section through the gills of *Oreochromis niloticus* at Soba station in Blue Nile Notice sloughing of secondary lamellae .H&E X400

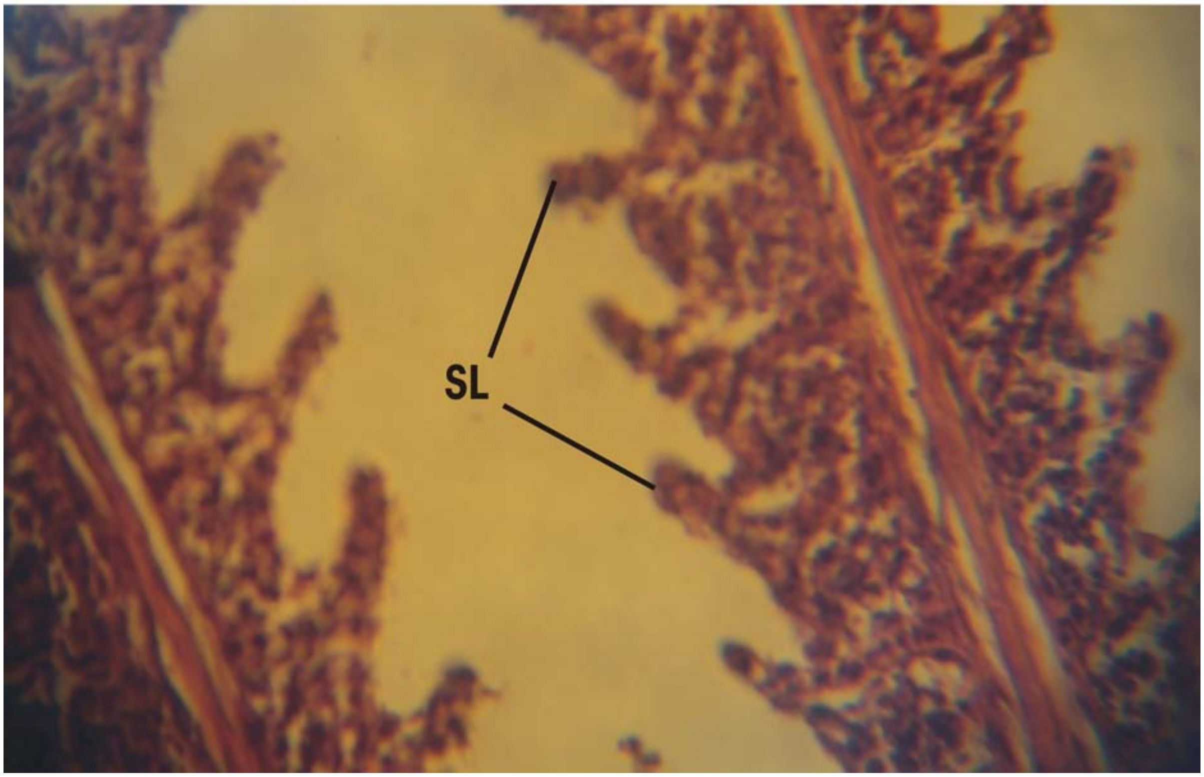


Plate 20.

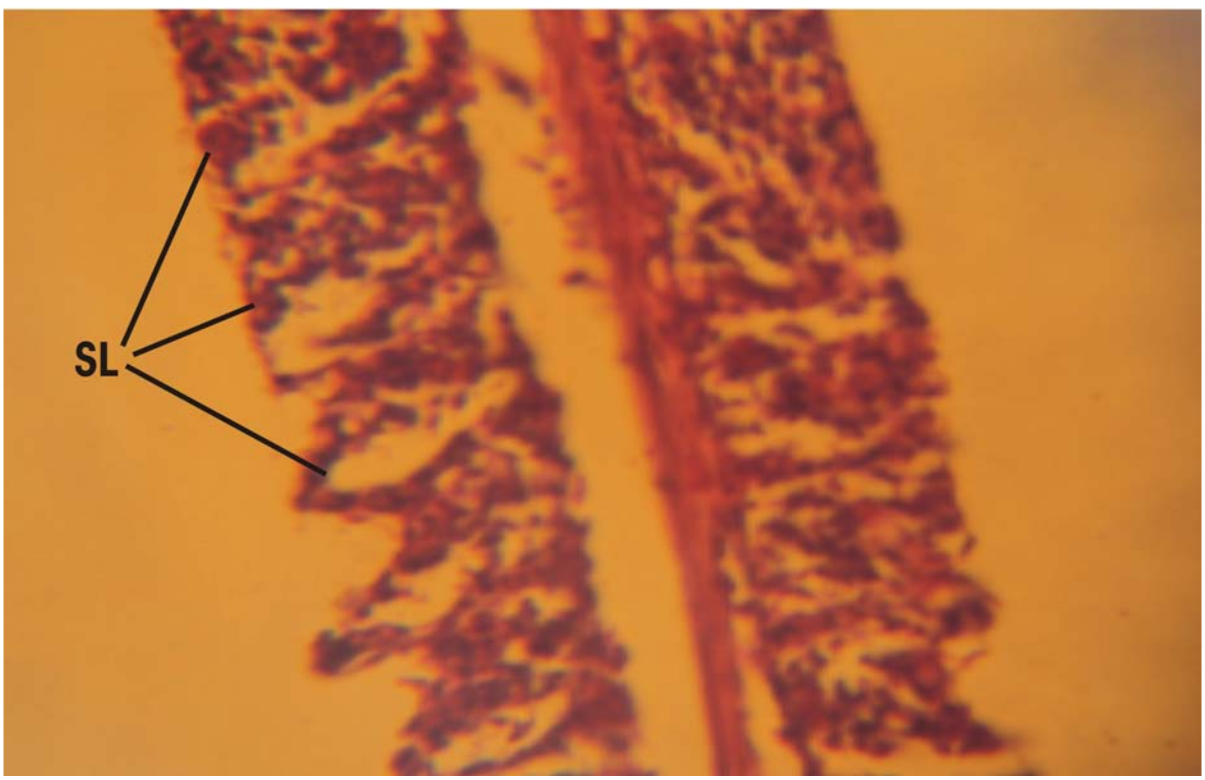
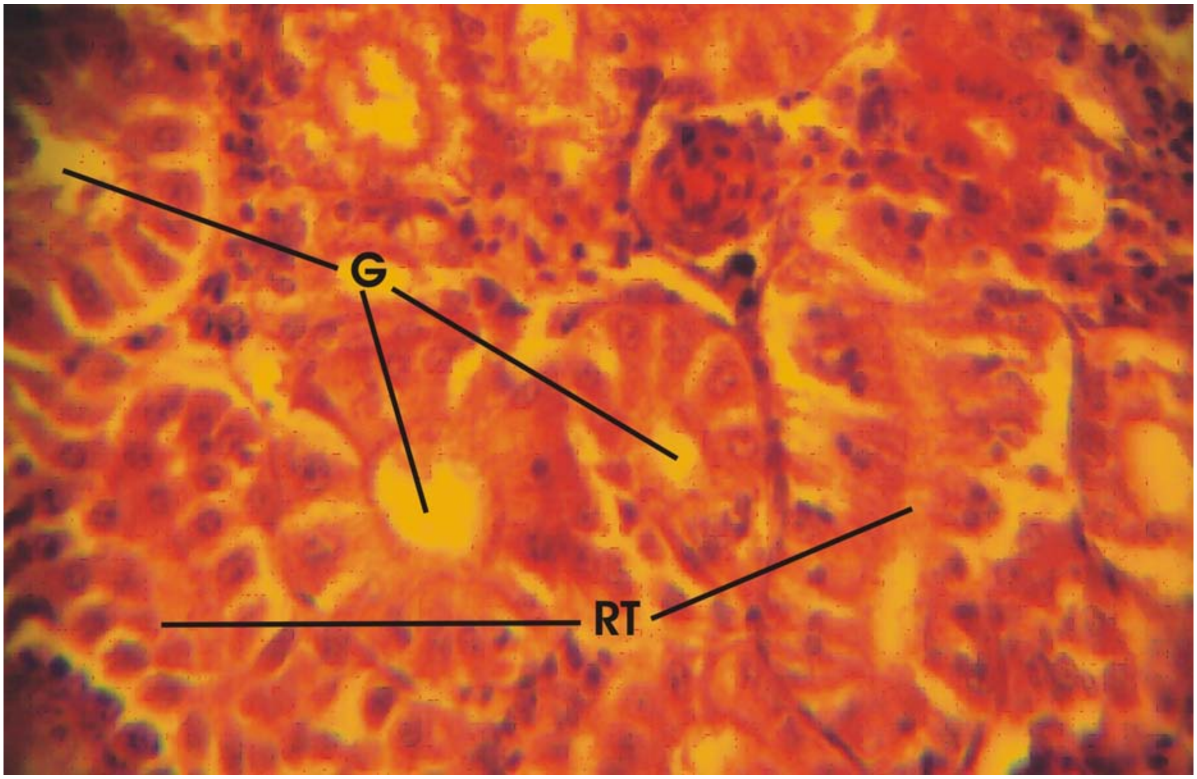


Plate 21.

Plate 22. Section through the kidney of *Clarias gariepinus* at Algreef station in Blue Nile Notice normal structure renal tubules, (RT), glomerulus (G).H&E X400

Plate 23. Section through the kidney of *Oreochromis niloticus* at Algreef station in Blue Nile Notice normal structure.H&E X200



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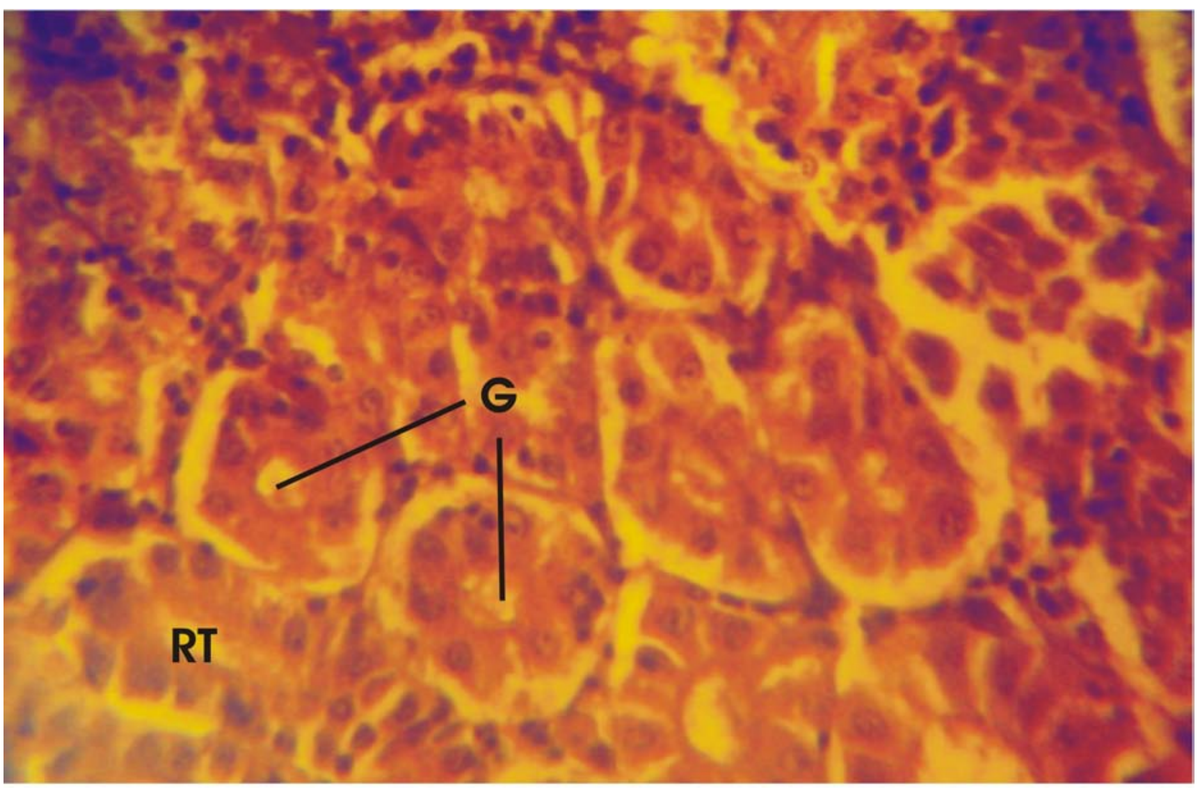
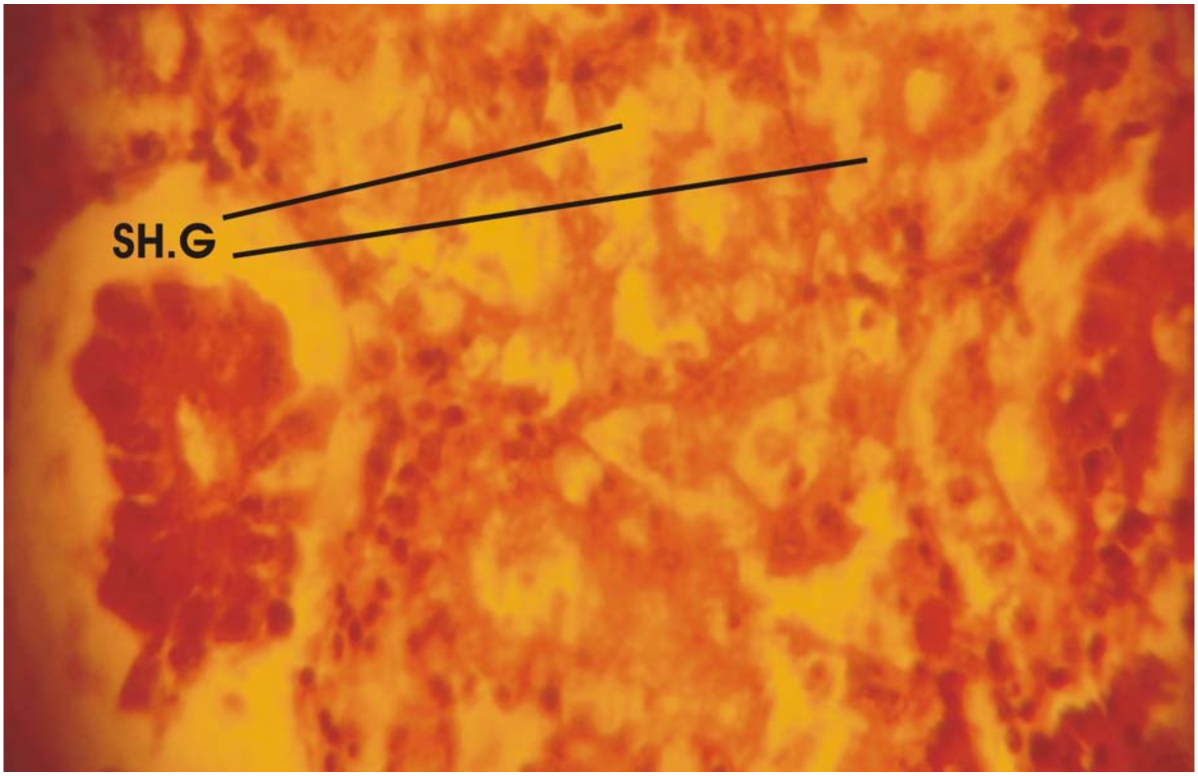


Plate 23.

Plate 24. Section through the kidney of *Oreochromis niloticus* at Soba station in Blue Nile Notice shrinking of glumeruli (Sh G).H&E X400

Plate 25. Section through the kidney of *Oreochromis niloticus* at Adobaseeen station in White Nile Notice less cellular in glomerulus (LC).H&E X400



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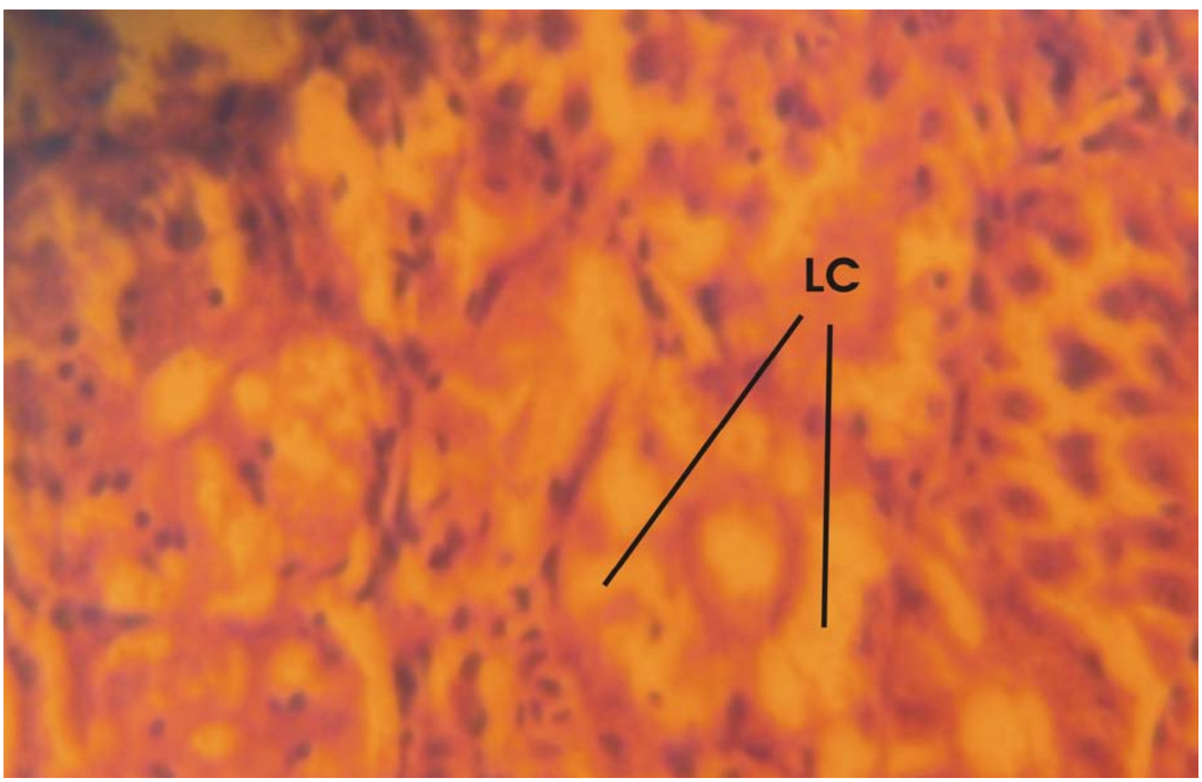

Plate 25.

Plate 26. Section through the kidney of *Oreochromis niloticus* at Alamab station in White Nile Notice edema in Bowman's capsule (E).H&E X400

Plate 27. Section through the kidney of *Clarias gariepinus* at Jebal Awlia station in White Nile Notice Blood congestion (BC).H&E X400

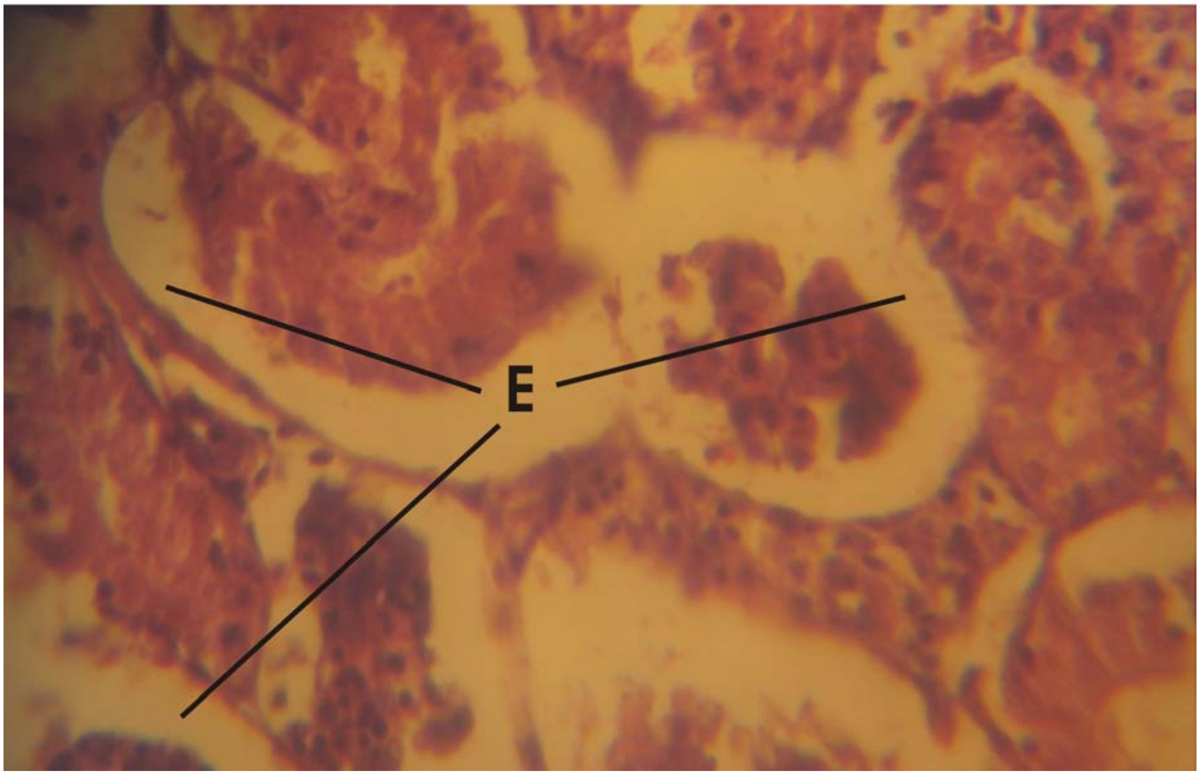

Plate 26.

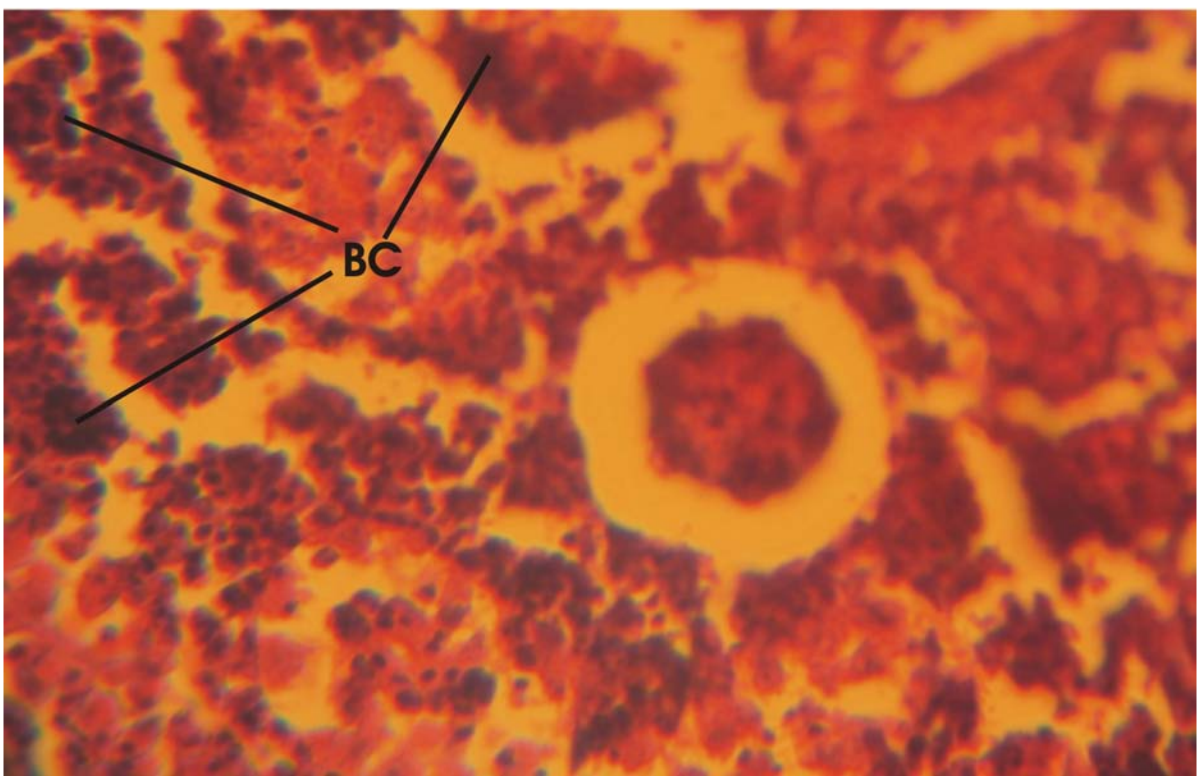

Plate 27.

Table (24). Showing the main types of histopathological changes detected In *Oreochromis niloticus*& *Clarias gariepinus*

organ	*Histopathological changes*	*%of fish in which the effect was detected*	
		Oreochromis	*Clarias*
muscle	Edema between muscle bundles	*20%*	*15%*
	Focal areas of necrosis	*9%*	*11%*
	Splitting of muscle fibers	*5%*	*11%*
liver	Lymphocytic infiltration	39%	*45%*
	Ruptured hepatocytes and vacuolation	*30%*	*32%*
	Focal areas of necrosis	*22%*	*51%*
	Cell damage	*45%*	*45%*
	Dilation and thrombosis formation in central vein	*50%*	*56%*
gills	Edema in secondary lamellae and gill filaments	30%	*18%*
	Dilation and congestion in blood vessels of gill filaments and atrophy of secondary lamellae	*50%*	*50%*
	Proliferation in the epithelium of gill filaments	*61%*	*54%*
	Sloughing of secondary lamellae	*12%*	*9%*
Kidney	Edema in Bowman's capsule	*18%*	*30%*
	Shrinking of glumeruli	*30%*	*10%*
	Vacuolation in glomerulus	*23%*	*30%*
	Blood congestion	*10%*	*25%*

Chapter five

CHAPTER FIVE

DISCUSSION

Physic-chemical parameters:

The physic-chemical parameters are considered as the most important principles in the identification of nature, quality and type of water (fresh, brackish, saline) for any aquatic ecosystem.

Temperature is a factor of great importance for aquatic ecosystem, as the chemical and physical characteristics of water. It's a major regulating factor for distribution of aquatic organisms.

The recorded temperature of water ranged from 25.1 C^o to 26.7C^o in White Nile in different stations and season which was 25.1 C^o in Jabal Awlila and 26.0 C^o in a Dobassen and 26.7 C^o in Allamab station. And it ranged from 29.2 C^o to 31.2 C^o in Blue Nile in different stations and season which was 31.0 C^o in Soba station and 29.2 C^o in Algreef and 31.2 C^o in Bori station. (Table 2, 3 and fig.2, 3, 4, 5) however, differences between sites might be due to difference in regional and sampling season. Which is slightly higher than the recommended limits according to Guidelines for Canadian Drinking Water Quality? But here and in this subtropical country this temperature might be a normal temperature.

These results were higher than the results of (**Bastawy. 2006)** who found that water temperature of white and Blue Nile was 21 C^o but was in the same line of **(Egbal. 2010)**who found that the Blue Nile temperature ranged between 31 C^o to 37 C^o and the white Nile ranged between 26C^o to 26.3 C^o Which is slightly higher in the Blue Nile than the White Nile, this may be due to the presence of two thermal electric power stations that discharge cooling waters into the Blue Nile in the vicinity of the sampling station The surface water temperature of all stations was within the normal range, reflecting the seasonal variation.

Water temperature and dissolved oxygen content are, due to metabolic processes, vital factor in water for organisms. The mechanism whereby heavy toxicity increases with higher water temperature can be explained by elevated respiratory activity. Moreover metal solution itself causes increased respiratory activity. The adsorption and release of metals can also depend on temperature.

Result of dissolved oxygen shows slight seasonal variation form Distribution of dissolved oxygen is affected by solubility of many inorganic nutrients and it decreases with elevated high water temperature.

The concentrations of dissolved oxygen occurred within a wide range of 7 to 7.7 mg O_2/ L in White Nile and from 6.9 to 7.5 mg O_2/ L in the Blue Nile. The White Nile and Blue Nile were fairly oxygenated waters at Khartoum although the dissolved oxygen sometimes decreased. It occurred at times of phytoplankton abundance in autumn.

Excessive phytoplankton growth may cause a decrease in oxygen through the removal of oxygen from the water column as a result of microbial breakdown of decaying algae.

Talling *et al* 1966, Hill and Rai 1984 and Talling. 2009) have noted that oxygen in fresh water decreases in regions with abundant phytoplankton in autumn and summer. So the dissolved oxygen concentrations were at the saturation level, with no oxygen deficiency.

The pH of pure water is 7. In general, water with a pH lower than 7 are considered acidic, and with a pH above 7 alkaline. The normal range for pH in surface water systems is 6.5 to 8.5, **(APEC, 2007)**

The pH values Ranged from 6.6.2 to 7.2 in White Nile and from 6.5 to 7.0 in the Blue Nile. Higher values were recorded in hot periods (summer) while the lower values are found in cold periods (winter). This decrease in pH values during cold periods is mainly related to high bicarbonate content, while the uptake of carbon dioxide by phytoplankton decrease as a result of increasing in concentration of HCO_3**.(Abdo,2005).**

The results of this study agree with the finding of (**Egbal. 2010) w**ho found that the Blue Nile pH ranged between 6.5 to 7.2 and the White Nile ranged between 6.9 to 7.4 Fresh water fishes can live normally in water with pH between 5.0 and 9.0. Acidic and alkaline waters have various effects on fish, and it was found that young Fish are more sensitive to pH fluctuation than adult ones **(FAO, 1975).**
The results from the pH measurements showed that all the samples were in the range considered normal for surface water.

Nitrates, together with phosphates are the main ingredients in fertilizers but can also come from sewage water. Nitrate, is potentially harmful if its concentration is high in water and serve as a good indicator of chemical polluted water **(Peter, 1998).** High concentrations have a harmful effect on living organisms **(Peter, 1998).** Ingestion of nitrite, e.g. through nitrate preserved meet, can pose great risk to public health by transformation to nitrosamines, which are carcinogenic **(Huang *et al,* 1996).** Since nitrite and nitrate are nutrients, their presence in high Concentrations can nurture the growth of algae in the water and consequentially impair the Water quality **(Bastawy *et al,* 2006).**

Nitrate concentration ranged from 0.23 **(ml/l)** to0.96 **(ml/l)** in White Nile and from 0.30 (**ml/l)** to 0.74 **(ml/l)** in Blue Nile (Table 2, 3 Fig, 2, 3, 4, 5) seasonal and regional variation could be mainly attributed to phytoplankton uptake and the oxidation of existing ammonia by nitrifying bacteria and biological nitrification, yielding nitrite as intermediate state especially in abundant oxygen during winter.

On the other side, the relative decrease in their content during hot period due to their reduction to ammonia.

The results in this study were lower than the finding of. **(Bastawy *et al*, 2006)`**who found that The concentration of NO3 varied between < 20 and 388 **(ml/l)**. High concentrations in the range of 140–388 **(ml/l)** occurred during the wet season.

Hall *et al.* (1977) suggested that NO3 comes mainly from the atmosphere, entering the River Zambezi with rain water. In addition, agricultural runoff may be another source of nitrate input into the Nile water.
Also our results was agree with **Ahmed *et al.* (1986)** who reported high values of dissolved NO3 during the rainy season in River Nile (Egypt).

Phosphates concentration recorded were raged between 1.80 to 3.11 in White Nile and1.59 to 1.80 **(ml/l)**in the Blue Nile .Phosphorus cycle was dynamic and complex involving adsorption and precipitation reactions, interchange with sediments and uptake by aquatic biota. The variation recorded in present study could be attributed to seasonality of sampling. The decrease in phosphate values during summer were probably due to distinct drop in phytoplankton on which nutrient regeneration process depends. In addition, the recycling processes of nutrients depend on the nutritional status of algal cells. On the other side the relative increase in phosphate values during winter can be related to the complete mixing of the water and more phosphorus release from the sediment especially in the presence of dissolved oxygen**.**

Heavy metals:

Anthropogenic sources such as agriculture run-off industrial and sewage have created both localized and regional Water pollution problems in nearly every country Around the world **(Adeogun, *et al*, 2011)** In some cases the pollution has been extensive enough to lead to environmental disasters and ecosystem shutdown **(Adeogun, .2012).**

In the present study, the concentration of heavy metals in water of White and Blue Nile from different station was as: Cu > Zn> Fe> Pb> Cr> Cd
For Cr, Cd, Pb and Zn the maximum concentrations in both White and Blue Nile were observed in summer, while the minimum values of Cr and Cd were detected in winter while the minimum values of Pb in autumn and the minimum values of Zn was observed in winter. Cu concentration was highest in winter lowest in autumn. And for Fe had high concentration in autumn and lower in winter and summer. These results comply with those recorded by (**Saeed 2000)** who found that heavy metals concentration showed seasonal variations, being greater in summer and lowest in winter and autumn. This may be attributed to the high temperature which is result in increasing water evaporation from the Nile. Or may be back to phytoplankton growth which was higher in autumn season that can absorb large quantities of heavy metals from water.

Moreover, the highest concentrations of all heavy metals were detected in water samples collected from Aldubaseen and Alamab station in the White Nile and Soba and Bori station in the Blue Nile. chromium (chromium oxide is used as inorganic color additive) which are used in soap and paint manufacture which may be attributed to wastes of industrial activities and wastewater around indesdirial zone around the sampling station in soba and allabab and exit of the sewage discharge nearest the (aAdobaseen bridge area). The sewage water was partially domestic, other sources of water were from hospitals, military factory, tanneries and others High levels of lead may be attributed to presence of industrial and agricultural discharges, direct sewage bonds discharge, motor boat traffics Or may be attributed to the fact that Blue and White Nile are the main collectors of all materials spread by human industrial and agriculture activities around Khartoum city. Also Blue And White Nile rivers are characterized by large amounts of silt especially in the flood seasons like many rivers in the tropical regions.

Cd is used in Nickel- Cadmium rechargeable batteries and for planting, also used in some paints, Plastic and ceramic **(WHO, 1993).** All these activities are found in Khartoum city Industrial areas nearest Soba and Alamab station and discharged in White Nile through sewage ponds or access the Nile throw the rain folds.

Present results showed that, most of the heavy metals concentrations in surface water of White and Blue Nile water were found within the permissible limits of the World Health Organization 2011. These results are in agreement with **(El Bouraie et *al,* 2010)** who studied heavy metals in five drain outfalls and found that the level of metals is within the permissible limits of Egyptian law and **(WHO)**, **(Lasheen *et al,*2012)** stated that the average concentrations of heavy metals in El-Moheet drain; which discharge in El- Rahawy drain; are within the permissible range according to **(WHO, 2011)**.

Sediments:

Sediments represent an important sink for trace metals in aquatic systems, and metal concentrations in sediment can be of several orders of magnitude greater than in the overlying water. Sediment-associated metals pose a direct risk to detrital and deposit-feeding benthic organisms, and may also represent long-term sources of contamination to higher tropic levels **(Eimers, Evans, & Welbourn, 2001; Luoma, 1983, 1989),**Sediment are important hosts for pollutant heavy metals, therefore they have been used to monitor the pollution in aquatic environment.

The maximum concentrations of trace metals in sediment in White and Blue Nile rivers from different station. For all studied metals were observed in summer, while the minimum values were detected in autumn.Moreover, the highest concentrations of all heavy metals were detected in sediments samples collected from Aldubaseen and Alamab station in the White Nile and Soba and Bori station in the Blue Nile.

The Fe and Cd contents of the White and Blue Nile sediments are lower than literature reports by **(Ashraf *et al.*, 1991; Park & Presley, 1997; Tariq *et al.*, 1991)**. Which were reported to be 61–218 and 146– 182 mg/kg respectively. The reports for Zn and Cu were found to be 23.3–38.9 mg/kg and 3.7–8.2 l mg/kg respectively **(Ashraf *et al.*, 1991; Aucoin *et al*, 1999).** These levels are higher than the present study**.** Pb, Cr contents in the reports ranged from 2.7 to 7.0 mg/kg and 4.4 to 10.7 mg/kg respectively. **(Tariq *et al.*, 1991).** Our Pb, Cr concentrations are lower than these results.

In the present study, the concentration of heavy metals in sediments of both White and Blue Nile was in the order: Cu > Fe> Pb> Cd> Cr> Zn.

Suspension of sediments into the water body may increase the metal concentration in the water. Sediment is the major depository of metals in some cases, holding more than 99% of total amount of a metal present in the aquatic system **(Odiete, 1999).** In addition, heavy rain fall leads to farm draining. Large amounts of pesticides containing metal compounds are brought via surface runoff from the farms to the river, contributing highly to the agricultural pollution. Chemical fertilizers containing Pb is used in agricultural industries of the regions around the two river coast

Metals content of selected fish species:

Fish in the present study were collected from different habitat and have various morphometric parameters that results showed that fish exhibited wide inter-specific variations in metals accumulation in all organs.

Elements from water are taken by fish through gills and the gastrointestinal tract, where they can be accumulated in inner organs, leading to pathological changes **(Cengiz *et al,* 2006)**

Fish have frequently been used as bioindicators for trace metal contamination. **(Fowler ,2002)** mentioned that many different fish species have been analyzed all have greatly different trace element physiologies and feeding strategies. Three major aspects must be considered in investigation of fish: first, the use of fish as indicator organisms for the heavy metals pollution of their environment and their possible unfitness for human consumption from a toxicological point of view. It is mainly the muscle tissue, which to be investigated in this context. Second is the use of fish to study the physiological behavior of heavy metals. Here the most important factor are distribution of heavy metals in body organs and respective affinity of these organs for metals, uptake kinetics, regulatory mechanisms, and effect on the metabolism by heavy metals which is to be investigated in this context also. Third, fish as the end consumer in the aquatic food chain and thus their use as indicator of heavy metals enrichment. Knowledge of biologic factors such as age and size, life cycle and life history, seasonal and local variations of heavy metals content in the animal and the tropic level of the species.

Generally, whatever the precise uptake mechanism, it may be concluded that most trace metals are accumulated by aquatic biota as a result of this is the potential for toxic effects, as all such metals are toxic at high level(even those which are of an essential nature). At least two mechanisms have been involved in aquatic species to address this problem. The first of these involves the capability to excrete trace elements at relatively high rates, in most cases matching the rate of metals uptake; the net result of this has been termed regulation of trace metals, and is important where consideration is given to the use of species as potential biomonitors of trace metals pollution **(Phillips and Rainbow, 1989).**

There are many routes for the entry of heavy metals into the body of fish, namely oral ingestion, absorption through gills **(Lloyd, R. 1992)** general body surface and gastrointestinal tract. After absorption the metal makes its way into the target organ, where it produces various types of disturbances. The gills which carry out the functions of respiration, osmoregulation and excretion, remain in contact with external environment and are particularly sensitive to changes in the quality of water so they are considered to be the primary target of the contamination.

Variations in organs ability to accumulate elements Fish of the present study always showed the lowest concentration of metals in muscle. The essential elements Cu, Zn and Fe were accumulated mainly in the liver, while Pb and Cr and Cd exhibited their highest concentrations in gills. The accumulation pattern of Cd differed between species where the highest concentrations were fluctuated between the liver and gills and kidney.

Muscle tissue:

The study of heavy metal concentrations in fishes was important with respect to human consumption of fish. Several studies shows heavy concentration in tissue of fishes may vary considerably among the different species. This was possibly due to differences in metabolism and feeding patterns of the fishes.

Concentrations of trace metals in fish muscles in White and Blue Nile rivers from different stations . Showed that the maximum concentration of Cu, Cr, Pb and Zn were observed in summer, while the minimum values were detected in autumn. While Cd and Fe showed no significance different in all seasons. Moreover, the highest concentrations of Cu were found in Jabal Awlia station and the lowest value was found in Alamab in the White Nile while Cr represents the opposite. But Cd concentration was high in Adubaseen and lower in Alamab. In the Blue Nile all heavy metals were detected as the highest concentration from Soba station.

The present study revealed that all studied elements were lower than the permissible levels recommended by **(WHO ,1984 & 1993, 2011)**, (**USEPA, 1986)**, (**ANZECC, 2000)** and **(Abbasi *et al.* ,1997)**. Also the results were lower than those recorded by(**Dobaradaran *et al,*2010)**, the data were nearly similar to those of(**Schlotfeldt and Alderman ,1995)**, (**Uluozlu *et al,*2007),** (**Mohamed ,2008)**, (**Miclean *et al*.,2009) and (Saeed and Shaker,2010).** Results were lesser than those reported by (**Gabriel *et al*, 2006),** (**Morshdy *et al*, 2007),** (**Al-Bader, 2008), (Dobaradaran *et al,* 2010).** These variations may be attributed to the differences between the localities, and the amount and source of pollution from one area to another.

In the present study, the concentration of heavy metals in the studied fish muscle from different stations in the order:

Cu > Cr> Zn> Pb> Cd> Fe

Liver:

Liver represented the highest site of concentrations of heavy metals. This may be attributed to the major role of liver in detoxification and protection from heavy metal exposure, both by producing metallothionines (metal binding-proteins) and by acting as storage site for bound metals **(Pratap et al., 1989).** Also, the liver concentrates these metals from the blood circulatory system of the fish.

The maximum concentration was observed in summer, while the minimum values were detected in autumn. Moreover, the highest concentrations of Cu were found in Jabal Awlia station and the lowest value was found in Alamab in the White Nile while Cr represents the opposite. But Cd concentration was high in Adubaseen and lower in Alamab. In the Blue Nile all heavy metals were detected in the highest concentration from Soba station.

The essential metals Cu, Zn and Fe were accumulated mainly in the liver, while Pb and Cr and Cd exhibited their highest concentrations in gills.

The present results are in agreement with (**Velcheva, 2006)** who reported that heavy metals were significantly higher in fish viscera, including liver tissue and kidney than in the edible muscle tissue.

The high heavy metal content in liver of fish collected from the two sources of water can be related to accumulation of such heavy metals from the water primarily through fish gill where metallothionine enhances that bioaccumulation in gills and its uptake could be controlled by the amount of water passing through the gills **(Saeed, 2000).** Fish collected from area of industrial activity (Alamab& Soba) and sewage areas (Adobaseen) significantly greater concentrations of heavy metals than fish collected from areas of agricultural activities (ALgreef). These variations in heavy metal concentrations may be due to the nature of water source. Moreover, it is obvious that the average heavy metal concentrations in different tissues *of Oreochromis niloticus* were higher than those of *Clarias gariepinus*. This may be due to the difference of feeding habits of the fish species, where the former fish is mainly omnivorous feeding on fish, insect larvae, mollusks, planktonic organisms and water weeds which accumulate large amounts of heavy metals.

Also, *Oreochromis niloticus* feeds mainly on phytoplanktons which accumulate large amounts of heavy metals while *Clarias gariepinus* feeds mainly on, insects and crustaceans. Moreover, *Clarias gariepinus* lives mainly in muddy or semi-muddy bottom and *Oreochromis niloticus* wanders in water from surface to bottom, being frequently in contact with soil particles **(Saeed, 2000).**

The accumulation of essential metals in the liver is likely linked to its role in metabolism **(Zhao, Feng ,2012)** high levels of Zn and Cu in hepatic tissues are usually related to a natural binding proteins such as metallothioneins **(Gorur , Keser ,2012)**which act as an essential metal store (i.e., Zn and Cu) to fulfill enzymatic and other metabolic demands **(Roesijadi.1996 Amiard, 2006)** In the same way, Fe tends to accumulate in hepatic tissues due to the physiological role of the liver in blood cells and hemoglobin synthesis**(Gorur , Keser ,2012)** On the other hand, the liver also showed high levels of non-essential metals such as Cd; this finding could be explained by the ability of Cd to displace the normally associated essential metals in hepatic tissues **(Amiard, 2006).**

Similar results of high Zn, Cu, and Cr, Cd in the liver were observed in many field studies **(Zhao, Feng .2012; Eisler, 2010, Dural, 2007).**

In the present study, the concentration of heavy metals in both fish species liver from both White and Blue Nile had the order:

Cu > Zn> Pb> Cr> Cd> Fe

Gills:

Gills are the main route of metal ion exchange from water **(Qadir, 2011**) as they have very large surface areas that facilitate rapid diffusion of toxic metals **(Dhaneesh, 2012)**Therefore, it is suggested that metals accumulated in gills are mainly concentrated from water.

The maximum concentrations of trace metals in fish gills in White and Blue Nile rivers from different stations in different seasons. were observed in summer, while the minimum values were detected in autumn. While Pb and Zn show high concentration in winter and low in autumn. This seasonal variation could be attributed to its bioavailability in the environment **(Lawani and Alawode,1987).**

Moreover, the highest concentrations of Cu, Pb was found in Jabal Awlia station and the lowest value found in Alamab in White Nile while it was found in high level in soba and low in Bori. But for Cr and Cd were found in highest value in Allamab and lower value in Jabal awlia in the White Nile. It was found in high value in Soba and lower in Algreef in the Blue Nile also Zn and Fe had the same trend in the Blue Nile while they were found in the highest concentration in Adobaseen and lower in Jabal Awlia station.

This is in agreement with the findings of **(Moore and Ramamoorthy, 1984)** they reported Cu, Cr, Cd, and Fe, Zn and pb and other trace element are accumulated in gills of aquatic organisms. Similar results for high heavy metals concentrations in gills were recorded by **(Karan, 1998) (Avenant-Oldewage and Marx ,2000) (Abu Hilal and Ismail ,2008) and(Qadir and Malik , 2011) Also, (Eisler, 2010)** who reported that fish's gills had consistently higher accumulations of Cr,and Cu and pb than muscles tissues.

In the present study, the concentration of heavy metals in both fish species Gills from White and Blue Nile in the order:

Cu > Pb> Zn> Cr> Cd> Fe

Kidney:

The maximum concentrations of trace metals in fish kidney in White and Blue Nile Rivers from different stations were observed in summer, while the minimum values were detected in autumn. While Cd ‹Zn and Fe show no significanc different in all seasons. Moreover, the highest concentrations of Cu was found in Jabal Awlia station and the lowest value was found in Adobaseen in White Nile while Cr was found in high concentration in Alamab and low in Jabal Awlia . But Cd concentration was higher in Adubaseen and lower in Alamab. in the Blue Nile Cu concentration was highest in Bori and low in Soba and Cr ,Zn and Cd were higher in Soba lower in Bori While Fe was higher in Soba and lower in Algreef and there was no significant difference in the accumulation of Pb in all stations in Blue Nile.

The concentration of heavy metals in both fish species kidney from all stations in White and Blue Nile were as:

Cu> Zn > Pb > Cd > Cr > Fe

Our obtained data were somewhat closer to (Abdallah **.2008)** recorded the concentrations of Cd, Pb, Cu and Zn in kidney of *Sardinella aurita*, *S. rivulatus* and *Synodus saurus* from two main harbors in Alexandria, Egypt.

In addition, metal levels in kidney in the present study were generally within the ranges of those found in the fish of the river Nile recorded by**(Hanna, 1989),(Abdelmoneim and El-Deek ,1992) (Emara *et al.*1993), (Ahmed *et al.* 1996),(El-Moselhy ,1996) and(Ali *et al,* 2011).**

Histopathlogical results:

Histological biomarkers of toxicity in fish organs are a useful indicator of environmental pollution (**Peebua, 2008)** Several histopathological changes have been reported in the gills liver, kidneys and muscles of fish in response to agricultural, sewage and industrial pollutants **(Mohamed, 2003)**

Results of the present study revealed that *Oreochromis niloticus& , Clarias gariepinus* from White and Blue Nile rivers, manifest histopathological changes in muscles, liver, gills and kidney during winter, summer and autumn seasons. It is possible that the pathological alterations in the tissues of both studied fish could be a direct result of the heavy metals, and sewage. This entered to the rivers with the drainage water.

Muscles:

The histopathological alterations in the muscles of both studied fish (plates1-6) are in agreement with those observed by many investigators who have studied the effects of different pollutants on fish muscles **(Sakr, and Gabr, 1991. Abo Nour, and Amer, 1995, Das, and Mukherjee, 2000.)** Focal areas of necrosis were seen in the muscles of O. *spilurus* exposed to contra/insect 500/50E.C. (**Elnemaki, and Abuzinadah, 2003**). at the same time, (**Abbas and Ali, 2007**) observed destruction and vacuolation and splitting of the muscle cells in *Oreochromis spp*. exposed to chromium.

Similar alterations in muscles of *Oreochromis* and *Clarias* were observed in several species of fish Exposed to heavy metals and these alterations were described by **(Oliveira Ribeiro *et al.* 2002**), **(Jiraungkoorskul *et al.* 2003),** (**Thophon *et al*. 2003)** and **(Gupta and Srivastava.2006).**

Liver:

The organ most associated with the detoxification and biotransformation process is the liver and due its function, position and blood supply, it is also one of the organs most affected by contaminants in the water.

Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment, or in other organs **(Heath, 1995).**

The liver is particularly susceptible to damage from a variety of toxicants. One of the most important functions of liver is to clean pollutants from the blood, so it is considered as indicator of aquatic environmental pollution **(Soufy, 2007).**

The liver of both studied fish showed Lymphocytic infiltration, focal areas of necrosis, thrombosis formation in central veins, ruptured hepatocytes and vacuolation and Cell damage These changes may be attributed to the direct toxic effects of pollutants on hepatocytes, since the liver is the principal organ responsible for detoxification in vertebrates generally and in fish particularly **(Freeman *et al*, 1983).**

The present study suggests a strong link between heavy metals and lesions in the liver. **(Sorensen, 1991**) cited that heavy metals might cause liver damage. **(Aly *et al,* 2003)** obtained similar results after exposure of *Clarias gariepinus* to lead pollution. Similar alterations in the liver of *Tilapia zillii* and *Clarias gariepinus* were observed in fishes living in Nile water polluted with heavy metals **(Ibrahim, *et al,* 2005, Tayel *et al*, 2008**).

(**Yacoub and Abdel Satar, 2003)** observed histopathological effects of heavy metals on some fishes inhabiting Bardawil lagoon. (**Ptashynski *et al,* 2002)** reported histopathological alteration after exposure of the lake whitefish *(Coregonus clupeaformis)* to nickel.

(**Olojo *et al*., 2005)** observed liver histopathological lesions after exposure of *Clarias gariepinus* to Pb for 9 days. Several histopathological changes in the liver were also observed in *Oreochromis niloticus* and *Tilapia zillii* collected from the southern region of Lake Manzalah contaminated with domestic, industrial and agricultural pollutants **(Mohamed, 2001).**

thus, the histopathological alterations observed in the liver of the studied fish may be attributed to the effects of the agricultural, industrial and sewage wastes discharge into White and Blue Nile.

Gills:

Fish gill is a multifunctional organ responsible for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion. Fish gills are exposed directly to aquatic media and sensitive to any change of water components, since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water **(El-Serafy et *a*, 2009)**

Thus, changes in fish gills are among the most commonly recognized responses to environmental stressors and are indicative of physical and chemical stress .Therefore, gill histology is used extensively as indication of environmental pollution **(El-Serafy*et al*, 2009).**Gill histopathological lesions as indicators of exposure to a wide range of contaminants including heavy metals have previously been used in numerous laboratory and field studies around the world.

The gills of both studied fish(plates 16-12) showed dilation and congestion in blood vessels of gill filaments and atrophy of secondary lamellae, proliferation in the epithelium of gill filaments and edema in gill filaments and secondary lamellae and congestion in blood and, sloughing of secondary lamellae.

These pathological changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants thorough the gill surface. The observed alterations like proliferation of the epithelial cells is defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants.

The observed edematous changes in gill filaments and secondary lamellae probably due to increased capillary permeability (**El-Serafy *et al*, 2009).**

The present results are in agreement with those observed in other fish species under the influence of different pollutants **(Fernandes, 2008)** In this respect **(MASON, 1991)** observed dilation and congestion in blood vessels of gill filaments and atrophy of secondary lamellae, sloughing of secondary lamellae, lifting of the lamellar epithelium and blood congestion in the gills of *P. lineatus* caged in Cambé stream, Brazil, polluted by industrial, domestic and agricultural wastes. Also, (**El-Serafy, *et al*.2009)** noticed epithelial lifting proliferation of epithelial cells of primary and secondary lamellae, hyperplasia of mucous cells and necrosis of epithelial cells in the gills of *C. nasus* and *L. cephalus* from River Mures, Western Romania, polluted by heavy metals.

Kidney:

The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal of wastes from blood but it is also responsible for selective reabsorption, which helps in maintaining volume and pH of blood and body fluids (**Iqbal *et al*, 2004)**.The kidney is one of the first organs to be affected by contaminants in the water **(Thophon, *et al*, 2003).**

The common alterations found in the kidney of both studied fish were edema in Bowman's capsule and Shrinking of glumeruli and Vacuolation in glomerulus.

The present results are in agreement with those observed in C. *carpio* exposed to sewage(**Adams, *et al* ,1994).** P. *lineatus* exposed trichlorfon (Authman**, 2008)** and L. *calcarifer* exposed to cadmium **(**Authman***, et al*** **.2008)** While**, (Finerty et *al*., 2006).** Found degeneration in the epithelium of renal tubules in the kidney of *P. lineatus* caged in Cambé stream, Brazil, polluted by industrial, domestic and agricultural wastes.

From the present data, it is obvious that liver, kidney and gills accumulated higher concentrations of the studied heavy metals than the muscles.

This means that it will be less hazardous to man if the fish muscles are the only to be eaten **(Abbas and Mohamed, 2013).**

Conclusions:

- All surface water characteristics; temperature, pH, dissolved oxygen were in the normal range
- The study clearly indicated significant accumulation of heavy metals in the organs of the two fish species from White and Blue Nile Rivers.
- It could be concluded that the environmental contamination of the White and Blue Nile induced several histopathological alterations in the tissues of *Oreochromis niloticus&, Clarias gariepinus*. Consequently, it is recommended to coordinate different efforts to rescue White and Blue Nile from the environmental pollution problems. The overcoming of these problems can be possible by subjecting the drainage waters discharged into the rivers to technical treatments that fulfill its safety In conclusion the present study showed that histopathology is a useful biomarker for environmental contamination.
- The results also showed that metal accumulation varied between organs and species depending on species specific factors like feeding behavior and geographical distribution that caused variation in metals accumulations between fish.
- Heavy metals in the edible parts of the fish indicated safe levels for human consumption and concentrations in the muscles are generally accepted by the international legislation limits so the two studied fish species can be safely used and consumed by human.
- It is logical to say that the high concentration of metals in river become gradually accumulated on the sediments and in due course get transferred to fish. Finally, the high level Cu, Cr, Cd, Pb Zn and Fe in gills ,liver and kidney of the two fish species shows that they were good bio-indicator to monitor pollution in the rivers

- The results of this study indicated that, Soba, Adobaseen stations were more polluted compared with JEbal Awlia and Algreef stations this fact is confirmed through differences of heavy metal concentration in fish, water and sediments between the study stations.
- Although the results obtained does not show any form of danger posed to consumers of fish and water from these Nile but the possibility of deleterious effects after long period cannot be ruled out.

Recommendations:

- The general research environment in fisheries sector must be improved, through technical support; instruments availability, trained technicians, and laboratories equipments and collaboration.
- Chemical analyses alone may not suffice to describe the adverse effects of the heavy metals present at contaminated sites. Therefore, the use of a set of biomarkers for assessment of environmental quality has been recommended.
- Health risk analysis of fish should be continuously monitored in potential polluted areas since fish showed a tendency to accumulate heavy metals in liver, gills and kidney and Since virtually all metals investigated were found in higher concentration, so government should intact laws that will ensure that industries make use of standard waste treatment plants for the treatment of their wastes before they are being discharged into water bodies
- Also, it is recommended that treatment of all kinds of wastewaters, sewage and agricultural wastes must be conducted before discharge into the aquatic systems. Also, enforcement of all articles of laws and legislations regarding the protection of aquatic environments must be taken into considerations.
- This is as a result of the fact that this water body serves as the receptor for domestic wastes as well as runoff from agricultural lands where phosphate fertilizers and other agrochemicals are frequently used. There is therefore the need for continual assessment of the level of pollution of this stream with metals from the mentioned sources with a view to reducing the level of pollution via education and public enlightenment.

- Biomarkers can offer additional biologically and ecologically relevant information valuable tool for the establishment of guidelines for effective environmental management. So, it can be stated that fish biomarkers are necessary for monitoring environmentally induced alterations to assess the impact of xenobiotic compounds (i.e. heavy metals) on fish.

Chapter six

CHAPTER SIX

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Appendix

APPENDIXES:

Appendix (1)

Permissible limits of heavy metals in water and fish according to international organization.

Metals	Water (mg/L)		Fish (mg/g)	
	FDA	WHO	EPA	WHO
Pb	0.005	0.01	0.05	1.5
Zn	-	3.0	5.0	150
Cu	1.0	1-2	1.0	-
Fe	-	0.3	0.1	2.5
Cd	0.05	0.03	-	0.2
Cr	-	0.1-0.5	0.05	-

Appendix (2):

Permissible limits of heavy metals in fish muscle according to International organization

Metal	Permissible	Country and reference
Copper	1.00 ppm 20.0 ppm	WHO (1984) South Africa (Foodstuffs, cosmetics
Lead	0.05 ppm 0.1 mg/kg 0.5 ppm	WHO (1984) Egypt "E.O.S.Q.C. (1993) FAO/WHO (1992)
Cadmium	0.005 ppm 0.05 ppm 0.1 mg/kg	WHO (1984) FAO/WHO (1992) Egypt "E.O.S.Q.C. (1993)
Mercury	0.001 ppm 0.5 mg/kg 0.5 ppm	WHO (1984) Egypt "E.O.S.Q.C. (1993) FAO/WHO (1992)

Appendix (3):

The heavy metal concentrations in water guidelines (mg/L).

Guidelines/Locality	Cd	Cr	Cu	Fe	Ni	Pb	References
TSE-266	0.005	0.05	2	0.2	0.02	0.01	TSE-266, 2005
WPCL	0.003	0.02	0.02	0.3	0.02	0.01	WPCL, 2004
CIW	0.01	0.1	0.2	5	0.2	5	Anonymous, 1997
WHO	0.01	0.05	2	-	0.02	0.05	WHO, 1993
EPA	0.01	0.05	1.3	0.3	-	0.05	EPA, 2002
EC	5	50	2	0.2	20	10	EC, 1998

Appendix (4): Direct discharge of untreated effluent from Assalaya sugar factory into White Nile. Source: UNEP.

Appendix (5): Access of sewage water to White Nile

Appendix (6): Untreated water and its way to Blue Nile

Appendix (7):

Oil, plastic and wooden scrapes around the study station in Jebal Awlia

Appendix (8):

Oil, scrapes around the study station in Jebal Awlia

Appendix (9): Untreated water in its way to White Nile near Allamab station

Appendix (10):

Untreated water in its way to White Nile

Appendix (11): Untreated water in its way to White Nile

Appendix (12):

Oil, scrapes around the study station in Bori

Appendix (13): Oil, from agricultural actvites around the study station in Bori

Appendix (14):

Cat Fish, *Clarias gariepinus*

Appendix (15): NileTilapia, *Oreochromis niloticus*

Appendix (16): Untreated water in its way to White Nile through khor Abou Adam

Appendix (17): Physiochemical parameters of the water samples from White and Blue Nile.

Appendix (18):

Physiochemical parameters of the water samples from White and Blue Nile.