1. Introduction

The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years,because they are indestructible and most of them have toxic effects on organisms. Among environment pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems (Goldstein ,1990 ,Gledhill et al., 1997).

There is increasing concern about the quality of foods in several parts of the world. The determination of toxic elements in food has prompted studies on toxicological effects of them in food. Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by marine organisms (Malik, 2004).

Heavy metals may enter aquatic system from different natural and anthropogenic (human activities) sources. Figure 1.1, including industrial or, domestic waste water, application of pesticides and inorganic fertilizers storm runoff, leaching from landfills shipping and harbor activities, geological weathering of the earth crust and atmospheric deposition.(Yilmaz, 2009).

Heavy metals are commonly found in natural water and some are essential to living organisms, yet they may become highly toxic when present in high concentrations .These metals also enter ecosystem through anthropogenic source and distributed in the water body, suspended solids and sediments during the course of their mobility (Olaijire, et al., 2003).

Like in other organisms, heavy metals are not destroyed in humans. Instead, they tend to accumulate in the body and can be stored in soft and hard tissues such as liver, muscles and bone and threaten the health of humans. Therefore, the heavy metals are among most of the pollutants, which received attention in various countries and considered the most dangerous category of pollutants in the water (Hassaan,et al.,2007). An early example of an environmental problem due to heavy metal accurred in 1952, in the vicinity of Japanese fishing harbour of Minimate .This disease (minimata) was a result of consuming fish and shrimps contaminated with mercury from the waste water discharged by factories. Another example is the (Ita-Ita)disease in Fugawa, Japan in 1955,it was the result of consuming rice ,fish and bivalves that were Cd –contaminated from waste water discharged by nearby mining .(Dural, et al., 2007).

Mostly the variation in elemental concentration in fish tissue has been attributed to variation in size. Many studies have focused attention on the dependence between the contents of .(metals and size of these fish (Kostecki 2000

Importance of fish 1.2

The real importance of fish in human is not only in its protein content, but also to the two kinds of omega-3 fatty acids: eicosapentenoic acid (EPA) anddocosahexenoic acid (DHA) .Omega-3 fatty acids are so important for growth where they reduce cholesterol levels and the heart disease, stroke, and preterm delivery (Burger, and Gochfeld, 2005). Fish also contain vitamins and minerals that play essential role in human health. Since diet is the main way of exposure to heavy metals, and fish is a part of human diet, it is not surprising that polluted fish could be a dangerous source of toxic metals. (Bogut, 1997. Burger and .(Gochfeld, 2005

In the last two decades there has been a growing interest in assessing the levels of heavy metals in food such as fish, and .interest aimed at the safety of the food supply

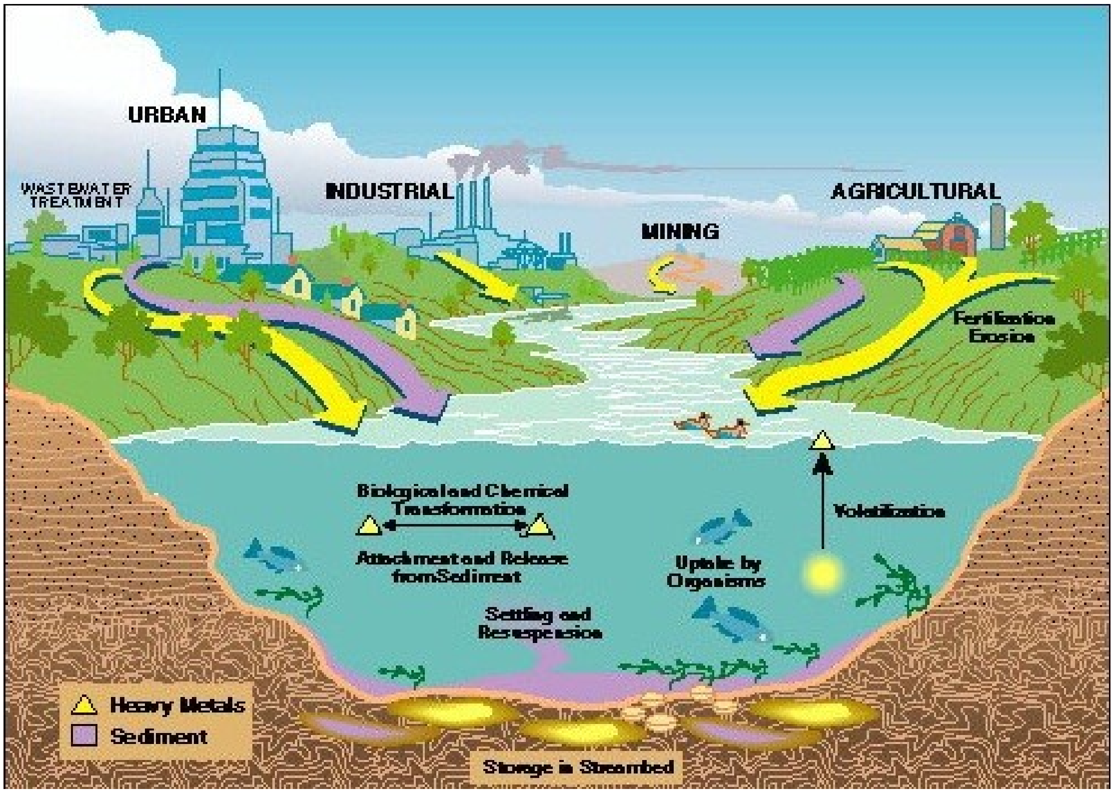


Figure 1.1 sources of Heavy metals

Hazards of Heavy metals contamination 1.3

Introduction 1.3.1

Heavy metals have variously been used to denote (1)metals with atomic number 23 (i.e.Vanadium) onwards except Rb ,Cs ,Ba and Fr (2) metals with density greater than 5 ,and (3) metals which are toxic to man and other life forms when found in the environment .The eight most common pollutant heavy metals listed by the Environment protection agency (EPA)are As ,Cd ,Cr, .(Cu,Hg, Ni, Pb and Zn. (Athar and Vohora ,2001

Biosphere is the natural environment of living object .It envelops the earth and contains surficial parts of the lithosphere, the lower part of the atmosphere and the hydrosphere. A relative homeostatic environment is essential for the survival of organisms in ecosystem .This necessitates the study of the chemical composition of air, water, and soil in order to monitor any abnormal changes due to industrial progression and .(consequent advancement of society.(Athar and Vohora, 2001

Heavy metals are diluted and affected by various surface water components (carbonate, sulphate, organic compounds – humic, amino acids) that formed complexes. These salts and complexes are predicted to be not harmful to aquatic organisms .Part of them sink and are accumulated in bottom sediments. However when water pH has declined (during acidic rains or other acidic episodes)heavy metals can be mobilized and released into the eater column and become toxic to aquatic biota .In addition , low concentrations of heavy metals can cause a chronic stress which may not kill individual fish, but lead to a lower body weight and smaller size and thus reduce their ability

(to compete for food and habitat. (Jadhav et al.,2010

Aquatic organisms, such as fish, accumulate pollutants directly

from contaminated water and indirectly via the food chain contamination of fish with these metals .The effect of pesticides either organophosphorous or chlorinated pesticides have been

.(extensively studied in fish .(Fagr 2008

Lead 1.3.2

1.3.2.1Occurrence, exposure and dose

Lead is a soft, silvery gray metal, melting at 327.5 C^0. It is highly

resistant to corrosion, but is soluble in nitric and hot sulfuric acids. The usual valence state in inorganic lead compounds is +2. Solubility in water varies, lead sulfide and lead oxides being poorly soluble and the nitrate, chlorate and chloride salts are reasonably soluble in cold water lead also forms salts with such organic acids lactic, and acetic acids, and stable organic compounds such as tetraethyl lead and tetra methyl lead . ((Black2001

Lead and its compounds may enter the environment at any

point during mining, smelting, processing, use, recycling or disposal. Major uses are in batteries, cables, pigments, petrol (gasoline) additives, solder and steel products. Lead and lead compounds are used in solder applied to water distribution pipes and to seams of cans used to store foods, in some traditional remedies, in bottle closures for alcoholic beverages and in ceramic glazes and crystal table ware .In countries where leaded petrol is still used ,the major air emission is from mobile and stationary sources of petrol combustion . Area in the vicinity of lead mines and smelters are subjected to high levels of air emissions. Air borne lead can be deposited on soil and water, thus reaching humans through the food chain and in drinking water .Atmospheric lead is also a major source of lead in household dust.(Hansen 1996).In the general non-smoking adult population, the major exposure path way is from food and water .Air borne lead may contribute significantly to exposure, depending upon such factors as use of tobacco, occupation, proximity to motorways, lead smelters etc. and leisure activities (e.g. arts and crafts, firearm target practice). Food, air, water and, dust⁄ soil are

the major potential exposure pathways for infants and children. For infants up to 4 or 5 months of age air, milk, and water are the significant sources of lead exposure .(Alexander et .(al .1998

Health Effects 1.3.2.2

Health effects of lead are caused by lead entering the water through corrosion of pipes. This occurs when the water is slightly acidic, and that is why public water treatment systems are now required to carry out PH. adjustment in water for drinking purposes. Lead is not essential to the body and is only a hazard encountered through the uptake from food, air, or water. Illnesses from high lead concentrations are disruption of the biosynthesis of hemoglobin and anemia. Other health risks linked to lead are a rise in blood pressure, kidney damage, miscarriage, and subtle a bortions, disruption of nervous system, brain damage, declined fertility of men through sperm damage, diminished learning abilities in children, and behavioral disruptions in children like aggression, impulsive behavior.(Chemical properties of lead-((2010) http: www. Lenntech.com/periodic/ elements/Pb. htm

1.3.3Cadmium

Occurrence, exposure and dose 1.3.3.1

Cadmium is typically a metal of 20th century ,even though large amounts of this by –product of zinc production have been emitted by non-ferrous smelters during the 19th century .Cd is mainly used in rechargeable batteries and for the production of special alloys .Although emissions in the environment have markedly declined in most industrialized countries, Cd remains a source of concern for industrial workers and for populations living in polluted areas, especially in less developed countries (Sethi and Khandelwal 2006).

Cd dispersed in the environment can persist in soils and sediments for decades. When taken up by plants. Cd concentrates along the food chain and ultimately accumulates in the body of people eating contaminated foods .Cd is also present in tobacco smoke, further contributing to human exposure .By far, the most salient toxicological property of Cd is its exceptionally long half – life in the human body .Once absorbed, Cd irreversibly accumulates in the human body, in particularly in kidneys and other vital organs such as the lungs or the liver . In addition to its extraordinary cumulative properties, Cd is also a highly toxic metal that can disrupt a number of biological systems, usually at doses that are much lower than most toxic metals.(Nordberg, et al.,2004).

Primary exposure sources of Cd for the general population include food and tobacco smoking. The highest concentrations of

Cd (10-100 ppm) are found in internal organs of mammals, mainly in the kidneys and liver (offals) as well as in some species of fish, mussels and oysters, especially when caught in polluted seas. Consumption of staple foods such as wheat, rice also significantly contributes to human exposure. In the industry, Cd exposure is mainly by inhalation although significant amounts of Cd can be ingested via contaminated hands or cigarettes. The amounts of Cd ingested daily with food in most countries are in the range of 10 to 20 µg per day. Tobacco smoking is an important additional source of exposure for smokers. Since one cigarette contains approximately 1 to 2 µg Cd, smoking one pack per day results in a daily uptake of Cd that approximates that derived from food. Absorption by the oral route varies around 5 per cent but can be increased up to 15 per cent in subjects with low iron stores. When exposure is by inhalation, it is estimated that between 10 to 50 per cent of Cd is absorbed, depending on the particle size and the solubility of Cd compounds. In the case of Cd in tobacco smoke (mainly in the form of CdO),an average of 10 per cent of Cd is absorbed. Absorption of Cd through the skin is negligible.

((Nordberg G. Nordberg et al., 2007

Health Effects 1.3.3.2

Toxic effects of Cd on the bones really become evident with the

outbreak of the Itai-Itai disease in the Cd –polluted area of Toyama, Japan, after World War 2. Itai-Itai disease patients presented, indeed, a severe osteomalacia accompanied with multiple bone fractures and renal dysfunction. They complained from pain in the back and in the extremities, difficulties in walking and pain on bone pressure (hence the name Itai-Itai meaning Ouch –Ouch in Japan).Recent studies in China have confirmed the

(bone toxicity of Cd .(Nordberg, et al., 2002

The bone mass density was decreased in postmenopausal

women with elevated Cd in urine or blood as well as among men with elevated Cd in blood . Bone lesions have been regarded for long as late manifestation of intoxication, occurring only after relatively high exposures in the industry or environment. Effects on the bone, especially at high exposure, are largely the consequence of Cd nephropathy, resulting in an altered vitamin D metabolism and a urinary waste of calcium and phosphate. According to studies on environmentally –exposure populations in Japan or China, the thresholds of urinary or blood Cd associated with bone effects are higher than those associated with renal

.(dysfunction (Qianet al., 2006

Cancer 1.3.3.3

Various regulatory bodies have concluded that there is sufficient evidence to classify Cd as a human carcinogen. The most convincing evidence comes from the finding of increased risk of lung cancer in workers exposed to Cd by inhalation as well as from animal data showing that Cd administered by various routes can produce cancer at multiple sites, including in the lung (Waalkes, 2003).Although the evidence from animal studies is undisputable, data from occupationally exposed populations require a more careful analysis because of the possible confounding by concomitant exposure to arsenic. Recent studies having adjusted for the concomitant exposure to arsenic and nickel have reported lower relative risks of lung cancer than in the past (Nordberg, et al 2007).

1.3.4 Nickel

1.3.4.1 Occurrence, exposure and dose

Nickel is a silvery white metal that takes on a high polish. It is a transition metal, hard and ductile. It occurs most usually in combination with sulphur and iron in pentlandite, with sulphur in millerite, with arsenic in the mineral nickeline, and with arsenic and sulphur in nickel glance (Nestle, et al., 2002).Nickel is one of five ferromagnetic elements. Nickel is also a naturally magnetostrictive material, meaning that in the presence of a magnetic field, the material undergoes a small change in length (Hathaway K, Clark AE.1993).Nickel is primarily found combined with oxygen or sulphur as oxides or sulphides that occur naturally in the earth's crust. Nickel is combined with other elements in all soils, in meteorites, and is emitted from volcanoes. As for most metals, the toxicity of nickel is dependent on the route of exposure and the solubility of the nickel compound.(Coogan, et

.(al.,1989

Health Effects 1.3.4.2

The adverse health effects of nickel depend on the route of

exposure and be classified according to systemic, immunologic, neurologic, reproductive, developmental, or carcinogenic effects following acute (01day), subchronic (10-100days), and chronic (100days or more) exposure periods. The most common harmful health effect of nickel in humans is an allergic skin reaction in those who are sensitive to nickel. Nickel is the most observed cause of immediate and delayed hypersensitivity noticed in occupationally exposed as immunotoxic agent in humans.(Das,

(2007

Iron 1.3.5

1.3.5.1Occurrence, exposure and dose

Iron is the second most abundant metal in the earth's crust, of which it accounts for 5%. Elemental iron is rarely found in nature, as the iron ions Fe^{2+} and Fe^{3+}readily combine hydroxides, carbonated, and sulfides. Iron is most commonly found in nature (in the form of its oxides (Elinder, C-G.1986

Health Effect 1.3.5.2

Iron is an essential element in human nutrition. Estimates of the minimum daily requirement for iron depend on age, sex, physiological status, and iron bioavailability and range from about 10 to 50 m/day (Requirements of vitamin A, iron, folate and vitamin B_{12}.Report of a joint FAO/WHO 1988). The average lethal dose of iron is 200-250 m/Kg of body weight, but death has occurred following the ingestion of dose as low as 40 m/Kg of body. Autopsies have shown haemorrhagic necrosis and sloughing of areas of mucosa in the stomach with extension into the submucosa .Chronic iron overload results primarily from a genetic disorder (haemochromatosis) characterized by increased iron absorption and from diseases that require frequent transfusions. Adults have often taken iron supplements for extended periods without deleterious effects and an intake of 0.4- 1 m/kg of body weight per day is unlikely to cause adverse effects in healthy .(persons. (WHO,1996

1.3.6 Copper

1.3.6.1 Occurrence, exposure and dose

Copper is an essential element in human health, and copper deficiency can result in a variety of disorder. Extremely high levels of copper can cause health problems, but this is rare. In nature, copper is usually associated with other metals such as zinc, nickel, molybdenum and gold. Copper is widespread in the environment and has been mined and used by humans for more than 5,000 years in utensils, coins, ornaments, and tools.

1.3.6.2 Health Effects

Copper is an essential element in human health, and health effects can result from both deficiencies and overexposure. Metallic copper has little toxicity, but coppers soluble salts are poisonous, low levels of copper are essential for maintaining good health. The recommended daily intake for adults = 2m/day. Extremely high levels (more than 15 m/day) of exposure can cause harmful effects such as: skin allergies, irritation of the nose, mouth, and eyes, nausea, diarrhea, vomiting, stomach cramps, and jaundice.

Longer periods of exposure can result in kidney/liver damage. It may decrease fertility in adults. Breathing copper containing dust or skin contact can cause death and liver /kidney diseases. .((National environmental health monographs 1997

Manganese 1.3.7

Occurrence, exposure and dose 1.3.7.1

Manganese is whitish-grey metal, very brittle, and oxidizes superficially in air. The atomic weight is 54.94, and a specific gravity of 7.2 gcm^{-3}.It resembles Fe in chemical behavior. Mn has oxidation states of 1,2,3,4,6, and 7.(Bradi, 2005). In natural environment Mn rarely occurs in free state, but mostly in combined form. Mn is frequently found in metamorphic, sedimentary, and igneous rocks. Its average content in the lithosphere is about 1000 ppm. As its ionic size is similar to Ca, the two elements can replace each other in silicate minerals. Although there are more than 100 Mn minerals such as sulfides, oxides, carbonates, silicates, phosphates, arsenates, and borates, the most important is manganese oxide, (MnO_2). Other main ores are rhodochrosite ($MnCO_3$),manganite (Mn_2O_3-H_2O). Manganese has many applications in industry, it is used for production of ferromanganese steels, electrolytic manganese dioxide for use in .batteries, and welding rods

Health Effects 1.3.7.2

Manganese is an essential element and present in all living organisms. The excess amount of manganese affects central nervous system, causes liver cirrhosis and a higher concentration of it produces a poisoning called Manganese (Parkinson disease) . .((Momtaz, 2002

1.4Literature Review

Distribution of some heavy metals in fish and water 1.4.1

The level of heavy metals in fish and its relation to fish size in Egypt 1.4.1.2

Heavy metal concentrations were determined in muscle of Oreochromisniloticus fish. In the water of Sabal drainage canal which is the largest drain in Al-Menoufiya. It was found that the concentrations of metals in fish were much higher than water. The relationship between concentrations of (Zn and Mn)metals and body size was observed with increasing fish length and weight. Correlations between concentrations of Cu, Fe, Pb, Mn, and Zn and body length and weight were also found. On the other hand, (a decreasing trend in Al, Cd (Authman, 2008

According to (Hussein and Ahmed, 2004)the level of heavy metals (Cd, Cu, Pb and Hg) in flesh of Mugil and Tilabianilotica as well as water sampl collected from different sites of El-Manzala lake. The result of this study revealed that the mean of Cd, Cu, Pb and Hg in the water was lower than levels in flesh of fish

Badr, et al., 2006) reported that the trace metals (Fe, Zn, Cd) and Pb) in surface and bottom water of El-Rahaway drain, at the point of its discharge withthe Nile water and also before and after the discharge. The results obtained showed the presence of high concentration of all elements studied which render the water unsuitable for both domestic and irrigation applications ,expected to kill most biotain the water, especially in autumn at the point of discharge .This bad effect generally continued till 300 meter after discharge point .Fe, Pb and Pb concentrations exceeded the upper limit of standard at most sites along the River Nile especially in .summer

The level of heavy metals in fish and water and 1.4.1.2

(relation to fish size in Saudi Arabia (KSA

Tuna fish are frequently and largely canned in KSA, so their toxic content should be of concern to human health. The result of analysis of samples of canned tuna fish has been reported by (Waqar2004), and it indicated the following concentrations: Cd varied from 0.08 to 0.66m/g with a mean of 0.16m∕g,andPb .ranged from 0.14 to 0.82 with a mean of 0.53m∕g

Bioaccumulation of Hg by fish in canned food items can be a rich source of metals. As a consequence of its known toxicity, as well as Pb and Cd, and of the serious contamination of foods that occurs from time to time during commercial handling and processing most countries monitor the levels of toxic elements in .food

In a study to correlate the heavy metal concentration with cuttle fish, they were divided into groups according to the mantle length increase. (Al- Farraj,et al., 2011).It was reported that heavy metal concentrations followed the order: Zn>Cu>Pb>Cr>Ni>Cd the concentration values obtained were low and well within maximum permitted concentrations imposed by various organizations so that they were within the safe limits for human .consumption and they did not pose any risk to the consumers

Statistically, significant positive correlations were detected between the concentrations of Ni, Pb, Cd and Cr and the mantle lengths of Sepia Pharaoniswhile Zn displayed a significant negative correlation .However, no significant correlation was observed between Cu concentration and length of studied specimens .The average concentration of Zn was significantly decreased by increasing the mantle length, this might suggest that small specimens were highly dependent on Zn metal to fulfill .their metabolic demands

The level of heavy metals in fish and water and 1.4.1.3 relation to fish size in Oman

Cadmium and Lead were analyzed by inductively coupled plasma atomic emission spectrometer.Lead in rock oyster collected from the Dhofar coastal areas in the Arabian sea ranged from 2.64 to3.80m kg^{-1} for cadmium 0.009to0.02 m kg^{-1}. The calculated annual mean concentrations of mercury ,cadmium and 0.02m $kg^{-1}$for lead and 0.01 to 0.02 m $kg^{-1}$for mercury. The presence of these metals in the rock oysters reflects the bioavailability for incorporation by oysters along the Dhofar coast of Arabian sea ,it has been bioaccumulating toxic metals in its soft tissues, and metal concentrations were ,on average variations . The metal concentrations in Dhofar coastal area were within the legal standards for mercury and lead (1.5m kg^{-1}wet weight).Cadmium concentrations in S.cucullata exceeded the maximum limits allowed for human consumption (1mkg^{-1}wet weight) under Eu (Ec,2001). There was distinct observed relationships between the size of oysters and metal uptake by the oyster (Al-Busaidi, et al., .(2013

Level of heavy metal in fish and water and relation to 1.4.1.4 fish size in Turkey

Heavy metal in some tissues and organs CapoetaCapoetaumble fish species from Keban Dam lake Elazing (Turkey)in relation to body size was determined . In this study no clear relationship between heavy metal concentration and fish size was observed . In relation to fish weight , Cu level was found to be significant ($p<0.05$)in gonad ,kidney and liver ,but insignificant ($p>0.05$)in gill and skin .Fe level was found to be significant in liver ($p<0.01$)and gill($p<0.001$).Mn level was of determined as significant in liver ($p<0.01$),gill and skin ($p<0.001$),but in significant ($p>0.05$)in gonad and kidney .Zn level was also found to be significant in kidney ($p<0.05$), liver($p<0.01$)and .gill($p<0.01$)but insignificant in skin and gonad

In relation to fish length, all tested metal levels were found to be insignificant in muscle tissue ($p>0.05$).Cu level was significant in gonad ($p<0.05$)and liver ($p<0.01$), but insignificant in all other tissue and organ samples ($p>0.05$) .Fe level was significant in skin , liver ($p<0.01$)and gill(p0.001), but insignificant in gonad and kidney ($p>0.05$).Mn level was significant in skin ,gonad ,kidney ,liver ($p<0.01$)and gill($p<0.001$).Zn level was only significant in kidney ($p<0.05$)and liver ($p<0.01$), but insignificant in gill ,skin and gonad ($p>0.05$). (Canpolat and Calta 2003)

1.4.1.5 Level of heavy metal in fish and water and relation to fish size in Malaysia

Kamaruzzaman, and Rina (2010) collected Fish samples from Kuantan, Kuala Pahang and Kuala Rompin of Pahang coastal water and they found that the high level accumulation of metals in some fish species could be due to heavy rainfall during monsoon season which increase the metal content of water by washing down the agricultural waste .Seasonal changes intrinsic factors such as growth cycle and reproductive cycle. The study focuses on the level of Zn ,Cu and pb in order to assess the environmental pollution by using fishes as an indicator .Concentrations of the heavy metals in examined fish species were found to be :Zn19.27µg g^{-1} for Zn 2.88µg g^{-1}andPb 0.26µg g^{-1}.The concentrations of Zn ,Cu and Pb were found to follow the order :stomach>muscle>gill. Significant correlations were found between fish weight and length significant positive correlation were found between body length and concentration with

Zn(r=0.713), for Zn(r=0.256)and forpb(r=0.079), where concentration of metals increased with increasing body length of .fish

Taweel 2011 reported that determination of heavy metals (m g^{-1} dry tissues) in the liver ,gills and muscles of the tilapia (Oreochromisniloticus) showed that the highest heavy metal concentration were detected in liver followed by gill and muscle .The heavy metal concentration in the tissues varied significantly depending upon the locations from where the fish was collected .In the liver, the highest Pb was detected in the Langat River (4.8±0.84µg⁄g) followed by that of Engineering Lake (3.28±1.15m g^{-1}) Cu and Ni levels were observed as the highest in the fish collected from Cempaka Lake with values of 449±37.7 and 20.9±5.7 m g^{-1} Cd the highest level was detected in the fish from Engineering Lake (0.70±0.17m g^{-1}) ,while the highest values of Zn was recorded in those from the Langat River (143±9.8m g^{-1}) .The metal accumulation in the liver of fish was found to be quite high in comparison to the gills and muscles .However , the concentration of heavy metals in the muscles of fishes collected from all the sites were within the permissible levels and safe for human consumption and public health

Level of heavy metal in fish and water and relation to 1.4.1.6 fish size in Pakistan

Chola is a fresh water fish. This fish is found in Pakistan .The mean of metals in fish have been found to be 170.89 for Zn 0.538 for Fe ,21.2for Cu,8.8forMnand 69.10 for Ca .It was observed that all metals except copper showed significant positive correlation ($p<0.001$) with total body weight or total length ,except Cadmium which was not quantifiable, were found to increase in direct proportion to an increase in total length indicating isometric relationships .Manganese showed positive allometry ,significant proportional increase in metal concentration with increase in body length which suggests that metal is probably accumulated at higher rate compared to its rate excretion as the fish grows .However Zinc, Iron and Copper showed negative allometry in an increase in total length ,significant proportional decrease in metal concentration with increase in body length which suggests that these metals are probably accumulated at lesser rate compared to its rate of excretion as the fish grows. It was observed that inter specific variations exist which could possibly be due the nature of their habitat ,feeding habits ,meat quality, gradual or quick accumulation of pollutants entering the aquatic ecosystem .Toxic metal such as cadmium did not appear to be either not or rarely accumulated in PuntiusChola which that might be due to rather the environment or species specific (Ansari, .(2006

Level of heavy metal in fish in Jordon 1.4.1.7

Cd, Cu and Zn concentrations were determinted in muscle,bone, skin ,scales and gills of three fish species (Oreochromisaureus. Cyprinuscarpio and Clariaslazera) .Heavy metals concentrations varied significantly depending on the type of thr tissue and fish species .Oreochromisaureus showed the lowest levels of both Cd and Cu metals in all tissues except gills. The other two fish species, Cyprinuscarpio and Clariaslazera showed less difference in their heavy metal levels, but still it was a significant difference ($p<0.05$). Cd and Cu recorded their lowest level in muscle and their highest level in gills. Oreochromisaureus accumulated the highest level with Zn in all organs (432.11±152.14 $mgkg^{-1}$). Other studied fish species showed a high values of Zn in their skin ,but not as high as that in Oreochromisaureus . The other two fish species showed no significant difference in Zn level between all their different organs .It was concluded that the level of heavy metals (Cd, Cu. and Zn) in muscles of the three fish species were within acceptable limits by FAO standards ,except for Zn concentration in muscles of Oreochromisaureus (70.76±31.21mg kg^{-1}),which might be due to the increase of agricultural influx and .(some other anthropogenic activity in that area.(AL-Weher , 2008

Level of heavy metal in fish and water in Sudan 1.4.1.8

Water and fish samples were taken from two stations in Khartoum city, one located on the Blue Nile River and the other on the White Nile River. Lead and Cadmium were analyzed using Atomic Absorption Spectrophotometer. The water of the Blue Nile contained high level of Pb concentration (9.658±0.445) mg cm^{-3} compared to the White Nile Pb ranged (2.430±0.246)mg cm^{-3}. Cd concentration in Blue Nile water ranged (0.480±0.018), while in the White Nile ranged (0.353±0.037)m cm^{-3}. Studied on different parts (gills, liver, flesh) of the fish revealed higher concentration of (5.352±0.106) mg g^{-1} in the liver followed by (3.001±0.028) mg g^{-1} in the gills, while flesh recorded low level of (1.877±0.050) mg g^{-1}Pb. The highest concentration 0f (4.304±0.037) mg g^{-1}Cd was detected in the liver, lowest concentration with value of

.((0.348±0.018) mg g^{-1} in the flesh(Ahmed, 2010

Description of the study area 1.5

JebalAulia Dam is a masonry dam (figure 1.2) , constructed in 1937. It sits across the White Nile with total length of 4.3Km and 22m in height The capacity of the reservoir is about $3.5\times10^8 m^3$ and the area of the reservoir is about $12\times2\times10^4$ m^3.The lake resulted from the dam is about 629Km in length with average dimension ranges between 6.0-2.3Km .The dam is situated in Khartoum state,about 50 Km south of Khartoum. It bounded between latitude 15° 36Nand longitude 32° 32E .It has been constructed as a storage reservoir for irrigation in Egypt, to hold back part of the White Nile while Blue Nile is in flood and to control the White Nile floods but, it has become redundant due to the construction of Aswan High Dam in 1964 ,it was handed over to Sudan government in 1977 .The valley above JebalAulia Dam is very flat and open, at maximum capacity the reservoir extends some 480 Km up stream; therefore, a great deal of water is lost

.(by evaporation and seepage.(Ezeldinm,etal.,2008

Figure 1.2 JebalAulia Dam

objectives of the study 1.6

to determine the concentration of heavy metal contaminants-1

.infish of JabalAulia lake

to investigate the effect of size on the contents of heavy metal-2

.in fish species of JabalAulia

Materials and Method .2

Samples 2.1

Collection of Water samples 2.1.1

Nile water samples were collected using polyethylene bottles which were prewashed with distilled water. The bottles were immersed to about 10 cm below the water surface . Immediately after collection of river water , it was filtered using filter paper, acidified with 2 cm^3 nitric acid to prevent precipitation of metal, reduce adsorption of the analytes on to the walls of the containers and to avoid microbial activity, then water samples were kept in cold place until time of analysis. The samples were .analyzed directly

Table (2.1) Dates and times of collection of White Nile Water .samples at different depths at JebalAulia

Location	Nile depth (in (cm	Time	Date of collection	Sample NO

JebalAulia	20	AM 00 :8	1/7/2013	1
JebalAulia	50	AM 9:00	1/7/2013	2
JebalAulia	100	AM 10:00	1/7/2013	3
JebalAulia	150	AM 10:30	1/7/2013	4
JebalAulia	200	AM 11:00	1/7/2013	5
JebalAulia	50	AM 10:00	11/2014 /17	6
JebalAulia	100	AM 10:00	17/11/2014	7

Collection of fish samples 2.1.2

Oreochromisniloticus (tilapia) were collected from the fisher men in JableAulia. After collection, the samples were rinsed with .distilled water and stored in deep freezers until analysis

Determination of heavy metals in fish and water 2.2 samples (Pb, Cd, Cu, Mn, Ni, Fe) by using Inductively Couple Plasma Emission Spectrometry

Principle 2.2.1

Inductively couple plasma atomic emission spectrometry (ICP-AES)(figure 2.1 is a very useful and sensitive analytical technique for determining the concentration of metallic elements. This technique can analyze several different elements and has good detection limits. ICP-AES are therefor, used in a wide by variety of applications. It also used to measure the concentrations of different metals within the sample. A high- energy plasma of an inert gas like argon is used to excite the analytes contained in the sample. The intensity of the spectral signals indicate the concentrations in the sample of the analytes in the sample.(www. Miplaxa.com/materials. 2008) .Fish samples were dry-ashed, treated with $HNO_3$and dissolved in HCL. Heavy metals (Pb, Cd, Cu, Ni, Mn, Fe) were determined by ICP-AES (Horwitz 2006 the official

.(method 2006

Reagents 2.2.2

Reagents of analytical grade and distilled water were used for -1 all experiment

Stock solutions . 1000mg dm^{-3} of (Pb, Cd, Cu, Ni, Mn, Fe) were -2 prepared

Standard solutions were prepared from stock solutions of each-3 metal by pipetting 1 cm^3 of stock solution into 100-ml volumetric flask and diluting to the mark with distilled water

ICP Instrument 2.2.3

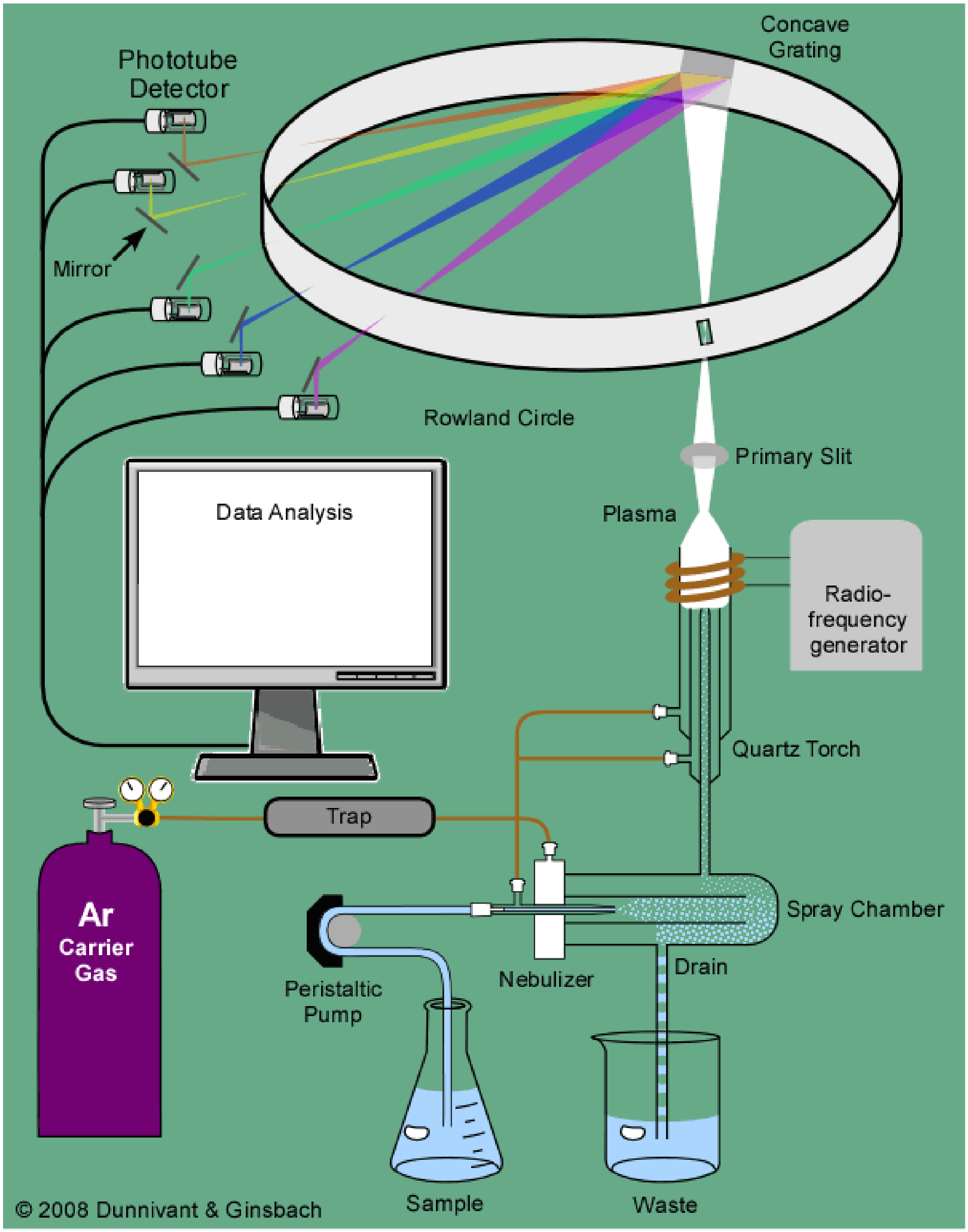
ICP-AES)model (ICP E-9000) Shimadzu, Japan (Table 2.2.2))

typical operating parameters forward power, 1.1 kilowatts, reflected power, <10 watts, aspiration rate 0.85- 3.5 ml/min, flush

.between test solutions, 15-45 s integration time 1-10 s

Table 2.2.2 spectral lines used in Emission measurements

(Wavelenght (nm	Element
228.8	Cd
324.7	Cu
259.4	Fe
257.6	Mn
231.6	Ni
220.4	Pb



figure(2.1) Inductively Couple plasma Atomic Emission Spectrometry

Dry ashing 2.2.4

About 1g dried from fish samples were weighed and grounded into glazed, high form porcelain crucible. Then, they were ashed for 2h at 500^0C and cooled,10 drops of H_2O were added and carefully 3-4ml of HNO_3(1+1) were added. The excess $HNO_3$was evaporated on hot plate set at 100^0-120^0C then the crucible was cooled, 10ml of HCL (1+1) were added to dissolve the ash, and the solution was transferred to 50 ml volumetric flask, and diluted to volume with H_2O

2.2.5Ashing Procedures

Ashing is a method in which a sample is heated to leave only noncombustible ash. The various forms of ashing are dry ashing, sulfated ashing, wet ashing, lowtemperatureashing, and closed system ashing (Gaines,2010). Dry ashing is performed by placing a sample in an open inert vessel and destroying the combustible (organic) portion of the sample by thermal decomposition using a muffle furnace, at temperatures from450 to 550 ºC; magnesium nitrate is typically used as an ashing aid. In dry ashing, charring the sample prior to muffling is recommended and preferred, and charring is accomplished by using an .open flame

ICP-Analysis 2.2.6

All standard solutions for a specific metal were prepared immediately before analysis to avoid adsorption of metals on to the containers and decomposition. The same instrumental conditions were used to run the standard solutions that were used for the samples for each metal. When the absorbance of sample exceeded that of the highest concentrated standard solution of a particular metal appropriate dilution was made to bring the sample concentration within the linear response range. Triplicate measurements of each standard solutions were taken along with

.triplicate measurements of each water and fish samples

Statistical analysis of data was carried out using spss statistical package programs and person analysis for culcalation of correlation

Results and Discussion .3

This research work studies the correlation between the levels of heavy metals (Pb, Cd, Fe, Ni, Mn and Cu)and sizes of fish (length(Cm), weight (g) and to investigate the concentration of heavy metals in both water samples and fish tissues of the fish .caught in this water

Heavy metal concentration in water samples 3.1

The mean of seven results and standard deviation values of heavy metals concentration in water samples of JADL are given in Table 3.1

Table 3.1 The concentration of heavy metals in JADL water samples

Maximum mgdm^{-3}	Minimum mgdm^{-3}	Std. Deviation mgdm^{-3}	Mean mg dm^{-3}	Metal
0.0210	0.0110	0.0037	0.0165	Pb
0.0002	0.0002	0.0000	0.0002	Cd
0.0157	0.0117	0.0020	0.0137	Mn
0.0014	0.0013	0.0001	0.0014	Ni
1.8700	0.9427	0.3819	1.4179	Fe

It was found that iron had the highest mean concentration in JebalAulia Lake water, while Cd had the lowest mean. Heavy metals concentrations were found to decrease in water samples in the following order Fe>Pb>Mn>Ni>Cd

The determined concentration of heavy metals in JADL water samples were evaluated according to the international standards ((Table 3.1.1

Table 3.1.1 comparison of concentrations of heavy metals in JADL water with those accepted by the international standards

EC(1998) $mgdm^{-3}$	EPA (2002) $mgdm^{-3}$	WHO (1993) $mgdm^{-3}$	JebalAulia Lake $mgdm^{-3}$	Elements
2	1.3	2	-	Cu
20	-	0.02	0.0014	Ni
5	0.01	0.01	0.0002	Cd
10	0.05	0.05	0.0165	Pb
-	-	-	0.0137	Mn
0.2	0.3	-	1.4179	Fe

In the present study all the metal concentrations were lower than concentration maximum limits set by WHO, EPA and EC except that for Fe which was above the maximum acceptable limit of EPA(0.3mg dm^{-3}) and EC(0.2 mg dm^{-3}) which limited the .maximum concentration of Fe in water

The determined concentration of heavy metals in JADL water samples were compared with means of those Turkey, Gladysher 2001,EgyptSamir (2008),andFidelis(2010), Nigeria

Table 3.1.2 Comparison concentration of the heavy metals in JADLwith those of Turkey, Egypt and Nigeria

Nigeria mg dm^{-3}	Egypt mg dm^{-3}	Turkey mg dm^{-3}	Sudan JebalAulia Lake mg dm^{-3}	Element
-	0.064	0.002	0.0165	Pb
0.0-0.0013	0.019	0.001>	0.0002	Cd
0.0-0.43	-	0.002	0.0014	Ni
0.06-0.97	0.186	0.002	-	Cu
0.1-3.67	0.244	-	0.0137	Mn
0.95-5.11	0.804	0.29	1.4179	Fe

Heavy metals Concentrations in fish 3.2

The mean and standard deviation values of heavy metals concentrations in fish of forty samples are given in Table. 3.1.3

Table 3.1.3 Heavy metal concentrations in fish meat of JADL

Maximum	Minimum	Std-Deviation	Mean	Metal
13.20	4.16	2.2429	8.9448	Pb
8.88	0.25	1.8028	2.1606	Ni
2.93	0.11	0.8030	0.8434	Cd
4.69	0.14	1.2867	1.8835	Mn
4.27	0.68	1.2218	2.0480	Cu
22.35	2.50	6.5689	6.8500	Fe

In this study Pb showed the highest mean concentration of fish .in JebalAulia Lake, while the Cd showed the lowest mean values

Accumulated metals were found to decrease in fish samples in .the following order:Pb>Fe>Ni>Cu>Mn>Cd

Compares the concentration of heavy metals in fish of JADL with those acceptable maximum concentrations stated by the international standards of WHO and EC

Table 3.1.4 A comparison of heavy metals concentrations in fish of JADL with maximum accepted concentrations of WHO and EC

EC (2005) mg kg^{-1}	WHO 1993mg kg^{-1}	JebalAulia Lake fish samples mg kg^{-1}	Element
0.2	2.00	8.9448	Pb
-	5.0	2.0480	Cu
-	-	2.1606	Ni
-	100.0	1.8835	Mn
0.05	0.5	0.8434	Cd
-	5	6.8500	Fe

The concentration of Pb in fish of JebalAulia Lake had much exceeded the maximum acceptable limits of (2.00 $mgkg^{-1}$)of WHO(1993) and (0.20$mgkg^{-1}$) of EC(2005).The concentration 0f Fe (6.8500), which is considered as an essential metal for biota, in that of JebalAulia Lake exceeded the maximum acceptable limit of .(Fe in fish muscles proposed by WHO(1993

The concentration of Cd (0.8434mg kg^{-1}),however, exceeded the maximum acceptable limits (0.5 $mgkg^{-1}$ and 0.05 mkg^{-1})in fish .muscles proposed by WHO(1993) and EC(2005) respectively

The determined concentrations of heavy metals in JADL fish samples were compared with means of those reported by Shareek(2004)in Gaza, Mokhtar(2009) Malaysia .andÖztÖrk(2008) inTurkey

Table 3.1.5 Comparison of concentration of heavy metals in JADL fish withthose of Gaza, Malaysia and Turkey

Turkey mg kg^{-1}	Malaysia mg kg^{-1}	Gaza Strip mg kg^{-1}	Sudan JebalAulia Lake mg kg^{-1}	Element
6.5	0.418	0.115	8.9448	Pb
15.1	0.313	0.638	2.0480	Cu
-	0.108	0.386	1.8835	Mn
14.3	0.053	0.892	2.1606	Ni
0.82	0.015	-	0.8434	Cd
15.68	-	-	6.8500	Fe

Figure 3.2 shows that the concentrations of heavy metals in

.water of the lake are lower than those in fish living in it

Figure3.2 The average determined concentration of metals in water and fish samples of JADL

Relationship between metal concentrations and body 3.3 size

An increasing trend in concentration values of Pb ,Ni and Cd,

was observed with increasing fish length and weight this was more conspicuous as the correlations are considered gave a .highly significant (p<0.01) and (P<0.05), respectively

the effect of heavy metals concentration Table 3.1.6Shows in fish of JADL on theirlength and weight

Maximum	Minimum	Std. Deviation	Mean	
32.00	14.00	4.6165	23.6175	Length ((cm
610.00	80.00	124.3764	268.5000	(Weight(g
13.20	4.16	2.2429	8.9448	Pb mg kg^{-1}
8.88	0.25	1.8028	2.1606	Ni mg kg^{-1}
2.93	0.11	0.8030	0.8434	Cd mg kg^{-1}
4.69	0.14	1.2867	1.8835	Mn mg kg^{-1}
4.27	0.68	1.2218	2.0480	Cu mg kg^{-1}
22.35	2.50	6.5689	6.8500	Fe mg kg^{-1}

Relationship between Pb concentration and length of fish 5.3.1 of JADL

Table 3.3.7 and figure 3.3.1 show the correlation between concentration of Pb in flesh of JADL fish andtheir body length was .found

Table 3.1.7Correlation between total lengths(cm) of fish of JebalAulia Lakeand Pb concentration in their muscles

**0.644	Pearson (Correlation(Pb
40	N

(Correlation is significant at the 0.01 level (2- tailed**

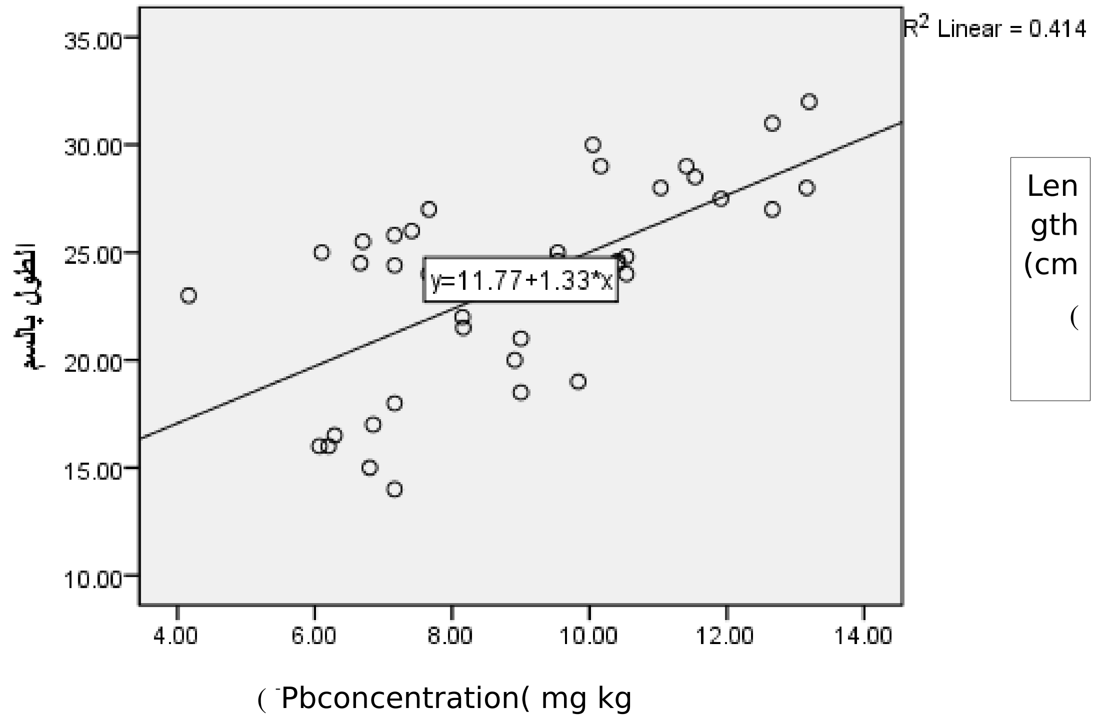


Figure 3.3.1 Pb concentration (mg kg^{-1}) in fish in JADL in relation to (total length (cm

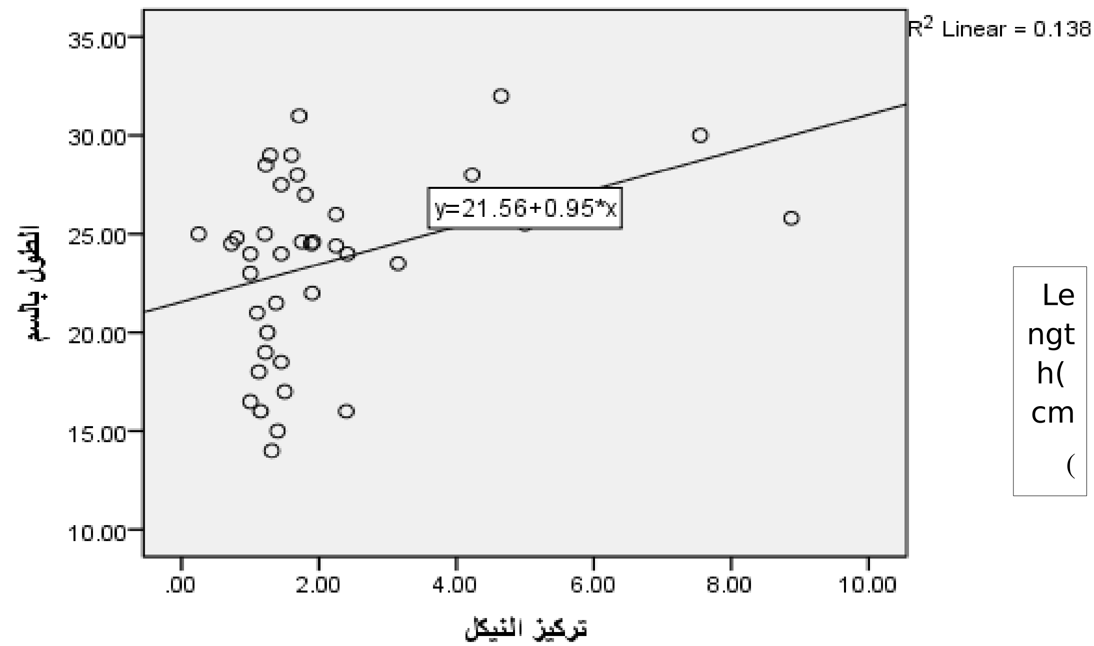
Relationship between Ni concentration and length of fish 3.3.2 of JADL

Table 3.3.8 and figure 3.3.2 show the correlation between concentration of Ni in flesh of fish in JADL and their body length .was found

Table 3.1.8 shows the correlation between total lengths (cm) of fish JADL and Ni concentration in their flesh

(Pearson Correlation(Ni	*0.371
N	40

(Correlation is significant at the 0.05 level (2-tailed*



Ni concentration mg

Figure 3.3.2 Ni concentration (mg kg^{-1}) in fish of JADL in relation (to their total length(cm

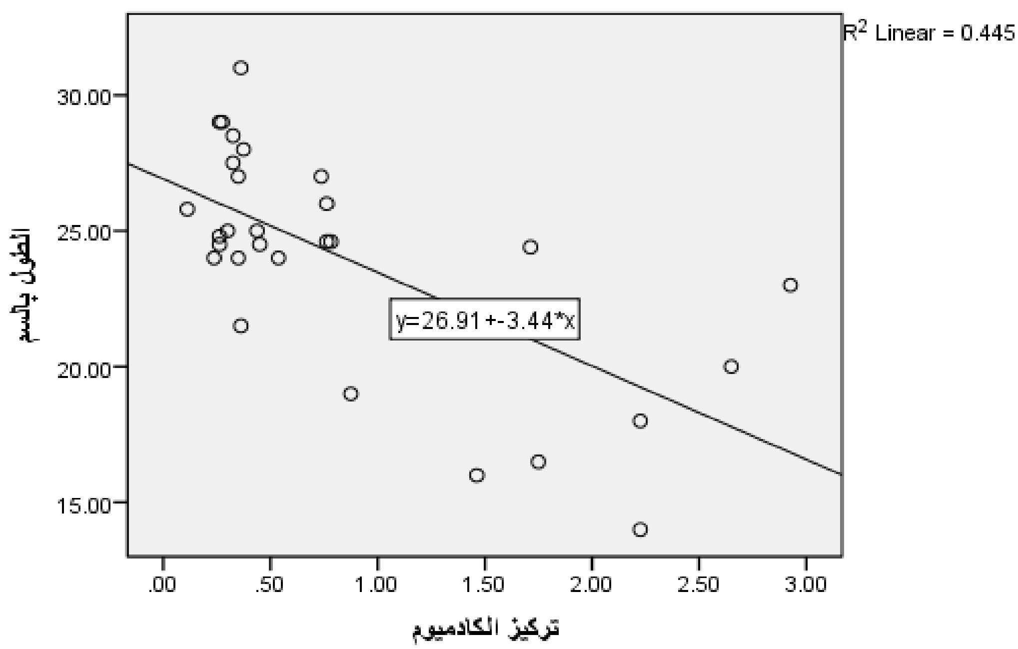
3.3.3 Relationship between Cd concentration and length of fish of JADL

Table 3.3.8 and figure 3.3.3 show correlation between concentration of Cd in flesh of JADL fish and their and body length .was found

Table 3.1.9 Correlation between total lengths (cm) and Cd concentration

Pearson Correlation	**0.667
N	29

(Correlation is significant at the 0.01 level (2- tailed**



Le
ngt
h(c
(m

Cd concentration
mg kg-1

Figure 3.3.3 Cd concentration (mg kg^{-1}) in fish in JADL of fish in (relation to their total length(cm

Relationship between Mn concentration and length of 3.3.4 fish of JADL

Table 3.1.10 and figure 3.3.4 show the correlation between

.concentration of Mn in flesh of JADL fish and their body length

Table 3.1.10 Shows there was no any correlation between Mn concentration and total length

(Pearson Correlation(Mn	0.372-
N	27

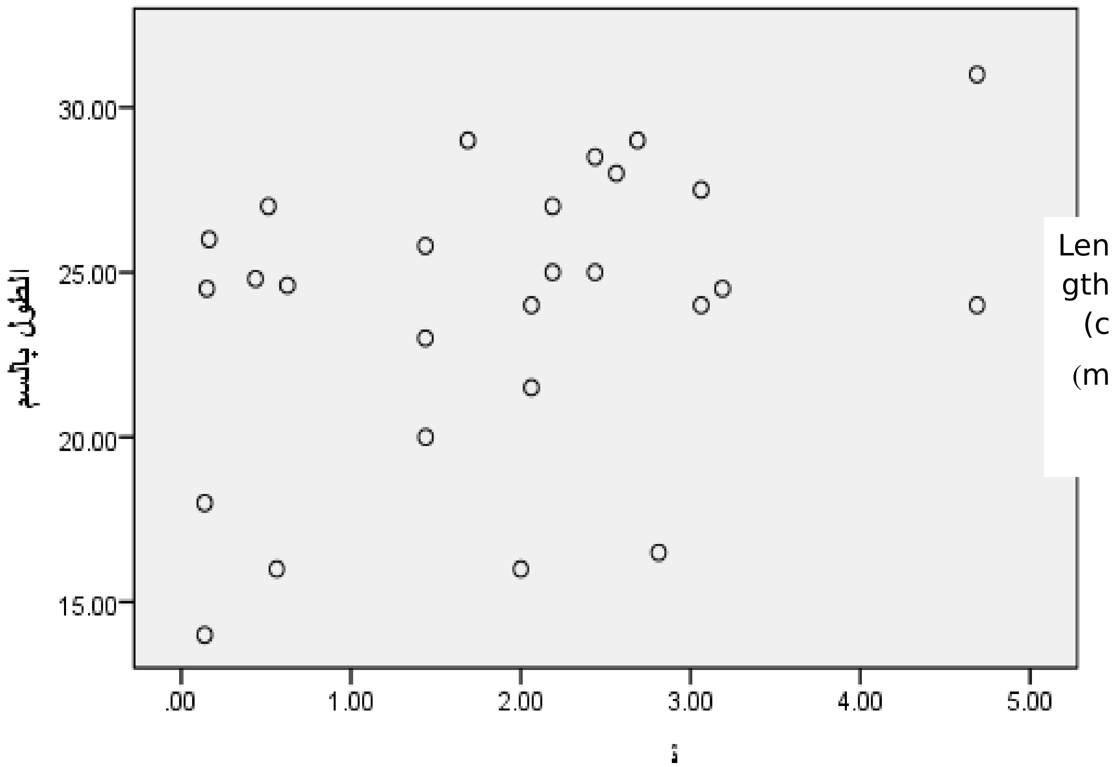


Figure 3.3.4 Mnconcentration (mg kg-1) in fishin JADL in relation to (their total length(cm

Relationship between Cu concentration andlengthof 3.3.5 fish of JADL

Table 3.1.11 and figure 3.3.5 show the correlation between .concentration of Cu in flesh of JADL and their body length

Table 3.1.11 shows there was no any correlation between Cu concentration and body length

0.573-	(Pearson Correlation(Cu

10 N

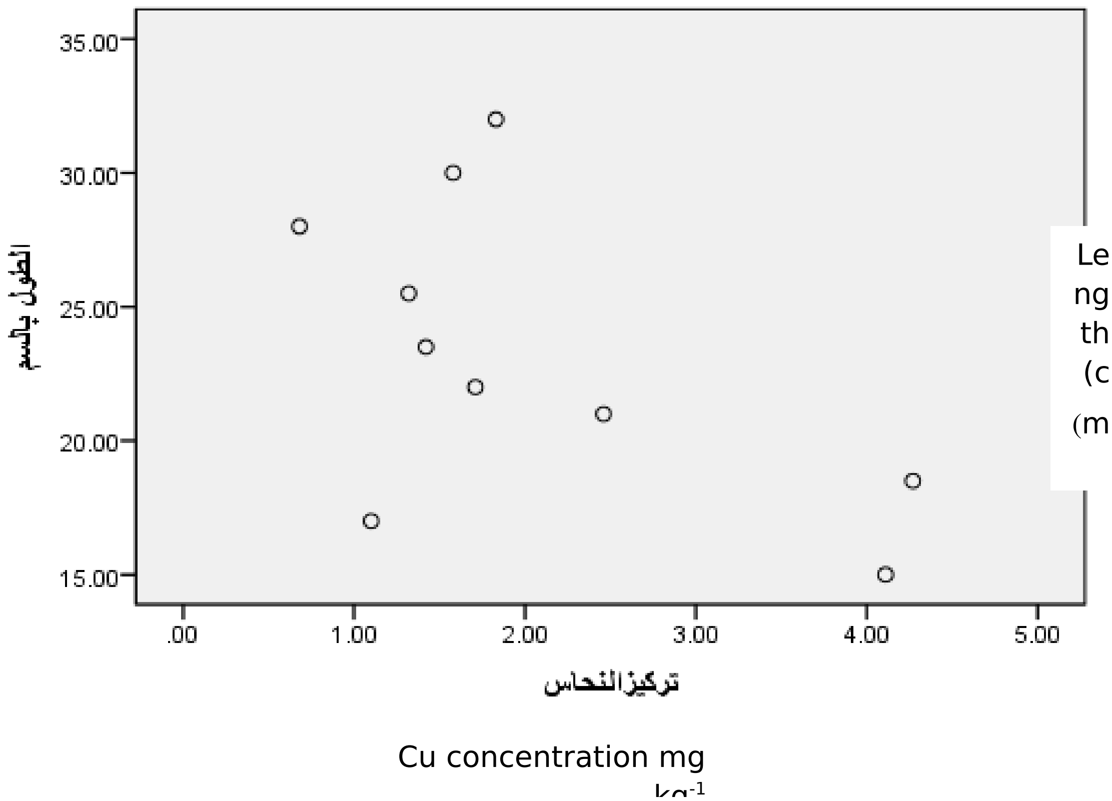


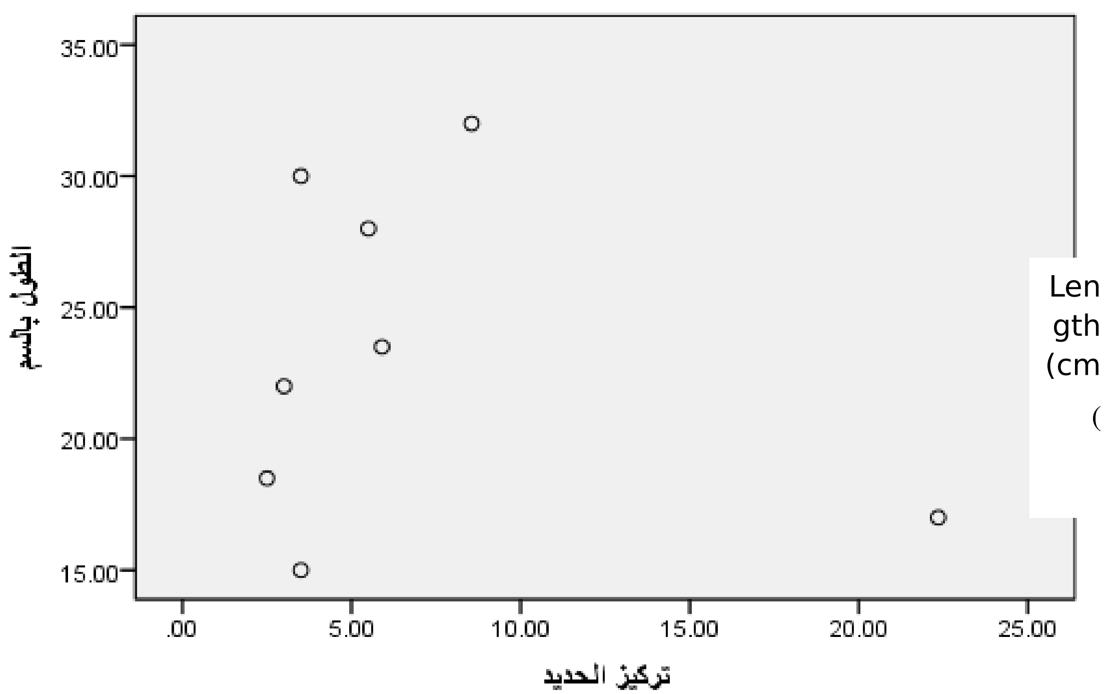
Figure 3.3.5 Cu concentration (mg kg^{-1}) in fish in JADL in relation to their total length (cm)

5.3.6 Relationship between Fe concentration and length of fish of JADL

Table 3.1.12 and figure 3.3.7 show the correlation between .concentration of Fe in flesh of JADL fish and their body length

Table 3.1.12 Shows there was no any correlation between Fe concentration and total length

(Pearson Correlation(Fe	0.201-
N	8



Fe concentration
mg kg-1

Figure 3.3.6 Fe concentration (mg kg^{-1})in fish in JADL in relation to (their total length(cm

Relationship between Pbconcentration and weight of 3.3.7 fish of JADL

Table 3.1.13 and figure 3.3.7 show the correlation between concentration of Pbin flesh of JADL fish and their bodyweight was found

Table 3.1.13 Correlation between total weight (g) and Pb concentration

**0.423	(Pearson Correlation(Pb
40	N

(Correlation is significant at the 0.01 level (2-tailed**

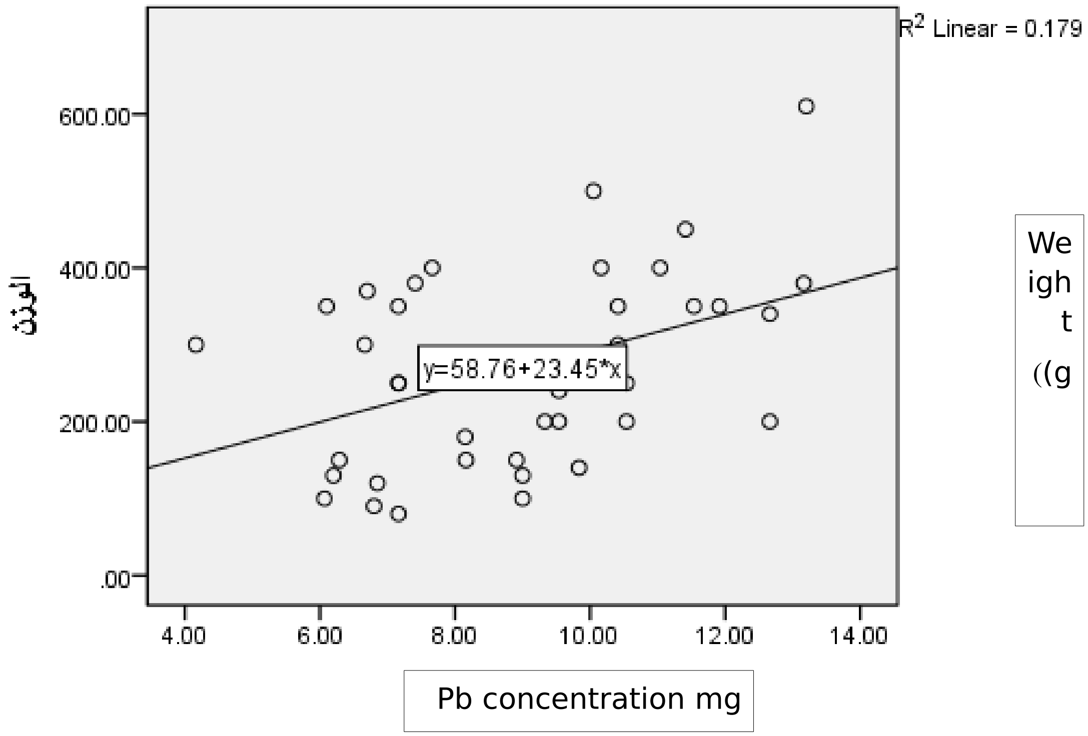


Figure 3.3.7 Pb concentration (mg kg^{-1}) in fish in JADL in (relation to their total weight(g

3.3.8Relationship between Ni concentration and weight of fish of JADL

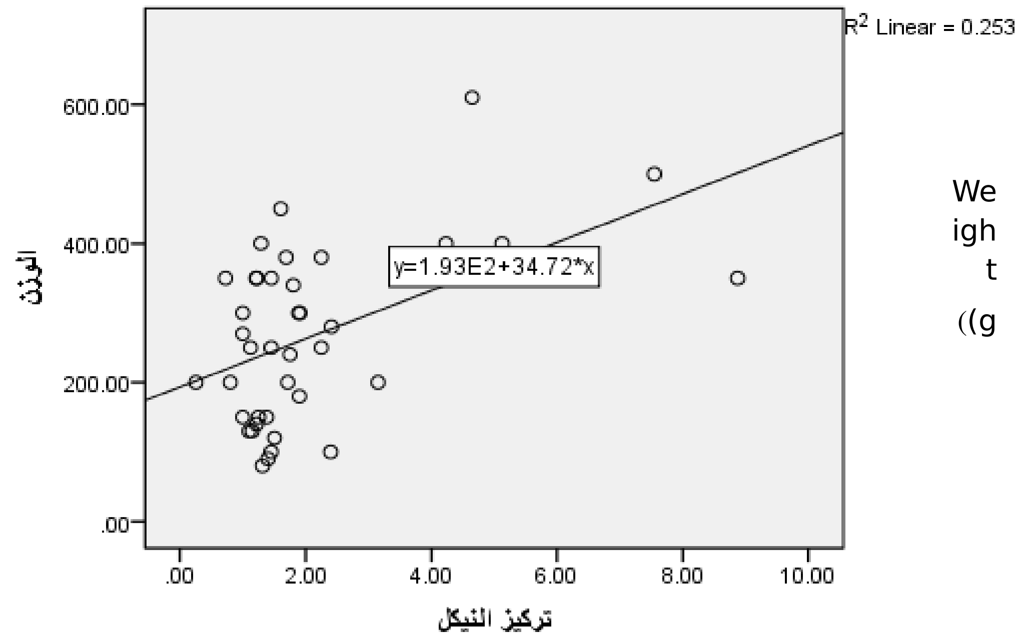
Table 3.1.14 and figure 3.3.8 show thecorrelation between concentration of Ni in flesh of JADL and their body weight was found

Table 3.1.14 Correlation between total weight (g) and Ni concentration

**0.503	(Pearson Correlation(Ni

40 N

(Correlation is significant at the 0.01 level (2-tailed**



Weight ((g

Ni concentration mg kg-1

Figure 3.3.8 Ni concentration (mg kg^{-1}) in fish of JADL in (relation to their total weight(g

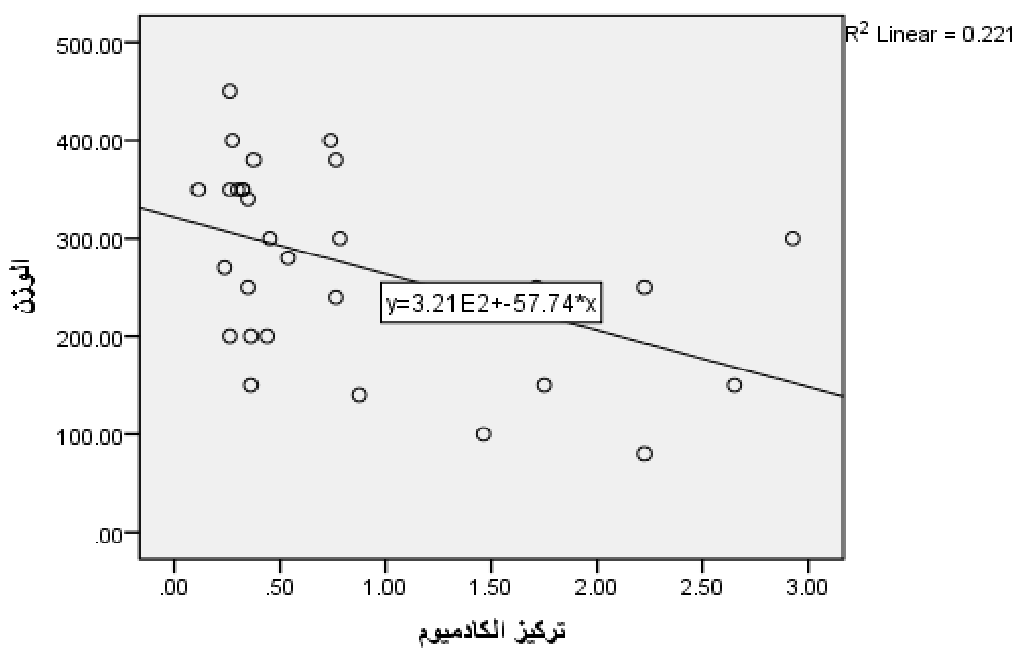
Relationship between Cd concentration and weight of 3.3.9 fish of JADL

Table 3.1.15 and figure 3.3.9 show the correlation between concentration of Cd in flesh of JADL fish and their body weight was .found

Table 3.1.15 Correlation between total weight and Cd concentration

(Pearson Correlation(Cd	**0.471
N	29

(Correlation is significant at the 0.01 level (2-tailed**



Weight ((g

Cd concentration mg kg^{-1}

Figure 3.3.9 Cd concentration (mg kg^{-1}) in fish of JADL in relation (to their total weight(g

Relationship between Mn concentration andweight of 3.3.10 fish of JADL

Table 3.1.16 and figure 3.3.10 show the correlation between .concentration of Mn in flesh of JADL fish and their body weight

Table 3.1.16 shows there was no any correlation between Mn concentration and body weight

(Pearson Correlation(Mn	0.063
N	27

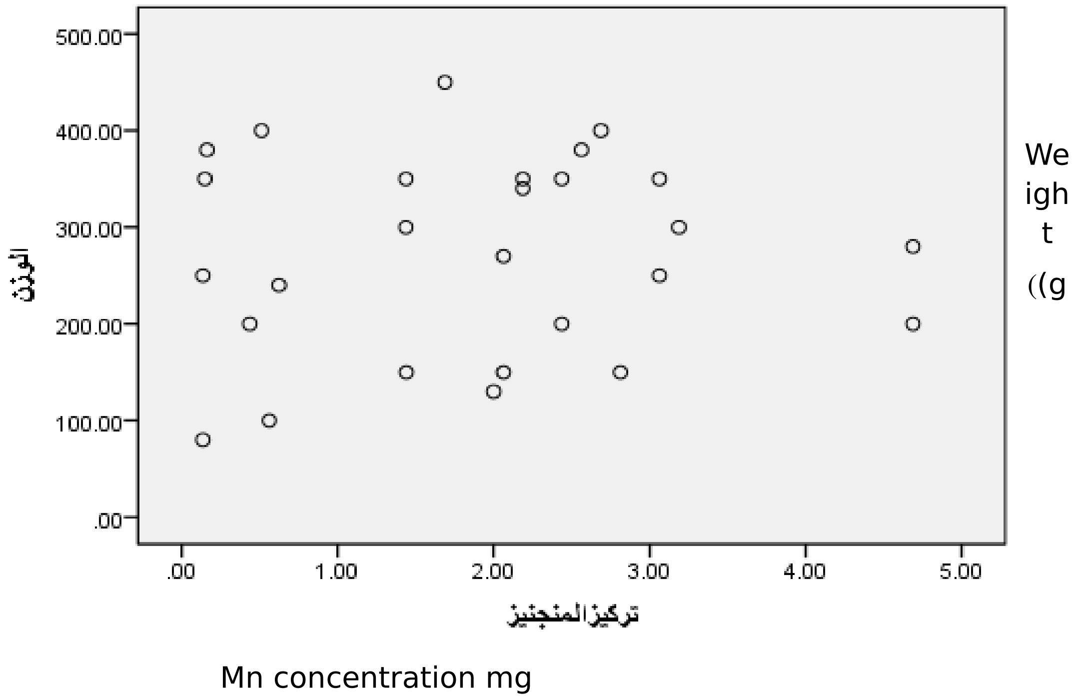


Figure 5.3.10 Mn concentration (mg kg^{-1}) in fish of in JADL in (relation to their total weight(g

Relationship between Cu concentration and weight of 3.3.11 **fish of JADL**

Table 3.1.17 and figure 3.3.11 show the correlation between .concentration of Cu in flesh of JADL fish and their body weight

Table 1.1.17 shows there was no any correlation between Cu concentration and body weight

(Pearson Correlation (Cu	0.500-
N	10

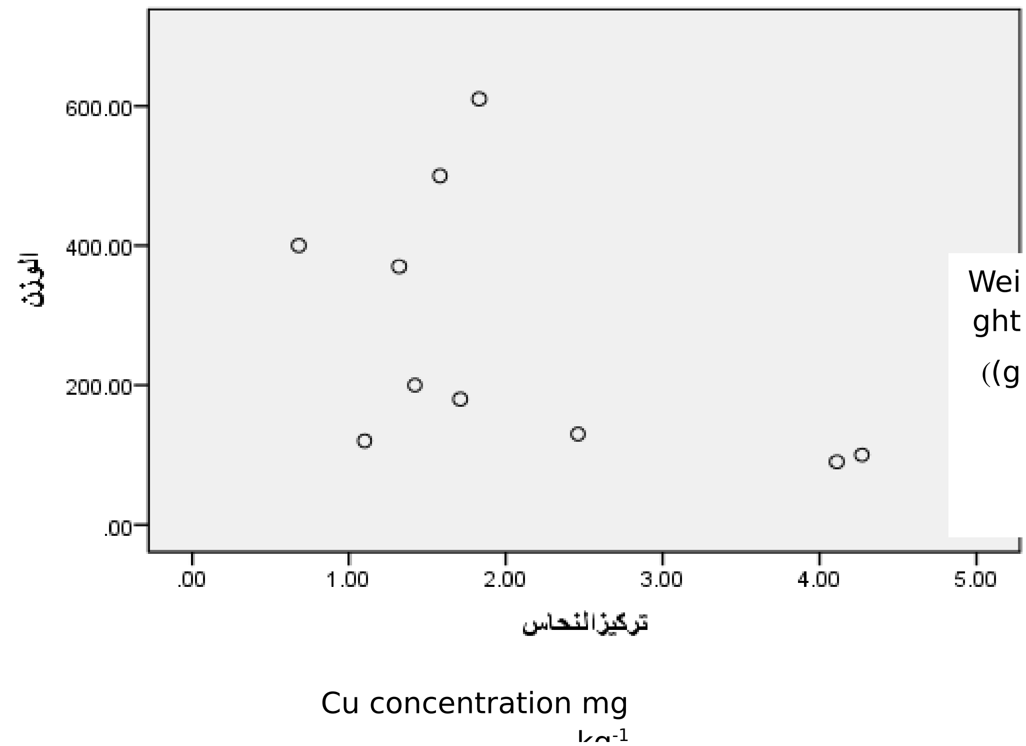


Figure 5.3.11 Cu concentration (mg kg^{-1}) in fish in JADL in relation (to their total weight(g

Relationship between Fe concentration and weight of 3.3.12 fish of JADL

Table 3.1.18 and figure 3.3.12 show the correlation between .concentration of Fe and body weight

Table 3.1.18 Shows there was not any correlation between Fe concentration and body weight

(Pearson Correlation(Fe	0.102
N	8

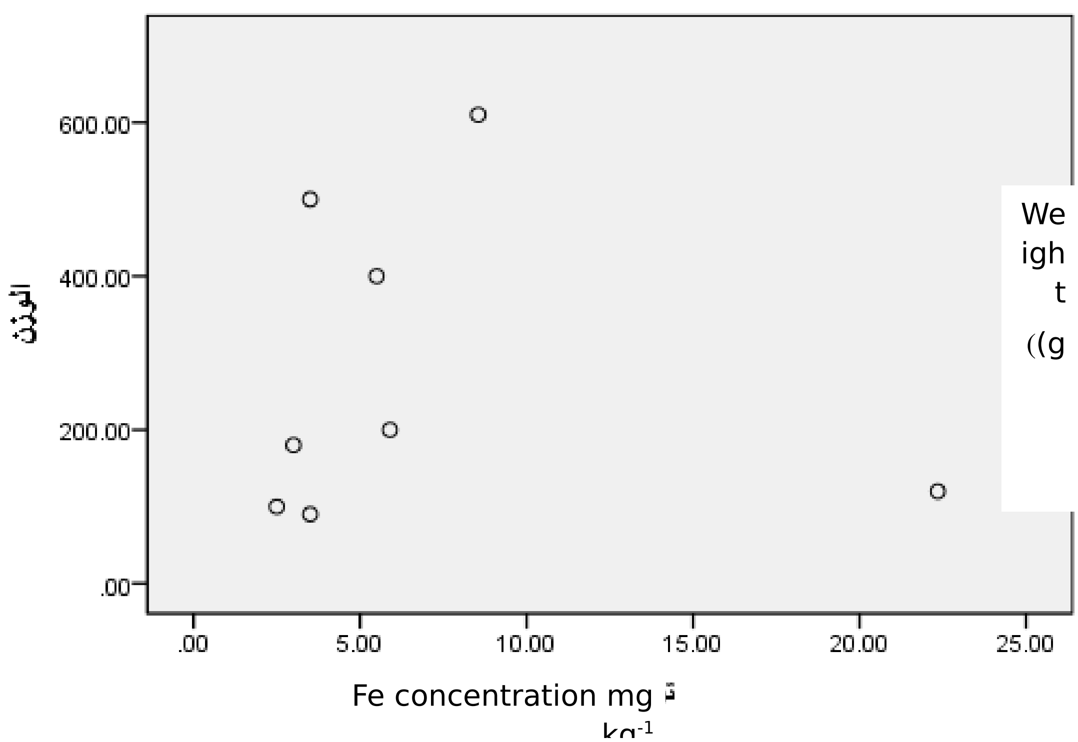


Figure 5.3.12 Fe concentration (mg kg^{-1}) in fish in JADL in relation (to their total weight(g

:Conclusion 3.4

The heavy metals (Pb, Cd, Ni, Mn, and Fe) pollution in JebalAuliaLakeis from both natural source and waste of human activities including agriculture and industry. Whereas the highest metal concentration in water of JebalAulia Lake was recorded for .iron, the lowest concentration was recorded for cadmium

Metals (Pb, Cd, Ni, Cu,Mn, and Fe) in their solutes in water of JADL are accumulated in the organs in the different species living in it. Consequently their concentration levels in their organs are much greater than those present in water. Lead, in particular, showed highest accumulation and cadmium showed the lowest. Whereas lead, nickel and cadmium showed a rather positive correlation between their concentration and both the total body length and weight, However, no correlation between them was shown in the .case of Fe, Cu and Mn metals

This suggests that the metals (Pb, Ni and Cd) are probably accumulated at higher rate compared to its rate of excretion as the fish grows. However (Fe, Mn and Cu) showed negative to an increase in body size which suggests that these metals are probably accumulated at lesser rate compared to its rate of .excretion as the fish grows

Recommendations and suggestions for future work 5.7

-1 The presence of heavy metal pollution in JebalAulia lake was significant as shown in the results and discussion. The heavy metal concentration in water, is low but we should not becomplacent. Awareness and effort for the containment of heavy metals should continue and monitoring at regular intervals should be maintained. Contaminants in fish can pose a health risk to the fish themselves, to their predators, and to the people who .consume them

Precaution means for the control of agricultural and industrial-2 pollution should be strictly followed to prevent pollution of the .water of the lake

Instrumental techniques for the determination of trace and ultra-3 trace elements in water and organisms which need for preconcentration should be applied such as argon coupled plasma- mass spectrometry, atomic fluorescence spectrometry .and stripping pulse voltammetry, should be developed

further work to study toxic metal contamination, not only -4 Oreochromisnilotcus fish but also in other species like chicken, sheep and cattle, controlled feed constituents and environmental conditions, and their harmful or useful effects on animal size(meat, eggs and milk), would be valuable, nutritional, health .and economic importance

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