A Study on Heavy Metal Pollutants in Water and Tilapia at Lake Temsah

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Abstract

A total of 120 *Oreochromis niloticus* were collected randomly and seasonally. Fish were examined freshly from western lagoon (Berket El-Siadin) which is branch from Lake Temsah in Ismailia governorate .the result revealed that post mortem lesions in liver was in the form of pale and enlargement in some cases, while in other cases showed variable degrees of congestion and hemorrhage spots. Spleen was dark in color and enlarged, kidneys appeared congested and the gall bladder was full and distended. The periodic difference of heavy metals concentration based on the data of water samples gathered from the same lagoon showed the following manner as: summer > autumn >winter. The results revealed the presence of metals in the following order: Fe > Cu >Pb > Zn > Cd. The concentration of heavy metals in fish liver, gills and musculature were always higher than of the water, indicating the bioaccumulation. The highest concentration of heavy metals in water and fish tissue was associated with the lowest RBCs count, Hb, PCV, leukocytosis, hypoproteinemia and elevation in serum AST and ALT levels than normal. So, there is accumulating evidence that some heavy metals are immunosuppressive and fish may be more exposed to disease.

Key words: *Oreochromis niloticus,* Berket El-Siadin, Lake Temsah, Heavy metals, Blood parameters.

Introduction

In Ismailia, as a coastal city where are people basically depend on fish as a main source of animal protein; most caught from Suez Canal and lake Temsah. In the same time, agriculture is considered one of the driving forces of Egypt's economy, thus intensive agriculture systems have been employed in order that agriculture can cope with the massive population, which led to the extensive use of pesticides resulting in various problems

(Abdel Fattah, 1992 and Senthilkumar et al, 2001).

Contamination of aquatic ecosystem by industrial and agricultural pollutants may affect the health of fish, either directly by uptake from the water, or indirectly through their diet of vegetation, invertebrates or smaller fish. While the obvious signs of group pollution, dead fish, have long been recognized, there is increasing evidence that low-level pollution may decrease the fecundity of fish population, leading to long term decline in fish numbers ***(Mona Zaki et al, 2015).***

Heavy metal contamination may have devastating effects on the ecological balance of the aquatic environment and a diversity of aquatic animals ***(Farombi, et al, 2007).*** Various studies on different fish species have confirmed that heavy metals may cause undesirable changes in the physiological activities and biochemical parameters both in tissues and in blood ***(Basa and Rani, 2003).*** Fish develop a protective defense mechanism against the harmful effects of xenobiotic like essential and inessential heavy metals that produce degenerative changes in the fish physiology ***(Abou EL-Naga et al, 2005).***

The present study aimed to investigate the water pollutants in Lake Temsah, focusing on heavy metal analysis of water, fish organs and tissues and it's reflect on blood parameters.

Materials and Methods

Fish:

In the present investigation, a total of 120 *Oreochromis niloticus* of total length (23±5 cm) and average body weight (257±10g) were collected randomly and seasonally. They were examined freshly from western lagoon which is a branch from Lake Temsah in Ismailia governorate.

Water sampling:

Atotal of 30 water samples were collected in pre-cleaned and acidified glass bottles during the period from summer to winter and subjected for heavy metals (Zn, Cu, Pb, Cd and Fe) analysis.

Clinical picture

All fish were clinically examined for detecting any abnormalities. The postmortem examination was performed on all freshly dead and moribund fish according to ***Conroy and Herman (1981).***

Heavy metals analysis

1-In water samples:

Water was collected in pre-cleaned and acidified glass bottles. The bottles were immediately brought to the laboratory and acidified with concentrated HNO_3 acid to pH less than 2.0. Water samples were then analyzed for the presence of heavy metals (Zn, Cu, Pb, Cd and Fe) according to the standard method for analysis of water and waste water ***APHA (2005).***The obtained clear filtrate was kept in clean dry bottles of 100 ml capacity and samples were kept at 4 °C till the time of analysis.

2-In fish tissue:

Sample Preparation for heavy metals analysis:

Each fish specimen was properly cleaned by rinsing with distilled water to remove debris and other external adherent then frozen at -20 °C prior to analysis. For analysis the fish specimens were defrosted for 2 hour then were dissected using stainless steel scalpels and Teflon forceps for their liver, gills and musculature. A part of the musculature without skin and the liver and gills were removed and transferred in polypropylene vials. All digested samples were filtered and analyzed for zinc, lead, cadmium, cupper and iron using Atomic Absorption Spectrophotom***(Massanyi et al, 2003).***eter (Model Thermo Electron Corporation, S. Series AA Spectrometer, UK,). The analytical procedure was checked using reference material

Haematological analysis

Blood was collected by heart puncture of the fish using heparinised syringe in vials containing EDTA as an anticoagulant, which was used to estimate RBC, Hb, PCV, MCV, MCH, MCHC, Total count of WBC and differential Leukocytic count by **Shah and Altindag (2004a)**. The collected blood samples were centrifuged to get sera for the following analyses of Serum total protein by ***King and Wootton (1959)*** and AST and ALT by ***Reitmans and Frankel (1957).***

Statistical analysis

The data was processed using the one-way analysis of variance (ANOVA). Duncan's test **(Duncan, 1955)** was further used to evaluate the mean differences at P <0.05 significant levels.

Results

Clinical picture

The external examination performed on Nile tilapia *O. niloticus* that some fish revealed no pathognomic clinical abnormalities, they showed slimy body with pale skin while other showed hemorrhagic areas on abdomen, base of dorsal fins and pectoral fins. P.M. examination of freshly dead fish revealed enlarged pale liver, enlarged full distended gall bladder and enlarged congested spleen. Otherwise, in some cases, showed enlargement and variable degrees of congestion in liver, congested gills and congestion of the kidneys.

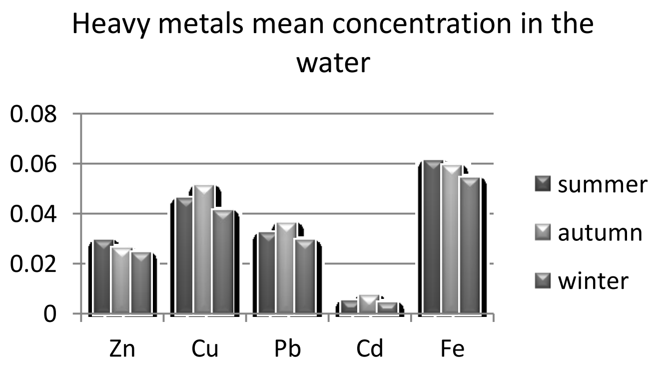


Figure 1: showing different mean concentration of heavy metals in water.

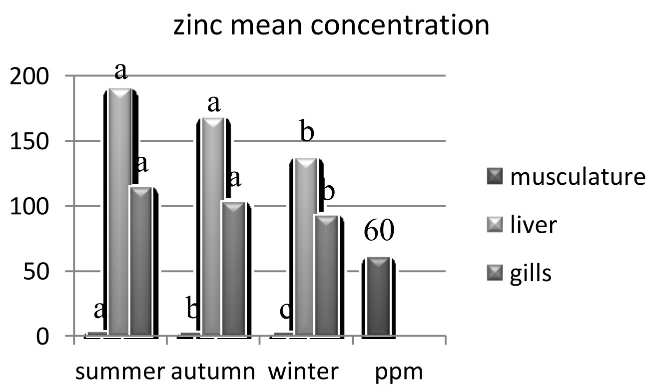


Figure 2: showing the mean concentration of Zn in *O.niloticus*.

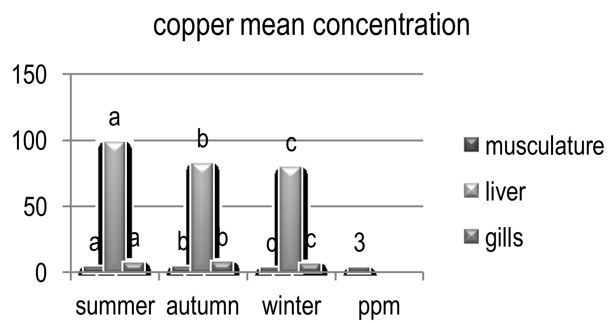


Figure 3: showing the mean concentration of Cu in *O. niloticus*.

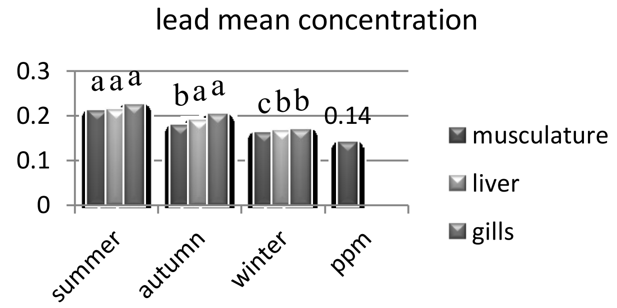


Figure 4: showing the mean concentration of Pb in *O. niloticus*.

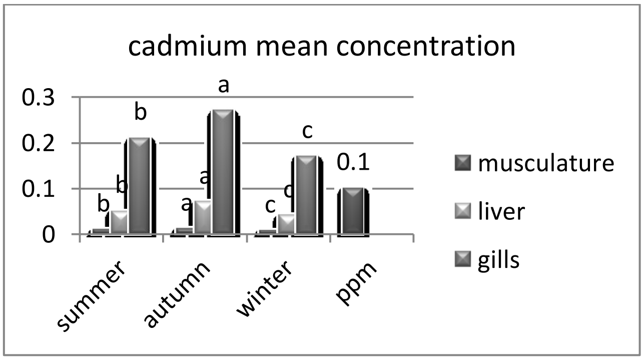


Figure 5: showing the mean concentration of Cd in *O.niloticus* .

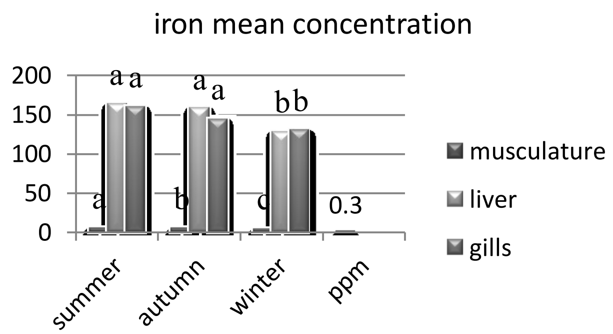


Figure 6: showing the mean concentration of the Fe in *O, niloticus*.

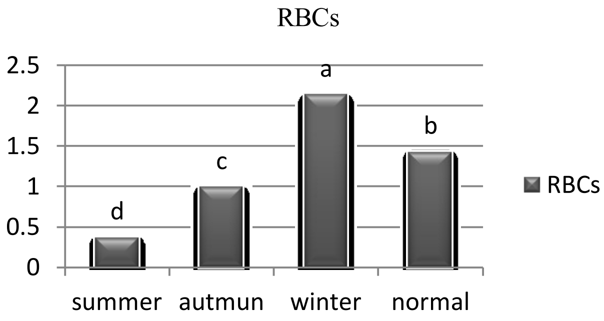


Figure 7: showing the mean concentration of the RBCs of *O. niloticus.*

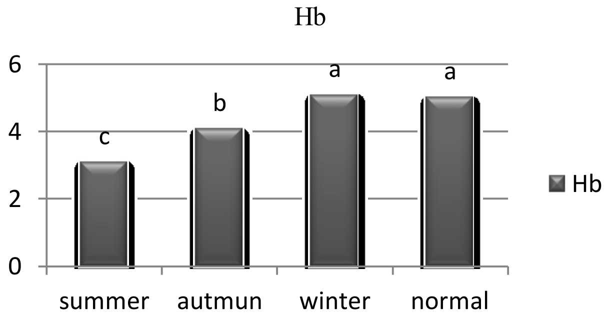


Figure 8: showing the mean concentration of the Hb in *O. niloticus*.

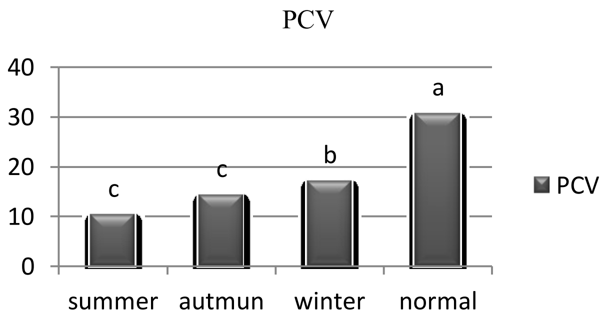


Figure 9: showing the mean concentration of the PCV of *O. niloticus*.

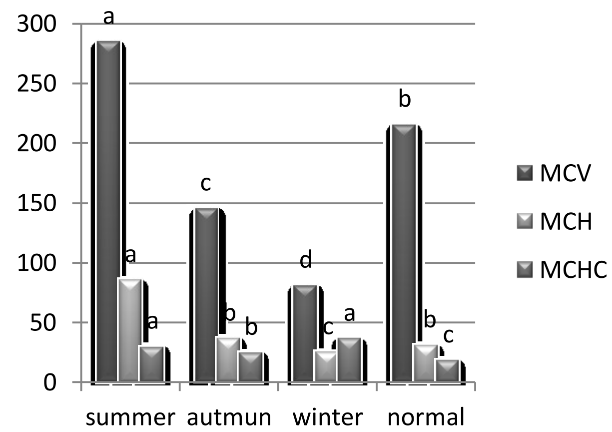


Figure 10: showing the mean concentration of the MCV, MCH, and MCHC of *O. niloticus*.
Column carry different superscripts letter are statically significantly different at (P≤ 0.05).

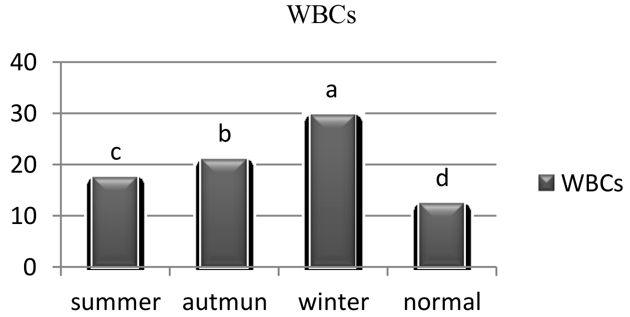


Figure 11: showing the mean concentration of the WBCs of *O. niloticus*.

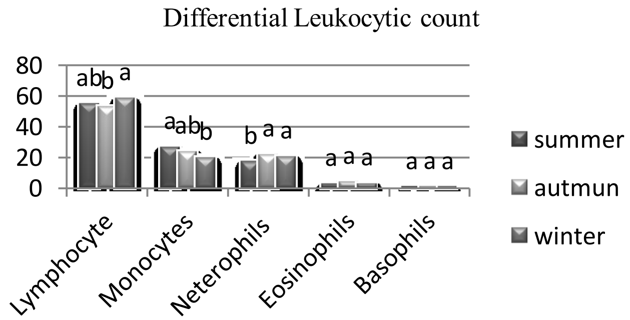


Figure 12: showing the mean concentration of the differential Leukocytic count of *O.niloticus*.

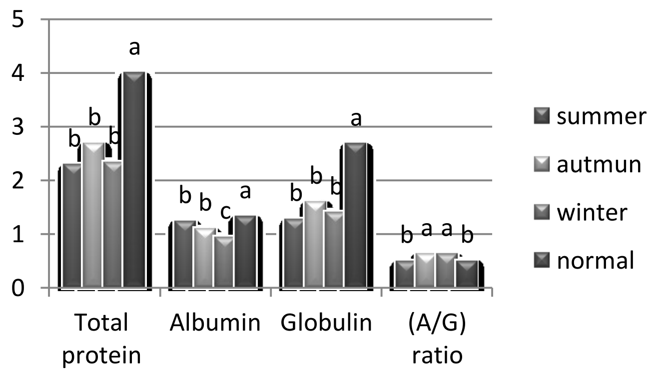


Figure 13: showing the mean concentration of the total protein, albumin, and globulin of serum of *O. niloticus*.

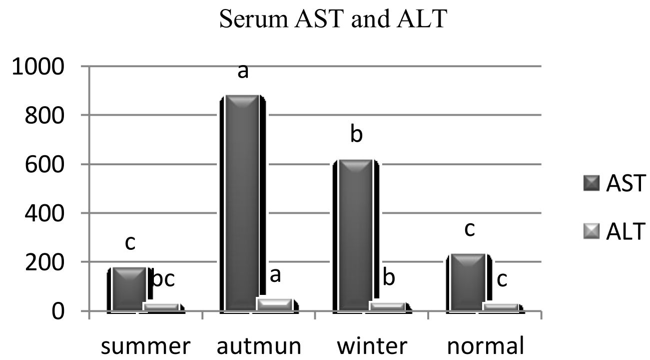
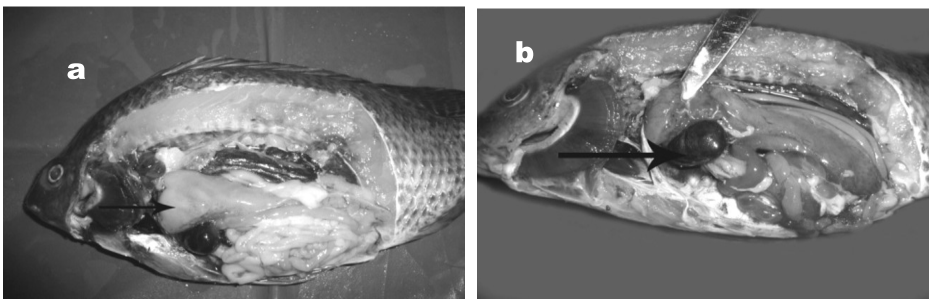


Figure 14: showing the mean concentration of the AST and ALT of serum of *O. niloticus*.



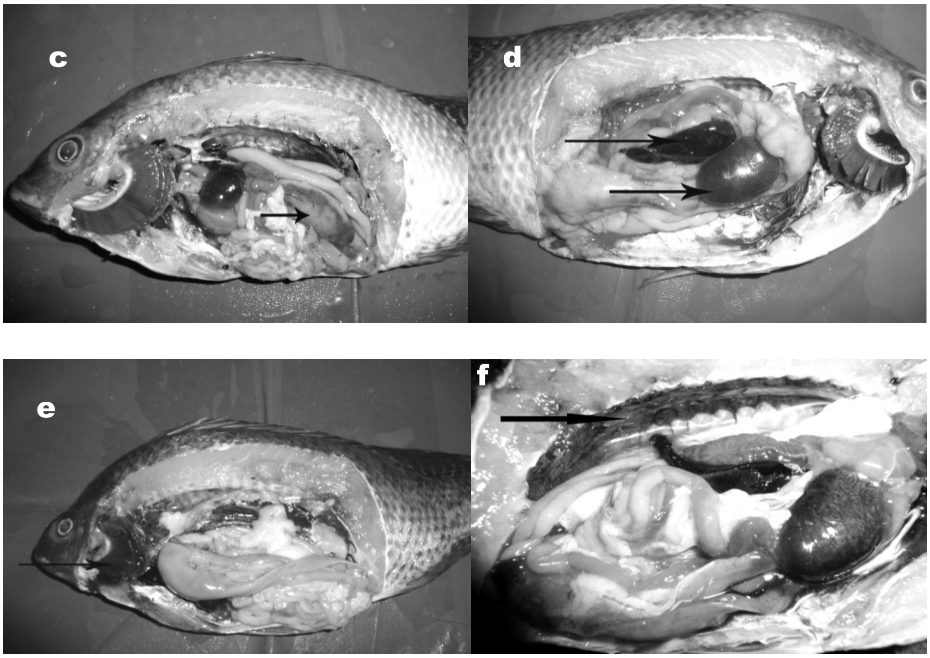


Plate 1: showing
a: *O. niloticus* showing enlarged pale liver naturally exposed to high level of copper.
b: *O.niloticus* showing enlarged full distended gall bladder naturally exposed to high level of cadmium.
c: *O. niloticus* showing congested inflammatory patches in liver naturally exposed to high level of lead.
d: *O.niloticus* has enlarged full distended gall bladder and enlarged congested spleen naturally exposed to high level of copper.
e: *O.niloticus* showing congestion of the gills naturally exposed to high level of iron and copper.
f: *Oreochromis niloticus* showing congestion of the kidney naturally exposed to cadmium.

Discussion

Fishes are the inhabitants that cannot escape from the detrimental effects of pollutants ***(Olaifa et al, 2004)*** and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components ***(Wilson and Taylor, 1993).***

In the present work, regarding post mortem lesions in cases suffering from heavy metal exposure, liver was in some cases pale and enlarged, while in other cases showing variable degrees of congestion and hemorrhagic spots. Spleen was dark in color and enlarged. Kidneys appeared congested. The gall bladder was full and distended.

Comparing the average concentrations of heavy metals (Zn,Cu, Pb, Cd and Fe) in water of lake Temsah in different seasons, the results revealed the presence of metals in the following order : Fe >Cu >Pb > Zn > Cd . This result was in agreement with the finding of ***Abdel -Sabour et al (1998)*** in Lake Temsah. The water samples collected during the present study, the mean concentrations of heavy metals were higher in the summer than in the winter that agree with the finding of ***El-Serehy et al (2012)*** in water surface in west of Port Said City where Deltaic Coast of the Mediterranean Sea.

In this study, the periodic difference of heavy metals concentration based on the data of samples gathered from the fish collected from Lake Temsah that exposed the following results: summer > autumn >winter and that result were in agreement with ***Afshan et al (2014).*** There was no significant different between mean concentrations of the heavy metals in the water of the lake in different seasons. This mean of concentration within the permissible limits guideline values for water quality from the World Health Organization ***(WHO, 1996)*** for zinc is (5ppm), copper (2ppm), lead (0.01ppm), cadmium (0.005ppm) and iron (0.2 ppm).

The mean concentration of heavy metals in the liver and gills of *O. niloticus* were markedly higher than those present in the edible parts of the fish and also the mean concentrations of heavy metals found in the lake water much lower than those obtained from different parts of the fish. This result agrees with finding of ***Mohammad (2008).*** The high accumulation potential of the liver is a result of the activity of metallothioneins, metal-binding proteins, which play an important role in metal regulation and detoxification of nonessential metals ***(Roesijadi, 1992).*** The pattern of metal distribution in the tissues has been suggested to reflect the route of metal uptake in fish, and this process is also strongly influenced by the water chemistry ***(Mager et al, 2010)***

In the present study, the mean concentration of Zn was higher in liver followed by gills then musculature of *O. niloticus.* There was significant difference between the mean concentration of Zn in fish tissue in different seasons, where the highest value found in summer followed by autumn then winter .The value of Zn in edible part within the permissible limits set by ***WHO (1989)*** which was 40 ppm. Other workers also reported the highest concentration of Zn in liver of *C. punctatus* and in the gills of *Labeo dyocheilus, Wallago attu* by **Murugan et al (2008***)*. However, highest accumulation was seen in other organs such as gills of *Tilapia zilli and O. niloticus as* **Akan et al** ***(2012 a).*** Zinc is capable of interacting with other elements and producing antagonistic, additive or synergistic effects ***(Baumann and May, 1984).***

In the present study, the mean concentration of Cu was seen high in liver followed by gills then musculature of O. *niloticus.* The highest value found in *O. niloticus* tissue is autumn followed by summer then winter .The value of Cu in edible part was more than the permissible limits (3 µg/g.) (Wet wt.) According to ***FAO / WHO (1999)*** value within set Permissible limits (20 µg/g). Copper revealed its maximum accumulation in the liver of O. *niloticus* study, while flesh shows the least level; this is in agreements with the findings in *Tilapia zillii* as ***Zyadah (1998)*** and in *C. punctatus as* ***Javed and Usmani (2013)***. The salinity decreases copper accumulation in fish tissues and inversely proportional to lead accumulation ***(Baldisserotto, 2004)***. Copper can combine with other contaminants such as ammonia, mercury and zinc to produce an additive toxic effect on fish ***(Rompala et al, 1984).***

Concerning the mean concentration of Pb, it was high in gills followed by liver then musculature of *O. niloticus.* The highest value found in summer and autumn followed by winter .The value of Pb in edible part was more than the permissible limits set as (0.14 µg/g.) according to ***FAO/WHO (1999)*** so there is a hazard from pollution with lead. It accumulates significantly in gills, livers and flesh tissue of all the fish species. The concentrations of lead were higher in the following order gills>liver> flesh. Similar findings were reported by ***Mohammad (2008)*** in O. *niloticus, L. niloticus,* also ***Akan et al (2012b)*** in *Heterotis niloticus, Lates niloticus and Citharinus citharium* . Lead is highly toxic to aquatic organisms, especially fish ***(Rompala et al, 1984).*** The biological effects of sublethal concentrations of lead include delayed embryonic development, suppressed reproduction and inhalation of growth, increased mucous formation, neurological problems, enzyme inhalation and kidney dysfunction ***(Rompala et al, 1984).***

In the present study, the mean concentration of Cd was seen in gills followed by liver then musculature. The highest value found in *O. niloticus* autumn followed by summer then winter .The value of Cd in edible part lower than the permissible limits set as (0.10µg/g) wet weight according to ***FAO/WHO (1999).*** The highest concentration of Cd in the gills and agree with ***Akan et al (2012b)*** finding in *O. niloticus, L. niloticus* .The highest concentration of Cd in gills because the main routes of cadmium uptake in fish are through the gills, gastrointestinal tract and olfactory rosette, also cadmium accumulates in fish olfactory system following water borne exposures ***(Scott et al, 2003).***

Iron was the most abundant metal in the studied tissues of *O. niloticus*. The highest Fe concentrations were found in summer and autumn while in the winter was in the gills. The present data showed that iron concentrations in the studied tissues were lower than US maximum permissible level for Fe (5µg/g) cited by ***Adeyeye (1993).*** In the fish analyzed, Fe accumulation was the maximum in all the organs but its highest value was observed in both gills and liver of. Studies reported in *C. gariepinus* ***(Osman et al, 2010)*** also revealed the maximum accumulation of Fe in liver. However, in other studies the highest accumulation was observed in the gills of O. *niloticus, L. niloticus* ***(Mohammad, 2008)***. Accumulation levels reported in the present study for gills and liver of corroborates to the findings in *O. niloticus* and *L. niloticus* ***(Mohammad, 2008).*** Earlier reports showed Fe to be normally high in gills ***(Philips, 1976)*** or in the liver ***(Charbonneau and Nash, 1993).***

Heavy metal exposure is known to induce changes in blood parameters of the fish ***(Heath, 1995).*** The significant decrease in RBCs, Hb and PCV values of collected fish from the lake when compared with the normal value according to ***Silveira-Coffigny et al (2014)*** may be due to reduction in red blood cells production in the haematopoietic organs under the action of high heavy metal concentrations recorded in water samples . These results are supported by ***El-Boshy et al (2014)*** in Nile catfish, by ***Zaghloul et al (2007)*** in *Oreochromis niloticus,* by ***Praveena et al (2013)*** in *Labeo Rohita*. Such a reduction may be also be due to the intrahepatic and intrasplenic haemorrage induced by the action of accumulated heavy metals reported by ***Zaghloul et al (2007).*** Prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants reported by ***Shalaby (2001).*** The alterations in these hematological indices may be due to a defense reaction against toxicity through the stimulation of erythropoises suggested by ***Praveena et al (2013).***

The anemic conditions in fish may be detected using haematocrit ***(Adakole, 2012).*** The PCV values always decrease when a fish loses appetite or become diseased or stressed. At present, the distinct decrease in the level of Haemoglobin and PCV after exposure to heavy metal iron clearly suggests a haemodilution mechanism possibly due to gill damage or impaired osmoregulation. The haemodilution has been interpreted as a mechanism that reduces the concentration of the irritating factor in the circulatory system. Our results are in line with **Smit *et al.* (1979)** who reported that heavy metal exposure results in the decrease in RBC count, Hb and PCV values are due to the impaired intestinal absorption of iron.

The WBCs counts of *O.niloticus* declared that, there was significant increase in different season from the lake summer <autumn< winter than in the normal value. In the present study, the increase in WBC has been attributed to several factors like increase in lymphocytes or squeezing of WBC's in peripheral blood. Increase in WBC count could be correlated with an increase in antibody production which helps in survival and recovery of fishes exposed to toxicant. High WBC counts indicate damage due to infection of body tissues, severe physical stress as well as Leukemia. This finding is in agreement with the finding of ***Nussey et al. (1995 a, b)*** in *O. mossambicus* after acute exposure to copper and ***Zaghloul et al. (2007)*** in *O. niloticus* **.**The increase in WBCs of fish was suggested to indicate alteration in defense mechanism against the action of the highly toxic and the bioaccumulated heavy metals in fish tissues as previously reported by ***Mazon et al(2002) and Zaghloul et al (2005).***

In the present study, a significant increase of the lymphocyte, monocyte than the normal value .The differential leukocyte count highlighted the higher synthesis of Lymphocytes and Monocytes in the unstressed and stress fishes. Reduction in the erythrocyte count associated with larger production of leucocytes might have triggered the immune system totally in the fishes due to the stress of chemicals**.** Our result agrees with ***Nagarajan et al, (2014)*** in *O. mossambicus.*

Significantly the mean corpuscular volume (MCV) significant increase in summer then significantly decreased in autumn and winter seasons. Moreover, mean corpuscular hemoglobin (MCH) significantly increased in the summer and significantly decreased in the winter while there was no significant difference from the normal value. Mean corpuscular hemoglobin concentration (MCHC) showed significant increase in the seasons more than normal value. Summer season has the highest concentration level of heavy metals in water and fish tissue associated with lowest RBCs count, Hb and PCV causing anemia also has leukocytosis where correlated with an increase in antibody production that indicate damage due to infection of body tissues, severe physical stress as well as Leukemia. The changes in red blood cell parameters suggested a compensatory response to the disruption of structural integrity of gills with consequent reduction of respiratory surface (tissue damage and cell proliferation), in order to increase O_2 carrying capacity and maintain the level of oxygen transference from water to tissues

(Zaghloul et al, 2005). The decrease in blood Hb content is accompanied by an increase in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). This may be due to the haemolytic action that led to fluid loss to the tissues with subsequent decrease in plasma volume ***(Swift, 1981).*** The MCV gives an indication of the status of size of Red blood cell and reflects an abnormal or normal cell division during erythropoises. The increase in MCV may be attributed to the swelling of erythrocytes as a result of Hypoxic condition or Osmotic stress or Macrocytic anaemia in fishes exposed to metal pollution ***Sinha et al (2000).***

The present study showed significant differences in the studied serum constituents through the highest values in serum AST and ALT of *O. niloticus* were within the normal range. Serum total proteins play an important role in the metabolism and regulation of water balance. Blood protein and enzyme levels, in rainbow trout, are affected by metals ***(Dethloff et al, 1999).*** The serum total protein concentrations in fish collected from the lake showed significant decrease, where the total protein serum to lower levels in summer , autumn and winter than normal value (3.9 g/dl) reported by **Hrubec *et al (*2000).** The recorded hypoproteinamiais may be explained on the basis of energy production during pollutant toxicity and/or due to other several pathological processes including renal damage and elimination in hepatic blood flow and/or plasma dissolution as reported by ***Gluth and Hanke (1985).*** Hypoproteinemia in this study was in agreement with the findings of Mekkawy ***et al (2011)*** who recorded that *O. niloticus* exposed to Cd for 15 and 30 days showed a reduction in serum protein levels This decrease of total protein may be due to the destruction of protein synthesizing subcellular structures and inhibition of the hepatic synthesis of blood protein ***(Fontana et al, 1998).*** Loss of protein from damaged kidneys could contribute further to the observed hypoproteinemia**.**

The elevation in serum AST and ALT activities of *O. niloticus* may be attributed to the damage of liver cells by the action of the accumulated heavy metals. The same results were previously reported in case of different fish species exposed to different heavy metals ***(Elghobashy et al, 2001 and Gad, 2005).*** The following cell damage, the membranes become permeable and the enzymes are found in the extracellular fluid and serum. So, determination of transaminases, (AST, ALT) has proven useful in the diagnosis of liver disease in fish ***(Sandnes et al, 1988).*** Also, Cell injury of certain organs leads to the release of tissue-specific enzymes into the blood stream ***(Chen et al. 2004)*** **.**The significant increase of transaminases (AST and ALT) activity in fish exposed to heavy metals may reveal possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver.

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الملخص العربي

دراسة عن التلوث ببعض المعادن الثقيلة في مياه واسماك البلطي في بحيرة التمساح

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تم تجميع ١٢٠ سمكة من أسماك البلطي النيلي بشكل عشوائي وفى المواسم المختلفة طازجة من البحيرة الغربية حيث تعتبر جزء من بحيرة التمساح بالإسماعيلية وقد تم رصد الصفة التشريحية الخاصة بتواجد المعادن الثقيلة كل على حده حيث وجود تضخم وشحوب في الكبد وفى حالات أخرى وجد دراجات مختلفة من النزيف واحتباس الدم في الكبد والخياشيم والكلى والطحال بالإضافة إلى تضخم وامتلاء المرارة وقد أظهر الاختلاف في المواسم لتركيز المعادن الثقيلة استنادا إلى البيانات من عينات المياه التي تم جمعها من نفس المكان وقد تبين من النتائج أن تركيز الملوثات في : الصيف> الخريف> الشتاء. أظهرت النتائج وجود المعادن في الترتيب التالي: الحديد> النحاس> الرصاص> الزنك> الكادميوم حيث أن تركيز المعادن الثقيلة في كبد السمك و الخياشيم والعضلات دائما أعلى من تركيزها في الماء مما يدل على تراكمها مع مرور الوقت. كما تبين أن أعلى موسم لتركيز المعادن الثقيلة في المياه والسمك يصاحب اقل عدد خلايا الدم الحمراء و الهيموجلوبين و زيادة في عدد خلايا الدم البيضاء بالإضافة إلى نقص بروتينات الدم وارتفاع في إنزيمات الكبد عن المعدل الطبيعي مما سبق تبين أن بعض المعادن الثقيلة أدت إلى تثبيط المناعة وإضعاف الأسماك مما يجعلها عرضة للإصابة بالأمراض .