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Variable changes of environmental and climatic factors on some endocrine disrupting compounds affecting thyroid hormones in Nile Catfish (*Clarias gariepinus*)

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Abstract

Global climatic change is receiving a particular attention because it is thought to cause habitat destruction and may increase exposure of humans to extreme temperatures, polluted air and water, extreme weather events and endocrine disrupters (EDCs) as heavy metals. The aim of the present study is to give new insight on the effect of some environmental and climatic factors that may affect the level of some heavy metals in the aquatic system and also the possible impact effects of these EDCs on thyroid function in male and female catfish (*Clarias gariepinus*).The study was conducted on the River Nile around Banha city (Kalubia governorate) and Idku Lake (Beheira governorate). Fishing trips to the studied areas were carried out every three months over a period of one year from (summer 2014–spring 2015). Ten fish samples (from each sex) were collected each season from every fishing site. Five water samples from each locality were collected seasonally. The climatic changes in both sites were recorded and heavy metals [(Zn); (Cu) and (Pb)] were determined in water and muscle samples. Quantitative determination of serum thyroid stimulating hormone (TSH); Triiodothyronine (T_3) and thyroxin (T_4) as well as T_4/T_3 ratio was also determined. The climatic changes in both sites were significantly ($P < 0.01$) influenced by season and locality. The three heavy metals were significantly ($P < 0.01$) affected with season except Pb was not detected in water from the two sites. Muscle heavy metals of both sexes from the two sites were significantly affected by season and location. TSH concentrations were significantly differed in all seasons for both fish sexes collected from Banha but only differed at autumn and spring for those from Idku Lake. Both sexes of fish showed significant variations in (T_4) concentrations with location all over the year except at spring for males and at winter for females. T_3 levels in the same site indicated significant variations between the two sexes at summer, autumn, spring and winter, spring in fish collected from River Nile and Idku Lake, respectively. Male fish from River Nile recorded lower T_4/T_3 ratios than females all over the year. The same significant lower ratios was

present also at winter in fish from Idku Lake, while at summer, autumn and spring male fish had higher ratio than females.

Introduction

Global climate change is receiving particular attention because it is thought to cause habitat destruction, desertification and biodiversity loss. Also, it has a possible serious impact on human health and society. Climate change may increase exposure of humans to extreme temperatures, polluted air and water, extreme weather events, wildfires, infectious disease, metals and metalloids, pesticides, dioxins, endocrine disrupters (EDCs) and other chemicals (**Noyes *et al*., 2009**). Moreover, it can enhance the toxicity, stability, bioavailability/uptake, transport, transfer, degradation, volatilization and adsorption of contaminants (**Kibria *et al*., 2013)**. Contaminants are biological, chemical, physical, or radiological substances which, in sufficient concentration, can adversely affect living organisms via air, water, soil, and/or food contamination (**Georgescu *et al*., 2006**). EDCs are defined as chemical substances with either agonistic or antagonistic endocrine effects on human or animals. These effects may be achieved by interferences with the biosynthesis or activity of several endogenous hormones. Recently, it was demonstrated that heavy metals such as (Cd), (Ni), (Pb) and (Zn) may exhibit endocrine-disrupting activity (endocrine disrupting metals) in animal experiments (**Kibria *et al*., 2010**). There are many endocrine-disrupting chemicals that can interfere with thyroid function by acting on different points of regulation of thyroid hormone synthesis, release, transport through the blood, metabolism of thyroid hormone, and thyroid hormone clearance .In addition, many natural substances are known to affect thyroid function, including low iodine as well as goitrogens in various foods **(Diamanti-Kandarakis *et al.,* 2009).**

Periodic changes of wet and dry climatic periods can lead to less stable forms of metal deposit and consequently, increase bioavailability of metals (**Carere *et al.,* 2011**). At higher temperatures the metabolism of aquatic organisms increases. Thus, the entrance of dissolved chemical pollutant(s) into the body increased (**Anawar, 2013**). Climatic change is projected to increase high rainfall events and thus run-off of pollutants will be increased (**Visser *et al*., 2012**). Fertilizers, farm yard manures, fossil fuel combustion, sewage sludge and incineration are sources of metals where aquatic organisms tend to concentrate trace metal to a high magnitude and man is the ultimate consume of these aquatic organisms (**Foran, 1990**).The capacity of some aquatic organisms to concentrate heavy metals up to 10^5 times the concentration present in the water **(Sander, 1997**). Heavy metals (Cu and Zn) are generally considered to be less toxic than non-essential metals (Pb), but they are toxic when present in elevated concentration in the environment (**Guimarães *et al*.,1982**).

Special concern for the possible effects that EDCs may impose on the ability of male and female Nile catfish (*Clarias gariepinus*) for adapting to environmental alterations caused by climatic changes (**Phoenix and Lee, 2004)**. It is possible that these chemicals may have a complex effect on the brain; hypothalamus; pituitary; gonad and liver in both sexes **(Singh *et al.,* 2004)**. The thyroid hormone seem to be among the most vulnerable endocrine systems with respect to disruption by EDCs in endothermic animals. As it regulates those metabolic processes, growth and differentiation of tissues, including the regulation of neuronal proliferation, cell migration and differentiation of the developing animal. There for there are a good reasons for being particularly worried about the possibilities that EDCs may affect endocrine processes involved in adaptations to climatic change in animals (**Zoeller *et al.,* 2002**). African catfish (*C. gariepinus*) is one of the most important tropical fish due to high growth rate, high consumer acceptability and high resistance to poor water quality and oxygen depletion (**Karami *et al.,* 2010**). Moreover, it has been used in fundamental research and considered as an excellent model for toxicological studies (**Mekkawy *et al.,* 2010**).

The aim of the present study is to give a new insight on the effect of some environmental and climatic factors that may affect the level of some endocrine disrupting compounds (heavy metals as Zn, Cu and Pb) in the aquatic system. Also, the possible impact effects of these EDCs on thyroid hormones (T_3; T_4 and TSH) function were studied in male and female catfish (*Clarias gariepinus*).

Material and Methods

Field collection:

The collection of samples was performed by commercial fishermen who fished the catfish *(Clarias gariepinus*) systematically. The positions of the two sampling sites are shown on the map (Fig. 1). The distance between the two sites is about 120 Km. The study was conducted on the River Nile around Banha city (Kalubia governorate) and Idku Lake (Beheira governorate). Fishing trips to the studied areas were carried out every three months over a period of one year (from summer 2014 to spring 2015). Ten fish samples (from each sex) were collected for each season and the average total body length (cm) from both fishing sites are outlined in Table 1.

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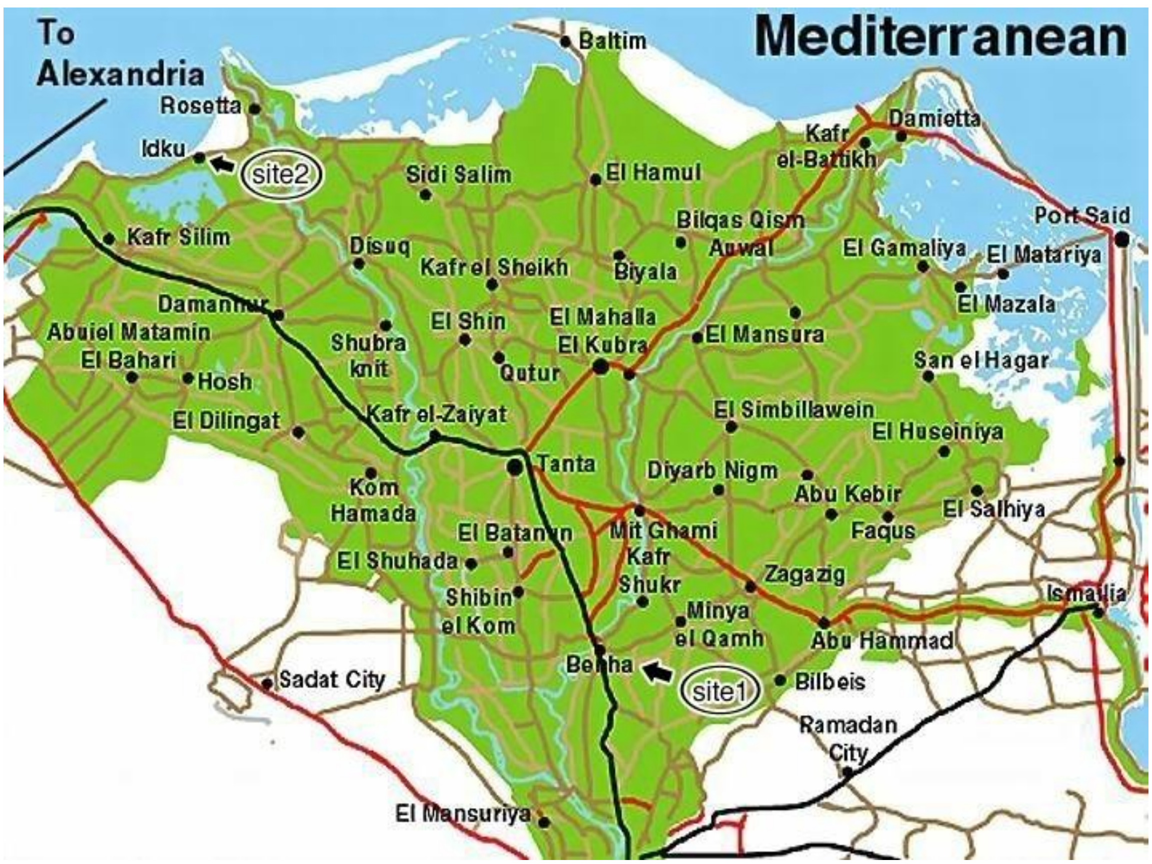
Table (1): Mean ± Standard deviation of the total body length (cm) of *Clarias gariepinus* collected from River Nile (Banha area) and Idku Lake regions.

	Summer season July 2014		Autumn season October 2014		Winter season January 2015		Spring season April 2015	
	Male	Female	Male	Female	Male	Female	Male	Female
River Nile (Banha area):								
Mean	36.6	35.6	34.7	34.3	32.5	31.4	37.1	37.3
± S.D.	1.896	3.180	1.380	1.569	2.045	1.290	2.224	3.104
Idku Lake:								
Mean	35.5	35.9	33.4	32.6	34.6	32.7	35.3	33.5
± S.D.	3.174	2.840	1.746	1.085	1.352	0.886	2.181	1.660

Fig. (1): The location of the two sites studied.



All fish were hauled and transferred directly into clean holding tanks to avoid the possibility of contamination and kept aboard the research vessel until blood sampling could be carried out within 24 hours in the hormonal unite, Animal Health Research Institute, Dokki, Giza. Five water samples from each locality were collected by a Hydro-Bios TPN water sampler at 30 cm depth as described by **Parker (1972)** using polyvinyl chloride Van Dorn plastic bottles of 1 liter capacity and preserved by addition of 1 ml concentrated nitric acid. The water samples from each site were filtered through ashless filter paper (0.45 um) and kept at 4^oC until the time of analysis of heavy metals. The climatic changes (i.e. minimum; maximum air temperatures; relative humidity and wind speed) in both sites were recorded by the local stations of the Central Laboratory of Climatic Research, Agriculture Research Center, Giza.

<u>Sampling:</u>

A - Blood Samples:

Blood was collected, after heart puncture, in sterile tubes, allowed for clotting overnight at 4° C and then centrifuged at 3000 rpm for 10 min. to separate serum. Serum samples were stored in polyethylene test tubes at -20°C until hormones analysis.

B - Muscle samples:

Each fish was washed with deionized water. Muscle samples were taken from above the lateral line, at the beginning of the dorsal fin of each fish and as deep as the backbone. Samples were minced and homogenized separately. The samples were placed on clean dry glass plates and dried in an oven at 105^oC for 48 hours **(Bohn and McElroy, 1976)**. The dry samples were wrapped in polyethylene bags and stored until heavy metal analysis.

<u>Analysis:</u>

A- Heavy metals determination in water and muscle samples:

Before use, all glassware and plastic utensils were previously soaked overnight in 5 M HNO_3 and rinsed with deionized water. One gram of dry muscle samples were digested separately in glass tubes with 10 ml of acid mixture (nitric acid/perchloric acid; 7:3 v/v). The digestion was facilitated by heating at 70^oC. After cooling, all digests were filtered through a Whatman 42 filter paper, and then diluted with deionized water to give a final volume of 25 ml. Both filtered water samples and filtered muscle digested samples were applied to analysis of zinc; copper and lead by atomic absorption spectrophotometer (Sens AA DUAL, GBC Scientific Equipment). The absorbance and concentration were recorded directly on digital scale of the spectrophotometer and the heavy metals levels can be estimated according to (**APHA**,**1998**) for water samples and (**Leung and Furness, 1999)** for muscle samples.

B- Hormone determination:

Quantitative determination of serum thyroid stimulating hormone (TSH); Triiodothyronine (T_3) and thyroxin (T_4) were determined by using enzyme immunoassay ELISA Kit. (Immunospec corporation, USA, catalog No.E29-227; E29-229 and E29-230, respectively).The assay is based on a solid phase enzyme-linked immunosorbent assay with sensitivity 0.2uIU/ml; 0.25 ng/ml and 0.5 ug/dl for TSH; $T_3$and T_4 respectively. The percentage change of particular concentration from River Nile, Banha (site I) to Idku Lake (site II) areas was determined according to the following formula: [(Conc. at site II - Conc. at site I) / Conc. at site I] X 100 .

***Statistical analyses*:**

Data entry and statistical analysis were done using **SPSS-20 (2009**) statistical software package. The basic statistics, means and standard deviations of the measured parameters were estimated. Comparisons of means were performed with *t*-tests **(Snedecor, 1971)**. Simple ANOVA was used to compare seasonal variability. In order to identify the independent predictors of various parameters, multiple linear regression analysis was used, and analysis of variance for the full regression models was done. Statistical significance was considered at P value < 0.05.

Results and discussion

Climatic measurements:

The climatic conditions during the experimental period (June 2014 – May 2015) in Banha and Idku Lake are summarized in table (2). ANOVA test indicated that minimum; maximum air temperature; relative humidity and wind speed in both sites are significantly (P < 0.01) influenced by season. The minimum air temperature and wind speed measures during the 12 months are significantly (P < 0.001) changed with the locations studied. Also, the maximum air temperature and relative humidity measures were significantly changed with the location during the year except in January and June respectively. In January, the lowest values for both minimum and maximum air temperatures were recorded in both locations but the highest records for the same items were recorded in July and September respectively in Banha area and in August for both in Idku Lake. In Banha, the lowest records for relative humidity and wind speed are detected in May and August respectively, while in Idku Lake these are recorded in June. The highest values for relative humidity and wind speed are reported in December and April respectively in the two sites studied. This agrees with **Rainbow and Dallinger (1993)** who reported that freshwater has been known to exhibit a high natural variability in their physical and chemical properties due to local differences in geology and climate. They are therefore more susceptible to anthropogenic influence than the marine environment. Temperature is a factor of great importance for aquatic ecosystem. It affects the organisms, as well as the chemical and physical characteristics of water **(Abdo, 2005).** As expected the water temperatures of the studied points are lower than that of the air. An increase in temperature affects the levels of dissolved oxygen in the water column where it is inversely proportional to temperature. Dissolved oxygen will elicit physiological regulatory mechanisms involved in the maintenance of oxygen gradient from water to tissues which is essential to maintain the metabolic aerobic pathways **(Adeogun, 2012).** Heavy metals enter an aquatic ecosystem from different natural and anthropogenic sources and atmospheric deposits **(Rajeshkumar and Munuswamy, 2011).** Thus seasonal atmospheric variability (air temperature, wind speed and relative humidity) affect heavy metals concentrations in an aquatic ecosystem.

Water heavy metals:

The seasonal surface water analysis of the three heavy metals (Zn; Cu and Pb) in both River Nile (Banha area) and Idku Lake are summarized in table (3). No records for Pb were detected in both sites all over the year. The bulk of Pb in surface water is particle-bound and it is relatively rapidly sediment **(Hutchinson and Fitchko, 1974)** and its concentration increased with depth **(Saleh *et al*., 1988).**The other two heavy metals (Cu and Zn) significantly ($P < 0.01$) affected with season in both locations. It is more clear in Idku Lake ($F = 2040.3$) than River Nile ($F = 1297.5$). **Barlas (1999)** indicated that seasonal variation effect differs according to the location of capture. **Pippy and Have (1969)** illustrated interrelationships among temperature, Zn-Cu pollution and bacterial infections. Previously significant changes in Cu concentrations between the two localities are monitored all over the year. The same significant change is detected in Zn values all over the year except at summer .The lowest and highest values of Zn are recorded at winter and autumn (River Nile) and autumn and spring (Idku Lake) respectively. Both levels of Cu and Zn wear within the maximum permissible limits (MPL)"1.0 ppm" all over the year in the two sites according to **Law No. 48 (1982)**. Working on Mariut Lake water, **Rizkalla *et al*. (2003)** recorded that concentrations of Cu and Zn were lower than the **Law No. 48 (1982)** and Pb recorded higher concentration.

Muscle heavy metals:

Copper and Zn are classified as essential heavy metals. These metals are known to help in growth and production of aquatic life. Alternatively, many minerals may be harmful to aquatic life, either through acute toxicity or chronic effects from accumulations in the organism over extended periods. Lead belongs to this group of heavy metals. It becomes common pollutant to freshwater from industrial, agricultural, sewage, mining effluents and the burning of fuels and car exhausts “climatic deposits” **(El Nabawi *et al*., 1987).** The toxic heavy metals affect biological functions, hormonal system and growth. In general, the hazardous effects of this toxic element depends upon the dietary concentration of the element, absorption of it by the digestive system and homeostatic control of the body for the element **(Rajaganapathy *et al*., 2011)*.*** The concentrations of zinc; copper and lead in muscles of both sexes of *Clarias gariepinus* from the two locations during the four seasons are tabulated in table (4) .The percentage of changes from Idku Lake (site II) relative to River Nile, Banha (site I) during the four seasons are figured in Fig.($2_{A\&B}$). ANOVA test illustrated that the three elements in both sexes from the two sites significantly ($P < 0.01$) affected by season. In view of the essential nature of Zn as cofactor for a number of enzymes (e.g. protein and carbohydrate digestions), it is known to be toxic to fish causing mortality, growth retardation, tissue alterations, respiratory and cardiac changes, inhibition of spawning ,and a multitude of additional detrimental effects which threaten survival of fish species **(Soransen, 1991).** Zinc concentrations in muscles of both sexes were significantly affected by location in the four seasons (except female in summer season). Also, significant higher values were recorded in male fish from Idku Lake than that in River Nile at autumn (107 % change) and winter (40.4 %) seasons, while at summer and spring seasons male fish showed significant lower values (-19.8 & -16.4 %) (Fig. 2_A). Female fish from Idku Lake showed significant higher values of Zn at autumn (189 %) and spring (20.9 %) and lower value at winter (-16.1 %) compared to females from River Nile (Fig. 2B). Significant sex differences ($P < 0.001$) in muscle Zn concentrations were obviously demonstrated in fish from River Nile at summer; winter and spring seasons, where values in males are higher than that in females at both summer and spring and lower at winter. Tissue distribution studies of Zn in *C. gariepinus* showed that relatively large amounts of Zn were present in liver **(Annune and Iyaniwura, 1993;Van den Heever and frey, 1994; Farombi *et al*., 2007 and Rizkalla *et al*., 2003 and 2012)** and that skeletal muscle accumulates little Zn (**Annune and Iyaniwura, 1993;Gbem *et al* .,2001 and Rizkalla *et al* ., 2003 and 2012). Baptist and Lewis**

(1967) found that fish accumulate 4- or 5- times more Zn from food-chain than from water.

Copper has numerous functions in cellular biochemistry including vital roles in cellular respiration and as a co-factor for 30 different enzymes **(linder, 1991)**. The presence of Cu in the aquatic environment at relatively lower concentration is known to be harmful **(petereson *et al*., 1991).** High concentration of this metal caused stomach and intestinal distress, liver and kidney damage and anemia **(U.S.EPA, 1991).** Other effects of Cu poisoning are increased ventilation rate and oxygen consumption, decreased antibody production, decreased osmolarity and plasma Na levels, disrupted trans epithelial ion exchange, inhibited Na-K-ATPase activity and reduction in viral clearance and serum protein levels **(Sellers *et al*., 1975 and Reid and MacDonald, 1988).** Muscle copper concentrations in male fish significantly affected by location at autumn and winter seasons, where males from Idku Lake were higher contents than that from River Nile (73.4 % change at autumn and 5.7 % at winter) (Fig. 2_A). Female fish from Idku Lake recorded significant higher values than that from River Nile at summer (43.3 %); autumn (152 %) and spring (22.4 %) (Fig. 2_B). Significant sex differences in the concentrations of muscle Cu were detected in the four seasons in both sites. Males from Idku Lake showed lower values than females all over the year. While, lower values were detected only in males from River Nile at autumn and winter and the higher values were recorded at summer and spring. **Ghazaly *et al*. (1992) and Rizkalla *et al*. (2003 and 2012)** indicated that Cu in fish appeared to be selectively accumulate in liver tissue and muscle tissues was poor indicator of increased metal availability. According to **Moore and Rammoorthy (1984),** fish muscle normally contains low concentration of Cu and, even at high levels of Cu, muscles does not often reflect increases in the external environment. **Ghazaly *et al*. (1992) and Rizkalla *et al*. (2003 and 2012)** found that Cu level in muscle of *Clarias gariepinus* was affected by the locality and an obvious significant seasonal effect was demonstrated by **Rizkalla *et al*. (2012).**

Highways pose a threat to fish because of Pb contamination from automobile exhausts **(Ney and Van Hassel, 1983). Soransen (1991)** reported that more Pb is present in benthic than pelagic fish and Pb poisoning in fish results in hematological alteration; respiratory changes; neuronal and muscular abnormalities; behavioral changes; growth retardation and inhibition of several enzymes. Lead disappeared from muscles of both sexes from River Nile at summer and in males from Idku Lake at spring. Significant ($P < 0.001$) location effect was demonstrated in both sexes all over the year except in male at autumn (table 4). **Rizkalla and AboDonia (1996)** said that Pb value in deboned flesh indicated significant variations with region. Male fishes from Idku Lake are higher than that from River Nile at summer and lower at winter (-43.4 %) and spring

(-100 %). On the other hand, female fishes from Idku Lake are higher than its values from River Nile at summer; autumn (48.2 %) and winter (185 %), while at spring it is lower (-38.6 %) (Fig. $2_{A\&B}$). Pb concentrations in muscles of male fishes from Idku Lake are significantly lower than that in females during the four seasons. Males from River Nile have significant higher values than females at autumn and winter seasons, while at spring it is lower. These result were confirmed by the result of multiple linear regression (Table ,5) which indicated that Pb is the only metal that is significantly not affected by its water level. **Larocque and Rasmussn (1998**) demonstrated that Pb concentration in fish are higher than water, which, indicates the bioaccumulation **(David *et al*., 2010)**. Lead was more concentrated in the non-edible parts of the fish where its concentration were several- fold higher than the edible muscle **(Saleh *et al*., 1998)**. Different authors reported various patterns of the highest concentration of Pb in different organs of *Clarias gariepinue*s follows: liver **(Soliman *et al*., 1998; Gbem *et al*., 2001; Farombi *et al*., 2007);** gills **(Abdel-Baky., 2001);** gonads **(Rizkalla *et al*., 2003)** and kidney **(Rizkalla *et al*., 2011) .** All the authors stated that Pb residues in muscle tissues were usually lower than these in other organs .This might attributed to either the low binding rate for Pb to sulphydryl groups in muscle proteins as well as low solubility of Pb salts **(Moore and Ramamoorthy, 1984)** and/or to the growth factor as growth may dilute toxicant concentration if growth is faster than accumulation (**Badsha and Goldspink, 1982 and Arnac and Lassus, 1985).**

All results of Zn and Cu concentrations of the this study were within the permissible limits (50 and 20 ppm, respectively) according to **FAO/WHO (1992)** .These results were in agreement with **Tag El-Din *et al*. (2009)** who reported that Zn and Cu concentrations in catfish were within the permissible limits and significantly decreased than that of basa catfish. Also, The Pb levels were higher than the MPL (0.5 ppm) according to **FAO/WHO (1992)** in female from Idku at summer and winter. This disagrees with **Tag El-Din *et al*. (2009)** who reported that Pb levels were within the MPL and significantly decreased than that of basa catfish (0.79 ppm). The multiple linear regression models between the climatic conditions and water heavy metals in one side and the concentrations of muscle heavy metals in *Clarias gariepinus* seasonally collected from River Nile (Banha) and Idku Lake on the other side are gathered in table (5). The concentrations of the three muscle heavy metals studied are significantly affected by the site of collection and the maximum air temperature. Two heavy metals are influenced by sex (Cu and Pb); relative humidity and wind speed (Zn and Cu). Muscle Zn and Cu are significantly affected by fish length and minimum air temperature respectively. The multiple linear regression analysis indicated that Pb is the only metal that non-significantly affected by its concentration in water. According to the values of standardized coefficient, muscle Zn; Cu and Pb concentrations are more influenced negatively by site/relative humidity; relative humidity/site and maximum air temperature respectively. Based on r-square value, the model explained 56.9; 69.0 and 59.6 % of the variation in the level of the muscle Zn; Cu and Pb respectively. This coincide with **Farkas *et al*.(2003)** who observed that the accumulation of heavy metals in fish muscle depends on the variety in size; age; sex; feeding behavior; ecological needs of the individuals and seasons of capture.

Serum thyroid hormones:

Thyroid hormones are among the major hormones playing important roles in the protection of the physiological balance of the body **(Nakamura and Nakao, 1993**). Malfunctions in these hormones directly affect the general situation of the living being (**Rose**, **2000**). The concentrations of serum thyroid hormones of both sexes of *Clarias gariepinus* under different environmental and seasonal conditions are illustrated in table (6) and the percentage of changes in its concentrations between Idku Lake and River Nile are graphed in Fig. ($3_{A\&B}$).

Effect of location:

Serum thyroid stimulating hormone levels (TSH) in both sexes of this study were significantly affected by the location except female fish at autumn season. Females from Idku Lake have significant higher (TSH) values at summer (104 %),winter (61.4 %) and autumn (17.4 %) and lower values at spring (-55.7 %) than that from River Nile (Fig.3B). Males from Idku Lake have significant higher values at autumn (37.7 %), spring (405 %) and lower values at summer (-52.9 %) and winter (-27.2 %) than that from River Nile (Fig. 3_A). Also, males from Idku Lake had significantly higher (T_4) levels (974 , 244 and 188 % change at summer; winter and autumn, respectively) but females had significantly higher levels at summer (33.7 %) and winter (6.8 %) and lower at autumn (-46.3 %) and spring (-90.9 %) than that in River Nile. These result show that location has a great effect on thyroid hormones results this agree with **Dupree *et al*. (1984)** who reported that thyroid activation in channel catfish may accompany maximum water temperature, weight gain and feeding where these factors exchange from location to other. Both sexes of fish showed significant variations in the thyroxin (T_4) concentrations with location all over the year except in spring for males and winter for females (Table 6). Fishes collected from River Nile recorded lower values in males than females while fishes from Idku Lake showed higher values in males than females. Triiodothyronine (T_3) concentrations in both sexes were significantly affected by location all over the year except male fish in autumn (Table 6). Significant variations also in T_4/T_3 ratio between the two sites were detected in males at summer and autumn in which males from Idku lake are higher than that from River Nile (421 and 191 % change, respectively). These results indicated that location affected on thyroid hormones levels in both sexes**.** This agrees with **Rizkalla *et al*. (2011)** who was working in Al-Aiat and Barrage areas in the River Nile. The levels of T_4 and T_3 in female Indian catfish *(Clarias batrachus*) during both prespawning and spawning phase of its annual reproductive cycle lower than that of Egyptian catfish *C. gariepinus* during the four seasons studied.Also,T_4/T_3 ratio between Al-Aiat and Barrage area in River Nile have significant lower values in female fish from Al-Aiat area at both winter ($P < 0.001$) and autumn ($P < 0.01$) seasons than that from Barrage area. On the contrary**,** female catfish from Al-Aiat area recoded significant ($P < 0.01$) higher values than that from Barrage area at spring season.

Effect of season:

ANOVA test indicated that all items studied were significantly ($P < 0.01$) affected by season except thyroid stimulating hormone (TSH) and triiodothyronine(T_3) in females and males respectively from Idku Lake (Table 6). Significant variations were detected in T_4/T_3 ratio of female fish at three seasons in which this ratio was higher in fish from Idku Lake at winter (37.4 %) but lower at summer (-46.7 %) and spring (-88.5 %) than that from River Nile (Fig. 3_B). T_3 levels were higher in male fish than female ones at summer and spring (from River Nile) and at winter and spring (from Idku Lake), while it was lower at autumn in fish from River Nile. So, the activity of the thyroid hormone fluctuated seasonally and was more active during the breeding phase than the non-breeding period. These results coincides with **Chouinard and Hanson (2000).** Also, vitellogenene have been identified as the major carriers of both T_4 and T_3 in the circulation **(Eales *et al*., 1982)**. So, the seasonal change in plasma or serum T_4 and T_3in female fish increased during previtellogenene and declined during vitellogenene and spawning period, which was related to the presence of high thyroid hormones levels in mature eggs. However the thyroid hormone levels in male fish were lower only in fish producing sperms **(Pavilidis *et al*.,1991).** The T_4/T_3 ratio is better item to study the temperature effect on thyroidal hormones status. It has not been possible to distinguish whether changes in thyroid activity were occasionally associated with reproduction state or whether they represented correlative seasonal variations. Reproductive in many teleosts is influenced by environmental factors that vary seasonally. Many of the same variables also affect thyroid hormones independently of reproductive state. These factors include temperature, salinity, photoperiod and lunar cycle **(Eales et al., 1982 and Browen, 1988).**

Effect of sex:

Comparison between sexes in the same site indicated that TSH concentrations were significantly ($P < 0.001$) differed in all seasons for fish collected from River Nile and at autumn and spring for those from Idku Lake. TSH levels in male fish from River Nile were significantly higher at summer and winter than females (Table 6). Significant sex difference in T_4 concentrations was illustrated. As the results indicated that T_4 level in the females from the River Nile and Idku Lake were significantly higher and lower than male all over the year, respectively. Also, **Rizkalla *et al.* (2011)** recorded that female fish from Barrage area was Significantly ($P < 0.001$) higher in serum T_4 levels at spring season than that of male and also T_4 levels were higher than that of T_3 levels all over the period of study. Meanwhile, sex comparison of T_3 levels in the same site indicated significant variations between the two sexes at 3 seasons (summer; autumn and spring) and at 2 seasons (winter and spring) in fish collected from River Nile and Idku Lake, respectively. T_3 levels in male fish from Idku Lake are higher at summer (92.5 % change) and winter (167 %) and lower at spring (-32.1 %) than that from River Nile. In female fish, T_3 values are higher at summer (154 %) and lower at autumn (-57.8 %), winter (-5.9 %) and spring (-23.6 %) than those from River Nile. Male fish from River Nile recorded lower T_4/T_3 ratio than females. The same significant lower ratio is presented also at winter in fish from Idku Lake, while at summer, autumn and spring male fish have higher ratio than females. **Eales *et al.* (1982)** mentioned that a growing body of evidence suggested that T_4 is generally regarded as relatively inactive precursor of T_3 binds with low affinity to nuclear receptors and has little direct action, while T_3 is metabolically the most active hormones and binds with high affinity. **Sefkow *et al*. (1996)** find that only 19 – 25% of the total T_3 production is actually produced through T_4 conversion to T_3 in extra thyroidal tissues and 75 – 81 % of T_3 is generated by secretion from the thyroid. Furthermore, both the intra thyroidal and extra thyroidal conversion of T_4 to T_3 may be coregulated together to maintain plasma T_3 in arrange appropriate for a particular physiological state. Thus T_4/T_3 ratio has been used as measures if thyroid status **(Eales *et al.*, 1992) .**

Effect of climatic factors:

Seasonal factors that affect serum thyroid hormones of *Clarias gariepinus* collected from River Nile (Banha) and Idku Lake are statistically analyzed by multiple linear regression models and tabulated in table (7). The minimum; maximum air temperature and wind speed significantly affected on the four thyroid parameters studied. Values of TSH; T_3 and T_4/T_3 ratio are significantly influenced by relative humidity and muscle Zn, while T_4; T_3 and T_4/T_3 are significantly affected by muscle Pb. Both sex of fish and muscle Cu significantly affect T_4 and T_4/T_3. Also, T_4/T_3 ratio was significantly affected by muscle Cd. Serum T_4 and T_3 significantly affected by fish length. The relative humidity and site of collection are the more influenced factors that affect negatively on serum TSH and T_4/T_3 ratio. Serum T_4 and T_3 values are more affected by minimum air temperature (positively); maximum air temperatures (negatively); wind speed (positively) and relative humidity (negatively) but in different arrangements. 20.9; 65.1; 48.1 and 67.4 % of the variations in the levels of TSH; T_4; T_3 and T_4/T_3 respectively are explained by r-square of the multiple linear regression models. **Fudllallah (2012)** demonstrated that Cd; Pb and Ni concentrations in the non-edible and edible organs of *C. gariepinus* fish in combination with the differences in the location of capture (before and after Cairo area); sex and season influence on serum T_3; T_4 and T_3/T_4 by 53; 29 and 30 % respectively.

Effect of heavy metals:

Endocrine disruption in fish provides a simple and extensive background to the field of fish endocrinology in order to assist toxicologists who have limited background for either mammalian or fish endocrinology. From the multiple linear regression models (Table 7), the values of TSH, T_3 and T_4/T_3 ratio were significantly influenced by muscle Zn, while T_4, T_3 and T_4/T_3 were significantly affected by muscle Pb. Muscle Cu significantly affects T_4 and T_4/T_3. The reason for an increase in T_4 and decrease in T_3, observed in females from the River Nile and males from Idku Lake might be due to a slowdown in the conversion of T_4 to T_3 in peripheral tissues. This may be due to the effect of endocrine disruptors (Pb) that limits the conversion of T_4 into T_3 causing the changes, mainly in 5-deiodinase as well as malic enzyme and 6-phosphogluconate dehydrogenase activities **(Diamanti-Kandarakis *et al.*, 2009)**. Also**, Abdel-Baky. (2001)** found that the concentration of thyroxin in *Clarias gariepinus* serum at different Egyptian ecological habitats (River Nile near Damietta, El-Salam canal and Lake Manzalla) was significantly different. Lead and cupper showed remarkable environmental stress on the level of T_4 hormone more than the effect exerted by Zn and Mn**.**

Conclusion:

- The present study provides evidence that heavy metal residues in fish tissues and its hazard effect on healthy people is a matter of great concern to food hygienists.
- T_4/T_3 ratio is a better item to study climatic effect (temperature) on thyroid status.
- Both sexes of fish showed significant variations in thyroid hormones levels due to the effect of seasons, location, sex, and heavy metals in the environment.
- Climatic changes have a great effect on heavy metal accumulation and thyroid hormones levels in catfish (*Clarias gariepinus*).

- Heavy metals as Zn, Cu and Pb can affect serum levels of thyroid hormone (T_3 and T_4) as well as T_4/T_3 ratio in catfish *C. gariepinus*.

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Table (2): Mean ± standard deviation of minimum; maximum temperature (°C); relative humidity (%) and wind speed (m/sec) of air measured monthly in River Nile (Banha) and Idku Lake.

Eason			Summer			Autumn			Winter			Spring		
Climatic items			June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May
River Nile (Banha)	Min. Temp.	Mean	28.4	31.5	30.8	23.1	19.1	14.5	11.6	8.9	9.4	13.3	14.4	19.5
		S.D. ±	1.933	0.892	0.843	1.311	1.694	1.941	2.180	2.278	2.299	2.364	1.918	2.009
	Max. Temp.	Mean	30.3	33.8	32.8	34.7	30.4	25.7	23.2	19.5	21.2	25.3	28.2	33.4
		S.D. ±	1.769	0.872	0.834	2.367	2.027	2.558	2.180	3.710	4.144	3.536	5.188	3.980
	Relative Humidity	Mean	48.578	57.161	57.874	54.733	57.548	64.133	64.710	55.903	55.571	57.300	49.323	48.300
		S.D. ±	8.445	3.448	4.504	4.025	6.582	8.328	10.912	12.692	10.258	9.487	8.272	7.800
	Wind speed	Mean	0.621	0.553	0.487	1.013	0.935	0.917	0.745	1.187	1.225	1.123	1.468	1.280
		S.D. ±	0.152	0.118	0.084	0.257	0.290	0.302	0.379	0.623	0.632	0.239	0.516	0.403
Idku Lake	Min. Temp.	Mean	10.7	20.0	21.3	19.3	15.1	12.4	8.5	6.8	7.0	9.2	10.7	14.7
		S.D. ±	4.656[c]	1.521[c]	1.514[c]	1.149[c]	1.721[c]	1.532[c]	2.004[c]	1.622[c]	1.988[c]	2.796[c]	2.445[c]	2.399[c]
	Max. Temp.	Mean	17.7	30.4	31.4	30.6	27.5	23.7	21.5	18.4	18.5	21.0	23.8	27.2
		S.D. ±	7.376[c]	1.438[c]	0.915[c]	1.756[c]	1.535[c]	1.911[b]	1.658[b]	3.035	3.405[a]	2.319[c]	4.639[c]	3.586[c]
	Relative Humidity	Mean	48.033	81.323	81.484	80.033	80.968	82.467	86.032	82.194	82.286	83.300	77.968	77.633
		S.D. ±	16.323	3.026[c]	4.242[c]	5.986[c]	5.370[c]	8.799[c]	7.199[c]	7.833[c]	9.424[c]	5.603[c]	9.308[c]	7.950[c]
	Wind speed	Mean	1.775	3.571	3.910	3.253	2.748	2.567	2.003	2.906	3.293	2.763	3.829	3.117
		S.D. ±	0.644[c]	0.695[c]	0.718[c]	0.743[c]	0.738[c]	0.971[c]	0.730[c]	1.18[c]	1.523[c]	0.819[c]	1.358[c]	0.790[c]
ANOVA			River Nile (Banha)						Idku Lake					
	Min. Temp.		F = 567.29			P < 0.01			F = 149.37			P < 0.01		
	Max. Temp.		F = 86.869			P < 0.01			F = 70.419			P < 0.01		
	Relative Humidity		F = 12.289			P < 0.01			F = 43.609			P < 0.01		
	Wind Speed		F = 21.239			P < 0.01			F = 14.97			P < 0.01		

Significant difference between two areas: a: P < 0.05 b: P < 0.01 c: P < 0.001

Table (3): Range and mean ± standard deviation of zinc, copper, and lead (ppm, μg l^{-1}) of water samples collected seasonally from River Nile (Banha) and Idku Lake.

Season		River Nile (Banha) (5)			Idku Lake (5)		
		Range	Mean	± S.D.	Range	Mean	± S.D.
Summer	Zn	0.000 – 0.000	0.000	0.000	0.000 – 0.000	0.000	0.000
	Cu	0.344 – 0.446	0.395	0.051	0.297 – 0.345	0.321	0.024[a]
	Pb	0.000 – 0.000	0.000	0.000	0.000 – 0.000	0.000	0.000
Autumn	Zn	1.300 – 1.430	1.366	0.060	0.840 – 0.940	0.890	0.041[c]
	Cu	0.780 – 0.820	0.800	0.016	1.050 – 1.170	1.110	0.047[c]
	Pb	0.000 – 0.000	0.000	0.000	0.000 – 0.000	0.000	0.000
Winter	Zn	0.440 – 0.560	0.500	0.047	1.160 – 1.200	1.180	0.016[c]
	Cu	0.700 – 0.800	0.750	0.045	0.654 – 0.666	0.660	0.004[b]
	Pb	0.000 – 0.000	0.000	0.000	0.000 – 0.000	0.000	0.000
Spring	Zn	1.210 – 1.270	1.240	0.022	1.470 – 1.590	1.530	0.047[c]
	Cu	0.600 – 0.760	0.680	0.063	0.500 – 0.580	0.540	0.032[b]
	Pb	0.000 – 0.000	0.000	0.000	0.000 – 0.000	0.000	0.000
ANOVA	Zn	F = 1297.541	P < 0.01		F = 2040.318	P < 0.01	
	Cu	F = 74.021	P < 0.01		F = 578.907	P < 0.01	
	Pb	-----	-----		-----	-----	

Significant difference between two areas: a: P < 0.05 b: P < 0.01 c: P < 0.001

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Table (4): Mean ± standard deviation of muscle zinc, copper and lead (ppm, μg g^{-1} dry weight) of *Clarias gariepinus* collected seasonally from River Nile (Banha) and Idku Lake.

Season	Item	River Nile (Banha)		Idku Lake	
		Male	Female	Male	Female
Summer	Zn	5.592 ± 0.561	4.320 ± 0.516***	4.484 ± 0.333c	4.650 ± 0.447
	Cu	0.469 ± 0.060	0.379 ± 0.052**	0.479 ± 0.074	0.543 ± 0.061*c
	Pb	0.000 ± 0.000	0.000 ± 0.000	0.411 ± 0.076c	0.919 ± 0.031***c
Autumn	Zn	1.015 ± 0.268	0.810 ± 0.166	2.101 ± 0.483c	2.340 ± 0.309c
	Cu	1.468 ± 0.082	1.710 ± 0.277*	2.545 ± 0.136c	4.300 ± 0.663***c
	Pb	0.389 ± 0.085	0.311 ± 0.026*	0.348 ± 0.073	0.461 ± 0.098**c
Winter	Zn	1.815 ± 0.145	2.471 ± 0.086***	2.549 ± 0.620b	2.074 ± 0.516a
	Cu	2.750 ± 0.142	3.453 ± 0.108***	2.907 ± 0.118a	3.515 ± 0.093***
	Pb	0.537 ± 0.049	0.274 ± 0.046***	0.304 ± 0.033c	0.781 ± 0.145***c
Spring	Zn	2.214 ± 0.111	1.630 ± 0.250***	1.852 ± 0.164c	1.979 ± 0.142b
	Cu	3.760 ± 0.492	3.215 ± 0.168**	3.494 ± 0.318	3.934 ± 0.123***c
	Pb	0.085 ± 0.038	0.228 ± 0.027***	0.000 ± 0.000c	0.140 ± 0.033***c
ANOVA	Zn	P < 0.01	P < 0.01	P < 0.01	P < 0.01
	Cu	P < 0.01	P < 0.01	P < 0.01	P < 0.01
	Pb	P < 0.01	P < 0.01	P < 0.01	P < 0.01

Significant difference between two sexes in the same location: *: P < 0.05; **; P < 0.01 and ***: P < 0.001
Significant difference between two locations in the same sex :a: P < 0.05; b: P < 0.01 and c: P < 0.001
Number of samples in each group = 10

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Table (5): Factors that affect on muscle heavy metals of *Clarias gariepinus* seasonally collected from River Nile (Banha) and Idku Lake (Multiple linear regression model).

Dependable factors	Zn			Cu			Pb		
	Unst. Coeff.		St. Coeff.	Unst. Coeff.		St. Coeff.	Unst. Coeff.		St. Coeff.
	B	S.E.		B	S.E.		B	S.E.	
Constant	13.573	1.179		18.780	1.534		0.860	0.069	
Site	-3.267**	0.307	-3.058	-3.650**	0.438	-2.262	0.077**	0.022	0.186
Sex				0.399**	0.071	0.247	0.099**	0.021	0.238
Fish length	0.062**	0.018	0.304						
Min. Air Temp.				0.179**	0.027	1.571			
Max. Air Temp.	-0.049**	0.011	-0.475	-0.153**	0.032	-0.972	-0.037**	0.003	-0.921
Relative Humidity	-0.087**	0.008	-2.114	-0.154**	0.012	-2.472			
Wind Speed	-0.387**	0.091	-0.864	0.424*	0.205	0.626			
Water Zn	-0.472**	0.057	-0.499						
Water Cu				1.169*	0.391	0.336			
Water Pb									
r-square	0.569			0.690			0.596		
Model ANOVA	F = 30.948, P < 0.001			F = 45.351, P < 0.001			F = 59.536, P < 0.001		

Unst.Coeff.: Unstandardized Coefficient St. Coeff.: Standardized Coefficient S.E.: Standard Error
*: Significanceat P < 0.05 **: Significance at P < 0.001

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Table (6): Mean ± S.D. of serum thyroid stimulating hormone (TSH, µIU/ml); thyroxine(T_4, µg/dl); triiodothyronine(T_3, ng/ml) and T_4/ T_3ratio of *Clarias gariepinus* seasonally collected from River Nile (Banha) and Idku Lake.

Season	Item	River Nile (Banha)		Idku Lake	
		Male	Female	Male	Female
Summer	TSH	0.350 ± 0.032	0.110 ± 0.011***	0.165 ± 0.036[c]	0.227 ±0.103[b]
	T_4	0.930 ± 0.082	1.444 ± 0.633*	9.990 ± 4.129[c]	1.930 ± 0.263***[a]
	T_3	1.030 ± 0.258	0.601 ± 0.101***	1.983 ± 0.722[c]	1.528 ± 0.218[c]
	T_4/ T_3	0.956 ± 0.267	2.405 ± 1.012***	4.982 ± 0.646[c]	1.281 ± 0.236***[b]
Autumn	TSH	0.114 ± 0.013	0.316 ± 0.090***	0.157 ± 0.047[a]	0.366 ± 0.090***
	T_4	0.890 ± 0.185	1.630 ± 0.715**	2.570 ± 0.863[c]	0.875 ± 0.122***[b]
	T_3	1.110 ± 0.357	2.392 ± 0.896***	1.115 ± 0.340	1.009 ± 0.273[c]
	T_4/ T_3	0.871 ± 0.281	0.717 ± 0.269	2.538 ± 1.160[c]	0.951 ± 0.367***
Winter	TSH	0.393 ± 0.060	0.119 ± 0.019***	0.286 ± 0.135[a]	0.192 ± 0.070[b]
	T_4	3.170 ± 1.137	6.770 ± 2.275***	10.920 ± 5.592[c]	7.240 ± 2.044
	T_3	1.135 ± 0.536	1.433 ± 0.464	3.034 ± 1.516[b]	1.062 ± 0.143***[a]
	T_4/ T_3	3.135 ± 1.370	4.965 ± 1.808*	4.211 ± 1.905	6.824 ± 1.733**[a]
Spring	TSH	0.120 ± 0.028	0.524 ± 0.221***	0.606 ± 0.236[c]	0.232 ± 0.100***[b]
	T_4	1.675 ± 0.461	7.160 ± 1.539***	1.615 ± 0.562	0.652 ± 0.044***[c]
	T_3	1.985 ± 0.294	1.205 ± 0.401***	1.348 ± 0.260[c]	0.921 ± 0.058***[a]
	T_4/ T_3	0.866 ± 0.305	6.185 ± 1.096***	1.234 ± 0.470	0.711 ± 0.069**[c]
ANOVA	TSH	P < 0.01	P < 0.01	P < 0.01	P < 0.05
	T_4	P < 0.01	P < 0.01	P < 0.01	P < 0.01
	T_3	P < 0.01	P < 0.01	P < 0.05	P < 0.01
	T_4/ T_3	P < 0.01	P < 0.01	P < 0.01	P < 0.01

Significant difference between two sexes in the same location: *: P < 0.05; **; P < 0.01 and ***: P < 0.001
Significant difference between two locations in the same sex :a: P < 0.05; b: P < 0.01 and c: P < 0.001
Number of samples in each group = 10

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Table (7): Factors that affect on serum thyroid hormones of *Clarias gariepinus* seasonally collected from River Nile (Banha) and Idku Lake (Multiple linear regression model).

Dependable factors	TSH			T_4			T_3			T_4/T_3		
	Unst. Coeff.		St. Coeff.	Unst. Coeff.		St. Coeff.	Unst. Coeff.		St. Coeff.	Unst. Coeff.		St. Coeff.
	B	S.E.		B	S.E.		B	S.E.		B	S.E.	
Constant	3.518	0.850		101.015	8.958		11.497	2.529		72.230	7.115	
Site	-0.618**	0.181	-1.454							-10.580**	1.598	-2.195
Sex				2.510**	0.395	0.356				1.948**	0.270	0.404
Fish length				0.238*	0.087	0.177	0.051*	0.023	0.173			
Min. Air Temp.	0.024**	0.007	0.798	3.227**	0.282	6.466	0.206*	0.078	1.896	0.654**	0.057	1.917
Max. Air Temp.	-0.026*	0.011	-0.626	-4.384**	0.339	-6.396	-0.461**	0.097	-3.086	-1.011**	0.098	-2.157
Relative Humidity	-0.025**	0.006	-1.552	-1.297**	0.104	-4.768	-0.082*	0.029	-1.376	-0.600**	0.050	-3.229
Wind Speed	0.095*	0.047	0.535	15.717**	1.333	5.320	0.820*	0.370	1.272	2.542**	0.392	1.258
Muscle Zn	-0.216**	0.041	-0.542				0.516**	0.130	0.358	-0.990*	0.328	-0.219
Muscle Cu				-1.696**	0.375	-0.388				-1.257**	0.268	-0.421
MusclePb				-6.167**	1.454	-0.364	-2.691**	0.363	-0.729	2.465*	0.874	0.213
r-square	0.209			0.651			0.481			0.674		
Model ANOVA	F = 7.014, P < 0.001			F = 27.921, P < 0.001			F = 15.753, P < 0.001			F = 31.146, P < 0.001		

Unst.Coeff.: Unstandardized Coefficient St. Coeff.: Standardized Coefficient S.E.: Standard Error

*: Significance at P < 0.05 **: Significance at P < 0.001

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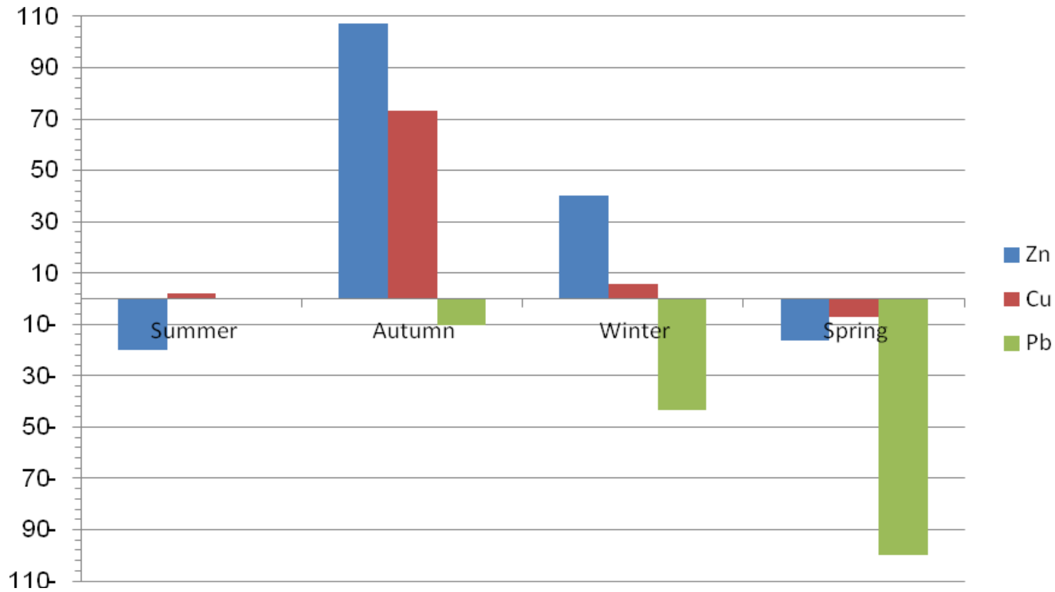
Fig. (2): The percentage change in the muscle zinc; copper; and lead concentrations of *Clarias gariepinus* from Idku Lake (site II) relative to River Nile – Banha (site I) during the four seasons.

A- Male:



B- Female:

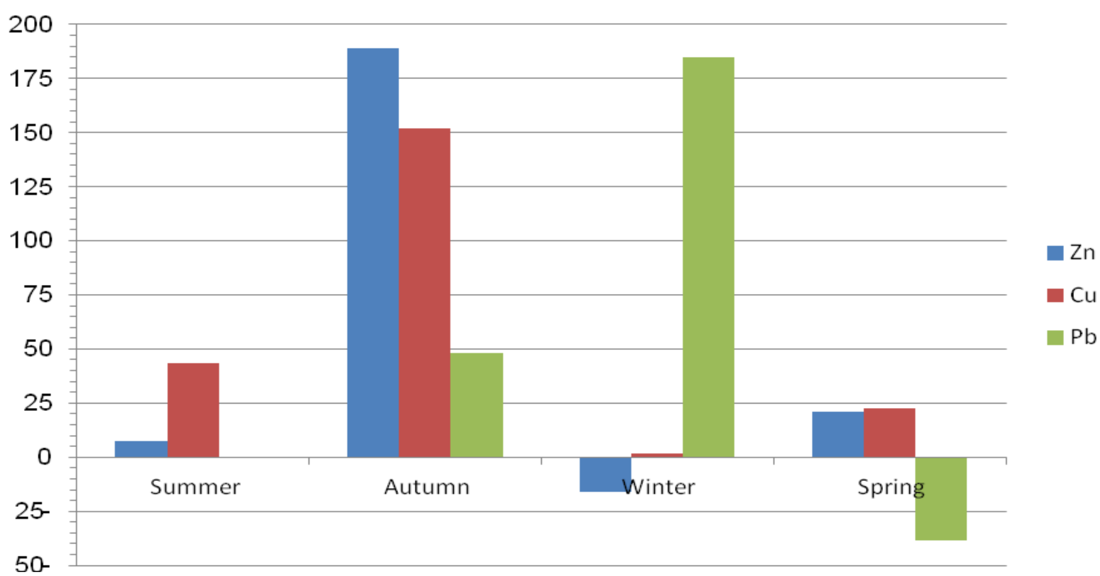


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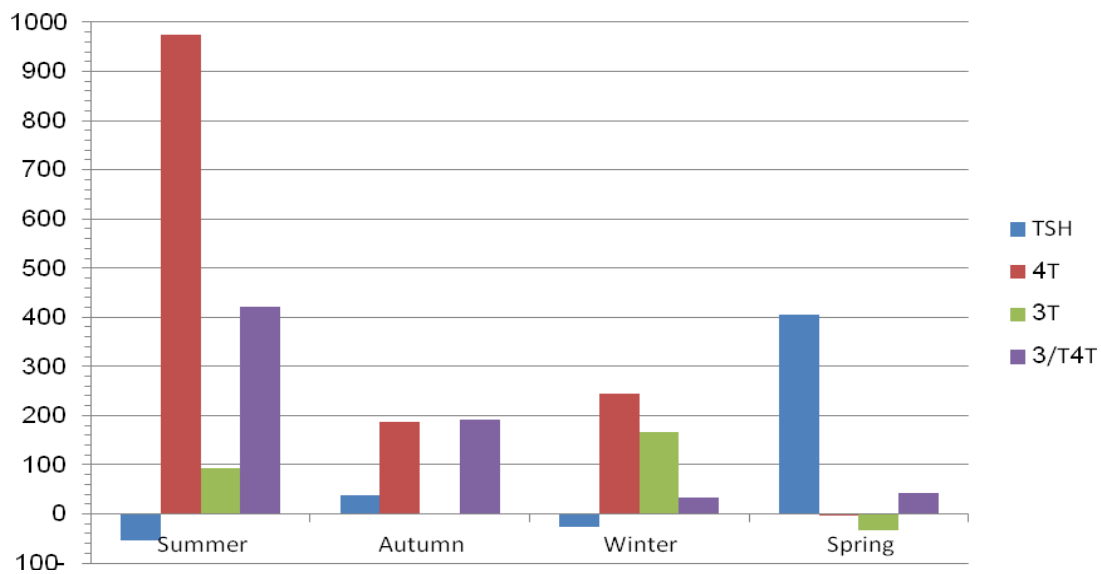
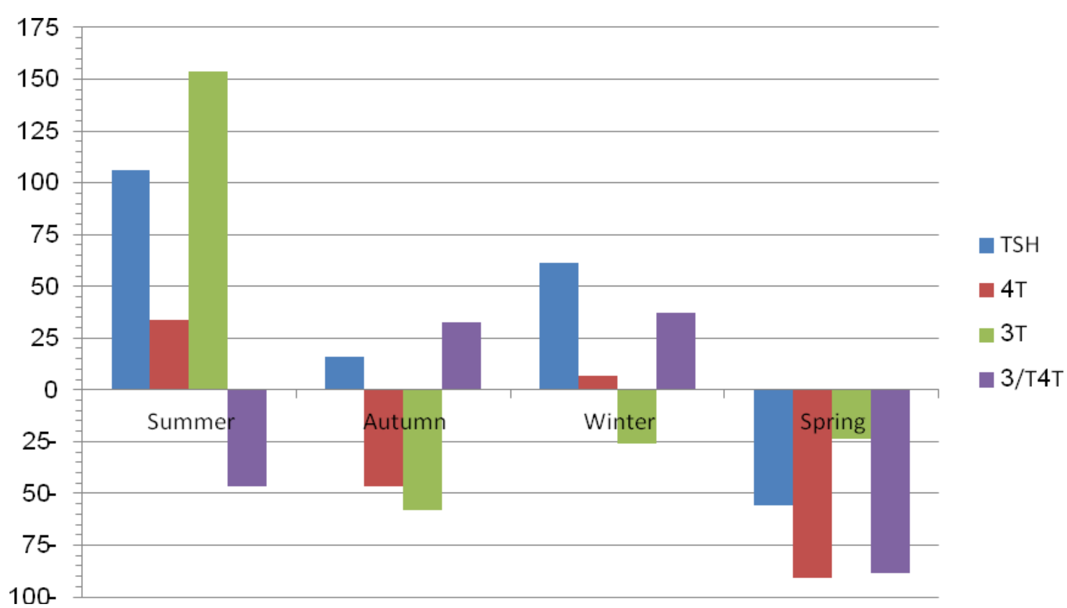
Fig. (3): The percentage change in the serum thyroid stimulating hormone (TSH); thyroxin (T_4) and triiodothyronine(T_3) concentrations and T_4/ T_3ratio of *Clarias gariepinus* from Idku Lake (site II) relative to River Nile – Banha (site I) during the four seasons.

A- Male:



B- Female:



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