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Environmental and physiological impacts of heavy metals on Nile tilapia (Oreochromis niloticus)

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Abstract

The present study was conducted to investigate the distribution of selected metals (Fe. Zn. Cd. Pb. and Cu) in tissues of Nile Tilapia (Oreochromis niloticus) grown along El- Khadrawia drain - Mubark industrial Zone. The tissues analyzed included muscle and liver. Results showed metal concentrations in water followed a richness of: Fe> Zn>Cu>Pb>Cd mg/l (p < 0.05) while Fe> Cu>Zn>Pb> Cd mg/Kg dry wt. (p < 0.05) in muscles and liver tissues in winter season. Metal levels in muscles follow the level: dry wt. (p < 0.05) in summer season. The presence of heavy metals led to significant decrease in nucleic acids (DNA & (p<0.05). So, the study showed that muscles of Nile Tilapia 2013). (Oreochromis niloticus) along El- Khadrawia drain are not safe from contamination with the metals investigated.

Keywords. Heavy metals, Nile tilapia, Liver, Muscle, DNA&RNA

1 Introduction

Industrial development and increase of urbanization resulted in rapid increase of domestic and industrial wastewater which has intensify environmental pollution in different environmental compartments. Industrial and agricultural runoffs are considered the primary source of metal poisoning to fish and other aquatic animals in Egypt (Eissa et al., 2009 and Eissa et al., 2013). Fish are excellent organisms to study different effects of pollutants that cause genotoxicity through their aquatic environments (Koca et al., 2008). Heavy metals are non-biodegradable and persistent contaminants in the environment causing serious illness in fish, animals and human. Heavy metals after entering into aquatic environment accumulate in tissues and organs of fish that enter the food chain and elevate up to the highest level of consumers (Akan et al., 2009). They

accumulated in aquatic organisms as an indicator of metal pollution (Karadede-Akin and Unlu, 2007) and affected them by changing the structural or biological functions of bio-molecules (Newman, 1998).

Prolonged exposure to water collected pollutants even in very low concentrations have been reported to induce morphological, histological and biochemical alterations in fish tissues, which may critically influence the quality and marketability of fishes (Kaoud and El-Dahshan, 2010). However, their persistence in the aquatic environment is surely related to the success of their immune system to Zn>Fe>Cu> Pb>Cd mg/Kg dry wt. (p < 0.05), while in counteract such impacts (Tort et al., 2003). It is also liver they follow the level: Fe > Zn > Cu>Pb>Cd mg/Kg possible that environmental toxicants may increase the susceptibility of aquatic animals to various diseases by interfering with the normal functioning of their immune, RNA) contents in liver tissue during summer season reproductive and developmental processes (Eissa et al.,

> Nile Tilapia (Oreochromis niloticus) is a teleost fish with a worldwide distribution; therefore it is a good model for assessing the impacts of different environmental pollutants on aquatic ecosystems. A comparative study of five economically important taxa of tilapia showed that Nile Tilapia (Oreochromis niloticus) presents a God gifted strong immune system that maximizes their capability to tolerate biotic and abiotic types of stress (Eissa et al., 2012). Further, the natural surface feeder omnivorous non predator behavior of Nile tilapia might grant them another natural tool that minimizes their possibility of contracting numerous types of pollutants including biological/chemical forms when compared to bottom feeder fishes (Eissa et al., 2013).

> Biomarkers such as DNA and RNA for water pollution are considered as early diagnostic tools for biological effect measurement and environmental quality assessment (Peakall, 1994). They are defined as a change in biological response that differs from molecular to organism level (Sanchez and Porcher, 2009). Therefore, this work aimed to measure heavy metals pollution rank in surface water and

Nile Tilapia (Oreochromis niloticus) along El- Khadrawia summer 2013 seasons from two sites that were: nondrain - Mubark industrial Zone.

2 Materials and Methods

The industrial wastewater had many negative environmental impacts on El- Khadrawia drainage water, aquatic environment organisms and others environmental components. Many physiological determinations were carried out to evaluate negative impact of industrial wastewater on El- Khadrawia drainage water quality and fish according standards quality.

Study Area

Khadrawia drain (between 19,600 to 29km of El-Khadrawia drain) at the intersection of the drain with the Cairo-Alexandria agricultural road as shown in Figure (1).



Fig. (1) Schematic Diagram for Mubarak Industrial Zone & El-Khadrawia Drain

El-Khadrawia drain starts in the Menoufiya governorate passing centers including Brket El-Saba, Quesna, and ends in the Western Province center Zifta. The length of El-Khadrawia drain is 30 km, and has an average disposition of the drain at the downstream 4.5 m³ / s (about 400,000 m³/day).

The El- Sahl canal in the east and El- Khadrawia canal in the west use large quantities of drain water with (El- Atf, Dhtorah and El- Karaneen) drains to mix with Abbasi Rayah water. This mixing compensate the shortage of irrigation water in periods of maximum agricultural needs deionized water was used for all the prepared reagent through the station of Menoufiya pumps and estimated annual energy quantities confused 57 million m³.

Sampling Program

Water and Nile Tilapia fish (Oreochromis niloticus) samples were collected during one year. The selection of sites was chosen to represent non-polluted area as control sample and polluted area (Mubarak Industrial Zone) wastewater effect on El- Khadrawia drain as shown in samples was determined gravimetrically at 105°C. Table (1).

Table (1). **Environmental** samples at Khadrawia Drain

No.	Code	Sampling sites			
1	A	Non-polluted site, River Nile - El-			
		Kanater El- Khairyia			
2	В	Drainage water before El-Khadrawia			
		drain with about 3 km.			

Water Samples

Water samples were collected in triplicates in various containers specialized to uniform the nature of tested parameters according to international standard methods for examination of water and wastewater (APHA, 2005). Water samples were collected through winter 2012 and

polluted area - River Nile - El- Kanater El- Khairyia as control sample (A) and drainage water before El-Khadrawia drain with about 3 km (B) (Table 1) to evaluate water characterization chemically, bacteriology and toxic pollutants impacts on biological indicator Nile tilapia, Oreochromis niloticus.

For laboratory analyses, water samples were collected in stopper polyethylene plastic bottles. All collected and examined samples, for physical, chemical biological quality on Nile tilapia, Oreochromis niloticus bacteriological were stored in an iced cooler box and delivered immediately to the laboratory for analyses. The plastic bottles were cleaned by soaking in 10% HNO3 and Mubarak Industrial Zone is located on the left El- the procedural blanks of standard solutions were prepared under clean laboratory environment.

Fish Samples

Fish samples (Oreochromis niloticus) were collected from two sites and transported to laboratory for dissection and analysis (Table 1). Nile tilapia (non- polluted samples) ranged with average weight 118.45 to 330.3 kg and the length from 18.4 to 28.8 cm. While fish samples collected from polluted area ranged with average weight from 70.06 to 246.94 kg, and the length from 15.2 to 25.2 cm. After dissection of fish, pieces from liver by digestion method of Oreochromis niloticus were taken and kept frozen at -20 °C for heavy metals analysis.

Analytical Procedures

In situ, field parameters: Temperature °C, pH, Electric conductivity (E.C), Salinity, Total dissolved solids (TDS) and dissolved oxygen (D.O) were measured in situ using multi-probe system, model Hydralab-Surveyor, and remeasured in laboratory using the following bench top equipment's to ensure data accuracy (APHA, 2005).

Laboratory analysis (APHA, 2005): for laboratory analysis, all reagents were used for analytical grade and solutions. Physico-chemical analyses including pH and EC were measured at 25°C using Info Lab meters, ammonium content using ammonia selective electrode ORION model 95-12 and checked by calorimetric techniques with formation of phenate. Also, turbidity (Turb) was measured by Turbidity meter with calibration solutions of 0.1, 15, and 100 NTU, Total dissolved solids (TDS) for filtrated water

El- Chemical analysis: Heavy metals (Cd, Cu, Fe, Pb, and Zn), the samples were filtered by filtration system through membrane filter of pore size 0.45μ and acidified with nitric acid to pH and were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) and their recovery studies for the trace elements ranged between 99 and 102%.

Fish samples: fish extracts for heavy metal determination were prepared by mixing 0.5 g of dry fish tissues (liver) for 24 hours at 70 °C with 6ml concentrated 65% HNO₃ and 1ml 30% hydrogen peroxide (H₂O₂) for digestion using microwave digestion system (model Milestone, MLS-1200 mega, Germany) then ventilated for 5 min and filtrated to 100 ml by using deionized water (DI). Three replications were performed for each fish sample. Nucleic acids contents DNA (deoxy ribonucleic acids) and RNA (ribonucleic acids) in liver were measured as described by Alexander et al., (1985).

Statistical analysis

Data were analyzed by analysis of variance using ANOVA Procedure, SAS System, Version 2004. All data is analyzed and presented as mean ± standard error.

3 Results

The minimum value of temperature recorded in winter was 17.8 °C in the River Nile and the maximum value recorded in the summer was 32.63 °C in polluted site (B). Table (2) represented significant changes at (P < 0.05) along polluted site B (drainage water before El-Khadrawia drain with about 3 km) in the winter and summer seasons.

pH values in non-polluted (A) and polluted (B) sites varied from 7.18 ± 0.012 to 7.87 ± 0.06 . The results recorded non-significant changes (P > 0.05) at polluted site (B) in the winter (7.18 \pm 0.012) and summer (7.46 \pm 0.08) as compared with non-polluted site (A).

E.C values in non-polluted (A) and polluted (B) sites varied from 0.361 ± 0.002 to 2.06 ± 0.03 . The results recorded significant changes (P < 0.05) at polluted site (B) in the winter (2.06 ± 0.03) and summer (1.418 ± 0.006) as compared with non-polluted site (A). In similar, salinity values ranged from 0.19 ± 0.011 to 1.09 ± 0.06 in non-polluted (A) and polluted (B) sites which noticed significant changes (P < 0.05) at polluted site (B) in winter and summer seasons in comparison with non-polluted site (A). Also, the results of TDS ranged from 232 ± 0.55 mg/l to 1318 ± 0.58 mg/l in non-polluted (A) and polluted (B) sites which showed highly significant increase (P < 0.05) during winter and summer seasons as compared with non-polluted site (A).

The minimum value of dissolved oxygen was 2.56 ± 0.13 mg/l in winter and 2.98 ± 0.03 in summer for polluted site (B) while the maximum value of dissolved oxygen was 3.66 ± 0.05 in summer and 3.33 ± 0.021 mg/l in winter for non-polluted site (A). The results clearly showed significant changes (P < 0.05) at polluted site (B) in winter and summer seasons as compared with non-polluted site (A).

Seasonal variations of turbidity in non-polluted (A) and polluted (B) sites varied from 0.14 ± 0.01 to 38 ± 0.23 which showed significant changes (P < 0.05) in winter and summer seasons as compared with non-polluted site (A).

Table (2) showed that ammonia concentrations ranged from 0.95 ± 0.005 to 2.11 ± 0.04 mg/l that were significantly different (P < 0.05) for polluted site (B) in winter season and non-significant in the summer season as compared with non-polluted site (A).

Table (2). Water Characterization of Non-polluted area and Polluted Area

Environmental Samples								
Physic-	Wi	nter	Summer					
Chemical	A	В	A	В				
Measurement								
S								
Temperature °C	17.8 ± 0.03	18.8 ± 0.06 *	30.43 ± 0.12	31.73 ± 0.28*				
pН	7.87 ± 0.06	7.18 ± 0.012	7.58 ± 0.017	7.46 ± 0.08				
E.C ds/m	0.434 ± 0.001	2.06 ± 0.03 *	0.361±0.002	1.418 ± 0.006*				
Salinity	0.22 ± 0.006	1.09 ± 0.06 *	0.19 ± 0.011	0.8 ± 0.02 *				
D.O (mg/l)	6.33 ± 0.021	2.56 ± 0.13 *	6.66 ±0.05	$2.98 \pm 0.03^*$				
TDS (mg/l)	280 ± 0.74	1318 ± 0.58 *	232 ± 0.55	908 ± 0 36 *				
<1000								
Turbidity	7 ± 1	38 ± 0 23 *	0.14 ± 0.01	6.8 ± 0.49 *				
(NTU)	7 – 1	30 - 0.23	0.11 = 0.01	0.0 = 0.17				
Ammonia	0.05 ± 0.005	2.11 ± 0.04 *	0.01	0.26 ± 0.003				
(mg/l)			****					
	Each value represents Mean \pm S.E. (*) P < 0.05 compared with non-polluted site (A).							
: D.O≥5 mg/l, TDS<1000 mg/l								

D.O mg/l

Limits

A

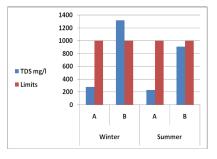
B

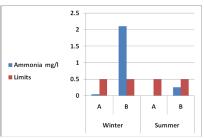
A

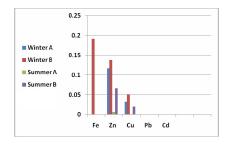
B

Winter

Summer







The values of iron concentrations in water samples of non-polluted and polluted sites (A) and (B) were observed in table 3. These results exhibited significant changes (p < 0.05) along polluted site (B) during winter season and <0.01mg/l in summer season in comparison with non-polluted site (A).

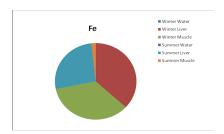
Zinc concentrations in water samples of non-polluted and polluted sites A and B were observed in table 3. It is apparent that zinc concentrations were significantly elevated (p < 0.05) at polluted site (B) during winter and summer seasons in comparison with non-polluted site (A).

Copper content in water samples of non-polluted and polluted sites A and B were observed in table 3. From these results, it is apparent that copper concentrations were significantly elevated (p < 0.05) at polluted site (B) during winter and summer seasons in comparison with non-polluted site (A).

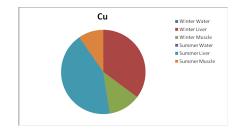
Table 3 clarified lead and cadmium concentrations that were measured in water samples collected from non-polluted and polluted sites (A and B). These results recorded under detection limits of ICP instruments (<0.005 and <0.0005 mg/l, respectively) during winter and summer seasons.

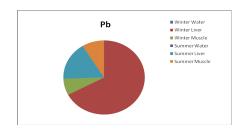
Table (3). Heavy Metals Concentrations (mg/l) in Water Samples along Non-polluted area and Polluted Area

Environmental Samples							
Heavy Metals	Wi	nter	Summer				
	A	В	A	В			
Iron - Fe	< 0.01	$0.192 \pm 0.001*$	< 0.01	< 0.01			
Zinc - Zn	0.117 ± 0.0012	$0.138 \pm 0.001*$	0.006 ± 0.0006	$0.067 \pm 0.0015*$			
Copper - Cu	0.033 ± 0.0012	0.051 ± 0.0007	< 0.002	$0.02 \pm 0.001*$			
Lead- Pb	< 0.005	< 0.005	< 0.005	< 0.005			
Cadmium- Cd	< 0.0005	< 0.0005	< 0.0005	< 0.0005			
Each value represents mean \pm S.E. (*) P < 0.05 compared with non-polluted site (A)							
Detection Limits for ICP Instrument: Fe: <0.01, Cu: <0.002, Pb: <0.005, Cd: <0.0005 mg/l							
Egyptian Environmental Law 48-decision 92/2013 (mg/l): Fe<3, Zn<2, Cu<1, Ph<0.5 and Cd<0.003							









The seasonal variations in the concentrations of trace metals that were Fe, Zn, Cu, Pb and Cd in different organs: liver and muscles fish of *Oreochromis niloticus* collected from the River Nile (non-polluted site – A) and El-Khadrawia drain (polluted site – B). All data of heavy metals accumulations in tissues (liver and muscles - mg/kg dry wt.) recorded in table 4.

The values of iron concentrations in the muscles of fish samples *Oreochromis niloticus* showed that highly significant increase (p < 0.05) at polluted site (B) during winter and summer seasons in comparison with non-polluted site (A). The iron concentrations in muscles reached the value of 61.908 ± 0.51 mg/kg dry wt. in summer to 1264.52 ± 0.51 mg/kg dry wt. in winter. While, the iron concentrations in the liver showed highly significant increase (p < 0.05) at polluted site (B) during the two seasons winter and summer in comparison with non-polluted site (A). The iron concentrations in liver reached the quantity of 961.197 ± 0.55 mg/kg dry wt. in summer to 1332.53 ± 0.5 mg/kg dry wt. in winter.

Zinc concentrations in the muscles of fish samples *Oreochromis niloticus* were highly significant increase at (p < 0.05) in polluted site (B) during winter and summer seasons in comparison with non-polluted site (A). Zinc concentrations in muscles reached the value of 55.469 \pm 1.18 mg/kg dry wt. in winter to 135.73 \pm 0.12 mg/kg dry wt. in summer; while, the concentrations of zinc in the liver of fish samples *Oreochromis niloticus* were highly significantly increased (p < 0.05) at polluted site (B) during winter and summer seasons in comparison with non-polluted site (A). Zinc concentrations in liver reach the value of 115.62 \pm 0.31 mg/kg dry wt. in winter to 273.22 \pm 0.48 mg/kg dry wt. in summer.

Copper content in the muscles and liver of fish samples *Oreochromis niloticus* were highly significant increase (p < 0.05) at polluted site (B) during winter and summer seasons in comparison with non-polluted site (A). Copper concentrations in muscles reached the quantity of 54.207 ± 0.4 mg/kg dry wt. in summer to 69.772 ± 0.48 mg/kg dry

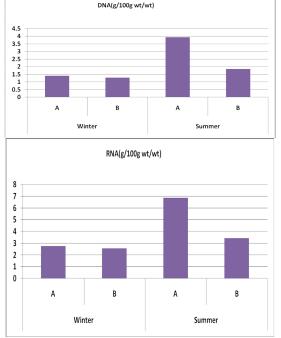
reached the value of 197.85 ± 0.49 mg/kg dry wt. in winter to 241.057 ± 1.13 mg/kg dry wt. in summer season.

The concentrations of lead in muscles and liver of fish samples *Oreochromis niloticus* showed highly significant changes (P < 0.05) at polluted site (B) during winter and summer in comparison with non-polluted site (A). Lead concentrations in muscles reached the value of 0.968 ± 0.02 mg/kg dry wt. in winter to 1.2 ± 0.04 mg/kg dry wt. in summer season while lead concentrations in liver reached the value of 2.259 ± 0.03 mg/kg dry wt. in summer to 9.102 in non-polluted and polluted Area \pm 0.1 mg/kg dry wt. in winter season.

Cadmium content in the muscles and liver of fish samples Oreochromis niloticus recorded <0.0005 mg/kg dry wt in non-polluted site (A) and polluted site (B) during winter and summer seasons.

Table (4). Heavy metals concentrations (mg/kg dry wt.) in fish tissues along non-polluted area and polluted area

Heavy Metals	Winter				Summer			
	A		В		A		В	
	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
Fe	22.47 ± 0.22	13.053 ± 0.3	1332.53 ± 0.5 *	1264.52 ± 0.51 *	17.646 ± 0.5	14.231 ± 0.41	961.197 ± 0.55 *	61.908 ± 0.51*
Zn	34.56 ± 0.42	25.564 ± 0.33	115.62 ± 0.31 *	55.469 ±1.18 *	36.583 ± 0.47	9.727 ± 0.37	273.22 ± 0.48 *	135.73 ± 0.12 °
Cu	27.814 ± 0.51	22.562 ± 0.49	197.85 ± 0.49 *	69.772 ± 0.48 *	20.828 ± 0.51	5.444 ± 0.04	241.057 ± 1.13 *	54.207 ± 0.4*
Pb	0.425 ± 0.0007	0.268 ± 0.01	9.102 ± 0.1 *	0.968 ± 0.02 *	0.297 ± 0.04	0.035 ± 0.03	2.259 ± 0.03 *	1.2 ± 0.04 *
Cd	<0.00 05	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	< 0.0005



Water pollution in El- Khadrawia drain caused variation of Nucleic acids content in the liver for Nile Tilapia, Oreochromis niloticus fish. The results clarified DNA (deoxy ribonucleic acids) was significant decrease changes (P < 0.05) during summer at polluted site (B) in comparison with non-polluted site (A). DNA (deoxy ribonucleic acids) concentrations (g/100g wt/wt) in liver reached of 1.285 ±

wt. in winter season while copper concentrations in liver 0.11 g/100 g wt/wt in winter to 1.844 ± 0.05 in summer as comparison with non-polluted site (A).

> It was apparent that RNA (ribonucleic acids) content showed significant decrease (P < 0.05) during the summer in comparison with non-polluted site (A). The maximum decrease RNA (ribonucleic acids) concentrations (g/100g wt/wt) in liver reached of 2.582 ± 0.22 g/100g wt/wt in winter to 3.429 ± 0.25 g/100g wt/wt in summer as comparison with non-polluted site (A).

Table (5). Nucleic acids content in Liver tissue of fish

Environmental Samples							
Nucleic	Winter		Summer				
acids content	A	В	A	В			
DNA (g/100g	1.409	1.285	3.921	1.844 ±			
wt/wt)	± 0.08	± 0.11	± 0.44	0.05 *			
RNA (g/100g	2.749	2.582	6.884	3.429 ±			
wt/wt)	± 0.24	± 0.22	± 0.38	0.25 *			
Each value represents Mean \pm S.E, n=10. (*) P < 0.05 compared with non-polluted site (A)							

4 Discussion

The results of trace elements in water and fish samples were compared with Egyptian Environmental Law 48-Decision 92/2013 and FAO 1983 to evaluate certain food additives and contaminates.

Soluble species of heavy metals are dangerous to fish in natural water that affected fish tissues by external and internal factors. High metal concentrations in aquatic environment caused high accumulation metals in Nile Tilipia Oreochromis niloticus. Metal concentrations in water of polluted site (B) as compared with non-polluted site (A) followed a richness of: Fe> Zn> Cu> Pb >Cd mg/l while Fe> Cu > Zn > Pb > Cd mg/Kg dry wt. in muscles and liver tissues in winter season. Metal levels in muscles follow the level: Zn>Fe>Cu> Pb>Cd mg/Kg dry wt., while in liver follow the level: Fe > Zn > Cu>Pb>Cd mg/Kg dry wt. in summer season.

The presence of heavy metals in El- Khadrawia drain was mainly of allochthonous origin of industrial wastewater, drainage water and sewage wastewater. The obtained mean values of all heavy metals in water not exceeded Egyptian Environmental Law 48-Decision 92/2013 in winter and summer seasons. While, all metals in fish tissues (muscles and liver) exceeded FAO, 1983 limits except Cd (<0.0005 mg/kg dry wt).

The higher heavy metals concentrations were found in liver and muscles of Nile Tilipia Oreochromis niloticus fish tissues more than the aquatic environment (El- Khadrawia drain). Nile Tilipia Oreochromis niloticus fish adsorb soluble heavy metals causing accumulation in liver and muscles with highly significant quantities, thus showing caused toxicological effects (Osman et al., 2009).

Trace elements in fish tissues were always higher than water that confirmed with Chale, 2002, Abumourad et al., and Fowler, S.W. (2003): Relative importance of dissolved 2014 and Riani, 2015 may be due to the variety of occurrences on fish body and other aquatic biota, including the regular diffusion, bio-magnifications, and bioconcentration (Boisson et al., 2003). The lowest concentrations of metals were found in muscle tissues than liver (Yacoub and Gad, 2012); this may due to the little blood supply to the muscular tissues (Kalkan, et al., 2015) and related to lower metabolic activities of muscle. The lowest concentrations of metals were found in muscle tissues than liver and brain; this may due to the little blood supply to the muscular tissues (Kalkan, et al., 2015) and related to lower metabolic activities of muscle. Liver is the main centre for various metabolisms where detoxification and drug metabolism take place which makes it greatly vulnerable to damage by toxic substances (Čelechovská et

The presence of heavy metals in El- Khadrawia drain (site B) led to significant decrease in nucleic acids (DNA & RNA) contents of Nile Tilipia Oreochromis niloticus fish tissues (liver) during summer season in comparison to nonpolluted (site A). The data were similar to those of Yousafzai and Shakoori, (2011) for freshwater fish, Tor putitora samples caught from River Kabul and Habib and Field assessment of the mid winter mass kills of trophic Abou Shehatta, (2013) for catfish, Clarias gariepinus of Sites Kafr El-Bateekh and Talkha. Significant decrease in nucleic acids contents may be due to proteolysis and 2008) or may be due to its utilization to mitigate the energy demand when the fish are under stress (Venkataramana et al., 2006). DNA damage of Nile tilapia Oreochromis niloticus fish occurred due to exposure to agricultural and industrial wastewater (Lima et al., 2006 and Farag et al., 2006).

Conclusion

The present study was done through the research plan of the National Institute of Oceanography and Fisheries, Cairo, Egypt and National Water Research Center (NWRC) - Center Laboratory Environmental Monitoring Quality (CLEQM). In order to gain information's about the environmental status of industrial wastewater quality that discharges in drains and River Nile streams and their negative impacts on aquatic organisms, especially fresh water Nile Tilapia, Oreochromis niloticus.

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