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Bioaccumulation of heavy metals and their histopathological impact on muscles of *Clarias gariepinus* from El-Rahawy drain, Egypt

Seham A. Ibrahim[1]; Mohammad M. N. Authman[2*]; Hanan S. Gaber[3] and Midhat A. El-Kasheif[3]
1- Department of Zoology, Faculty of Science, Benha University, Benha, Egypt.
2- Department of Hydrobiology, National Research Centre, Dokki 12622, Giza, Egypt.
3- National Institute of Oceanography and Fisheries, Cairo, Egypt.

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ABSTRACT
Some physico-chemical parameters and heavy metals (Cu, Fe, Pb, Cd, Mn and Zn) concentrations were studied in the water and muscles of the African catfish *Clarias gariepinus* collected seasonally during the period from winter 2010 to autumn 2011 from El-Rahawy drain and River Nile at El- Kanater El-Khyria to assess the effects of water pollution upon fish and human health. Moreover, biochemical and histological changes of the muscles of the same fish species were studied. The results revealed depletion in dissolved oxygen and transparency and increasing in the levels of ammonia, nitrite, nitrate and heavy metals in water and muscles of the samples collected from El-Rahawy drain. In comparison, *C. gariepinus* fish collected from El-Rahawy drain exhibited higher levels of ash and water content and lower levels of total protein and total lipids content in muscles. Several histopathological alterations and some parasites cysts in the muscles of fish collected from El-Rahawy drain were found. It could be concluded that, *C. gariepinus* fish; inhabiting El-Rahawy drain; were found to accumulate high concentrations of heavy metals in their muscles and its meat quality is deteriorated to the point that it could be hazardous to humans. So, a recommendation is given for treatment of wastewaters, especially sewage wastes before discharging into the natural water bodies to protect the fish and the people from the dangerous effects of such pollution.

1. INTRODUCTION
Anthropogenic disturbances lead to the deterioration of water quality and are the main threat to aquatic fauna worldwide. In particular, indiscriminate disposal of sewerage, industrial waste and plethora of human activities that has not been appropriately treated is the main cause of this deterioration (Maceda-Veiga *et al.*, 2012) as in El-Rahawy drain, Egypt.

Corresponding Author: E-mail: mmauthman@yahoo.com
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El-Rahawy drain is one of the main drains, which is far from El-Kanater (The Barrage) by about 15 km. It starts at Rahawy Pump Station on Mansouria Rayah, lies at 30 Km, North to Cairo at El-Kanater El-Khayria area, Egypt. El-Rahawy drain lies between latitudes 30º 10’ N to 30º 12’ N and longitudes 31º 2’ E to 31º 3’ E. It is about 12.41 km^2. It passes through El-Rahway village and many villages distributed along it receiving agricultural and domestic wastes without treatment, in addition to sewage of El-Giza governorate that discharged directly into Rosetta branch of the River Nile (El Bourie *et al.*, 2011). The drain is surrounded by high density of population area and wide agricultural lands. The surface level of the drain is 12.37 m above sea level. This drain receives wastewater from El-Moheet drain that passes by a deep under El-Nassery sub-branch of the River Nile to open into a concrete reservoir of about 20m high at El-Rahawy drain. From this reservoir, the drainage wastewater runs to about 4 km through El-Rahawy village and opens into Rosetta branch.

The urban sewage and industrial wastewater contain a mixture of pollutants such as drugs, household products and heavy metals (Pinto, 2009). Also, sewage contains a large proportion of organic matter and nutrients as well as numerous microorganisms (bacteria and viruses) and parasitic worms (Tayel *et al*., 2007). This led to increase microorganisms and parasites contamination in the water system, hydrophytes and fish.

Among these toxic substances, heavy metals are one of the greatest threats to biota because of their persistence, and possible bioaccumulation and biomagnification in food chains (Uysal *et al*., 2009; Ricart *et al.*, 2010). Other sources of metal pollution are direct atmospheric deposition, geologic weathering and the discharge of agricultural waste products (Demirak *et al.*, 2006; Uysal *et al.*, 2009). Although the presence of metals in the environment may also have a natural origin, most of the heavy metals in water bodies come from anthropogenic activities (Boscher *et al.*, 2010; Ricart *et al.*, 2010).

Heavy metals such as copper, iron, chromium and nickel are essential metals since they play an important role in biological systems, where cadmium and lead are non-essential metals, as they are toxic, even in trace amounts (El-Naggar *et al.*, 2009). Heavy metal concentrations in aquatic ecosystems are usually monitored by measuring their concentrations in water, sediments and biota (Lasheen *et al.*, 2012) which generally exist in low levels in water and attain considerable concentration in sediments and biota (Rashed, 2001).

Water analyses may be inefficient to identify metal inputs to fluvial systems because of the inherent variability of flow and contaminant concentrations (Ricart *et al.*, 2010). With this regard, fish can be considered as one of the most significant indicators in freshwater systems for the impact of metal pollution (Lasheen *et al.*, 2012) because they occupy various trophic levels, and are the key species in trophic chains, and concentrate large amounts of some metals, and some are widely consumed by humans or wild predators (Gupta *et al.*, 2009; Uysal *et al.*, 2009; Barata *et al.*, 2010).

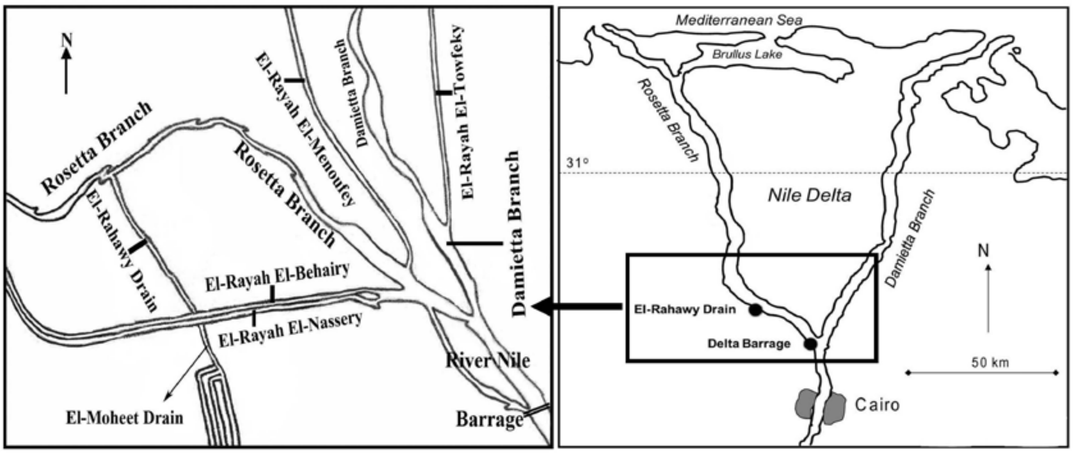
Fish muscles are commonly analyzed to determine contaminant concentrations, and heavy metals have been quantified in muscle tissues from a variety of fish species (e.g. Andreji *et al.*, 2006; Soegianto and Hamami, 2007). These studies have been done for various reasons; many of them concerning food safety and public health interests where muscle tissues are generally the major edible portion of the fish (Ashoka *et al.*, 2011). Moreover, bioaccumulation of heavy metals in fish critically influences the growth rate, physiological and biochemical status and consequently the meat quality of fish (Haggag *et al.*, 1999; Elghobashy *et al.*, 2001).

In addition, histopathological alterations can also be used as indicators for the effects of various anthropogenic pollutants on aquatic biota and are a reflection of the overall health of the entire population in the ecosystem (Mohamed, 2009; El-Bakary *et al.*, 2011). Degenerative changes in muscular tissues have been reported as symptoms of exposure to environmental contaminants such as pesticides or metals (Wang *et al.*, 2004; Koca *et al.*, 2005). So, histological changes in fish muscular tissue might therefore be a reliable indicator of water pollution.

The overall objective of this study is to evaluate the impact of wastewater effluents on the water quality of El-Rahawy drain, Nile delta, Egypt, and its effect on levels of heavy metals in muscles of the freshwater fish *Clarias gariepinus* inhabiting this drain. In addition, it is focused on some chemical parameters and histopathological lesions of *C. gariepinus* muscles.

2. MATERIALS AND METHODS

The present study was conducted during the period from winter 2010 to autumn 2011, for four successive seasons. Two sites (Map 1) were selected to carryout the present study; the first one was located in River Nile at Delta Barrage in front of El-Kanater El-Khayria City (used as a reference point) and the second was selected in El-Rahawy drain at El-Rahawy village. Samples were collected from River Nile at Delta Barrage and different locations of El-Rahawy drain to represent the drain ecosystem.



Map 1**:** The study area at El-Rahawy drain and River Nile at Barrage.

2.1. I- Water samples collection and analysis

Sampling, preservation and experimental procedure of the water samples were carried out according to the standard methods for examination of water and wastewater (APHA, 1998).

2.1. I. a) Field observations

In situ, air and surface water temperatures (ºC) were measured by a dry mercury thermometer, transparency (cm) by Secchi disc, electrical conductivity (EC)(µmohs/cm) by using conductivity meter model (S.C.T. 33 YSI) and hydrogen ion concentration (pH) by Orion Research Ion Analyzer 399A pH meter.

Water samples were collected at 60 cm depth from different sites (10 samples/ season); using polyvinyl chloride Van Dorn plastic bottles (1.5 liter capacity). For trace elements analysis, water samples were collected in one-liter plastic bottles, and preserved with 5 ml concentrated nitric acid on the spot and stored in refrigerator (APHA, 1998). One-liter plastic bottles were also filled with water samples for undertaking the rest of chemical analysis. The samples were preserved in an icebox and returned immediately to the laboratory.

2.1. I. b) Laboratory analysis

Dissolved oxygen was measured using the modified Winkler method, and biochemical oxygen demand (BOD) was determined with the 5-days incubation method. Concentration of ammonia, nitrite and nitrate were determined by using the colorimetric techniques. All previous analyses were carried out according to the standard methods for examination of water and wastewater (APHA, 1998).

Heavy metals (copper, iron, lead, cadmium, manganese and zinc) in water samples were determined using atomic absorption spectrometry (Perkin-Elmer 3110, USA) with graphite atomizer HGA-600, after using the digestion technique by nitric acid (APHA, 1998).

2. 2. II-Fish samples collection and analysis

Samples of African catfish *Clarias gariepinus* were collected seasonally (30 fish/ season) from each site. The fishes were transposed alive back after catching to the laboratory for subsequent analysis. In the laboratory, the total length and total weight for each fish were recorded. Fish total length and total weight were from 250 to 440 mm and from 290 to 500 g, respectively.

2. 2. II. a) Heavy metals analysis in muscles

After the dissection of fish samples, parts of dorsal muscles were taken and stored in a deep freezer (-20°C) until processing for metal analysis. Tissue samples were digested using HNO_3 (4 ml per gram tissue) at 70°C on a hot plate until NO_2 evaporation ceased (Chernoff, 1975). A volume of reagent grade 10% H_2O_2 equal to the initial HNO_3 was added to the digested samples until the sample becomes clear and then allowed to cool to ambient temperature. After cooling, the solution was filtered and the filtrate made up to a known volume (100 ml) with distilled water. The samples were stored cool at 4°C till metals analyzed. Levels of metals in samples were measured by atomic absorption spectrophotometry (Perkin-Elmer 3110, USA) with graphite atomizer HGA-600. The results were expressed as µg/g wet weight of the tissue.

2. 2. II. b) Accumulation factor (AF)

The accumulation factor (AF) is the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in water. It gives an indication about the accumulation efficiency for any particular pollutant in any fish organ. AF was calculated using the following equation (Authman and Abbas, 2007):

AF = Pollutant concentration in fish muscle µg g^{-1}/ Pollutant concentration in water µg l^{-1}

2. 2. II. c) Muscle chemical composition (Meat quality)

After the dissection of fish samples, muscle samples were taken and muscle chemical composition parameters were determined. Muscle water content (moisture) was determined according to Sidwell *et al.* (1970) where muscle samples rapidly transferred directly to weighing bottles and accurately weighed. The bottles were then placed in drying oven thermostatically regulated at 105°C for 72 hours. The loss in weight was taken as equivalent to the weight of water content (moisture) of the samples. Muscle total protein content was determined using the semi-microkjeldahl method as reported by Josyln (1950). Muscle total lipids content was determined by the standard method reported in AOAC (1980) where extraction was carried out in Soxhlet apparatus using petroleum ether. Muscle ash was determined by heating samples in a muffle furnace at 200°C for two hours, then the temperature was raised gradually every two hours to reach 550-600°C (Sidwell *et al.*, 1970). The samples were left in the furnace at this temperature for 6 hours. The weight of the residue represents the ash content.

2. 2. II. d) Histopathological examination

Other part of muscles from each fish sample were carefully removed and fixed in Bouin's solution for 24 hr and washed with 70% alcohol. The tissues were routinely dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 4-6 µm thick were cut, processed and stained with hematoxylin and eosin (H&E). They were examined according to Roberts (2012) by a complex Olympus light microscopy and photographed by a built in camera.

3. Statistical analysis

The basic statistics, means and standard deviations of the measured parameters were estimated. Pearson's correlation coefficients matrix among the different parameters was computed as well. Comparison of muscle chemical composition data were statistically analyzed using one way analysis of variance (ANOVA) test. All statistical analyses were done, using the computer program of SPSS Inc. (version 17.0 for Windows) at the 0.05 level of significance.

3. RESULTS

3.1. A) Physico-chemical Parameters

Table (1) shows the mean values of physico-chemical parameters of the sampling sites. It is obvious that, the mean values of the different parameters of the water collected from El-Rahawy drain were very high as compared to the reference site of the River Nile, with the exception of DO and transparency. The present results are shown depletion in oxygen content and transparency and increasing in ammonia, nitrate and nitrite concentrations at El-Rahawy drain.

Table 1: Physico-chemical parameters (mean ± standard deviation) at various sampling sites.

Site	Season	Air Temperature (ºC)	Water Temperature (ºC)	DO (mg/L)	BOD (mg/L)	Transparency (cm)	pH	Ammonia (mg/L)	Nitrite (NO_2) (µg/L)	Nitrate (NO_3) (µg/L)	Conductivity (µmohs/cm)
River Nile at El-Kanater El-Khyria	Winter	19.25 ± 0.53	17.36 ± 0.39	8.36 ± 0.34	3.18 ± 0.26	90.05 ± 2.17	7.32 ± 0.06	0.59 ± 0.02	11.80 ± 1.71	32.28 ± 2.92	457.65 ± 5.41
	Spring	27.30 ± 0.32	25.13± 0.28	7.88± 0.17	5.77 ± 0.17	107.63 ± 5.28	7.48 ± 0.18	0.57 ± 0.01	14.70 ± 1.67	35.18 ± 3.55	346.95 ± 8.41
	Summer	32.45 ± 0.58	30.48 ± 0.40	6.98 ± 0.17	1.93± 0.06	118.93± 2.05	7.68 ± 0.16	0.65 ± 0.06	17.85 ± 1.52	27.55 ± 2.34	382.68 ± 6.01
	Autumn	26.98 ± 0.38	24.95 ± 0.34	8.15 ± 0.26	4.10 ± 0.21	79.85 ± 1.55	7.36 ± 0.17	0.52 ± 0.04	18.28 ± 2.86	41.23 ± 1.98	375.08 ± 6.82
	Mean	**26.49 ± 4.88**	**24.48 ± 4.83**	**7.84 ± 0.59**	**3.75 ± 1.46**	**99.11 ± 15.90**	**7.46 ± 0.20**	**0.58 ± 0.06**	**15.66 ± 3.25**	**34.06 ± 5.68**	**390.59 ± 42.71**
El-Rahawy Drain	Winter	18.98 ± 0.35	16.96 ± 0.21	4.18 ± 0.47	14.10 ± 0.29	29.24 ± 1.02	8.16 ± 0.32	10.08 ± 0.29	40.08 ± 0.92	39.43 ± 1.46	930.20 ± 11.45
	Spring	27.80 ± 0.19	25.86 ± 0.29	4.88± 0.18	13.92 ± 0.26	21.62 ± 2.01	8.11 ± 0.28	12.50 ± 0.56	36.04 ± 0.92	60.26 ± 1.07	537.60 ± 23.97
	Summer	32.38 ± 0.53	30.16 ± 0.21	3.38 ± 0.08	12.66 ± 0.21	20.78 ± 0.90	8.35 ± 0.31	7.14 ± 0.23	41.80 ± 1.16	59.20 ± 1.11	587.58 ± 14.85
	Autumn	26.90 ± 0.43	25.10 ± 0.34	3.66 ± 0.11	13.04 ± 0.24	24.98 ± 0.41	7.87 ± 0.23	9.70 ± 0.25	33.23 ± 0.96	76.25 ± 1.39	724.74 ± 17.09
	Mean	**26.52± 4.96**	**24.52± 4.90**	**4.03 ± 0.63**	**13.43± 0.66**	**24.16± 3.60**	**8.12 ± 0.31**	**9.86 ± 1.98**	**37.79 ± 3.56**	**58.79 ± 13.45**	**695.03 ± 156.85**
Permissible limits (mg/l)	**Egyptian law No. 48 (1982)**	**NA**	**NA**	**>5**	**<6-10**	**NA**	**6.5-9**	**<0.5**	**NA**	**40**	**NA**

NA = not available.

3. 1. B) Heavy Metals in water

Data reported in Table (2) indicated that the values of the detected heavy metals in El-Rahawy drain were appreciably higher than those in the River Nile water. The mean values of the elements at different sites showed that Fe is the most abundant metal in water, whereas Cd is the least one.

Table 2: Heavy metals concentrations (mean ± standard deviation) in water and the African catfish *Clarias gariepinus* muscle and the accumulation factor (AF) at various sampling sites.

Site	Season	Copper (Cu)	Iron (Fe)	Lead (Pb)	Cadmium (Cd)	Manganese (Mn)	Zinc (Zn)
Heavy metals concentrations in water (µg/L)							
River Nile at El-Kanater El-Khyria	Winter	11.65±0.52	298.20±8.64	29.18±1.13	7.02±0.32	145.50±4.16	15.80±0.73
	Spring	14.12±0.53	804.14±15.67	33.15±1.87	7.98±0.20	155.82±4.26	12.94±0.36
	Summer	15.88±0.28	942.24±83.48	35.67±0.83	11.31±1.00	120.68±1.99	15.16±0.44
	Autumn	13.77±0.39	718.44±20.46	31.47±1.41	7.15±0.26	170.32±2.95	19.66±0.75
	Mean	**13.86±1.60**	**690.76±249.81**	**32.37±2.74**	**8.37±1.85**	**148.08±18.85**	**15.89±2.54**
El-Rahawy Drain	Winter	20.16±0.94	851.90±32.57	71.37±1.84	7.14±0.33	79.10±0.93	61.72±2.44
	Spring	29.82±0.90	951.80±33.60	63.18±3.26	8.54±0.15	176.87±4.88	80.44±1.36
	Summer	26.92±0.98	1002.92±7.57	49.12±1.47	9.08±0.28	169.44±2.70	51.00±1.84
	Autumn	24.77±0.54	904.50±7.34	42.58±1.49	8.95±0.16	181.66±1.89	46.80±1.01
	Mean	**25.42±3.70**	**927.78±61.48**	**56.56±11.80**	**8.43±0.82**	**151.77±43.36**	**59.99±13.43**
Permissible limits (µg/l)	**Egyptian law No. 48 (1982)**	**1000**	**1000**	**50**	**10**	**500**	**1000**
Permissible limits (µg/l)	**U.S.EPA (2009)**	**9.0**	**1000**	**2.5**	**0.25**	**NA**	**120.0**

NA = not available.

3. 1. C) Heavy Metals in Muscles:

Table (3) shows the mean and SD values of the tested heavy metals in African catfish *C. gariepinus* muscles in the selected sites (El-Rahawy drain and River Nile water). For all heavy metals, the mean concentrations in the muscles of fish collected from El-Rahawy drain were higher than that in the muscles of fish collected from the Nile water.

Table 3: Heavy metals concentrations (mean ± standard deviation) in the African catfish *Clarias gariepinus* muscle and the accumulation factor (AF) at various sampling sites.

Site	Season	Copper (Cu)	Iron (Fe)	Lead (Pb)	Cadmium (Cd)	Manganese (Mn)	Zinc (Zn)
Heavy metals concentrations in *C. gariepinus* muscles (µg/g wet weight)							
River Nile at El-Kanater El-Khyria	Winter	18.78±0.81	42.55±0.45	7.25±0.22	1.18±0.14	26.43±0.93	40.60±0.75
	Spring	2.96±0.20	58.74±1.68	8.50±0.07	1.90±0.10	29.98±1.00	60.63±0.81
	Summer	2.26±0.18	45.52±0.73	9.49±0.37	2.49±0.04	19.88±0.92	47.38±1.70
	Autumn	1.74±0.14	49.84±1.30	7.59±0.12	2.45±0.05	21.55±0.61	37.03±1.30
	Mean	**6.43±7.39**	**49.16±6.39**	**8.20±0.92**	**2.00±0.55**	**24.46±4.20**	**46.40±9.37**
El-Rahawy Drain	Winter	20.93±0.86	187.95±7.09	45.45±1.97	8.13±0.27	76.03±1.05	62.35±1.35
	Spring	12.94±0.37	208.55±14.63	26.48±0.75	15.03±0.31	38.53±0.63	70.03±1.05
	Summer	4.97±0.39	189.58±4.43	28.70±0.36	16.09±0.29	34.90±1.04	63.35±0.65
	Autumn	22.74±0.71	266.93±5.56	40.00±1.41	12.99±0.17	38.60±0.55	84.30±3.66
	Mean	**15.39±7.31**	**231.25±34.02**	**35.16±8.19**	**13.06±3.17**	**47.01±17.39**	**70.01±9.23**
Permissible limits (µg/g)	**FAO (1992)**	**NA**	**30.0**	**30.0**	**5.0**	**NA**	**50.0**
	EOSQC (1993)	**20**	**NA**	**0.1**	**0.1**	**NA**	**50.0**
Accumulation Factor (AF) of heavy metals in *C. gariepinus* muscles							
Site	Season	Copper (Cu)	Iron (Fe)	Lead (Pb)	Cadmium (Cd)	Manganese (Mn)	Zinc (Zn)
River Nile at El-Kanater El-Khyria	Winter	1.62±0.125	0.14±0.005	0.25±0.013	0.17±0.022	0.18±0.010	2.60±0.127
	Spring	0.21±0.022	0.10±0.061	0.26±0.016	0.25±0.035	0.19±0.010	4.48±0.520
	Summer	0.15±0.004	0.05±0.005	0.27±0.017	0.26±0.054	0.17±0.007	3.37±0.296
	Autumn	0.11±0.022	0.06±0.007	0.23±0.022	0.25±0.057	0.13±0.005	2.29±0.350
	Mean	**0.61±0.703**	**0.09±0.048**	**0.25±0.021**	**0.23±0.056**	**0.17±0.027**	**3.18±0.928**
El-Rahawy Drain	Winter	1.04±0.093	0.22±0.003	0.64±0.024	1.13±0.015	0.96±0.021	1.02±0.020
	Spring	0.49±0.088	0.23±0.011	0.42±0.047	1.88±0.173	0.22±0.006	0.92±0.113
	Summer	0.18±0.022	0.19±0.005	0.51±0.091	1.78±0.097	0.20±0.004	1.02±0.265
	Autumn	0.85±0.062	0.27±0.018	0.84±0.053	1.45±0.024	0.21±0.002	1.70±0.123
	Mean	**0.64±0.350**	**0.23±0.032**	**0.60±0.172**	**1.56±0.319**	**0.40±0.334**	**1.17±0.349**

NA = not available.

On the other hand, the correlation coefficient matrix (r) of the investigated water parameters and heavy metals in water with heavy metals in muscles of *C. gariepinus* collected from El-Rahawy drain (Table 4), demonstrated some significant positive and negative correlations.

Table 4: Pearson correlation matrix between metals concentrations in muscles of *Clarias gariepinus* and different physico-chemical parameters and heavy metals concentrations in water (µg/l) at El-Rahawy drain.

	CuM	FeM	PbM	CdM	MnM	ZnM
AT	-0.269	0.529*	-0.385	0.694**	-0.797**	0.505*
WT	-0.308	0.505*	-0.411	0.717**	-0.803**	0.476
DO	-0.324	-0.493	-0.297	0.058	0.113	-0.485
BOD	-0.006	-0.492	-0.080	-0.271	0.432	-0.468
Transparency	0.307	-0.443	0.496	-0.714**	0.755**	-0.394
pH	-0.197	-0.163	-0.124	0.095	-0.097	-0.099
Ammonia	-0.266	-0.414	-0.392	0.138	0.019	-0.360
NO_2	0.183	0.025	0.346	-0.265	0.216	-0.045
NO_3	-0.266	0.531*	-0.487	0.737**	-0.822**	0.547*
Conductivity	-0.039	0.011	-0.193	0.146	-0.109	0.057
CuW	-0.465	0.310	-0.610*	0.794**	-0.808**	0.279
FeW	-0.387	0.428	-0.399	0.708**	-0.772**	0.374
PbW	-0.043	-0.660**	0.117	-0.431	0.604*	-0.659**
CdW	-0.324	0.458	-0.381	0.689**	-0.752**	0.429
MnW	-0.081	-0.239	-0.183	-0.001	0.079	-0.173
ZnW	-0.460	-0.443	-0.525*	0.307	-0.121	-0.417

M = muscles. W = water. **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

3. 1. D) Accumulation Factor (AF):

Table (3) shows the AF values of detected metals in muscle of *C. gariepinus* collected from El-Rahawy drain and River Nile. It was found that, AF mean values of heavy metals in the muscles of fish collected from El-Rahawy drain were higher than those in the muscles of fish collected from the River Nile water with the exception of Zn. It could be seen from the results that while Zn and Cu accumulated the highest concentrations, Mn and Fe were accumulated at the lowest levels in Nile fish muscles. Considering El-Rahawy drain fish muscles, it was found that Cd and Zn accumulated the highest concentrations, while Mn and Fe were accumulated at the lowest levels.

3. 1. E) Muscle chemical composition (Meat quality)

The measurements concerning the muscles chemical composition of *C. garipinus* fish collected from different sites are presented in Table (5). It was found that, the muscle total protein and total lipids contents of fish collected from El-Rahawy drain showed high significant (F = 10.584, P = 0.002) and insignificant (F = 2.125, P = 0.149) decrease, respectively, as compared with the values of the River Nile fish. Meanwhile, Table (5) revealed a significant (F = 9.274, P = 0.003) and insignificant (F = 0.611, P = 0.437) increase in muscles water content and ash, respectively, of fish collected from El-Rahawy drain as compared with the values of the River Nile fish.

Table 5: Changes of muscle chemical composition (% of fresh weight) (mean ± standard deviation) of the African catfish *Clarias gariepinus* at various sampling sites.

Site	Season	Water Content (Moisture) (%)	Total protein Content (%)	Total lipids Content (%)	Ash (%)
River Nile at El-Kanater El-Khyria	Winter	78.53±0.64	14.75±0.33	4.30±0.39	2.49±1.05
	Spring	78.56±0.79	15.29±0.51	5.43±0.97	1.38±0.66
	Summer	78.17±0.40	15.03±0.71	5.88±0.77	1.53±1.10
	Autumn	78.22±0.90	14.82±0.60	5.06±0.64	2.52±0.43
	Mean	**78.37±0.70**	**14.97±0.58**	**5.17±0.91**	**1.98±0.98**
El-Rahawy Drain	Winter	79.47±0.61	13.82±0.95	4.06±0.37	3.39±1.59
	Spring	79.97±0.67	14.54±0.56	4.25±0.43	2.77±1.42
	Summer	78.51±0.77	14.53±0.48	5.81±0.86	1.26±0.68
	Autumn	78.01±0.99	15.02±0.57	5.32±0.79	1.39±0.97
	Mean	**78.99±1.08**	**14.48±0.77**	**4.86±0.97**	**2.20±1.49**

On the other hand, the correlation coefficient matrix (r) of the investigated water parameters and heavy metals in water with muscle chemical composition parameters of *C. gariepinus* collected from El-Rahawy drain (Table 6), demonstrated some significant positive and negative correlations.

Table 6: Pearson's correlation coefficient matrix between different muscle biochemical composition parameters of the African catfish *Clarias gariepinus* and different physico-chemical parameters and heavy metals concentrations at El-Rahawy drain.

	Water content	Protein content	Fat content	Ash
Air Temperature	0.258	0.692**	0.516*	0.598**
Water Temperature	0.229	0.714**	0.533*	0.626**
DO	-0.592**	-0.361	-0.454*	-0.127
BOD	0.133	-0.448*	0.462*	0.161
Transparency	-0.573**	-0.693**	-0.443	-0.570**
pH	0.134	0.040	-0.442	0.150
Ammonia	0.232	-0.436	-0.489*	0.118
NO_2	-0.216	-0.310	-0.565**	-0.348
NO_3	0.036	0.711**	0.582**	0.615**
Conductivity	0.125	-0.492*	0.656**	0.218
CuW	-0.124	0.685**	0.249	0.633**
FeW	0.180	0.528*	0.391	0.549*
PbW	0.015	-0.595**	-0.596**	-0.433
CdW	0.173	0.767**	0.610**	0.628*
MnW	0.207	0.122	0.492*	0.178
ZnW	0.051	-0.014	-0.068	0.078
CuM	0.026	-0.260	-0.619*	-0.385
FeM	0.040	0.561*	0.237	0.341
PbM	0.044	-0.427	-0.215	-0.657**
CdM	-0.100	0.623**	0.344	0.803**
MnM	0.177	-0.683**	-0.377	-0.866**
ZnM	-0.163	0.609*	0.334	0.423

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
W = water. M = muscle.

3. 1. F) Histopathological study

The muscles are composed of segmental myomeres. Each myomere is regarded as apparent muscle and its fibers are parallel to the long axis of the body. This muscular layer covered with skin which formed of three layers (epidermis, dermis and hypodermis). Also, this skin is covered with an epithelial layer. Several histopathological alterations were detected in the muscles of *Clarias gariepinus* collected from El-Rahawy drain. The pathological results included degeneration in muscle bundles and focal areas of necrosis (Figs. 1 and 2). A focal necrosis and hyaline degeneration were seen in muscles (Fig. 2). Edema and splitting of muscle bundles can be detected (Fig. 3). Also the distribution of metacercarial cysts in most regions of the fish muscles were recorded (Fig. 4). The muscles of fish *C. gariepinus* showed round parasitic cyst in subcutis causing pressure atrophy of the surrounding muscle bundles. This cyst was appearing with irregular thin wall (Fig. 5). The cyst wall was oval in shape, thick and composed of two layers. Clear refractive granules were present inner to the cyst wall. The body of the metacercaria was folded and the oral sucker was present (Fig. 6).

4. DISCUSSION

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

The elevated levels in physico-chemical properties observed in El-Rahawy drain implicate pollution as the source of alteration in water quality. The negative impact of different sources of pollutants discharged into this drain was further confirmed by the highest values in all physico-chemical parameters with a concomitant decrease in DO.

Temperature is a factor of great important for aquatic ecosystem, as it affects the organisms, as well as the chemical and physical characteristics of water (Abdo, 2005). As expected the water temperature of the studied points followed more or less that of the air. The relative increase in temperature of El-Rahawy drain water has potential implications on the oxygen retention capacity of the water (UNEP, 2006) as increases in temperature affects the levels of dissolved oxygen in the water column where DO is inversely proportional to temperature (Adeogun, 2012). In addition, Veado *et al.* (2000) reported that the introduction of excess of organic matter may result in a depletion of oxygen from an aquatic system mainly during warm stagnant condition. Similarly, the reduction in dissolved oxygen content may be due to decomposition of suspended organic matter of sewage in this drain (Tayel *et al.*, 2007). Prolonged exposure to low dissolved oxygen level (<5-6 mg/L) will increase organisms susceptibility to other environmental stress (Osman *et al.*, 2010) and has dire consequences for the survival of fish and other aquatic animals as reduced DO 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).

BOD measures the dissolved oxygen consumed by microorganisms present in the studied samples to stabilize any biodegradable organic matter, as well as the quantity of oxygen used in its respiration (APHA, 1998; Elewa *et al.*, 2007). Increase in BOD values monitored in El-Rahawy drain environment being affected by quantity and quality of discharges, as well as seasonal and spatial effects (Abdel-Hamid *et al.*, 1992).

On the other hand, the increase of turbidity (low transparency) may be due to the disposal of domestic and industrial effluent in this drain (Osman *et al.*, 2010).

The pH value is considered to be an important factor in the chemical and biological system of aquatic environment (Osman *et al.*, 2010). The relatively high pH of El-Rahawy drain water can be attributed to the large amounts of different pollution sources discharged in this drain. pH has profound effects on water quality affecting the ability of bacteria which require slightly acidic pH to degrade toxic substances to less harmful forms (Adeogun, 2012).

Dissolved inorganic nitrogen is the summation of the ammonia, nitrate and nitrite (Tayel *et al.*, 2007; Osman *et al.*, 2010). These parameters were found in high concentrations in El-Rahawy drain water which may be due to sewage outfalls, as recorded by Tayel *et al.* (2007). The higher contents of nitrite in El-Rahawy drain water are indication of the microbial activity. The recorded increase in NO_3 comparing to in River Nile water might be attributed to the fast conversion of NO_2-NO_3-ions by nitrifying bacteria (Osman *et al.*, 2010). The increase in ammonia level in water samples collected from El-Rahawy drain water is indicator of the presence of pollutants of high activity viz.: sewage discharge, industrial effluents and agriculture-runoff, and could be attributed to the increase in the oxygen consumption of the decomposing organic matter and oxidation of chemical constituents (Elghobashy *et al.*, 2001). The presence of large concentration of NO_2 and NO_3 in water can create a large oxygen demand. High concentration of nitrate and nitrite can cause algae to grow in large quantity. Dead algae can cause oxygen depletion problems which in turn can kill fishes and other aquatic organisms (Osman *et al.*, 2010). Although mean ammonia value in El-Rahawy drain water exceeded acceptable limits by the Egyptian governmental law No. 48 (1982), the value in River Nile water was considerably elevated.

The high conductivity values observed in El-Rahawy drain water suggests possible sources of run-off from adjacent land and strongly implicates industrial and sewage sources. This agrees with reports of UNESCO-WHO-UNEP (1996) about the conductivity being a direct measure of anthropogenic impact.

It was found that the values of water samples collected from El-Rahawy drain were higher than that collected from Nile water, but generally the detected values of the water samples from both sites are in the permissible levels set by the Egyptian governmental law No. 48 (1982).

Heavy metals may enter an aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills, shipping and harbor activities and atmospheric deposits (Rajeshkumar and Munuswamy, 2011).

Present results showed that, most of the heavy metal concentrations in surface water of El-Rahawy drain and River Nile water were found within the permissible limits of both the Egyptian governmental law No. 48 (1982) and U.S.EPA (2009). These results are in agree with El Bouraie *et al.* (2010), who studied heavy metals in five drain outfalls and found that the level of metals is within the permissible limits of Egyptian law 48/1982. Also, Lasheen *et al.* (2012) stated that the average concentrations of heavy metals in El-Moheet drain; which discharge in El-Rahawy drain; are within the permissible range according to the Egyptian law 48/1982.

Generally, lower mean value of DO and higher mean values of turbidity, conductivity, BOD, NO_2, NO_3 and trace metals in El-Rahawy drain comparing to the Nile water prove the presence of large quantities of organic and inorganic pollutants in El-Rahawy drain. This was expected due to the fact that the water of such drain receives large quantities of domestic, agricultural and industrial effluents.

Contamination of aquatic ecosystems with heavy metals has seriously increased worldwide attention (Authman, 2008; Osman *et al.*, 2010). Fish are one of the most indicative factors in freshwater systems and may concentrate large amounts of some metals, such as lead, cadmium, copper, mercury, zinc and iron (Papagiannis *et al.*, 2004; Yilmaz et *al.*, 2007). These metals accumulate differentially in fish organs and cause serious health hazards to humans.

For all heavy metals the mean concentration in the muscle of fish collected from El-Rahawy drain was higher than that in the muscle of fish collected from the River Nile water. These results agree with those obtained by Bahnasawy *et al.* (2009) who demonstrated that fish surviving at highly polluted areas accumulate higher levels of heavy metals than those surviving at less polluted area. This was expected due to the fact that the water of such drain receives large quantities of domestic, agricultural and industrial effluents (Osman *et al.*, 2010). Ibrahim and Mahmoud (2005) and El-Naggar *et al.* (2009) anticipated the heavy metals increase to industrial, drainage and sewage effluents. It was found that the concentrations of some metals in muscles of the *C. gariepinus* collected from El-Rahawy drain were very higher than the permissible limits issued by Egyptian (EOSQC, 1993) and international (FAO, 1992) organizations, so, they are regarded as potential hazards that can endanger both animal and human health. Therefore, muscles of *C. gariepinus* fish caught from El-Rahawy drain are considered harmful for human consumption.

The AF or the relative index of the concentration of the metal in the fish muscles to that in water showed lower values when compared to the real concentrations of those pollutants in water. These results were in contrast with that of Khallaf *et al.* (1998) and Authman *et al.* (2013). On the other hand, the significant positive correlations (Pearson's correlation coefficients) observed between the concentrations of some metals in the water and those in the fish muscles suggest that the detected metals somewhat follow the same pattern of variations and the tissue levels are influenced by water concentrations. Adding to that the negative correlations between some metals concentrations in water and fish muscles may reflect the increase of metals in muscles as their concentrations in water decreases (Authman *et al.*, 2012).

Regarding the muscle chemical composition, the decrease in total muscles protein and total lipids content of fish collected from El-Rahawy drain may be attributed to the change in water quality by the action of pollutants and heavy metals that may critically influence the growth rate and the quality of fish (Elghobashy *et al.*, 2001). The increase in fish muscles water content is in agreement with Weatherly and Gill (1987) who reported that depletion of body total protein and total lipids results in tissue hydration as an inverse dynamic relationship between protein, lipids and water content in the muscles. Moreover, the increase in fish muscle ash may be attributed to the bioaccumulation of heavy metals in fish as previously reported by Haggag *et al.* (1999) and Elghobashy *et al.* (2001). On the other hand, the significant positive and negative correlations (Pearson's correlation coefficients) observed, in the present study, between the water physico-chemical parameters and some metals concentrations in El-Rahawy drain water and muscle chemical composition parameters indicated the influence of water pollution upon fish meat quality.

Several investigations had concerned with the effect of metals on the levels of the muscle protein and lipid. Reduction in protein levels was noticed in the muscles of *Sarotherodon mossambicus* exposed to mercury (Ramalingam and Ramalingam, 1982); in the muscles of grass carp exposed to lethal concentration of cadmium (Salah El-Deen *et al.*, 1996) and in the muscles of *O. niloiicus*, *T. zillii* and *C. lazera* fish collected from Abu Za'baal Lakes (Mohamed and Gad, 2005). Similarly, a decrease of lipid levels was observed in the muscles of the grass carp exposed to lethal and sublethal concentrations of cadmium (Salah El-Deen *et al.*, 1996) and in the muscles of *C. lazera* fish collected from Abu Za'baal Lakes (Mohamed and Gad, 2005).

Histopathology, with a broad range of causes, are increasingly being used as indicators of environmental stress since they provide a definite biological end-point of historical exposure **(**Stentiford *et al.*, 2003). Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on aquatic biota and are a reflection of the overall health of the entire population in the ecosystem (Mohamed, 2009).

Although muscles are the most edible part of fish body, it is the most exposed part to be damaged by several types of pollution (El-Serafy *et al.*, 2005; Sitohy *et al.*, 2006). If fish have epithelial lesion in polluted water, they would probably be invaded by microorganisms which cause severe epidermal pathology resulting in musculature destruction (Saad *et al.*, 2012).

Several changes were observed in the muscles of *C. gariepinus* collected from El-Rahawy drain such as; sever edema, splitting of muscle bundles and cysts of parasites. The cysts were found in the dermis and between muscle bundles in histopathological examinations and that in consonance with those reported by Mahdy *et al.* (1995). Degenerative and necrotic changes observed in muscle bundles could be attributed to pressure atrophy induced by the encysted metacercariae, in addition to the effect of toxic parasitic metabolites in muscle cells as observed by Mahdy *et al.* (1995). The tissue reaction in the skin and muscles were possibly due to mechanical irritation effect of the parasitic cysts during its migration and /or due to their toxic products (Mahmoud *et al.*, 1989; El Reid, 1994).

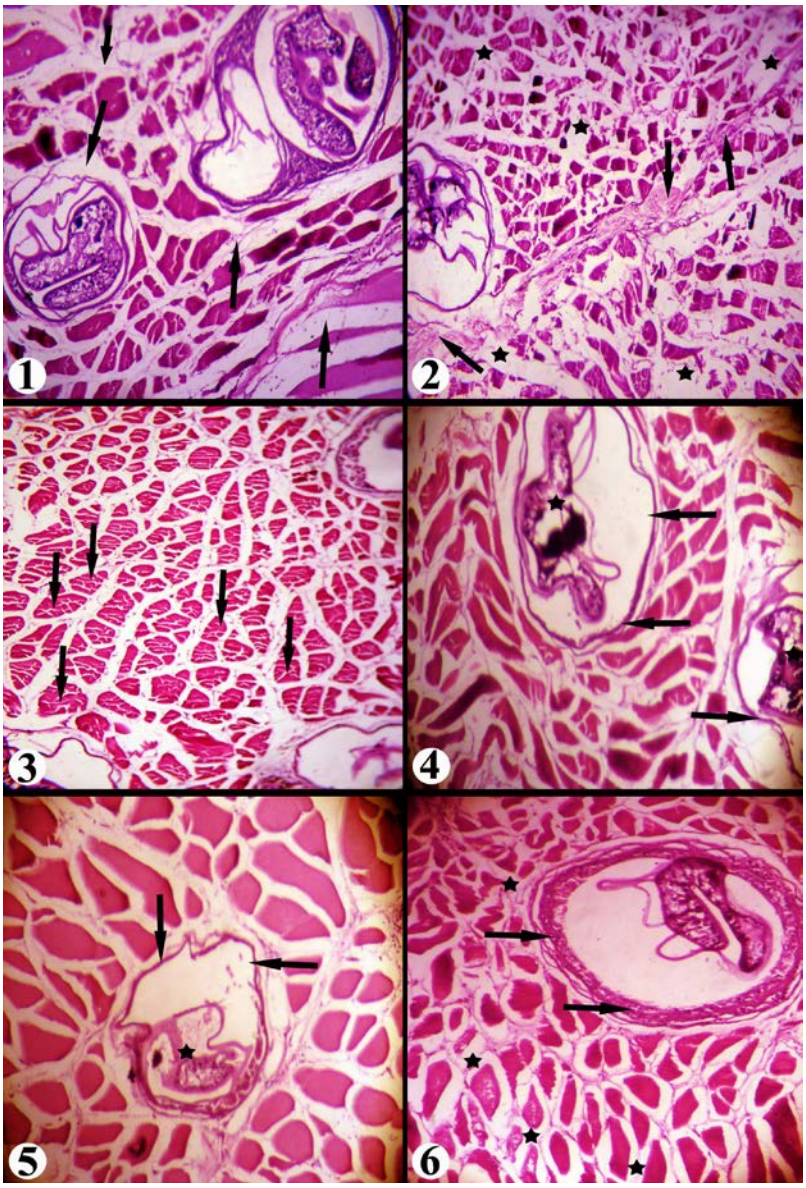


Fig. 1: Section in muscles of *C. gariepinus* showing edema (arrows) (H&E, X100).

Fig. 2: Section in muscles of *C. gariepinus* showing hyaline degeneration (arrows) and edema (stars) (H&E, X100).

Fig. 3: Section in muscles of *C. gariepinus* showing edema and splitting muscle bundles (arrows) (H&E, X100).

Fig. 4: Section in muscles of *C. gariepinus* contains more than one cyst (arrows) of parasite (stars) (H&E, X100).

Fig. 5: Section in muscles of *C. gariepinus* showing edema, with cyst of parasite has thin wall (arrows) and parasite (star) (H&E, X100).

Fig. 6: Section in muscles of *C. gariepinus* showing edema (stars) and cyst of parasite around with fiber tissue (arrows) (H&E, X100).

The high parasites infection can be explained as the suppressive effect of the pollution to the immunity system of the fish (Marcogliese *et al.*, 2005). Also, these histopathological changes in the muscles of *C. gariepinus* collected from El-Rahawy drain may be due to high heavy metal concentrations in water and their accumulation in fish muscles. These results agree with that obtained by Sitohy *et al.* (2006) in muscles of *C. gariepinus* fish collected from River Nile at Helwan region.

Histopathological alterations in the muscles of *C. gariepinus* collected from El-Rahawy drain are in agreement with those observed by many investigators who have studied the effects of different pollutants on fish muscles (Sakr and Gabr, 1991; Das and Mukherjee, 2000). Focal areas of myolysis were seen in the muscles of *O. spilurus* exposed to contra/insect 500/50E.C. (Elnemaki and Abuzinadah, 2003). Abbas and Ali (2007) observed destruction and vacuolation of the muscle cells in *Oreochromis* spp. exposed to chromium.

5. CONCLUSION

In conclusion, based on the physico-chemical properties of water, the present study revealed that water at El-Rahawy drain region are affected by industrial, agricultural and sewage effluents and contaminated with some heavy metals which have toxicological effects on the chemistry and histology of fish muscles. High concentrations of heavy metals accumulate in muscles of *Clarias gariepinus*; inhabiting this drain; deteriorating its quality to the point that it could be hazardous to humans. Muscles also contained parasites that are of public health concern.

6. RECOMMENDATIONS

Finally, according to the obtained results, it could be recommended that, treatment of wastewaters especially sewage wastes before discharging to the natural water bodies is a necessary procedure to protect the fish and the people from the dangerous effects of such pollution. Since fish are certainly caught from this drain and sold for consumers, the concerned authorities should take firm regulation for prohibiting fishing from such drain.

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