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Some Studies on Heavy Metal Affecting Wild Catfish in Different Regions In Egypt

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Key words
Catfish, heavy metal, muscles, Egypt.

ABSTRACT:

Fish is considered one of the most important sources of animal protein in Egypt due to the extreme need for sources of animal proteins particularly with the gradual increase of the population. In this study about 200 catfish were collected from four different canals (El Maruttia, El Mansouria, El Ismailia and El Khanater) of the metropolitan area of Cairo include (Cairo - Giza - Kalyobia) in Egypt. The results of this study point to the existence of a significant differences in the concentrations of copper, iron, zinc, chromium, nickel, lead, cadmium and mercury in the muscles of catfish among all the groups of the study. In a comparison between all the groups of the study for iron (Fe) levels in muscles of the catfish (males and females) it was found that 100% of the fish in El Maryottia canal have concentrations of iron higher than allowed levels. with regard to the copper (Cu) it was found that its levels in the muscles of fish collected from El Maruttia (males and females) were higher than admissible limits as the following (19.16 , 21.28) µg / g , respectively, while samples from all the other places such as El Mansouria, El Ismailia , El Khanater were contain different concentrations of copper, but within the permissible levels either in males or females .With reference to lead (Pb) it was found in all the muscles of catfish from all canals with rate 100% higher than the permitted levels except for El Khanater its levels in muscles were within the permissible limits, but lead was found in the liver samples of all groups from all canals. For mercury (Hg) it was noticed that the muscles of catfish collected from El Maruttia canal and El Mansouria contain a higher concentrations than the allowed levels. Also the results of this study refer to presence of significant differences in the concentrations of (iron, copper, lead and mercury) in between the liver samples of catfish from different groups of the study.

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1. INTRODUCTION

Fish considered one of the main sources of the national income that Egypt depends on, stimulating local market economies, and important source of foreign exchange. In Egypt there is increasing rate of water pollution by the heavy metals due to an upsurge interest of construction of industrial plants on the banks of Nile river and its tributaries that discharge their chemical wastes in these canals resulting in pollution of the aquatic resources and accumulation of these metals in the meat of aquatic organisms like cat fish leading to serious health hazards for human consumers (Stephen et al., 2000).

Therefore, we have to explore this field to know how we can protect the Egyptian fresh water culture against these pollutants which will be reflected on human being. The present work aimed for monitoring of some heavy metal concentrations in both fish tissues and water in which fish is living.

In aquatic systems, heavy metals have received considerable attention due to their toxicity and accumulation in biota (Robenson, 2011) mentioned that metals generally enter the aquatic environment through atmospheric deposition, erosion of the geological matrix, or due to anthropogenic activities caused by industrial effluents, domestic sewage, and mining wastes. Some of these metals, such as Cd and Pb, are toxic to living organisms even at quite low concentrations, whereas others, such as Zn and Cu, are biologically essential and natural constituents of aquatic ecosystems, and generally only become toxic at very high concentrations. The effects of heavy metals on human health and the environment are of great interest today, especially for aquatic products (Uluozlu et al., 2007).

Symptoms related to prolonged low-level exposure may not be apparent until later in life and, when they do occur, may be chronic and irreversible. Serious illness due to long-term exposure to various

toxic chemicals may include damage to the immune and nervous systems, impairment of reproductive function, cancer and organ-specific damage (WHO 1988).

2. MATERIALS AND METHODS

1-Materials:

1.1. Fish sampling:

Total number of two hundred fish of male and female freshwater catfish (clarias lazera) were collected from different locations along the Nile basin.The fish divided into four groups as following :

Twenty five male fish + twenty five female fish collected from Al Maruttia canal (Giza governorate).
Twenty five male fish and twenty five female fish from Al Mansouria canal (Giza governorate).
Twenty five male fish and twenty five female fish collected from El Ismailia canal (Cairo governorate).
Twenty five male fish and twenty five female fish from El Kanater El Khayria (Kalyobia governorate).

All fish samples were varied in length (between 25-30 cm.) and varied in weight (600-800 g.) .

1.2. Muscular tissues sampling and preparation:

Dorsolateral muscles were rapidly removed from the killed fish and were stored in plastic container at -20 °C for heavy metals residues analysis (Jonsson et al., 2012).

1.3. Liver sampling and preparation :

Livers were divided into two parts. The first of which connected to the dorsolateral muscles was stored in plastic container and kept at -20 °C for heavy metals analysis. The second part was washed in water then cut into small fragments and homogenized with deionized water (1g/ ml.) in a potter elvehjem homogenizer. The homogenate was used for measurement of different types of antioxidants (Mason, 1991).

1.3. Biochemical analysis :

A) Biochemical analysis of muscular tissues were used in the determination of (iron, copper, lead and mercury) residues.
B) Biochemical analysis of liver tissues were used in the following biochemical assays:
   Detection of heavy metals residues.
   Determination of different types of antioxidants markers (malondialdehyde MDA, reduced glutathione GSH, glutathione peroxidase GPX and glutathione-S-transferase GST).
C) Biochemical analyses of water were used for detection of heavy metals constituents.

2-Methods:

2.1. Heavy Metals (iron, copper, lead and mercury) in muscles of catfish :

Atomic absorption spectrophotometric method was used for the determination of copper, zinc, iron, chromium, mercury, cadmium, selenium, nickel and lead as described in Perkin Elemer catalogue of atomic absorption model 2380, U.S.A., (Jonsson et al., 2012).

2.2. Biochemical analysis of liver tissues for determination of (Heavy metals residues) :

A wet digestion procedure (Mason, 1991) was followed using pure conc. Nitric and perchloric acids in a ratio of 5:1 for each one gram of tissue in a Kjeldahl flask. The mixture was heated on a hot plate to a total colorless solution, then diluted with deionized water up to 50 ml. blank was treated in the same manner as samples.

Atomic absorption spectrophotometric method was used for the determination of copper, iron, zinc, chromium, mercury, cadmium, selenium, nickel and lead as described in Perkin Elmer catalogue of atomic absorption model 2380, U.S.A (1982). Atomic absorption with a single slot burner head that is capable of operating at the following wave length in (nm): 324.8, for Cu, 248.3 for Fe and Pb.

2.3. Hg residues determination in liver Tissues :

Accumulation of Hg in liver was measured colorimetrically by dithizone method as the following

Principle :

Sample was digested with HNO_3 and H_2SO_4 under reflux in special apparatus, Hg was isolated by dithiozone extraction, Cu was removed and Hg was estimated by photometric measurement of Hg dithizonate (Ates et al., 2008).

2.4. Biochemical analysis of muscular tissuesfor determination of heavy metals residues:

Accumulation of the heavy metals in the muscular tissues were measured using atomic absorption spectrophotometric method to determine copper, iron, zinc and other metals as previously described in liver tissues. Also mercury was measured calorimetrically as previously described in serum.

2.5. Biochemical analysis of liver fordetection of anti-oxidants (MDA, GSH, Gpx, GST) :

Determination of malondialdehyde MDA(Allen et al., 2004)**:**

Calculation:

The absorbance was converted into nM/ml using standard curve and expressed as nM MDA/ml plasma.

For the tissues, the total protein was determined in tissue homogenate according to Loucks et al. (2012) and the results were expressed as nM of MDA/mg protein.

Determination of reduced glutathione GSH (Ciardullo, 2010**):**

Method :

1- 0.2 ml of whole blood or tissue homogenate was added to 1.8 ml of deionized dist. water. 0.2 ml of the standard glutathione solution was added to 1.8 ml of dist. water.
2- Allow to stand for no more than 3 minutes.
3- 3.0 ml of precipitating solution was added to test and standard and mixed well. At the same time prepare a reagent blank by adding 3.0 ml of precipitating solution to 2.0 ml of deionized dist. water.
4- Allow to stand for 10 minutes.
5- Filter test, standard and blank solutions through Whatman no. 1 paper.
6- 0.5 ml filtrate was added to 2.0 ml of 0.3 M Na_2HPO_4 and place in 3 ml cuvette of 1 cm light path.
7- Read the optical density (OD) of the test and standard solutions at 412 nm against the reagent blank (OD_1).
8- Add 0.25 ml of DTNB solution to blank, standard and test solutions. Mix well by inversion and immediately take a second OD reading against the reagent blank (OD_2).

Calculation:

As standard GSH solution concentration = 30 mg/L and Molecular weight of GSH = 307 then:

GSH level in blood = $\frac{OD_2 - OD_1 test}{OD_2 - OD_1 std.} x \frac{30x1000}{307}$ µM/ml

$= \frac{OD_2 - OD_1 test}{OD_2 - OD_1 std.} x97.72$ µM/ml

GSH concentration in tissue homogenate = $\frac{OD_2 - OD_1 test}{OD_2 - OD_1 std.} x97.72$ µM/ml.

Total protein was determined in tissue homogenate according to **Lowry et al. (1951).**

GSH conc. in tissues = $\frac{GSH\ conc.(\mu M/ml)}{Protein\ conc.(mg/ml)}$ µM/mg protein.

Determination of glutathione peroxidase (Gpx, EC 1.11.1.9) (Pandeyet al., 2001)**:**

Procedure:

Wave length: 340 nm. Light path: 1 cm.

Final volume: 3 ml. Temp. : 20ºC.

Reading occurred against blank containing buffer and sample solution (hemolysate or homogenate) for test cuvette and buffer with dist. water for control cuvette. This was measured at 340 nm at 20ºC.

Determination of glutathione-S-transferase (GST, EC 2.5.1.18) according to (Hajeb et al., 2012):

Procedure:

Light path 1cm, pH 6.5 and final volume 3ml read at 25ºC against blank.

3. RESULTS and DISCUSSION

In this study we used 200 catfish (100 male and 100 female) from four water sources in Delta and Cairo (Al Maruttia canal, Al Mansouria canal, El Ismalia canal, El Kanater Elkhayria), in addition to four water samples from the same water sources and the results would be classified as the following:

1- Determination of heavy metals (iron, copper, lead and mercury) in the muscles of cat fish that would be shown in the following tables (1, 3, 5, 7)

2- Determination of heavy metals (iron, copper, lead and mercury) in the livers of cat fish that would be shown in the following tables (2, 4, 6, 8) .

3- Determination of anti-oxidants MDA ,GSH ,GPX , GST) in the livers of cat fish that would be shown in the following tables (9, 10, 11, 12) .

Table (1): Analytical results of the Iron ((µg /g) wet weight) of the examined samples of 25 cat fish meat.

Water Channels	Sex	Minimum	Maximum	Mean±SEM	Samples above the permissible limits	
					Number	Percentage
EL Maruttia	Male	31	34	32.60±0.48[a]	25	100
	Female	29	35	32.82±1.02[a]	25	100
El Mansouria	Male	15	20	17.52±0.81[bc]	0	0
	Female	15	20	17.96±0.84[b]	0	0
EL Ismailia	Male	14	18	15.56±0.70[c]	0	0
	Female	16	19	16.92±0.59[bc]	0	0
EL Khanater	Male	10	13.6	11.32±0.68[d]	0	0
	Female	10	14	12.40±0.68[d]	0	0

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (2): Analytical results of the Iron ((µg /g) wet weight) of the examined samples of 25 cat fish liver.

Water Channels	Sex	Minimum	Maximum	Mean±SEM	Samples above the permissible limits	
					Number	Percentage
EL Maruttia	Male	82	90	85.50±1.47[c]	25	100
	Female	85	91	88.24±1.07[c]	25	100
El Mansouria	Male	215	230	222.40±2.56[a]	25	100
	Female	209	230	219.60±4.00[a]	25	100
EL Ismailia	Male	70	82	74.40±2.54[d]	25	100
	Female	72	81	77.20±1.66[d]	25	100
EL Khanater	Male	198	205	201.80±1.24[b]	25	100
	Female	200	210	204.80±1.77[b]	25	100

Number of the samples = 25.
SEM = Standard error for mean.
Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (3): Analytical results of Copper ((µg /g) wet weight) of the examined samples of 25 cat fish meat.

Water Channels	Sex	Minimum	Maximum	Mean±SEM	Samples above the permissible limits	
					Number	Percentage
EL Maruttia	Male	15.9	21.4	19.16±0.93[a]	25	100
	Female	19.8	23.1	21.28±0.62[a]	25	100
El Mansouria	Male	4.9	6.8	5.76±0.37[c]	0	0
	Female	3.9	6.2	5.12±0.38[cd]	0	0
EL Ismailia	Male	3.2	4.2	3.74±0.19[e]	0	0
	Female	3.8	4.9	4.12±0.20[de]	0	0
EL Khanater	Male	1.1	1.7	1.40±0.11[f]	0	0
	Female	1.2	1.9	1.54±0.13[f]	0	0

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (4): Analytical results of Copper ((µg /g) wet weight) of the examined samples of 25 cat fish liver.

Water Channels	Sex	Minimum	Maximum	Mean±SEM	Samples above the permissible limits	
					Number	Percentage
EL Maruttia	Male	60	70	64.60±1.72[a]	25	100
	Female	61	72	65.60±2.25[a]	25	100
El Mansouria	Male	39	48	42.60±1.60[b]	25	100
	Female	39	44	41.80±1.02[b]	25	100
EL Ismailia	Male	5	9	7.40±0.81[c]	11	44
	Female	10	12	11.00±0.45[c]	25	100
EL Khanater	Male	40	43	41.20±0.58[b]	25	100
	Female	40	44	41.80±0.66[b]	25	100

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (5): Analytical results of Lead ((µg /g) wet weight) of the examined samples of 25 cat fish meat.

Water Channels	Sex	Minimum	Maximum	Mean±SEM	Samples above the permissible limits	
					Number	Percentage
EL Maruttia	Male	0.85	1.3	1.07±0.09[bc]	24	96
	Female	1	1.3	1.16±0.05[bc]	25	100
El Mansouria	Male	1.8	2.9	2.30±0.18[a]	25	100
	Female	2	2.7	2.48±0.13[a]	25	100
EL Ismailia	Male	1.5	2	1.80±0.09[ab]	25	100
	Female	1.9	2.3	2.04±0.07[a]	25	100
EL Khanater	Male	0.299	3.52	0.95±0.64[cd]	0	0
	Female	0.219	0.332	0.30±0.02[d]	0	0

Table (6): Analytical results of Lead ((µg /g) wet weight) of the examined samples of 25 cat fish liver.

Water Channels	Sex	Minimum	Maximum	Mean±SEM	Samples above the permissible limits	
					Number	Percentage
EL Maruttia	Male	6.5	8.2	7.40±0.31[cd]	25	100
	Female	7.2	8.2	7.76±0.18[bc]	25	100
El Mansouria	Male	7.8	9	8.34±0.21[ab]	25	100
	Female	8.1	9.3	8.90±0.23[a]	25	100
EL Ismailia	Male	6	7	6.44±0.17[e]	25	100
	Female	6.2	7.8	7.04±0.33d[e]	25	100
EL Khanater	Male	0.321	0.41	0.37±0.02[f]	4	16
	Female	0.3	0.45	0.38±0.03[f]	7	28

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (7): Analytical results of Mercury ((µg /g) wet weight) of the examined samples of 25 cat fish meat.

Water Channels	Sex	Minimum	Maximum	Mean±SEM
EL Maruttia	**Male**	0.12	0.18	0.15±0.01
	Female	0.11	0.18	0.14±0.01
El Mansouria	**Male**	0.00	0.16	0.11±0.03
	Female	0.13	0.17	0.15±0.01
EL Ismailia	**Male**	0.03	0.14	0.07±0.02
	Female	0.01	0.07	0.03±0.00
EL Khanater	**Male**	0.00	0.04	0.02±0.00
	Female	0.00	0.03	0.02±0.00

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (8): Analytical results of Mercury ((µg /g) wet weight) of the examined samples of 25 cat fish liver.

Water Channels	Sex	Minimum	Maximum	Mean±SEM
EL Maruttia	**Male**	0.07	0.12	0.09 ± 0.01^{a}
	Female	0.05	0.09	0.07 ± 0.01^{a}
El Mansouria	**Male**	0.01	0.07	0.03 ± 0.01^{b}
	Female	0.01	0.03	0.02 ± 0.00^{b}
EL Ismailia	**Male**	0	0.05	0.01 ± 0.00^{b}
	Female	0	0.06	0.01 ± 0.00^{b}
EL Khanater	**Male**	0	0.1	0.02 ± 0.00^{b}
	Female	0	0.09	0.02 ± 0.00^{b}

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (9): Analytical results of Malondialdehyde (MDA) of the examined samples of 25 cat fish liver.

Water Channels	Sex	Minimum	Maximum	Mean±SEM
EL Maruttia	**Male**	0.91	1.46	1.18 ± 0.10^{cd}
	Female	0.89	1.97	1.34 ± 0.20^{bc}
El Mansouria	**Male**	1.9	3.5	2.82 ± 0.27^{a}
	Female	1.9	3.5	2.78 ± 0.26^{a}
EL Ismailia	**Male**	1.3	2.1	1.72 ± 0.15^{b}
	Female	1.5	2.1	1.76 ± 0.11^{b}
EL Khanater	**Male**	0.71	0.95	0.81 ± 0.04^{d}
	Female	0.61	0.96	0.79 ± 0.06^{d}

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (10): Analytical results of Reduced glutathione (L-γ-glutamyl-L-cysteinylglycine, GSH) of the examined samples of 25 cat fish liver.

Water Channels	Sex	Minimum	Maximum	Mean±SEM
EL Maruttia	**Male**	85.5	98.2	90.46±2.79[a]
	Female	69.3	102.6	89.46±6.15[a]
El Mansouria	**Male**	90	100	95.20±1.93[a]
	Female	90	100	93.80±1.74[a]
EL Ismailia	**Male**	82	90	87.40±1.47[a]
	Female	81	99	87.40±3.20[a]
EL Khanater	**Male**	60	77	68.20±3.68[b]
	Female	55	72	65.40±2.94[b]

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (11): Analytical results of (selenium dependent glutathione peroxidase, Gpx) of the examined samples of 25 cat fish liver.

Water Channels	Sex	Minimum	Maximum	Mean±SEM
EL Maruttia	**Male**	102	110	106.00±1.30[b]
	Female	104	111	108.20±1.24[b]
El Mansouria	**Male**	92	104	98.40±2.38[c]
	Female	90	100	93.80±1.69[c]
EL Ismailia	**Male**	89	100	96.20±1.98[c]
	Female	85	100	95.00±2.90[c]
EL Khanater	**Male**	120	125	122.20±1.02[a]
	Female	119	125	122.80±1.11[a]

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

Table (12): Analytical results of glutathione-S-transferases catalyses (GST) of the examined samples of 25 cat fish liver.

Water Channels	Sex	Minimum	Maximum	Mean±SEM
EL Maruttia	**Male**	206	217	210.40±1.86[b]
	Female	214	225	218.20±1.98[a]
El Manouria	**Male**	215	222	218.60±1.21[a]
	Female	207	225	217.60±2.96[a]
EL Ismailia	**Male**	200	206	203.60±1.03[b]
	Female	205	210	207.80±0.86[b]
EL Khanater	**Male**	174	195	185.60±4.23[c]
	Female	175	190	181.00±2.53[c]

Means carry different superscripts within the same column are significantly different (P≤ 0.05).

The present work deals with the occurrence and distribution of heavy metals in water and their accumulation in the muscles and livers in **Clarias lazera** collected from four different locations **(El Maruotia, El Mansouria, El Ismailia and El khanater)**, also the effects of exposure to pollutants including metals on some biochemical parameters in muscles and livers were investigated in this study.

(A) Detection of heavy metals (Fe, Cu, Pb and Hg) in muscles and livers of Catfish :

The results presented in this study revealed a wide significant variation in (Fe, Cu, Pb and Hg) burdens in livers among the different investigated groups , as well in this study a high significant changes in (Fe, Cu, Pb and Hg) were reported in the muscular tissues of Claria lazera trapped from different locations.

In the present study when the previous heavy metals contents for the investigated parts of cat fish (Livers and muscles) were compared with water concentrations of these metals at the investigated areas was shown to be highest for muscles for females as the following (32.82±1.02, 17.96±0.84, 16.92±0.59 and 12.4±0.68) respectively.

Fe levels in livers of male fish collected from (El Maruttia, El Mansouria, El Ismallia and El Khanater) were (85.5±1.47, 222.4±2.56, 74.4±2.54 and 201±1.24) respectively, but in livers of female fishes collected from (El Maruttia, El Mansouria, El Ismallia and El Khanater) were (88.25±1.07, 219.6±4, 77.2±1.66 and 204.8±1.77) respectively, but Fe accumulated only in the muscles of fishes over permissible limits was from El Maruttia males and females, only with the following levels (32.6±0.48 and 32.82±1.02) respectively, while Fe levels in the muscles of (♂,♀) fishes collected from (El Mansouria, El Ismallia and El Khanater) were reported without significant flesh iron accumulation. The presence of a direct relationship between the concentration of iron (Fe) in the flesh and its levels in the livers and muscles may confirm the previous observation. Also the highest bioaccumulation level of iron in spite of the relatively low level in water may be due to affinity of iron to chelate and precipitation with the sediment as recorded by (basholaf, 2009).

All of these above results are agree with the finding of (Sunderlandet al.,2012) who demonstrated that (Fe, Cu, Pb and Hg) and other heavy metals mostly accumulated in liver followed by muscles, other investigations have also shown that the concentration of heavy metals were higher in the liver than in muscles (Sanchez, 2005).Fe % is small and not affect human public health but with time meneral accumulation in fish muscle my accumulated in human liver causing toxicity and cancer. (Amaldin, 2008).

(B) Anti-oxidants (MDA, GSH, Gpx and GST) in livers of catfish:

I- Malondialdehyde (MDA):

Malondialdehyde (MDA) is a lipid degradation product (Roméo et al., 2000). It is formed by peroxidation of ω 3 PUFAs (polyunsaturated fatty acids) and ω 6 PUFAs, and is used as a biological marker of oxidative stress (Dural et al., 2006).

Concerning the effect of heavy metals on MDA, our results agree with that recorded by Farag et al. (2006), they found that lipid peroxidation was increased by 30% in the liver of Oreochromis hornorum fish directly intoxicated with lead. Similar results was recorded by Gravato et al. (2006), they found that cadmium increased hepatic lipid peroxidation in Atlantic croaker and Clarias batrachus fish respectively. On the other hand our result disagree with that observed by Ternjej et al. (2010), they found that liver thiobarbituric acid reactant substances in rainbow trout remained unaffected by cadmium.

II- Reduced glutathione (GSH):

Reduced glutathione (L-γ-glutamyl-L-cysteinylglycine, GSH) is a major cellular antioxidant with numerous key functions (Orun et al., 2008). It plays an important role in metabolism of peroxides and free radicals, protecting cells from lipid peroxidation. It aids in maintenance of mitochondrial calcium content (Heath, 1995).

Kasheir et al.,(2004) attribute the increase of glutathione concentration observed in lead exposed fish to the increase in glutathione synthesis rather than a decrease in utilization.

III- Glutathione peroxidase (Gpx) activity:

Gpx (selenium dependent glutathione peroxidase) is the major effector in relieving oxidative stress by the conversion of GSH to GSSG with concomitant reduction of hydrogen peroxide (H_2O_2) (Sivaperumal et al., 2007). Selenium dependent glutathione peroxidase reduces H_2O_2 (generated by superoxide dismutase) and organic peroxides (produced by the reaction of organic

macromolecules with ROS (Reduced Oxidative Submutase) to water and alcohol respectively (Stephen et al., 2000).

IV- Glutathione-S-transferase (GST) activity:

The glutathione-S-transferases catalyses the conjugation of a wide variety of electrophilic compounds (endogenous substrates as well as xenobiotics) with

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