Effect of industrial waste discharges including heavy metals in Burullus lake on some physiological parameters and antioxidants in *Tillapia niloticus* and *Siluriformes* fish

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Abstract: Background: Most heavy metals have toxic effects and impair function of many animal organs when accumulates inside. In case of fish which consider source of protein to human, heavy metals which accumulates inside its organs transported to human and accumulates inside his organs resulting in organ injury threaten his live. Because burullus lake is polluted with industrial waste products and because fish present in this polluted water and can't escape so, **the aim** is to assess effect of this pollution on fish organs and assessment of heavy metals impairments. **Materials and Methods:** two fish species from two different water Burullus lake and Bahr Tira canal as control used to assess kidney and liver function and antioxidants in muscles and gills also heavy metals were assessed in water of two different regions and both muscles and gills. **Results:** show increase of liver and kidney function tests level and decrease in catalase and glutathione reduced activity in both gills and muscles in addition to elevation of heavy metals levels in burullus lake specimens than Bahr Tira canal specimens. Also some heavy metals show elevation in gills and muscles of fish taken from Burullus lake as general.
[Ahmed D.F.I. **Effect of industrial waste discharges including heavy metals in Burullus lake on some physiological parameters and antioxidants in *Tillapia niloticus* and *Siluriformes* fish.** *N Y Sci J* 2013;6(4):85-92]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 13

Keywords: Industrial waste; Heavy metals; Burullus lake; Antioxidants; Kidney; Liver; Fish

1. Introduction

Fish, apart of being a good source of digestible protein, vitamins, minerals and polyunsaturated fatty acids, are also an important source of essential heavy metals (Irwandi and Farida, 2009).

Unlike organic contaminants that loose toxicity by the time with biodegradation, heavy metals cannot be degraded; their concentration can be increased by bioaccumulation (Aksoy, 2008). Metal bioaccumulation is largely attributed to differences in uptake and depuration period for various metals in different fish species.

Industrial development ; fertilizers; livestock manure; air pollution; increases in pesticide usage and mining have led to increasing levels of heavy metals in aquatic environments (Cooper, 1993).

2. Material and Methods

2.1. Site description

Lake Burullus is one of the largest nile delta brackish water lakes. It occupies more or less the central position of northern delta along the Mediterranean coast of Egypt between longitudes 30º 30 and 31º 10 E and latidudes 31º 21 and 31º 35 N. the water depth in the lake is subjected to strong fluctuation variations from day to day, so that depth varies annually from 50 to 160 Cm. generally the depth increases from east to west and from south to north. In the northeast, the lake is permanently connected to the Mediterranean sea by an opening (EL Boughaz) 50-60m width. The lake receives its water from two main sources the drains and Lake-sea connection. Drain water is discharged through seven drains and Brembal canal, which connects the lake to Rosetta estuary. The in flow of seawater and the drainage water play a predominant role in the hydrographic and chemical conditions of the lake.

2.2. Study areas

lake Burullus is an important lake located in the northern part of Kafr elsheikh governorate, there are seven drains pour in the lake with fresh water one of this drains is the kotshiner drain which bears fresh water but with industrial waste products coming from el Garbeya governorate specially from El Mahalla El Kubra the surface area of the lake is about 460 Km^2 . Samples were collected from two sites one of them from the water area where the Kotshiner canal drains its water, the second site is from another canal called Bahr Tira which contains fresh water coming from the Nile river that pass through Al Mansoura city in Dakahleyya governorate, Bahr Tira samples are for comparing . Fish were collected by fishermen nets and directly after fishing dissected in the field to take different samples from the organs we need to study then the organ samples were put in containers containing ice for preservation to reaching the laboratory for analysis before dissection each fish was measured and weighted using scale for *Tillipia Oreochromis niloticus* the weight vary between 125-140 gm, the length vary between 15-20 cm and for

Siluriformes (catfish) the length 20-25 cm and the weight vary between 200-250 gm. Before analysis in the laboratory, For kidney,liver function testes parts (1.0 gm) of kidney and liver homogenized in 1.0ml (10 % KoH) then centrifuged at 4000 rpm. For 10 min. The supernatant used for the testes.

2.3. Sample Preparation

Fish samples for heavy metals were put onto a dissection tray and thawed at room temperature. They were dissected using stainless steel scalpels and Teflon forceps using a laminar flow bench. A part of the muscle (dorsal muscle without skin) and the gills (1 g) were removed and transferred in polypropylene vials. Before acid digestion, a porcelain mortar was employed to grind and homogenize the dry tissue samples. Aliquots of approximately 1 g wet gills and muscle were digested in Teflon beakers for 12 h at room temperature, and then for 4h at 100°C with 5ml ultrapure nitric acid (65%, Merck) (Zheng et al., 2007).

2.4. Determination of heavy metals in water

Concentrations of heavy metals were determined after the digestion by nitric acid according to (APHA, 1992). Atomic absorption spectrophotometer (Perkin Elmer model 3150.) was used for measuring the optical density for each element.

2.5. Oxidative stress parameters

The gill tissue and muscles were weighted 1.0 gm and homogenized in potassium phosphate buffer solution; homogenates were centrifuged at 4000 rpm, the supernatant was removed, placed on ice, and immediately used for oxidant enzyme measurement. All analysis were performed at room temperature (25-27^{o}C).

2.5.1. Catalase assay

Determined according to Aebi (1984) using Biodiagnostic kits

2.5.2. Glutathione reduced

Assayed according to Beutler (1963) using biodiagnostic kits

2.6. Liver function tests

2.6.1. Colorimetric measurement of tissue glutamic oxaloacetic transaminase GOT (ASAT) and tissue glutamic pyruvic transaminase GPT (ALAT) in tissue activity

Tissue ASAT and ALAT is determined spectrophotometrically using (Bio-Adwic) kit according to Reitman and Frankel (1957).

2.6.3. Measurement of tissue alkaline phosphatase activity

Alkaline phosphatase was determined using kit of Bioadwic and the method of John (1982).

2.6.4. Determination of tissue total protein level

Tissue total proteins were colorimetrically measured according to the method of Doumas (1975), using Bio-Adwic kit.

2.6.5. Estimation of tissue albumin level

Tissue albumin was measured using bromocresol-green (BCG) method according to the method of Doumas (1971) using the kits of centronic GmbH-Germany.

2.6.6. Determination of tissue total bilirubin concentration

Serum bilirubin was measured using Bio-Adwic kit and method of Wooton (1964).

2.7. Kidney function tests

2.7.1. Enzymatic determination of serum urea concentration

Tissue urea estimated according to the colorimetric method described by Patton and Crouh (1977) using the kits of Bio-Adwic.

2.7.2. Enzymatic assessment of tissue uric acid level

Tissue uric acid colorimetrically measured according to the method of Fossati *et al.* (1980).

2.7.3. Colorimetric determination of createnine concentration

Createnine were measured using spectrophotometer according to Bartels *et al.* (1972), using Bio-Adwic kit.

Statistical analysis was carried out using Excel software of Microsoft office.

3. Results

Data in table (1) shows very high significant increase in GOT and GPT in tilapia where the mean was (43.00, 51.30 respectively) and siluriformes(64.60, 62.60 respectively) taken from burullus lake, also data in the same table shows very high significant increase in each of total protein where the mean was (18.00), bilirubin (9.24), and high significant increase in albumin (8.70) and alkaline phosphatase (172.34) in tilapia and very high significant increase in each of total protein (17.70), bilirubin (9.88), albumin (15.30) and alkaline phosphatase (62.78) in siluriformes taken from Burullus lake.

By studying effects of heavy metals on kidney function tests data in table (2) shows very high significant increase in urea and uric acid in siluriformes fish taken from burullus lake where the mean was(91.29) and (16.82) respectively. On the same direction urea shows high significant increase in tilapia fish taken from Burullus lake and the mean was (177.25). Also uric acid and createnine in tilapia taken from burullus lake shows significant increase and the means were (38.75) and (200.80) respectively.

By data analysis using T-test table (3) shows significant decrease in catalase enzyme in gills of tilapia and siluriformes taken from burullus lake when compared with bahr tera canal where means was (0.81) and (0.53) respectively. On the same way glutathione reduced decreased significantly in gills and muscles of both tilapia and siluriformes fish taken from burullus lake when compared with fish taken from bahr tera canal where means were (4.08), (3.62) and (2.39), (1.14) respectively.

Heavy metals shows increase in muscles and gills of tilapia and siluriformes taken from burullus lake as in table (4).

Data in table (5) shows high levels of the following heavy metals Hg, Ni,Co,Pb, Mn,Cu, Zn and Fe in water taken from burullus lake than water taken from Bahr Tera canal.

Table 1. liver function tests measured in liver tissue in *Tilapia oreacromes* and siluriformes taken from Burulluslake and Bahr Tera canal

PARAMETER	Fish / Region	TILLAPIA		SILURIFORMES	
		BAHR TERA	BURULLUS LAKE	BAHR TERA	BURULLUS LAKE
ASAT (U/g)	MEAN ± SD	17.90 ±5.23	43.00*** ±11.21	26.20 ±12.86	64.60** ±31.31
ALAT (U/g)	MEAN ± SD	19.00 ±7.85	51.30*** ±6.00	15.90 ±3.54	62.60*** ±15.84
TOTAL PROTEIN (mg/g)	MEAN ± SD	10.79 ±0.62	18.00*** ±0.64	9.81 ±0.49	17.70*** ±2.32
BILIRUBIN (mg/g)	MEAN ±SD	1.78 ±0.75	9.24*** ±3.02	3.33 ±0.77	9.88*** ±1.35
ALBUMIN (mg/g)	MEAN ± SD	2.15 ±0.54	8.70** ±6.34	4.52 ±1.23	15.30*** ±4.64
ALP (U/g)	MEAN ± SD	27.09 ±7.41	172.34** ±103.33	27.93 ±11.13	62.78*** ±12.31

Comparison of data is between Bhr Tera and Burullus Lake
*** = very highly significant; P≤ 0.001
**= highly significant; P≤0.01
*=significant; P≤0.05 using T-test

Table 2. Kidney function tests measured in kidney tissue in *tilapia oreacromes* and siluriformes taken from Burullus lake and Bahr Tera canal

PARAMETER	Fish / Region	TILLAPIA		SILURIFORMES	
		BAHR TERA	BURULLUS LAKE	BAHR TERA	BURULLUS LAKE
UREA (mg/g)	MEAN ± SD	6.96 ±2.04	177.25** ±137.75	27.58 ±5.77	91.29*** ±27.72
URIC ACID (mg/g)	MEAN ± SD	3.66 ±1.55	38.75* ±48.04	8.91 ±2.65	16.82*** ±2.67
CREATENINE (mg/g)	MEAN ± SD	8.42 ±4.89	200.80* ±234.37	5.43 ±7.11	40.66 ±72.80

Comparison of data is between Bhr Tera and Burullus Lake
*** = very highly significant; P≤ 0.001
**= highly significant; P≤0.01
*=significant; P≤0.05 using T-test

Table 3. Levels of some antioxidants in muscles and gills of *Tillapia oreocromes* and siluriformes taken from Burullus lake and Bahr Tera canal

Fish / Region		TILLAPIA				SILURIFORMES			
		BAHR TERA		BURULLUS LAKE		BAHR TERA		BURULLUS LAKE	
Antioxidant		muscles	gills	muscles	gills	muscles	gills	muscles	gills
CATALASE (U/gm)	**MEAN ± SD**	0.07 ±0.03	1.27 ±0.19	0.15 ±0.13	0.81* ±0.20	0.16 ±0.25	1.02 ±0.19	0.30 ±0.05	0.53* ±0.13
GLUTATHIONE REDUCED (mg/g)	**MEAN ± SD**	5.52 ±0.15	7.11 ±0.79	3.62** ±0.89	4.08** ±0.15	2.66 ±0.53	3.30 ±0.46	1.14** ±0.13	2.39* ±0.31

Comparison of data is between Bhr Tera and Burullus Lake
*** = very highly significant; P≤ 0.001
**= highly significant; P≤0.01
*=significant; P≤0.05 using T-test

Table 4. Levels of some heavy metals in muscles and gills of *tilapia oreocromes* and siluriformes taken from Burullus lake brackish water and Bahr Tera frish water canal

Fish / region	TILLAPIA				SILURIFORMES			
	BAHR TERA		BURULLUS LAKE		BAHR TERA		BURULLUS LAKE	
Heavy metal	**MUSCLES**	**GILLS**	**MUSCLES**	**GILLS**	**MUSCLES**	**GILLS**	**MUSCLES**	**GILLS**
Hg ppb	32.76	34.42	128.90	53.22	36.23	32.98	38.80	90.39
Ni ppm	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Co ppm	00.00	00.00	00.00	0.78	0.29	00.00	00.00	0.11
Pb ppm	9.60	6.83	10.75	8.81	7.28	7.78	8.53	8.44
Mn ppm	1.45	14.60	1.06	13.70	1.69	3.62	1.25	7.85
Cu ppm	0.02	3.33	1.77	1.25	1.12	9.86	1.18	1.19
Cd ppm	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Zn ppm	6.14	12.24	7.44	19.13	6.09	17.44	8.65	30.39
Fe ppm	83.10	159.70	63.20	173.10	82.70	32.40	82.80	31.40

ppb= part per billion
ppm= part per million

Table 5. Levels of some heavy metals in Burullus lake brackish water and Bahr Tera fresh water

Metal / region	Hg PPb	Ni ppm	Co ppm	Pb ppm	Mn ppm	Cu ppm	Cd ppm	Zn ppm	Fe ppm
Burullus lake	0.5413	0.0031	0.0013	0.0075	0.0275	0.0212	0.0011	0.0072	0.4140
Bahr Tera	0.2710	0.0021	0.0007	0.0033	0.0176	0.0072	0.0018	0.0027	0.2060

Ppb= part per billion
Ppm= part per million

4. Discussion

Metal residues problems in the fish tissues are serious, as reflect by the high metal concentrations recorded in the water and sediments (Wong et al., 2001). The gills are directly in contact with water. Therefore, the concentration of metals in gills reflects their concentration in water were the fish lives, whereas the concentration in liver represent storage of metals in the water (Romeo et al., 1999).

Heavy matals are known to reduce the activities of antioxidant enzymes (e.g. superoxide dismutase, Catalase). When the fish were contacted with water containing a single pollutant, superoxide dismutase activity was affected by the presence of Cd but not by methyl parathionine or Zn. However catalase activity were sensitive to all three pollutants. The combined treatment showed that the enzymes could be chosen as biomarkers of joint pollution by both metals and organophosphate.(Ling et al., 2011).

Metallic mercury may cause kidney damage, which is reversible after exposure has stopped. It has also been possible to detect proteinurea at relatively low levels occupational exposure (Langworth et al., 2002).

In organic arsenic is acutely toxic and intake of large quantities leads to gastrointestinal symptoms, severe disturbance of the cardiovascular and central nervous systems, and eventually death. Populations exposed to arsenic via drinking water show excess risk of mortality from lung. Bladder and kidney cancer, the risk increasing with increasing exposure. One of the suggested mechanisms by which arsenic exerts its toxic effect is through an impairment of cellular respiration by inhibition of several carbohydrates enzymes (i.e. gluconeogenesis and glycolysis pathways and the uncoupling of oxidative phosphorylation. There are indications that chronic lead exposure may affect systemic lipid metabolism. Current evidence on lead- induced oxidative stress based mostly on invitro experiments or studies conducted in animals. Many studies have focused on metal-induced toxicity and carcinogenicity, emphasing their role in the generation of reactive oxygen and nitrogen species in biological systems. Metal-mediated formation of free radicals may enhance lipid peroxidation and changes in calcium and sulfhydryl homeostasis. By promoting reactive oxygen species production, lead may trigger a cycle of oxidative stress and inflammation in the target tissues. Depletion of cells major sulfhydryl reserves seems to be an important indirect mechanism for oxidative stress that is induced by redox in active metals, cited by Eman and Gordon (2011).

The decreasing of liver enzymes may be due to effect of heavy metals on liver and kidney which accumulates inside cells and stop or decrease its function. Also may be there is a synergistic effects to these heavy metals to exerts these effects on live and kidney.

My results is supported with decreasing of catalase and glutathione reduced in gills and muscles due to the efficiency of these antioxidants in scavenging heavy metals which lead to decreasing levels of free catalase and glutathione reduced.

Exposure to cobalt resulted in increased levels of lipid peroxides in brain and liver . the activities of primary antioxidant enymes, superoxide dismutase and catalase, were substantially suppressed in brain and liver as a result of Co^{2+} exposure, whereas in kidney catalase activity was unchanged and SOD activity increased. The activities of glutathione – related enzymes, glutathione peroxidase and glutathione –S – transferase, did not change as a result of cobalt exposure, but glutathione reductase activity increased in brain and kidney. Taken together, these data show that exposure of fish to CO^{2+} ions results in the development of oxidative stress and the activation of defense system in different gold fish tissues (Olha et al.; 2011).

Hepatptoxiity is the most common finding in patients with iron overload since the liver is the major recipient of iron excess, even though the kidney could be a target of iron toxicity. The effect of iron overload was studied in the early stages after iron-dextran injection in rats, as a model for secondary hemochromatosis. Total hepatic and kidney iron content was markedly elevated over control values 20 h after the iron administration. Plasma GOT, GPT, AND LDH activities were not affected, suggesting that the liver cell permeability was not affected by necrosis. The activities of catalase, and glutathione peroxidase were determined. Enzymatic activities declined in liver homogenates after iron injection. These activities were not affected in kidney as compared to control values (Galleano and Puntarulo, 1994).

The level of kidney GSH was not changed at either 0.5 or 1 hr. after lead exposure, but increased 3,6,12 and 24h after a single injection of lead. MDA levels (a marker of lipid peroxidation) did not change in kidney following lead injection. We conclude that the increases in GST levels in kidney following lead exposure were not depended on oxidative stress. This study demonstrates that acute lead exposure causes dramatic changes in the subcellular distribution and expression of rat kidney GSTS, and that these changes are not a result of oxidative stress (Daggett et al., 1998).

High-dose cobalt chloride significantly increased AST, ALT, the concentrations increasing in parallel with treatment duration pathological evaluation showed that high-dose cobalt chloride had toxic effects on the heart and liver; however no significant effect was apparent in the kidney (Liu et al., 2010).

The liver tissue was conspicuously damaged and degenerative and necrotic changes were observed in almost every area of the mercury exposed liver tissue . ALT, AST, BIL, CREATENINE, UREA were significantly increased in $HgCl_2$ exposed animals (Mohammad, 2009).

It has also been reported that mercury exposure increased the activities of AST, urea and createnine (Jadhav et al., 2007).

Elevation in the activity of serum ALT, a liver cytoplasmic enzyme, indicates for necrotic lesions in the liver, while a decrease in serum ALP level indicates for no congestion or cholestasis (Rus et al., 2003).

Copper in the blood exist in two forms: bound to ceruloplasmin (85-95%0 and the test “free”

loosly bound to albumin and small molecules. Free copper causes toxicity, as it generates reactive oxygen species such as superoxide, hydrogen peroxide, and the hydroxyl radical. These damage proteins, lipids and DNA (Brewer, 2010).

Mercury is a widespread environmental and industrial pollutant, which induces severe alterations in the tissues (Sener et al., 2007). Mercury poisoning can result from inhalation, ingestion, or absorption through the skin and may be highly toxic and corrosive once absorbed into blood stream. Furthermore, it combines with proteins in the plasma or enters the red blood cells but does not readily pass into the brain or fetus and instead, may enter other body organs (El-Shenawy and Hassan, 2008). The liver is a major site of metabolism for mercury and it can accumulate in the liver, resulting in sever hepatic damages. The results suggest that Hg has detrimental effects on steroid hormone synthesis also at very low concentrations and consecutively on reproductive physiology (Knazicka, 2013).

There is substantial evidence for lead – induced carcinogenesis in experimental animals. Lead acetate and basic lead acetate are carcinogenic when fed to rats and mice, with tumors arising most commonly in the kidneys (Boyland et al., 1962).

Many environ mental pollutants are capable of inducing oxidative stress in aquatic animals, including fish. The oxidative stress resulting from the production of reactive oxygen species has gained considerable interest in the field of ecotoxicology (Lemaire et al., 1996).

Under natural conditions, fish gills are the first target of waterborne pollutants, as gills are the primary sites of uptake for substances dissolved in water and exhibit large surface areas that are in direct contact with noxious substances (Perry and Laurent, 1993). Gills are highly susceptible to adverse environmental conditions, and damage in gill epithelia has been considered a good indicator of the effects of xenobiotics on fish (Korkmaz et al., 2009).

The apparent decrease in the glutathione detoxification system in the gills- the first point of contact with environmental Xenobiotics-indicates that this system is a sensitive biochemical indicator of environmental pollution (Faromi et al., 2007) in fish. A decline in tissue GSH content during exposure to pollution may be due to (1) an increased utilization of GSH, which can be converted to GSSG; and (2) in efficient GSH regeneration.

Increases in SOD and CAT activity are usually observed in the presence of environmental pollutants since the SOD-CAT system represents the first line of defense against oxidative stress. However enzymes in fish can also decrease after exposure to xenobiotics, as was reported by Pandey et al. (2008) the decreased CAT activity may be due to the flux of superoxide radicals, which have been shown to inhibit CAT activity (Kono and Fridovich, 1982).

High concentrations of copper have also been reported to inhibit CAT in liver, gill, and muscle of common carp *Cyprinus carpio* after a 24-h exposure (Radi and Matkovies, 1988).in field conditions, a complex interaction among pollutants can occur, and these interactions can be synergistic or antagonistic (Vasseur and cossu-Leguille, 2003).

5. Conclusion

The study concluded that industrial waste discharges affect greatly on fish that harbor in burullus lake which in turn effects on human due to eating this fish leading to accumulation of heavy metals in human body threaten his life.

6. Recommendation

After these results we recommend with stopping industrial waste to discharges in Burullus lake and until this action happens we advise with avoiding eating fish catches from Burullus lake.

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20/3/2013