Investigating the Oxidative Stress of Heavy Metal's Pollution in *Clarias Gariepinus*

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Abstract

In the current study, abnormal high levels of lead, cadmium, copper, zinc, iron, nickel and chromium were detected in Ismailia channel water. Significant elevation in the levels of such metals was observed in liver and musculature of *Clarias gariepinus* living in this area. Malondialdehyde and reduced glutathione concentrations as well as glutathione-S-transferase and superoxide dismutase activities were significantly increased in the liver of the fish while glutathione peroxidase and catalase activities were significantly decreased.

Introduction

Increasing generation of hazardous wastes is one of the main environmental problems in most countries in the world **(Li, et al., 2015).** Heavy metals are a common type of typical environmental pollutants from effluent and industrial activities, and are hazardous to public health and ecological safety **(Zhu, et al., 2013)**.

Soil serves as the most important sink for heavy metal pollutants in terrestrial ecosystems **(Li, et al., 2013),** and soil heavy metals pollution is a worldwide problem **(Liu, et al., 2006)**. Heavy metals originate from two primary sources: natural background sources and anthropogenic inputs including metalliferous mining and industries, agrochemicals and mineral fertilizers, vehicle exhaust, sewage sludge and industrial wastes **(Zhang, 2006)**. It also results from using metals containing plastics and pesticides. Coal and oil combustion participate in metal pollution **(Shibamoto and Bjeldanes, 1993).**

Heavy metals, when present in excess or under the wrong conditions, can produce multiple toxic effects **(Zhan et al., 2014).** Water pollution by heavy metals impedes fish production. It injures the fish health and disturbs the physiological functions of their organs. Due to their toxicity, long persistence, bio-cumulative and non biodegradable properties in the food chain **(Yuan et al., 2012)**, metals constitute a core group of aquatic pollutants **(El-Bouraie et al., 2011)**. Their accumulation in fish organs and flesh, leads to serious healthy hazardous to the consumers **(Daoud et al., 1999).**

Fish are very nutritious; rich in many vitamins, minerals and all of the essential amino acids in the right proportions **(Savikko et al., 2014).** However, fish absorb heavy metals from the surrounding environment depending on a variety of factors, such as characteristics of a species, exposure period and concentration of the element, as well as biotic factors such as temperature, salinity, pH and seasonal changes **(Ginsberg and Toal, 2009).**

Heavy metals transform O_2 into reactive oxygen species (ROS), which are highly toxic and mutagenic. Heavy metals catalyze the reaction of superoxide anion and H_2O_2 to form a hydroxyl radical, a highly reactive and deleterious ROS that damages many biomolecules **(Winston and Di Giulio, 1991).**

Oxidative stress arises when the rate of ROS production exceeds the rate of quenching, detoxification and biomolecule repair **(Rodríguez-Ariza et al., 2003)**. ROS may react with membrane lipids and start a complex sequence of lipid peroxidation reactions resulting in the formation of lipid degradation products such as malondialdehyde (MDA), alkanals, alkenals, hydroxyalkenal and ketones **(Roméo et al., 2000).**

Antioxidant defense detoxifies ROS. Its major constituents include non enzymatic components such as the vitamins, E and C and the endogenous reducing agents like reduced glutathione (GSH) and other thiols. The enzymatic components of antioxidant defense involve superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx) and glutathione-S-transferase (GST) **(Winston and Di Giulio, 1991** and **Gustafson et al., 1993)**.

The present work was planned to assess heavy metals pollution in Ismailia channel water and *Clarias Gariepinus* fish. In addition, it is aimed to elucidate the effect of this pollution on MDA as a measure of lipid peroxidation, GSH and the antioxidant enzymes; GPx, GST, SOD and CAT in the liver of *Clarias Gariepinus*.

Materials and Methods

1- Water samples and analysis:
Ten water samples were collected in clean and sterile glass bottles from Ismailia channel and from a relatively clean region of Nile River at Al-Kanater Al-Khayreya as reference site. Each water sample was subjected to chemical analysis for the pH, hardness, phosphorus, nitrate, nitrite and ammonia according to **Fresenius *et al.* (**1988*)* and for lead, cadmium, iron, copper, zinc, nickel and chromium by atomic absorption spec-

trophotometer using Unicam 929 AA spectrometer as the procedures described by **Jackson (1973)**.

2- Fish:

Ten Clarias gariepinus of 150-200 g body weight were collected alive from the sites of water collection. The fish were immediately transported in prepared aerated bags to the Fish Disease Res. Dept. in Animal Health Res. Inst. Each group of fish was placed in separate fully prepared glass aquaria (100x50x50 cm$^{3)}$ containing aerated chlorine free tap water. Samples of muscles and livers were collected from each fish for biochemical analysis.

3- Clinical and postmortem examination:

The fishes were clinically examined using the methods described by **Lucky** *(*1977*)* for determining any abnormalities on the external body surface. Gross pathological lesions in gills, abdominal cavity, and internal organs were carried out according to **Amalacher (1970).**

4- Determination of heavy metals:

The fish's livers and musculature samples of both sites were digested according to **Cottenie (1980)** to investigate the lead, cadmium, iron, copper, zenc, nickel and chromium concentrations by atomic absorption spectrophotometric technique using Unicam 929 AA spectrometer according to **Jackson (1973)**.

5- Biochemical analysis:

Liver, samples from each fish of both groups were homogenized according to **Combs** *et al.* **(1987).** The tissue homogenates were subjected to determination of malondialdehyde according to **Albro** *et al.* **(1986),** reduced glutathione according to **Chanarin (1989),** glutathione peroxidase (EC 1.11.1.9) as described by **Paglia and Valentine (1967),** glutathione-S-transferase (EC 2.5.1.18) as adopted by **Habig** *et al.* **(1974),** superoxide dismutase (EC 1.15.1.1) after **Misra and Fridovich (1972)** and catalase (EC 1.11.1.6) according to **Aebi (1974).** The protein was measured in tissue homogenate according to **Lowry** *et al.* **(1951).**

6- Statistical analysis:

Data were presented as mean ± standard error (SE) and the significance of differences was estimated using student t-test by the computer program **SPSS 14 (2006)**

Results and Discussion

Chemical properties of water:

Table (1) shows the pH value and the levels of phosphorus, nitrate, nitrite, ammonia, lead, cadmium, iron, copper, zinc, nickel and chromium in the water of Ismailia channel and Al-Qanater Al-Khayreya (reference**)**. Lead, cadmium, iron, copper, zinc, nickel and chromium levels in Ismailia water were higher than normal limits that stated by **UNEP-GMES (2006) and Swann (1997).**

Clinical signs:

Clarias gariepinus fishes of Ismailia channel exhibited restlessness, increased frequency of opercular beatings, slime secretion exhaustion and paleness of fish body. Lateral disposition and eroded skin were also observed in some fish.

Postmortem findings:

Postmortem (P.M.) findings in Ismailia channel *Clarias gariepinus* included corrosions of gills, pale anemic internal organs as well as loose and soften musculature.

The clinical signs and P.M. findings indicate that the fish undergo stresses as heavy metal's pollution (**Schäperchaw,** *et al.***, 1992**). The heavy metals pollution was confirmed by their limits in water and their residues in fish liver and musculature.

Heavy metals concentration:

Table (2) reveals that the lead, cadmium, iron, copper, zinc, nickel and chromium concentrations in the liver and musculature of *Clarias gariepinus* fish of Ismailia channel were significantly increased against the values of reference fish. These levels are higher than the safe limits according to **FAO (1992)** and **FAO (1983)**.

Toxicological studies have shown that the impact of contaminants on aquatic ecosystems can be evaluated by measuring biochemical parameters in the liver of the fish that respond specifically to the degree and type of contamination **(Barhoumi et al., 2012)**.

Lead can induce oxidative damage through direct effects on the cell membrane, interactions between lead and hemoglobin, which increase the auto-oxidation of hemoglobin. **Maiti** *et al.* **(2010)***,* suggest that the manner and duration of exposure are important factors in lead-induced oxidative stress.

Excessive uptake of iron or disturbances in its regulation can be toxic which is related to its ability to catalyze ROS formation, the deleterious effects of iron include DNA damage, lipid peroxidation (LPO), and oxidation of proteins (**Valko *et al.*, 2005**).

Biochemical analysis:

Table (3) demonstrates that the concentrations of malondialdehyde and reduced glutathione as well as the activities of glutathione-S-transferase and superoxide dismutase enzymes were significantly increased in the liver, of *Clarias gariepinus* comparing with reference fish, while glutathione peroxidase and catalase activities significantly decreased.

The increased malondialdehyde concentration indicates increased lipid peroxidation due to oxidative stress developed by heavy metals. Antioxidant enzyme activities showed an adaptive response and increase in the more polluted areas (**Yildirim et al., 2011**). Lipid peroxition has been suggested to be one of the primary mechanisms of cell injury by xenobiotics (**Recknagel and Glende, 1977**). Transition metals are thought to initiate the lipid peroxidation by catalizing the production of reactive oxygen species (**Green and Hill, 1984**). The stimulatory effect of xenobiotics on lipid peroxidation may also be attributed to impairment of cystolic protective systems including low molecular weight antioxidants such as ascorbic acid and cystolic enzymes such as glutathione peroxidase and catalase (**Palace *et al.*, 1993**)

Reduced glutathione (GSH) plays an important role in metabolism of reactive oxygen species, protecting cells from lipid peroxidation (**Heath, 1995**). The reduced gluthathione levels in the liver of Ismailia catfish were significantly higher than those of the reference fish. Our result is nearly agreed with that of **Allen (1995)** who found that cadmium and lead significantly elevated reduced glutathione concentration in the kidney of *Oreochromis niloticus* fish. The increase of reduced glutathione concentration in the present study may be due to induction of the synthesis of the rate-limiting enzyme, γ-glutamyl-cysteine synthetase by heavy metals (**Zalups and Lash, 1996**).

Glutathione peroxidase (GPx) is the major effector in relieving oxidative stress by the conversion of reduced glutathione to oxidized glutathione with concomitant reduction of hydrogen peroxide (**Stephensen et al., 2002**). Glutathione peroxidase activity in the liver of Ismailia *C. gariepinus* was significantly lower than the reference fish values. The decline in glutathione peroxidase activity contributes greatly to peroxidative damage (**Sugiyama, 1994**). The inhibition in the glutathione peroxidase enzyme activity may be due to alteration of the tertiary and quaternary structure of the enzyme by the

heavy metals (**Bem *et al.*, 1985**) and interaction of heavy metals with tissue selenium necessary for the enzyme synthesis (**Omaye and Tappel, 1975**). Contrary to our results, **Mohsen and Mohammed (2008)** and **Siraj and Usha (2003)** reported that the chronic Cd administration in *Oreochromis mossambicus* resulted in a gradual rise in hepatic antioxidant defense. They also reported that the increase in hepatic GPx shows a possible shift toward a detoxification mechanism under long-term exposure to Cd.

Hepatic glutathione-S-transferase (GST) activity in Ismailia *C. gariepinus* was significantly higher than the reference fish. Glutathione-S-transferase conjugates glutathione to heavy metals as a tool of detoxification (**Nakagawa, 1991**). In addition to detoxification, the induction of Glutathione-S-transferase bearing peroxidase activity during oxidative stress could compensate the loss of selenium dependent glutathione peroxidase activity (**Steinberg et al., 1989**) Hence the increased in the enzyme activity may be an adaptive response protecting the cells from the noxious effects of the heavy metals.

Superoxide dismutase (SOD) enzyme activity was significantly increased in the liver of Ismailia *C. gariepinus* against the reference fish. Cadmium does not generate ROS directly, but can alter glutathione (GSH) levels and influence cell thiol status, inducing the expression of metallothioneins (MTs). Changes in GSH and MTs can lead to lipid peroxidation (LPO) of the cell membrane, and also affects antioxidant enzymes, especially superoxide dismutase (SOD) and catalase (CAT) (**Ercal et al., 2001**). The stimulation of the superoxide dismutase activity may be an adaptive response elicited by the increased production of superoxide radicals in the presence of heavy metal pollution (**Pedrajas et al., 1993**).

Catalase (CAT) enzyme activity was significantly decreased in the liver of Ismailia *C. gariepinus* fishes comparing with the reference fishes. The reduction of the catalase enzyme activity may be due to direct metal mediated structural alteration of the enzyme (**Arillo et al., 1982**), depression of catalase synthesis (**Pruell and Engelhardt, 1980**) and inhibition of the enzyme activity by superoxide radicals (**Kono and Fridovich, 1982**).

The apparent decrease in glutathione detoxification system (glutathione, and glutathione peroxidase), may demonstrate the inefficiency of fish organs in neutralizing the impact of peroxides, resulting in increased lipid peroxidation, or may be related to the decreased availability of glutathione needed to reduce the reactive oxygen species (**Barim et al., 2009**).

Also the decreased CAT activity may be due to the flux of superoxide radicals, which have been shown to inhibit CAT activity **(Stanic et al., 2005)**. Maintenance of high constitutive levels of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) is essential to prevent oxyradical-mediated lipid peroxidation.

Conclusion

Ismailia channel was found to be polluted with lead, cadmium, iron, copper, zinc, nickel and chromium metals. Residues of such metals were found in the liver and musculature of the channel *Clarias gariepinus*. This pollution had tremendous effect on the antioxidants in the fish hepatic tissues and increased lipid peroxidation in these tissues. So it is advisable to protect the water from heavy metal pollution, avoid disposal of waste products in Ismailia channel, prevent use of insecticides and pesticides containing heavy metals adjacent to the channel and prohibit industrial and agricultural drainage in water.

Table (1): Chemical properties of water in Ismailia channel

Parameter	Reference site	Ismailia	Optimal limits
pH	7.0±0.12	8.1±0.23	6.6-9[#]
Hardness(mg/L as CaCO$_3$)	58.7±2.3	99.0±4.1	50-400[#]
Phosphorus(mg/L)	0.00	0.00	3[#]
Nitrate (mg/L)	0.2±0.012	0.5±0.02	3[#]
Nitrite (mg/L)	0.02±0.001	0.3±0.013	0.1[#]
Ammonia (mg/L)	0.001±0.0002	0.005±0.00032	0.0125[#]
Lead (mg/L)	0.05±0.001	0.6±0.023	0.03[#]
Cadmium (mg/L)	0.002±0.0004	0.05±0.001	0.004[#]
Iron (mg/L)	0.052±0.001	0.909±0.012	0.5[#]
Copper(mg/L)	0.193±0.0014	6.21±0.078	0.006[#]
Zinc(mg/L)	0.43±0.003	2.8±0.041	0.05[#]
Nickel (mg/L)	0.001±0.0002	0.011±0.001	0.02[##]
Chromium(mg/L)	0.000	0.092±0.001	0.05[##]

[#]: **Swann (1997)**
[##]: **UNEP-GMES (2006)**

Table (2): Heavy metals concentration in the liver and musculature of *Clarias gariepinus* living in Ismailia channel

Group ⟍ Heavy metal (ppm)	*Liver*		*Musculature*		Maximum Desirable Limits
	Reference	**Ismailia**	**Reference**	**Ismailia**	
Lead (μg/g)	0.37 ± 0.02	6.74 ± 0.20*	0.32 ± 0.05	1.92 ± 0.07*	2.0[#]
Cadmium (μg/g)	0.45 ± 0.07	1.49 ± 0.08*	0.11 ± 0.02	0.49 ± 0.07*	2.0[#]
Iron (μg/g)	75.80 ± 1.50	203.30±1.13*	11.86 ± 0.49	16.24±0.49*	30.0[#]
Copper (μg/g)	9.2 ± 0.74	41.50±0.43*	1.47±0.08	3.91 ± 0.15*	30.0[#]
Zinc (μg/g)	22.3 ± 0.70	29.60±0.82*	3.00 ± 0.12	5.49 ± 0.37*	40.0[#]
Nickel (μg/g)	0.26 ± 0.02	1.00 ± 0.04*	0.16 ± 0.01	0.46 ± 0.03*	0.6[##]
Chromium (μg/g)	0.20 ± 0.06	0.66 ± 0.02*	1.56 ± 0.07	3.18 ± 0.07*	1.0[##]

Each value represents mean ± S.E.; n = 10.
*: Significant difference against reference by t-student test at $p \leq 0.01$.
[#]: **FAO (1992)**
[##]: **FAO (1983)**

Table (3): Malondialdehyde and antioxidants in the liver of *Clarias gariepinus* living in Ismailia channel

Organ ⟍ Parameter	Liver	
	Reference	**Ismailia**
Malondialdehyde (nM/mg protein)	0.802 ± 0.034	1.74 ± 0.087*
Reduced glutathione (μM/mg protein)	66.8 ± 2.27	87.4 ± 1.66*
Glutathione peroxidase (U/mg protein)	122.5 ± 0.72	95.6 ± 1.76*
Glutathione-S-transferase (U/mg protein)	183.3 ± 2.45	205.7 ± 0.94*
Superoxide dismutase (U/mg protein)	23.5 ± 0.52	37.1 ± 0.92*
Catalase (U/mg protein)	55.4 ± 1.15	37.1 ± 0.89*

Each value represents mean ± S.E.; n = 10.
*: Significant difference against reference by t-student test at $p \leq 0.05$

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