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Assessment of Toxicity Induced by Different Domestic and Industrial Wastes in Nile Tilapia (*Oreochromis niloticus*)

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Abstract: The River Nile represents the principle freshwater resource. It represents the major source for the potable water which is susceptible to be polluted by different metals. Heavy metals are the most common pollutants in aquatic environment. The present study aimed to evaluate the adverse effect of heavy metals on in liver and kidney tissues of Nile Tilapia (*Oreochromis niloticus*) that were collected from El-Kanater El-Khayria and two discharge points (El-Rahawy drain and Kafr El-Zayat industrial area) at Rosetta branch, River Nile, Egypt. During the present study, it was found that concentration of Lead (Pb), Cadmium (Cd), Iron (Fe) and Zinc (Zn) elevated significantly ($P<0.05$) in livers of fishes living in Rahawy and Kafr El-Zayat with respect to liver of fishes living in water of kanater. No differences observed significantly in concentration of Nickle (Ni), Chromium (Cr), Cobalt (Co) and Copper (Cu). On the other hand, it was noticed that concentration of Pb, Cd, Ni, Fe and Zn increased significantly ($P<0.05$) in kidney of fishes living in Rahawy and Kafr El-Zayat with respect to kidney of fishes living in water of kanater. There were no significant differences in concentration of Co and Cu. Bioaccumulation of the various heavy metals in liver and kidney tissues of fishes caused different mutagenicity in the native protein and isoenzymes detected electrophoretically. These alterations were represented qualitatively by hiding of the normal bands or appearance of abnormal bands with different relative mobilities and quantities. Moreover, these abnormalities might occur quantitatively by changing quantities of the qualitatively normal bands. The study concluded that presence of the heavy metals with higher concentration could be toxic and affect fishers and other aquatic organisms and this is worrisome in view of the health implications for the population that need fish resources to cover the food requirements.

Keywords: Heavy Metals, *Oreochromis niloticus*, Electrophoresis, Protein, Isoenzymes

Introduction

River Nile is the life donor to Egypt. It represents the principle resource of freshwater that required to cover nearly all demands for drinking water and irrigation [1,2]. It is the main source for the potable water which is susceptible to be polluted by different metals [3,4].

Geology of the catchments soil is considered as the main source of heavy metals that enter river water. Heavy metals are considered as common pollutants in aquatic environment. Because of the different toxicities induced by heavy metals, the exposure to heavy metals is a serious problem for human life [5]. Heavy metals that are more widely distributed include Iron (Fe), Zinc (Zn), Cadmium (Cd) and Lead (Pb). Fe plays an essential role for growth and wellbeing of living organisms. It is a component of iron-porphyrins haem and ferroxins and Zn exhibits low toxicity to human, but relatively high toxic to fish. It was found that Cd appeared with high toxicity to fish and other aquatic organisms [6]. Furthermore, Cd causes human toxicity leading to many diseases [7].

Although some heavy metals (Fe, Zn, Nickle (Ni), Manganese (Mn), Cobalt (Co) and Copper (Cu)) exhibit beneficial effect on metabolic pathways and normal growth, they exhibit adverse effect leading to toxicity when their concentrations rises to supra-optimal values. Furthermore, they alter various physiological processes at cellular/molecular levels through inhibiting enzymes, blocking functional groups of important macromolecules, displacing or substituting for essential elements and disrupting integrity of the cellular membranes. Consequently, the toxicity induced as a result of the exposure to heavy metals was expressed by enhancing production of Reactive Oxygen Species (ROS) and this was attributed to the interference with activities of the electron transport system [8,9].

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The pollution sources which potentially affect water quality at Rosetta branch were categorized into two main sources. The El-Rahawy drain is the first pollution source that receives all sewage of El-Giza governorate in addition to agricultural and domestic wastes of El-Rahway village. These wastes discharge directly without treatment into the branch [10]. The second source is Kafr El-Zayat industrial area which includes the industrial effluents from the factories of super phosphate and sulfur compounds, oil and soap industries and pesticides factories [11].

In aquatic system, fishes are considered as one of the most important biomonitors for estimating degree of metal pollution [12]. Liver and kidney are the most specific organs that are responsible for major vital functions, such as producing antibody against blood born antigens, excretion, accumulation and biotransformation of xenobiotics in the fish [13,14]. For this reason, these organs were selected to be under the current study. The present study aimed to evaluate the adverse effect of heavy metals on in liver and kidney tissues of Nile Tilapia (*Oreochromis niloticus)* that were collected from El-Kanater El-Khayria and two discharge points (El-Rahawy drain and Kafr El-Zayat industrial area) at Rosetta branch, River Nile, Egypt.

Materials and Methods

Collection of samples

The samples were consisting of fishes (Tilapia) isolated from different areas with various sources of contamination. According to the complete environmental analysis, three areas (Kanater, Rahawy and Kafr El-Zayat) were selected to be under the present study. The fishes were collected from these areas and arranged as three groups then prepared for the analytical and electrophoretic studies.

Preparation of the samples

Liver and kidney tissues were excised from fishes (Tilapia) isolated from three different areas. After washing the tissues in cold phosphate buffered saline, they freezed rapidly with liquid nitrogen and ground then homogenized in 0.05 M Tris-HCl buffer (pH 7.4). The homogenates left in refrigerator overnight and vortexed for 15 s then centrifuged at 10,000 rpm for 15 min. The supernatants with water-soluble proteins were transferred to new eppendorf tubes and kept at deep-freeze until the electrophoretic analysis.

Determination of heavy metals in the tissue homogenates

Heavy metals (Lead (Pb), Cadmium (Cd), Nickle (Ni), Iron (Fe), Manganese (Mn), Zinc (Zn), Chromium (Cr), Cobalt (Co) and Copper (Cu)) were digested by adding 1 mL of concentrated nitric acid (HNO_3) purchased from Aristar Co to acidify pH<2. The digestion process was carried out in the microwave using ultraviolet light to convert these metals to the nitrated form to be suitable to be measured. This assay was carried out using Inductive Coupled Plasma–Mass spectrometer System (ICP-MS) manufactured by Varian Co according to method suggested by The metals were measured by the instrument from the calibration curve plotted automatically by software.

Statistical analysis

All data were expressed as mean ± standard error (SE). They were statistically evaluated by one-way analysis of variance test (one-way ANOVA) followed by Least Significant Difference (LSD) test and confirmed by Benferoni test. A "P" value of less than 0.05 was considered to indicate statistical significance.

Assay of protein concentration

Samples were collected from each group and pooled together then used as one sample. Protein concentration was estimated in all pooled samples according to method of [15] using standard of bovine serum albumin.

Electrophoretic protein pattern

To determine the relative mobility (Rf), molecular weight (Mwt) and band percent (B %) of the isolated proteins, the native 10% polyacrylamide gel electrophoresis of samples was carried out based on method described by Laemmli (1970) using Mini-gel electrophoresis (BioRad, USA), with the modification that samples, gels and running buffers were lacking Sodium Dodecyl Sulphate (SDS). The gels contained Acrylamide/Bis (30% T, 2.67% C) (Acrylamide: bis-acrylamide=29.2:0.8) and 10% glycerol. The gel was run in buffer containing Tris (24 mM) and glycine (194 mM) at room temperature. Five microletre of the marker loaded in the first well with the samples each run. After completing the electrophoretic run, protein bands were visualized by staining with Coomassie Brilliant Blue G-250 and destained overnight with 7% (v/v) glacial acetic acid after documentation [16]. The molecular weights of the separated proteins were estimated in comparison to marker of standard molecular weights with regularly spaced bands ranging from 6.458 to 195.755 KDa. Also, the native gels were stained for lipid and calcium moieties with Sudan Black B (SBB) [17] and Alizarin Red 'S' [18,19] respectively.

Electrophoretic isoenzyme pattern

The native gel was stained for electrophoretic

catalase and peroxidase patterns according to methods suggested by [20,21] respectively. After developing the colored bands of enzyme activity, the reaction was stopped by fixing the gels in 7% glacial acetic acid for 30 min, followed by preserving the gel in 5% acetic acid prepared in 10% methanol.

Data analysis

The gel plates were photographed by gel documentation system then analyzed using Quantity One software (Version 4.6.2). The Similarity Index (SI) and Genetic Distance (GD) were calculated using equation suggested by [22] for comparing all groups.

Results

Data compiled in Table 1 showed that concentration of Pb, Cd, Fe and Zn increased significantly ($P<0.05$) in liver of fishes living in Rahawy and Kafr El-Zayat with respect to liver of fishes living in water of kanater. While Mn concentration increased significantly ($P<0.05$) in liver of fishes living in Kafr El-Zayat as compared to liver of fishes living in kanater water. No differences were statistically observed in concentrations of Ni, Cr, Co and Cu. On the other hand, it was emphasized that concentration of Pb, Cd, Ni, Fe, Mn and Zn elevated significantly ($P<0.05$) in kidney of fishes living in Rahawy and Kafr El-Zayat with respect to kidney of fishes living in water of kanater. While Cr concentration elevated significantly ($P<0.05$) in kidney of fishes living in Rahawy as compared to kidney of fishes living in kanater water. There were no significant differences in concentration of Co and Cu.

As compared to the electrophoretic protein pattern in liver of fishes living in kanater water (Figure 1a), elevation of the heavy metals in Rahawy water caused mutagenicity represented qualitatively by hiding 3 normal bands. Moreover, the heavy metals in Kafr El-Zayat water caused disappearance of one normal band with existence of 2 abnormal ones at Rf 0.40 (B% 11.70 and Qty 97.26) and 0.72 (B% 11.79 and Qty 24.06). On the other hand, with respect to the electrophoretic protein pattern in kidney of fishes living in kanater water, elevation of the heavy metals in Rahawy water caused qualitative mutagenicity represented by disappearance of one band with appearance of 2 abnormal bands at Rf 0.30 (B% 15.99 and Qty 12.67) and 0.59 (B% 15.77 and Qty 12.41). Moreover, the heavy metals in Kafr El-Zayat water caused hiding one normal band with appearance of abnormal one at Rf 0.59 (B% 18.90 and Qty 13.81).

With respect to the electrophoretic lipid meioty of native protein pattern in liver of fishes living in kanater water (Figure 1b), elevation of the heavy metals in Rahawy water caused mutagenicity expressed qualitatively by hiding of one normal band. Moreover, the heavy metals in Kafr El-Zayat water caused disappearance of one band with appearance of one abnormal band at Rf 0.67 (B% 25.03 and Qty 34.82). Furthermore, as compared to electrophoretic lipoprotein pattern in kidney of fishes living in kanater water, elevation of the heavy metals caused qualitative abnormalities with the same degree in Rahawy and Kafr El-Zayat water represented by hiding one normal band.

As compared to the electrophoretic calcium

Table 1: Heavy metals in liver and kidney tissues of tilapia fishes collected from three different areas (Kanater, Rahawy and Kafr El-Zayat).

		kanater		Rahawy		Kafr El-Zayat	
		Liver	Kidney	Liver	Kidney	Liver	Kidney
Pb	mg/L	0.003 ± 0.000	0.002 ± 0.00	0.005 ± 0.000*	0.004 ± 0.000*	0.012 ± 0.000*	0.006 ± 0.000*
Cd		0.000 ± 0.000	0.000 ± 0.000	0.003 ± 0.000*	0.003 ± 0.000*	0.005 ± 0.000*	0.004 ± 0.000*
Ni		0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.003 ± 0.000*	0.028 ± 0.000*	0.006 ± 0.001*
Fe		0.394 ± 0.024	0.344 ± 0.003	0.980 ± 0.020*	0.628 ± 0.018*	0.883 ± 0.013*	0.500 ± 0.004*
Mn		0.052 ± 0.032	0.012 ± 0.000	0.024 ± 0.000	0.016 ± 0.000*	0.057 ± 0.003	0.022 ± 0.001*
Zn		0.002 ± 0.000	0.002 ± 0.000	0.004 ± 0.000*	0.004 ± 0.000*	0.005 ± 0.001*	0.004 ± 0.000
Cr		0.000 ± 0.000	0.011 ± 0.001	0.000 ± 0.000	0.014 ± 0.000*	0.004 ± 0.000*	0.013 ± 0.000
Co		0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.002 ± 0.000*	0.000 ± 0.000
Cu		0.015 ± 0.000	0.000 ± 0.000	0.016 ± 0.000	0.000 ± 0.000	0.021 ± 0.001*	0.000 ± 0.000

*: p < values compared to control sample (kanater water) (significant $p<0.05$)

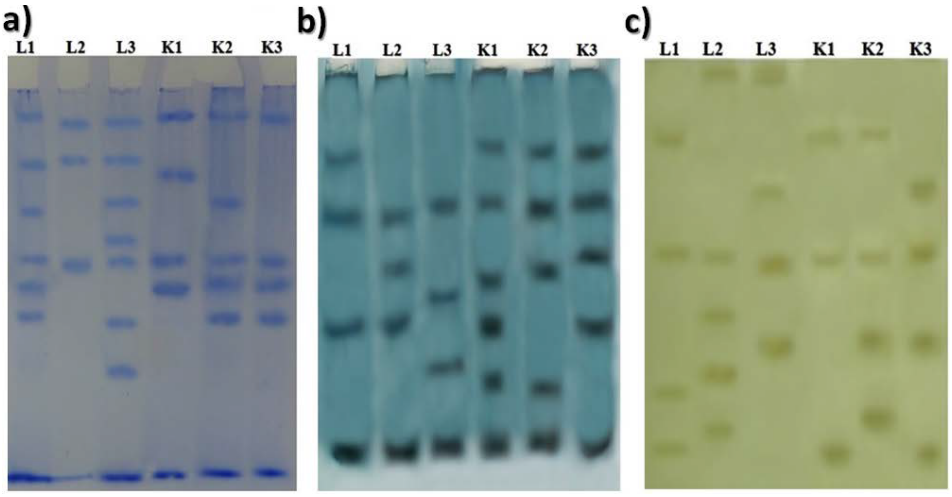

Figure 1: Native electrophoretic patterns showing a) native protein pattern, b) lipid meioty of native protein and c) calcium meioty of native protein pattern in liver and kidney tissues of tilapia fishes collected from different contaminated areas with different enviromental conditions.
L1 and K1: Liver and kidney tissues collected from fishes living in Kanater water
L2 and K2: Liver and kidney tissues collected from fishes living in Rahawy water
L3 and K3: Liver and kidney tissues collected from fishes living in Kafr El-Zayat water

meioty of native protein pattern in liver of fishes living in kanater water (Figure 1c), elevation of the heavy metals in Rahawy water caused mutagenic disturbances expressed at the qualitative level through disappearance of 3 bands with appearance of 5 bands at Rf 0.02 (B% 17.25 and Qty 84.11), Rf 0.47 (B% 15.98 and Qty 70.76), Rf 0.61 (B% 17.25 and Qty 100.00), Rf 0.76 (B% 17.25 and Qty 93.30) and Rf 0.89 (B% 17.25 and Qty 92.65), respectively. Moreover, the heavy metals in Kafr El-Zayat water caused disappearance of 3 bands with appearance of 3 abnormal bands at Rf 0.05 (B% 24.63 and Qty 76.99), 0.31 (B% 23.82 and Qty 74.17) and 0.69 (B% 25.71 and Qty 100.00). As compared to electrophoretic calcium meioty of native protein pattern in kidney of fishes living in kanater water, elevation of the heavy metals in Rahawy water caused mutagenicity represented qualitatively through hiding one normal band with appearance of 2 abnormal ones at Rf 0.67 (B% 26.21 and Qty 100.00) and 0.86 (B% 26.85 and Qty 95.56). Moreover, the heavy metals in Kafr El-Zayat water caused disappearance of one band with appearance of 2 abnormal bands at Rf 0.30 (B% 24.60 and Qty 92.60) and 0.67 (B% 25.09 and Qty 100.00).

As compared to the electrophoretic catalase pattern in liver of fishes living in kanater water (Figure 2a), presence of heavy metals in Rahawy water at these elevated levels exhibited mutagenicity expressed at qualitative level through hiding one normal band with existence of another one at Rf 0.57 (B% 34.24

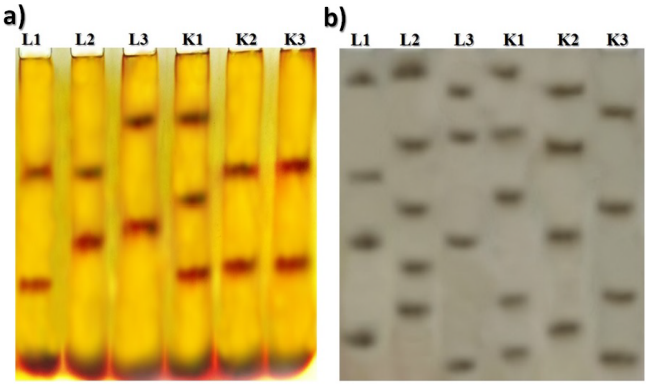

Figure 2: Native electrophoretic isoenzyme pattern showing a) catalase pattern and b) peroxidase pattern in liver and kidney tissues of tilapia fishes collected from different contaminated areas with different enviromental conditions.
L1 and K1: Liver and kidney tissues collected from fishes living in Kanater water
L2 and K2: Liver and kidney tissues collected from fishes living in Rahawy water
L3 and K3: Liver and kidney tissues collected from fishes living in Kafr El-Zayat water

and Qty 35.68). Moreover, the heavy metals in Kafr El-Zayat water caused disappearance of one band with appearance of 2 abnormal bands at Rf 0.21 (B% 36.72 and Qty 100.00) and 0.53 (B% 33.87 and Qty 74.13). As regard to the electrophoretic catalase pattern in kidney of fishes living in kanater water, elevation of the heavy metals in Rahawy and Kafr El-Zayat water media caused mutagenicity with the same qualitative degree through disappearance of 2 bands with appearance of one abnormal at Rf 0.36 (B% 35.15 and Qty 71.97) and 0.35 (B% 36.68 and Qty 97.08), respectively.

As compared to the electrophoretic peroxidase pattern in liver of fishes living in kanater water (Figure 2b), elevation of the heavy metals in Rahawy water caused mutagenicity expressed through hiding 3 normal bands with presence of 4 abnormal ones at Rf 0.28 (B% 19.83 and Qty 99.53), 0.48 (B% 19.70 and Qty 90.17), 0.66 (B% 19.70 and Qty 94.67) and 0.79 (B% 19.70 and Qty 100.00). Moreover, the heavy metals in Kafr El-Zayat water caused disappearance of all bands with appearance of 4 abnormal bands at Rf 0.12 (B% 25.25 and Qty 100.00), 0.26 (B% 25.25 and Qty 97.09), 0.58 (B% 24.42 and Qty 95.01) and 0.97 (B% 25.08 and Qty 90.58). As regard to electrophoretic peroxidase pattern in kidney of fishes living in kanater water, elevation of the heavy metals in Rahawy and Kafr El-Zayat water matrix caused abnormalities represented qualitatively by differences in arrangement and number of bands.

Discussion

It is well known that heavy metals accumulate in an organism's body with different rates vary from organ to organ. These findings were supported by results of the present study that showed that the heavy metals accumulate with different concentrations in liver and kidney tissues. There was an equilibrium between concentration of the heavy metals in an organism's environment and its rate of ingestion and excretion [23,24]. Bioaccumulation of metals within an organism results from interactions between physiological factors (growth, weight loss, absorption and accumulation), chemical factors (metal concentration, speciation and bioavailability) and environmental factors (temperature and food concentration) [25]. The fish liver plays a primary role in the metabolism and excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions [26]. The current study revealed the electrophoretic alterations in the fish liver and kidney tissues. This might refer to ability of the metals to change activities of the hepatic enzymes leading to histopathological hepatic changes. Furthermore, the adverse effect of heavy metals depends on the metal type and concentration and length of exposure [27]. The alterations were detected electrophoretically in native protein and lipoprotein patterns and these might be due to effect of the heavy metals especially Pb and Cd which have high affinity for thiol groups, turn proteins and peptides susceptible to structural modifications in sub-cellular compartments and tissues [28]. In the present experiment, alterations in the electrophoretic calcium meioty of native protein pattern in liver and kidney tissues. This may be related to contamination with Cd that alters calcium homeostasis [29]. Moreover, the abnormalities detected electrophoretically in CAT and Gpx patterns might be attributed to presence of Cd that exhibits the ability to alter the cell adhesion and the cellular antioxidant defense mechanisms [30]. On the other hand, accumulation of Cu in fish organs when exposed to toxic concentrations [31] which can lead to redox reactions generating free radicals and, therefore, may cause biochemical and morphological alterations [32,33]. Also, Pb residues could result in various biochemical dysfunctions in the fish organs that selected to be under the present study. The prolonged exposure to Pb may also cause chronic liver and nephropathy. Alterations of the different electrophoretic patterns may refer to effect of Pb which inhibits enzymes and alters cellular calcium metabolism [34]. In parallel, Cd is a widespread environmental pollutant that is highly toxic and is considered to have no biological function [35]. It causes severe membrane integrity damage with a consequent loss of membrane-bound enzyme activity which can result in cell death [36,37]

Conclusion

The present results showed that, the fishes, based on the higher levels of metal bioaccumulation, could be unsafe for human consumption. The finding is worrisome in view of the health implications for the population that need fish resources to cover the food requirements. Consequently, it is necessary to monitor the heavy metal in the areas which are considered as sites for fish collection and susceptible to be contaminated with heavy metals.

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