Environmental Research Journal 4 (3): 235-243, 2010
ISSN: 1994-5396

Evaluation of the Use of Protein Electrophoresis of the African Catfish *Clarias gariepinus* (Burchell, 1822) for Biomonitoring Aquatic Pollution

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Abstract: Water pollution is one of the principal environmental and public health problems Egyptian river Nile are facing. The objective of this study was to investigate pollution induced changes in the protein electrophoresis of the African catfish (*Clarias gariepinus*) from El-Madapigh canal (one of the river Nile tributary at Assiut which receives quantities of sewages, industrial and agricultural effluents) and river Nile at Assiut for comparison. The present research recorded lower mean value of dissolved oxygen and higher mean values of turbidity, conductivity, alkalinity, chemical oxygen demand, nitrate, nitrite and trace metals in the water of El-Madapigh canal comparing to the Nile water. Orthophosphate and sulfide were detected only in the water of El-Madapigh canal. For all heavy metals (except Cu and Zn) the detected concentration in the tissues of *Clarias gariepinus* collected form El-Madapigh canal were higher than those collected from Nile water. This was expected due to the fact that the water of such canal receives large quantities of domestic, agricultural and industrial effluents. This heavy pollution in El-Madapigh canal also explains the presence of female fishes only in the water of this canal. The results of the present study revealed that both quantitative and qualitative differences in the banding pattern of serum and muscles proteins of the African catfish *C. gariepinus* collected from Nile water and El-Madapigh canal were recorded. A remarkable reduction in the number of protein bands was recorded in fishes collected form El-Madapigh canal comparing to those collected from the Nile water. Also, the present results recorded a remarkable increase in the intensity of α-Globulin, β-Globulin, γ-Globulin in the serum of fishes collected from El-Madapigh canal. The alteration in protein banding patterns and intensity observed in the present study may be attributed to the pollutant induced inhibition of protein synthesis. These results clearly show that protein electrophoreses is a sensitive tool for biomonitoring aquatic pollution.

Key words: Protein electrophoresis, African catfish *Clarias gariepinus*, rive Nile, aquatic pollution

INTRODUCTION

Contamination of aquatic ecosystems has been receiving increased worldwide attention and the literature has many publications on this (Mansour and Sidky, 2002; Ozoh, 1980; Ali and Soltan, 1996). Water pollution is one of the principal environmental and public health problems Egyptian river Nile are facing (Anwar, 2003). The river Nile and its tributaries are the principal freshwater resource for the country, meeting nearly all demands for drinking water, irrigation and industry (Mohamed *et al*., 1998). The Nile is also a primary receptor of wastewater and irrigation return flow (Mohamed *et al*., 1998). During its transit through Egypt, the river Nile and its tributaries receive numerous non-point and point source discharges (Mohamed *et al*., 1998). The most possible sources of water pollution are domestic and industrial effluents. El-Madapigh canal is one of the river Nile tributary at Assiut and it receives quantities of sewages, industrial and agricultural effluents.

In aquatic ecosystems, fish are regarded as bio-indicators of overall system health. Fish can be affected directly or indirectly. The direct effects are initiated at the lower level of biological organization (molecular level). Indirect affects are where the effect is on the food chain and the behavior of the organism (Osman *et al*., 2007a, b). The African catfish *Clarias gariepinus* is among the most widespread freshwater fishes in Africa (Nguyen and Janssen, 2002). It inhabits tropical swamps, lakes and rivers (De Graaf and Janssen, 1996). The economic importance of this species has increased tremendously in recent years as a result of its

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extensive use in aquaculture (De Graaf and Janssen, 1996). Besides being an excellent candidate for aquaculture, *C. gariepinus* has also been used in fundamental research and for ecotoxicological studies (Nguyen *et al*., 1997, 1999; Nguyen and Janssen, 2002; Olaifa *et al*., 2003; Osman *et al*., 2007a, b, 2008; Zimmerman, 1975).

Many studies have been used protein electrophoresis as a valid method of determining intra and inter-specific variation among species, identifying population, determining the genetic variation between natural populations of various animals and for the taxonomic purposes (Zimmerman, 1975; Kilpatrick and Zimmerman, 1976; Jeng *et al*., 1973; Nakagawa *et al*., 1988). In contrast, very few literatures are available concerning with the use of protein electrophoresis in the monitoring of pollution including John and Jayabalan (1993), Kekic and dos Remedios (1999), Pan and Dutta (2000), Muthukmaravel *et al*. (2007) and Osman *et al*. (2009).

The present study was aimed to evaluate the use of protein electrophoreses in the monitoring of aquatic pollution by studying the electrophoretic patterns of serum and muscle protein of the African catfish *Clarias gariepinus* collected form El-Madapigh canal (which receive large quantities of domestic, agricultural and industrial effluents) and Nile water which for comparison.

MATERIALS AND METHODS

Study area: The required water and fish samples were collected in triplicates from:

- El-Madapigh canal (one of the river Nile tributary at Assiut) which receives quantities of sewages, industrial and agricultural effluents
- The river Nile at Assiut Governorate

Samples collection: In the present study, 60 live specimens (245-430 mm in SL) of *Clarias gariepinus* were collected from the Nile and from El-Madapigh canal at Assiut, Egypt. The Standard Length (SL), Weight (W) and sex were recorded for each specimen. The randomly collected fishes from the Nile water gave us some males and some female fishes. In contrast all the collected fish from El-Madapigh canal were females only. Fishes were transported a live to the laboratory for subsequent tissues and electrophoretic analysis. Water sample were collected by polyvinyl chloride Van Dorn bottle 2 m depth at the selected sites. Water samples were kept into a 1 L polyethylene bottle in ice box and analyzed in the laboratory.

Water analysis: Some of the physicochemical parameters including the electrical Conductivity (Cond), pH, water temperature (Temp.) and Turbidity (Terb.) measured by using water checker U-10 Horiba Ltd. The other water criteria (Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Nitrite (NO_2), Nitrate (NO_3), Alkalinity (Alkal), Chloride (CL) Florid (F) Sulfate (SO_4), Sulfide (S), Orthophosphate (O-PO $_4$) measured according to the traditional manual methods of American Public Health Association (APHA, 1995).

Total Pb, Cu, Cr, Mn, Zn, Hg, Fe, Cd were measured after digestion using Graphite Furnace AA (GFAA) spectroscopy. A mixture of nitric acid and the material to be analysed refluxed in a covered Griffin beaker. After the digestate has been brought to a low volume, it cooled and brought up in dilute nitric acid (3% v/v). The sample filtered, allowed settling and preparing it for analysis. It is to be noted that the results obtained for most parameters in the river Nile water relatively closed to each other, therefore the average calculated by the end of the measurement period. Also, the correlation coefficients between the quality parameter pairs of the river water samples calculate in order to indicate the nature and the sources of the polluting substances.

Tissues analysis: Fresh fish samples (*Clarias gariepinus*) collected by using long line or nets from the selected sites. Muscles, gills, gonads and liver were transported in liquid nitrogen container to the laboratory for chemical analysis. These organs washed with tap water (previously analyzed for Pb and Cd) followed by bi-distilled water, then oven-dried to constant weight at 105°C. The dried fish crushed and powdered in an agate mortar, then they were kept in polyethylene bottles for analysis. One gram portions of fish tissues digested by means of a microwave after addition of nitric acid and hydrogen peroxide.

The results are calculated in milligram per kilogram wet weight. Chemicals concentration analysed according to German industrial standard, DIN 38406-6 (DEV, E6) with an Atomic Absorption Spectrometer using flame and graphite furnace technique.

Electrophoresis: Blood samples were taken from the caudal vein into a centrifuge tube and were left to coagulate for 15-20 min. Blood was centrifuged for 12 min at 3000 rpm. After centrifugation, the fresh serum was subjected immediately to electrophoresis. From each fish specimen a sample of raw flesh (5 g) was taken from the dorso-lateral part of the body (at the level below the dorsal fin). As recommended by Partington and Mills

(1988), this raw flesh was homogenized with 0.01 M Tris-HCl (pH 8.6) equal to one and half times its weight in a chilled glass homogenzer. The homogenates were centrifuged at 12.0 rpm for 5 min and the supernatant stored under refrigeration for electrophoresis.

Blood serum and skeletal muscle proteins were analyzed by electrophoresis according to the procedures mentioned in Helena Laboratories (1984). In such procedure, Titan III cellulose acetate plates, Electra HR buffer of a pH of 8.6, Ponceau S stain were used. The electrophoresis was performed at 300 V across the plates for 30 min. Electrophoretic protein were scanned and graphed by Auto Scanner Flur-Vis and emphasized by Gel Pro Analyzer (Media-Cybernetics, 1988) to reveal the densitometry tracings and to estimate the relative concentration (%) of protein bands.

Statistical analysis: All values from chemical analyses and electrophoresis were presented as mean±SD. Data obtained from the experiment were subjected to one way Analysis of Variance (ANOVA) test using the Statistical Package for the Social Sciences (SPSS, 1998). The correlation coefficients between the quality parameter pairs of the water samples were calculated by the application of Pearson correlation analysis (SPSS, 1998).

RESULTS AND DISCUSSION

Water analysis: The results of means and SD of the studied physical and chemical parameters for water samples from the selected sites are given in Table 1. The pH measurement is one of the most important and frequently used tests in water chemistry (APHA, 1995). The pH value is considered to be an important factor in the chemical and biological system of aquatic environment. pH of the Nile water was (8.2) and for El-Madapigh canal was (7.5). The relatively lowest pH of El-Madapigh canal water can be attributed to the discharge of effluents which loaded with a large amount of organic acids.

The mean value of the turbidity of the water collected from the EL-Madapigh canal was very high (33.7 NTU) comparing to the mean value of the turbidity of the Nile water (6.5 NTU).

Such increase may be due to the disposal of domestic and industrial effluent in this canal. Table 1 shows that the conductivity values of water samples collected from El-Madapigh canal was higher than that collected from Nile water and generally the detected conductivity values of the water samples from both sites are in the permissible level. The results of the present work revealed that the Dissolved Oxygen (DO) of the water from El-Madapigh canal was 2.00 and it was 5.5 for the Nile water. The decrease in DO might be due to increase in oxidative processes of organic matter (Abdel-Satar and Elewa, 2001). The introduction of excess of organic matter may result in a depletion of oxygen from an aquatic system (Maria *et al*., 2000). Prolonged exposure to low dissolved oxygen level will increase organisms susceptibility to other environmental stress. Low levels of dissolved oxygen can be associated with excessive algae and plant productivity.

Table 1: Mean and SD of some physical and chemical parameters of the water samples collected from El-Madapigh canal and Nile water at Assiut, Egypt

Parameters	Nile water	El-Madapigh canal
Temperature (°C)	26.4±1.8	16.33±0.58
PH	8.23±0.25	7.52±0.19
Turb. (NTU)	6.52±0.11	33.67±22.03
Cond	0.22±0.16	0.68±0.24
DO	5.5±0.0	2.00±1.32
COD (ppm)	9.25±0.96	107.00±79.16
TDS (ppm)	224±50.03	358.33±180.02
TSS (ppm)	17.25±6.55	46.67±22.55
TS (ppm)	221.5±20.24	405.00±170.88
NO_2 (ppm)	0.003±0.001	0.04±0.03
NO_3 (ppm)	0.64±0.29	4.42±1.01
Alkal (ppm)	120.0±5.72	208.33±38.19
Cl (ppm)	8.95±2.90	28.33±76.38
F (ppm)	0.41±0.35	0.47±0.25
S (ppm)	Not detected	2.00±0.50
SO_4 (ppm)	52.75±5.12	76.67±22.55
O-PO_4 (ppm)	Not detected	3.18±2.26
Fe (ppm)	0.12±0.16	0.27±0.21
Mn (ppm)	0.050±0.006	0.06±0.04
Cd (ppm)	0.0068±0.0087	0.008±0.00
Pb (ppm)	0.01±0.009	0.09±0.00
Cu (ppm)	0.032±0.039	0.01±0.01
Zn (ppm)	0.05±0.01	0.02±0.01
Cr (ppm)	0.003±0.002	0.01±0.01
Hg (ppm)	Not detected	0.0002±0.00

This result also can explain the detection of the Orthophosphate (O-PO_4) in the water of El-Madapigh canal because it is generally considered to be the primary nutrient limiting algal and plant growth in fresh waters.

The Chemical Oxygen Demand (COD) is used as a measure of oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by strong chemical oxidants (Abdel-Satar, 2005). According to the data shown in Table 1, the mean value of Chemical Oxygen Demand (COD) was (107 ppm) in the water of El-Madapigh canal and it was (9.25 ppm) in the Nile water. The mean value of the total dissolved solid was increased from 224 ppm in the Nile water to 358.3 ppm in El-Madapigh canal. Also, the concentration of the total suspended solids was increased from 17.25 ppm in the Nile water to 46.7 ppm in El-Madapigh canal. The mean values of the total solids in El-Madapigh canal recorded a remarkable increase (405 ppm) comparing to the Nile

water (221.5 ppm) (Table 1). Such increase in TDS, TS and TSS values in the water of the canal is probably due to the phytoplankton blooming.

With respect to nutrient, nitrate is often the limiting element restricting biological productivity of water. Nitrogen is nutrient and occurs in many forms including ammonia, nitrate and nitrite. Data shown in the present results revealed that the average value of Nitrite (NO_2) and Nitrate (NO_3) in the water of El-Madapigh canal was higher than that of the Nile water especially for NO_3 which recorded an extreme increase in El-Madapigh canal. 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. The concentration of NO_2 was lower than NO_3 in all sites (Table 1). The recorded increase in NO_3 comparing to in all sites might be attributed to the fast conversion of NO_2-NO_3-ions by nitrifying bacteria.

Alkalinity of water is its acid-neutralizing capacity. It is the sum of all titratable bases. It is taken as an indication of the concentration of carbonate, bicarbonate and hydroxide content in water. According to the present results, total alkalinity was very high in El-Madapigh canal (208.33 ppm) comparing to the Nile water (120 ppm). Cholride, Floride and Sulphate were the dominant anions in the water samples from the Nile and El-Madapigh canal.

They are the most common inorganic anion found in water and wastewater. The concentration of Cholride, Floride and Sulphate were higher in the water of El-Madapigh canal than the Nile water (Table 1). High anion content may indicate pollution by sewage or industrial wastes. Sulfide (S) was not detected in the Nile water during the sampling period but they were recorded in the water of El-Madapigh canal (Table 1). Sulfide often is present in wastewater comes partly from the decomposition of organic matter, sometimes from industrial wastes. The detection of Sulfide (S) in the water of El-Madapigh canal can improve the presence of industrial wastes of this canal.

Heavy metals enter rivers and lakes from a variety of sources. The rocks and soils directly exposed to surface water are the largest natural sources. In addition to, the discharge of various treated and untreated liquid wastes to the water body can introduce large amounts of trace metals rivers. Data reported in Table 1 indicated that the water samples collected from the Nile and from the canal contained trace of Fe, Mn, Cd, Pb, Cu, Zn, Cr, Hg. Generally, the values of the detected heavy metals in El-Madapigh canal were higher than that in the Nile water (except for Zn and Cu). These values might be attributed to the direct inputs from different sources (industrial wastes and atmospheric inflow of dust containing car exhaust).

By the application of Pearson correlation analysis we recorded a significant positive correlation between the COD and TS, TSS, TDS, NO_2, Cl, O-PO_4, SO_4, alkalinity.

Lower mean value of OD and higher mean values of turbidity, conductivity, alkalinity, COD, NO_2, NO_3 and trace metals and the detection of orthophosphate and sulfide in El-Madapigh canal comparing to the Nile water prove the presence of large quantities of organic and inorganic pollutants El-Madapigh canal. This was expected due to the fact that the water of such canal receives large quantities of domestic, agricultural and industrial effluents. This heavy pollution in El-Madapigh canal also explains the presence of female fishes only in the water of this canal. This phenomenon is called feminization. In recent years there has been considerable concern over the ability of substances discharged into the environment to disrupt the normal endocrine function of wildlife. In particular, the apparent widespread feminization of male fish in rivers has received significant attention (Melanie *et al*., 2006). These pollutants are known as endocrine disruptor compound. Endocrine disruption has been reported in freshwater fish populations in various parts of the world (Jobling *et al*., 1988). The future research will concern with the identification and screening of these endocrine disruptor compound in the river Nile and its tributaries.

Tissues analysis: Fish are one of the most indicative factors in freshwater systems and may concentrate large amounts of some metals, such as lead, cadmium, chromium, 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 this reason, many studies (Evans *et al*., 1993; Rashed, 2001) on different fish species at different localities were carried out to assess the problem of fish contamination by toxic metals. The present study provides data about the contamination potential of fish from El-Madapigh canal and Nile water at Assiut Governorate (Egypt) by a number of heavy metals. The concentration of the selected heavy metals were investigated in liver, gills, gonads and muscles of the African catfish *Clarias garipienus* to detect the most appropriate tissues for heavy metal monitoring and to detect the distribution of such heavy metals in the selected sites. Table 2 shows the mean and SD values of the tested heavy metals in African catfish organs in the selected sites (El-Madapigh canal and Nile water). For all

Table 2: Mean and SD of the concentration of the selected heavy metals (mg kg^{-1}) in the tissues of the African catfish *Clarias gariepinus* collected from El-Madapigh canal and Nile water at Assiut, Egypt

Site	Organ	Cu	Mn	Cr	Zn	Pb	Cd	Fe	Hg
Nile water	Liver	12.76±1.61	4.2±0.680	4.3±4.870	156.17±74.63	8.00±1.000	1.45±0.07	254.94±0.0800	Not detected
	Gill	1.66±0.71	5.4±0.500	8.0±0.000	80.17±28.81	12.00±0.000	1.00±0.00	175.20±5.7200	0.26±0.015
	Gonad	9.03±0.63	3.3±3.820	3.6±0.470	178.95±20.62	34.00±1.000	1.05±0.00	385.00±49.500	0.19±0.001
	Muscle	1.44±0.08	4.5±0.030	7.0±0.000	104.80±12.45	8.50±0.710	0.45±0.07	51.50±0.7100	0.12±0.007
El-Madapigh canal	Liver	6.53±1.06	10.0±0.070	13.5±0.710	89.33±9.500	44.82±20.83	1.90±0.10	344.03±39.650	0.27±0.007
	Gill	3.68±0.25	8.5±0.710	12.1±0.860	107.53±19.52	62.10±27.74	1.15±0.07	258.78±102.70	0.27±0.021
	Gonad	6.25±1.77	5.0±0.000	7.7±0.580	152.36±15.92	90.47±17.41	1.56±0.04	352.39±124.81	0.12±0.007
	Muscle	2.10±0.14	5.2±0.420	11.5±1.020	61.67±32.15	58.85±28.41	0.90±0.00	201.88±107.03	0.17±0.028

heavy metals (except Cu and Zn) the mean concentration in the tissues of fish collected form El-Madapigh canal was higher than that in the tissues of fish collected from the Nile water. This was expected due to the fact that the water of such canal receives large quantities of domestic, agricultural and industrial effluents. The accumulation levels vary considerably among tissues, organs, metals and species (Heath, 1996). Table 2 shows concentrations of the selected heavy metals in the African catfish *Clarias gariepinus* and their distribution in different organs of the body (liver, gills, gonads and muscles) from both sites. The metals were differentially distributed in the different parts of fish but the pattern of distribution of some metals (Cu, Pb, Cd, Fe) was similar for Nile and El-Madaligh fishes. Cu and Cd were mostly accumulated in liver followed by gonads, gills and muscles. For Pb, the higher concentration was detected in gonad followed by gills and then muscles and liver. Fe was mostly accumulated in gonad followed by liver and finally gills and muscles. The pattern of distribution of the other metals (Mn, Cr, Zn and Hg) was different for Nile and Al-Madapigh fishes. For example, the manganese and chromium in the body of fishes collected from Nile water were mostly accumulated in gills followed by muscles and then liver and gonad. In case of the fishes collected from El-Madapigh canal Mn and Cr were mostly accumulated in liver followed by gills, muscles and gonads. The highest concentration of Zn was detected in gonads of fishes collected from both sites but it followed by liver, muscles and gills in case of fishes collected form the Nile water and followed by gills, liver and muscles in case of fishes collected from El-Madapigh canal. Accordingly, liver have the highest concentration of most metals in both sites due to its ability to produce metal binding protein metallothionein. The liver followed by gills which act as primary site of some other metals due to its branched organization and increased surface area. The lowest concentration of most metals were in the muscles which may reflect the low level of the metal-binding proteins in the muscles. Such difference in the accumulation patterns of heavy metals in tissues of fish is dependent upon of exposure concentration and duration as well as other factors as temperature, hardness and metabolism of the animals (Heath, 1996). So, the heavy metal concentration in tissues reflects past exposure via water and/or food (Velcheva, 2006).

Fig. 1: Diagrammatic serum protein patterns of the African catfish *Clarias gariepinus* (Patterns I and II male fishes collected from the Nile water at Assiut), (Patterns III, IV, V, VI female fishes collected from the Nile water at Assiut), (Patterns VII, VIII female fishes collected from El-Madapigh canal)

Electrophoresis: In the present reseach, different patterns of serum and muscles proteins were identified in *C. gariepinus* collected from the Nile water and El-Madapigh canal denoted polymorphism or heterogeneity of this species. Polymorphism of protein patterns was reported by several researchers including Christofferson *et al.* (1978) and Eckwert *et al.* (1997). Accordingly, such polymorphic proteins provided the most clearly discernible measure of biochemical-genetic relationship. The results of the present study revealed that both quantitative and qualitative differences in the banding pattern of serum and muscles proteins of the African catfish *Clarias gariepinus* collected from both sites were recorded.

Results of blood serum proteins are given in Fig. 1, 2 and Table 3. Six patterns of blood serum proteins were identified in *C. gariepinus* collected from the Nile water. Two of these patterns were specific for males (patterns I and II) and the rest were specific for females (patterns III, IV, V and VI). The electrophoretic patterns of the serum proteins of *C. gariepinus* collected from El-Madapigh canal exhibited two patterns (Patterns VII and VIII). In the aforementioned species, the differences between patterns included number, mobility and concentration of the bands. The First pattern composed of 6 bands namely:

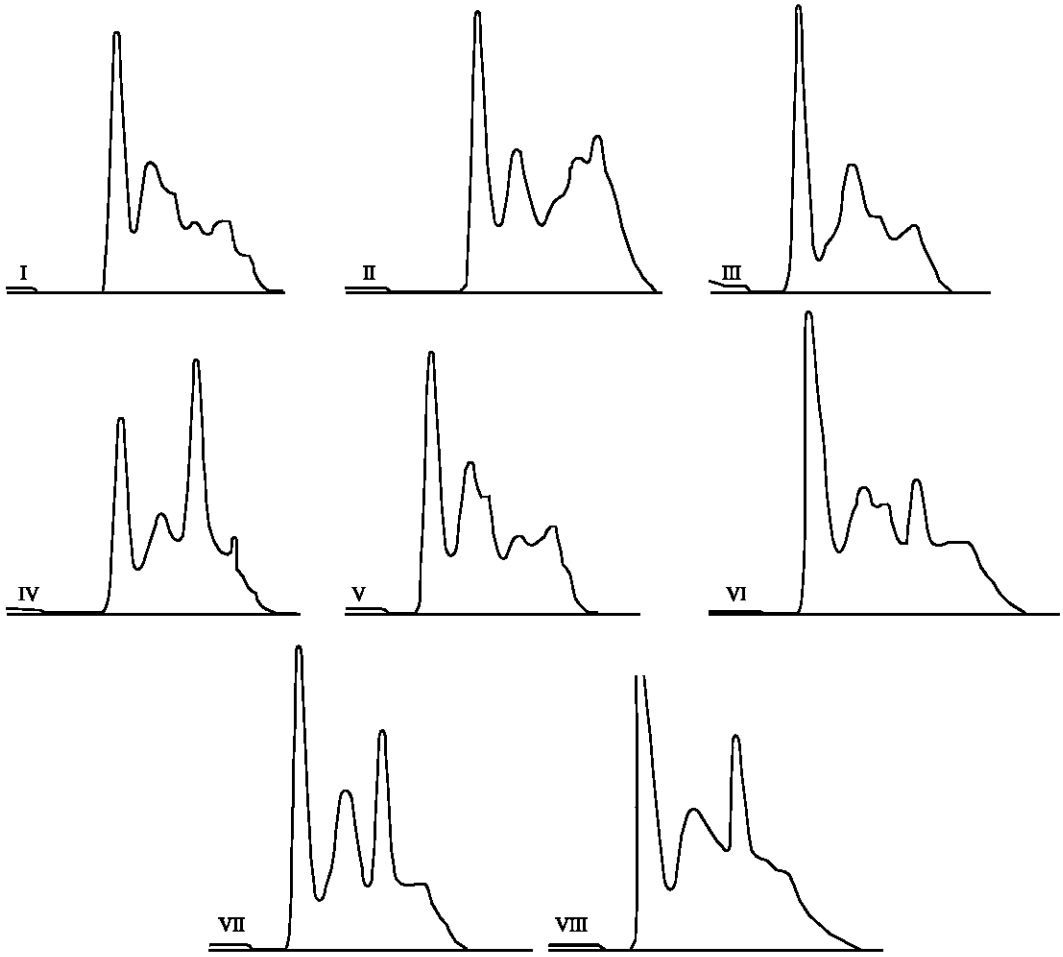

Fig. 2: Densitometer tracing of serum protein patterns of the African catfish *Clarias gariepinus* (Patterns I and II male fishes collected from the Nile water at Assiut), (Patterns III, IV, V, VI female fishes collected from the Nile water at Assiut), (Patterns VII, VIII female fishes collected from El-Madapigh canal)

Table 3: The mean and Standard Deviation (SD) of the concentration of the observed bands in each blood serum pattern of *Clarias gariepinus* from El-Madapigh canal and Nile water at Assiut, Egypt as percentages of the total protein

Observed band pattern	Prealbumin	Albumin	Transferrin	α-Globulin	β-Globulin	γ-Globulin	SL (mm)	W (g)
1	33±0.0	27±0.0	12±0.0	11±0.0	14±0.0	3±0.0	350±0.0	380±0.0
2	-	27±0.0	23±0.0	10±0.0	15±0.0	25±0.0	270±0.0	184±0.0
3	-	35±0.0	35±0.0	13±0.0	-	17±0.0	430±0.0	600±0.0
4	-	23±1.4	-	21±4.3	44.3±2.6	11.7±2.8	318.5±56.5	359±169
5	-	30±3.0	22.5±0.5	13.5±0.5	17.0±5.0	17.0±2.0	340±10.0	309±13.0
6	-	31.5±3.5	19.5±0.5	11.5±0.5	21.5±0.5	16.0±1.0	336±1.0	399±21.0
7	-	28.8±2.8	-	29.7±2.6	25.0±5.4	16.4±2.6	330.4±24.2	338.8±67.1
8	-	-	-	29.5±2.5	29.0±4.0	41.5±6.5	327.5±22.5	326.0±96.0

Prealbumin, Albumin, Transferrin, α-Globulin, β-Globulin, γ-Globulin. This pattern was recorded only in the male fishes. The second, the fifth and the sixth patterns composed of 5 bands namely: Albumin, Transferrin, α-Globulin, β-Globulin, γ-Globulin. The third patterns composed of Albumin, Transferrin, α-Globulin, γ-Globulin. The fourth and the seventh ones composed of Albumin, α-Globulin, β-Globulin, γ-Globulin. The remaining eighth pattern, which specific for fish collected from El-Madapigh canal composed only of three bands namely α-Globulin, β-Globulin, γ-Globulin. This means that the first two fractions (albumin and transferring) were missing in the female fishes collected from El-Madapigh canal comparing to the females collected from Nile water. Also, the present results recorded a remarkable increase in the intensity of α-Globulin, β-Globulin, γ-Globulin in

the serum of fishes collected from El-Madapigh canal water comparing to that collected from the Nile water (Table 3). Table 3 shows that fish size had no impact on the patterns revealed by the species under investigation.

Four different patterns of skeletal muscle proteins were detected in *C. gariepinus* collected from Nile water (Fig. 3, 4 and Table 4); two patterns (I and II) were specific for males and two patterns (III and IV) were specific for females. Only one pattern (V) was recorded for the muscle protein of *C. gariepinus* collected from El-Madapigh canal.

Table 4 shows no association between the patterns of the detected proteins and fish size. Six and five different protein bands were detected in the muscles of the fishes (male and females) collected form the Nile water.

Only four protein bands were detected in the muscles of fishes collected from El-Madapigh canal. This means that one or two protein fractions were missing in the muscles of fish collected from El-Madapigh canal comparing to those collected from Nile water.

Reduction in the number of bands and band intensity under the action of pollution has been reported in several studies (Mohan and Hosetti, 1997; Muthukmaravel *et al.*, 2007).

The increase in the concentration of globulin fraction has been previously reported in some animals due to the exposure to chronic diseases, bacterial and parasitic infection (Cray and Tatum, 1998; Kaneko *et al.*, 1997; Gordon *et al.*, 1998). Stress induced a decrease in energy charge, which probably contributed to the decrease in protein synthesis (Rhodes *et al.*, 1987). Also such reduction in the number of band could be attributed to pollutants stimulating hydrolytic enzyme activities. The reduction of proteins could be due to the impact on the protein synthetic pathway or due to the depletion of reserve proteins to over come to stress caused by heavy metal (Muthukmaravel *et al.*, 2007).

Fig. 3: Diagrammatic muscle protein patterns of the African catfish *Clarias gariepinus* (Patterns I and II male fishes collected from the Nile water at Assiut), (Patterns III, IV female fishes collected from the Nile water at Assiut), (Patterns V female fishes collected from El-Madapigh canal)

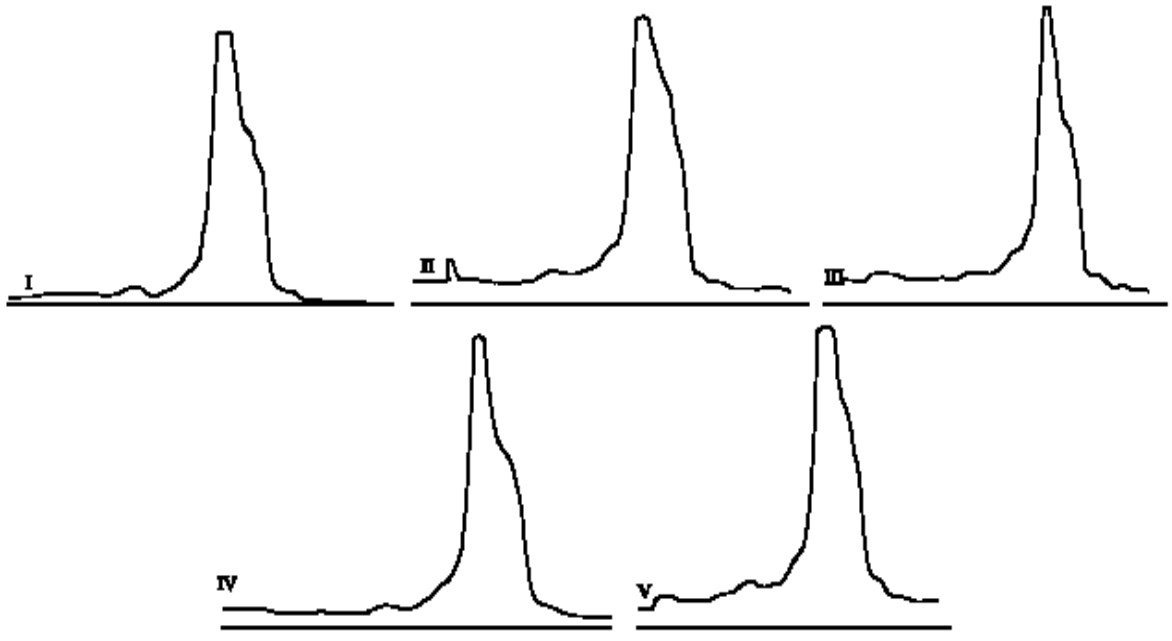

Fig. 4: Densitometer tracing of muscle protein patterns of the African catfish *Clarias gariepinus* (Patterns I and II male fishes collected from the Nile water at Assiut), (Patterns III, IV female fishes collected from the Nile water at Assiut), (Patterns V female fishes collected from El-Madapigh canal)

Table 4: The mean and Standard Deviation (SD) of the concentration of the observed bands in each muscle pattern of *Clarias gariepinus* from El-Madapigh canal and Nile water at Assiut, Egypt as percentages of the total protein

Observed band pattern	1	2	3	4	5	6	SL (mm)	W (g)
1	13.7±0.3	21.0±1.0	13.0±5.0	36.3±9.3	8.5±0.5	7.5±3.1	325±25.0	290±45.0
2	12.6±2.6	26.0±6.2	47.7±5.7	7.7±2.2	6.7±1.2		320±15.3	260±33.3
3	11.9±206	16.7±3.3	20.9±3.3	38.7±4.0	6.9±0.9	4.6±1.2	276.6±26.6	203.3±49.9
4	7.4±2.8	6.3±0.94	46.89±6.3	25.8±5.5	13.6±4.2	-	328.7±15.3	291±27.8
5	22.3±9.8	37.6±6.2	34.0±16.5	5.9±8.0	-	-	321±8.8	280±19.8

CONCLUSION

The present study indicates that water pollution might have detracted the proteins. The polluted water of El-Madapigh canal lead to an inhibition of some fractions which reflects a genetic damage due to the pollutants. The alteration in protein banding patterns and intensity of *Clarias gariepinus* observed in the present study may be attributed to the pollutant induced inhibition of protein synthesis. These results clearly show that protein electrophoreses is a sensitive tool for biomonitoring aquatic pollution.

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