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Effect of Heavy Metals Pollution on Protein Biosynthesis in Catfish

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ABSTRACT

The present study was carried out to evaluate the pollution and its effect on the quality of catfish. Four sites in Eygpt were chosen for the research, Ras El-Bar (Site 1) as control, Shatta (Site 2), Kafr El-Bateekh (Site 3), and Talkha (Site 4). The research was carried out on water, sediments and catfish (serum and muscles). Nitrite, nitrate and ammonia were determined in water and sediment. Also, RNA and DNA were determined in serum samples and the muscles of the catfish. In addition, the concentrations of heavy metals (Pb, Cd, Fe, Zn and Cu) were estimated in water, sediments and the muscles of catfish. Also, hepatosomatic index, liver water content, condition factor, lipid and protein contents were determined in the fish. The concentrations of nitrite, nitrate and ammonia in water and sediment of Site 4 and the levels of heavy metals especially Pb and Cd in water, sediment and muscle of catfish from Sites 3 and 4 were highly elevated compared to those of the control. On the other hand, DNA, RNA and protein contents in the catfish of Sites 3 and 4 decreased. The results illustrated that, Cd and Pb levels in the muscle of catfish were negatively correlated with DNA, RNA and with the protein contents. In conclusion, the accumulation of heavy metals in catfish tissues therefore, can cause health problems in human after catfish intake.

Keywords: Catfish; Protein Biosynthesis; Heavy Metals; Pollution

1. Introduction

The world suffers from deficiency of protein sources. Fish are considered as an important source of high quality animal protein as they contain large amounts of essential amino acids. Also, fish contain crude lipids, which supply the body with energy and essential fatty acids that are necessary for life and play an important role in regulation of the cardio-vascular system and for reducing cholestrol level in the blood. Moreover, fish are rich in fat-soluble vitamins, iodine and phosphorous [1].

Humans now have a strong influence on almost every major aquatic ecosystem, and their activities have dramatically altered the quality of receiving waters worldwide. Thus, there is a continuous need to develop and apply novel and effective technologies to detect, manage, and correct human-induced degradation of aquatic systems [2].

Pollution is the changes that occur in chemical, physiccal and biological characters of the environmental system. Environmental contamination of air, water, soil and food

have been still the most important subject in recent years because it causes threat extend to many plants and animals and may ultimately threaten the survival of humanity, and results from direct and indirect human activities [3]. Since intensive farming practices are essential to produce enough food for the increasing population, farmers have been using more inorganic fertilizers, pesticides and herbicides [4].

Many of heavy metals such as lead and cadmium have no nutritional importance, and their presence in relatively high concentration in body tissues can result in health problems in human as well as in animals [5]. The over accumulation of these heavy metals in tissues of animals has received considerable attention, partly because lethal and sub-lethal effect of such accumulation [6]. The levels of these metals in water may not be lethal to these organisms but the concentration of such metals in their tissues creates hazards when used as food for human consumption [7].

From the analytical data of physico-chemical parameters, it is indicated that the river water is contaminated

mainly due to the industrial and municipal effluents [8]. Some heavy metals such as Zn, Pb, Cd, Hg, Fe and Cu when discharged into the water can enter the food chain and bioaccumulate in fish and hence became a threat source to man [9]. The concentration of these metals in muscles is much higher ten times in some cases, than that found in the surrounding water [10]. Some metals are essential for life, and others have an unknown biological function and the increase in such heavy metals more than the allowable limit cause toxicity [11]. Also, Ayyat *et al.*, reported that Pb can be absorbed by the body and takes the place of Ca or Zn [12]. Chronic poisoning is more dangerous as it is very difficult to revert back to normal conditions after chronic exposure to this insidious metal present in our life [13].

Hayes, reported that chronic inhalation and oral exposure to Cd can be accumulated in the kidney and therefore, he stated that kidneys were susceptible to Cd toxicity [14]. In addition, Mohan and Hosetti stated that the catalase and protease activities were significantly decreased in the presence of Cd, with a simultaneous increase in peroxidase and superoxide dismutase activities, and decreases in the levels of protein and carbohydrate [15].

Damietta area is a region in Egypt that heavily exposed to pollution. Domestic and agricultural sewage coming from surrounding villages, and industrial wastes from factories enter the environment. The ship building industry in the village of Ezbit El-burg, together with the building of Damietta harbor, led to increase shipping activities that contribute in water pollution of the area [16]. So, the present work aims at evaluation of the levels of some pollutants in the River Nile and their effects on the quality of catfish via measurements of physical, chemical and biochemical changes of water, sediment and fish (*clarias lazera*). Also, heavy metals (Pb, Fe, Cu, Zn, and Cd) have to be determined in the same samples.

2. Materials and Methods

Four sites were chosen for the research, Ras El-Bar (Site 1) as control, as it lies far from the sources of pollution, Shatta (Site 2) located in Port Saied-Damietta road, its water source is the Manzala lake, Kafr El-Bateekh (Site 3) it lies in Damietta Governorate and receives the discharge of Electric Power Station, and Talkha (Site 4) it lies directly closed to Talkha Fertilizer Factory and receives the main outlets of factory drainage. The research was carried out on water, sediments and catfish (serum and muscles). Nitrite, nitrate and ammonia were determined in water and sediment. RNA and DNA were determined in serum and muscles. The concentrations of heavy metals (Pb, Cd, Fe, Zn and Cu) were estimated in muscles, sediments and water. Hepatosomatic index, liver water content, condition factor, lipid content and

protein content were determined in the fish.

3. Methods

Nitrite was determined according to the method of Blaek [17], and nitrate as described by [18]. Ammonia was determined as described by Merck [19]. Heavy metals were determined by Atomic Absorption Spectrophotometery [20]. Water content was determined according to the method of Sidwell *et al.*, [21]. Hepatosomatic index was calculated according to the method of Kohla [22]. Total lipids were determined according to AOAC [23]. Protein content of homogenized samples was performed according to the method described by Peters [24]. Protein electrophoresis was carried out using polyacrylamide gel electrophoresis [25]. Determination of nucleic acids according to the method of Schnieder *et al.* [26].

The Statistical analysis of the results was carried out using Instate software computer program, version 2.03 (Graph pad, USA), Origin software computer program, Inc. version 6.0 (Northampton, Ma 01060 USA), and Gel Pro Analyzer program (Media Cybernetices, Georgia, USA).

4. Results

The results were illustrated in **Tables 1-6** and **Figures 1** and **2(a)-(c)**.

In **Table 1**, the levels of nitrite, nitrate and ammonia in water and sediment of site 4 are highly significant increased compared to those of the control. Similar results are observed in the concentration of nitrite and nitrate in water and sediment of Site 2 and ammonia in water of Sites 2 and 3. Moreover, the concentration of ammonia in water and sediment of Site 4 was nearly 900 and 60 times respectively more than that of the control.

No detectable amount of Pb is observed in water of Sites 1 and 2 but its levels in water of Sites 3 and 4 were 0.223 and 1.12 ppm respectively. However, the concen-
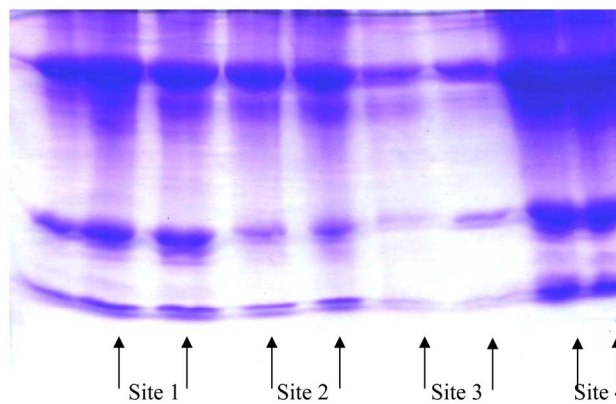

Figure 1. The electropherograph of different proteins of catfish from Sites 1, 2, 3, and 4.

Table 1. Concentrations of nitrite, nitrate and ammonia in water and sediment from locations 1, 2, 3 and 4.

Parameter Sites	Nitrite (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Nitrite (mg/kg)	Nitrate (mg/kg)	Ammonia (mg/kg)
	In water			In sediment		
Site 1	0.01 ± 0.001	0.023 ± 0.006	0.221 ± 0.056	0.03 ± 0.01	0.03 ± 0.01	0.35 ± 0.05
Site 2	$0.03 \pm 0.004^*$	$0.035 \pm 0.013^*$	$0.336 \pm 0.111^*$	$0.04 \pm 0.01^{**}$	$0.04 \pm 0.01^{**}$	0.42 ± 0.11
Site 3	$0.02 \pm 0.005^*$	0.018 ± 0.005	$0.323 \pm 0.108^*$	0.030 ± 0.004	0.03 ± 0.01	0.32 ± 0.1
Site 4	$0.04 \pm 0.003^{**}$	$0.23 \pm 0.066^{**}$	$188.33 \pm 15.9^{**}$	$0.058 \pm 0.006^{**}$	$0.92 \pm 0.11^{**}$	$19.38 \pm 5.91^{**}$

Number of samples = 10, *Significant P<0.05; **Highly significant P < 0.001, Site 1: Ras EL-Bar, Site 2: Shatta, Site 3: Kafr EL-Bateekh and Site 4: Talkha.

Table 2. Concentrations of heavy metals (Pb, Cd, Fe, Zn and Cu) in water from sites 1, 2, 3 and 4.

Sites	Pb ppm	Cd ppm	Fe ppm	Zn ppm	Cu ppm
Site 1	0	0.01 ± 0.001	0.13 ± 0.034	0.003 ± 0.0004	0.063 ± 0.008
Site 2	0	0.012 ± 0.004	$0.20 \pm 0.02^*$	0.043 ± 0.009	0.073 ± 0.016
Site 3	0.22 ± 0.08	$0.071 \pm 0.009^{**}$	0.182 ± 0.037	$0.086 \pm 0.018^{**}$	$0.115 \pm 0.040^*$
Site 4	1.12 ± 0.11	0.013 ± 0.004	$0.269 \pm 0.055^*$	$0.436 \pm 0.070^{**}$	0.062 ± 0.018

Number of samples = 10, *significant P<0.05, ** highly significant P < 0.001, site 1-Ras EL-Bar, site 2-Shatta, site 3-Kafr EL-Bateekh and site 4-Talkha.

Table 3. Concentrations of heavy metals (Pb, Cd, Fe, Zn and Cu) in sediments from Sites 1, 2, 3 and 4.

Sites	Pb ppm	Cd ppm	Fe ppm	Zn ppm	Cu ppm
Site 1	1.78 ± 0.76	0.54 ± 0.14	487.03 ± 51.78	9.93 ± 1.12	9.65 ± 0.73
Site 2	$2.86 \pm 0.67^*$	$0.86 \pm 0.27^*$	$651.70 \pm 11.90^{**}$	10.16 ± 1.2	$11.87 \pm 2.37^*$
Site 3	$31.06 \pm 5.52^{**}$	$5.43 \pm 1.19^{**}$	$645.90 \pm 55.04^{**}$	$18.06 \pm 2.28^{**}$	$74.29 \pm 6.82^{**}$
Site 4	$26.47 \pm 4.39^{**}$	$0.75 \pm 0.09^*$	$696.70 \pm 25.37^{**}$	$34.49 \pm 5.94^{**}$	$51.32 \pm 6.16^{**}$

Number of samples = 10, *Significant P < 0.05, **Highly significant P < 0.001, Site 1: Ras EL-Bar, Site 2: Shatta, Site 3: Kafr EL-Bateekh and Site 4: Talkha.

Table 4. Heavy metals concentrations (Pb, Cd, Fe, Zn and Cu) in muscles of catfish from Sites 1, 2, 3 and 4.

Sites	Pb ppm	Cd ppm	Fe ppm	Zn ppm	Cu ppm
Site 1	1.22 ± 0.33	0.07 ± 0.02	11.57 ± 1.89	1.33 ± 0.43	0.23 ± 0.079
Site 2	1.19 ± 0.36	0.07 ± 0.01	11.60 ± 1.20	1.34 ± 0.22	0.23 ± 0.03
Site 3	$11.90 \pm 1.92^{**}$	$0.17 \pm 0.05^{**}$	$27.80 \pm 4.50^{**}$	$5.78 \pm 0.69^{**}$	$1.05 \pm 0.04^{**}$
Site 4	$11.24 \pm 1.36^{**}$	0.07 ± 0.01	$42.70 \pm 5.90^{**}$	$8.092 \pm 1.47^{**}$	$1.04 \pm 0.05^{**}$

Number of samples = 10, *Significant P < 0.05, **Highly significant P < 0.001, Site 1: Ras EL-Bar, Site 2: Shatta, Site 3: Kafr EL-Bateekh and Site 4: Talkha.

Table 5. RNA, DNA and RNA/DNA ratio in serum and muscle of catfish of Sites 1, 2, 3 and 4.

Parameter Sites	RNA (µg/mL)	DNA (µg/mL)	RNA/DNA	RNA µg/mg	DNA µg/mg	RNA/DNA
	In serum			In muscle		
Site 1	42.58 ± 5.41	35.68 ± 3.24	1.20 ± 0.16	8.53 ± 1.21	4.46 ± 1.17	1.86 ± 0.54
Site 2	41.19 ± 5.33	35.28 ± 3.18	1.18 ± 0.2	8.23 ± 1.47	4.95 ± 1.16	1.78 ± 0.68
Site 3	$31.08 \pm 6.21^{**}$	$32.16 \pm 2.81^*$	$0.97 \pm 0.22^*$	$5.45 \pm 1.46^{**}$	4.38 ± 0.374	$1.01 \pm 0.23^*$
Site 4	40.95 ± 1.242	35.04 ± 3.4	1.17 ± 0.10	7.61 ± 1.44	4.59 ± 0.98	1.72 ± 0.53

Number of samples = 10, *Significant P < 0.05, **Highly significant P < 0.001, Site 1: Ras EL-Bar, Site 2: Shatta, Site 3: Kafr EL-Bateekh and Site 4: Talkha.

tration of Cd in water of Site 3 is higher (nearly 6 times) than that of the control. In addition, the levels of Zn in water of Sites 3 and 4 and Cu in water of Site 3 are highly significant increased compared to that of the control. Also, Fe levels in water of Sites 2 and 4 are significantly increased compared to that of the control **Table 2**.

The concentration of Pb, Cd , Fe, Zn and Cu in sediments of Sites 3 and 4 and Pb, Cd and Fe in sediment of Site 2 are highly significant increased compared to those of the control. Moreover, the levels of Cd in Sites 3 and 4 were 18 and 14 times, respectively more than that of the control **Table 3**.

In **Table 4**, the concentrations of Pb, Fe, Zn and Cu in muscles of catfish from both Site 3 and Site 4 are highly significant increased compared to control. Also, the levels of Cd in muscles of catfish from Site 3 are highly significant increased compared to that of the control.

RNA and RNA / DNA ratio is highly significant decrease in both serum and muscles of catfish from Site 3 and significantly decreased in Site 3 compared to that of the control.

In **Table 6**, there is a highly significant decrease in muscles protein content, condition factor and Hepatosomatic index of catfish from Sites 3 and 4 compared to those of the control. Also, there is a highly significant decrease in protein content and condition factor of catfish from Site 2 compared to that of the control. On the other hand, there is a highly significant increase in lipid and liver water contents in catfish of Sites 3 and 4 compared to those of the control.

The correlations between the levels and contents of protein, RNA and DNA are illustrated in **Table 7**. The Table shows negative correlation between both Pb and Cd levels and DNA, RNA and protein contents.

The electrophoresis and Gel pro-analysis of protein in serum of catfish from the different sites is illustrated in **Figures 1** and **2(a)-(c)**. It is appeared from **Figure 2(b)** (Gel pro-analysis of protein of catfish from Site 3 that, two protein peaks disappeared.

5. Discussion

The present study is mainly concerned with the effect of pollution on catfish quality. The obtained results illustrate highly significant increases in levels of nitrite, nitrate and ammonia in water and sediment of Site 4 compared to that of the control. Similar results were also ob served in the concentration of nitrite and nitrate in water and sediment of Site 2 and ammonia in water of Sites 2 and 3. Wang *et al.*, showed that, NO_3 was mainly associated with agriculture activities [27] and nitrites are intermediate products in the nitrification process of ammonia to nitrates and they are toxic to fish [28]. The toxicity of nitrite may be due to the reaction of nitrite with sec ondary amines to produce the carcinogenic nitrosamine [29]. These results agree with that recorded by other invistigators [30] including Mohammed *et al.*, who re corded a high level of nitrite (0.30 - 0.32 mg /L) in water of Qarun Lake [31].

The concentration of ammonia in both water and sediment of Site 4 is nearly 900 and 60 times, respectively more than that of the control. This can lead one to conclude that, the outlets of Talkha Fertilizers Factory is the source of ammonia elevation in Site 4 (188.3 mg/1). Abdelhamid, recorded that the toxic level of NH_3 for fish is 0.6 - 2.0 mg/1 [32]. Taking together one can expect muscle and nuclear damage of the catfish living in such area which was already the case in this study.

Table 6. Lipid and protein content, condition factor, liver water content and hepatosomatic index of catfish from Sites 1, 2, 3 and 4.

Parameter Sites	Lipid content g% of dry weight	Protein content g % of dry weight	Condition Factor (%)	Liver water Content (%)	Hepatosomatic index (%)
Site 1	4.81 ± 0.63	76.61 ± 7.56	0.93 ± 0.06	67.15 ± 3.95	0.52 ± 0.14
Site 2	4.87 ± 1.56	70.86 ±7.04*	0.81 ± 0.07**	70.72 ± 4.71*	0.47 ± 0.14
Site 3	5.31 ± 0.55*	68.75 ± 6.03*	0.71 ± 0.06**	74.81 ± 5.53**	0.37 ± 0.05*
Site 4	5.80 ± 0.64*	70.42 ± 6.40*	0.58 ± 0.14**	77.37 ± 4.92**	0.30 ± 0.06**

Table 7. Correlations between Pb and Cd, and DNA, RNA and protein.

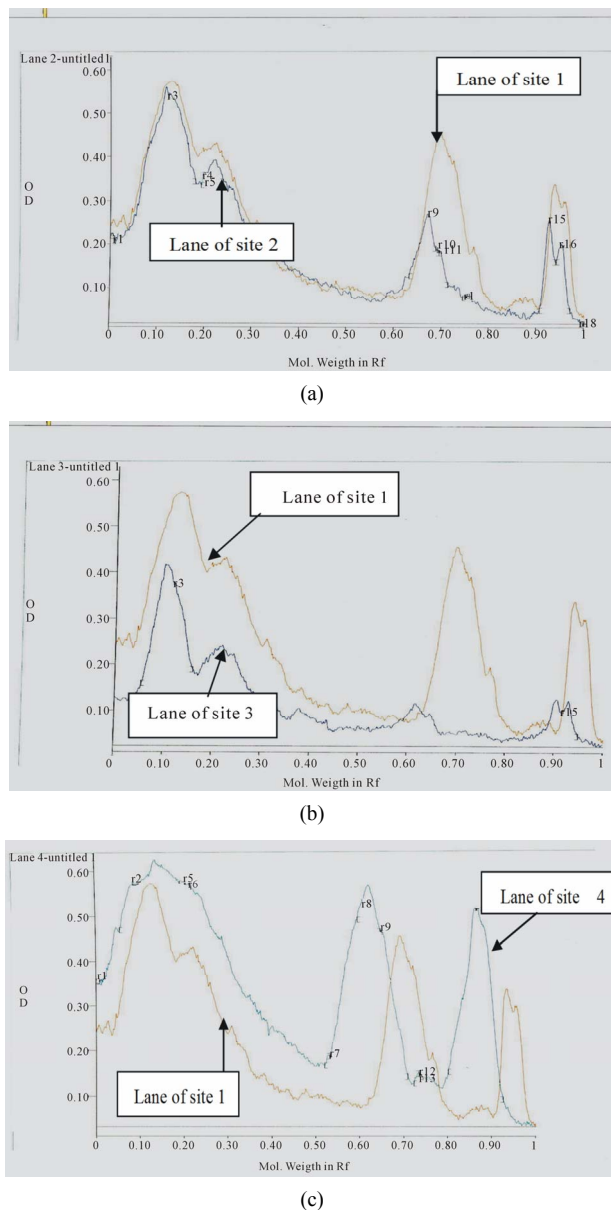| Correlation between	R value	P value
Pb (ppm) and protein percentage in muscles of catfish of Site 4	−0.709	0.02
Pb (ppm) and RNA content (μg/mg) in muscle of catfish of Site 3	−0.828	0.003
Cd (ppm) and RNA content (μg/mg) in muscle of catfish of Site 3	−0.797	0.017
Cd (ppm) and DNA content (μg/mg) in muscles of catfish of Site 3	−0.813	0.004

(a)

(b)

(c)

Figure 2. (a) A comparison between Pro analysis of electropherograph of protein patterns in serum of catfish from Sites 2 and 1; (b) A comparison between Pro analysis of electropherograph of protein patterns in serum of catfish from Sites 3 and 1; (c) A comparison between Pro analysis of electropherograph of protein patterns in serum of catfish from Sites 4 and 1(control).

The concentrations of Pb in water of Sites 3 and 4, were 2.23 and 1.12 ppm, respectively these levels were above the permissible limit according to Mance, who reported that the critical concentration of Pb in fresh and salt waters may range from 0.005 to 0.02 ppm [33]. In addition the concentration of Cd in water of Site 3 (0.071 ppm) was higher elevated than that of the permissible limit (0.05 ppm) as reported by FAO/WHO [34] and similar to that reported by Zaghloul [35] (0.063 and 0.097 ppm) in

water samples from different zones at Hel- wan city. Also, Fe level was significantly increased in water of Sites 2 and 4 compared to that of the control (Site 1). Zyadah, found high levels of Fe in the sediment and fish, which exceed the allowable limit in River Nile Estu-ary-Damietta branch, Egypt [36]. In addition, there were highly significant increases in zinc in water of Sites 3 and 4 and copper in Site 3 when compared to that of the control. Copper is an essential trace metal for physio-logical functions, while, copper overload causes cytotox-icity in living organisms including human [37]. Mo-hammed *et al.*, recorded 0.096 - 0.12 ppm for zinc and 1.25 - 2.59 ppm for Cu in water of Qarun Lak'e [31]. The latter results lead one to conclude that the distribution patterns of Cu and Zn in the Lake water increased in hot seasons where the heavy metals will be released from sediments to the overlying water under the effect of both high temperature and decomposition of organic matter by fermentation [38]. On the contrary, the values of Cu and Zn decrease in the water during cold period due to pre-cipitation of heavy metals from water to the sediments under slightly high pH and the adsorption of heavy met-als onto organic matter as was reported by Goher [39].

The present findings of Cd level in sediment (5.4 ppm) and muscle of catfish (0.174 ppm) from site 3 were more than that of the control. Mohammed, recorded 0.05 - 6.14 ppm for Cd in sediments from different locations of Egypt [1]. Abdelhamid, mentioned that cadmium level in foods must not exceed 0.135 ppm and fish not exceed than 0.05 ppm in wet weight [28].

Moreover, the obtained data showed high elevation in the concentrations of Pb (9 times), Fe, Zn (5 times), and Cu (4 times) was more than that of the control in muscles of catfish of Sites 3 and 4. Similar results were reported by Sorensen, who stated that, the Pb uptake by aquatic organisms depend upon exposure time, aqueous Pb con-centration, pH, temperature and diet [40]. These expecta-tion is based on the finding of Somero *et al.*, who stated that, fish live at 20 - 25 C for 24 days accumulate Pb from two to twenty times more than fish live at 10 C [41]. In the present study, iron concentration in fish muscle is nearly similar to that reported by Zaghloul [35] (10.7 - 30.6 and 8.6 - 21.3 mg/kg wet weight).

Zinc is soluble in water and may cause illness by drink-ing water containing with such metal [42]. In the same line Abdelhamid showed that the Increase in zinc con-centrations in fish depends on its levels in water, and reduce fish growth rate and destroy the gills leading to hypoxemia and hypoxia in tissues [43]. In addition, zinc is absorbed and transported through blood to various body tissues and either storage in liver, spleen, and go-nads or excreted out by the kidney [44]. Therefore, one can expect damage of such organs in human after catfish intake.

Nucleic acids play a major role on growth and development of the organism. The amount of DNA, the genetic information carrier, reflect the cell numbers [45,46]. The amount of RNA in the cell is directly proportional to the amount of protein synthesis occurring. The relationship between RNA and DNA is an index of the cell's metabolic intensity and has been used to measure recent growth in fishes [47].

The decrease in RNA in muscle and serum of catfish of Site 3 in the present study may be due to the effect of heavy metals overload which was reported in this study especially in Sites 3 and 4. These increases in heavy metals can disturb the biological activity of catfish, as well as nucleic acids and ribosomal activity [48]. The latter decreases can in turn cause decreases in protein biosynthesis including those of the muscle content of catfish which were collected from these sites. The results of the present study confirm those reported by Muhammad *et al.*, who concluded that the decreased in RNA/DNA ratio could be due to decreases in the ribosomal activity [49] and also that of Buckley and Bulow, who reported that the heavy metals have an inhibitory effect on protein biosynthesis via its effect on RNA and ribosomal activity [50].

Gel Pro analysis of the protein electrophoresis of different sites revealed the disappearance of two protein peaks from serum of catfish of Site 3 and the appearance of new protein peaks in serum of catfish from Site 4. In addition, El-Demerdash and Elagamy, showed differences in electrophoretic patterns of proteins prepared from Maryout and Nozha fish samples. The nutritional quality of Maryout fish proteins was lower than that of Nozha samples this may be due to lowering in the essential amino acids contents as a result of the presence of high concentrations of Hg and Cd in Maryout samples than Nozha samples [51].

These findings were confirmed by the inverse relationship between heavy metals concentration and protein content in muscles of catfish. In the same line Salem, concluded that Cd and Pb negatively affected fish body gain, protein efficiency ratio, chemical composition and muscles area [3]. These two metals were found to be responsible for different types of chromosomal aberrations and lower mitotic index in the fish [52]. Demuynck added that cadmium-binding protein level in the cells of intestine was increased after exposure to Cd, so it appears that this protein will be synthesized as a response to Cd over exposure [52]. Abdelhamid and El-Ayouty, [11] mentioned that the muscles protein content was decreased while that of fat and Pb contents in the fish muscles were increased in proportion to the pollution levels.

Condition factor is the percentage of whole live body weight divided by cubic length of fish. It is one of the most important growth condition of fish because it gives

an idea about the fish health and subsequently, the water quality [42]. A highly significant decrease in condition factor was found in Site 3 and Site 4. The lower percentage in condition factor of fish which more collected from Sites 3 and 4 indicated that the pollution affects the water quality and subsequently, the growth of fish. As reported by Weatherley and Gill, the condition factor changes may reflect fairly and faithfully the changes in body protein and lipid content [53]. It was appeared from the present study that, there was an inverse correlation between the hepatosomatic index and pollution. Therefore, a similar effect may occur in human intake such fish. These results are similar to that obtained by Abbas, who reported that, there was a significant decrease in the hepatosomatic index of lead exposed fish [9]. This decrease may be attributed to the decomposition of liver tissues by the heavy metal overload. Finally, it can be concluded that the protein content and catfish quality are markedly affected by water pollution. Also, the heavy metal overload of such fish can cause sever health problems in man especially kidney and liver.

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