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SUBLETHAL EFFECTS OF COPPER SULFATE, MALATHION AND PARAQUAT ON PROTEIN PATTERN OF *OREOCHROMIS NILOTICUS*

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ABSTRACT

The present study deals with the effect of some water pollutants; copper sulfate as a fungicide, malathion as an insecticide and paraquat as a herbicide, on serum total protein, serum albumin and globulin. Furthermore, proteinogram of blood serum *niloticus* in *Oreochromis* was done using electrophoresis. It was found that the control fish have nine serum protein fractions. The exposure of fish to the examined pollutants induced a disappearance of some fractions and consequently changes of relative mobility and area that indicate genetic damage.

INTRODUCTION

Nowadays, with the extensive use of pesticides and herbicides, some of these compounds have been drained either directly or indirectly to the aquatic environment. In such way, the chemical composition of the water ecosystem was changed. Thus, pollution of such habitat has been generated. The degree of pollution in any area in the River Nile depends on the quantity of wastes washed down (Siliem, 1993; 1994 and Badawy & El-Serafy, 1998). Serum protein fractions of *Clarias gariepinus* were used by Badawy and El-Serafy (1998) to detect the pollution level of the aquatic habitat. Therefore, the present study aims to investigate laboratory effect of some water pollutants on protein polymorphism of Nile tilapia (*O. niloticus*).

MATERIALS & METHODS

Nile tilapia fishes were reared in large acquaria in the fish biology laboratory, Benha Faculty of Science, in a healthy status. The fishes selected for this study nearly measure 17 – 23 cm of both sexes. The photoperiod in the laboratory was adjusted to 14L : 10D. The fishes then were transferred to glass acquaria for the experimental work. One fish group was left as a control. The other acquaria were used for the pollution study. The insecticide, malathion (Kafr El-Zayat insecticide Co., Egypt) and the herbicide, paraquat (methyl viologen) (Sigma) were added separately at levels of 0.1, 0.01, 0.001 and 0.0001 mg/L each in separate tanks. Copper sulfate, anhydrous (Spectrum quality products Inc., USA) was added at levels of 1.0, 0.5, 0.1 and 0.01 mg/L in separate tanks. Ten fishes were raised in each tank. The fishes were fed twice a day with a standard fish food. The levels of the pollutants were approximately kept constant throughout the experiment. The experiment continued for four days. The fishes of the control and polluted exposed groups were decapitated by heart puncture and the blood samples were collected into non-heparinized tubes. The tubes were left overnight at 4°C to complete the clotting. The blood samples were centrifuged at 4000 rpm for 5 min and serum samples were separated and stored in deep freeze until analysis.

The serum total protein was determined following the Biuret principle, using Biomereux kit (Sentinel diagnostic kit cat No 17261A). The serum globulin level was calculated by substracting the albumin from total protein levels.

Protein fractionation was done using SDS polyacrylamide gel electrophoresis (SDS-PAGE) (Herzberg & Pasteur, 1975). The gels were stained in Comassie Brilliant Blue and the excess dye was washed using destaining solution (methanol & acetic acid). The gel bands were scanned using densitometer and the date analysed using Hoefer 365 software (Heofer Scientific Instruments, CA. USA). The t-student test was used as a test of significance between the control and treated groups (Pipkin, 1984).

RESULTS

The data presented in table (1) shows the changes of serum total protein, albumin and globulin levels of the control as well as the fishes subjected to the pollutants. Evidently, the serum total protein

was generally increased in case of the fishes subjected to the examined pollutant. All malathion concentrations induced a significant increase of protein level. The serum of fishes exposed to 0.0001 mg malathion /L showed the highest elevation (6.33%) of serum total protein.

The serum albumin was significantly reduced in case of fish reared in malathion and $CuSO_4$ polluted water, whereas paraquat induced a significant elevation of albumin level that was evident at the lowest concentration 0.0001 mg/L.

A general increase of globulin level was recorded in the blood of tilapia fish reared in water polluted with malathion and $CuSO_4$ (Table 1). The highest value (1.71±0.14 mg/100ml) was recorded in case of 0.01 mg/L malathion subjected fishes. This value differed from the control by 25% percentage difference. The paraquat at a dose level of 0.01 mg/L induced a significant rise of globulin level with a percentage difference 17.65%, when compared with the control group, whereas the lowest tested levels of this insecticide (0.0001 mg/L) induce a marked reduction of the circulating globulin.

The electrophoretic serum protein fractionations of *O. niloticus* reared in non-polluted water displayed 9 fractions (Table 2; Fig.1). By the exposure of fishes to the highest tested copper level (1.0 mg/L), the protein pattern showed three fractions only (i.e six fractions were missing)(Table 2 & Fig.2). The rest of the examined copper levels showed no effect. One fraction only was missing as a result of 0.1, 0.01 and 0.001 mg/L malathion exposure (Table 2 & Fig.3). However, the lowest tested malathion level (0.0001 mg/L) showed more or less the same pattern as control. In treatment with all paraquat concentrations two fractions were missing (Table 2 & Fig.4). Fractions number 2, 3 and 1, 2 were missing in case of paraquat exposure levels 0.1, 0.01 and 0.001, 0.0001 mg/L, respectively.

The percentage of frequency appearance of various serum protein fractions of *O. niloticus* in different groups is presented in table (2). This study was confined mainly on the polymorphic fractions. The polymorphism (appearance > 50%) of protein fractions of control group was noted for all fractions except fraction number 1. This phenomenon was also noted for all exposed groups.

Table (3) shows the relative mobility of protein fractions which indicates the relative genetic distance in which fraction

migrates from the application sample's point to its position in the electropherogram through the gel. Fractions number 1, showed a significant mobility in all fish groups reared in copper polluted water. The lowest Cu concentration induced a significant mobility of fraction numbers 2, 4 and 5. Fraction number 1 showed a significant reduction of relative mobility after exposure to 1.0 mg/L Cu.

The relative mobility of protein fraction number 1 was significantly different from the control value. This was reported in case of malathion concentrations 0.01 and 0.001 mg/L and 0.1 mg paraquat /L subjected groups (Table, 3). Fractions number 2, 4, 5 and 8 showed a significant increase in mobility after exposure to concentrations 0.001, 0.01 and 0.1 mg malathion /L. The malathion exposure levels of 0.1 and 0.01 mg/L induced a marked significant increase in mobility of fraction number 7. No significant changes in fish exposed to 0.0001 mg malathion /L.

Paraquat exposure levels (0.1 and 0.01 mg/L) induced a significant difference of relative mobility of fractions number 1, 4, 5 and 9. Fractions number 4 and 5 showed a significant reduction in mobility as a result of exposure to 0.001 and 0.0001 mg paraquat/L, whereas, fraction number 4 was significantly increased over the control levels at the lowest two paraquat levels (0.001 and 0.0001 mg/L).

The relative areas of all protein fractions were significantly changed after the exposure of fish to the highest copper concentration (1.0 mg/L) (Table 4). Fractions number 1, 2, 3 and 6 showed a higher relative area after all tested copper levels. Conversely, the bands number 4, 5, 7 and 9 showed a reduced area (Table 4). The malathion and paraquat concentrations induced a marked increase of relative area of bands number 5, 6, 7, 8 and 9 showed a reduced value.

DISCUSSION

The studies on protein metabolism were found to be of a vulnerable value of fish population. The structure of blood proteins, muscle proteins, haemoglobins as well as enzymes in the blood and some organs appear to be variable (Boyd, 1964 and Kirpichinkov, 1973 & 1981). The determination of protein content in the blood plasma is a good indicator for the ecological stress, physiological homeostasis and aquatic pollution (Abdel-Hamid, 1994). The results obtained in the present investigation showed an elevated serum protein content after the exposure to the tested pollutants. This

phenomenon reflects the hazardous effect due to such pollutants (Abdel-Hamid, 1994 and Sharafeldeen, 1999). Moreover, Sharafeldeen (1999) reported that the histological investigation of *O. niloticus* subjected to sublethal concentrations of $CuSO_4$, malathion and paraquat showed expanding hepatic sinusoids, necrosis and vacuolation of hepatocytes. So, as a result of increasing hepatocellular damage, the total serum protein content was elevated. This observation was previously reported by Poleksic and Karan (1995).

The pollution of aquatic habitat by ametryn (herbicide) caused a reduction of the plasma total protein content in the grass carp (*Ctenopharyngodon idella*) (Abo-Hegab *et al.*, 1990). Also, the molluscicide and urea exhibit a similar pattern of effect. This phenomenon gets the support from Patterson (1976) who reported that the pollutants react with the cell nucleoproteins and nucleic acids and consequently affect protein synthesis and cell integrity.

The albumin level showed a general reduced level in all treated groups. This may reflect a reduced blood viscosity. This phenomenon runs parallel with those obtained by Ramalingam (1982) and Awasthi *et al.* (1984).

Sanders (1964) found inter and intraspecific differences in protein compounds. Many authors studied the protein polymorphism which is mainly due to genetic disturbances of pollution (Payne *et al.*, 1971; Badawy and El-Serafy, 1998 and Salama, 2001).

The present investigation found that six protein fractions were missing due to copper exposure (high level, 1.0 mg/L). This result revealed a high genetic damage that was occurred due to copper. This phenomenon was previously reported by Badawy and El-Serafy (1998) and Salama (2001). Avtalion & Wojdani (1971) reported that, in the serum proteinogram of some tilapia, some fractions are confined to transferrin (B-globulin) which is an important genetic area. Also, Bus *et al.* (1977) described that paraquat induced a damage of membranes, protein and DNA.

Khud-Bukhsh *et al.* (1987) mentioned that polyacrylamide gel electrophoretic bands of glutamine, albumin globulin and muscle protein of X-radiated tilapia differed significantly with respect to number, mobility and density of bands than that of the control.

The significance in the relative areas of protein fractions reported in the present study as a result of pollution is mainly due to

the polyorphism and disappearance of some fractions. This explanation was suggested previously by many authors (El-Sharkawi *et al.*, 1978; Siliem, 1994; Yacoup, 1994 & Awad and El-Serafy 1998).

The protein electrophoresis revealed a high difference between control and polluted samples due to the production or activation of a new sequence of DNA responsible for synthesizing new types of protein as concluded by El-Bermawy *et al.* (2000)

The present study indicates that the examined pollutants; copper sulfate, malathion and paraquat induce a rise of circulating protein content, protein polymorphism and inhibition of some fractions which reflects a genetic damage due to the pollutants.

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Table 1. Changes of the serum total protein content, albumin and globulin in blood serum of control *O. niloticus* as well as those of fishes exposed to different levels of $CuSO_4$, Malathion and Paraquat.

Groups		Total protein (g/100ml)		Albumin (g/100 ml)		Globulin (g/100 ml)	
		Mean ± SD	% change	Mean ± SD	% change	Mean ± SD	% change
Control		3.2 ± 0.04	—	1.8 ± 0.08	—	1.4 ± 0.04	—
$CuSO_4$ (Mg/L)	1.0	3.1 ± 0.1	- 1.26	1.9 ± 0.2	3.89	1.3 ± 0.3	-8.09
	0.5	3.3 ± 0.1*	5.38	1.7 ± 0.1*	- 6.11	1.6 ± 0.03*	20.59
	0.1	3.1 ± 0.05	- 0.63	1.7 ± 0.1	- 3.33	1.4 ± 0.1	4.41
	0.01	3.1 ± 0.2*	- 1.26	1.8 ± 0.1	- 2.78	1.4 ± 0.1	0.73
Malathion (Mg/L)	0.1	3.3 ± 0.1*	2.85	1.7 ± 0.1*	- 6.67	1.6 ± 0.1*	16.91
	0.01	3.3 ± 0.1*	4.43	1.6 ± 0.01*	- 11.67	1.7 ± 0.1*	25.73
	0.001	3.2 ± 0.03*	2.21	1.7 ± 0.1	- 5.0	1.6 ± 0.1*	19.12
	0.0001	3.4 ± 0.09*	6.33	1.7 ± 0.1*	- 6.67	1.7 ± 0.04*	22.06
Paraquat (Mg/L)	0.1	3.1 ± 0.2	- 2.21	1.9 ± 0.04	2.78	1.3 ± 0.2	- 8.09
	0.01	3.3 ± 0.05*	4.75	1.7 ± 0.01	- 3.33	1.6 ± 0.1*	17.65
	0.001	3.1 ± 0.2	- 1.9	1.7 ± 0.1	- 3.89	1.3 ± 0.1	- 0.73
	0.0001	3.2 ± 0.1	0.95	2.3 ± 0.1*	25.55	0.9 ± 0.1*	- 31.62

* Significant at $P < 0.05$

Table 2. Percentage appearance of serum protein fractions of control *O. niloticus* as well as those of fishes exposed to different levels of $CuSO_4$, Malathion and Paraquat.

Groups		Fractions number								
		1	2	3	4	5	6	7	8	9
Control		33.33	50	83.33	100	66.67	100	100	100	100
$CuSO_4$ (Mg/L)	1.0	-	-	-	100	-	-	-	100	100
	0.5	100	66.67	100	66.67	66.67	100	100	100	100
	0.1	50	50	100	50	100	50	100	100	50
	0.01	66.67	100	66.67	33.33	66.67	100	66.67	100	66.67
Malathion (Mg/L)	0.1	-	66.67	100	66.67	100	100	100	100	66.67
	0.01	33.33	100	100	-	66.67	100	66.67	33.33	100
	0.001	100	100	100	-	100	100	50	50	100
	0.0001	33.33	100	100	66.67	100	100	66.67	100	66.67
Paraquat (Mg/L)	0.1	50	-	-	100	100	100	100	100	100
	0.01	33.33	-	-	33.33	66.67	33.33	100	100	100
	0.001	-	-	66.67	100	66.67	66.67	100	100	100
	0.0001	-	-	33.33	100	66.67	66.67	100	100	100

Table 3. Relative mobility of serum protein fractions of control *O.niloticus* as well as those of fishes exposed to different levels of $CuSO_4$,Malathion and Paraquat.

Groups		Fractions number								
		1	2	3	4	5	6	7	8	9
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control		13.1 ± 1.0	25 ± 1.5	37.1 ±2.1	45.2±1.5	56.1±1.4	67.02±1.62	77.0±1.26	86.0±2.27	93.43±2.44
$CuSO_4$ (Mg/L)	1.0	-	-	-	41.7±2.4*	-	-	-	88.8±1.9	96.3±2.1
	0.5	16.5±1.5*	24.5±3.5	33.8±3.08	45.1±1.2	56.3±2.05	67.3±1.7	78.5±1.11	88.13±0.5	95.8±0.51
	0.1	17.7±1.0*	25.5±2.5	35.5±3.5	44.3±4.2	56.0±2.1	69.9±2.6	78.1±2.4	85.1±1.34	91.1±1.9
	0.01	17.5 ± 0.5*	28.8±0.7*	36.5±1.5	41.1±1.0*	54.0±1.0*	66.3±2.5	75.0±4.3	88.16±0.88	93.8±3.0
Malathion (Mg/L)	0.1	-	27.0±1.7	33.6±3.4	42.1±1.43*	57.5±4.5	68.7±4.1	72.4±2.4*	82.4±2.7*	94.0±4.3
	0.01	4.09 ± 0.1*	25.33±2.49	36.33±3.77	-	53.33±0.47*	66.67±1.24	79.05±0.05*	88.2±1.2	93.43±1.02
	0.001	8.6 ± 1.16*	28.3±0.7*	36.5±2.45	-	55.6±1.9	67.55±1.15	77.0±1.78	84.21±2.11	92.55±0.55
	0.0001	13.8 ± 1.5	25.0±0.7	35.0±0.77	44.0±0.74	55.7±3.75	66.67±6.76	74.41±4.11	85.5±5.7	94.3±4.91
Parnquat (Mg/L)	0.1	17.4 ± 1.57*	-	-	43.57±2.09	53.0±1.6*	67.5±0.3	77.85±0.15	87.0±0.9	94.0±3.0
	0.01	12.1 ± 0.75	-	-	84.3±0.79*	55.45±2.85	69.8±2.6	76.23±1.43	84.83±3.67	96.17±0.46*
	0.001	-	-	38.7±0.8	47.93 ± 0.62*	50.33±0.41*	66.5±1.5	76.5±1.51	85.6±2.16	96.4±0.4*
	0.0001	-	-	39.1±1.11	48.1 ±1.42*	54.90±4.0	66.0±3.1	75.47±1.11	85.77±3.74	96.37±0.33*

* Significant at $P < 0.05$

Table 4. Relative area of serum protein fractions of control *O.niloticus* as well as those of fishes exposed to different levels of $CuSO_4$,Malathion and Paraquat.

Group		Fractions number								
		1	2	3	4	5	6	7	8	9
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control		16.01 ± 0.35	6.81 ± 0.45	8.86 ± 0.62	11.13 ± 2.92	8.06 ± 1.32	9.36 ± 1.96	10.98 ± 1.82	11.5 ± 2.1	17.66 ±3.62
$CuSO_4$ (Mg/L)	1.0	-	-	-	70.9 ± 3.95*	-	-	-	15.5 ± 1.1*	13.6 ± 1.11*
	0.5	21.8 ± 3.31*	8.25 ± 3.55	23.4 ± 5.02*	6.5 ± 1.0*	7.6± 1.8	8.5 ± 1.42	5.2 ± 1.9*	8.63 ± 2.7	16.53 ± 1.93
	0.1	22.8 ± 3.51*	12.7 ± 1.47*	18.9 ± 4.41*	-	4.6 ± 0.5*	14.70 ± 1.4*	3.5 ± 0.11*	7.2 ± 0.37*	16.30 ± 1.14
	0.01	23.7 ± 0.6 *	11.8 ± 1.3*	13.65 ± 0.45*	13.7 ± 1.1	4.25 ± 0.45*	12.4 ± 2.99	13.7 ± 4.1	9.7 ± 0.3	14.20 ± 1.80
Malathion (Mg/L)	0.1	-	38.05 ± 6.15*	13.9 ± 1.15*	10.35 ± 1.05	5.53 ± 1.19*	9.6 ± 1.3	5.47 ± 1.61*	17.65± 3.45*	14.80 ± 1.11
	0.01	17.1 ± 1.5	29.7 ± 3.45*	26.25 ± 1.75*	-	5.55 ± 0.15*	14.07 ± 3.23*	13.2 ± 0.8*	4.1 ±0.1*	10.70 ± 1.14*
	0.001	20.14 ± 2.13*	27.1 ± 0.9*	17.05 ± 1.45*	-	7.6 ± 0.3	11.25 ± 4.75	9.1 ± 0.91	7.5 ± 0.45*	13.0 ± 0.20*
	0.0001	18.6 ± 0.41*	18.55 ± 0.05*	17.7 ± 1.42*	8.1 ± 1.11	6.07 ± 0.93	13.1 ± 2.08*	7.2 ± 0.73*	8.14 ± 0.91*	13.6 ± 0.34*
Paraquat (Mg/L)	0.1	49.3 ± 1.41*	-	-	9.4 ± 0.45	6.45 ± 1.15	6.05 ± 1.75*	8.45 ± 0.35*	6.80 ± 0.70*	12.55 ± 1.7*
	0.01	10.35 ± 0.1*	-	-	20.8 ± 0.44*	6.50 ± 2.11	7.1 ± 0.41*	6.0 ± 2.6*	6.85 ± 1.15*	13.4 ± 2.01
	0.001	-	-	48.4 ± 3.4*	8.05 ± 2.95	4.8 ± 0.6*	4.15 ± 0.75*	9.17 ±1.54	9.73 ± 1.24	15.7 ± 1.06
	0.0001	-	-	30.1 ± 2.11*	34.85 ± 2.8*	3.75 ± 0.45*	4.8 ± 1.4*	5.67 ± 1.43*	8.53 ± 2.21	12.40 ± 1.85*

* Significant at $P < 0.05$

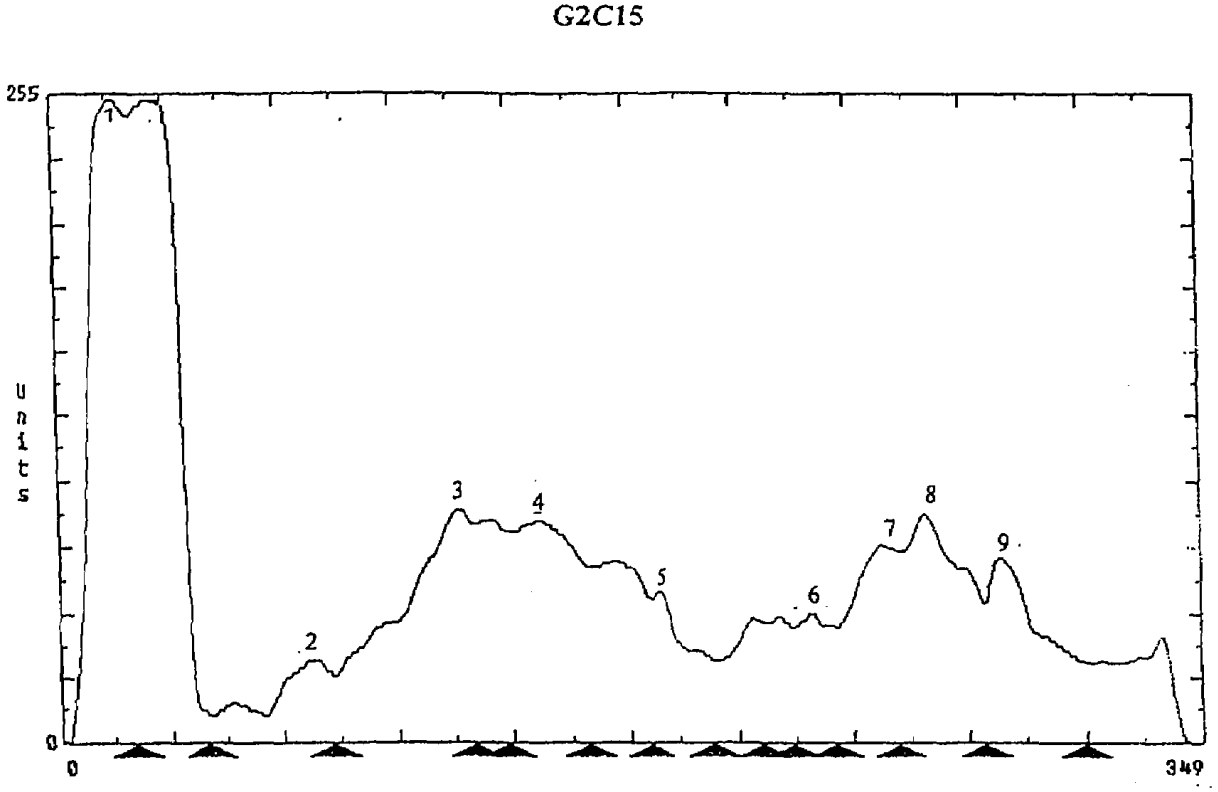

Fig. (1): Serum proteinogram of control *O.niloticus*.

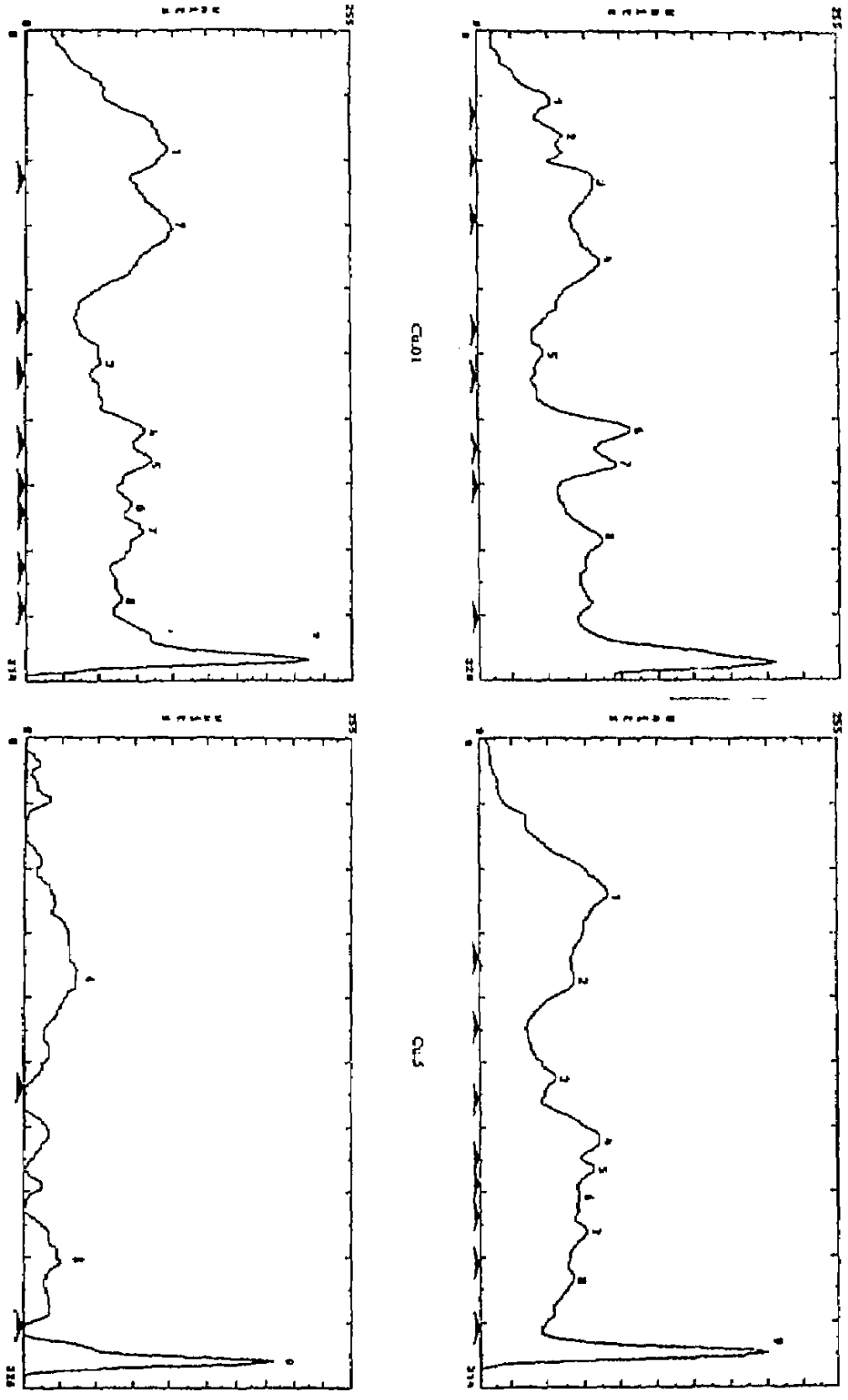

Fig. (2): Serum proteinogram of *O. niloticus* subjected to 0.01, 0.1, 0.5 and 1.0 mg / L copper (Cu).

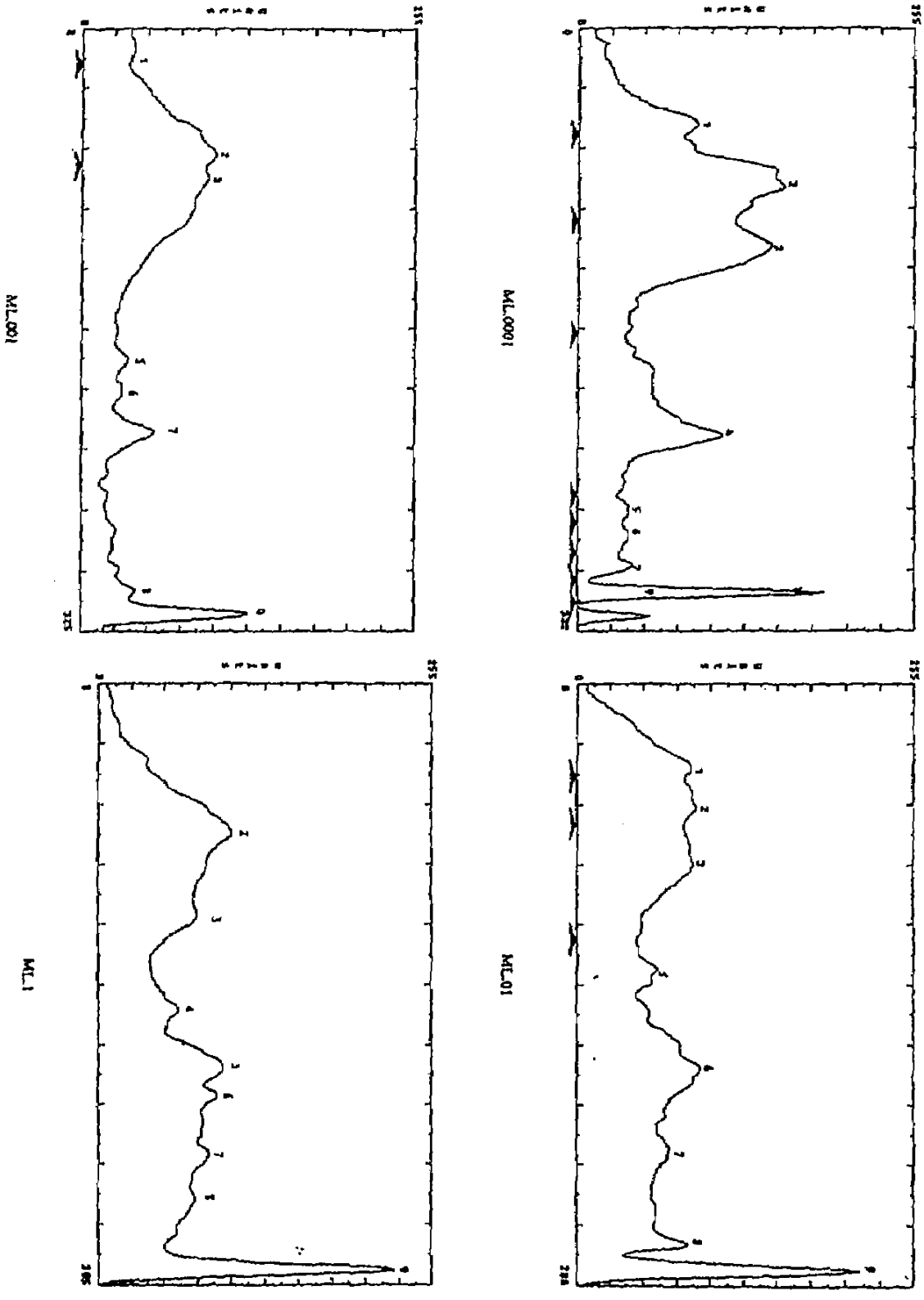

Fig. (3): Serum proteinogram of *O.niloticus* subjected to 0.0001, 0.001, 0.01 and 0.1 mg / L malathion (ML).

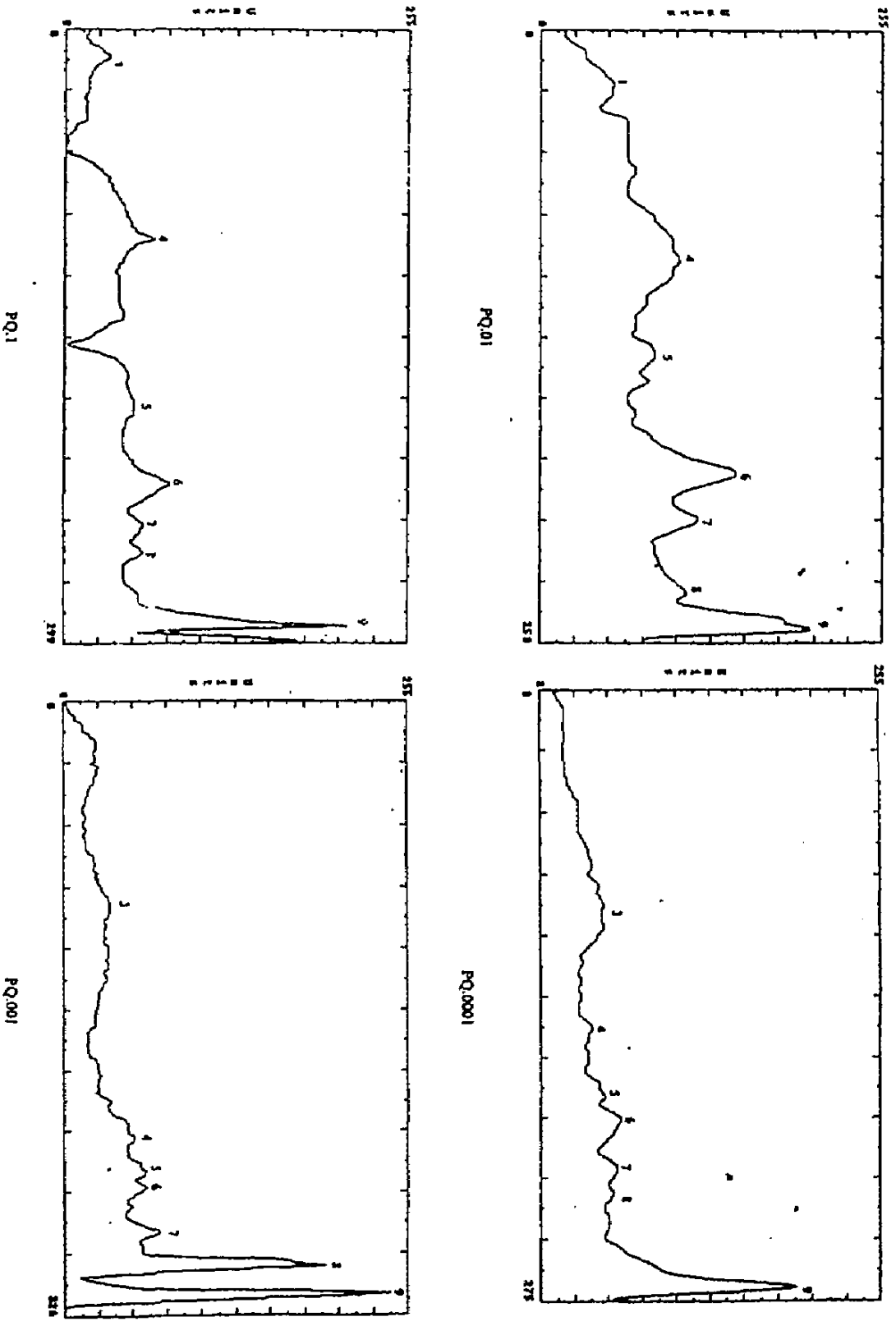

Fig. (4): Serum proteinogram of *O.niloticus* subjected to 0.0001, 0.001, 0.01 and 0.1 mg / L paraquat (PQ).