

## **Protein Profile Changes in *Chroococcus dispersus*, *Microcystis flos-aquae* and *Microcoleus steenstrupii* in Response to Cadmium Treatments**

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*Abstract.* The electrophoretic studies were carried out on three selected algal species, namely *Chroococcus dispersus*, *Microcystis flos-aquae* and *Microcoleus steenstrupii* exposed to two different concentrations (low = 0.5 & high = 2 ppm) of cadmium. Protein profile analyzed by SDS PAGE gel electrophoresis showed differential expression of several proteins. Considerable changes in the number and the percentages of protein fractions are found for each alga. In addition, each organism reacts so differently and individually in response to heavy metal exposure.

### **Introduction**

Heavy metals are difficult to remove from the environment and unlike many other pollutants cannot be chemically or biologically degraded and are ultimately indestructible<sup>[1]</sup>. Today, many heavy metals constitute a global environmental hazard. For example, environmental pollution by cadmium (Cd), arising mainly from mining and smelting, dispersal of sewage sludge and the use of phosphate fertilizers is increasing<sup>[2]</sup>. Thus, the use of microorganisms, algae and plants for decontamination of heavy metals has attracted growing attention<sup>[2-5]</sup>. Algae show immense ability to grow in the presence of heavy metals (HM) concentrations as a result of variety of tolerance mechanisms, *e.g.* binding at cell wall, precipitation in vacuole and synthesis of HM binding compounds such as proteins, organic acids and phenolic compounds<sup>[6]</sup>.

One of the most abundant mechanisms of HM binding seems to be the synthesis of phytochelatin (PCs). PCs are small peptides have high cysteine content, these peptides are able to chelate ions. The introduction and overexpression of metal-binding proteins have been widely exploited to increase the metal binding capacity, tolerance or accumulation. Chelation is prevailing mechanism through the induction of metal binding peptides and the formation of metal complexes. These peptides are enzymatically derived and synthesized on exposure of the cell to toxic metals <sup>[1]</sup>.

Little is known about the effect of heavy metals on the protein profile of algae. Proteins are the primary effectors molecules that can be affected by environmental, physiological or pathological conditions. Therefore, this study aimed to show the protein expression variation in response to heavy metals by examining the influence of two different concentrations of cadmium on three cyanobacterial strains isolated from Ismailia-Port Said Desert Roads, Egypt.

## Materials and Methods

### *Isolation of Algae*

Three algal species were isolated and identified according to <sup>[7-9]</sup>. The isolation was carried out from Ismailia-Port Said Desert Road at Km 14, 52 and 82, respectively. The first site was described as alkaline, while the second was sand textured more or less neutral, on the contrary the third was saline with slightly acidic reaction.

### *Culture Conditions and Growth Evaluation*

The optimization of growth conditions, the effect of Cd metal on the growth of three algal species as well as percentage of metal uptake by organisms have been carried out a previously described <sup>[10]</sup>. Briefly, the algae were used in their growth phase being, 28 days for *Chroococcus dispersus*, 35 days for *Microcystis flos-aquae* and 42 days for *Microcoleus steenstrupii*. The optimum temperature for growth of the three algal species was 35 °C. Meanwhile, the maximum Light intensities were 3000 Lux for both *Chroococcus dispersus* and *Microcystis flos-aquae*, while it was 2000 Lux for *Microcoleus steenstrupii*. Optimum pH values were 7, 10 and 9 for *Chroococcus dispersus*, *Microcystis flos-aquae* and *Microcoleus steenstrupii*, respectively.

### ***Cd Metal Treatments Experiments***

The experiment was conducted according to the following course:  
-10 ml of algal suspension of each species was inoculated in 250 ml conical flask containing 150 ml of medium C <sup>[11]</sup>. The flasks were in triplicate and divided according to the followings:

- 1) 0.5 ppm cadmium + *Chroococcus dispersus*
- 2) 2.0 ppm cadmium + *Chroococcus dispersus*
- 3) 0.5 ppm cadmium + *Microcystis flos-aquae*
- 4) 2.0 ppm cadmium + *Microcystis flos-aquae*
- 5) 0.5 ppm cadmium + *Microcoleus steenstrupii*
- 6) 2.0 ppm cadmium + *Microcoleus steenstrupii*

On the other hand, the control set of three algae was handled with the same manner without any metal treatments. The experiment was carried out under suitable condition of growth parameters for 28-35 days of incubation period.

### ***Preparation of the Three Studied Algal Species for Protein Gel Electrophoresis Method***

#### ***Preparation of Sample***

Algal suspension for each control and treatment was centrifuged at 3000 rpm for 15 minutes. The supernatant was washed by distilled water and centrifuged several times to get rid of any excess salts. The supernatant was decanted and 0.5g of algal material was homogenized in 500 µl of distilled water, centrifuged at 1000 rpm, 400 µl from the supernatant were taken in glass tube treated with 10 µl 10% SDS (Sodium Dodecylsulfate) and 50 µl Mercaptoethanol. The tubes were then heated in boiling bath for 5 min after samples cool down, a drop of tracking dye (Amedo-blue-black) was added for each tube and the samples stored frozen till time of application. 30 µl of the sample was loaded to the gel <sup>[12]</sup>.

#### ***Gel Electrophoresis and Data Analysis***

Samples were subjected to SDS PAGE analysis in a vertical system with gels of 14×16 cm area, 1 mm thick, using the method described by <sup>[12]</sup>. The slab gel of proteins was photographed, scanned using Hooper scanning densitometry G 300. Sigma protein marker wide range used for

determination the approximated molecular weight protein fractions (Fig.1). The scanned gel plates were analyzed using Statistica (Ver.5.1 Stat soft, 1997).

## Results

### *Effects of Cadmium on Chroococcus Dispersus*

The data showed considerable changes in the number and the percentages of protein fractions of each organism. Also, each organism reacts differently with Cd metal and individually with each concentration. The data of gel scanning of normal protein pattern of *Chroococcus dispersus* as control (Tab.1& Fig.2) showed that twenty seven bands represent a percentage ranged between 0.9% and 14% of total protein. Bands 6, 21, 43 and 48 represent the major protein bands, where two bands of them are in the high and middle molecular weight areas and two bands in low molecular weight area. Also, six bands showed middle molecular weight marked by band no. 2, 8, 9, 17, 18 & 30. The rest of bands (seventeen bands) was represented by less than 4% of total protein. Changes in protein profile of *Chroococcus dispersus* were investigated following the exposure to low concentration of cadmium (0.5 ppm). Figure 3 shows the SDS PAGE analyses of algal protein; the profile comprises twenty two bands, relevant to twenty seven bands recorded for control. Eleven bands were categorized as a new band; three of them (band no. 7, 10, 15) being in high molecular weight area, two bands (23 & 24) located in the middle molecular weight area and six bands (28, 36, 38, 39, 40 & 42) existing in the low molecular weight area. The rest bands (eleven bands) were registered for both control and treated samples. Almost these bands show in part a gradual increase or decrease in protein content in comparison with control. High dose of cadmium (Fig.4) resulted in appearance of only twenty four bands compared to twenty seven bands in control. The data showed that almost complete elimination of fifteen protein bands and appearance of twelve new bands. The new bands have the numbers 5, 10, 15, 16, 22, 28, 29, 36, 38, 39, 40 and 41, with percentage range between 1.3 and 8.7% of total protein. Another twelve bands were detected in either control or treated protein. Eight bands of them increase in the levels of proteins after cadmium treatment. The other four bands were of low concentration percentage as compared with control sample and almost two bands (no. 21 & 43) were totally eliminated (Table 1).

### ***Effects of Cadmium on Microcystis Flos-Aquae***

The SDS PAGE protein profile of *Microcystis flos-aquae* of control and treated samples is presented in Table 2 and Fig. 5- 7. It is evident from the results that there is difference in the appearance of the protein bands in treated samples in comparison with control. The gel scan of control showed that the protein profile of *Microcystis flos-aquae* consists of twenty nine bands ranged in their percentage between 0.4% and 7.6%. The major protein bands were excelled by the numbers 12, 22, 24, 30, 40 and 44. Five bands are found in low molecular weight area with percentages of 7, 7.6, 5.1, 5.4 and 5.5%. There was only one band in the middle molecular weight area. Another eleven bands were located in the middle molecular weight bands. These were bands no. 6, 14, 15, 18, 26, 32, 35, 36, 42, 43 and 47. They are distributed as the following: one band in the high molecular weight area, three in the middle one and seven in the low molecular weight area. The rest of bands (twelve bands) were represented by low percentages of total protein (less than 3 % of total protein). The results of SDS PAGE gel of both treated samples exposed to low and high concentrations of cadmium (0.5 & 2 ppm) comprise twenty five bands in comparison to control (twenty nine bands). The scanning detected eleven and twelve new bands for low and high dose cadmium treated specimens, respectively. The detected eleven new bands of numbers 10, 13 and 16 are located in the middle molecular weight area while band no. 19, 21, 25, 27, 38, 39, 45 and 49 are located in low molecular weight area. The scanning of *Microcystis flos-aquae* exposed to high dose of cadmium showed loss of seventeen bands and appearance of twelve new bands. These bands represent between 0.7-17.6 percent of total protein. Concerning the rest bands of protein, Table (2) shows that fourteen and twelve bands are found for both control and treated samples in case of low and high cadmium treatments, respectively.

### ***Effects of Cadmium on Microcoleus Steenstrupii***

The SDS PAGE protein profile of *Microcoleus steenstrupii* as control (Fig.8) showed that it consists of twenty six bands ranged in their percentage between 0.4 and 12.5 %. Two bands of them located in high molecular weight area and another two bands in the middle and low molecular weight areas with protein percentage between 6.9-12.5%. There were another six bands located in middle molecular weight in percentage between 4-6% of total proteins. These were distributed as follows: three at high molecular weight area and two in the middle molecular weight and one in low molecular weight area.

**Table 1. SDS-PAGE gel scanning of protein fractions of *Chroococcus dispersus* control and after treated with 2 different concentrations of Cd metal. (L=Low;H=High).**

Number	Rf.	Control	LCd%	HCd%
1	0.035	-	-	-
2	0.05	4.3	-	13.3
3	0.075	-	-	-
4	0.08	0.9	10.7	1
5	0.09	-	-	5.4
6	0.1	6.2	5.7	-
7	0.12	-	3.8	-
8	0.14	5.3	-	-
9	0.16	4.2	4.5	-
10	0.18	-	3.2	1.5
11	0.2	1.2	1.8	-
12	0.22	1.4	-	-
13	0.24	2.8	4.7	-
14	0.27	1.9	-	6.3
15	0.29	-	3.2	2.2
16	0.33	-	-	1.8
17	0.34	4.4	-	1.4
18	0.35	4.4	9.2	-
19	0.37	1.7	-	4.6
20	0.38	2.5	-	-
21	0.4	7.2	28.1	1.9
22	0.42	-	-	6.3
23	0.44	-	5.3	-
24	0.46	-	1.4	-
25	0.47	3.2	-	-
26	0.48	-	-	-
27	0.5	2.3	2	-
28	0.52	-	2.1	8.7
29	0.55	-	-	6.6
30	0.6	4.1	3.9	-
31	0.62	2.7	-	3.2
32	0.63	2.3	-	-
33	0.64	1.6	-1.7	27
34	0.66	2.3	-	1.8
35	0.69	1.3	-	1.6
36	0.7	-	0.9	3.3
37	0.72	-	-	-
38	0.74	-	1.9	2.5
39	0.76	-	0.6	1.3
40	0.79	-	0.4	2.5
41	0.8	-	-	6.5
42	0.82	-	0.4	-
43	0.84	14	4.5	6.2
44	0.86	-	-	-
45	0.89	3.8	-	-
46	0.91	1.8	-	7.2
47	0.92	1.7	-	-
48	0.98	7.5	-	-

The rest bands (sixteen bands) represent less than 4% of total protein. Exposure to treatments of cadmium (Table 3 & Fig. 9&10) creates thirty bands and twenty two bands for low and high concentrations of cadmium (0.5 and 2 ppm), respectively. The scanning detected twelve new bands (7, 9, 11 and 14 appeared in high while, 15, 21, 24 in middle and 33, 35, 36, 37 & 44 in low molecular weight area ) for samples exposed to low cadmium concentration. The other eighteen bands were registered for both control and treated samples, out of them eight bands were of high percentages and they located as follow: one in high molecular weight area (band no. 2), four in the middle (band no.19, 22, 27 & 28 ) and three in low molecular weight area (band no. 32, 40 and 43 ). On the other hand, ten bands were of lower percentages protein content in comparison with control.

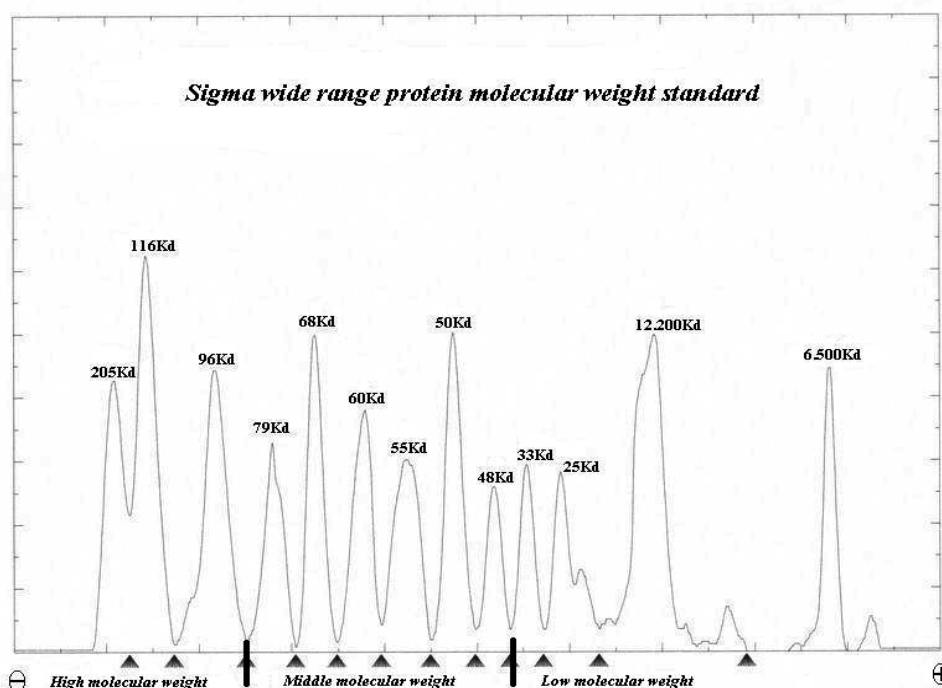
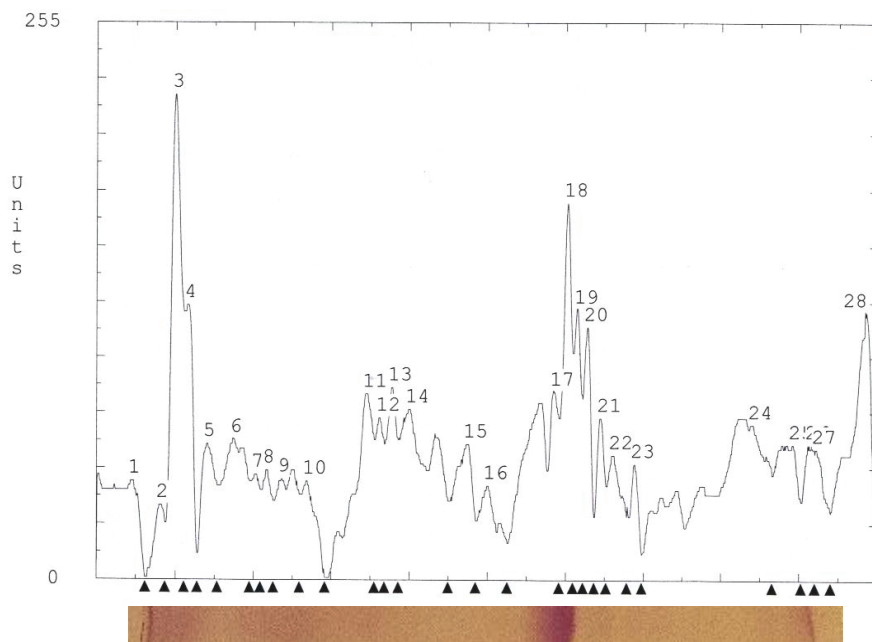
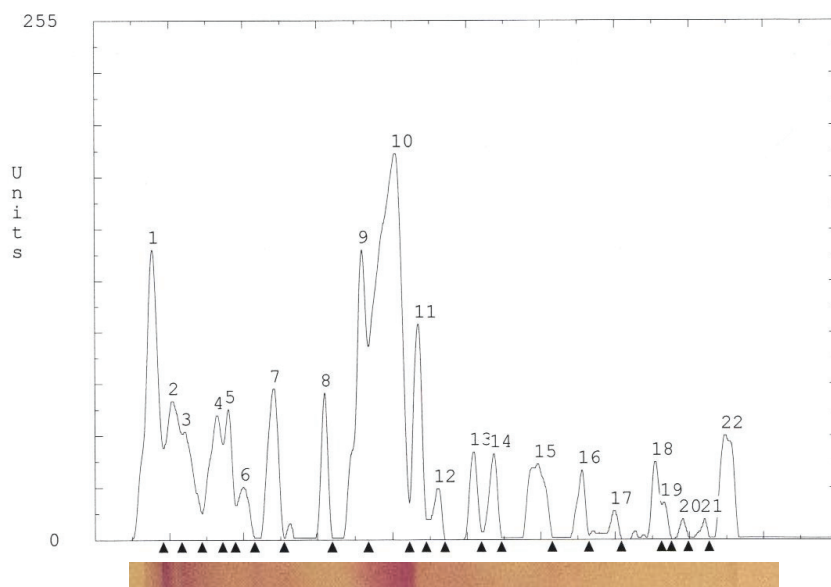


Fig.1. Result of scanning sigma wide range molecular weight protein.

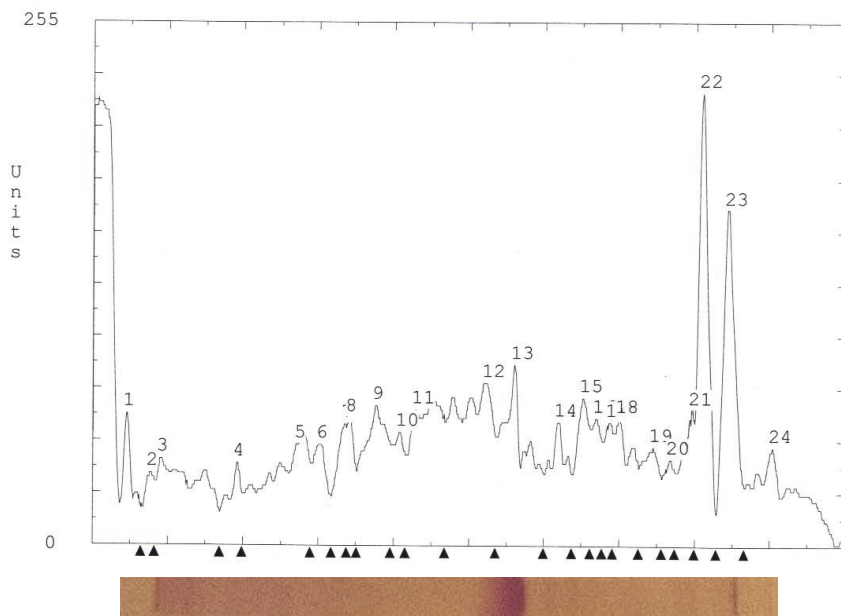


**Fig. 2.** Electropherogram showing the results of scanning of control protein fractions of *Chroococcus dispersus* and corresponding SDS PAGE gel.

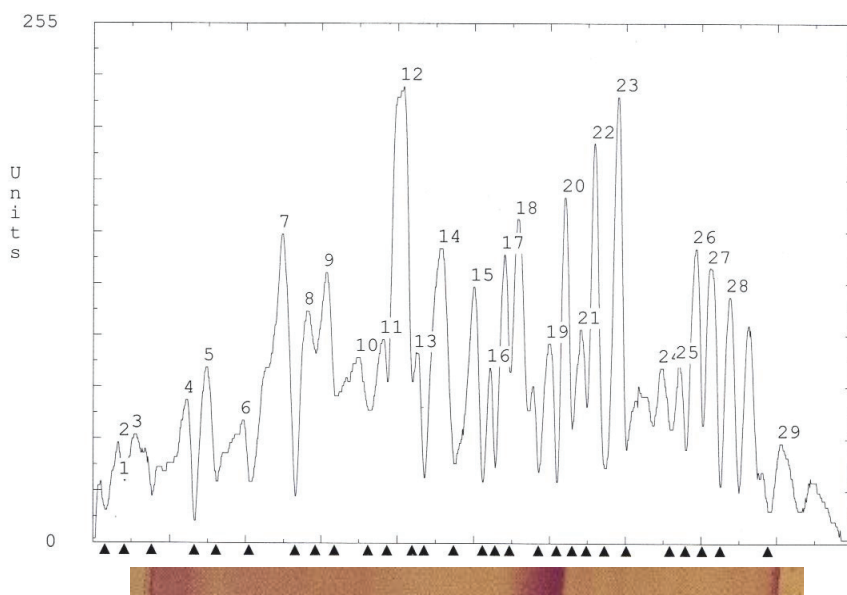


**Fig. 3.** Electropherogram showing the results of scanning of exposed protein fractions of *Chroococcus dispersus* to low concentration of cadmium and corresponding SDS PAGE gel.





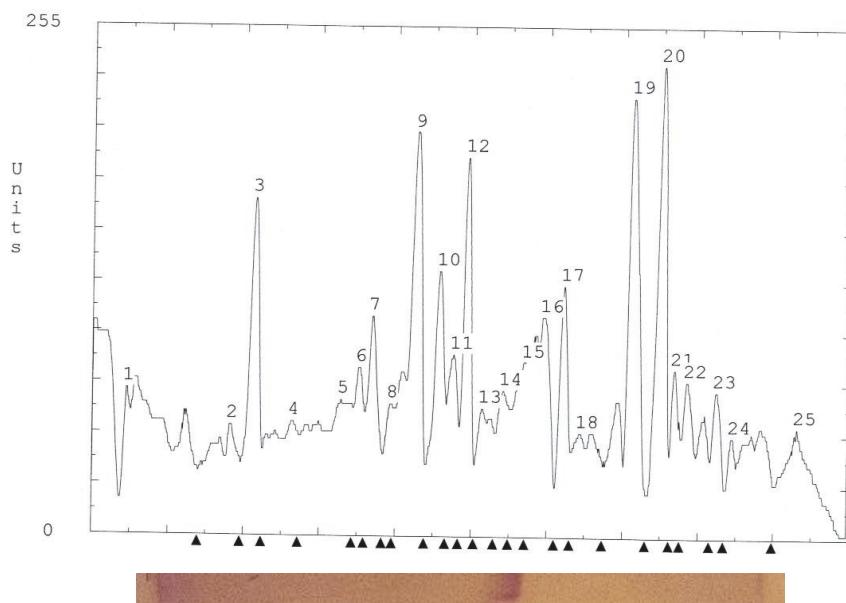
**Fig. 4.** Electropherogram showing the results of scanning of exposed protein fractions of *Chroococcus dispersus* to high concentration of cadmium and corresponding SDS PAGE gel.



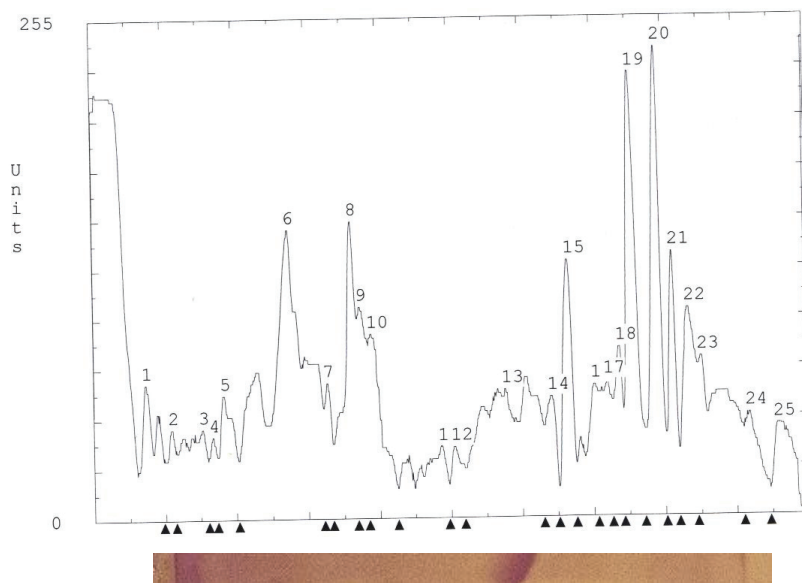
**Fig. 5.** Electropherogram showing the results of scanning of control protein fractions of *Microcystis flos-aquae* and corresponding SDS PAGE gel.

**Table 2. SDS-PAGE gel scanning of protein fractions of *Microcystis flos-aquae* control and after treated with 2 different concentrations of Cd metal.(L=Low;H=High).**

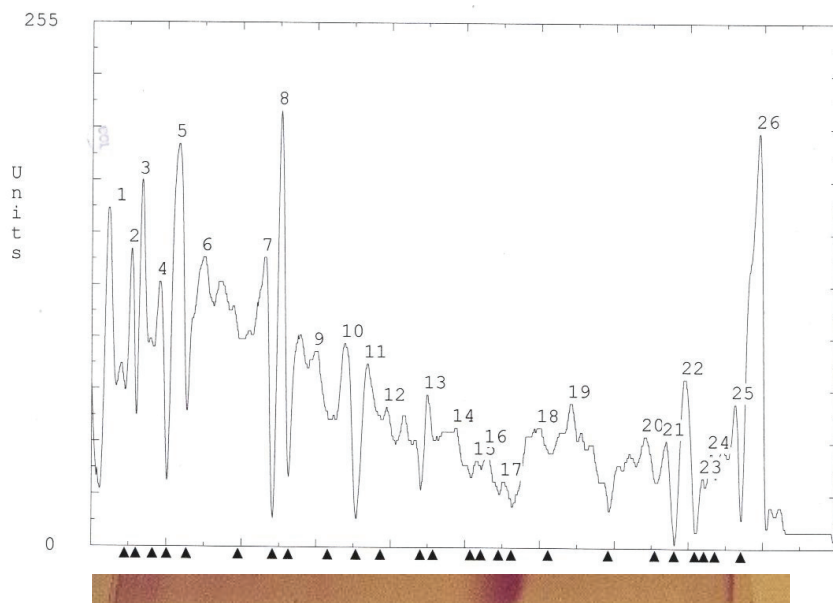
Number	Rf.	Control	LCd%	HCd%
1	0.01	0.4	-	-
2	0.03	1.2	-	-
3	0.05	2.1	13.1	-
4	0.08	-	-	17.6
5	0.10	-	-	1.0
6	0.12	3.2	-	-
7	0.15	2.2	-	2.7
8	0.17	-	-	0.7
9	0.19	2.8	3.7	2.1
10	0.22	-	4.1	-
11	0.24	-	-	-
12	0.25	7.0	-	-
13	0.26	-	3.8	-
14	0.28	3.2	-	13.8
15	0.30	3.8	-	-
16	0.32	-	6.2	1.1
17	0.34	-	-	-
18	0.35	4.8	1.8	-
19	0.36	-	2.8	4.3
20	0.38	2.8	-	2.4
21	0.39	-	1.2	3.2
22	0.41	7.6	-	-
23	0.43	1.6	7.2	-
24	0.45	5.1	3.4	-
25	0.47	-	2.1	-
26	0.49	3.8	3.6	2.8
27	0.51	-	2.2	1.0
28	0.53	1.3	-	-
29	0.54	2.6	2	-
30	0.57	5.1	2.6	8.9
31	0.60	2.3	5.3	-
32	0.62	3.0	2.5	-
33	0.64	2.2	3.1	1.6
34	0.65	-	-	-
35	0.66	3.8	-	2.9
36	0.69	4.6	-	-
37	0.70	-	-	2.1
38	0.72	-	7.7	2.0
39	0.74	-	5.4	1.8
40	0.76	5.4	-	5.6
41	0.77	2.0	1.5	-
42	0.79	3.1	3.4	5.5
43	0.81	3.2	-	2.3
44	0.83	5.5	1.6	3.2
45	0.84	-	4.4	5.9
46	0.86	-	-	-
47	0.90	4.3	-	-
48	0.92	-	-	1.9
49	0.93	-	5.3	-



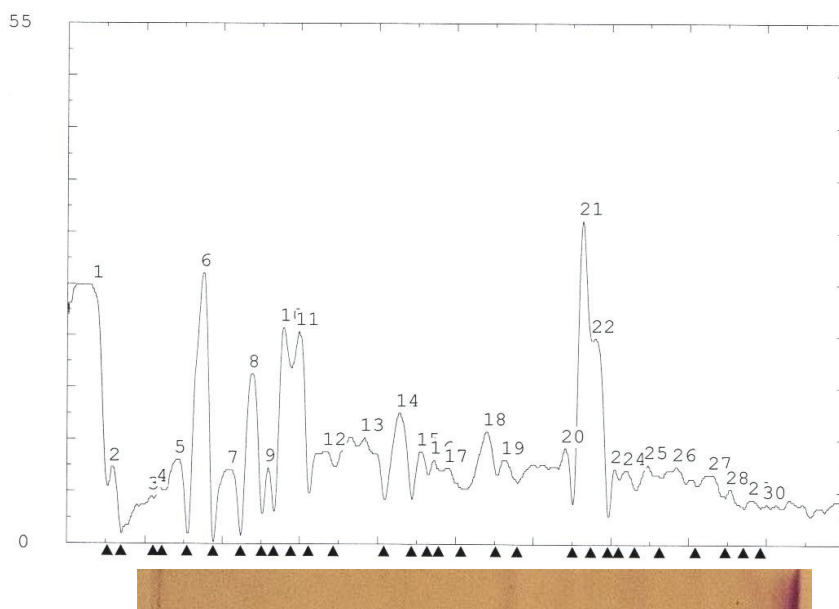
**Fig. 6.** Electropherogram showing the results of scanning of exposed protein fractions of *Microcystis flos-aquae* to low concentration of cadmium and corresponding SDS PAGE gel.



**Fig. 7.** Electropherogram showing the results of scanning of exposed protein fractions of *Microcystis flos-aquae* to high concentration of cadmium and corresponding SDS PAGE gel.



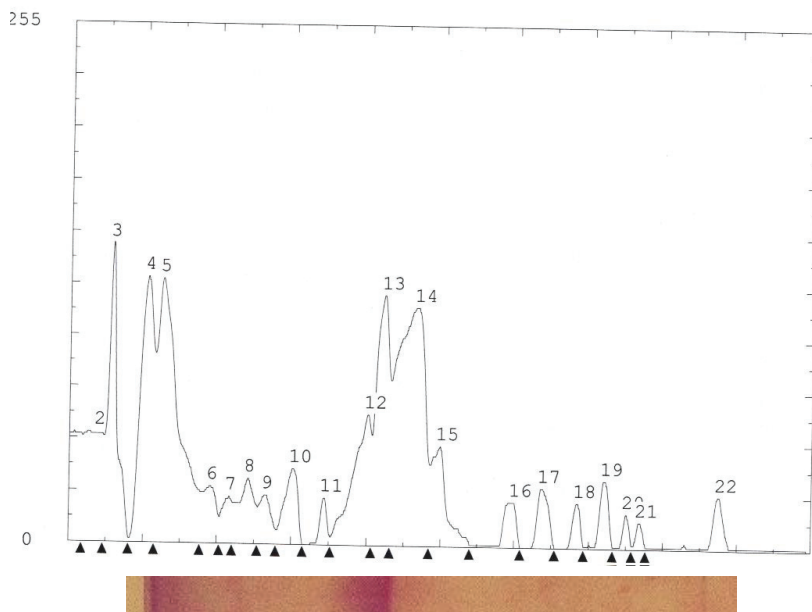
**Fig. 8.** Electropherogram showing the results of scanning of control protein fractions of *Microcoelus steenstrupii* and corresponding SDS PAGE gel.



**Fig. 9.** Electropherogram showing the results of scanning of exposed protein fractions of *Microcoelus steenstrupii* to low concentration of cadmium and corresponding SDS PAGE gel.

**Table 3. SDS-PAGE gel scanning of protein fractions of *Microcoleus steenstrupii* control and after treated with 2 different concentrations of Cd metal . (L=Low;H=High).**

Number	Rf.	Control	LCd%	HCd%
1	0.007	-	-	3.4
2	0.022	5.8	13.4	5.6
3	0.05	2.5	-	-
4	0.06	4.1	1.1	7.5
5	0.09	2.9	-	-
6	0.11	5.4	1.5	9.4
7	0.12	-	0.7	15.6
8	0.15	12.5	2.4	-
9	0.17	-	5.8	-
10	0.19	-	-	2.3
11	0.21	-	2.2	1.2
12	0.23	7.3	3.3	-
13	0.25	3.7	1	2.9
14	0.27	-	4.1	1.7
15	0.29	-	4.4	-
16	0.31	6.9	-	2.8
17	0.34	4	3.1	0.9
18	0.37	3.2	-	-
19	0.39	4.6	6.7	6.2
20	0.40	-	-	-
21	0.43	-	3.8	8.8
22	0.45	1.5	1.8	-
23	0.46	-	-	-
24	0.47	-	1.3	18.3
25	0.49	3.9	2.3	4.4
26	0.52	0.9	-	-
27	0.53	1.4	4.1	-
28	0.56	0.8	2.4	-
29	0.59	-	-	1.6
30	0.60	3.4	-	-
31	0.62	-	-	-
32	0.64	6	6.2	1.8
33	0.66	-	5.9	-
34	0.67	-	-	-
35	0.68	-	4	0.9
36	0.70	-	1	-
37	0.72	-	1.7	1.6
38	0.74	3.9	2.6	0.6
39	0.76	1.4	-	0.5
40	0.78	2	3.7	-
41	0.80	-	-	-
42	0.82	0.4	-	-
43	0.83	0.9	2.7	-
44	0.85	-	1.3	-
45	0.88	2.5	1.1	2.1
46	0.89	8.3	4.7	-
47	0.91	-	-	-



**Fig. 10. Electropherogram showing the results of scanning of exposed protein fractions of *Microcoleus steenstrupii* to high concentration of cadmium and corresponding SDS PAGE.**

## Discussion

Using SDS PAGE, the present study demonstrated the presence of twenty seven protein bands separated from *Chroococcus dispersus* as untreated control. While, treated organism by high dose and low dose of cadmium resulted in appearance of twenty four and twenty two protein bands, respectively. The appearance of a definite decrease in protein band numbers in cadmium treatment organism in comparison to control, indicating that the cellular proteins are one of the main targets of the heavy metals treatments. In contrast, some authors reported that the exposure of *Cyprinus carpio* to different levels of heavy metals (Hg, Pb, Cu and Ni) at lethal and sub-lethal concentrations affected only the magnitude of protein profile, while did not affect the behavior of the protein pattern<sup>[14]</sup>. The present results reflect in part a gradual decrease or increase in protein contents in comparison with control and this is consistent with the previous behavior of *Cyprinus carpio* exposed to metals exposure<sup>[14]</sup>.

Previous experimental findings indicated that cadmium treatments result in complete elimination of some protein bands in one hand and creation of new ones on the other hand [3, 4, 5, 15]. Also the present results revealed that treated organism by low cadmium dose results in disappearance of sixteen protein bands and appearance of eleven new bands. On the other hand, a high dose of cadmium treatments resulted in a complete elimination of fifteen protein bands and creation of twelve new bands. These findings agree well with the results by [3, 5, 15]. They reported that the treated test organisms respond to stress by synthesizing a new set of proteins. Indeed, other authors observed that the synthesis of protein was repressed under heavy metals tested [15-17].

Present work on *Microcystis flos-aquae* showed that cadmium treatments (both low and high doses) result in twenty five protein bands in comparison to control (twenty nine protein bands). Furthermore, the scanning studies revealed eleven new protein bands and twelve new protein bands in treated organism with low and high doses of cadmium, respectively. On the other hand, *M. flos-aquae* exposed to high dose of cadmium showed disappearance of seventeen protein bands. The SDS PAGE protein profile of *Microcoleus steenstrupii* (as control) revealed twenty six protein bands. While organism treated with low and high doses of cadmium showed thirty and twenty two protein bands, respectively. Moreover, scanning studies demonstrated twelve and ten new protein bands in treated organism with low and high cadmium doses, respectively. On the other hand, fourteen protein band disappeared in organism exposed to high cadmium dose. The foregoing data showed that each organism reacts differently against heavy metals treatments. These responses of organism could be attributed to the reaction against the heavy metal as well as its concentration, by dissociation of some protein fractions, which moved to the lower molecular weight area. These findings are consistently with the results of the previous results [2, 18]. They reported that the impact of some environmental conditions affects the protein profiles expressed under stress.

The effects of physiological factors such as heat, salinity, UV irradiation and different concentrations of cadmium, copper, nickel, zinc were studied world-wide [3, 13]. Similar studies were carried out concerning higher plants [4, 5]. Despite of the numerous studies concerning the effects of cadmium on the growth parameters of cyan bacteria [2, 18], very little is known about the impact of the heavy metals on protein

profile of cyanobacteria isolated from Egyptian soils. Therefore, the present data permit certain generalizations on control mechanism of the algae against heavy metal stress. At present, this control can be described as a protein regulation. In conclusion, although the physiological roles of protein profile of the algae against heavy metal stress are not clear, it is slowly becoming evident that some protein bands have been utilized for a variety of purposes including antagonist of the heavy metal stress. As far as we know, the observations presented here provide a baseline as an aspect of relationship between protein profile of algae and the heavy metals.

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تغييرات في النمط البروتيني الكمي والكيفي لثلاثة طحالب هي  
 كروؤكس\_ديسبرسس، ميكروسيستس فلوس-اكوا  
 وميكروكولياس ستينستروكي نتيجة للمعاملات بمعدن الكاديوم

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المستخلص. تناول البحث دراسة تأثير تركيزين مختلفين من الكاديوم (٥،٠ - ٢ جزء في المليون) على النمط البروتيني لثلاثة أنواع من الطحالب كروؤكس ديسبرسس، ميكروسيستس فلوس-اكوا وميكروكولياس ستينستروكي أظهرت نتائج التفريد الكهربائي (SDS PAGE) تباين أنماط المحتوى البروتيني للطحالب الثلاثة، مقارنة بالكنترول، كما بينت الدراسة أن الكائنات الثلاثة محل الدراسة قد استجابت بآليات مختلفة واستقلال تام مع التركيزين المختلفين من الكاديوم وقد خلصت النتائج أن هذه التغييرات ما هي إلا صورة من الصور الميكانيكية لتحمل الإجهاد الناشئ من استخدام المعادن الثقيلة.