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Toxicity effect of Cobalt on total protein and carbohydrate of cyanobacteria *Spirulina platensis*

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Abstract: *The nano-planktons are considered to be the most important marine phytoplankton components since they constitute the basic food of almost all the marine larval organisms and other marine creatures. They are considered to be the first organisms affected by heavy metals pollutants discharged in aquatic environment because they are directly in contact with the medium separated only by the cell wall and the cytoplasmic membrane. Regarding content of protein and carbohydrates under the effect of the different concentrations of this element (Co^{+2}) the results provided that they affected the content of these metabolites with different degrees. Under lower concentrations of these elements the content of the measured metabolites increased but with different responses. The increase in content of these metabolic products with low concentrations of the tested heavy metals may be attributed to inhibition to export out these metabolites from the cells by heavy metals. On the other hand, inhibition in their accumulation induced by higher concentrations of heavy metals may be attributed to the toxic action of these heavy metals on the enzymatic reactions responsible for biosynthesis of these metabolites.*

Keywords: *Cyanobacteria, heavy metal, toxicity, total protein and total carbohydrate.*

I. Introduction

Heavy metals can enter aquatic environment from point sources such as industrial premises and sewage treatment plants or from diffuse sources which include road and agricultural runoff and aerial deposition [1]. Alloway [2] illustrated that heavy metals or trace metals are the term applied to a large group of trace elements that are both industrially and biologically important. Micronutrients are another term used to call heavy metals because some of these elements are essential in small but critical concentrations, such as Co, Mo, Mn and Zn, although they are toxic at high concentrations. Algal communities in fact have been used in toxicity tests for environmental monitoring of heavy metal pollution [3]. Although dead algae have been utilized successfully in heavy metal adsorption experiments [4 and 5] , living algae remove significantly more metal ions than non-living algae at all metal concentrations examined, probably due to metabolic uptake and continuous growth [3 and 6]. Moreover, living algae possess intracellular polyphosphates which participate in metal sequestration, as well as algal extracellular polysaccharides that serve to chelate or bind metal ions [7, 8, 9 and 10].

Cobalt, a transition element, is an essential component of several enzymes and co-enzymes. It has been shown to affect growth and metabolism of plants. The unicellular green alga *Monoraphidium minutum* and the diatom *Nitzschia perminuta* were cultured under different concentrations of Co^{+2}, growth and pigment content were slightly increased at low concentrations and inhibited by high Co^{+2} concentrations. The results concerning the effect of different concentrations of Co^{+2} on photosynthetic O^2 evolution showed a reduction in the amount of O^2 evolved by each alga in response to increasing Co^{+2} concentrations [11]

Lustigman *et al* [12] studied the effect of Co^{+2} on *Chlamydomonas reinhardtii*, they observed reduction of growth at 10 ppm Co^{+2} and without change in the morphology of the cells or pH. At 20 ppm Co^{+2}, on the other hand, growth was considerably reduced compared to the control and the color of the organism became paler and the cells were clumped. In cyanobacteria, $CoCl^2$ inhibits the formation of heterocyst, ammonia uptake, and nitrate reductase activity

The most prominent food algae are cyanobacteria (*Spirulina*). It can supply the human body with most of vitamins and essential inorganic and organic elements. It gets its own sun protective defense from the higher carotene production in intensive sun. *Spirulina* may contain from 65-70% protein. That means that all the eight essential amino acids are present in their correct ratios. It also contains chlorophyll of three times the amount of highly developed green plants. Chlorophyll is mentioned for the prevention and treatment of gastric duodenal ulcers, acne; to strengthen the heart muscles, build up immunity and energy, as a possible anti-bactericide and still unknown others [13]. This *Spirulina* can be used against certain illnesses for example, Acne, AIDS, Allergies, Anemia, Arthritis, Cancer, Depression, Protection against Radiation, Heavy metal detoxification, Cholesterol, Hypertension, Arteriosclerosis Pancreatitis, Loss of Vision, Cataracts and Glaucoma, Hepatitis and Cirrhosis, Gastric and Duodenal Ulcers, Sexual Vitality, Anti-ageing, Weight loss and a Slimming agent. However, the highest content of tyrosine may be the reason which suppresses hunger [13]. In our research we

aimed to estimate the amount of proteins and carbohydrates in the blue-green alga *spirulina* under the effect of different concentrations of Co^{+2}.

II. Material and methods

S. platensis was grown in Spirulina medium [14]. It consists mainly of the following: Macronutrients (Solution A) (NaCl: 1g, MgSO4. 7H2O: 0.2g, CaCl2. 2 H2O: 0.04g, FeSO4.7 H2O: 0.01g, Na-EDTA: 0.08g, K2HPO4: 0.5g, NaNO3: 2,5g,K2SO4:1.0g and NaHCO3:16.8g, in one liter distilled H2O). Stock solution B was prepared as following (NH4NO3: 0.023g, K2Cr2 (SO4)2. 27 H2O: 0.096g, NiSO4. 7H2O: 0.044g, Na2SO4. 7H2O: 0.018g, Ti (SO4)3: 0.040g and Co (NO3) 2. 6H2O: 0.044g in one litter of distilled water). Stock solution C was prepared as following (H3BO3: 2.820g, MnCl2. 4H2O: 1.810g, ZnSO4. 7H2O: 0.222g, CuSO4. 5H2O: 0.077g andMoO3: 0.015g, in one liter distilled water). One ml from stock solution B + 1.0 ml from stock solution C were added to each 1000ml of solution A.

The cultures were grown under controlled laboratory conditions. Culture experiments were conducted under a regime of 16 hour light/ 8 hour dark. The culture period lasted for 18 days. The cells of *S. platensis* were harvested by centrifugation at 5000 rpm for 30 min using angle rotor centrifuge. The supernatants were discarded and the remaining pellets were used for the determination of carbohydrates, protein consequently, the following selected concentrations were 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l respectively for Co^{+2}.

Measurements of total proteins and its fractions:
In this investigation protein was determined by the method described by [15] which is the modification of the original Folin-phenol method of Lowry *et al* [16].

Preparation of algal extract for protein analysis:
Protein was extracted from the algal cells according to the method described by Rausch, [17].

Determination of total proteins:
Reagent A: 2.0 g potassium sodium tartrate and 100g sodium carbonate dissolved in 1 liter of 0.5 N NaOH (0.9 ml used for each determination).
Reagent B: 2.0 g potassium sodium tartrate and 1.0 g CuSO4.5H20 dissolved in 100 ml 0.1 N NaOH (0.1 ml was used for each determination).
Reagent C: one volume Folin ciocalteu reagent diluted ten-fold with distilled water (3.0 ml were used for each determination).

Reagent A was added first to one ml of clear extract and to the blank or standard solution (for the standard curve) in a test tube and heated in water bath at 50°C for 10 min. cooled rapidly in ice. Reagent B was then added and the tube was left for at least 10 min at room temperature. Reagent C was then added and mixed well by whiter mixer, placed in water bath at 50°C for 10 min., then cooled rapidly. The intensity of the blue color developed in each sample was measured using a spectrophotometer at 650 nm and readings were then related to the standard curve.

Determination of water soluble proteins:
Aliquots (10 ml) of algal suspension were centrifuged. The algal precipitate was boiled in 10 ml distilled water for two hours. After cooling, the water extract was centrifuged and the supernatant was decanted and completed to a definite volume by distilled water and analyzed for determination of soluble proteins following the same method for estimation of total protein content.

Determination of water-insoluble proteins:
It was calculated as the difference between the total and water-soluble protein fractions of the same sample.

Measurements of carbohydrates content:
Carbohydrates content was estimated according to the method described by Dubois *et al*, [18].

Determination of total carbohydrates:
In a test tube two ml of N HCl were added to the-cell pellets -obtained from 5ml culture after centrifugation and shaken well for complete homogeneity. The mixture was transferred to a clean test tube and then boiled for 20 min. After cooling at room temperature, the extract is clarified by centrifugation for five minutes at 2000 rpm. In a clean test tube 0.5 ml of the clear supernatant and 0.5 ml of 5% phenol solution was added and mixed well. To each tube 2.5 ml of 98% H2S04 were added rapidly so that the stream hit the liquid surface directly to produce good mixing. Tubes were equally agitated during the acid addition. Blank was also treated in the same manner, but 0.5 ml distilled water was used instead of algal extract. After ten minutes, the

tube were re-shaken and placed in a water bath at 25-30°C for 20 min. The developed yellow-orange color was stable for several hours. The optical density was determined at 488 nm and compared to calibration standard curve.

Determination of water- soluble carbohydrates:

To estimate water-soluble carbohydrates, 5 ml culture from each algal culture was centrifuged and the supernatant was decanted. After decantation, the algal precipitate was extracted by distilled water for two hours in a boiling water bath. After cooling, the soluble fraction was separated by centrifugation and completed to a definite volume. Then the water soluble carbohydrates were determined by the method mentioned previously in case of total carbohydrates.

Determination of water- insoluble carbohydrates:

It was calculated as the difference between the total carbohydrates and the water soluble carbohydrates of the same sample.

Statistical analysis:

All estimates of sample variability were given in terms of the SD. The data were means ± SD of at least three independent experiments. The obtained data were analyzed statistically using the least significant difference (LSD) at 0.05.

III. Results

Effect of different concentrations of Co^{+2} ion on protein content of *S. platensis*:

Results obtained for protein content in *S. platensis* revealed that, although the values of total protein contents that obtained in control increased gradually till the end of the experiment (16 days), yet under the effect of tested element, It is clear from these data that protein content under the effect of 1.0, 1.5 and 2.0 mg/l increased gradually till the end of the experiment while at concentrations 2.5 and 3.0 mg/l Co the total protein content began to decrease after the 12th day of culturing (Table 1 and Fig1)

Effect of different concentrations of Co^{+2} ions on carbohydrates content of *S. platensis*:

On the light of the obtained experimental results for the effect of different concentrations of Co^{+2} ions on carbohydrates content of *S. platensis* after 4, 8, 12 and 16 day of culturing. The data recorded in Table (2) and graphed in Fig (2) showed that the content of total carbohydrates decreased gradually under the stress effect of Co^{+2}. The degree of increase or decrease in the content of carbohydrates depends mainly on the length of culturing period.

IV. Figures and Tables

Table 1: Protein content (µg/ml) of *S. platensis* after 4, 8, 12 and 16 days of culturing under the effect of different concentrations of Co^{+2} (mg/l).

Time (days)	Parameters	Control	Different Concentrations of Co^{+2} (mg/l).				
			1.0	1.5	2.0	2.5	3.0
4	Soluble	14.22 ± 0.05 [a]	15.12 ± 0.02 [b]	10.23 ± 0.12 [a]	10.12 ± 0.11 [d]	9.89 ± 0.06 [f]	9.06 ± 0.06 [f]
	Insoluble	15.22 ± 0.09 [a]	15.31 ± 0.06 [a]	14.25 ± 0.06 [b]	13.91± 0.07 [c]	12.05 ± 0.07 [e]	11.99 ± 0.05 [d]
	Total	29.44	30.43	24.48	24.13	21.94	21.05
8	Soluble	20.63 ± 0.12 [a]	20.77 ± 0.05 [a]	20.63 ± 0.03 [a]	18.22± 0.10 [b]	16.27 ± 0.10 [c]	16.02 ± 0.05 [d]
	Insoluble	32.46 ± 0.18 [a]	24.96 ± 0.05 [b]	28.66 ± 0.01 [c]	15.73 ± 0.08 [c]	15.02 ± 0.01 [d]	14.47 ± 0.07 [d]
	Total	53.09	45.73	49.29	33.95	31.29	30.49
12	Soluble	24.34 ± 0.03 [a]	24.83 ± 0.07 [c]	23.12 ± 0.06 [c]	23.05 ± 0.04 [b]	20.62 ± 0.07 [c]	18.20 ± 0.09 [d]
	Insoluble	32.12 ± 0.07 [a]	35.93 ± 0.08 [e]	33.12 ± 0.02 [b]	29.12 ± 0.02 [c]	22.16 ± 0.05 [d]	19.62 ± 0.12 [e]
	Total	56.46	60.76	56.24	52.17	42.78	37.82
16	Soluble	26.41 ± 0.03 [a]	27.75 ± 0.06 [a]	29.33 ± 0.10 [c]	25.00 ± 0.05 [a]	24.38 ± 0.08 [c]	20.07 ± 0.07 [d]
	Insoluble	40.31 ± 0.09 [a]	41.75 ± 0.08 [a]	43.18 ± 0.10 [b]	33.44 ± 0.10 [c]	23.26 ± 0.03 [d]	21.85 ± 0.05 [d]
	Total	66.72	69.5	72.51	58.44	47.64	41.92

Data are expressed in mean ± SD Different superscripts are significant

Table 2: Carbohydrates content (µg/ml) of *S. platensis* after 4, 8, 12 and 16 days of culturing under the effect of different concentrations of Co^{+2} (mg/l).

Time (days)	Parameters	Control	Different Concentrations of Co^{+2} (mg/l).				
			1.0	1.5	2.0	2.5	3.0
4	Soluble	3.60 ± 0.08 ^{a}	5.18 ± 0.03 ^{d}	6.68 ± 0.02 ^{c}	6.75 ± 0.03 ^{c}	4.79 ± 0.07 ^{c}	4.32 ± 0.03 ^{b}
	Insoluble	23.49 ± 0.04 ^{a}	24.14 ± 0.09 ^{b}	28.69 ± 0.07 ^{d}	20.43 ± 0.12 ^{c}	18.76 ± 0.04 ^{c}	14.76 ± 0.12 ^{d}
	Total	27.09	29.32	35.37	27.18	23.55	19.18
8	Soluble	5.29 ± 0.05 ^{a}	6.55 ± 0.10 ^{b}	8.20 ± 0.01 ^{b}	8.89 ± 0.15 ^{c}	8.03 ± 0.03 ^{d}	7.92 ± 0.15 ^{c}
	Insoluble	41.27 ± 0.07 ^{a}	42.67 ± 0.07 ^{b}	37.69 ± 0.07 ^{c}	35.37 ± 0.11 ^{c}	32.62 ± 0.10 ^{d}	29.34 ± 0.11 ^{d}
	Total	46.56	43.22	45.89	44.25	40.65	37.26
12	Soluble	11.47 ± 0.10 ^{a}	12.14 ± 0.06 ^{a}	13.34 ± 0.03 ^{b}	13.39 ± 0.10 ^{b}	11.13 ± 0.07 ^{a}	8.02 ± 0.08 ^{c}
	Insoluble	47.13 ± 0.07 ^{a}	48.77 ± 0.03 ^{c}	48.80 ± 0.01 ^{a}	45.15 ± 0.08 ^{b}	33.19 ± 0.02 ^{d}	26.05 ± 0.10 ^{e}
	Total	58.6	60.91	62.14	58.54	44.32	34.07
16	Soluble	12.14 ± 0.08 ^{a}	12.30 ± 0.04 ^{a}	12.01 ± 0.02 ^{a}	10.06 ± 0.07 ^{b}	8.50 ± 0.04 ^{c}	6.68 ± 0.07 ^{c}
	Insoluble	48.01 ± 0.11 ^{a}	49.55 ± 0.07 ^{b}	42.30 ± 0.15 ^{c}	39.61 ± 0.05 ^{c}	27.44 ± 0.12 ^{d}	19.01 ± 0.03 ^{e}
	Total	60.15	61.85	54.31	49.67	35.94	25.69

Data are expressed in mean ± SD Different superscripts are significant

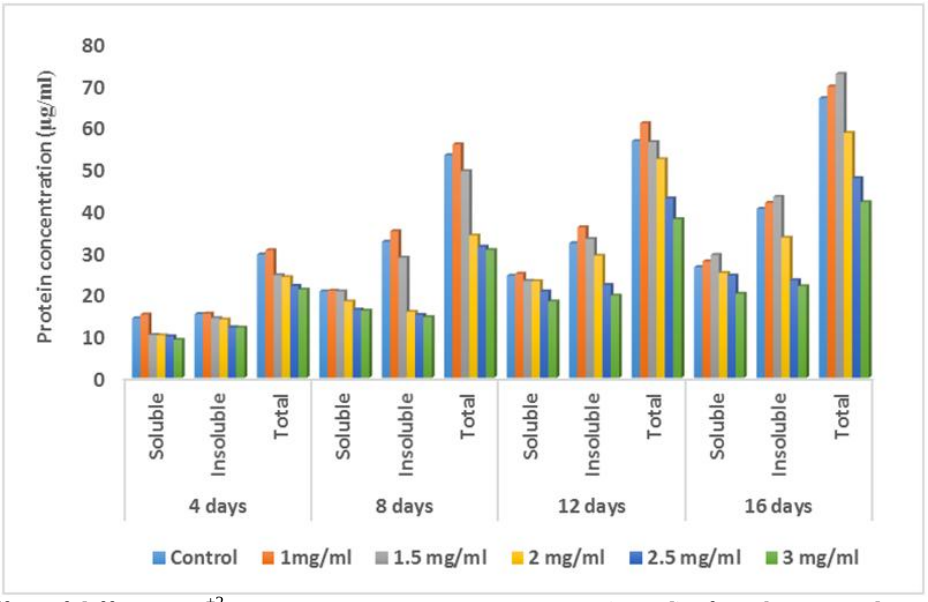

Figure 1: Effect of different Co^{+2} concentrations on proteins content (µg/ml) of *S. platensis* cultured for 4, 8, 12 and 16 days of culturing

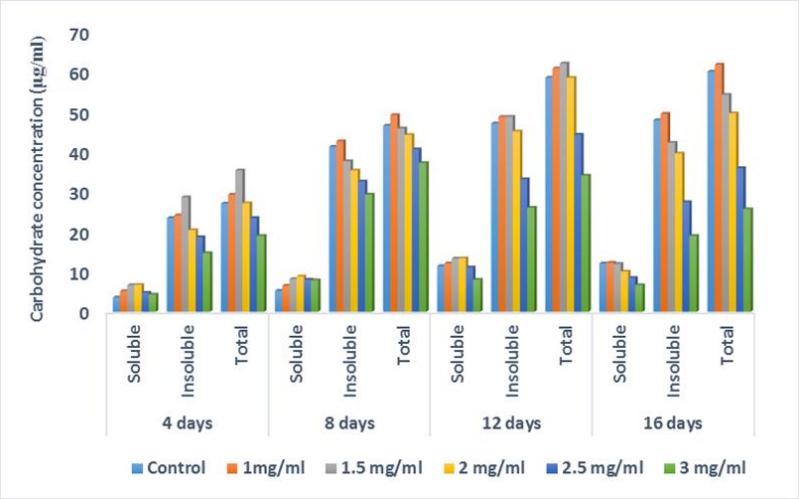

Figure 2: Effect of different Co^{+2} concentrations on carbohydrate content (µg/ml) of *S. platensis* cultured for 4, 8, 12 and 16 days of culturing.

V. Discussion

This work dealt with the effect of different concentrations of Co^{+2} ion on some important metabolites of the blue green alga *S. platensis*. Anent results obtained from protein content of *S. platensis* (soluble, insoluble and total) comparing to control, it is clear that protein content whether soluble, insoluble or total increased gradually with increasing the period of culturing till the 16th day. That agrees with Thomas and Dumas, [19] who reported that protein content is usually higher in the early stages of growth and falls as the culture ages. However, several studies found that protein content of microalgae depends on the nitrogen source used in the culture medium [20, 21, 22, 23 and 24]. Another study reported that elemental and biochemical composition of *Dunaliella primolecta* were correlated positively with nitrogen concentration of the medium and this composition was influenced by the availability of the nitrogen which could be the reason for this phenomena [25].

Concerning the effect of Co^{+2} ions on the content of total protein, the obtained results indicated that low concentrations of Co^{+2} (1.0 and 1.5 mg/l) induced a significant increase in protein content of *S. platensis* till the 4th day of culturing. Further increase in Co^{+2} levels was accompanied by a progressive reduction in protein content of the tested alga. These results are in agreement with those obtained by Stillwell and Holland [26], El-Mazally [27] who reported that high concentrations of Ni^{+2} significantly decreased all proteins of *Cricosphaera cartera*. Additionally, Angadi and Mathad [28] found that total proteins content were inhibited at higher concentrations of Co^{+2} while lower concentrations showed stimulatory effect in *Scenedesmus quadricauda*. Also, [29] found that 100 µM Co^{+2} reduced the protein content of *Haematococcus lacustris*. Accordingly, accumulation of protein at low heavy metal concentrations may be one of the ways through which the algae can abolish their toxic effects, and/or may be due to increased respiration leading to the utilization of carbohydrates in increasing nitrogen metabolism. On the other hand, inhibition of protein accumulation induced by higher concentrations of heavy metals may be attributed to the toxic action of these heavy metals on the enzymatic reactions responsible for protein biosynthesis and the extent of such inhibition was concentration and pH dependent [30, 31, 32 and 33].

Regarding protein content under the effect of different concentrations of Co^{+2}, the results cleared that under lower concentrations of Co^{+2} (1.0 and 1.5 mg/l) its content increased at the 4th day of culturing. Further increase in the concentration of Co^{+2} the content of protein decreased gradually till the end of the experiment. These results coincides with those results obtained previously, that increase in protein content at low concentration of Co^{+2} and perhaps other heavy metals may be attributed to inhibition of protein export out of cells by heavy metals[29, 30, 31, 34 and 35]. On the other hand, the decrease of protein under higher concentrations of heavy metal ions in case of Co^{+2} may be attributed to the toxic action of these heavy metals on the enzymatic reactions responsible for biosynthesis of proteins [36, 37, 38 and 39].

Regarding carbohydrates content in cultures amended by Co^{+2}, the present results revealed that different concentrations of Co^{+2} induce gradually decrease in total carbohydrates content till the 16th day of culturing. These findings are in agreement with those obtained by [40] who reported that concentrations of Co^{+2} accelerated carbohydrates content of *Chlorella*. Also [32] reported that the reduction pattern in the various carbohydrates in response to high concentrations of heavy metals could be accounted for inhibitory effect of heavy metals upon photosynthesis activity and/or utilization of carbohydrates as substrate in increasing nitrogen metabolism.

VI. Conclusion

The overall map of our results that content of protein and carbohydrates under the effect of the different concentrations of Co^{+2} the results proved that they affected the content of these metabolites with different degrees. Under lower concentrations of these elements the content of the measured metabolites increased but with different responses. These may be attributed to the toxic action of this heavy metal on the enzymatic reactions responsible for biosynthesis of these metabolites.

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