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EFFECT OF VITAMINS ON GROWTH CRITERIA, PHOTOSYNTHETIC PIGMENTS AND SOME METABOLIC PRODUCTS OF COBALTE CHLORIDE STRESSED *SCENEDESMUS OBLIQUUS* CULTURES

Desouky, S.A., USAMA, M. A. and AHMED, W. A.

Botany and Microbiology Department, Al-Azhar University, Faculty of Science, Assiut, 71524

ABSTRACT:

This study show the effect of applied vitamins ascorbic acid (vitamin C) and thiamine (vitamin B_1) on growth criteria, total photosynthetic pigments total, carbohydrate, total protein, free amino acids and proline contents of stressed *Scenedesmus obliquus* cultured for 7 days. The growth criteria (cell count and dry weight), total photosynthetic pigments, total carbohydrate contents, total protein contents, free amino acids and proline of stressed *Scenedesmus obliquus* cultures were significantly increased, when the algal cultures subjected to lower doses (1.5 and 3 ppm) of $CoCl_2$ only. On the other side, under relatively higher concentration (4.5 ppm) of $CoCl_2$ the cell count, dry weight, total photosynthetic pigments, total carbohydrate contents, total protein contents and free amino acids of $CoCl_2$ stressed *Scenedesmus obliquus* cultures were significantly decreased. However, the soluble carbohydrate contents, soluble proteins and praline contents of stressed *Scenedesmus obliquus* cultures were significantly increased.

On the other hand, the parameters tested were significantly increased, when the algal cultures were subject to various doses (1.5, 3 and 4.5 ppm) of $CoCl_2$ and treated with 200 ppm applied vitamins (ascorbic acid or thiamine). However, the different carbohydrate fractions (soluble, insoluble and total carbohydrate), soluble proteins of $CoCl_2$ stressed *Scenedesmus obliquus* cultures were significantly decreased.

INTRODUCTION:

It well known that the pollutions of fresh water by heavy metals are considered the main problem in the development countries. Cobalt is one of these elements, which cause the water pollution. Cobalt is an essential element for the growth of marine algal species, including diatoms, chrysophytes, and dinoflagellates (Bruland *et al.*, 1991). Cobalt has also been shown to enhance growth of some plants at low concentrations. In higher concentration, cobalt is toxic to human, terrestrial and aquatic animals and to plants. In this respect, cobalt is one from heavy metals group, which play an important role in the growth parameters and metabolic activities of higher plants and algae. In this regards, Co^{+2} constituting 4% of vitamin B_{12}, appears to be universally essentially within the plant kingdom as a micronutrient. It's only

known biological function is associated with this vitamin; although organic compounds containing Co^{+2} are know which reversibly bind oxygen (Wang and Schaeffer 1969 and Bunt 1970). Cobalt also activates several enzymes, especially those which are necessary for metabolic process (Bowen, 1966 and Skholnik 1974).

Cobalt has been found to inhibit transmembrane ion movement and cell division (Hagiwara *et al.* 1969). Ultra-structurally, Co^{+2} caused enlargement of the cell vacuole (Blankenship and Wilbur (1975). Also, Kumar and Kumar (1985) found growth inhibition of a blue green alga *Nostoc linkia* to be directly proportional to increase concentration of cobalt chloride. Also, Agrawal and kumar (1977) studied the response of a blue green alga *Anacystis nidulans* to cobalt. They found that no significant growth inhibition at the lower concentration of (58.9 µg L^{-1}) of Co^{+2}, while, the growth inhibition was recorded at higher level (589 µg $L^{-1)}$ of Co^{+2}, and cell division stopped at concentration (5.9 mg L^{-1}) of Co^{+2}. In this context, Rachlin *et al.* (1982) found that, cobalt caused significant decreased in cell size.

It is well known that algal cells exposed to heavy metals may suffer serious morphological and biochemical alterations (Rocchetta *et al.*, 2006). In this context, Afkar *et al.*, (2010) found that the pigment contents of *Chlorella vulgaris* gradually increased, when the algal cultures were subjected to low concentration 10^{-9} M of some heavy metals (Co^{+2}, Cu^{+2} and Zn^{+2}) during exposure periods, whereas other concentrations 10^{-8} and 10^{-9} M of some heavy (Co^{+2}, Cu^{+2} and Zn^{+2}) cause a clear reduction in the pigments content. Generally, the growth rates decreased in respect of increasing metals concentration more than 10^{-9} M of these heavy metals.

The total protein contents of the green alga *Chlorella vulgaris* gradually decreased in a manner dependent on the metal concentration in the medium (Afkar *et al.*, 2010). On the other hand, the supplementation of copper and zinc by concentration 10^{-9} M increases the total protein content as compared to the control. However, no marked change in total protein contents occurred in cells of *Chlorella vulgaris* which exposed to cobalt, Osman *et al.*, (2004).

The total free amino acids of *Chlorella vulgaris* gradually increased with increasing of heavy metals concentration (Afkar *et al.*, 2010). The same authors found that, the most pronounced stimulation was detected at the culture supplemented with 10^{-7} M copper in comparison to the other tested metals. On the other hand, cobalt and zinc also stimulated the biosynthesis of the total free amino acids, but the stimulatory effect is less than that obtained with copper).

Proline accumulation under heavy metal stress has also been reported earlier in some higher plants (Bassi and Sharma, 1993), although the mechanism of accumulation of proline in plants or plant parts exposed to stress. Thus, Maggio *et al.*, (2002) found that the depicted an inverse relationship between biomass and proline accumulation in the test algae under stressed conditions.

The vitamin is an organic compound required as a nutrient in tiny amounts by an organism Lieberman and Bruning (1990). Vitamins have diverse biochemical functions. Some have hormone-like functions as regulators of mineral metabolism (e.g., vitamin D), or regulators of cell and tissue growth and differentiation (e.g., some forms of vitamin A). Others function as antioxidants (e.g., vitamin E and sometimes vitamin C) Bender (2003). The largest number of vitamins (e.g., B complex vitamins) functions as precursors for enzyme cofactors that help enzymes in their work as catalysts in metabolism. In this context, Desouky (2008) working with *Scenedesmus obliquus* cultures subjected to 200 and 400 mM NaCl and treatment with 100 and 200 ppm Salicylic acid (vitamin S) found that the growth criteria (cell number and dry weight), total photosynthetic pigments, photosynthesis, respiration, proline, free amino acid and catalase enzymes were significantly increased.

This study aim to elucidated the role of some external addition of vitamins (ascorbic acid "vitamin C" and thiamine "vitamin B_1") to alleviate the adverse effect of $CoCl_2$ on the growth criteria, total photosynthetic pigments and some metabolic activities of Scenedesmus obliquus cultures.

MATERIALS AND METHODS:

Tested alga:

***Scenedesmus obliquus* was collected from the River Nile and used as a test organism. Beijerinck's nutritive culture was used as a medium for enrichment and growth of the tested alga, (Stein, 1966).**

Treatments:

***Scenedesmus obliquus* cultures subjected to 00 (control) and 200 ppm of ascorbic acid (vitamin C) and thiamine (vitamin B_1) in the absence or presence of different levels (0, 1.5, 3, and 4.5 ppm) of cobalt chloride for 7 days incubations.**

ANALYTICAL METHODS:

1-Determination of cell number:

One drop of the algal suspension was pipette on the Haemocytometer (0.1 mm depth), covered and left two minutes for algal setting. The mean counts of four replicates were taken into consideration and the results measured as cells ml^{-1} algal suspension.

2-Determination of dry weight:

A definite volume (100 ml.) of algal suspension was filtered through weighed glass fiber filter. The cells after being precipitated on the filter were washed twice with distilled water and dried over night in an oven at 105 ^{o}C. The data were expressed as µg 100 ml^{-1} algal suspension.

3-Determination of total photosynthetic pigments:

The pigment fractions (µg $m1^{-1}$ algal suspension) chlorophyll a, chlorophyll b and carotenoids were calculated by using the equations mentioned by Metzner *et al.*, (1965).

- Chlorophyll a = $10.3E_{663} - 0.918E_{664}$

- Chlorophyll b = $19.7E_{664}$ - $3.87E_{663}$

- Carotenoids = 4.2 E_{452}-(0.0264 chloro. a + 0.426 chloro. b)

4-Determination of carbohydrate contents:

Carbohydrate contents were determined according to Badour (1959).

5-Determination of protein contents:

Protein contents were estimated according the method by Lowry *et al.,* (1951).

6- Determination of proline:

It was determined according to Bates *et al,* (1973) methods.

7- Determination of free amino acids:

Free amino acids were extracted from fresh water algal suspension and calorimetrically determined using the method of Moore and Stein (1948).

8- Statistical Analysis:

The data were statistically analyzed to calculate the Least Significant Difference (L.S.D) according to Snedecor and Cochran (1980).

RESULTS:

The growth criteria (cell count and dry weight) and total photosynthetic pigments of *Scenedesmus* cultures were increased up to the level 3 ppm of $CoCl_2$. However, under relatively higher level (4.5 ppm) of $CoCl_2$, all these parameters were decreased as compared with that of the control cultures.

The maximum values of growth parameters and total photosynthetic pigments of *Scenedesmus* were significantly increased, when algal cultures subjected to 3 ppm $CoCl_2$ only, Whereas, the minimum values of growth parameters were significantly decreased, when algal cultures subjected to 4.5 ppm $CoCl_2$ only, (Fig. 1-a&b).

The growth parameters and total photosynthetic pigments were significantly increased when algal cultures subjected to vitamins (200 ppm of either ascorbic acid "vitamin C" or thiamine "vitamin B_1"). This increase depending on concentration and level of $CoCl_2$ and type of applied vitamin.

Ascorbic acid treatments to non-polluted *Scenedesmus* cultures, the contents of cell number, dry weight and total photosynthetic pigments were raised (Fig. 1-a). On the other side, the contents of cell number, dry weight and total photosynthetic pigments were raised, when treated with thiamine, (Fig. 1-c).

Metabolic products:

The various contents of carbohydrate fractions under different levels (1.5, 3 and 4.5 ppm) of $CoCl_2$ exhibited higher contents than those of the control cultures. However, highest level of $CoCl_2$ (4.5 ppm) lowered in most cases the content of these carbohydrate fractions.

The maximum value of soluble carbohydrates content amounted t0 130% of that of the control cultures, when algal cultures subjected to 3 ppm $CoCl_2$. Also, the maximum values of insoluble and total carbohydrates content amounted to 139% and 134% of that of the control cultures, when algal cultures subjected to 1.5 ppm $CoCl_2$, respectively. But, the minimum values of soluble, insoluble and total carbohydrates content amounted to 92%,

93% and 93% of that of the control cultures, when algal cultures subjected to 4.5 ppm $CoCl_2$, respectively (Fig. 2-a).

Addition 200 ppm of either ascorbic acid or thiamine to different levels of $CoCl_2$ changed the content of carbohydrate fractions (soluble, insoluble and total carbohydrates).

When, the non-polluted *Scenedesmus obliquus* cultures treated with vitamins the content of soluble, insoluble and total carbohydrates were raised and amounted to 185%, 142% and 149% that of the control cultures, when the algal cultures treated with ascorbic acid, respectively (Fig. 2-b). Whereas, addition of thiamine to non-polluted *Scenedesmus* cultures significantly increased the content of soluble, insoluble and total carbohydrates (Fig. 2-c).

The maximum value of soluble carbohydrates content amounted to 92% of that the control cultures, when the *Scenedesmus* cultures subjected to 1.5 ppm $CoCl_2$ and treated with 200 ppm of ascorbic acid respectively. the maximum values of insoluble and total carbohydrates content were 105% and 99% as compared with that of the control cultures, when the algal cultures subjected to 3 ppm $CoCl_2$ and treatment with ascorbic acid, respectively (Fig. 2-b).

On the other side, the maximum value of soluble carbohydrates content amounted to 85% that of the control cultures, when the algal cultures treated with thiamine and subjected to 1.5 ppm $CoCl_2$, respectively. Also, the maximum values of insoluble and total carbohydrates content were reported, when the algal cultures subjected to 3 ppm $CoCl_2$ and treated with thiamine, respectively (Fig. 2-c).

The maximum value of soluble protein contents when treated with $CoCl_2$ amounted to 133%, that of the control cultures when algal cultures subjected to 1.5 ppm $CoCl_2$. Whereas, the maximum values of insoluble and total protein contents when treated with $CoCl_2$ only amounted to 132% and 125% of that of the control cultures, when algal cultures subjected to 3 ppm $CoCl_2$, respectively. In contrast, the minimum values of soluble, insoluble and total protein contents when treated with $CoCl_2$ only amounted to 81%, 67% and 70% of that of the control cultures when algal cultures subjected to 4.5 ppm $CoCl_2$, respectively (Table 1-a).

Treating of non-polluted *Scenedesmus* cultures with 200 ppm of either ascorbic acid or thiamine resulted in promotion of soluble, insoluble and total protein fractions giving amounts of 160%, (Table 1-b). While, it reached to 152%, 151% and 151% of that of the control cultures when algal cultures treated with thiamine, respectively (Table 1-c).

The results given in table (2) indicate that heavy metals exerted generally a stimulatory effect on the accumulation of proline in the test algal cultures. The highest proline level was consistently found in cultures incubated under the highest concentration of heavy metal used.

The maximum value of proline accumulation amounted to 574%, that of the control cultures, when algal cultures subjected to 4.5 ppm $CoCl_2$ (Table 2-a).

Table 1: Protein contents (µg mg^{-1} dry weight) of *Scenedesmus obliquus* cultures subjected to various combinations of $CoCl_2$ and 200 ppm of either ascorbic acid (vitamin C) or thiamine (vitamin B_1) for 7 days incubation

Treatments / $CoCl_2$ (ppm)	Water-soluble Proteins	% Control	Water-insoluble Proteins	% Control	Total Proteins	% Control
(a) $CoCl_2$						
00:00	33.00	100.00	142.00	100.00	175.00	100.00
1.5:00	44.20**	133.94	167.50 **	117.96	211.70**	121.00
3:00	31.10**	94.24	188.25**	132.60	219.35**	125.24
4.5:00	26.80**	81.21	96.00**	67.61	122.80**	70.17
L.S.D at 1 %	1.002		8.002		12.005	
L.S.D at 5 %	2.004		10.045		24.008	
(b) Ascorbic acid (vitamin C)						
$CoCl_2$ (ppm) &Ascorbic acid (ppm)						
00 : 200	53.05**	160.76	230.00**	162.00	283.05**	161.74
1.5:200	60.81**	180.30	259.37**	182.65	320.18**	182.96
3:200	66.00**	200.00	283.40**	199.30	349.40**	199.66
4.5:200	55.53**	168.30	205.00**	144.40	260.53**	148.87
L.S.D at 1 %	7.002		14.002		15.003	
L.S.D at 5 %	8.004		20.004		22.007	
(c) Thiamine (vitamin B_1)						
$CoCl_2$ (ppm) & Thiamine (ppm)						
00:200	50.43**	152.82	215.00**	151.40	265.43**	151.70
1.5:200	61.00**	184.85	250.37**	176.32	311.37**	178.00
3:200	63.42**	192.18	283.44**	199.60	346.86**	198.21
4.5:200	55.50**	168.18	194.76	137.15	250.26**	143.01
L.S.D at 1 %	7.025		20.124		11.123	
L.S.D at 5 %	9.125		25.456		15.123	

Table 2: Proline contents and free amino acids (µg mg^{-1} dry weight) of *Scenedesmus obliquus* cultures subjected to various combinations of $CoCl_2$ and 200 ppm of either ascorbic acid (vitamin C) or thiamine (vitamin B_1) for 7 days incubation

Treatments / $CoCl_2$ (ppm)	Proline	% Control	Free amino acids	% Control
00:00	0.93	100:00	10.88	100
1.5:00	1.88**	202.15	12.78**	117.46
3:00	3.45	371.06	17.32**	159.20
4.5:00	5.34**	574.20	10.00	92.00
L.S.D at 1 %	0.001		0.008	
L.S.D at 5 %	0.004		0.008	
2-a: Thiamine (vitamin B_1)				
$CoCl_2$ (ppm) &Ascorbic acid (ppm)				
00:00	1.36**	146.24	23.35**	214.60
1.5:00	1.80**	193.55	31.52.92	293.40
3:00	1.15**	123.65	38.91**	357.63
4.5:00	1.84**	197.85	2.04**	239.34
L.S.D at 1 %	0.005		8.124	
L.S.D at 5 %	0.007		10.450	
2-b: Thiamine (vitamin B_1)				
$CoCl_2$ (ppm) & Thiamine (ppm)				
00:00	2.82**	303.23	38.00**	349.36
1.5:00	1.75**	188.20	26.51**	234.66
3:00	1.56**	167.74	35.82	329.23
4.5:00	2.67**	287.10	21.61**	198.62
L.S.D at 1 %	0.057		9.245	
L.S.D at 5 %	0.054		11.456	

*** Significant difference** **** High Significant difference…………… as compared absolute control**

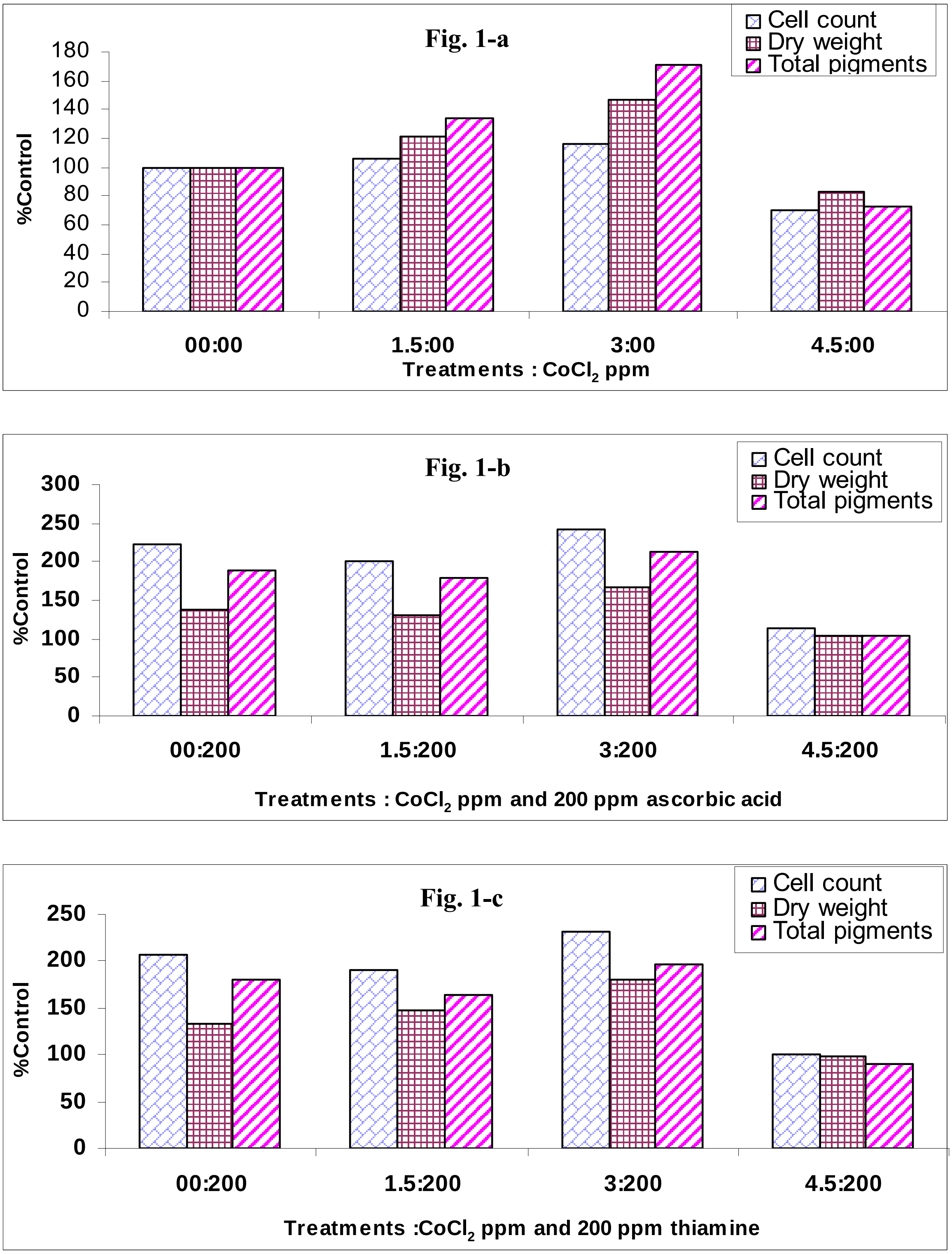

Figure 1: Cell count (cell ml^{-1} algal suspension), dry weight (µg ml^{-1} algal suspension) and total photosynthetic pigments (µg ml^{-1} algal suspension) of *Scenedesmus obliquus* cultures subjected to various combinations of $CoCl_2$ and 200 ppm of either ascorbic acid (vitamin C) or thiamine (vitamin B_1) for 7 days incubation

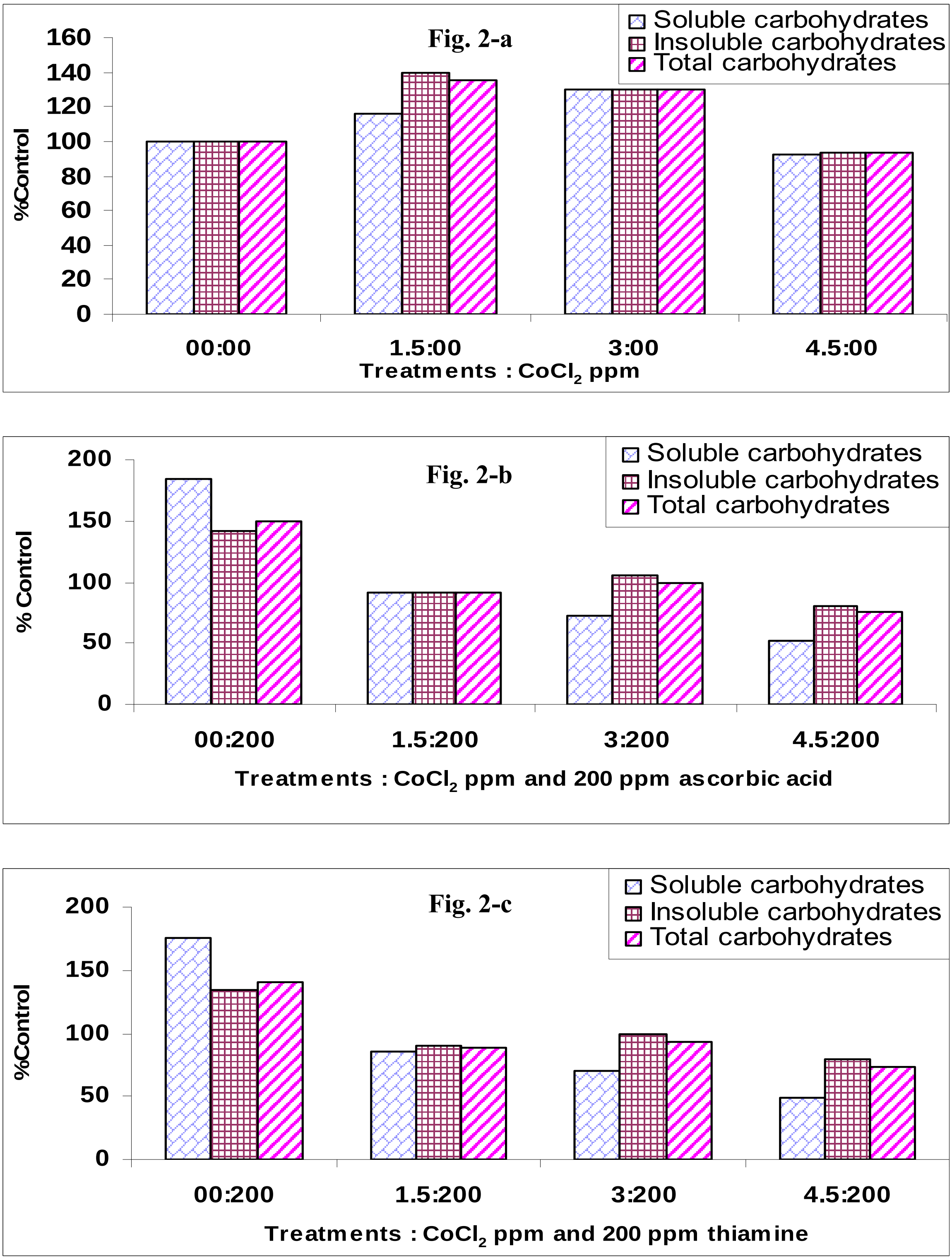

Figure (2): Carbohydrate contents (µg mg^{-1} dry weight), of *Scenedesmus obliquus* cultures subjected to various concentrations of $CoCl_2$ and 200 ppm of either ascorbic (vitamin C) acid or thiamine(vitamin B_1) for 7 days incubation

The highest proline content of *Scenedesmus obliquus* recorded at the level of 4.5 ppm $CoCl_2$ only was associated with a significantly decreased in the contents of free amino acids and thus the lower value was 92% of that of the control cultures, when algal cultures subjected to 4.5 ppm $CoCl_2$ only, whereas highest value of amino acids content was 159% in algal cultures subjected to 3 ppm $CoCl_2$ (Table 2-a).

Using of either ascorbic acid or thiamine as additional treatments to non-polluted *Scenedesmus* cultures resulted in an increase in the content of free amino acids. This content amounted to 214% and 349% of that of the control cultures when algal cultures treated with 200 ppm of either ascorbic acid or thiamine, respectively (Table 2-b&c).

The maximum value of proline accumulation amounted to 574%, of that of the control cultures, when algal cultures subjected to 4.5 ppm $CoCl_2$ only (Table 2-a). In this context, in polluted-*Scenedesmus* cultures, the maximum value of proline content were 287% of that of the control cultures, when algal cultures subjected to 4.5 ppm $CoCl_2$ and treated with 200 ppm of either ascorbic acid or thiamine, respectively. Also, the minimum values of proline contents were 177% of that the control cultures, when the algal cultures subjected to 1.5 ppm $CoCl_2$ and treated with 200 ppm of either ascorbic acid or thiamine (Table 2-b &c).

The important role played by applied vitamins (200 ppm of either ascorbic acid "vitamin C" or thiamine "vitamin B_1") in polluted algal cells by heavy metals is also expressed in changes in the biosynthesis of free amino acids. These changes are given in table (2). The inhibitory effect of heavy metals on the rat of biosynthesis of free amino acids than proline considerably varied.

The highest proline content of *Scenedesmus obliquus* recorded at the level of 4.5 ppm $CoCl_2$ only was associated with a significantly decreased in the contents of free amino acids and thus the lower value was 92% of that of the control cultures, when algal cultures subjected to 4.5 ppm $CoCl_2$ only, whereas highest value of amino acids content was 159% when algal cultures subjected to 3 ppm $CoCl_2$ only (Table 2-a).

Using 200 ppm of either ascorbic acid or thiamine as additional treatments to non-polluted *Scenedesmus* cultures resulted in increasing the content of free amino acids. This content amounted to 214% and 349% of that of the control cultures when algal cultures treated with 200 ppm of either ascorbic acid or thiamine, respectively (Table 2-b &c).

DISCUSSION:

Adverse effect of heavy metals on green algae may lead to disturbances in plant metabolism which consequently lead to a reduction in the growth of these algae (Desouky, "1995, 2001, 2003 and 2004" and Afkar *et al.*, 2010).

The results in this study showed the growth parameters and total carbohydrate contents of *Scenedesmus obliquus* cultures were

significantly increased, when the algal cultures subjected to lower level (1.5 and 3 ppm) of $CoCl_2$. While under higher dose of the carbohydrate contents were significantly decreased. However, the soluble carbohydrate contents were increased with the increasing of $CoCl_2$ levels in the medium cultures. These results present in this study are in agreement with Afkar *et al.*, (2010) that repotted the carbohydrate contents of the *Chlorella vulgaris* alga also declined in manner dependent on the metal concentration exist in the medium. In this context, Fathi *et al.* (2005) reported that the higher doses of heavy metals have severely attenuate chlorophyll synthesis coupled with severe drop in protein resulting in increased carbohydrates. In this respect, Desouky, (2004) found that the total carbohydrate contents of *Chlorella vulgaris* cultures were significantly decreased when; the algal cultures were subjected to various concentrations (5, 10, 20, 25 and 50 ppm) of $CoCl_2$.

The results in this study showed the protein contents of *Scenedesmus obliquus* cultures were significantly increased, when the algal cultures subjected to lower levels (1.5 and 3 ppm) of $CoCl_2$, only. However under higher doses of (4.5 ppm) of $CoCl_2$ the protein contents decreased. The soluble protein contents increased with the increased of treatment element in the medium cultures. Under higher concentrations of this element (Co^+), the total protein contents decreased, when compared with that the control cultures. The same authors reported that all the three metals (Co^{+2}, Cu^{+2} and Zn^{+2}) affected negatively the total protein contents at higher doses. On the other hand, the supplementation of copper and zinc by concentration 10^{-9} M increases the total protein content as compared to the control. However, no marked change in total protein contents occurred in cells of *Chlorella vulgaris* which exposed to cobalt. It could be suggested that accumulation of protein at low heavy metal concentrations may be one of the ways through which the algae can abolish their toxic effects, or to increase respiration leading to the utilization of carbohydrate in favor of protein accumulation (Osman *et al.*, 2004). These results in this investigation in accordance with Afkar *et al.,* (2010) reported that the total free amino acids of *Chlorella vulgaris* gradually increased with increasing metals concentration. On the other hand, cobalt and zinc also stimulated the biosynthesis of the total free amino acids, but the stimulatory effect is less than that obtained with copper.

Generally, the accumulation of amino acids in response to metals concentrations may lead to the assumption that suppressed protein biosynthesis encouraged free amino acids accumulation, or may be due to some counteracting chelating mechanism against heavy metals toxicity (El-Sheekh *et al.*, 2003, Osman *et al.*, 2004 and Fathi *et al.*, 2005).

Also, the results in this study showed the proline contents of *Scenedesmus obliquus* cultures were significantly increased, when the algal cultures subjected to low levels (1.5 and 3 ppm) $CoCl_2$. While under higher level (4.5 ppm) of $CoCl_2$, the proline contents were significantly increased, under heavy metal stress has also been reported earlier in some higher plants

(Bassi and Sharma, 1993), although the mechanism of accumulation of proline in plants or plant parts exposed to stress is still unknown.

Vitamins are nutritional organic compounds required for continued growth by phytoplankton species when studied in axinic cultures. These vitamins are required in very low concentrations Bender (2003), as cofactors by organisms which undergo autotrophic growth (Berland *et al,* 1978, Swift, 1980 and Desouky, 2011).

Plants generally synthesize large amounts of ascorbic acid which could be utilized in cell metabolism (Arrigoni *et al,* 1975 and Chinoy, 1984), and could activate the biological defense mechanisms (Arrigoni *et al*., 1979).

In this investigation, it was generally found that the concentrations of the applied vitamins (200 ppm ascorbic acid "vitamin C" or thiamine "(vitamin (B_1") were significantly increased, the growth parameters (cell number and dry weight) and total photosynthetic pigments of *Scenedesmus obliquus* cultures, when subjected to various doses (1.5, 3 and 4.5 ppm) of $CoCl_2$.

Ascorbic acid was also recorded to affect the chlorophyll contents (Choudhury *et al.,* 1993) through promoting the capacity of chlorophyll by stabilizing and protecting these molecules from being oxidized. This stimulation of growth of both organisms mentioned above in response to treatments with thiamine (vitamin B_1) could be attributed to the significant role played by thiamine (vitamin B_1) in cellular metabolism as coenzymes in the oxidative decarboxylation of pyruvate or of α-ketoglutarate (Harper, 1991 and Makled, 1995). In this context, Desouky (2001) found that the growth parameter of *Chlorella vulgaris* cultures (cell number and dry weight), total photosynthetic pigments and some metabolic activities increased when the algal cultures were subjected to 200 ppm applied vitamin (thiamine).

In this investigation, total carbohydrate contents of *Scenedesmus obliquus* cultures were significantly decreased when the algal cultures subjected to various doses of $CoCl_2$ and treatment with any vitamins (200 ppm of either ascorbic acid or thiamine). These results in this study in accordance with, Desouky, (2003 and 2004) recoded that the total carbohydrate contents of *Chlorella vulgaris* cultures were significantly decreased, when the algal cultures were subjected to various levels of either salinity or heavy metals and treatments with any vitamins (200 ppm of ascorbic acid or thiamine).

The counteractive effects of thiamine might link with the physiological role of this vitamin. Such vitamins can be used as coenzyme performed generally the major physiological function in the plant cells (Kefeli, 1981). Mohamed *et al*., (1988) found that thiamine increased the protein contents of *Chlorella vulgaris*, and the highest protein contents were produced in the presence of 100 ppm concentration of thiamine. Similarly, Makled (1995) working with two green algae namely *Chlorella vulgaris* and *Ankistrodesmus falcatus*, reported that the addition of thiamine, nicotinic acid or pyridoxine exerted considerable increase in growth rate, synthesis of total photosynthetic pigments, carbohydrate and protein contents.

In this study, total protein content of *Scenedesmus obliquus* cultures were increased, when the algal cultures were subject $CoCl_2$ and treated with any applied vitamins (200 ppm ascorbic acid or thiamine). In this respect, Mohamed *et al.* (1988) working with *Chlorella vulgaris,* found that thiamine (Vitamin B_1) increased the protein contents, and the highest protein content was produced in the presence of 10^{-4} M thiamine (Vitamin B_1).

In this investigation, the free amino acid and proline contents of *Scenedesmus obliquus* cultures were significantly increased, when the algal cultures were subjected with various doses (1.5, 3 and 4.5 ppm) of $CoCl_2$ and treated with any applied vitamins (200 ppm ascorbic acid or thiamine). These results in this investigations in accordance with, some other authors working with some types plants found that the growth parameters, total photosynthetic pigments, total carbohydrates, total protein free amino acid and proline contents were markedly raised, when exposed either salinity stress or heavy metals and treatment with any of the applied vitamins (200 ppm of ascorbic acid, thiamine or pyridoxine) (Arrigoni *et al.,* 1992, Desouky, 2001, 2003, 2004 and 2011).

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تأثير الفيتامينات علي النمو ، الأصباغ النباتية وبعض المنتجات الأيضية لمزارع طحلب "*السينيدسمس أوبليكس*"المجهدة بكلوريد الكوبلت

سيد عباس دسوقي، أسامة محمد عبدالرؤوف، أحمدورداني عبدالراضي
جامعة الأزهر - كلية العلوم - قسم النبات والميكروبيولوجي - أسيوط - ٧١٥٢٤
البريد الألكتروني: Desouky_alazhar@yahoo.com

اهتمت تلك الدراسة بإظهار تأثير الفيتامينات [(حمض الاسكوربيك (فيتامين ج) والثيامين (فيتامين ب١)] على معدلات النمو (عدد الخلايا والوزن الجاف)، الأصباغ النباتية، محتوي المواد الكربوهيدراتة، محتوي المواد البروتينة، الأحماض الأمينية والبرولين علي مزارع طحلب "*السينيدسمس أوبليكس*" المجهدة يكلوريد الكوبلت لمدة سبعة أيام.

ويمكن تلخيص النتائج التي أمكن الحصول عليها في هذه الدراسة، كما يلي :

١- عند معالجة طحلب مزارع طحلب "*السينيدسمس أوبليكس* المجهدة بتركيزات مختلفة من كلوريد الكوبلت (١,٥، ٣,٠، ٤,٥) جزء من المليون منفرداً. لاحظ أن معدل النمو متمثلاً في (عدد الخلايا والوزن الجاف) والأصباغ النباتية المحتوي الكلي لكل من المواد الكربوهيدراتية والبروتينية والأحماض الأمينية والبرولين لمزارع لطحلب "*السينيدسمس أوبليكس*" المجهدة بكلوريد الكوبلت تزداد زيادة معنوية حتى مستوى ٣ جزء من المليون.

٢- عند التركيز العالي (٤,٥ جزء من المليون) تتناقص معدلات النمو والأصباغ النباتية المحتوي الكلي لكل من المواد الكربوهيدراتية والبروتينية والأحماض الأمينية المحتوي الكلي لكل من المواد الكربوهيدراتية والبروتينية تناقصاً معنوياً. وعلي العكس من ذلك يلاحظ زيادة محتوي المواد الكربوهيدراتية والبروتينية الذائبة والبرولين.

٣- ازدياد المعدلات المختبرة تزداد زيادة معنوية، وذلك عند معالجة مزارع طحلب "*السينيدسمس أوبليكس* " المجهدة بتركيزات مختلفة (١,٥، ٣,٠، ٤,٥) جزء من المليون من كلوريد الكوبلت بواسطة ٢٠٠ جزء من المليون من أي من الفيتامينين. أما المحتوي إلى للمواد الكربوهيدراتية (الذائبة وغير الذائبة والكلية) ومحتوي المواد البروتنية الذائبة تتناقص تناقصاً معنوياً.