Minia Science Bulletin Volume 27 (1), 19-36, 2016

# COMPARISON BETWEEN THE PHYSIOLOGICAL RESPONSES OF *ANKISTRODESMUS FALCATUS* AND *CHLORELLA VULGARIS* TO COPPER

**Zeinab[1] A. Ahmed, Asmaa[2] B. Ahmed**

1,2 Sohag University, Faculty of Science, Botany Department

## ABSTRACT

The physiological and biochemical responses of two green algae *Ankistrodesmus falcatus* and *Chlorella vulgaris* to copper sulphate were investigated. In this experiment both *Ankistrodesmus* and *Chlorella* cultures were treated with copper sulphate concentrations ranging from 0. 5 to 250 µM. The influence of copper on growth and metabolism was determined. *Ankistrodesmus* grown at 250 µM copper sulphate, while *Chlorella* grown at 100 µM copper sulphate**.** Low copper sulphate concentrations (0.5, 5, 10 µM) induced growth, pigments, all metabolites (Total carbohydrates, total proteins, total free amino acids, and phenolic compounds) and nitrate reductase activity in *Ankistrodesmus* cells and then they were decreased with increasing copper sulphate concentrations. In *Chlorella* the above parameters were decreased under all doses of copper used . In *Ankistrodesmus*, soluble carbohydrates, soluble proteins and proline were decreased until 10 µM copper then they increased with increasing copper levels. While in *Chlorella* they increased in all copper concentrations. SDS-PAGE analysis of *Ankistrodesmus falcatus* and *Chlorella vulgaris* grown in different copper sulphate concentrations caused appearance of new polypeptides and disappearance of others. The treatments of *Ankistrodesmus* with high copper levels (250 µM) caused the synthesis of twenty new polypeptides bands. **.**The numbers of polypeptides bands in *Chlorella* cells were decreased at all copper concentrations when compared with control. There are a differential responses to copper treatments between *Ankistrodesmus falcatus* and *Chlorella vulgaris***.** *Chlorella vulgaris* more sensitive to copper toxicity than *Ankistrodesmus falcatus*

***Key Words:*** Physiological responses, *Ankistrodesmus falcatus*, *Chlorella vulgaris*,

# INTRODUCTION

Aquatic contamination is one of main problems derived from toxic effects of heavy metals. Many pollutants can get into aquatic environments after direct or indirect release from agriculture, industries and domestic waste cause pollution of aquatic ecosystem (Fathi *et al*., 2008; El-Sheekh *et al*., 2011 ). Microalgae are widely used in the regulations for metals (Levy *et al*., 2007). There are many authors studying the toxic effects of heavy metals on different algal species (El-Sheekh *et al*., 2003; Osman *et al*., 2004; Akira *et al*., 2005; Muwafq and Bernd, 2006). Copper, Zink, Cobalt and Manganese are essential micronutrients required for nutrition and metabolism in plants and algae (Reichman, 2002; Ma *et al*., 2003; Andrade et al., 2004 and Fathi, 2005). The high concentrations of the previous metals inhibited growth of higher plants and algae (Hall, 2002; Reichman, 2002; Lim et al., 2006 and Afkar *et al*., 2010). Copper toxicity decreased the activity of photo-system 11 and electron transfer rate of the photosynthetic apparatus in algae (Malick and Mohn, 2003 and Perales-Vela *et al.*, 2007).

Essential and non-essential heavy metals are known to cause inhibition of most growth parameters (An, 2004; Burzynski and Zurek 2007 and Gao *et al*., 2008) and productivity of plants (Kasim, 2001 and Wu et al., 2004) . Heavy metals stress was known to induce a pronounced alteration in nitrogen metabolism (Paraskevi Malea *et al*., 2006 and Kholoud 2009). Generation of reactive oxygen species (ROS) is the major response of plants to toxic concentrations of heavy metals including Cu (Schützendübel and Polle, 2002 and Maksymiee and Krupa, 2003).

At concentrations above those required for optimal growth, Copper can be toxic for most of algae with the exception of a few algal species that can hyper accumulate metals. This toxicity is dependent on algal species, the concentration of metal supplied (Afkar et al., 2010). In sensitive plant species copper was shown to inhibit growth and to interfere with important cellular processes such as photosynthesis and respiration (Prasad and Strzalka, 1999 and Yruela, 2005).

In this work, the physiological responses to lower and higher copper concentrations in two species of green algae *Ankistrodesmus falcatus* and *Chlorella vulgari*s were studied .

## MATERIALS AND METHODS

### Normal and salt stress algal cultures

The sample was collected from El-Dare wastewater treatment plant at Sohag governorate, Egypt. *Ankistrodesmus falcatus* and *Chlorella vulgari*s were isolated from the sample. The two algae were grown in liquid BG-11 medium according to Rippka *et al*., (1979) under the conditions of fluorescent illumination (2500 Lux) and room temperature (25±2°C). Filtered dry air was let to bubble in the culture vessels to provide carbon dioxide and to prevent settling of algal cells.

In this experiment, the *Ankistrodesmus falcatus* and *Chlorella vulgaris* were grown under different concentrations of copper sulphate ( 0.0, 0.5, 5, 10, 50, 100, 150, 200 and 250 µM) Each treatment was made in three replicates. At the end of incubation period, the algal cells were harvested and used for growth and metabolic determinations.

### A- Determination of Growth criteria

Cell number: The cell count of control and treated cultures was measured by Hemocytometer 0.imm deep, having improved Naubauer ruling (A.O. Spencer "Bright fine"). The count was expressed as cells/ml algal suspension.

Dry weight: Dry weight was determined according to Utting (1985) by filtering Culture aliquots (50 ml) through Whatman GF/C filters. The filters were dried and weighed. Data were given as µg/ml algal suspention.

Photosynthetic pigment extraction: Chlorophyll *a*, *b* and Caroteniods were extracted in 100% acetone at 65°C and their contents were determined spectrophotometrically (SPEKOL 11, CARL ZEISS, JENA, GERMANY) according to Metzner *et al*.(1965).

### B.Biochemical determinations

Estimation of Carbohydrate contents: Carbohydrate contents was determined in aqueous (soluble carbohydrate) and in HCl solutions (total carbohydrate) with anthrone sulphuric acid reagent according to Fales

(1951), using glucose as a standard. The blue green color developed was measured at the 620 nm using spectrophotometer.

Estimation of protein contents: Protein content was determined according to Lowry *et al*. (1951). The alga of 10 ml of algal suspension was extracted in distilled-water (soluble protein) and in NaOH (total protein) for 2 h at 90°C. The extract was centrifuged and the supernatants were pooled. The water-soluble protein was estimated by the Folin-phenol reagents and measured spectrophotometrically (SPEKOL 11, CARL ZEISS, JENA, GERMANY.) Bovine serum albumin was used as a standard.

Estimation of total free amino acids: Total free amino acids were determined according to Moore *et al.* (1958). The quantity of total free amino acids was calculated as µ gm/mg. dry weight.

Estimation of proline: Free proline content of algal suspension was determined according to Bates *et al*. (1973). The absorbance was measured at 520 nm. Proline was used as a standard.

Determination of phenolic compounds: Phenolic compounds content was determined according to Dai *et. al*. (1994). The absorbance was measured at 765 nm. Total phenolic compounds were expressed as a nano equivalents of gallic acid using a calibration curve prepared with 10-50 µ M of Gallic acid.

Nitrate reductase assay *in vivo*: For *in vivo* assay of nitrate reductase, the method of Jaworski (1971) was used. Algal cells of 10 ml algal suspension of diuron-treated alga and untreated were precipitated and incubated in anaerobic dark conditions for 1 hr. in 5 ml of 0.1 M K-phosphate (pH=7.5) containing 50 mM $KNO_3$ and 1% (v/v) n-propanol at 28 ºC. The reaction was stopped by boiling in water bath for 5 min and then centrifuged. The supernatant of one ml sample mixed well with two ml 1% w/v sulphonilamide in 1N HCl and two ml 0.1 % w/v N-(1- naphthyl) ethylenediamine dihydrochloride in distilled water. The absorbance was measured by using spectrophotometer (SPEKOL 11, CARL ZEISS, JENA, GERMANY) at 540 nm. Nitrate reductase activity was expressed as µg $NO_2$/mL algal suspension $h^{-1}$

Determination of protein electrophoretic pattern: The algal suspension containing $10^9$cells /ml. was centrifuged at 4000 rpm for 5 min. The pellet was used for the extraction of protein by the method cited by Laemmli (1970) using an extraction buffer as recommended by Hawkesford and Belcher (1991). The method of SDS vertical polyacrylamide gel electrophoresis (SDS- PAGE) was used as described by Laemmli (1970), for the determination of protein electrophoretic pattern. The gels were subjected to the staining solution for 1-2 h., followed by distaining solution overnight .There-after, the distained gels were photographed while wet.

## RESULTS AND DISCUSSION

In this study, we compared growth rate of *Ankistrodesmus falcatus* and *Chlorella vulgaris* under low and high copper sulphate concentrations. In low concentrations of copper sulphate (0.5 to 10 µM) significant increase in cell number and dry weight (157% and 208% respectively) and significant decrease at high concentrations from 50 to 250 µM were observed in *Ankistrodesmus falcatus.* In *Chlorella vulgaris* the cell numbers and dry weight were decreased under all copper concentrations from 0.5 to 100 µM. Fig.1. *Ankistrodesmus* cultures showed increase in chlorophyll a, b and carotenoids at low levels and gradual decrease at high levels from 50 to 250 µM. Fig.2. In, *Chlorella vulgaris* both dry mass and chlorophyll pigments contents decreased in all Copper concentrations. Azeez and Banerjee (1986) also reported decreased chlorophyll contents in two Cyanophytes, *Spirulina platensis* and *Anacystis nidulans* due to Copper toxicity. As the ionic Cupper increases, Copper bound to chloroplast membranes and other cell proteins causing inhibition in chlorophyll pigments (Rai et al., 1990 and Rijstenbil et al., 1994). Higher concentrations of Copper, produce irreversible damage to chloroplast lamellae (Overnell, 1975) preventing photosynthesis and eventually causing the death of cell.

In *Ankistrodesmus* cultures exposed to low copper concentrations from 0.5 to 10 µM the carbohydrates and proteins (total, insoluble) were increased but the soluble ones were decreased when compared with control. In *Chlorella* cultures the same constituents (total and

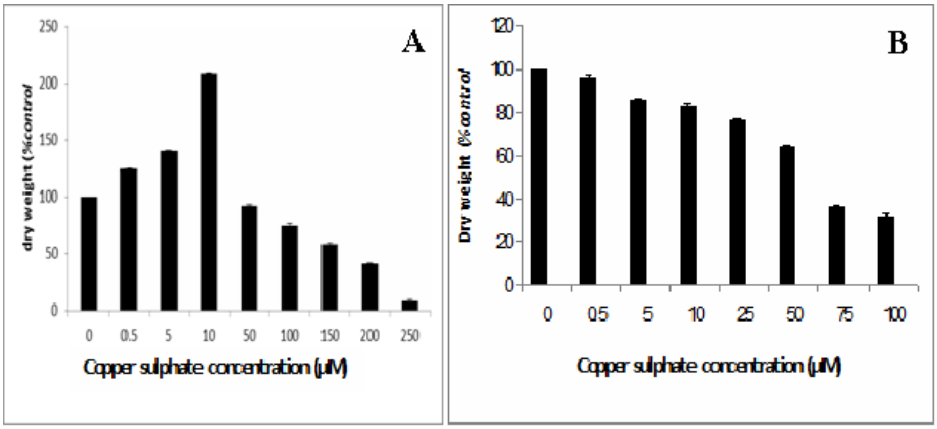


**Fig. 1.** Effect of copper sulphate (µM) on the dry weight (%control*) Ankistrodesmus falcatusnd* (A) *Chlorella vulgari*s (B) culture.

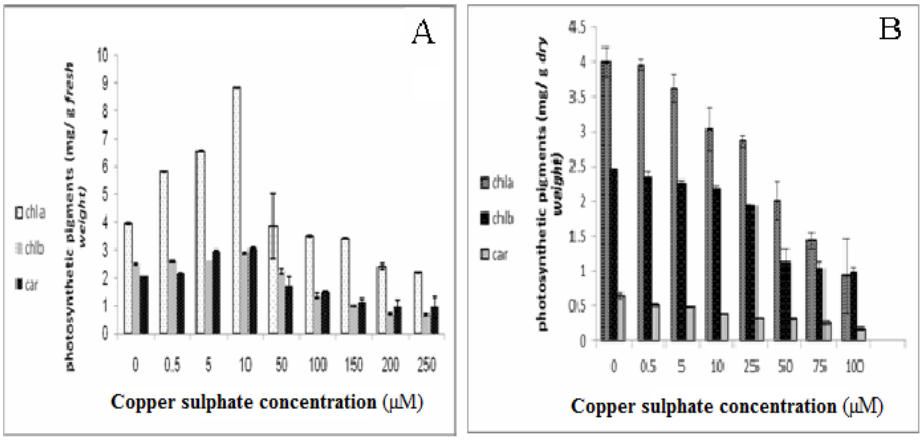


**Fig. 2.** Effect of copper sulphate (µM) on photosynthetic pigments (mg/ g dry weight) of *Ankistrodesmus falcatus* (A) and *Chlorella vulgaris* (B) cultures.

insoluble) were decreased and the soluble ones were increased in all copper levels (Fig.3 and Fig.4) . *Ankistrodesmus* grow in low concentrations of Copper has no toxic effect on growth and the biochemical composition. Copper affects the biochemical such as proteins, lipids and free fatty acids in algae (Lage et al.1994, 1996) . As reported by Lupi et al. (1998), both carbohydrate and protein contents declined in cultures exposed to higher concentrations of Copper. Some authors (Kobbia et al., 1985, Rai et al., 1994, Hart and Scaife, 1997) recorded that protein inhibited by high heavy metal contents and accumulated by low contents of metal. They explained the accumulation of protein at low heavy metal concentrations as one of the ways through which the algae can alleviate their toxic effects, or to increase the utilization of carbohydrate through respiration.

The data in Fig.5 showed that, the total free amino acids were increased in the first algal cells and decreased in the second one at all copper dose. Some amino acids metabolism are stimulated and certain others are inhibited due to pollution effects (Hammouda et al.,1995). Similarly, the biosynthesis of amino acids in cyanobacteria and in eukaryotic phytoplankton cells found in the water polluted with insecticides were enhanced or inhibited (El-Ayouty and Ezzat, 1991; Fathi, 2003) . Oxidative stress directly damages proteins, amino acids, nucleic acids and membrane lipids (Nagalakshmi and Prasad, 1998). As shown in Fig.6, the proline contents in the cells of *Ankistrodesmus* exposed to low copper concentrations were lowered and gradually increased above 10 µM until 250 µM $CuSO_4$. Proline contents of *Chlorella* cultures were elevated at all low and high copper levels. Copper stressed *Ankistrodesmus falcatus* used proline as osmo-regulation. This is in agreement with the conclusion of El-Naggar, (1993). Increased accumulation permits osmotic adjustment and provides protection for enzymes, biological membranes and polyribosomes (Sharma *et al.*, 1998, Basak *et al*., 2001).

In *Ankistrodesmus falcatus* the content of phenolic compounds slightly increased until 10 µM $CuSO_4$ and then decreased with increasing $CuSO_4$ dose while in *Chlorella vulgaris*, the content of phenolic compound reduced at all concentrations of $CuSO_4$(Fig7). The antioxidant compounds are much agree to those recorded for other macro-algae exposed to various abiotic stresses (Burritt, *et al*., 2002; Contreras, *et al.*, 2005). ROS levels

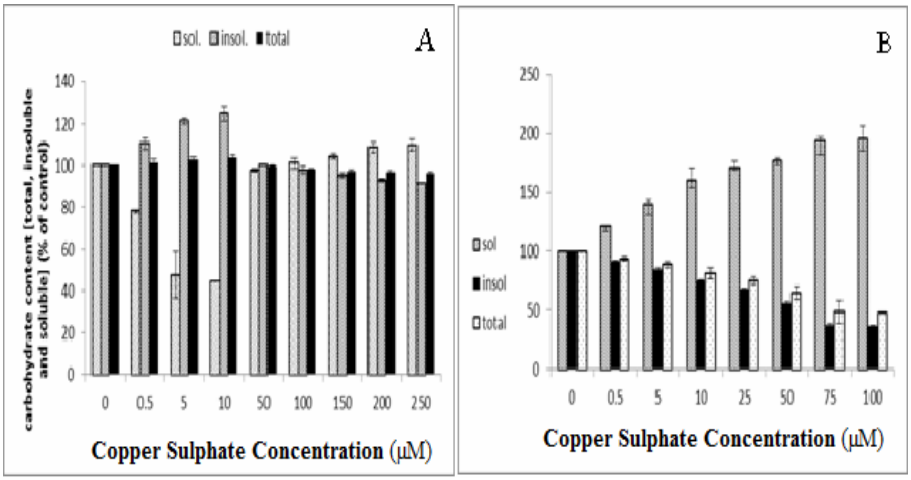


**Fig. 3**. Effect of copper sulphate (µM) on carbohydrate content [total, insoluble and soluble] (% of control) of *Ankistrodesmus falcatus*(A) and *Chlorella vulgaris* cultures (B).

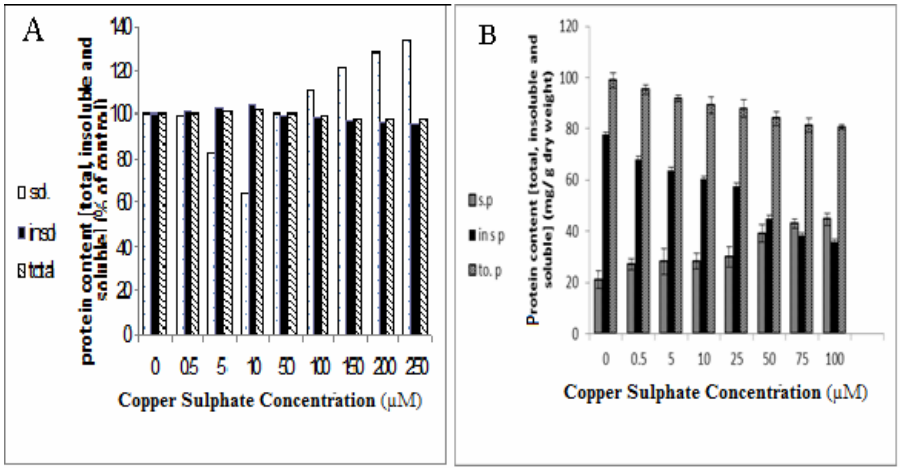


**Fig. 4**. Effect of copper sulphate (µM) on protein contents [total, insoluble and soluble] (% of control) of *Ankistrodesmus falcatus*(A) and *Chlorella vulgaris* cultures (B).

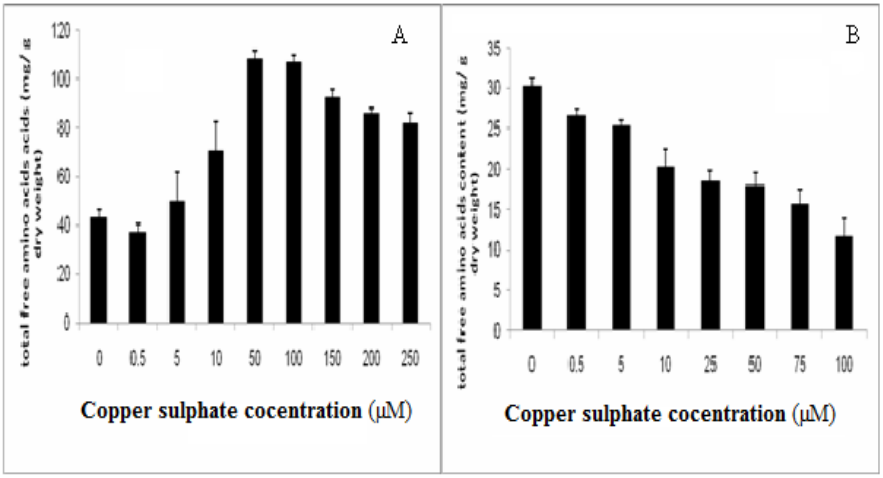


**Fig. 5**. Effect of copper sulphate (µM) on total free amino acids (mg/ g dry weight) of *Ankistrodesmus falcatus* (A) and *Chlorella vulgaris* cultures (B).

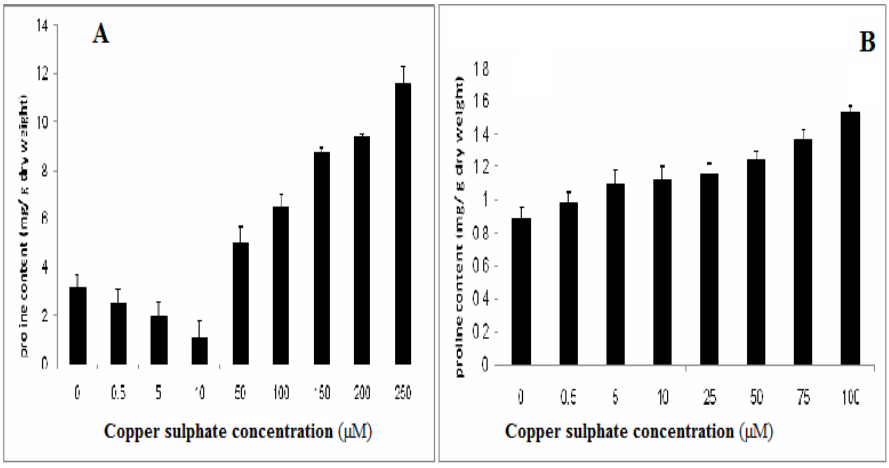


**Fig. 6.** Effect of copper sulphate (µM) on proline content (mg/ g dry weight) of *Ankistrodesmus falcatus* (A) and *Chlorella vulgaris* cultures (B).

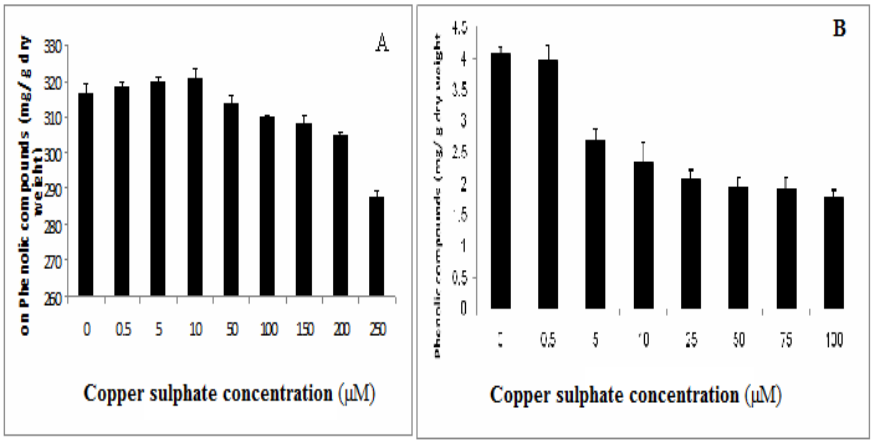


**Fig. 7.** Effect of copper sulphate (µM) on Phenolic compounds (mg/ g dry weight) of *Ankistrodesmus falcatus* (A) and *Chlorella vulgaris* cultures (B).

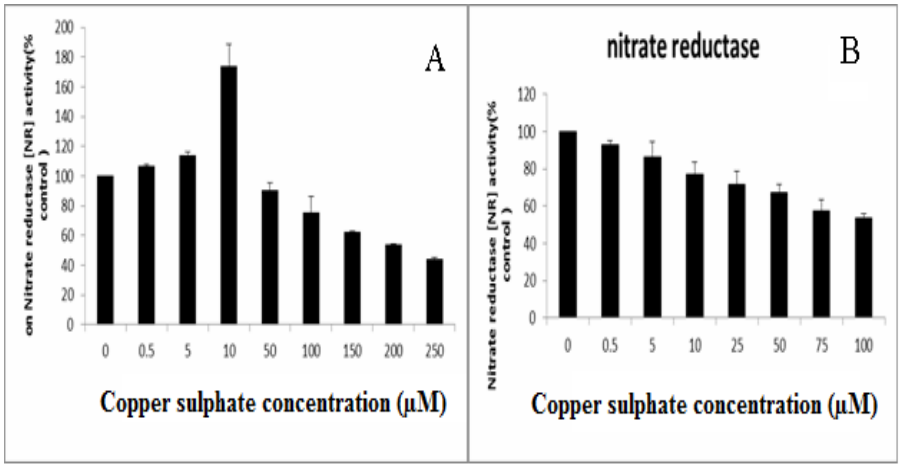


**Fig. 8.** Effect of Copper sulphate (µM) on Nitrate reductase [NR] activity **(**% of control) of *Ankistrodesmus falcatus* (A) and *Chlorella vulgaris* cultures (B).

are controlled by secondary metabolites (Inmaculada, 2005). Fig.8 revealed that, the activity of nitrate- reductase of *Ankistrodesmus falcatus* was increased up to 10 µM copper sulphate and then it was reduced with increasing copper dose until 250 µM. Nitrate- reductase activity of stressed *Chlorella* cells was decreased under all concentrations used. The inhibition of N-R activity in the two algae is likely due to impaired $NO_3^-$ uptake in the presence of elevated levels of the test metals. Proteins did not affected with the pronounced drop in the activity of nitrate reductase especially at the higher doses of copper. This might indicated that, the two processes (the activity of Nitrate reductase and the machinery of protein synthesis ) did not necessary linked. This agree well with the previous reports (Campbell 1999, Klobus, *et al*. 2002) . The other possibility could be direct inhibition of N-R activity by the test metals (De Filippis and Pallaghy, 1994). Copper stress either stimulated or inhibited the biosynthesis of some polypeptides in the cells of the two investigated chlorophyta. The results in all cultures treated with different copper sulphate concentrations were quite different from that of the untreated control culture.

SDS-PAGE analysis of the total protein in *Ankistrodesmus falcatus* (Fig.9 (A) showed that, Copper stress resulted in the appearance of new polypeptides bands. The numbers of polypeptides in control were 15 polypeptides. They were 35 at 250 µM $CuSO_4$. (more than 2 fold ) which fluctuated between the low molecular weight polypeptides to high molecular weight polypeptides. For example 37, 34 and 22 kDa polypeptide appeared only under copper stress also the higher molecular weight polypeptides 208 kDa to 71 kDa appeared only in copper treated *Ankistrodesmus falcatus*. In addition the polypeptide bands (45, 42 and 15 kDa) increased in densities. The investigated alga produced low molecular weight protein bands as a possible tolerance mechanism to Copper stress.

SDS-PAGE (Fig. 9 (B) showed that,. The number of polypeptides in *Chlorella vulgaris* was higher in control (22 bands) than in copper treated alga (4, 3, 3) bands at higher doses of metal. This alga was not produce any new polypeptides especially under high copper stress which might be a suitable marker for the great sensitivity of this alga when compared to *Ankistrodesmus falcatus*. The appearance and disappearance of different protein bands due to metal stress were recorded by other authors (Perez-Rama et al., 2001; Osman et al., 2004).

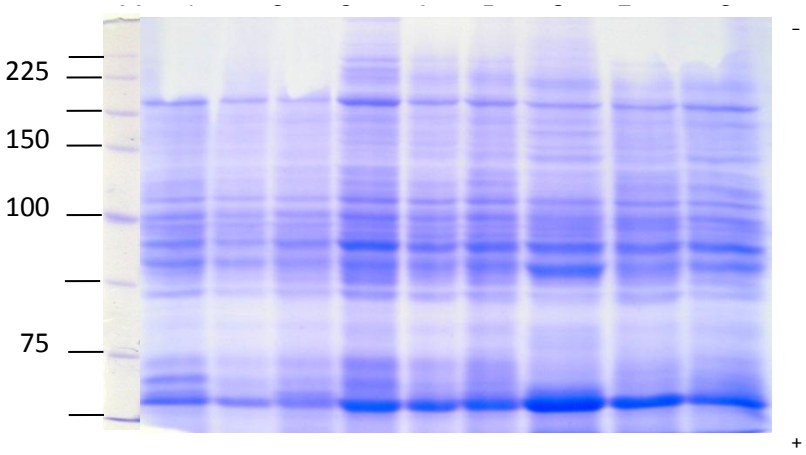


**Fig.(9-A).** Coommassie blue-stained SDS polyacrylamide gel of polypeptides of *Ankistrodesmus falcatus* cultured under copper sulphate concentrations (0.0, 0.5, 5, 10,50, 100, 150, 200, 250 µM) . Lane M is protein marker, Lane 1 represents the control.

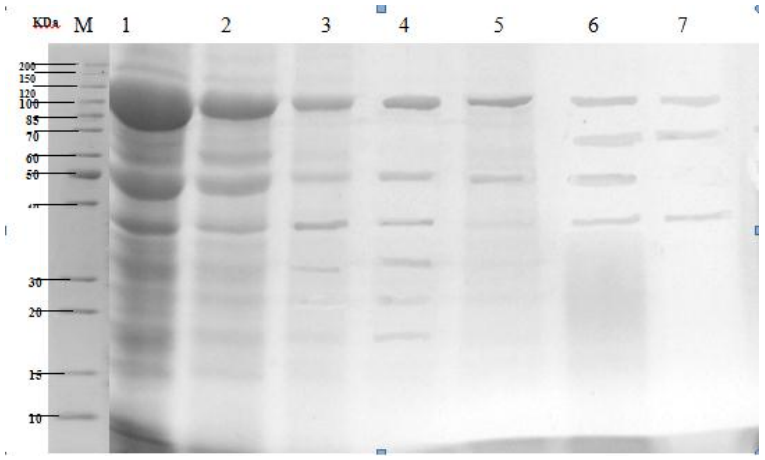


**Fig. (9-B).** Coommassie blue-stained SDS polyacrylamide gel of polypeptides of *chlorella vulgaris* cultured under copper sulphate concentrations (0.0, 0.5, 5, 10, 50, 100, 150 µM). Lane M is protein marker, Lane 1 represents the control.

## REFERENCES

Afkar, E., Ababna , H. and Fathi, A. A. 2010. Toxicological Response of the Green Alga *Chlorella vulgaris*, to Some Heavy Metals American Journal of Environmental Sciences 6 (3): 230-237.

Akira, S., Litiana, Q.V. Norihide, K. and Shigetoh, M. 2005. Evaluation of the sensitivity of marine microalgal strains to the heavy metals, Cu, As, Sb, Pb and Cd. Environ. Int., 31: 713-722. DOI: doi:10.1016/j.envint.2005.01.001.

An, Y.J. 2004. Soil ecotoxicity assessment using cadmium sensitive plants. Environ. Poll., 127: 21-26.

Andrade, I.. R.; Farina, M. and Filho, A.M.G. 2004. Effects of copper on *Enteromorpha flexuosa* (Chlorophyta) in vitro. Ecotox. Environ. Safe, 58: 117-125.

Azeez, P.A. and Banerjee, D.K. 1986. Effect of copper and cadmium assimilation and uptake of heavy metals. Bull. Environ. Chem. 12: 77-86. .

Bates L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid determination of free proline for water-stress studies, *Plant Soil*, 39: 205–207.

Burzynski, M. and Zurek, A. 2007. Effects of copper and cadmium on photosynthesis in cucumber cotyledons. Photosynthetica, 45: 239-244.

Burritt, D. J., Larkindale, J. and Hurd, K. 2002. Antioxidant metabolism in the intertidal red seaweed *Stiosiphonia arbuscula* following desiccation. Planta, 215: 829–838.

Campbell, W.H. 1999. Nitrate reductase structure, function and regulation: Rev. Plant Physiol. Plant Mol. Biol., 50: 277–303.

Chaudiere, J. 1994. Some chemical and biochemical constraints of oxidative stress in living cells. In: Free radical damage and its control (Rice-Evans C. A. and Burdon R. H., eds). New Comprehensive Biochemistry, 28: 25-66.

Contreras, L., Moenne, A. , Correa, J. 2005. Antioxidant responces in *Scytosiphon lomentaria* (phaeophyta) inhabiting copper enriched coastal environments .J. Physiol., 41: 1184-1195.

Dai, G.H., Andary, C., Cosson, L. M. and Boubals, D. 1994. Polyphenols and resistance of grapvienes ( Vitis spp.) to downy mildew (*Plasmopara viticola)*. Acta. Horticulture, 381:763-766.

De Filippis, L. F., Pallaghy, C.K. 1994. Heavy metals: sources and biological LC, Gaur JP, Soeder CJ (eds) Algae and Water Pollution. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgartpp 31–77.

El-Ayouty, Y.M. and Ezzat, S.M. 1991. Effect of the herbicide prometryn on the metabolic activity of the Cyanobacterium *Nostoc musocorum*. *Egypt. J. Microbiol*., 26: 194-208.

El-Naggar, A. H. 1993. Growth and Some Metabolic Activities of *Chlorella* and *Scenedesmus* in Relation to Heavy Metal Water Pollution in Gharbia Governorate. Ph. D. Thesis Botany Department, Faculty of Science, Tanta University, Egypt.

El-Naggar, A.H., Osman, M.E.H., Dyab, M.A., and El- Monhsenawy, E.A. 1999. Cobalt and lead toxicities on *Calothrix fusca* and *Nostoc muscorum*. J. Union Arab biol., Cairo. Botany (Physiology and algae). **7**: 421-441.

El-Sheekh, M. M., El-Naggar, A.H., Osman, M.E.H. and El-Mazaly, E. 2003. Effect of Cobalt on growth, pigments and the photosynthetic electron transport in *minutum* and *Nitzchia perminuta*. Braz. J. Plant Physiol.,15:159-166.

El-Sheekh, M.M., Ahmed Mohamed El-Otify,A. M. and Saber, H. 2011. Alterations in Proteins and Amino Acids of the Nile Cyanobacteria *Pseudanabaena limnetica* and *Anabaena wisconsinensein* Response to Industrial Wastewater Pollution. Brazilian Arch. of Biolo. And Technolo. Vol.54, n. 4: pp. 811-820. ISSN 1516- 8913.

Fales, F.W. 1951. The assimilation and degradation of carbohydrates by yeast cells. *J.Biol. Chem*., 193: 113–124.

Fathi, A.A. 2003. Some metabolic activities in the green alga *Scenedesmus bijuga* as affected by the insecticide trichlorfon. *Protistology*., 3: 92-98.

Fathi, A.A., Zaki, F.T. and Ibraheim, H.A. 2005. Response of tolerant and wild type strains of *Chlorella vulgaris* to Copper with special references to Copper uptake system. Protistology, 4: 73-78. http://protistology.ifmo.ru/num4_1/fathi.pdf

Fathi, A.A., El-Shahed, A.M. Shoulkamy, M.A., Ibraheim, H.A. and Abdel Rahman, O.M. 2008. Response of Nile water phytoplankton to the toxicity Cobalt, Copper and Zinc. Res. J. Environ. Toxicol., 2: 67-76. DOI: 10.3923/rjet.2008.67.76.

Gao, S., Yan, N., Cao, M., Yang, W., Wang,S. and Chen, F. 2008. Effects of copper on growth, antioxidant enzymes and phenylalanine ammonia-lyase activities in Jatropha curcusl. Seedlings. Plant Soil Environ ., 54(3): 117-121.

Hawkesford, M.J.and Belcher, A.R., 1991. Differential protein synthesis in response to sulfate and phosphate deprivation: identification of possible components of plasma membrane transport systems in cultured tomato roots. Planta, 185:185-323.

Hammouda, O., Gaber, A., and Abdel Raouf, N. 1995. Microalgae and wastewater treatment. *Ecotoxicol Environ. Safety*., 31: 205-210.

Hart, B.A. and Scaife, B.D. 1997. Toxicity and bioaccumulation of cadmium in *Chlorella* pyrenoidosa . Environ. Res., 14: 401-413.

Hall, J.L.2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany, 53: 1-11.

Inmaculada, Y. 2005. Copper in plants. Braz. J. Plant Physiol., 17: 145-156.

Jaworski, E.G.1971. Nitrate reductase assay in intact plant tissues, Biochem.Biophys. Res. Co*mmun*., 43: 1274–1279.

Kasim, W.A. 2001**.** Effect of copper and cadmium on some growth criteria and physiological aspects of *Sorghum bicolor*. Egypt. J. Biotechnol., 9: 298 -310.

Klobus, G., Burzynski, M. and Buczek, J. 2002. Heavy metals and nitrogen metabolism. In:. Prasad MNV, Strazalka K (eds) Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants Kluwer Academic Publishers. The Netherlands PP. 325–355.

Kobbia, I.A., Hofner, W., Naguib, M.I. and Ibrahim, Z.K. 1985. Uses of laboratory cultures of some algae to predict heavy metal toxicity VI. Effect of various concentrations of Cd, Cr, Ni and Zn on the composition of amino acids and soluble proteins in *Chlorella fusca*. *Bull. Fac. Sci., Cairo Univ*., 53: 249-263.

Kholoud, N.S.A 2009**.** Effect of Some Metabolic Inhibitors on the Growth and Some Relative Metabolites of Some Broad Bean Cultivars. M.Sc. Minia Univ., Minia, Egypt, 1-191.

Kopponel, W.H. 1994**.** Chemistry of iron and copper in radical reactions. In: Free radical damage and its control (Rice-Evans C. A. and Burdon R. H., eds New Comprehensive Biochemistry, 28: 3-24.

Laemmli, U.K. 1970. Cleavage of the structural proteins during the assembly of the head of the bacteriophage T4. Nature 277, 680.

Levy, J. L., Stauberand, J. L. and Jolley, D.F. 2007. Sensitivity of marine microalgae to Copper: The effect of biotic factors on Copper adsorption and toxicity. Sci. Total Environ., 387: 141-154. DOI10.1016/J.SCITOTENV.2007.07.016

Lage, O.M., Parente, A.M., Soares, H.M. V.M., Vasconcelos, M.T.S.D. and Salema, R. 1994. Some Amphidinium carterae and Prorocentrum micansin batch culture. Eur. J. Phycol., 29: 253-260 .

Lage, O. M., Parente, A. M., Vasconcelos, M. T. S. D. and Gomes, C. A. R. 1996. Potential tolerance mechanisms of Prorocentrum mican Lage, O.M., Parente, A.M. M.T.S.D. Vasconcelos and C.A.R.Gomes Dinophyceae) to sub lethal levels of copper. J. Phycol., 32: 416-423.

Lowry, O.H., Rosebrough N.S., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin-phenol reagent, *J. Biol. Chem*., 193: 265–-275.

Lupi, F.M.,. Fernandes, H.M.L and Correia, I.S. 1998. Increase of copper toxicity to growth of *Chlorella vulgaris* with increase of light intensity. Microb. Ecol., 35, 193-198.

Ma M., Zhu W, Wang Z, Witkamp, G. 2003. Accumulation, assimilation and growth inhibition of copper on fresh water alga (*Scenedmus subspicatus 86.81S AG)* in the presence of folic acid and EDTA Aq.Toxicol.63, 221-228.

Maksymiec, W. and Krupa, Z. 2003. Oxidative processes under heavy metal stress, the influence of methyljasmonate.Acta physiol. Plant, 3:95

Metzner, H., Rau, H. and Singer, H. 1965. Untersuchungen zur Synchronisier barkeit einzelner Pigmentmangel Mutanten von Chlorella. Planta, 65: 186–94.

Moore, S., Spachman, D..H. and Steins,W.1958. Chromatography of amino acids on sulphonated polystyrene resins.Anal.chem.30:11-85.

Muwafq, H.M. and Bernd, M. 2006. Toxicity of heavy metals on *Scenedesmus quadricauda* (Turp.) de brebisson in batch cultures. Environ. Sci. Pollut. Res., 13: 98-104. DOI: 10.1065/espr2005.07.274.

Nagalakshmi,N. and Prasad, M., 1998. copper -induced oxidative stress in *Scenedesmus bijuggatus*: protective role of free radical scavengers. Bull. Environ. Contam . Toxicol. 61: 623-628.

Osman. M.E.H., El-Naggar, A.H., El-Sheekh, M.M., and El-Mazally, E.E. 2004. Differential effects of $Co^{+2}$ and $Ni^{+2}$ on protein metabolism in *Scenedesmus obliquusand* and *Nitzschia perminuta*. Environ. Toxicol. pharmacol ., 16: 169-178.

Overnell, J. 1975. The effect of heavy metals on photosynthesis and loss of cell potassium in two species of marine algae. Marine Biol., 29: 99-103.

Paraskevi, M., Rijstenbil, J. W. and Haritonidis, S. 2006. Effects of cadmium, zinc and nitrogen status on non-protein thiols in the macroalgae *Enteromorpha spp* . From the Scheldt Estuary (SW Netherlands, Belgium) and Thermaikos Gulf (N Aegean Sea, Greece). Marine Environ. research, 62: 45-60.

Prassad, D. and Prassad, A. 1987. Altered aminolaevulinic acid metabolism by lead and mercury in germinating seedlings of Bajra(*Pennisetum typhoideum*). J. Plant Physiol., 127: 241-249.

Perez-Rama, M., Herrero Lopez, C., AbaldeAlonso, J. and Torres Vaamonde, E. 2001. Class III metallothioneins in response to

cadmium toxicity in the marine microalga *Tetraselmis suecica* (Kylin) Butch. *Environ. Toxicol. Chem*., 20, 2061-2066.

Rai, L.C., Singh, A.K. and Mallick, N. 1990. Employment of CEPEX enclosures for monitoring toxicity of Hg and Zn on in situ structural and functional characteristics of algal communities of river Ganga in Varanasi. Ecotoxicol. Environ. Safe, 20, 211-221.

Rai, P.K., Mallick, N., and Rai, L.C. 1994. Effect of Cu and Ni on growth, mineral uptake, photosynthesis and enzyme activities of Chlorella vulgaris at different pH values. Biomed. Environ. Sci ., 7: 56–67.

Reichman, S.M. 2002. The responses of plants to metal toxicity: A review focusing Copper, Manganese and Zinc. Australian Minerals and Energy on Environment Foundation, 1-54.

Rijstenbil, J.W., Derksen, J.W.M., Gerringa, L. J. A., Poortvliet, T.C.W. , Sandee, M., van den Berg, Van Drie, J. and Wijnholds, J. A. 1994. Oxidative stress induced by copper defence and damage in the marine planktonic diatom Ditylum bright well grown in continuous cultures with high and low zinc levels. Marine Biol., 119: 583- 590.

Rippka, R., Derulles, J., Waterburry, J. B., Herdman, M. and Stanier, P. Y.1979. Generic Assignments, strain histories and pure cultures of Cyanobacteria. J.Gen. Microbiol. 111: 1-61.

Sharma, S. S., Schat, H. and Vooijs, R. 1998. In vitro alleviation of heavy metal induced enzyme inhibition by proline, Phytochemistry.49: 1531-1535.

Schützendübel, A. and Polle, A. 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp. Bot., 53: 1351-1365.

Utting, S.D. 1985. Influence of nitrogen availability on the biochemical composition of three unicellular marine algae of commercial importance. Aquacult. Eng., 4:175- 190.

Wu, F., Wu, H., Zhang, G. and Bachir, D.M.L. 2004**.** Differences in growth and yield in response to cadmium toxicity in cotton genotypes. J. Plant Nutr. Soil Sci., 167: 85-90.

Yruela, I. 2005. Copper in plants. Brazilian Journal of Plant Physiology, 17: 145- 156.

# مقارنه بين الاستجابة الفسيولوجيه لطحلب انكستروديسماس فالكاتس وطحلب كلوريللا فولجاريس لعنصر النحاس .

١- زينب عبد الفتاح احمد –٢- اسماء بكر احمد
جامعه سوهاج - كليه العلوم – قسم النبات

في هذا البحث تمت دراسة تأثيرعنصرالنحاس على طحلب انكستروديسماس فالكاتس وطحلب كلوريللا فولجاريس من الناحية الفسيولوجية والبيو كيمائيه. لقد تمت تنمية مزارع من طحلب انكستروديسماس فالكاتس وطحلب كلوريللا فولجاريس تحت تأثير تركيزات مختلفة ( ٥و. – ٥ -١٠- ٥٠-١٠٠-١٥٠-٢٠٠-٢٥٠ميكرو مول) من كبريتات النحاس. لقد نمى طحلب انكستروديسماس فالكاتس حتى تركيز ٢٥٠ ميكرو مول كبريتات النحاس أما مزارع طحلب كلوريللا فولجاريس نمت حتى تركيز ١٠٠ ميكرو مول كبريتات النحاس . أدت التركيزات المنخفضة (٥.و . – ٥ - ١٠ ميكرو مول كبريتات النحاس) إلى زيادة النمو والإصباغ النباتية وكل نواتج الايض (المواد الكربوهيدراتيه الكلية والبروتينات الكلية والأحماض الامينيه والمركبات الفينوليه) ونشاط إنزيم مختزل النترات في مزارع طحلب انكستروديسماس فالكاتس أما في طحلب كلوريللا فولجاريس انخفض كل ما سبق تحت تأثير جميع التأثيرات من كبريتات النحاس. لقد زاد محتوى الكربوهيدرات الذائبة والبروتينات الذائبة والحمض الامينى برولين في طحلب كلوريللا فولجاريس بزيادة تركيز كبريتات النحاس من ٥٠ ميكرو مول حتى ٢٥٠ ميكرو مول أما في طحلب كلوريللا فولجاريس تزداد كل هذه المركبات عند كل التركيزات من ٥و. حتى ٢٥٠ ميكرو مول كبريتات النحاس. لقد أدى تنمية طحلب انكستروديسماس فالكاتس عند تركيز ٢٥٠ ميكرو مول من كبريتات النحاس إلى ظهور ٢٠ من الببتيدات الجديدة . أما في طحلب كلوريللا فولجاريس انخفض عدد الببتيدات عند كل التركيزات من كبريتات النحاس. يوجد اختلاف من حيث الاستجابة لتأثير النحاس بين طحلب انكستروديسماس فالكاتس وطحلب كلوريللا فولجاريس .