EFFECT OF SOME HEAVY METALS ON GROWTH OF *SCENEDESMUS OBLIQUUS* (TURPIN) KÜTZING

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Abstract

The effect of concentrations of copper (0.5, 1.5 mg l^{-1}), lead (10, 40, mg l^{-1}) and zinc (0.05 and 0.5 mgl^{-1}) singly or in combination on growth of *Scenedesmus obliquus* was studied. The results showed that there is a difference between the effects of each metal singly and in combinations with each others. Generally, heavy metals decreased the growth of *Scenedesmus obliquus*. High concentration of copper uptake exceeds its uptake in low concentration (58 and 42%, respectively). While the uptake of lead and zinc in low concentrations exceeds their uptake in high concentrations with percentages of (87%, 86%, 13% and 14%, respectively). Zinc uptake in combinations was dominant over the two other metals. The effect of heavy metals on ultrastructure of *Scenedesmus obliquus* by using the Transmission Electron Microscope was investigated.

Key Words: Heavy metals, *Scenedesmus obliquus*, Ultrastructure.

Introduction

Heavy metal pollution is an issue of great environmental concern. Heavy metals are released from prevalent municipal industrial, agricultural and domestic wastes; they modify the structure and productivity of aquatic ecosystems (**Magdaleno *et al.*, 1997; Osman *et al.*, 2004**). Also, heavy metals are known to disrupt algal metabolism either by inactivating the photosynthetic machinery, enzymatic pathways or altering the nutrient transport and availability (**Mallick and Rai, 1992; Rai *et al.*, 1998**).

A number of publications discussed water quality and phytoplankton distribution of the River Nile in Egypt (**Kobbia *et al.*, 1993; Toulibah, 1996; and Shaaban-Dessouki *et al.* 2004**).

Much research information has been amassed on the effect of heavy metal toxicity on growth and uptake of microorganisms. While, the knowledge of the toxicity and general uptake of single species of heavy metals by algae is increasing, relatively little is known about the combined effects of two or more metals (**Ting *et al.*, 1991**). The present work aims to verify the ability of magnitudes of *Scenedesmus obliquus*, isolate from the River Nile, to tolerate single and combined heavy metal concentrations.

(ISSN: 1110-8649)

Materials and Methods

S. obliquus (Turpin) Kützing sample was collected during 2004, spring season from El Maadi Yakht Club in River Nile. Elimination of contaminants was done in the field by filtration method, for obtaining axenic culture was carried out according to (**Guillard**, **1995**). Where, the identification was according to (**Bourrelly, 1968**). The best growth conditions were at the seventh day, 2000 Lux light intensity pH 8 at 30 ^{O}C using BG-11 medium (**Allen and Stanier, 1968**) modify No. 11 (**Hughes *et al*.**, **1958**) copper, lead and zinc, in two concentrations of each heavy metal were used in singly or in combination. The concentrations of the metals ware 0.5, 1.5 mg.l^{-1} for copper, 10, 40 mg.l^{-1} for lead and 0.05 and 0.5 mg.l^{-1} for zinc. Determination of dry weight, chlorophylls *a* and *b* was carried out according to the method of **Jeffrey and Humphrey** (**1975**). The total soluble carbohydrates were determined using anthrone technique according to **Umbriet *et al.*** (**1969**). Total soluble proteins were determined according to the method of **Lowry *et al*.** (**1951**) and the determination of crude lipid was determined according to the method of **Padmini Sreenivasa Rao *et al*.** (**1986**). The preparation of algal cell for transmission electron microscope was carried out according to the method of (**Palade *et al*., 1952)** and the sections were then examined photographed by a JEOL 1200 EXII transmission electron microscope.

Results

Under different doses of copper and lead the photosynthetic activities of *S. obliquus* cell varied considerably with various doses accompanied with a remarkable decrease in dry weight (Table 1). With regard to chlorophyll *a* and *b*, it was evident that the highest concentration of copper and lead induced decrease in both chlorophylls contents.

It was clearly evident that chlorophyll *a* and *b* content in *S. obliquus* cells increased under low concentrations of zinc (2.03 ± 0.04 and 0.69 ± 0.05 mg l^{-1}, respectively) compared to the influence of copper and lead and it decreased under high dose.

All the parameters studied for *S. obliquus* were decreased gradually with increasing concentrations of heavy metals mixture (Table 1). However a conspicuous variation was registered in the magnitudes the uptake of each metal in the mixture relevant to that for each metal alone. Also, the data indicate that weigh gain, chlorophylls *a* and *b* in *S. obliquus* decrease dramatically with low and high concentrations of the three combined metals as compared to control, On the other hand, mixture 3 (0.5 mg l^{-1} Cu + 0.05 mg l^{-1} Zn) and mixture 6 (40 mg l^{-1} Pb + 0.5 mg l^{-1} Zn) increased the amount of chlorophylls *a* and *b* of *S. obliquus* compared to the other mixtures (Table 1).

Table (1) Effect of different heavy metals concentrations supplemented singly or in combination to the algal culture media on dry weight, chlorophylls a and b of *Scenedesmus obliquus*, grown for 7 days.

Heavy metals Concentration (mg l^{-1})		Dry weight (mg l^{-1})	Chlorophyll *a* (mg l^{-1})	Chlorophyll *b* (mg l^{-1})
Control		**820 ± 10**	**2.75 ± 0.01**	**0.92 ± 0.04**
Copper	**0.5**	**600 ± 5**	**1.55 ± 0.02**	**0.45 ± 0.05**
	1.5	**490 ± 8**	**1.06 ± 0.01**	**0.35 ± 0.06**
Lead	**10**	**460 ± 6**	**0.96±0.02**	**0.29 ± 0.01**
	40	**420 ± 5**	**0.82±0.02**	**0.27 ± 0.02**
Zinc	**0.05**	**791 ± 6**	**2.03 ± 0.04**	**0.69 ± 0.05**
	0.5	**435 ± 10**	**1.03 ± 0.02**	**0.31 ± 0.01**
Mixture 1 0.5 Cu + 10 Pb		**630 ± 8**	**1.82 ± 0.01**	**0.61 ± 0.02**
Mixture 2 1.5 Cu + 40 Pb		**490 ± 6**	**1.35 ± 0.02**	**0.39 ± 0.03**
Mixture 3 0.5 Cu + 0.05 Zn		**700 ± 9**	**2.4 ± 0.1**	**0.78 ± 0.01**
Mixture 4 1.5 Cu + 0.5 Zn		**480 ± 7**	**0.78 ± 0.01**	**0.24 ± 0.01**
Mixture 5 10 Pb + 0.05 Zn		**410 ± 6**	**0.83 ± 0.01**	**0.28 ± 0.02**
Mixture 6 40 Pb + 0.5 Zn		**530 ± 7**	**1.73 ± 0.2**	**0.57 ± 0.04**
Mixture 7 0.5 Cu + 10 Pb + 0.05 Zn		**360 ± 8**	**0.73 ± 0.01**	**0.21 ± 0.5**
Mixture 8 1.5 Cu + 40 Pb + 0.5 Zn		**320 ± 6**	**0.57 ± 0.04**	**0.17 ± 0.9**

The data in Table (2) indicate that the soluble carbohydrates and soluble proteins of *S. obliquus* decrease dramatically with high concentrations of copper, lead and zinc compared to control. Also, under different doses of copper, lead and zinc the lipid behave almost similarly to protein and carbohydrates.

Combination of the three metals with two different concentrations induced retardation in carbohydrate, proteins and lipid. Irrespective, of some minor fluctuations slightly stimulated in lipid biosynthesis production with mixture 7 (0.5 mg l^{-1} Cu + 10 mg l^{-1} Pb + 0.05 mg l^{-1} Zn) and mixture 8 (1.5 mg l^{-1} Cu + 40 mg l^{-1} Pb + 0.5 mg l^{-1} Zn) (Table 2).

Table (2) Effect of different heavy metals concentrations supplemented singly or in combination to the algal culture media on carbohydrates, protein and lipid of *Scenedesmus obliquus*, grown for 7 days.

Heavy metals Concentration ($mg.l^{-1}$)		Carbohydrates (mg.g dry weight^{-1})	Protein (mg.g dry weight^{-1})	Lipid (mg.g dry weight^{-1})
Control		**9.2 ± 0.2**	**0.85 ± 0.04**	**0.45 ± 0.01**
Copper	**0.5**	**7.9 ± 0.5**	**0.68 ± 0.05**	**0.35 ± 0.01**
	1.5	**6.8 ± 0.9**	**0.59 ± 0.06**	**0.30 ± 0.05**
Lead	**10**	**6.2 ± 0.2**	**0.53 ± 0.01**	**0.27 ± 0.01**
	40	**5.4 ± 0.5**	**0.47 ± 0.02**	**0.22 ± 0.01**
Zinc	**0.05**	**8.3 ± 0.1**	**0.75 ± 0.05**	**0.39 ± 0.01**
	0.5	**5.9 ± 0.5**	**0.55 ± 0.01**	**0.21 ± 0.02**
Mixture 1 0.5 Cu + 10 Pb		**8.4 ± 0.1**	**0.7 ± 0.02**	**0.35 ± 0.01**
Mixture 2 1.5 Cu + 40 Pb		**6.8 ± 0.9**	**0.49 ± 0.03**	**0.27 ± 0.05**
Mixture 3 0.5 Cu + 0.05 Zn		**7.4 ± 0.5**	**0.68 ± 0.01**	**0.32 ± 0.01**
Mixture 4 1.5 Cu + 0.5 Zn		**5.5 ± 0.3**	**0.48 ± 0.04**	**0.22 ± 0.03**
Mixture 5 10 Pb + 0.05 Zn		**5.2 ± 0.2**	**0.46 ± 0.02**	**0.30 ± 0.02**
Mixture 6 40 Pb + 0.5 Zn		**6.7 ± 0.1**	**0.51 ± 0.01**	**0.28 ± 0.05**
Mixture 7 0.5 Cu + 10 Pb + 0.05 Zn		**2.6 ± 0.1**	**0.24 ± 0.02**	**0.23 ± 0.01**
Mixture 8 1.5 Cu + 40 Pb + 0.5 Zn		**2.0 ± 0.1**	**0.15 ± 0.01**	**0.20 ± 0.05**

In Figure (1 A - K) revealed the synergism and antagonism between metal to each other. In spite of concentration of single metal uptake high concentration of cupper slightly increased the percentage of uptake to 58% compared to 42% at low concentration. In contrast, the uptake of low concentrations of lead and zinc (87%, 86%, respectively) were higher than their corresponding high doses uptake (13% and 14% respectively) (Figure 1A - C).

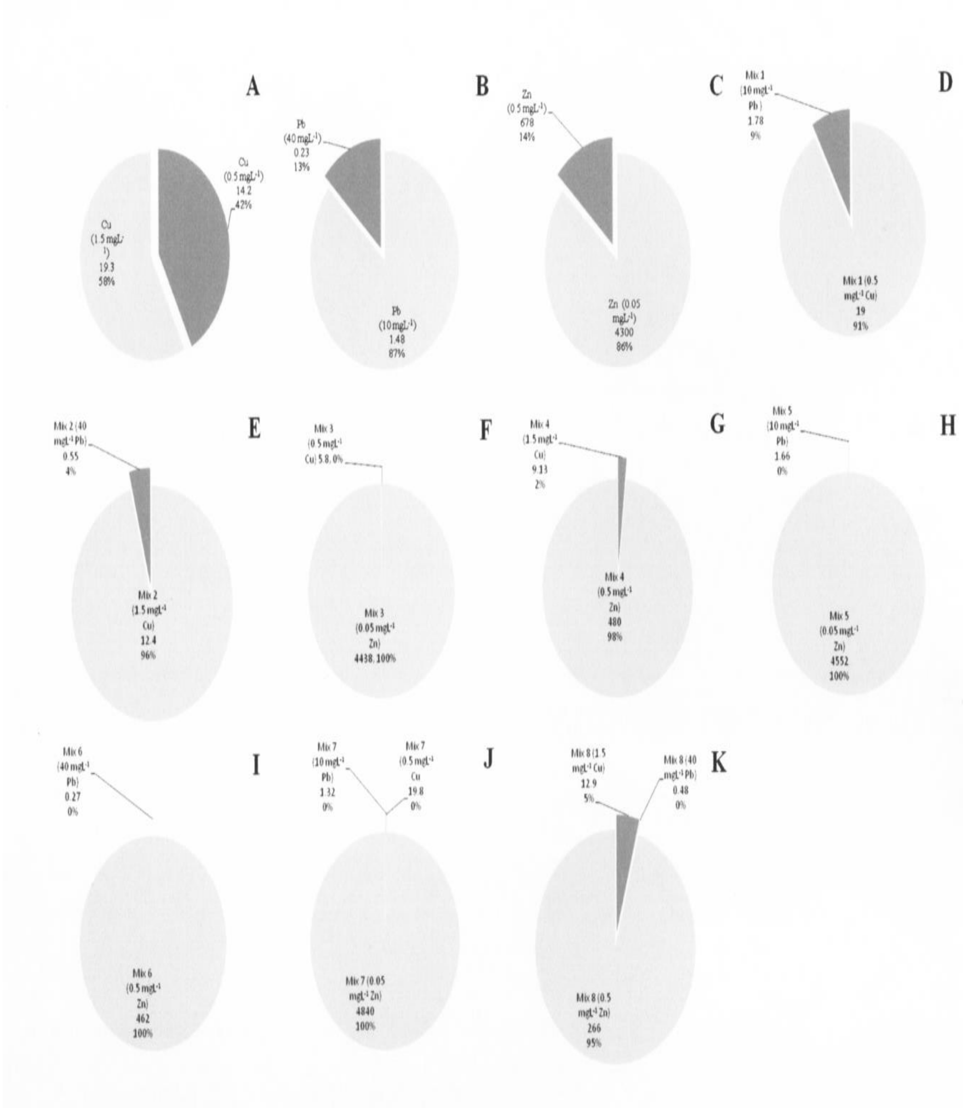


Figure (1 A- K) synergism and antagonism of heavy metals absorption related to each other.

On the other hand, the two metal combinations Figure (1 D - K) behave differently, the low and high concentrations of copper uptake in mixture was dominated over the lead with percentage of (91 and 96% respectively). Meanwhile, the presence of copper and lead at the combination with zinc was almost neglected with an amount of 100% zinc and 0.00% copper and lead at low concentration. While, at high concentration the percentages of uptake were 98% zinc and 2% copper.

In regard, the three metal combinations zinc was dominated over the two other metals and behave almost as two metals combination, at low and high concentrations zinc uptake were 100% and 95% respectively.

Plate I (A - G) reveal effect on the internal structure of the algal cells under the stress of copper and zinc compared to the control. The ultrastructural appearance of *S. obliquus* cultured in 1.5 mgl^{-1} copper (Plate 1C, D and E) was relatively normal except for focally increased vacuolation in the cytoplasm. These vacuoles appeared occupied by electron-opaque precipitates of the metal. Damage of cytoplasm contained abundant dense bodies. Also, shrinking phenomenon was observed in treated cells.

On the other hand, the electron photomicrographs of thin sections of *S. obliquus* cells growing in low concentration of zinc 0.05 mgl^{-1} (Plate 1F and G) revealed shrinking and malformations of the cells as well as tangled mass of the cell contents in net format (6000× and 70k×) magnifications. The cytoplasm contained abundant dense bodies and lipid droplets. Uneven surface texture along with lot of irregular surface format was observed as roughly stellate outline.

Plate I (A-B)
Control Cell
Under Transmission Electron Microscope

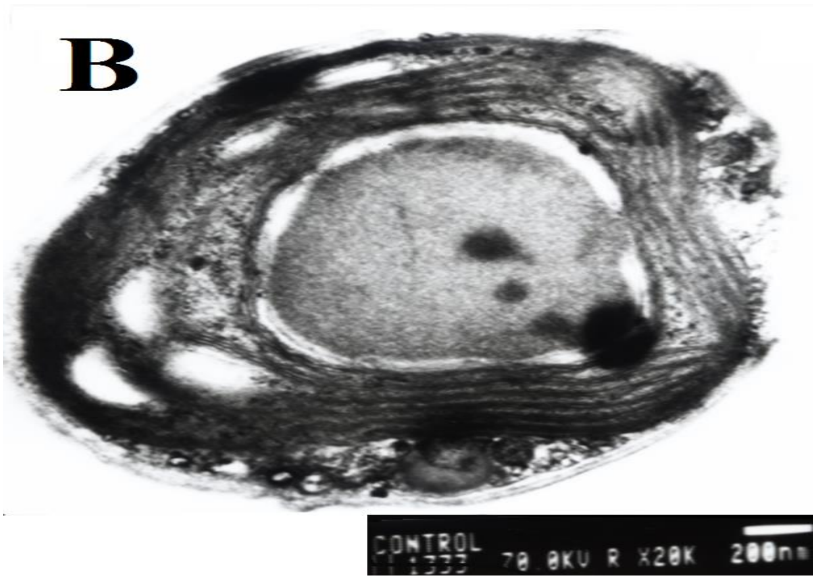


Plate I (C-E)

Treated Cell: Under High Concentration of Copper

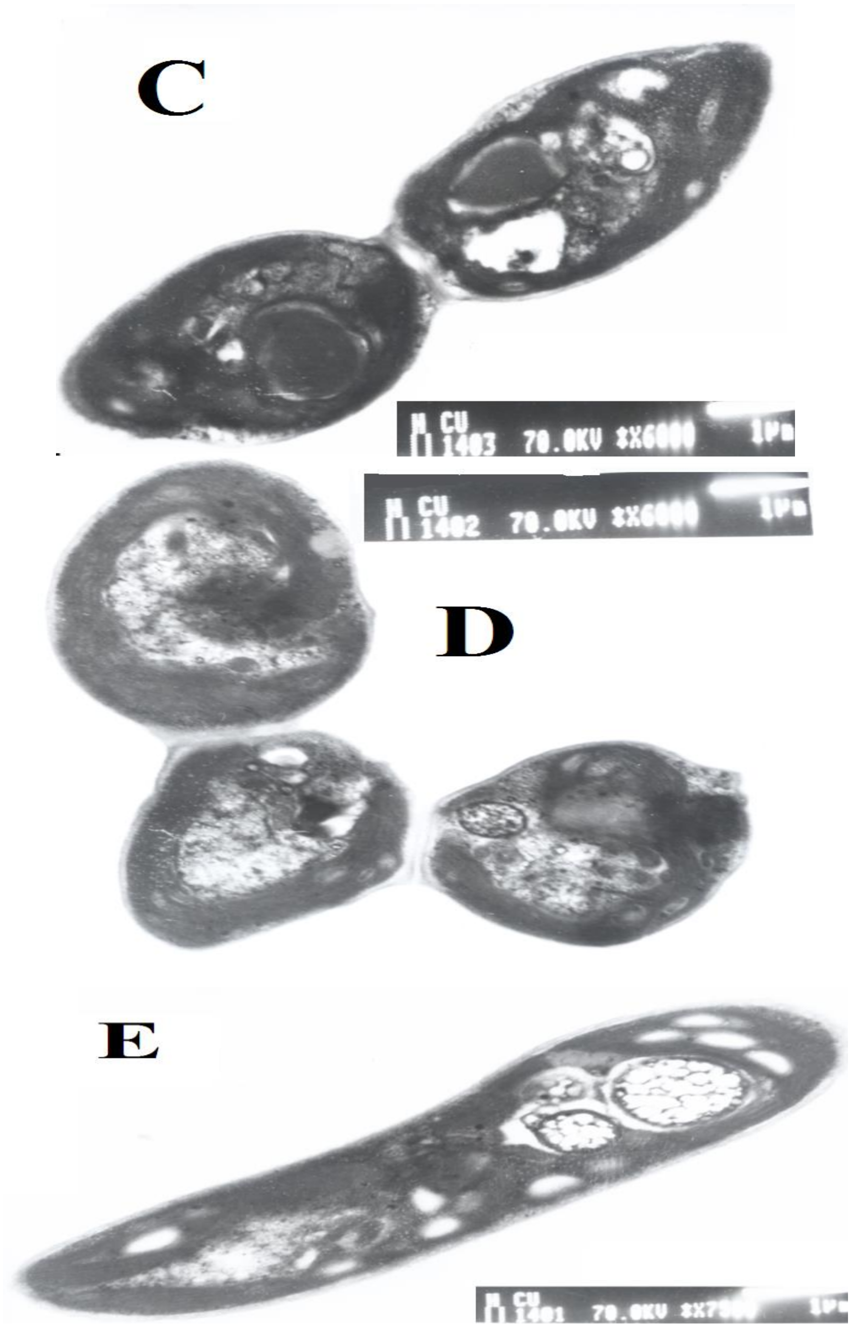
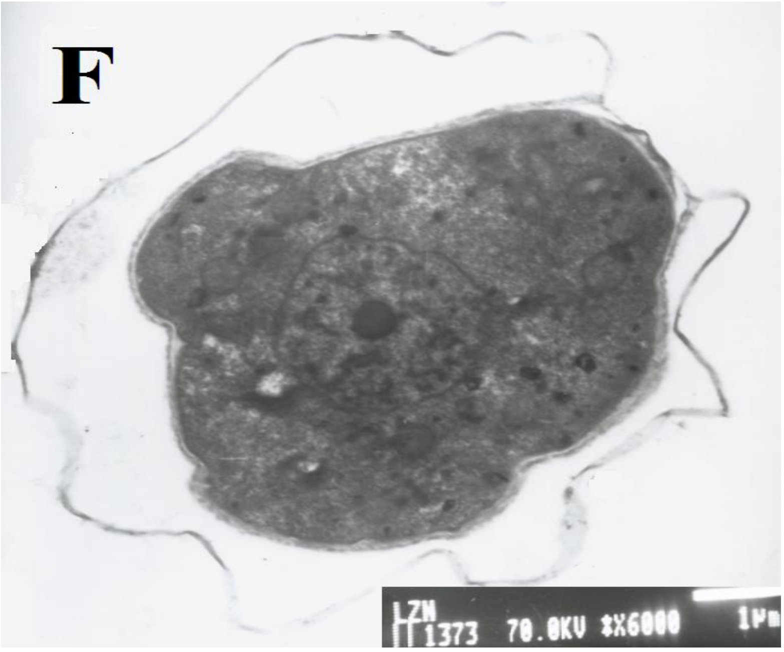
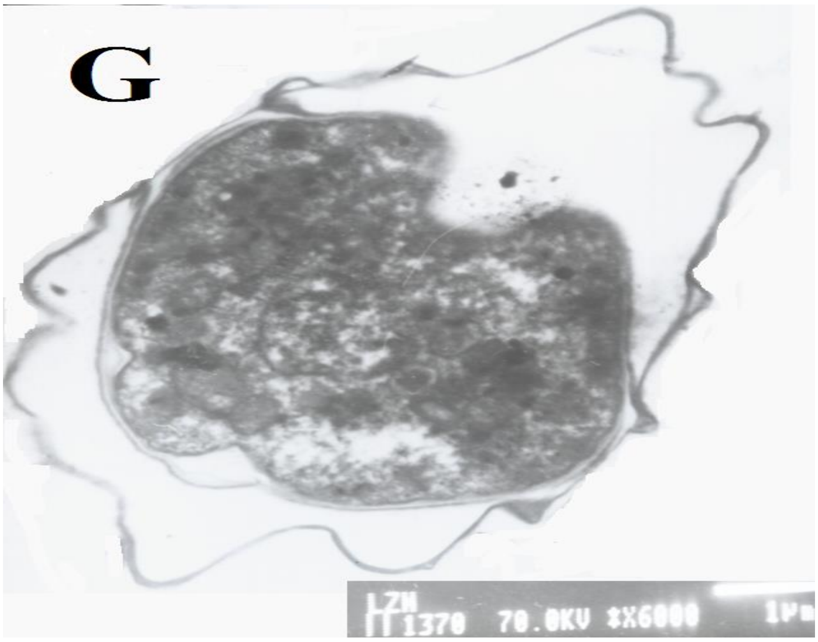


Plate I (F-G)
Treated Cell: Under Low Concentration of Zinc





Discussion

The reduction in chlorophyll *a* contents of *S. obliquus* recorded in this study may be due to the substitute of the central Mg^{2+} in the chlorophyll molecule, a process that lowers the fluorescence quantum yield and results in a shift in fluorescence spectrum, an interpretation that appears in harmony with the finding of **Küpper *et al*. (1996**). Heavy metals, may probably act on PSII at a common site(s) located at either the oxidative side (**Krupa *et al*., 1993**) or the reducing side (**Jegerschöld *et al*., 1995**).

The data demonstrated in this study reveals variability in sensitivity of *S. obliquus* toward the applied heavy metals a phenomenon that may be attributed to the physiological status of the organism and the mode of action of applied heavy metals. In this connection **Olsson *et al*. (1979**) had clarified the importance of proteins and lipoproteins in trapping metals as means of detoxifying them within the cell. **Bariaud *et al*. (1985**) concluded that the mechanism of the resistance depends upon the algae and the metal involved.

In general, more than one heavy metal found in the environment polluted. Also, toxins are rarely present in isolation and they may interact with other substances. The combined effect of several toxins may be the addition of one mortality to another; one increases the mortality caused by the other (synergy) that mean if the toxic effects are additive, the sum of the growth reductions for each of the metals acting alone should be the same as the reduction caused by all metals acting together. In contrast to the synergistic effect, one reduces the mortality caused by the other is antagonism (**Clark *et al*., 1997**). Only few and limited information concern with the combined effects of heavy metals on algae is available (**Hollybaugh *et al*., 1980; Ahmed, 2003**). The effect of combinations of different heavy metals should be studied in order to assess the impacts of heavy metals pollution on the primary producers of the food chains.

Results in the present study indicated that most of the combinations of the three heavy metals showed severe inhibition on algal growth. At the same time our results indicated that these heavy metals interact sometimes synergistically and other time antagonistically with each other on the inhibition of algal growth. Also, the data clearly documented concentration dependent toxic effects of the three heavy metals on growth, protein and chlorophyll contents of *S. obliquus*. The results are in agreement with the published data on algae by (**Franklin *et al*., 2002**). They studied the individual and combined effects of copper, cadmium and zinc on the cell division rate of the tropical fresh water alga *Chlorella* sp., that significant interactions were observed for all metal combination. An equitoxic mixture of Cu + Cd was synergistic to the growth of *Chlorella* sp., while combination of Cu+ Zn, Cd+ Zn and Cu+ Cd+ Zn were antagonistic effect on the growth of *Chlorella* sp., which is in favor with our results. **Pratima *et al*. (2001**) found a degree of antagonism on the growth rate of the cyanobacterium

Hapalosiphon stuhmanni and the photosynthetic green alga *Scenedesmus quadricauda* due to the combination of lead-nickel and cadmium-zinc. They explained the variations in antagonistic behavior to be due to the adsorption of one of the metal hindering the sorption of the other. Where the varied interactive behaviors were possibly because of either the formation of complexes with the algal exudes or the coagulation and co-precipitation of the metals with each other and their finding go parallel to our results.

The biotic effects of copper and zinc pollution are of particular interest because, although copper and zinc are essential trace element for both plants and animals. Where, copper it is strongly toxic at quite low concentrations. Although the toxicity of copper to different algal types varies greatly (**Maloney and Palmer, 1956**), the development of copper-tolerant strains has been reported for several algal species (**Fitzgerald and Faust, 1963**).

Silverberg *et al*. (1976) stated for the first time the copper tolerance mechanism in *Scenedesmus* sp. cells by incorporated into the nuclei. Furthermore, their study adds to the growing list of presently known metal pollutants capable of being sequestered in the form of intranuclear inclusions. Where, the formation of insoluble, intranuclear metal protein complexes is characteristic of some forms of heavy metal poisoning, particularly lead, bismuth and zinc, they have been reported in a variety of animal tissues and in moss leaf cells (**Choie and Richter, 1972; Hsu *et al*., 1973; Skaar *et al*., 1973**). Whereas, the origin of the protein in the inclusion bodies has been of great interest but has remained speculative (**Goyer *et al*., 1970**; **Goyer and Rhyne, 1973**).

It has been suggested by **Moore and Goyer (1974**) that the binding of such metals that occurs in the inclusion body serves as an adaptive or protective mechanism during transcellular transport. This mechanism has the effect of maintaining a relatively low cytoplasmic concentration of the metal and, therefore, reducing the toxic effects of metals on sensitive cellular functions. On the other hand, suggestions concerning the relative metabolic inertness of intracellular metal complexes receive some support in view of the finding that copper inclusions in the nucleus of B-4 cells are found at a concentration of copper lower than that which produces any signs of copper toxicity (**Stokes *et al*., 1973**). The above interpretations may explain the presence of abundant dense bodies in the form of central dense-core complexes for *S. obliquus* experimented with in the present investigation.

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تأثير بعض العناصر الثقيله على نمو السنيدزمس أوبليكس (تربين) كوتزينج

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2- قسم النبات و الميكروبيولوجى، كلية العلوم، جامعة حلوان.
3- مدرسه الفرير بالظاهر.

تم دراسة تأثير النحاس بتركيز 0.5، 1.5 مليجرام / لتر و الرصاص 10، 40 مليجرام / لتر و الزنك 0.05 و 0.5 مليجرام / لتر لكل عنصر منفرد أو فى خليط من العناصر على نمو طحلب سنيدزمس أوبليكس. أوضحت النتائج أن هناك تباينا واختلافا بين التأثير المنفرد للعنصر عنه في الخليط. ولكن بصفة عامة أثر دخول العناصر الثقيلة بصوره منفردة او فى مخلوط تأثيرا سلبيا على نمو الطحلب. أما فيما يتعلق بمقدرة الطحلب علي أمتصاص العناصر الثقيلة بتركيزات مختلفة لكل عنصر فلقد لوحظ ان نسبة الامتصاص الكبري لعنصر النحاس كانت عند التركيز العالي دون التركيز المنخفض. وجدنا أن نسبة امتصاص الزنك تغلبت علي نسبة أمتصاص العنصرين الاخريين بالنسبه للخليط. كما *تم دراسة تأثير المعادن الثقيلة علي التركيب الدقيق لطحلب السنيدزمس* أوبلبكس *باستخدام الميكروسكوب الإليكتروني الناقد.*