



Effect of some heavy metals ions on the chlorophyll a pigment of *Nostoc linkia* and *Hapalosiphon aureus*

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Abstract

Two Cyanophyt A species : *Nostoc linkia* and *Hapalosiphon aureus* were exposed to some heavy metals ions {silver (Ag^{1+}), cobalt (Co^{2+}) and lead (Pb^{2+}) } in different concentrations (1, 5 and 10) mg/l to study their effects on the growth (i.e. Chlorophyll A) of these species. Chlorophyll A pigment was extracted from these species after different exposure periods (0, 1, 2, 3 and 4) weeks for using as physiological parameter in the toxicological studies with Cyanophyta.

Results showed that the effect of heavy metals has not significant differences ($p > 0.05$) between these two algal species, the effect was

Significantly ($p < 0.05$) depended on the metal type and its concentration in the growth medium so as the exposure periods, the effect of heavy metals on the growth and pigment of these two species was in the following sequences:- $\text{Ag} > \text{Co} > \text{Pb}$

1-Introduction

The ubiquity of heavy metals in the biosphere results in the introduction of high amounts of toxic metals into the food chain from various sources including : municipal , agricultural and industrial effluents, and they

modify the structure and productivity of aquatic ecosystems (Magdaleno *et al.* ,1997) .

From a biological point of view, heavy metals can be divided into two categories : essential and non-essential ; however, essential which have been reported to be toxic at high concentrations, for example, some heavy metals

including cobalt , copper , zinc , and manganese ,are essential for growth at very low concentrations but toxic at slightly higher levels (Al-Hejuje, 1997 ; El-Sheekh *et al.*, 2003 ; Awasthi and Das, 2005 ; and Okmen *et al.*, 2007) .

Whereas the other category (non- essential metals) have been reported to be toxic even at low concentrations such as silver, lead , mercury and cadmium (Lee *et al.*, 2005 ; Al- Hejuje , 1997) .

Algae are generally considered as the best bioindicator of aquatic bodies contamination by heavy metals (Chmielewska and Medved , 2001

Cyanophyta, a group of prokaryotic , photosynthetic nitrogen fixers are present in every ecological niche and therefore ,exposed to the toxic effects of the heavy metals .

The effects of heavy metals have been studied with respect to growth , chlorophyll , nitrogenase activity and carbon fixation (Bohme, 1998 ; Okmen *et al*, 2007). Cavet *et al.* (2003) established that cyanophyta has metal requirements; copper in thylakoidal plastocyanin, zinc in carboxysomal carbonic anhydrase, cobalt in cobalamin , magnesium in chlorophyll, molybdenum in heterocystous nitrogenase and manganese in thylakoidal water-splitting oxygen evolving complex .

The influences of heavy metals on algal growth and other biochemical as well as physiological properties have been studied by a

number of authors (Danilov and Ekelund , 2001 ; Lee *et al.* ,2005 ; and Awasthi and Das , 2005) and they found that lethal concentrations of heavy metals vary enormously between species and within ecotypes , and the extent of toxicity for different heavy metals is usually based on the inhibition of growth or photosynthesis .

In the present study , two of the strongest nitrogen fixing cyanophyta : *Nostoc linkia* and *Hapalosiphon aureus* were subjected to different concentrations of cobalt , silver and lead stress and the effect on the chlorophyll (a) pigment was investigated . The aim of this study is to determine if chlorophyll (a) pigment can be used as sensitive physiological parameter in toxicological studies of heavy metals on cyanophyta.

2-Materials and Methods

Organisms and growth condition :-

Two local strains of cyanophyta : *Nostoc linkia* and *Hapalosiphon aureus* were obtained from the algal research lab.(College of Science , University of Basrah ,Iraq)which were isolated from moist lands in Basrah city , and cultured in Chu-10 D medium . These cultures were kept in an air-conditioned room , which was illuminated by white fluorescent tubes at a distance of 50 cm from culture.

The cultures were shaking and randomized daily at room temperature ($25\pm 3^{\circ}\text{C}$).

Cells in the logarithmic phase of growth were collected from stock cultures and used as inocula for experiments .

Metal solutions :-

Cobalt , silver and lead stock solutions (1000 mg/l)were prepared by dissolving $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, AgNO_3 and $\text{Pb}(\text{NO}_3)_2$ respectively , in deionised distilled water and were filtered with 0.45 μm Millipore filter paper before adding to the medium . Different concentrations (1 , 5 , and 10 mg/l) of each stock solution were made for experiments .

Chlorophyll (a) measurement :-

Growth experiments were carried out in culture bottles, each one containing 250 ml basal medium with one concentration of metal , one of these bottles was prepared without metal addition to represent control treatment.

Cultures were incubated under the experimental conditions after inocula adding . Samples (50 ml) were taken from each bottles, after the incubation periods (0 , 1 , 2 , 3 and 4) weeks , then filtered by GF/C filter papers to measure the growth rate by chlorophyll a concentration.

Chlorophyll a was extracted in 10 ml. of 90% acetone , and placed overnight at 4°C in dark so as to ensure complete extraction . The optical density of the extract was measured (after centrifugation at 3000 rpm for 15 min.) with a spectrophotometer at 665 and 750 nm.

Before and after the adding of two drops of HCl (2N) . Duplicates were used for each experiment . The amount of chlorophyll a extracted was calculated according to the equation of Lorenzen (Vollenweider , 1974) .

3-Results

The statistical analysis of variance (ANOVA test) revealed that significant differences in the chlorophyll a pigment were occurred by different metals types .

The effect of silver ions (Ag^{1+})

The most inhibition effect of metal was caused by the silver ions. When *N. linkia* were exposed to different concentrations of silver ions , the chlorophyll a pigment was decreased significantly ($p < 0.05$) at all those concentrations as compared with control treatment. While there was non-significant differences ($p > 0.05$) in the pigment content caused by the different exposure period , except several growth appeared at 1mg/l treatment in the 4th week of experiment , that stilled less than those in the control treatment (fig.1) .

In contrast , the chlorophyll a pigment in *H. aureus* were also decreased significantly ($p < 0.05$) at all silver treatments , and no growth appeared along the experiment periods (fig.2). The effect of silver ions on *H. aureus* didn't differed with that on *N. linkia* .

The effect of cobalt ions (Co^{2+})

Fig.(3) showed that all cobalt concentrations used were inhibited the growth of these two species. In *N. linkia* the chlorophyll a pigment was inhibited significantly ($p < 0.05$) at 1st and 2nd weeks incubation of 1mg/l and 5mg/l treatments, after this period several growth was appeared at 1mg/l treatment, but not at 5mg/l treatment. While at 10mg/l treatment no growth appeared along the experiment period.

On the other hand, the effect of cobalt ions on the growth and chlorophyll (a) pigment content in *H. aureus* were less than the effect on *N. linkia* fig.(4), and the growth and pigment of *H. aureus* were appeared at all treatments, but they are still less than control treatment. There were non-significant differences ($p > 0.05$) between the effect of 5 mg/l and that of 10 mg/l treatments along the experimental periods.

The effect of lead ions (Pb^{2+})

When *N. linkia* was exposed to different concentrations of lead ions, the growth and chlorophyll a pigment content were increased significantly ($p < 0.05$) as compared with the control treatment (fig.5).

These increasing in the growth and pigment was reached to the stationary phase at 1 mg/l and 10 mg/l treatments at 2nd and 3rd weeks incubation respectively, whereas increasing in the growth was continuous at 5 mg/l treatment along the exposure periods. While, the growth and pigment content of *H. aureus* were inhibited at the 1st week exposure period at all lead concentrations as compared with the control treatment (fig.6), at 2nd week exposure the growth and pigment showed a slight increasing only at 1mg/l treatment, at 3rd week exposure the growth at all treatments were less than those in control treatment, so that at 4th week, except 1mg/l treatment which showed the maximum significant ($p < 0.05$) increasing in the growth and pigment content at 4th week exposure as compared with the other treatments

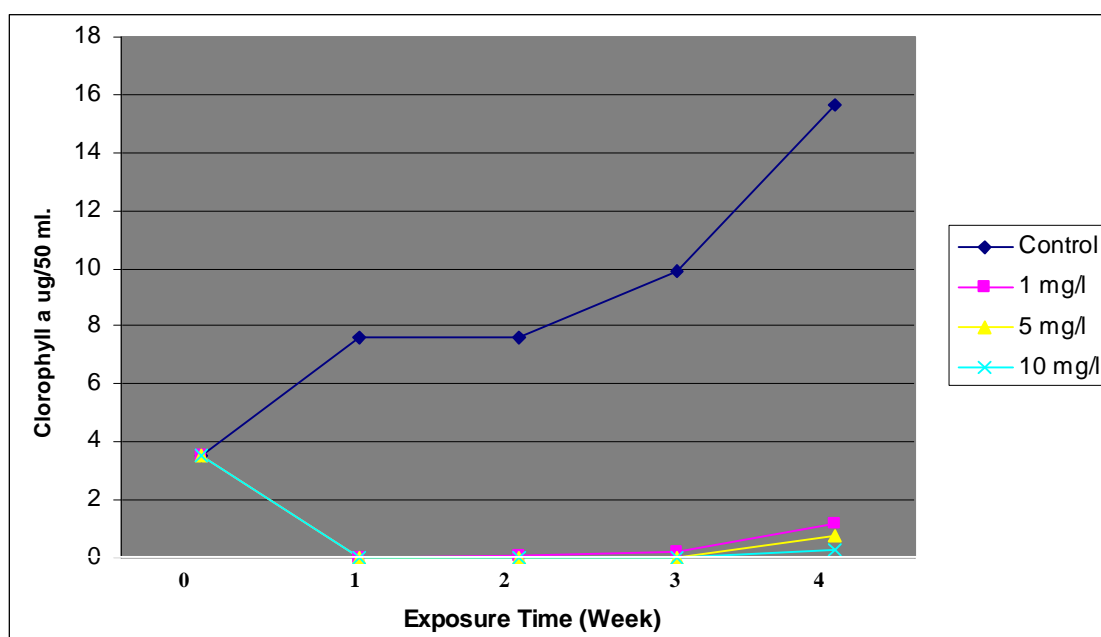


Fig. (1): The effect of silver ions on the chlorophyll a pigment in *N. linkia*

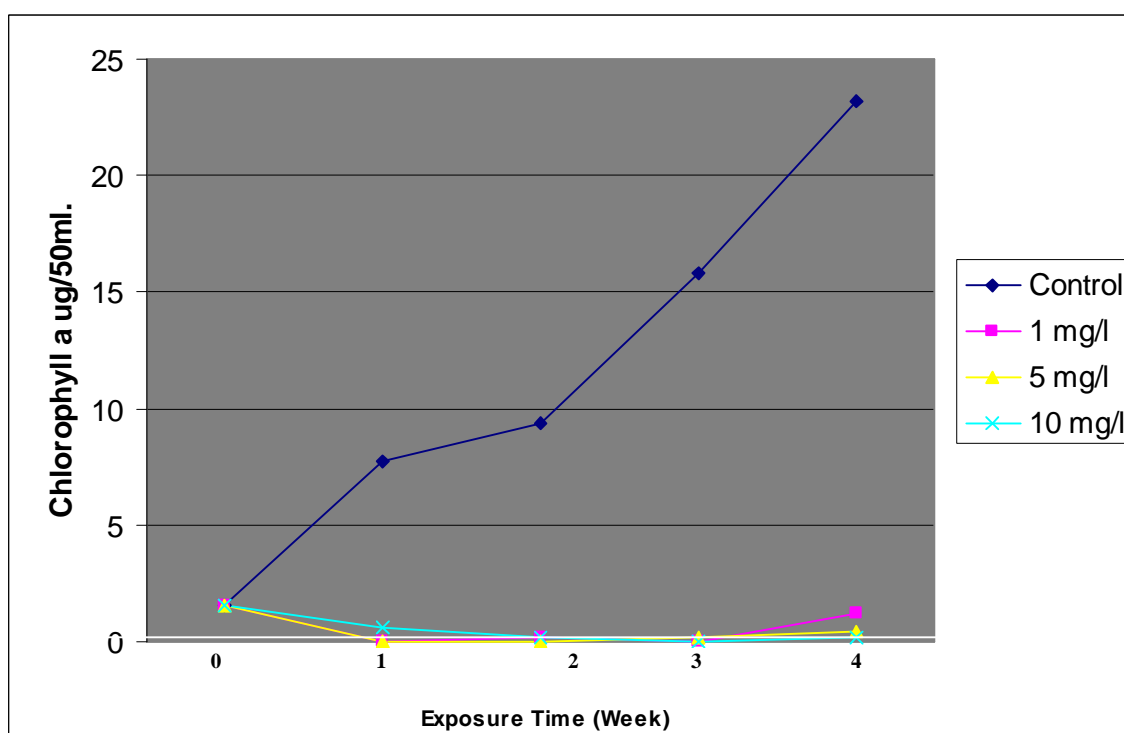


Fig. (2): The effect of silver ions on the chlorophyll a pigment in *H. aureus*

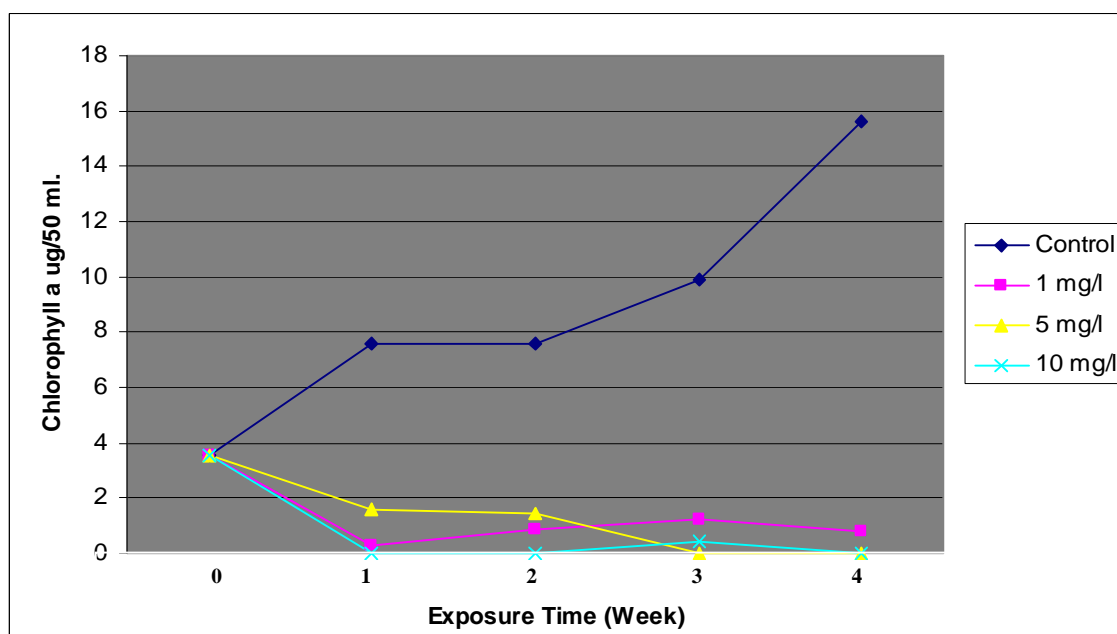


Fig. (3): The effect of cobalt ions on the chlorophyll a pigment in *N. linkia*.

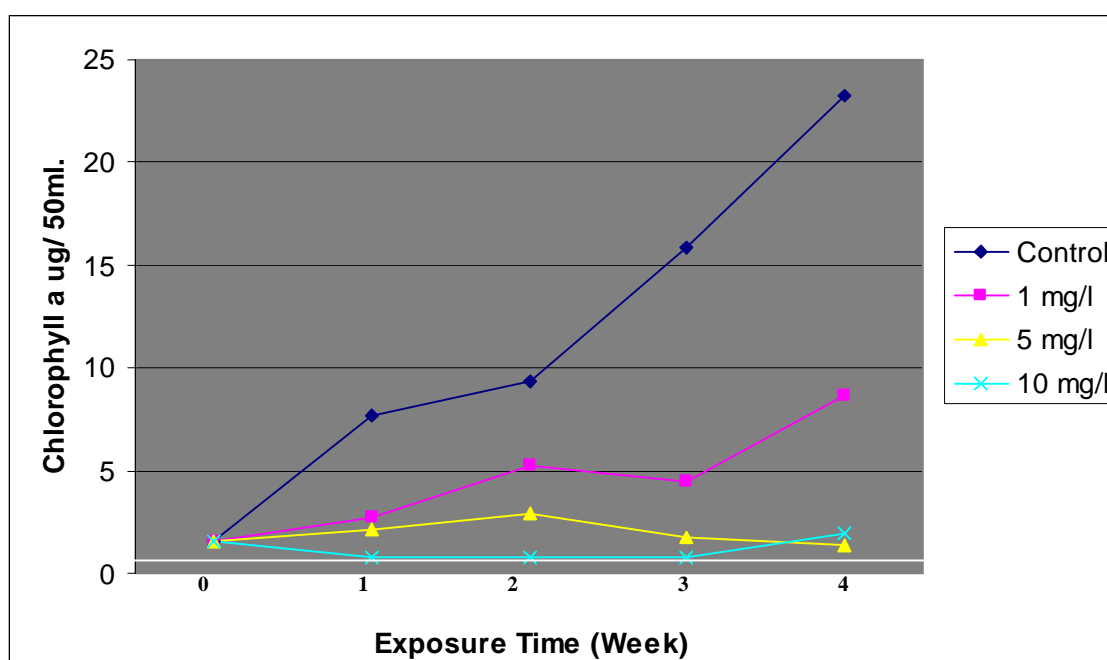


Fig. (4): The effect of cobalt ions on the chlorophyll a pigment in *H. aureus*

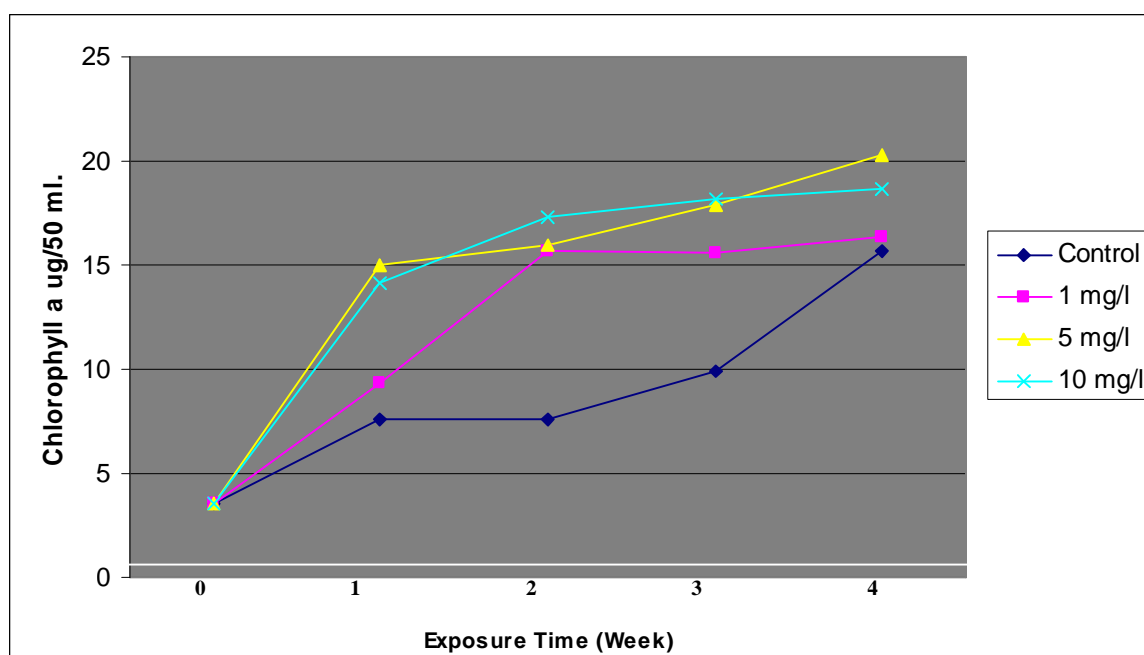


Fig. (5): The effect of lead ions on the chlorophyll a pigment in *N. linkia*.

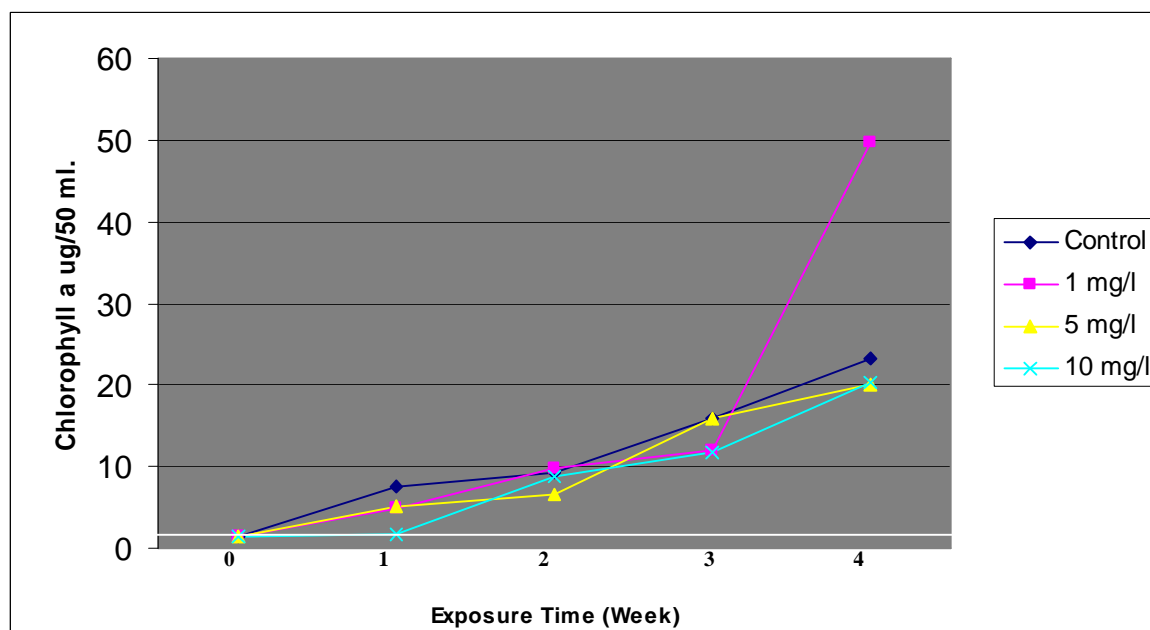


Fig. (6): The effect of lead ions on the chlorophyll a pigment in *H. aureus*

4-Discussion

In the present study , only several differences in the growth and chlorophyll a pigment of two cyanophyta species were observed , this may explain the different distribution of these genera in fields , or due to the different in cell structures .

As reported by Xue *et al.* (1988), the adsorption capacity of algae is linked to the presence on the cell wall of aminic acid and carboxylic groups as well as the functional side chains of amino acids ,mainly histidine and cysteine , which are reported to have a strong affinity for metal ions . However , the adsorption efficiency strongly depends on the type of metal ions , the number of charges and the affinity of the binding site for each metal (Converti *et al.* , 2006) .

Most reports demonstrated that the inhibitory effect of stress become greater with an increase in metal concentrations and suggested that the reduction in the growth rate of algae due to a decrease in algal photosynthesis caused by the inhibition of synthesis of chlorophyll , the most important pigment in algal cells for collecting solar energy for photosynthesis .

The mechanism of toxicity of heavy metals to cyanophyta are not fully known but several heavy metals retard the flow of electrons in electron transfer reaction in mitochondria and chloroplast and thus can be expected to have a

detrimental effect on respiration , photosynthesis and other processes related to it (Takamura *et al.*,1990) .

The other mechanism proposed for the inhibition is the replacement of magnesium in the chlorophyll molecule , consequently cells accumulate protoporphyrin and synthesis of chlorophyll is blocked ,this may be attributed to inhibition of reduction step in the biosynthetic pathways of this pigment (El-Sheekh *et al.* , 2003).

Stratton *et al.*(1979) showed that the damage of cell membrane at high metal concentrations will lead to uncontrolled efflux / influx of electrolytes or even other vital ions which may be responsible for the inhibition of growth .

These behaviors may not be a scribed only to the limited supply of energy or reductants but also to the poisoning of intracellular enzyme systems by displacement of an essential metal ion forming the central and functional part of the enzyme protein , or due to the interference of heavy metals with sulphhydryl (- SH) groups which often determine the secondary and tertiary structure of the proteins (Awasthi and Das , 2005).

The present results are in agreement with those obtained by El-Sheekh *et al.* (2003) who reported that high concentrations of Co^{2+} were decrease the chlorophyll a content in two algal species : *Monoraphidium minutum* (1 , 2 and 3

mg/l) and *Nitzschia perminuta* (2.5, 3.5 and 5 mg/l) and they suggest that decrease in pigment is due to the inhibitory of O₂ uptake.

The highest toxicity caused by silver ions in the present study may be due to the charge's numbers, which was one in silver and two in the others (cobalt and lead). Also Lee *et al.* (2005) showed that silver toxicity is a direct result of intracellular accumulation rather than cell surface interaction, in two fresh water algae: *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii*.

In contrast, lead ions were stimulated the growth and pigment content, and this was in agreement with the result of Al-Hejuje (1997) who found the similar results on *Oscillatoria amoena* and *Chlorella vulgaris* when exposed to Pb(NO₃)₂ salt. My opinion may be due to release organic substances, by some algal species, that can chelate free metal ions as a detoxification mechanism, while the increasing in the growth and pigment content may be due to the nitrate (NO₃) which was added to the medium as lead nitrate salt Pb(NO₃)₂.

5-References

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تأثير ايونات بعض المعادن الثقيلة على صبغة اليخضور أ في الطحليين
Hapalosiphon aureus و *Nostoc linkia*

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الملخص

تم تعريض نوعين من الطحالب الخضر المزروعة هما *Hapalosiphon aureus* و *Nostoc linkia* لايونات بعض المعادن الثقيلة (الفضة Ag^{1+} ، الكوبلت Co^{2+} و الرصاص Pb^{2+}) بتركيزات مختلفة (1 ، 5 و 10) ملغم /لتر ، لدراسة تأثيرها على نمو (اليخضور) هذين الطحليين ، وتم استخلاص صبغة اليخضور أ من هذين الطحليين بعد فترات تعريض مختلفة (0 ، 1 ، 2 ، 3 و 4) أسابيع لاستخدامها كمقياس فسيولوجي في دراسة سمية المعادن الثقيلة على الطحالب الخضر المزروعة .
أظهرت النتائج إن تأثير تلك الايونات لم يختلف معنوياً ($p > 0.05$) ولم يختلف التأثير باختلاف نوع الطحلب ، ولكنه اختلف معنوياً ($p < 0.05$) باختلاف نوع المعدن الثقيل والتركيز المستخدم من العنصر في وسط النمو و فترات التعريض المختلفة ، وكان تأثير تلك العناصر على نمو الطحليين ومحتواهما من صبغة اليخضور أ بالتسلسل التالي : $Pb < Co < Ag$