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Growth response of Cyanobacterium: *Chroococcus* sp. To copper (Cu^{+2}) and Nickel (Ni^{+2}) ions, singly and in dual combination

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Abstract

A cyanobacteria strain *Chroococcus* sp. was isolated from moist land (Abo Al-Khasib) in Basrah city purified and exposured to different concentrations (3, 5 and 10ppm) of Copper(Cu^{+2}) and Nickel (Ni^{+2}) ions as singly and in dual combinations.

Chlorophyll-A was extracted at different exposure periods (0, 1, 2, 3 and 4) weeks as indicat the growth response.

Results showed that growth response of cyanobacterium *Chroococcus* sp. was depending on the ions concentration and the exposure periods the effect of Nickel ions on growth response of *Chroococcus* sp. isolate better than Copper ion expertly in 1st week exposure while the antagonistic (increasing of growth) effect was appeared at most treatments in dual compaction and synergestic (decreasing of growth) effect in some, the synergistic effect of dual compaction in increasing of Copper ion concentration compared with Nickel ion also, the antagonistic effect was appeared at most treatment in dual combinations. Synergestic or antagonistic effect was depending on the concentration of each ion and the exposure periods.

1- Introduction:

The production, utilization and disposal of chemicals have increased dramatically in recent years. The release of heavy metals from multi-sources such as: industries, agriculture and sewage into the environment has resulted in many problems for both human health and aquatic ecosystems (Inthorn *et al.*, 2002). and can alter macro and microbiological communities (Ivorra *et al.*, 2000). Some of heavy metals such as: Nickel and Copper at low concentrations have an important role in microbiological activity (Cavet *et al.*, 2003), but have a toxic effect at high concentration (Mehtar *et al.*, 2008), and can affect normal flora in ecosystems and there toxic for human beings (Rodriguez *et al.*, 2006).

Convential chemical methods for heavy metals removal from wastewater Precipitation, Filtration, ion-exchange and reduction-oxidation are expensive and ineffective, particularly when metal concentration is low One strategy to reduce heavy metal solution is to use microorganisms. (Vieire and Volesky, 2000).

Microalgae, specially cyanobacteria can sequester heavy metal ions by adsorption and by absorption, as do by other microorganisms, therefore , they used for metal removal as a chief greater performance at a lower cost than conventional wastewater treatment technologies , this is consistent with the recent trend for growing interest in biosorbent technology for removal of trace amounts of toxic metal from aqueous waste (Inthorn *et al.*, 2002).

Converti *et al.* (2006) used *Spirulina platensis* biomass as adsorbent for Copper removal from water solution while El-Sheekh *et al.* (2005) found that Copper was removed by 12.5-81.8% from wastewater by using cyanobacterial cultures of *Nostoc muscorum* and *Anabaena subcylindrica* , and they found that single culture in most cases was better than the mixed cultures in heavy metal removal.

The hazardous chemicals that have been released into the aquatic environment do not exist in single pure form, but occur in mixtures in the environment consequently, their effects on organisms living in the aquatic environment are primarily due to the combined toxicities (Aoyama *et al.*, 1987). Lasheen *et al.* (1990) found that the inhibitory effect of some heavy metals on Nile water algae was in the following order: Cd-Cu> Cd-Cr > Cd > Cu > Cr, Also Hutchinson (1973) has shown that the interaction between Copper and Nickel ions has a synergistic effect on the growth of *Chlorella* species.

There are many paper on the evaluation of the toxicity of a single chemical on cyanobacteria , However, there are a limited number of papers on the interaction effect caused by mixed heavy metals, therefore, the puorposes of this study are to evaluate the toxic effect of a single, and the combined toxic effect, i.e. , the interaction effect of two heavy metals ions (Nickel and Copper) on the growth of a strain of cyanobacterium: *Chroococcus* sp.

2- Materials and methods

A strain of cyanobacterium *Chroococcus* sp. was isolated from moist land Abo Al-Khasib in Basrah city, and transported to the laboratory, the samples were then streaked on agar medium (Chu-10-D) plates and incubated under the air conditioned room which was illuminated with while fluorescent tub at room temperature (25±3)°C, until the appearance of algal colonies. Single algal; colonies were selected and re-streaked on agar medium plates several time to obitain unialgal cultures.

Single colonies were maintained in 250cm^3 classified according to Desikachary (1959), of Chu-10-D medium for stock culture under the same conditions classified according to Desikachary (1959), purified according to (Stein, 1973).

Copper (Cu^{+2}) and Nickel (Ni^{+2}) ions as stock solutions (1000 ppm) were prepared separately by dissolved $Cu(NO_3)$. H_2O and $Ni(NO_3)_2$. H_2O respectively , in deionised distilled water. Different concentrations (3, 5 and 10ppm) of each stock solution were used (singly and in dual combinations).

Growth experiments were carried out in conical flask 500cm^3, each one containing 250cm^3 basal medium supplied with one concentration of metal (for singly effect) and two concentrations of metals (for dual combinations effect). Two conical flasks, which didn't receive metal, served as control. Experiments were carried out in duplicate. Each bottle was inocubated with 5cm^3 of old culture (one-week), and incubated under the experimental conditions, sample 50cm^3 of each treatment was taken at different exposure periods (0, 1, 2, 3 and 4) weeks for chlorophyll a determination (Al-Hejuje, 2008). Chlorophyll a was calculated according to the equation of lorenzen (Vollenweider, 1974).

All statistical analysis were performed in the computer package Minitab 11.0 by using ANOVA test.

The method modified by Stratton was used for determination the modes of interaction effects of the chemical combinations. (Aoyama *et al.*, 1987).

3- Results

The statistical analysis (ANOVA test) revealed a significant ($P<0.05$) growth response that caused by the different concentrations of Copper and Nickel ions, as they used singly or in dual combination, on the growth of a strain of cyanobacterium *Chroococcus* sp. The effect of Copper ions was not significant differences ($P>0.05$) as compared with the effect of Nickel ion.

The effect of Copper ion (Cu^{+2}) as singly:-

Fig (1) showed that all the Copper concentrations (3, 5 and 10ppm) were lead to inhibition growth of *Chroococcus* sp. and the maximum inhibition growth was appeared at 3ppm and 10ppm treatments, which have no detectable chlorophyll a, at the first week exposure periods, whereas the growth at 5ppm treatment was not different significantly ($P>0.05$) as compared with control at the same time.

As the exposure period was increased (second week), the growth response was increased, too, at all treatments, and reached maximum at 5ppm and 3ppm treatments respectively. After this period, an inhibition effect was found at 5ppm and 3ppm treatments at the third week, while didn't affected at 10ppm treatment was found as compared with control group. At the end of the experiment (fourth week) the growth was decreased gradually at all treatment specially at 10ppm treatment.

The effect of Nickel ion (Ni^{+2}) as singly:-

Nickel ion was have inhibition growth of *Chroococcus* sp, these effects were clearly appeared at the first week exposure (fig. 2), then, the growth was increasing with the exposure period increasing (second week). At the third week exposure, the growth at 10ppm treatment was inhibited significantly ($P<0.05$) whereas the other treatments (5, 3 ppm) have slight decreasing. The fourth week xposure showed an increasing in the growth rate at all treatment specially (3ppm) treatment which was stimulated significantly at these periods but still less than control group.

The effect of Copper and Nickel ions as dual combination :-

As Copper (3ppm) was used as dual combination with different concenterations of Nickel (3, 5 and 10ppm), the growth of the studied strain *Chroococcus* sp. was antagonistically affected on growth at all treatments except three (3Cu, 0Ni, 3Cu, 3Ni, 3Cu, 5Ni) treatment which appeared synergistic effects at the first week exposure only (Fig. 3), whereas an antagonistically effects were appeared at all treatments that supplied with Copper (5ppm) and Nickel at different concentration (3, 5 and 10ppm) along the experiment periods (Fig. 4) except two (5Cu, 10Ni) treatments which appeared synergistically effect at the first and fourth week exposure.

On the other hand, when Copper ion (10ppm) was added with different concentrations (3, 5 and 10ppm) of Nickel, the effect was synergistically at all treatments at the first week exposure periods and the Chlorophyll a was undetectable at this period (Fig. 5), than, the growth was appeared, and the effect became antagonistic at all treatments. From the above experimental results we found that the synergistic or antagonistic effect was depended on the concentrations of each metal ions and the exposure periods for each dual combination of Copper and Nickel. The synergestc effect of dual combination increase with increasing of

Copper ion concentration compared with Nickel ion

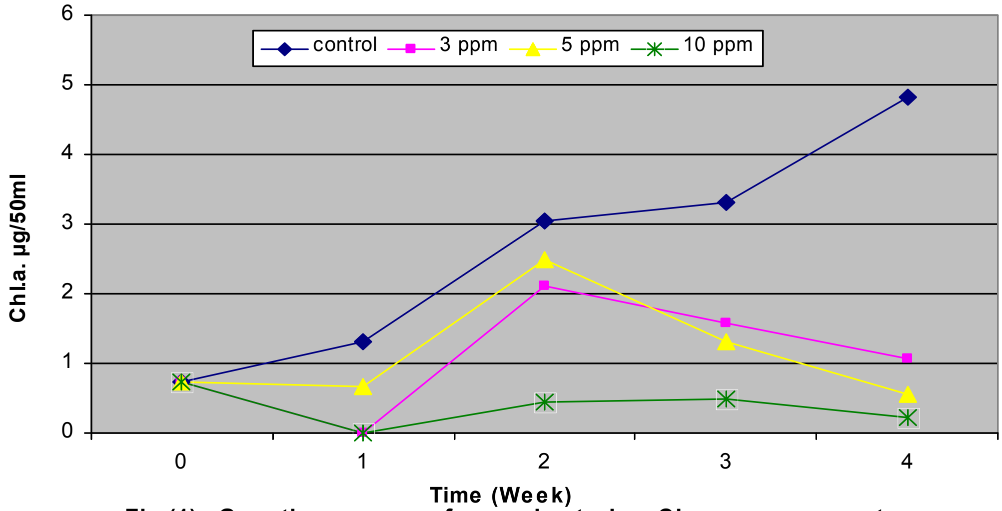

Fig.(1): Growth response of cyanobacterium Chroococcus sp. at different concentration of copper ions as singly on the exposure time.

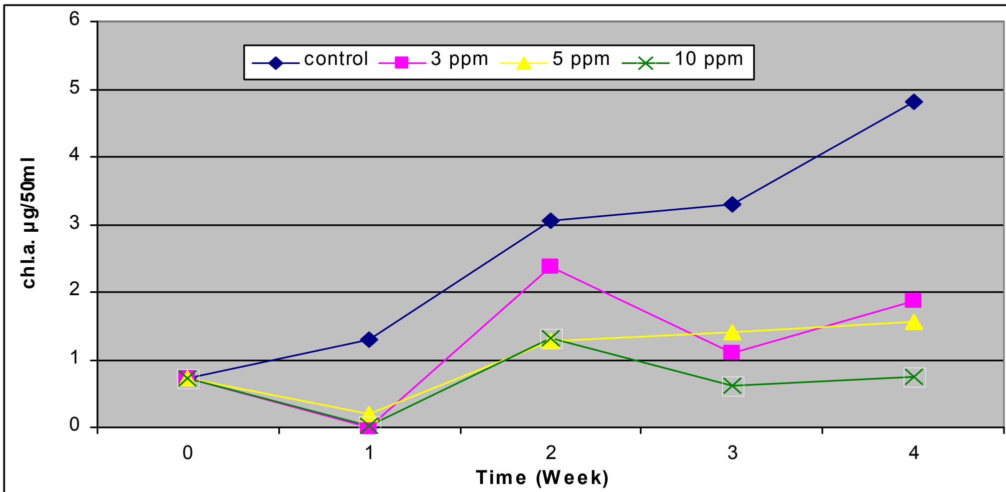

Fig (2):Growth response of cyanobacterium Chroococcus sp. at different concentration of Nickel ions as singly on the exposure time.

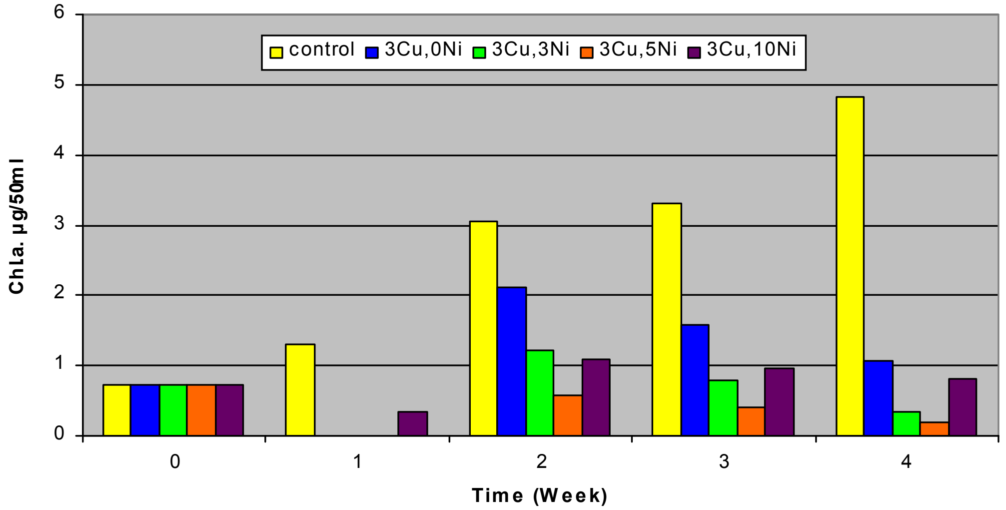

Fig.(3): The effect of (3ppm) concentration of copper with different concentration Nickel ions (3,5 and 10ppm) on the growth rate of cyanobacterium *Chroococcus* sp. at different exposure

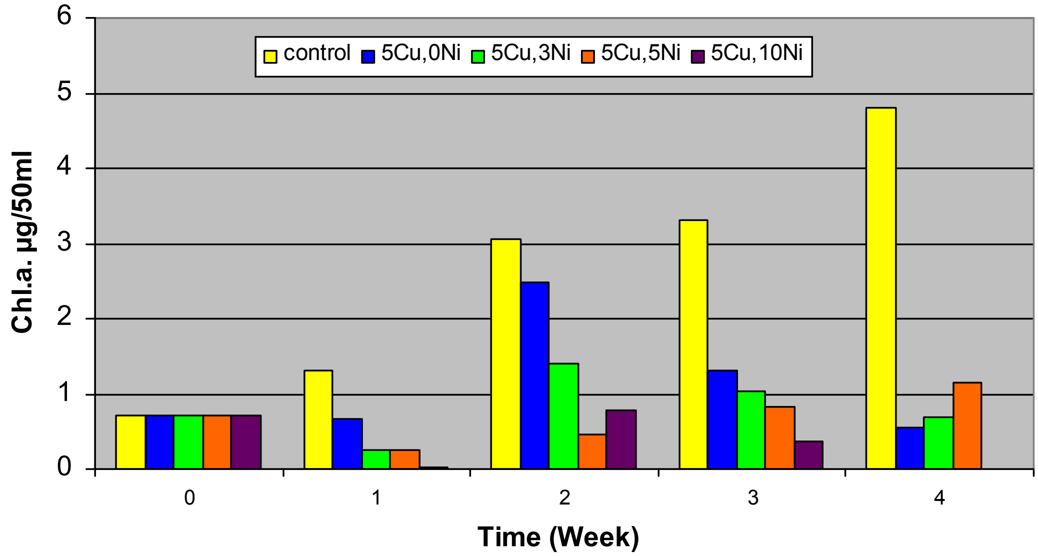

Fig. (4): The effect of (5ppm) concentration of copper with different concentration Nickel ions (3,5 and 10ppm) on the growth rate of cyanobacterium *Chroococcus* sp. at different exposure

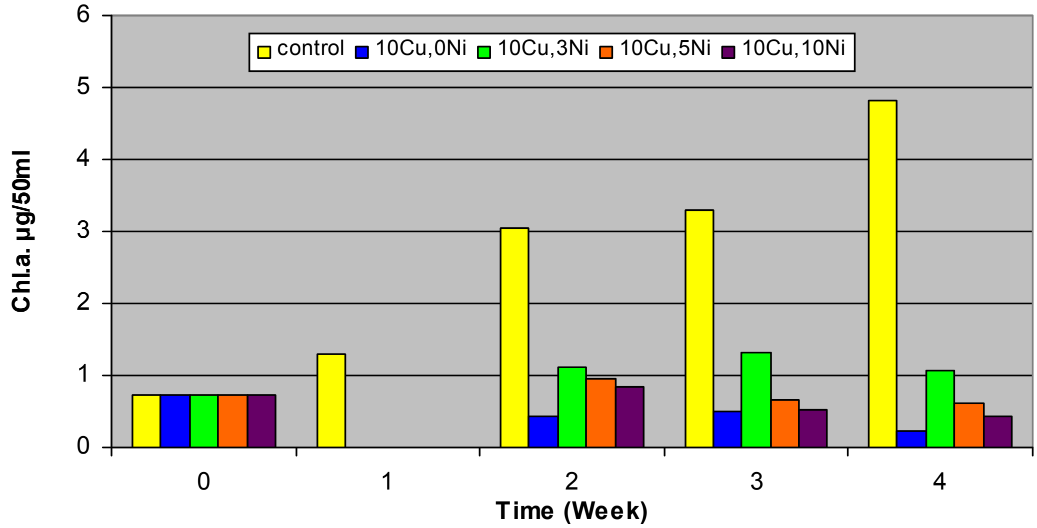

Fig. (5): The effect of (10ppm) concentration of copper with different concentration Nickel ions (as dual combination) on the growth rate of cyanobacterium *Chroococcus* sp. at different exposure

4- Discussion

The role of algae in waste water treatment and their affinity for heavy metal cations, based on high negative surface change. Cyanobacterial cell wall is provided with amine, carboxylic, thiol, sulphydryl and phosphoric functional groups which can bind metal ions. However, the adsorption efficiency strongly depends on the type of metal ions, their number of charges and the affinity of the binding site for each metal (Converti *et al.*, 2006). As reported by Xue *et al.* (1988), histidine which found on cell wall was able to bind Copper Cu^{+2} because furnishes a bidentate site. Amine and Carboxylic groups can also interact bidentately with copper. The presence of methionine as one of the amino acids is very significant, which may result in binding of the metals with sulphydryl groups. Further, the polysaccharides may act as chelators (Caire *et al.*, 1997).

Heavy metal uptake by microbial cell has been shown in laboratory cultures to be dependent on the free ions activity (Moffett and Brand, 1995). Cyanobacteria are known to release organic substances that can chelate free metal ions, this interaction may influence their availability or toxicity to the organism for example, copper, which is known to complex with the peptide band , was complexed by a peptide-containing fraction of a concentrated culture filtrate and in this form was found to be anti-toxic activity to the alga (walsby, 1974). Exclusion of copper from the cell interior due to the high Cu-adsorptive capacity of the cell surface is suggested as the primary tolerance mechanisms for the Cu-tolerant strain (Twiss *et al*., 1992), also, it is possible that some internal detoxification mechanism might also be operating in this strain. Les and Walker (1984) found that Cu was bound rapidly by the sheath forming cyanobacteria *Chroococcus paris*, approximately 90% of the total amount of the added metal was bound within 1 min, further significant binding occurred at a slower rate. Converti *et al*. (2006), during their study on copper biosorption by *Spirulina*, found that during the first hour, a certain tendency of the cell material to aggregate, thus exposing a small specific surface to metal binding. After several hourse the suspension appeared to be well dispersed, thus increasing the available surface and the number of adsorption sites.

Heavy metals stress caused reduction in growth, photosynthetic pigments (Al-Hejuje, 2008), nitrate reductase (Okmen *et al*., 2007), alkaline phosphatase (Awasthi and Das, 2005), and uptake of nutrients or damage of cell membrane (Fathi, 2002), also Gupta and Agrawal (2007) found that heavy metals (Ni, Cu, Zn, Co, Fe and Hg)at (1-200 ppm) concentrations were affected the survival and motility of algae.

Van and Clijsters (1999), proposed two possible mechanisms for the effect of heavy metals on algae: first, the displacement of an essential metal ion the central and functional part of the enzyme protein, and secondly, interference with sulphydryl (-SH) groups which often determine the secondary and tertiary structure of the proteins.

Cavet *et al.* (2003)showed that cyanobacteria was Cu requirement at low concentration (in thylakoidal plastocyanin), also Awosthi and Das (2005) reported that Ni was stimulated CO_2^{14}- Fixation in algae at low concentration, too. During this study found that all concentrations used have a toxic effect on the growth of this strain, this result was in agreement with Noriko *et al*. (1989) who demonstrated that high concentration of Cu^{+2} caused 50% inhibition of photosynthesis in 118 isolates of algae, this may be attributed to inhibition of reduction steps in the biosynthetic pathways of the pigments (De-Filippis *et al*., 1981). Also, we are in agreement with Rai and Raizada (1989) who reported that Ni (1mg/L) was inhibited the nitrogenase activity in *Nostoc* sp. after 48hrs. Mohanty *et al.* (1989) reported that the effect of Ni^{2+} is possibly due to its role in modifying the function of QB (the primary acceptor of PSII) thereby impairing the PSII activity.

Ni^{2+} can impair the QB function in three possible ways: Interruption of electron flow between QA and QB, directs modification of QB, or alteration of components beyond QB, which leads to the impairment of PSII activity at the QB site.

The effects of Cu^{2+} and Ni^{2+}, as they added singly, was depending on its concentration and the exposure periods, this finding in agreement with Al-Hjuje (2008).

A synergistic effect which found at most treatments that applied with dual combination (Cu^{2+} and Ni^{2+}) was in agreement with Lasheen *et al.* (1990) who found the same effect caused by dual combination of (Cu and Cd) on the growth of Nile water algae. Also, agreement with Aoyama *et al.* (1987), who observed a synergistic effect for combination of (Cu and Cr) or (Cu and Cd) on the growth of *Chlorella ellipsoidea.* In contrast, Antagonestic effect was appeared at another treatments, this effect may be due to displacement of Ni^{2+} ions caused by Cu^{2+} ions and the Cu^{2+} was accumulated as same extent as if applied singly (Munda and Hudnik, 1986). Also, Danilov and Ekelund (2001) found that Cu^{2+} has a toxic effect on photosynthetic efficiency (PE) of *Chlamydomonas reinhardtii*, while Ni^{2+} has stimulated.

The PE at the same concentration (1-2 mg/L), and they found that increasing in Cu^{2+} concentration led to decrease in the maximum values of oxygen evolution compared to the control, whereas Ni^{2+} caused increasing in maximum values of oxygen evolution. This interaction between Cu^{2+} and Ni^{2+} my be led to the above results.

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استجابة نمو الطحلب الاخضر المزرق ***Chroococcus* sp.** لايونات النحاس (Cu^{+2}) والنيكل (Ni^{+2}) بصورة منفردة ومجتمعة

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الخلاصة

تم الحصول على عزلة الطحلب الأخضر-المزرق *Chroococcus* sp. من الترب الرطبة في ابي الخصيب – محافظة البصرة، وعزلت ونقيت ثم عرضت لتراكيز مختلفة (3 ، 5 و 10) جزء بالمليون من ايونات النحاس (Cu^{+2}) والنيكل (Ni^{+2}) بصورة مفردة ومجتمعة.

تم استخلاص صبغة اليخضور أ بعد فترات تعريض مختلفة (0، 1، 2، 3، 4) أسابيع للاستدلال على استجابة النمو. أشارت النتائج إلى أن استجابة الطحلب الأخضر-المزرق *Chroococcus* sp. اعتمد على تركيز الايونات وفترات التعريض، اذ كان عنصر النيكل اكثر تاثيراً على تثبيط نمو الطحلب المعزول مقارنة مع عنصر النحاس وخاصة في الاسبوع الاول من التعريض لذلك العنصر، بينما كان تاثير التداخل لتلك الايونات متضاداً (زيادة نمو) في معظم العاملات وتعاونياً (تثبيط نمو الطحلب المعزول) في بعضها وقد ازداد الفعل التعاوني للايونات المتداخلة بزيادة تركيز عنصر النحاس اكثر مقارنة مع عنصر النيكل.

أن التأثير المضاد او التعاوني كان معتمداً على تركيز كل عنصر وفترة التعريض.