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Effects of Heavy Metals (Copper, Cobalt and Lead) on the Growth and Photosynthetic pigments of the Green Alga *Chlorella pyrenoidosa* H. Chick

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ABSTRACT

The present study was carried out to investigate the effect of different concentrations (0.05, 0.5, 1, 2, 4, 6 and 8 mg/L) of Copper Cu^{2+}, Cobalt Co^{2+} and Lead Pb^{2+} on growth, pigment contents, protein and antioxidant enzyme activities of superoxide dismutase (SOD) and catalase (CAT). The data show that the lower doses of Co^{2+} had stimulatory effect on biomass of *Chlorella pyrenoidosa*, whereas the higher doses were inhibitory depending on the type of the metals. The inhibitory effect of copper to growth parameters of *Chlorella pyrenoidosa* was more pronounced than other two tested metals. The total protein content of the tested green alga gradually decreased in a manner dependent on the metal concentration in the medium. Our results showed reduction in the antioxidant enzymes (SOD) and (CAT) in *Chlorella pyrenoidosa* after exposure when compared with activities in the control. The inhibitory effects of either of the used heavy metals depend on concentration and time of exposure. These results provide some additional information that can lead to better understand consequences of heavy metal poisoning in microalgae.

Keywords: Growth, Pigments, Heavy metals, Antioxidant enzymes, *Chlorella pyrenoidosa*.

INTRODUCTION

Many pollutants like pesticides, oil hydrocarbons, heavy metals as well as thermal radioactive pollution can get into aquatic environments after direct and indirect release from industries, agriculture and households (Fathi *et al*., 2008). As an important group of these different chemical substances, heavy metals may be deposited into all ecosystem (Mutlak *et al*., 1979). The disturbance of aquatic ecosystems provoked by heavy metals pollution from industrial and domestic sources has as consequence the loss of biological diversity and increased bioaccumulation of toxicants in food chain (Pena-Castro *et al*., 2004). Toxic heavy metals have harmful effects even at very low concentration on the aquatic organisms including plankton, aquatic plants, invertebrates and vertebrates (Atici *et al.,* 2008).

Microalgae are sensitive indicators of environmental change and as the basis of freshest water and marine ecosystems, are widely used in the assessment of risk and developmental regulations for metals (Levy *et al.,* 2007). There are a noteworthy number of investigations demonstrating the toxic effect of heavy metals on different species of algae (Lutz-Meinndl *et al*.,2015, Vona *et al*., 2013; Kaoutar and Mourad, 2013; Cherifi *et al.,* 2012; Fathi *et al.,* 2010 and El-Sheekh *et al.,* 2003). Microalgae, due to their widespread occurrence in nature and metabolic uptake with continuous growth (Sobhan and Sternbery, 1999), have gained a paramount attention. The cell walls components of microorganisms such as polysaccharides, proteins and lipids offer many functional groups which can bind metal ions (Ari *et al.,* 1999).

However, the metal uptake process is complex and dependent upon not only the specific surface properties of the organisms but also cell physiology (Arunakumara and Zhang, 2009) and other abiotic factor as PH and temperature. Copper (Cu^{2+}) and cobalt (Co^{2+}) are two essential trace elements, while lead (Pb^{2+}) is non-essential. These heavy metals are toxic for many living organisms. They accumulate in algae (Debelius *et al.,* 2009). These metals are frequently found as fresh water pollutants. Szivak *et al.* (2009) detected that Cu, Pb and Co are strongly phototoxic, causing growth inhibition and even death (Deckert, 2005). In general, the toxic effects of metal pollutants in plant cell are related to their strong reactivity, resulting in inhibition of enzyme activity and oxidative damage. For these reasons, heavy metal ions are present in the cytoplasm mostly in a bound form (Sirko and Gotor, 2007).

The aim of this study deals with the toxicity of some heavy metals to the unicellular *Chlorella pyrenoidosa.*

MATERIALS AND METHODS

Organism and culture condition

The fresh water green microalga *Chlorella pyrenoidosa* was obtained from the Institute of Oceanography and Fisheries in Alexandria (ARE). Alga used for experimental studies was axenic. *Chlorella pyrenoidosa* was cultivated in a liquid medium, which was prepared as described in Kuhl (1962). Cultures of algae were grown at 25 ± 1°C in a temperature-controlled room. Illumination was provided with an irradiance of 200 µmol m^{-2} s^{-1}, under a 16h / 8h light / dark regime. All cultures were shaken twice daily to prevent cells from clumping. Seven days old cultures were spun down at 4000 g for 10 min and the pellets were re-suspended in fresh medium in order to use for the metal treatments.

Chemical and analytical methods

The chemicals were of analytical grade and used without further purification. De-ionized water obtained from a Millipore Milli-Q system was used throughout

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the experiment. Copper sulphate ($CuSO_4$. $5H_2O$), Cobalt chloride ($CoCl_2$) and Lead nitrate ($Pb(NO_3)_2$) were used as the sources of Cu^{2+}, Co^{2+} and Pb^{2+} respectively. The stock solutions (1000 mg/L) were prepared and kept in a refrigerator at 4°C until use.

Metal treatments

A standard initial inoculum $1.2x10^4$/ml cell number of the tested alga was inoculated to culture flasks (500 mL each) that contained 200 mL of sterile nutrient medium The culture flasks were supplied with various concentrations of Copper, Cobalt and Lead (0, 0.05, 0.5, 1, 2, 4, 6 and 8 mg/L). At the end of the incubation period (6 days) cultures were filtered and washed several times by distilled water. Three replicates for each sample and controls were used.

Determinations of pigment content

Chlorophyll a and carotenoid content were estimated in acetone extract according to Jeffrey and Humphery (1975). The content of the pigments fractions (µg/ml algal suspension) was then calculated under consideration of the dilution factors.

Algal counting

Cell number was determined using a Haemocytometer Chamber. Haemocytometer 0.1 mm deep, having improved Neubauer ruling was used.

Calculation of growth rate

The growth rate of the algal growth was calculated by the following equation (Nichol's, 1973). $K(day) = [3.322/(t_2-t_1)]. (\text{Log } N_2/N_1)$.

Biochemical analysis

a) *Protein estimation*

Total protein was estimated by using the methods of Lowery *et al.* (1951).

b) *Determination of antioxidant enzymes*

Enzyme extract for superoxide dismutase (SOD) and catalase (CAT) was prepared by homogenization of 0.15 gram fresh tested algae with 10 ml of chilled buffer in a pre-chilled mortar and pestle. For SOD and CAT, the extraction medium was 0.1 M potassium phosphate buffer (pH7.5) containing 0.5 m EDTA. The extract was centrifuged in a Beckman12-21 refrigerated operation at 4°C.

c) *Superoxide dismutase (SOD) activity*

Superoxide dismutase (SOD) activity was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977). The reaction mixture (3ml) contained 50mM phosphate buffer (pH 7.8), 0.1µM riboflavin enzyme extract. Riboflavin was added last and tubes were shaken and illuminated with two 20 W fluorescent tubes. The reaction was allowed to proceed for 15 minutes after which the lights were switched off and the tubes covered with a black cloth. Absorbance of the reaction was measured spectrophotometrically at 420 nm.

d) *Catalase (CAT) activity*

Catalase (CAT) activity was measured by the decrease in absorbance at 240nm due to H_2O_2 consumption (extinction coefficient; 40 $M^{-1}cm^{-1}$) according to Aebi (1974). The reaction volume was 1 ml, which contained 50mM phosphate buffer, pH 6.5, 50 mM H_2O_2. The reaction was started by the addition of sample.

e) *Metal uptake*

For the analysis of metal contents, the cultures were centrifuged to harvest the algal mass (50 mL). The pellet was digested 5 mL mixture containing HNO_3 (70%), H_2O_2 (30%) and deionized water in 1:1:3 ratio (Bates et al., 1982). After digestion the samples were analysed for metal content with a Perkin-Elmer atomic absorption spectrophotometer.

Statistics

Results were tested by one –way Analysis of variance (ANOVA). ANOVA effects and treatments differences were considered significant when $p<0.05$.

RESULTS

Effect of copper, cobalt and lead on the growth

The effect of copper, cobalt and lead on the growth and growth rate of *Chlorella pyrenoidosa* are shown in figure 1 and 2 respectively. There was no significant effect under the 0.05mg/L treatment when Co^{2+} was added to the medium ($P> 0.05$). The number of cells gradually increased in the culture supplemented by 0.05mg/L of Co^{2+} during exposure periods. Though, an inhibiting effect was found when the same concentration of Cu^{2+} and $Pb^{2+}$was added ($P< 0.05$). Whereas other concentrations of the three metals (0.5, 1, 2, 4, 6 and 8 mg/L) cause a clear reduction in the cell number of *Chlorella pyrenoidosa*.

The cell numbers were lower than the blank in all cases when exposure concentration increased to 8 mg/L indicating that specific concentration had an inhibiting impact on algal growth. The inhibition effect becomes weaker with increase of exposure time. Cu^{2+} has the most inhibition effect on the growth of *Chlorella pyrenoidosa* followed by Co^{2+} and the least was Pb^{2+}. The same effect was observed with respect to growth rate, as indicator of algal growth, as shown in fig. (2). the growth rates decreased in respect of increasing metals concentrations.

Effect of copper, cobalt and lead on the pigment contents

The pigment contents (chlorophyll a and carotenoid) of *Chlorella pyrenoidosa* treated with different concentrations of Cu^{2+}, Co^{2+} and Pb^{2+} as illustrated in table (1) showed clear differences between control and

treated ones when algae were exposed to different concentrations of the three tested metals. The pigment contents were decreased gradually with increasing metal concentration.

In contrast, inhibitions caused by Cu^{2+} were considerably higher than those of Co^{2+} and Pb^{2+}. The values of chlorophyll a and carotenoid of algal cells treated with 0.05 mg/L of Co^{2+} were nearly the same values obtained in control. The results also show that the inhibitory effect of copper on the growth and pigment contents is more pronounced than for the other two tested metals.

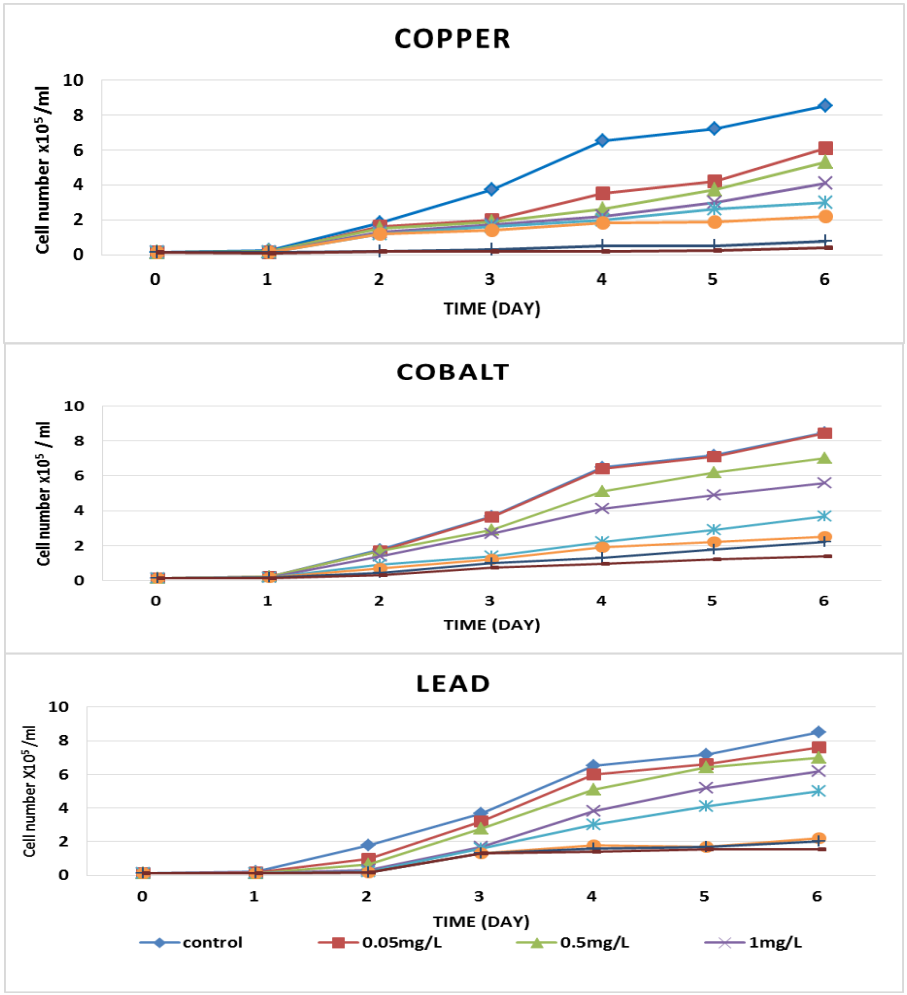

Figure (1): Effect of various concentrations of copper, cobalt and lead on the growth of *Chlorella pyrenoidosa* after six days incubation period. (Mean and standard deviation of three replicates are shown)

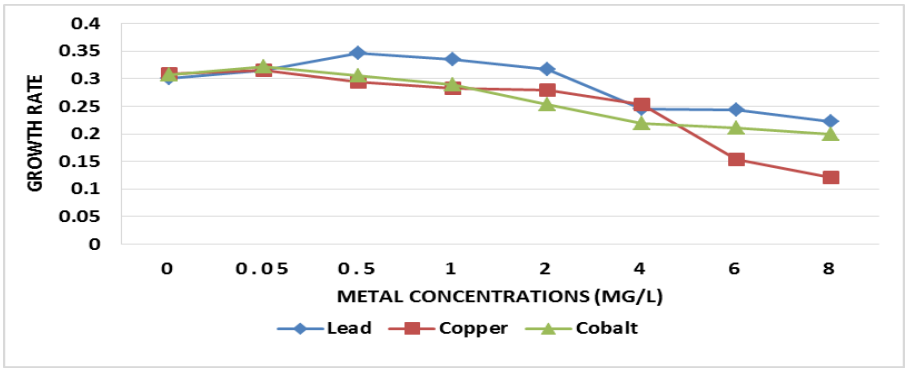

Figure (2): Effect of various concentrations of copper, cobalt and lead on the growth rate of *Chlorella pyrenoidosa* after six days incubation period. (Mean and standard deviation of three replicates are shown).

Table (1): Effect of metals (Cu^{2+}, Co^{2+} and Pb^{2+}) on the pigment contents (chlorophyll a and carotenoid µg/ml) in *Chlorella pyrenoidosa* (mean ± S.E.)(n=3).

Metal	Treatment (mg/L)	Chlorophyll a (µg/ml)	Carotenoid (µg/ml)
Control	0.00	0.428±0.023	0.230±0.015
Cu^{2+}	0.05	0.351 ±0.021	0.189±0.023
	0.50	0.316±0.012	0.172±0.006
	1.00	0.291±0.007	0.150±0.011
	2.00	0.215±0.006	0.128±0.012
	4.00	0.206±0.013	0.113±0.014
	6.00	0.139±0.010	0.084±0.003
	8.00	0.113±0.011	0.056±0.008
Co^{2+}	0.05	0.425±0.071	0.228±0.023
	0.50	0.337±0.071	0.177±0.011
	1.00	0.311±0.047	0.157±0.021
	2.00	0.266±0.083	0.140±0.003
	4.00	0.213±0.047	0.126±0.021
	6.00	0.168±0.047	0.096±0.006
	8.00	0.124±0.048	0.067±0.011
Pb^{2+}	0.05	0.375±0.011	0.192±0.021
	0.50	0.355±0.012	0.180±0.021
	1.00	0.320±0.011	0.160±0.007
	2.00	0.269±0.009	0.144±0.011
	4.00	0.244±0.015	0.132±0.007
	6.00	0.179±0.008	0.102±0.008
	8.00	0.167±0.008	0.073±0.007

Effect of copper, cobalt and lead on the protein content

The bioassay results as illustrated in Fig. 3 showed clear differences in protein content of *Chlorella pyrenoidosa* treated with different concentrations of Cu^{2+}, Co^{2+} and Pb^{2+}. The data show that the total protein content of the green alga *Chlorella pyrenoidosa* gradually decreased in a manner dependent on the metal concentration in the medium. The data also shows that all the three metals affected negatively the total protein content at higher doses. On the other hand, the supplementation of copper and lead by 0.05mg/L increases the total protein content as compared to the control.

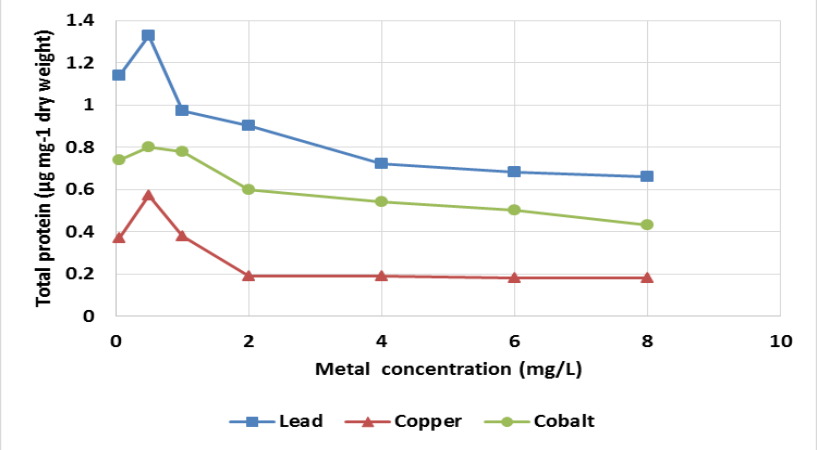

Figure (3): Effect of various concentrations of copper, cobalt and lead on total protein content (µg mg^{-1} dry weight) of *Chlorella pyrenoidosa* after six days incubation period. (mean and standard deviation of three replicates are shown).

Effect of copper, cobalt and lead on antioxidant response

Enzyme activity catalase and superoxide dismutase responses of *Chlorella pyrenoidosa* when exposed to the three metals after six days of incubation period presented in Figure 4 (A and B) respectively. Results of antioxidant enzymes activity catalase (CAT) and superoxide dismutase (SOD) (µ mg^{-1}protein) revealed similar behaviour. Both enzymes showed reduction in their activities in *Chlorella pyrenoidosa* after exposed to each of the tested metals (Cu^{2+}, Co^{2+} and Pb^{2+}) compared to their activities in the control. Significant

decreases in CAT and SOD activities were observed only when metals concentrations were greater than or equal to 1 mg/L, the most pronounced decrease was recorded in Cu followed by Co, and the least was Pb.

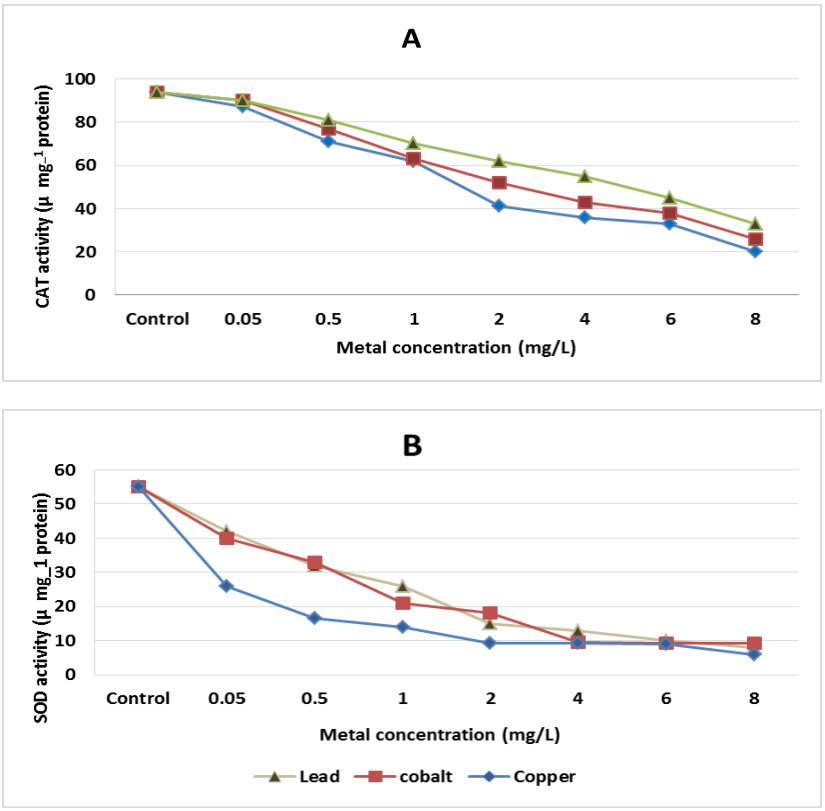

Figure 4 (A and B): Levels of antioxidant enzymes activities: catalase (CAT) and (SOD) (µ mg^{-1}protein) in *Chlorella pyrenoidosa* exposed to tested metals (copper, cobalt and lead) after six days of incubation period. (mean and standard deviation of three replicates are shown).

Metal accumulation

The data of table 2 performed that accumulation of copper, cobalt and lead by *Chlorella pyrenoidosa* cells were parallel to increasing the concentrations in the culture medium. Also, it can be seen that the tested alga *Chlorella pyrenoidosa* accumulated an appreciable amounts of copper more than other that observed with cobalt and lead.

However, no significant difference was observed between each of cobalt and lead. Metal accumulation by *Chlorella pyrenoidosa* were shown to be in an order Cu^{2+}> Co^{2+}> Pb^{2+}.

Table (2): Copper, Cobalt and Lead biosorbed by *Chlorella pyrenoidosa* after six days exposure for different concentrations (mean ± S.E.)(n=3).

Initial metal concentration (mg/L)	Metal accumulation (mg/g DW)		
	Cu^{2+}	Co^{2+}	Pb^{2+}
0.05	1.2±0.3000	0.63±0.400	0.84±0.300
0.50	2.21±0.200	1.65±0.400	1.55±0.500
1.00	5.82±0.400	3.65±0.200	3.52±0.300
2.00	22.63±1.20	19.2±0.500	18.56±0.50
4.00	71.98±1.50	53.8±1.200	53.58±1.10
6.00	143.45±1.3	96.21±1.10	94.53±1.40
8.00	275.51±3.1	134.32±1.9	118.66±1.7

DW mean dry weight

DISCUSSION

It is well known that algal cells exposed to heavy metals may suffer serious morphological and biochemical alteration (Rocchetta *et al.,* 2006). In general, changes in cell number, growth rate, chlorophyll content or, absorbance are used in assessing the algal responses to metal exposure. Studies of the effects of Cd^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+} on the green alga *Chlorella sorokiniana* revealed that the toxicity for all the observed parameters including the growth were increased in a dose-dependent manner (Vona *et al.*,2013), which is in good agreement with the present results of growth. We observed that the inhibitory effect of Cu^{2+} on the growth, growth rate and pigments content is more pronounced than for the other two tested metals. These findings are in agreement with several previously published data (El-Sheekh *et al*., 2003; Osman *et al.*, 2004; Fathi *et al.,* 2005; Muwafq and Bernd, 2006; Romera *et al.,* 2007; Cecilia *et al.,* 2007; Deng *et al.*, 2007 ; Fathi *et al.,* 2010 and Priyadarshani and Rath., 2012).

Regards to the stimulatory or inhibitory effect of Co^{2+} showed on this investigation, the present results are in agreement with those obtained by (El-Naggar *et al*. (1999) who found that a lower Co^{2+} concentration stimulated growth of *Nostoc muscorum.* However, higher Co^{2+} concentrations were inhibitory for the alga. On the other hand, growth promotion at low Co^{2+} concentrations may be due to Co^{2+} substitution for Zn^{2+} in some metalloenzymes in *vitro and* in *vivo* (El-Sheekh *et al*., 2003; Osman *et al.,* 2004). Moreover, high concentrations of cobalt were inhibitory for pigment content of tested alga. These results are in agreement with those obtained by El-Naggar *et al.* (1999) who reported inhibition of chlorophyll biosynthesis as a

result of Co^{2+} treatment is the replacement of magnesium in the chlorophyll molecule. In this regard, De Filippis *et al.* (1981) reported that reduction of chlorophyll a content is a common symptom of heavy metals toxicity.

Although Cu^{2+} is essential metal for living organisms, this metal can be toxic and can cause algal cell death at elevated concentrations. The toxic effect of copper at higher concentrations (4, 6 and 8 mg/L) detected in the present study may be due to the oxidative potential of Cu^{2+}, that causes reduction of chlorophyll and decrease of oxygen evolution rates and depletion of ATP by inhibition of enzymes (nitrate reductase and alkaline phosphatase), which are involved in nitrate and ammonia cellular metabolism (Muwafq and Bernd, 2006). The reduction of Cu^{2+} toxic effects with lower concentrations can be accounted of some organic compound, which decreases metal toxicity. Albergoni *et al* (1980) and Rijstenbil *et al* (1998) suggested that some algae capable to produce metal binding compounds get the ability to bind and sequester copper ions in the cytoplasm and reduce toxicity.

The growth of *spirulina* was severely inhibited by Pb^{2+} at high concentrations (Slotton *et al*, 1989). Regarding changes in pigment composition (chlorophyll a and carotenoid), in autotrophic cultures of *Chlorella pyrenoidosa*, grown under different concentrations Cu^{2+}, CO^{2+} and Pb^{2+} (table 1). Chlorophyll biosynthesis was reported to be inhibited by Cu^{2+}, CO^{2+} and Pb^{2+} leading to the lowered chlorophyll contents (Pahlsson, 1989; Arunakumara and Zhang, 2009 Fathi *et al* 2010; and Cherifi *et al.,* 2012). Studies with *Cladophora fracta* revealed that Pb^{2+} at high concentrations can destroy the chloroplasts (Lamaia *et al.,* 2005). Prassad and Prassad (1987) found that heavy metals inhibit the enzymes that are responsible for the chlorophyll synthesis. Biosynthesis of chlorophyll and carotenoid were also proved to be affected by the heavy metals as reported by Atri and Rai (2003).

Taking into account all the reports, we confirm here that poor carbon assimilation due to loss of pigments is the major reason for the growth inhibition observed in the present study. In addition, heavy metals could interrupt routing metabolic processes by competing for the protein binding sites, active enzymes and various biological reactive groups, causing poor growth.

The data concerning the cell contents of proteins indicated 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 favour of protein accumulation (Osman *et al.,* 2004). Whereas, the suppression of protein accumulation may be attributed to shortage of carbon skeleton results from low photosynthetic rate. Such results are in accordance with those of Fathi *et al.* (2000). However, some authors (Osman *et al.,* 2004; Tripathi and Gaur, 2006 and Fathi *et al*., 2010) reported that the toxic action of heavy metals on the enzymatic reactions responsible for protein biosynthesis.

Microalgae represent an almost untapped resource of natural antioxidants, due to their enormous biodiversity, much more diverse than higher plants (Li *et al.,* 2006). Takekoshi *et al.* (2005) have reported that the unicellular microalgae *Chlorella vulgaris* expresses various pharmacological effects both in animals and humans. Antioxidant enzymes such as superoxide dismutase (SOD) and Catalase (CAT) play a key role in the removal of reactive oxygen species produced in microalgae during various physical-chemical stress responses (Santos *et al*. 1999).

Our results show reduction in the antioxidant enzymes SOD and CAT activities in the *Chlorella pyrenoidosa* after exposed to each of the tested metals compared to their activities in the control. It is well known that reactive oxygen species such as superoxide (O_2^-), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) are produced in cells when exposed to environmental stress, e.g. exposure to high light intensities, UV radiation, metals, etc.(Li *et al.*2006). The SOD-CAT system provides the first defences against oxygen toxicity (Barata *et al*., 2005). SOD catalyses the dismutation of the superoxide anion radical to water and hydrogen peroxide, which is detoxified by the CAT activity. Usually, a simultaneous induction response in the activities of SOD and CAT is observed when exposed to pollutants (El-Bassat *et al.,* 2011). The low levels of CAT could be attributed to

high production of superoxide anion radical, which has been reported to inhibit CAT activity in case of excess production (Kumar *et al.*, 2014). An additional reason for the low antioxidant enzymes activities in the present study was the enzymes activities that measured by the end of the acute experiment, which means high stress on the *Chlorella pyrenoidosa* after exposure to high metals concentrations.It was observed that SOD and CAT responded to exogenous metal concentrations. Some studies have reported antioxidant enzyme responses including CAT and SOD to metals exposure (Alonzo *et al*., 2006).

In addition, the physiology itself may have an overall effect on the way in which the metal is accumulated in the cell. It is demonstrated that there are two phases in metal adsorption by microalgae: a first phase, not dependent on cellular metabolism, where metal binds to the cellular surface and the second, slower phase, dependent on metabolism, where metal is accumulated in the interior of the cell (Moreno-Garrido *et al.,* 2000). Table (2) shows the total amount of three metal element biosorbed by *Chlorella pyrenoidosa* as function of different metals concentrations in the medium after six days of exposure. The higher Cu^{2+}accumulated in the exposed *Chlorella pyrenoidosa*, could be due to induction of heavy metal peptides sequestration (phytochelatin) and detoxifying metals in vegetal cells. The ability of microalgae to accumulate metals from aqueous solution is well- documented (Fathi *et al.,* 2000). Algae take metals up both passively and actively.

Some metals, such Pb and Sr, may be passively adsorbed by charged polysaccharides in cell wall and intercellular matrix (El-Sheekh *et al.,* 2003 and Osman *et al.,* 2004). Other metals (Zn and Co) are taken up actively against large intracellular concentration gradients. Hamdy (2000) reported that metal uptake dependent on the type of biosorbant, with different accumulation affinities towards the tested elements and the amount of metal uptake increased steeply by increasing the weight of the biomass. Fathi *et al.* (2005) reported that the uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the water.

CONCLUSION

The inhibitory effects of copper, cobalt and lead on the growth of *Chlorella pyrenoidosa* depend on concentrations of metal and time of exposure. Biosynthesis of chlorophyll and carotenoid were also proved to be affected by the heavy metals. Also all the three metals affected negatively the total protein content at higher doses. This study indicates the *Chlorella pyrenoidosa* which is widely available can be used as biosorbent material for removal of heavy metals, which can accumulate high amounts of copper, cobalt and lead. Practical applications of such techniques at larger scales would be useful for bioremediation of heavy metal polluted wastewaters since there is a lack of industrial wastewater treatment.

Therefore, *Chlorella pyrenoidosa* and possibly other microalgae may have the potential to be used as an eco-friendly and economic biosorbent cheap material for the removal of toxic metals in polluted waters.

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تاثير المعادن الثقيلة (النحاس والكوبلت والرصاص) على نمو ومحتوى الصبغات في الطحلب الاخضر الكلوريللا بيرينويدوزا ح (تشيك)

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الملخص العربي

ان اضطراب النظم المائية الناتج عن وجود المعادن الثقيلة يسبب فقدان التنوع البيولوجي وكذلك يسبب مشاكل خطيرة في البيئة فضلا عن زيادة تراكم المواد السامة في السلسلة الغذائية وهذه المشاكل يمكن حلها جزئيا باستخدام النباتات والطحالب المائية لازالة ملوثات البيئة وقد اجريت التجارب باستخدام تركيزات مختلفة (٠.٠٥ و٠.٥ و١ و ٢ و٤ و٦ و٨ ملغ\لتر) من النحاس والكوبلت والرصاص على نمو ومحتويات الاصباغ والبروتين وبعض الانشطة الانزيمية كالكاتاليز وفوق اكسيد ديسميوتيز وقد اظهرت النتائج الى ان التركيزات الضعيفة من الكوبلت لها تاثيرات تنشيطية في زيادة نمو الطحلب في حين ان التركيزات العالية مثبطة لنمو الطحلب تبعا لنوع المعدن حيث وجد ان معدن النحاس اكثر المعادن تثبطا لنمو الكلوريللا عن غيره من المعادن المستخدمة. وتشير النتائج الى انخفاض واضح في محتوى البروتينات وكذلك الانزيمات المضادة للاكسدة. وقد اكدت التجارب تراكم كبيرلمعدن النحاس عن غيره من المعادن المستخدمة.