Effect of some heavy metals and nutrients upon the growth of green alga *Chlorella vulgaris*

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ABSTRACT

The present study contained manifestation of toxic effect of two heavy metals (Cadmium and Lead) separately (as single) and in a combination at different concentrations on the growth of green alga *Chlorella vulgaris* ,also studying effect of adding nutrients (Nitrogen and Phosphorus) in different concentrations on this toxicity. The study was carried out depending on the biomass represented by the total number of cells and then taking into account of the growth rate and doubling time .

The results showed the differences of the toxic effect of heavy metals (as separately) were significantly different according to the difference of type, concentration of metals and exposing period, and there were cases of antagonistic effect and others of synergistic effect when the alga was exposed to the alloy of both metals depending on used concentrations and exposing period.

The addition of nutrients (Nitrogen 1 and 3 mg/l) with cadmium (1 mg/l) and lead (50 mg/l) had a positive effect on a growth of studied alga and decreased the toxic effect of both metals .The results showed there was a significant increase in the growth rates by increasing the added phosphorus concentrations (0.1-- 0.5 mg/l) with cadmium (1 mg/l) and lead (50 mg/l) in compared with control group.

تضمنت الدراسة الحالية بيان سمية المعدنين الثقيلين الكادميوم والرصاص بصورة مفردة ومجتمعه وبتراكيز مختلفة على نمو الطحلب الأخضر *Chlorella vulgaris*، كذلك دراسة تأثير أضافة المغذيات (النيتروجين والفسفور) بتراكيز مختلفه ايضا على سمية هذين المعدنين ، وتمت الدراسة بالأعتماد على الكتلة الحيوية ممثلة بالعدد الكلي للخلايا ومنها تم حساب معدلات النمو وزمن التضاعف.

أظهرت النتائج أختلاف التأثير السمي للمعادن الثقيلة (منفردة) معنويا بأختلاف نوع المعدن ، تركيزه وفترة التعرض ، وظهرت حالات من التأثير التضادي وأخرى من التأثير التعاوني عند تعريض الطحلب لخليط من المعدنين أعتمادا على التراكيز المستخدمه وطول مدة التعرض.

وكان لأضافة المغذيات :النيتروجين(١ و ٣)ملغم/لتر مع الكادميوم (١) ملغم/لتر والرصاص (٥٠)ملغم/لتر أثر ايجابي في نمو الطحلب وفي تقليل سمية المعدنين، وبينت نتائج الدراسة حصول زيادة معنوية في معدلات النمو بزيادة تركيز الفسفور المضاف (٠.١ ــ ٠.٥)ملغم/لتر بوجود الكادميوم (١)ملغم/لتر والرصاص (٥٠) ملغم/لتر بالمقارنه مع معاملة السيطرة.

INTRODUCTION

Heavy metals are considered from the essential elements for growth and development of organisms including human, some of them enter in the structure and activity of enzymes as activators (Al-Saadi *et al*.,1995), in the same time, when the human expose to acute or high concentrations more than what he needs, it will be toxic.

There are more physiological and cytological changes coupled with the toxic exposure to the heavy metals including the changes in structure of cellular membrane and the osmosis swelling and rending of organelles as plastids and mitochondria ; inhibition of important enzymes; competition with important elements, or decrease the surface area of thylakoids (Horcsik *et al*.,2006) ; and more researchers found the heavy metals may cause inhibition of the photosynthesis and growth of phytoplankton, the mechanism of heavy metals effect on algae may be summarized as : a/detriment which was produced by obstruction of active groups sites or change of enzymes shape ,genetic material and transport systems,and then change them to inactive shape. b/ detriment which was produced by rending of cell or organelles membrane (Nakanishi *et al*., 2004).

The researchers were studied the effect of selected heavy metals on the growth of some of algae, Lamas *et al*., (1996) referred to the toxic effect of Cadmium on the three strains of *Euglena gracilis* , and showed the (1.2) mg/l of Cd cause inhibition of cell growth rate by (5%) and cause more changes in structure of membrane of chloroplasts and mitochondria ; also Delmotte, (1980) mentioned when the *Anabaena cylindrical* alga was exposed to (2) mg/l of Cd that affected on the nitrogen fixation process; also there was a significant increase in the growth of *Oscillatoria omaena* where it exposed to different low concentrations of lead but the growth decreased when increasing the concentrations more than (3)mg/l (Al-Hajaj,1997); the photosynthesis was fully inhibited when the *Macrocystis pyrifera* exposed to (9.1)mg/l lead (Stewart,1976); also the growth of *Chlorella* Sp.was inhibited when it cultured in media supported with lead ions (10)mg/l (Al-Aarajy *et al*.1992); and the addition of iron to media in concentration (0.11) mg/l was produced reduction in photosynthesis pigments and unsaturated fatty acids for *Microcystis aeroginosa* alga, and the high concentrations of iron (3 and 4)mg/l caused total loss of pigments and reduction all important fatty acids during three hours (Walsh *et al*.,1998); also Al-Hayalee (2001) noticed there was a clear effect of heavy metals on the growth of *Microcystis aeroginosa* especially the high concentrations and the effect of a level was depended on the concentrations and exposing period .

The interpenetration (in combination) among heavy metals may affect on their toxicity by increasing or decreasing their toxicity ,for example the toxicity of vanadium was reduced by chromium, and the toxicity of cadmium was decreased by zinc and selenium ,also the toxicity of mercury, silver and cupper reduced by selenium effect ;also toxicity of lead is reduced by iron effect(Ellwood and Hunter,2000).Generally,the effect of interpenetration among chemicals including

heavy metals were classified into: Synergistic ;Additive and Antagonistic effect (Sunda and Huntsman,2000).

The hydrogen ion and other cations and anions which resulted from analysis of heavy metals salts may increase or decrease the toxicity of metals for aquatic organisms like algae (Macfie *et al*., 1994); whereas Haritonidis *et al*., (1994) noticed when the *Enteromorpha prolifera* alga was exposed to (0.45) mg Cd/l and was added some concentrations of iron ,lead ,zinc and cupper there is a weak synergistic effect between lead and cadmium from side and between cupper and cadmium on the other hand ,and there is a good synergistic effect between cadmium and iron , also in the same time there is a antagonistic effect between cadmium and zinc.

The toxicity of heavy metals may be reduced by adding some organic legends or chelating agents into the media , and may be increased too by adding some ions by destruction metals complexes; by ultraviolet radiation or by analytical microorganisms (Rijstenbile and Poortvliet,1992; Masoud *et al*., 2006)).

Stauber and Florence,(1989) noticed the effect of Pb and Cu on the cell diffusion rate of *Chlorella* may be differ by changing of used media composition, also noticed the toxic effect of theses elements were decreased when they used media supported with more phosphate and nitrate as compared with other media which was poor with nutrients. Other researchers were noticed the toxicity of some of heavy metals like mercury which was effected on the protein and chlorophyll content of *Chlorella vulgaris* these toxicity may be affected clearly by finding some of amino acids like Alanine,Cystine, Histidine,Aspartic acid and Glutamine in media, that's may be due to the ability of these amino acids to formation some of bonds or legends with mercury ions and these amino acids may be support the algae by energy, also the toxicity of Cadmium was effective by changing of pH value of media (Mohapartra *et al*.,1999).

The present study aimed to determine the effect or toxicity of Cadmium and Lead as a single , and synergistic and antagonistic effect of these metals (as coupled) on the growth of *Chlorella vulgaris* alga. Also the present study include detect the effect of adding two essential nutrients (nitrogen and phosphorus) on the toxicity of selected metals.

METERIALS AND METHODS

Pure isolations of *Chlorella vulgaris* alga were obtained from water samples from marshes region in Thi-Qar governorate (southern of Iraq), by using isolation and purification methods (Streaking method) which were described by (Stein ,1973) Beijerinek media as described in (Stein,1973) was used as a culture for growth in experiments, the purity of isolations was tested as (Patterson,1983), and cultures were incubated at (27 ±2)°c and light density (250) $\mu E/m^2$ /sec. .

Standard solutions of heavy metals were prepared as (1000)mg/l for cadmium and lead by dissolving pure salts for each $CdCl_2.2.5H_2O$ and $Pb(NO_3)_2$ respectively, in deionized distilled water , and the studied concentrations were prepared by

diluting method, as the following concentrations were prepared as: (0.1 , 0.5 , 1 , 2 , and 3) mg Cd/l and (30 , 40 , 50 , 75 and 100) mg Pb/l, for studying the effect of the interpenetration (in combination) between the metals (synergistic or antagonistic effect) the metals were incorporated in the following concentrations (30 Pb+0.1 Cd), (40 Pb+0.5 Cd) and (50 Pb+1 Cd) mg/l. And the studying of the effect of nutrients addition to the media on the toxicity of metals ,the (1)mg Cd/l and (50)mg Pb/l concentrations were used because these concentrations cause inhibition ratio more than (50%) ,and nutrients were added in the following concentrations (0.1 , 0.15 , 0.3 and 0.5) mg p/l for phosphorus and (1 , 3 , 5 and 10) mg N/l for nitrogen .

Standard curve of the studied alga was detected by use (10) ml as inoculums which contain (2 -5) $X10^6$ cell /ml placed in (1) L. from used media, the growth were monitored in a control and experimental samples by calculating the cells number by using Haemocytometer slide and the calculating of growth rate; doubling time and inhibition rate for treatments as a percentage for response according to equations which described in (Nyholm,1985), and active medium concentration according to (Matsumura,1975).

RESULTS

1-Effect of different concentrations of Lead and Cadmium (as a single) on the growth Of alga

The results showed(Table 1&2) there was a clear effect for each metal on the growth of alga when it was exposed to different concentrations of lead and cadmium as a single, there was a clear significant decrease in the growth rate of alga with increases of concentrations of each metal.

The results showed a significant differences ($p<0.05$) between treatments for each concentration of both metals, so there was a negative correlation coefficient showed between the increase of used concentrations and the growth rate of lead ($r= -0.99$) and cadmium ($r= -0.97$) ,{$p<0.05$; $n=20$}, and there was a positive correlation coefficient between the increase of concentrations and the doubling time ($r= 0.98$) and ($r=0.90$) respectively .

The use of different concentrations of each metal as a single cause a significant reduction in the cell numbers in compared with control group, and the reduction rate was increased gradually with the increase of metal concentrations, so that, there was an acute reduction in cell numbers when the following concentrations were used : (75 and 100)mg/l lead (Table 1), and (2 and 3)mg/l cadmium (Table 2).

2-Effect of both metals (as in combination) on the growth rate of alga.

The results (Table 2) showed the addition of both metals (in combination) have a antagonism and synergism effect of metals on the growth of studied alga depending on the exposing time, in that same time the lead has a antagonism effect during the first three days from exposing , the incorporate of lead with cadmium causes decrease of lead effect in comparison with lead effect as single (as alone) ,the effect

of both metals were synergistic during the first four days of experiment. Cadmium as single, its effect on alga was antagonistic only during the beginning of the experiment and synergistic during the last seven days, whereas the growth rate was significantly decreased in the metals coupled treatments , and the doubling time was increased in the incorporate treatments as compared with the single metals treatments(Table 2).

3- Effect of nutrients addition on the metals toxicity

A- Effect of Nitrogen addition

The addition of nitrogen in different concentrations (1 -10) mg/l with both metals as single ,cadmium (1 mg/l) and lead (50 mg/l) (Table 3) causes an improvement on the alga growth by increase the total cell number especially with nitrogen concentrations (1 , 3) mg nitrogen/l more in comparison with other added nitrogen concentrations added(5 , 10)mg/l, the highest cell numbers were recorded when add (3)mg N/l (9.1 and 10.2) cel1 x 10^6/ml after (120)hrs for Cd and Pb respectively, but the lowest cell numbers were recorded when adding nitrogen concentration (10) mg N/l were (5.1 and 4.4) after (72) hrs. for both metals respectively, the growth rate increase with the decrease of the doubling time for all treatments in comparison with control treatment, and the highest growth rate were (4.493 and 4.879) cell/hr and the lowest doubling time were(3.702 and 3.409) hr. for both metals respectively were recorded with addition of nitrogen concentration (3) mg/l; in the same time, the lowest growth rate and the highest doubling time were recorded with the addition of nitrogen concentration (10)mg/l (Table 3).

The statistical analysis results showed there are a significant differences ($p<0.05$) between these treatments as compared with the control treatment , the results showed a weak negative relation between the increase of nitrogen concentrations with the growth rate ($r= -0.10$) for both metals, and a weak positive relation with doubling time ($r=0.21$; $r=0.09$) for Cd and Pb respectively ($p<0.05$; $n= 20$).

B- Effect of phosphorus addition

The addition of phosphorus in different concentrations (0.1 – 0.5)mg p/l with both metals concentrations (as above in A) as single (Table 4) causes increase the cell numbers with the increase of added phosphorus concentration, the highest cell numbers were recorded with addition of phosphorus (0.5) mg p/l was (10.5X 10^6) cell/ml and (11.0 X10^6)cell/ml with Cd and Pb respectively , the lowest cell number was recorded when the addition of phosphorus concentration (0.1)mg p/l were (5.0X10^6) cell/ml and (7.9X10^6) cell/ml with Cd and Pb metals respectively , that's indicate ,the increase of phosphorus concentration causes decreasing the effect of both metals,the results of statistical analysis showed there are a significant differences ($p<0.05$) between treatments (0.15 – 0.5) mg p/l.

Also the concentration (0.5)mg p/l was recorded the highest growth rate was (4.699) and (5.224) cell/hr. with Cd and Pb respectively ,the same concentration was recorded the lowest doubling time were (3.540) and (3.184) hr. respectively too;

There was a positive relation between increase of used concentrations of phosphorus with growth rates (r=0.99) for both metals, and negative relation with doubling time (r= – 0.98) for both metals also ($p<0.05$; n= 20).

Table (1): Effect of different concentrations of Lead on the growth of *Chlorella vulgaris* alga.

Lead conc. [mg/l]	Cell No. [cell x 10^6/ml] after:		Growth rate(μ) [cell/hr.]		Doubling time (G) [hr.]	
	72 hrs.	120 hrs.				
00	8.0	14.2	5.998^a	±0.003	2.774^a	±0.02
30	6.1	9	4.439^b	±0.003	3.747^b	±0.08
40	4.8	8.1	4.065^b	±0.003	4.092^b	±0.04
50	4.6	6.5	3.342^c	±0.002	4.977^c	±0.07
75	3.1	5	2.442^d	±0.002	6.812^d	±0.12
100	3.1	4.1	1.860^e	±0.004	8.914^e	±0.61

Similar litters in the same column means no significant differences ($p<0.05$)
LSD for growth rate (μ) = 0.191 at ($p<0.05$)
LSD for doubling time (G) = 0.494 at ($p<0.05$)

Table (2): Effect of different concentrations of Cadmium on the growth of *Chlorella vulgaris* alga, and the effect of interpenetration (in combination)both metals.

Cadmium conc. [mg/l]	Cell No. [cell x 10^6/ml]after:		Growth rate(μ) [cell/hr.]		Doubling time (G) [hr.]	
	72 hrs.	120 hrs.				
00	8.0	14.2	5.998^a	±0.003	2.774^a	±0.02
0.1	7	12	5.220^b	±0.004	$3.186^{a\,b}$	±0.06
0.5	6.2	8.2	4.088^c	±0.002	4.069^b	±0.04
1.0	6	6.5	3.111^d	±0.003	5.347^c	±0.11
2.0	3.5	4	1.207^e	±0.002	13.78^d	±0.45
3.0	3.1	2.9	0.329^f	±0.006	50.56^e	±0.27
0.1 Cd+ 30 Pb	4.1	7.3	4.100	±0.001	4.057	±0.03
0.5 Cd+ 40 Pb	2.9	6	3.509	±0.007	4.740	±0.07
1 Cd+ 50 Pb	1	4.1	2.797	±0.004	5.947	±0.19

Similar litters in the same column means no significant differences ($p<0.05$)
LSD for growth rate (μ) = 0.006 at ($p<0.05$)
LSD for doubling time (G) = 0.959 at ($p<0.05$)

Table (3):Effect of Lead (50 mg/l) or Cadmium (1 mg/l) on the growth of *Chlorella vulgaris* alga with finding different concentrations of Nitrogen.

Conc.of Nitrogen (mg/l)	Cell No. * [cel1 x 10^6/ml]after :				Growth rate(μ) [cell/hr.]		Doubling time (G) [hr.]		Inhibition rate (%)					
	Pb		Cd		Pb	Cd	Pb	Cd	Pb (50 mg/l)			Cd (1 mg/l)		
	72 hrs.	120 hrs.	72 hrs.	120 hrs.					24 hrs.	72 hrs.	120 hrs.	24 hrs.	72 hrs.	120 hrs.
0.12** (control)	4.6	7.2	5.8	6.0	3.551^{a} ±0.002	3.139^{a} ±0.003	4.684^{a} ±0.07	5.299^{a} ±0.04	28	41	49	21	32	53
1	6.6	10.0	6.0	5.5	4.798^{b} ±0.005	4.256^{b} ±0.004	3.467^{b} ±0.03	3.908^{b} ±0.08	3	21	31	19	28	41
3	6.9	10.2	6.3	9.1	4.879^{b} ±0.002	4.493^{c} ±0.002	3.409^{b} ±0.08	3.702^{c} ±0.04	6	16	30	18	24	37
5	5.1	8.8	5.8	7.1	4.390^{c} ±0.006	3.766^{d} ±0.004	3.789^{c} ±0.12	4.417^{d} ±0.09	16	33	40	21	31	47
10	4.4	7.5	5.1	6.2	3.919^{d} ±0.002	3.561^{e} ±0.002	4.254^{d} ±0.06	4.671^{e} ±0.05	19	41	46	24	37	51

*Cell numbers in the start of the experiment (Time=0) is (2.5 x 10^6 /ml)

**A comparison with nitrogen with its normal concentration in media (0.12 mg/l)

Similar litters in the same column means no significant differences at ($p<0.05$)

LSD for growth rate (μ) :with cadmium (0.042) ; lead (0.054) at ($p<0.05$)

LSD for doubling time (G) :with cadmium (0.179) ; lead (0.132) at ($p<0.05$)

Table (4):Effect of Lead (50 mg/l) and Cadmium (1 mg/l) on the growth of *Chlorella vulgaris* alga with finding different concentrations of Phosphorus.

Conc.of Phosphorus (mg/l)	Cell No. * [cell x 10^6/ml]after :				Growth rate(μ) [cell/hr.]		Doubling time (G) [hr.]		Inhibition rate (%)					
	Pb		Cd						Pb (50 mg/l)			Cd (1 mg/l)		
	72 hrs.	120 hrs.	72 hrs.	120 hrs.	Pb	Cd	Pb	Cd	24 hrs.	72 hrs.	120 hrs.	24 hrs.	72 hrs.	120 hrs.
0.02** Control	4.9	7.2	6.0	6.8	3.532^{a} ±0.002	3.160^{a} ±0.003	4.709^{a} ±0.077	5.264^{a} ±0.047	28	41	49	21	32	53
0.1	5.0	7.9	6.0	6.9	4.009^{b} ±0.004	3.263^{a} ±0.003	4.049^{b} ±0.098	5.098^{a} ±0.122	25	39	45	18	31	52
0.15	5.9	8.4	6.1	7.1	4.183^{c} ±0.004	3.586^{b} ±0.003	3.976^{c} ±0.090	4.638^{b} ±0.107	22	32	41	15	30	49
0.3	6.2	9.6	6.3	9.1	4.686^{d} ±0.007	4.188^{c} ±0.010	3.540^{d} ±0.124	3.972^{c} ±0.051	13	24	34	12	26	38
0.5	7.0	11.0	6.5	10.5	5.224^{e} ±0.003	4.699^{d} ±0.003	3.184^{e} ±0.071	3.540^{d} ±0.049	3	17	25	5	21	33

*Cell number in the beginning of the experiment (Time=0) is (2.5 x 10^6 /ml)
**A comparison with phosphorus with its normal concentration in media (0.02 mg/l)
Similar litters in the same column means no significant differences at ($p<0.05$)
LSD for growth rate (μ) :with cadmium (0.077) ; lead (0.008) at ($p<0.05$)
LSD for doubling time (G) :with cadmium (0.899) ; lead (0.140) at ($p<0.05$)

Table (5): Cadmium toxicity and active medium concentration (Ec_{50}) for *Chlorella vulgaris* alga with the use of different concentrations of cadmium in various times. *Response (R) (%). **Probability unit (P.U.)

Cadmium		Time (hour)									
		24		48		72		96		120	
Conc. (mg/l)	Log of conc.	R*	** P.U.	R.	P.U.	R.	P.U.	R.	P.U.	R.	P.U.
0.0	---	00	---	00	---	00	---	00	---	00	---
0.1	– 1	2	2.931	8	3.589	13	3.880	17	4.051	21	4.188
0.5	– 0.3	8	3.589	16	4.010	22	4.230	31	4.499	43	4.815
1	00	21	4.196	29	4.451	32	4.541	43	4.833	53	5.080
2	0.3	35	4.620	50	5.010	56	5.161	60	5.266	68	5.471
3	0.47	41	4.769	57	5.180	58	5.210	70	5.515	76	5.698
EC_{50} (mg/l)		4.44		2.2		1.80		1.75		0.9	

Table (6): Lead toxicity and active medium concentration (Ec_{50}) for *Chlorella vulgaris* alga with the use of different concentrations of lead in various times. *Response (R) (%). **Probability unit (P.U.)

Lead		Time (hour)									
		24		48		72		96		120	
Conc. (mg/l)	Log of conc.	R.*	** P.U.	R.	P.U.	R.	P.U.	R.	P.U.	R.	P.U.
0.0	---	00	---	00	---	00	---	00	---	00	---
30	1.447	13	3.869	19	4.130	22	4.230	29	4.450	32	4.529
40	1.60	20	4.161	30	4.480	35	4.621	39	4.719	41	4.768
50	1.69	28	4.420	39	4.722	41	4.769	46	4.901	49	4.981
75	1.87	40	4.750	48	4.948	54	5.110	58	5.200	61	5.282
100	2	50	5.003	56	5.160	56	5.329	63	5.329	67	5.462
EC_{50} (mg/l)		98		79		72		60		53	

DISCUSSION

Algae and aquatic plants were used as bioindicators in wide range for water quality(Nakanishi *et al*., 2004), and the heavy metals have a clear effect on the water quality, in the present study Cadmium and Lead were used in the study of effect of water toxicity (by these metals) on the algae growth in view of increase uses of these metals in the industry; also the study included using nitrogen and phosphorus to study the effect of these nutrients on the metals toxicity because of these nutrients were considered a limiting factors to the algae growth and because these nutrients were found in large amounts in polluted aquatic ecosystems by sewage and industrial wastes.

At this study, was noticed decrease of cell numbers and growth rate of *Chlorella vulgaris* alga when adding both Cd and Pb as a single (as alone) in different concentrations, that's may be due to the effect of the metals especially Cd on the photosynthesis (Horcsik *et al*.,2006);respiratory and metabolism process, or to the reduction of number and size of polyhydral bodies, also the toxicity of Cd causes changes in cellular membranes permeability and causes disorders of ionic balance (Rai *et al*.,1990),.The reduction of growth rate may be due to the interaction between the ions of the metal with essential elements for growth on the attachment sites or these ions attached with active sites of important enzymes, also the Cd works on inhibit the entering of some elements like Fe and causes decrease of growth (Sunda and Huntsman,2000), also Rai *et al*.,(1990) noticed that effect on growth of *Anabaena flos-aquae* when used (0.13) mg/l concentration , also present results agree with other researcher results (Rachlin *et al*., 1984 ; Barque *et al*.,1995; Al-Hajaj,1997)

The toxicity of lead which was noticed in this study may be due to the high tendency of lead for conjugation by ⎯SH groups for active molecules and inhibit its work , also may be attached with phosphate groups of nucleic acids which causes genetic mutations,or may be due to the ability of lead to affect on the photosynthesis by the change of surface area of thylakoids in chloroplasts (Jackson *et al*.,1991)

The results was indicated to the negative relation between increase of Pb concentrations with growth rates ,that was with accordance authers (Al-Aarajy *et al*.,1992 ;Bajguza,2000).Also,the present study showed a direct relation between increase of Pb concentrations with doubling time,that agreed with (Kassim *et al*., 2000).

Experiments of the effect of incorporation of both metals, the study was showed an antagonism effect for both metals was found in compare with the addition of each metal as alone ,that's agree with (Kassim *et al*., 2000; Al-Hayali 2001), this effect may be due to the antagonism effect between lead and cadmium ions, then both metals showed a synergistic effect when they combine for longer period because increase of toxicity of these metals during exposing period, that was also noticed by (Haritonidis *et al*., 1994) which showed there was a weak antagonism relation between Pb and Cd ions in growth of *Enteromorpha* alga.

For the study of the effect of interpenetrate between metals and essential nutrients (Nitrogen and Phosphorus) ,the study showed, the addition of N and P in different concentrations causes decrease the toxicity effect of used heavy metals and causes a significant increase in growth rates in compared with the control, that's may be explained on the basis of the nutrients like N and P work on reduction of the toxicity of heavy metals by the effect of (N and P) on the mechanism of metals absorption by algae or by making a physiological changes in algal cells , or as indirect effect by consider the Nitrogen as an important compound for constructing the amino acid and proteins which are considered as phytochelating for hunting (as chelating agents) the heavy metals ions and form a metals complex (Ahner and Morel,1995).

Also for phosphorus especially the excess from uses converts to polyphosphate bodies which were considered as one of the important mechanisms in resistant of toxicity effects of heavy metals because of the ability of these bodies to restrict the metal ions and exclude its effect away from active sites in the cells (Jensen and Corre, 1993) ; also may be due to the type of competition or mass antagonism between the metal ions on the entrance sites by the untoxic ions replace the other ions which are more toxic and has a similar charge and similar in chemical formula (Al-Sukkari *et al*.,1988). And may be due to the occurrence of anions or phosphorus in growth media may be work on sedimentation of heavy metals in the media which causes unavailable theses metals for algae , these results were agree with (Bajguza, 2000).

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