Antagonism or Synergism effect of heavy metals (Cadmium and Lead)upon growth of green alga *Chlorella vulgaris*

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ABSTRACT

The antagonism or synergism effect of some heavy metals (both Cadmium and Lead) in different concentrations upon growth of green alga *Chlorella vulgaris* were studied, firstly, the effect of each metal alone was carried out by adding the cadmium metal (0.1 , 0.5, 1, 2, 3) mg/L to culture, the concentration (3 mg/L) was more toxicity for alga, and the lowest chlorophyll content was in second day (1.9) µg/100 ml in comparison with control(2.4 µg/100 ml). For the Lead ,was added as(30 ,40, 50, 75 and 100) mg/L, and the concentration (100 mg/L)was more toxicity for alga and the chlorophyll content reduced to (1.6) µg/100 ml in comparison with control.

In the other experiments , metal ions were mixed and added together to the culture media as concentrations (30 Pb + 0.1 Cd ; 40 Pb + 0.5 Cd and 50 Pb + 1 Cd) mg / l , the concentration (30 Pb + 0.1 Cd) was the lowest toxicity, and recorded the maximum of dry weight which reached to(63.3) mg/gm in *C. vulgaris* and chlorophyll content (11.5 µg/100 ml) . Some cases of antagonism effect were happened depending on used concentration and exposure period , and the growth rates were increased , while the doubling time were decreased.

INTRODUCTION

The pollution has clear effect on organisms in general and human especially in present decades, so, there is increased importance in the study of causes and effects of pollution on organisms and try to discard from its negative effects. The pollution of aquatic ecosystem by heavy metals is consider most important mode of pollution and most dangerous on organisms, and the artificial wastes formed the major sources of pollution.

the Algae are one of important components of aquatic ecosystem because they composed the base of food web, the (algae) expose to effects of pollution such as by heavy metals which consider the start of effect on most important component of aquatic ecosystem which is food web (especially if the algae expose to mix of heavy metals), the effect of heavy metals on algae represented in different modes like: morphological, physiological and biochemical content changes, here are many studies interested with study of heavy metals effects and its existence of in aquatic ecosystem and its effect on diversity and numbers of algae [1;2;3]

And other experimental studies were carried out to study the aggregated effects of heavy metals [4], and the local studies include the effect of ions on biology of some algae like [5;6;7].

The cadmium consider the most importance in it toxicity [8], so, there are many studies refer to it effects on algae(cytological effect and its effect on growth, photosynthesis process and pigments contents [9 ; 10].

And the toxic exposure of lead has effect on genetic structure for animals and plants communities [11], and the lead is cause of decrease the surface area of thylakoids in plastids 12 ; 13] and also inhibit the growth.

Lead has high intimacy for engagement with –SH group in active molecules and inhibit its, and lead engagement with phosphate group which found within chemical structure of genetic substance [11 ; 6].

Some of aquatic organisms like algae have the ability to increase the concentrations and accumulation of heavy metals in its bodies more than its concentrations in surrounding medium [14], the accumulated amounts of heavy metals depended on some factors like species, physiological state, mechanism of toxicity resistance for these metals, type and concentration of metals in medium, time of exposure and effect of salinity ,pH and organic materials [15] and Al-Hajaj *et al.*, [16] notice the ability of *Chlorella vulgaris* on accumulation of heavy metals ions like Pb,Zn,Cu and Cd.

Many researchers used algae as bioindicators for aquatic ecosystem pollution by heavy metals because its ability to accumulate the metals in it bodies[17 ; 18].

Present study carried out to explain the …..

1-effects of two dangerous heavy metals are: cadmium and lead on growth of green alga *Chlorella vulgaris* (as chlorophyll content) when its added to medium as single (each metal is alone)in different concentrations .

2- effect of these metals on growth of studied alga when its added to medium together.

MATERIALS AND METHODS

Samples collected from Al-Garaf river in Al-Nassiria city, southern of iraq.Chu-10 medium used for isolation ,purification and the experimental studies, unialgal cultures were obtained by use of streaking method [19], and the cultures were purified according to [20 ; 21] which described in [22].

Standard solutions of Cd and Pb were prepared (1000 mg/l which mean 1 mg/ml) by dissolve the pure salts of Cd $(CH_3COO)_2$ and Pb$(CH_3COO)_2$ in deionized water and the experimental concentrations were prepared by adding appropriate volumes (ml)from standard solution to the media to prepare the following concentrations (0.1 , 0.5 , 1 , 2 and 3)mg/l for Cd and (30 , 40 , 50 , 75 and 100)mg/l for Pb; each concentration was added as single to the medium which contain an inoculums (10 ml) from stock culture in the start of experiment(time = 0) in the first part of study; and its added (together) in the second part of study as the following concentrations(1 mg Cd/l +50 mg Pb/l) ; (0.5 mg Cd/l+40 mg Pb/l) and (0.1 mg Cd/l+30 mg Pb/l); all additions were in three replicates, and the growth measurements were each 24 hrs for seven days (mid of stationary phase according to results of growth curve) . For growth measurements were followed the standard methods which described in [23 ; 22] by determination of chlorophyll content was according to Lorenzen equation [24]. And the growth rate was indicated by growth constant (K) and doubling time according to [25], The concentrations of heavy metals determined by Flam Atomic Absorption Spectrophotometer (Shimadzu (AA-670) with standard solutions. The results were statistically analyzed by analysis of variance (ANOVA).

RESULTS

(1)Effect of heavy metals (as single)

(A)Effect of Cadmium

Results of exposure the algae to different concentrations of Cd showed a significant decrease in chlorophyll content of green alga *Chlorella vulgaris* for treatments in comparison with control treatment (Fig.1).

The growth during exposure time which extended to 168hrs. (7 days) was gradually decreased as chlorophyll content with increase of concentrations (0.1 , 0.5 , 1)mg/l; while when use the concentrations (2 ; 3)mg/l the decreases was acute in chlorophyll content, and the lag phase extend for more than 48 hrs. in comparison with control group (24)hrs. (Fig.1).

The growth rate was decreased and increase of doubling time with increase of Cd concentrations (Table 1).the statistical analysis results showed there are a significant differences ($p<0.05$) between treatments, the correlation coefficient refer to a negative relationship between the used concentrations of Cd and growth constant [$r= -0.41$; $p<0.05$; $n=18$] and positive relation between increase of concentrations and doubling time [$r=0.55$;$p<0.05$; $n=18$].

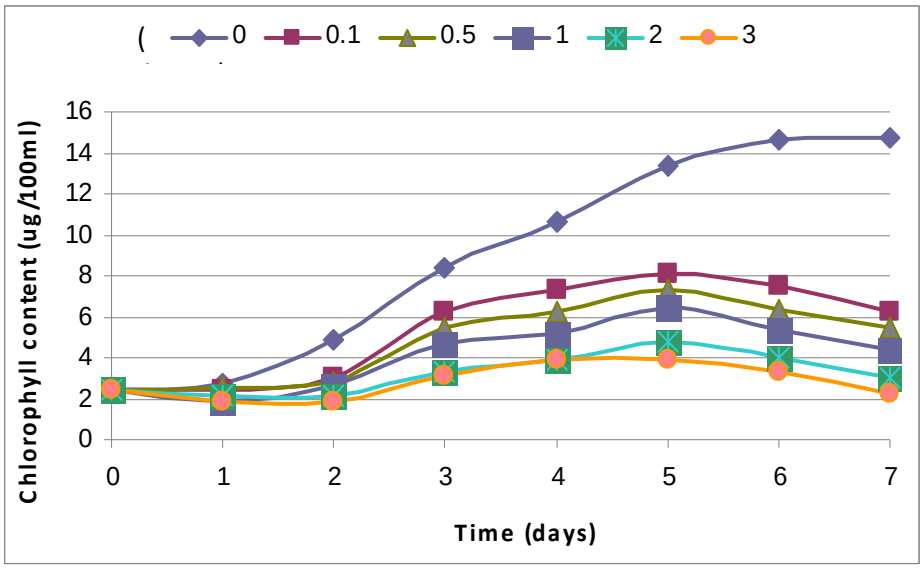

Figure 1: Variations in chlorophyll content of *Chlorella vulgaris* alga exposed to different concentrations of Cadmium

Table (1):Growth constant and doubling time for *Chlorella vulgaris* alga when it exposed to different concentrations of cadmium

Cadmium concentration (mg/L)	Growth constant (K)	Doubling time (day)
0	0.0056^{a} ±0.001	053.750^{f} ±2.1
0.1	0.0028^{a} ±0.0001	107.500^{e} ±4.2
0.5	0.0027^{a} ±0.0001	111.481^{d} ±5.1
1	0.0025^{a} ±0.0001	120.4^{c} ±4.7
2	0.0008^{a} ±0.0001	376.25^{b} ±6.2
3	0.0008^{a} ±------	3010^{a} ±8.7

(B)Effect of Lead

Results of effect of different concentrations of lead showed a decrease of chlorophyll content with increase of added concentrations in gradually during the exposure period (Fig.2) ;the lag phase extend to more than three days in comparison with control group (2 days), the death phase started in the 7^{th} day while in the control group was after the 8^{th} day.

The growth constant decreased with increase of concentrations while doubling time was increased with increase of concentrations and reach the highest value (602) in 100 mg Pb/l concentration (Table 2).

The statistical analysis showed a significant differences ($p<0.05$) between treatments , and a negative correlation coefficient between increase of concentrations and growth constant [r= – 0.77 ; $p<0.05$; n=18], and other positive relationship between increase of concentrations and doubling time [r= 0.63 ; $p<0.05$; n=18].

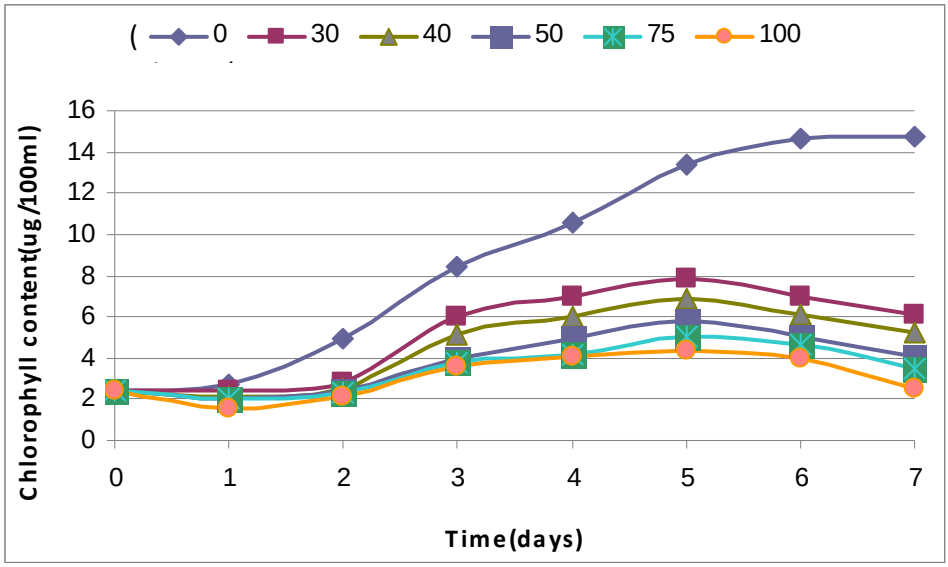

Figure 2: **Variations in chlorophyll content of *Chlorella vulgaris* alga exposed to different concentrations of Lead**

Table (2):Growth constant and doubling time for *Chlorella vulgaris* alga when it exposed to different concentrations of lead

Lead concentration (mg/L)	Growth constant (K)	Doubling time (day)
0	$0.0060^{a} \pm 0.001$	$50.166^{f} \pm 2.2$
30	$0.0034^{a} \pm 0.001$	$88.529^{e} \pm 2.5$
40	$0.0033^{a} \pm 0.001$	$91.212^{d} \pm 3.1$
50	$0.0024^{a} \pm 0.0001$	$125.416^{c} \pm 4.4$
75	$0.0012^{a} \pm 0.0001$	$250.833^{b} \pm 4.6$
100	$0.0005^{a} \pm 0.0001$	$602.000^{a} \pm 7.2$

(2) Combined effect of addition of heavy metals together

The results of effect of addition of heavy metals together mentioned to the best results were (the closer to control group) when use the concentration (Pb 30 + Cd 0.1) as chlorophyll content ,and each Cd and Pb were manifested the antagonism effect during

exposure period (168 hrs),this effect was reflect on growth rates which increased and the doubling time was decreased (Fig.3)(Table 3).

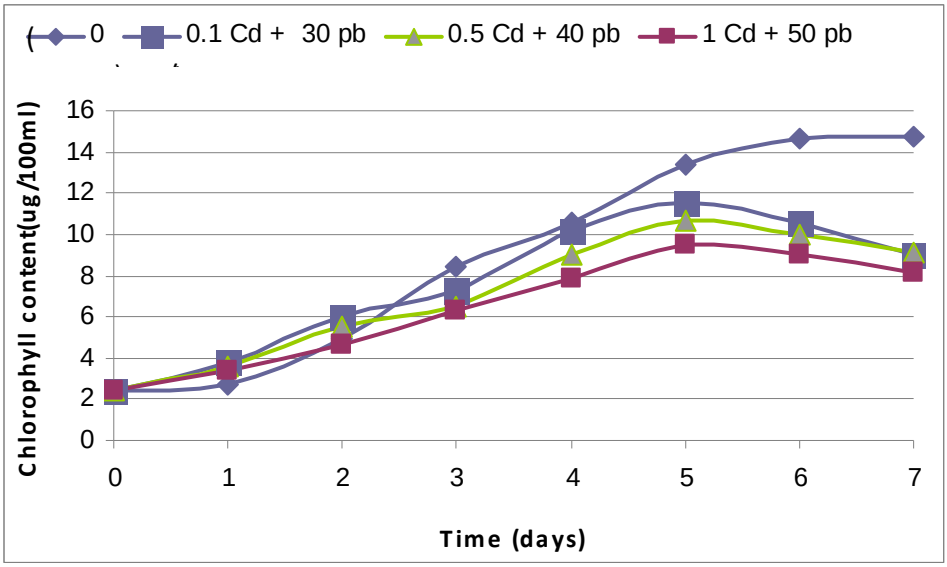

Figure 3: **Variations in chlorophyll content of *Chlorella vulgaris* alga exposed to different concentrations of Cadmium and Lead (together)**

Table (3):Growth constant and doubling time for *Chlorella vulgaris* alga when it exposed to different concentrations of lead +Cadmium (together)

Lead +Cadmium concentrations (mg/L)	Growth constant (K)	Doubling time (day)
0	$0.0059^{a} \pm 0.001$	$51.016^{c} \pm 3.5$
1 Cd + 50 pb	$0.0053^{a} \pm 0.001$	$56.792^{a} \pm 3.6$
0.5 Cd + 40 pb	$0.0056^{a} \pm 0.002$	$53.750^{b} \pm 2.7$
0.1 Cd + 30 pb	$0.0061^{a} \pm 0.001$	$49.344^{d} \pm 2.1$

(3)Accumulation of heavy metals by alga

Results (Table 4) showed ability of *Chlorella vulgaris* alga on increase of ions accumulation during exposure period, and decrease of accumulated concentrations of Cd with increase of exposure period.

The highest value of accumulation was recorded when use (1) mg Cd/ concentration which reached to (0.664) mg/gm, and the lowest value when use (3)mg Cd/l was (0.067)mg/gm, the statistical analysis showed a significant differences between treatments (Table 4).Also when the used alga exposure to different concentrations of Lead, the accumulated amounts of Pb was decreased with increase of exposure period too, the highest accumulated amount of lead was recorded when use (50) mg Pb/l was (6.726)mg/gm ,and the lowest amount when use (100)mg Pb/l (Table 5).

Table(4):Cadmium accumulation rate(mg/gm Dry weight) for *Chlorella vulgaris* alga when it exposed to different concentrations of cadmium

Cadmium concentration (mg/L)	Cadmium accumulation rate(mg/gm Dry weight)		
	24 hrs.	96 hrs.	168 hrs.
0.1	$0.627^{a} \pm 0.2$	$0.312^{b} \pm 0.01$	$0.171^{a} \pm 0.001$
0.5	$0.623^{a} \pm 0.2$	$0.445^{a} \pm 0.01$	$0.200^{b} \pm 0.001$
1	$0.664^{a} \pm 0.3$	$0.472^{a} \pm 0.04$	$0.212^{b} \pm 0.01$
2	$0.311^{b} \pm 0.01$	$0.222^{c} \pm 0.01$	$0.168^{a} \pm 0.001$
3	$0.121^{c} \pm 0.001$	$0.102^{d} \pm 0.001$	$0.067^{c} \pm 0.001$

DISCUSSION

(1)Effect of heavy metals (as single)

Heavy metals enters to algae bodies through the cell walls and plasma membranes and to settle inside cells , if its concentrations more than normal values in habitats its causes unfavorable changes in cells [26].

Results of present study showed decrease of chlorophyll content when addition of Cd in different concentrations into medium, and that's was more clear after 5 days (Fig.1), and the reduction continues with time, that's may be due to Cd effect on respiratory organelles for alga because it effect on structure of phospholipids of plastids and mitochondria membranes, that's consider as the main effect of Cd toxicity [10] . In addition to that the Cd has ability to replacement of iron which linked to photosynthesis cytochromes which caused negative effect on photosynthesis process [27].

In present study the *Chlorella vulgaris* alga when exposure to Cd the adaptation phase (Lag phase) extended to for more than 2 days that's may be due to the alga was un adapted to these conditions because of it is isolated from unpolluted ecosystem with Cd and may be due to physiological and biochemical properties of studied alga .

The increase of reduction growth rate with increase of Cd concentration with time is agree with many studies [28] which noticed the increase of Cd concentration causes reduction of growth rate and increase doubling time the *Chlorella saccharophila* and *Anabaena flos-aquas* algae ,also noticed the algae cell sizes decreased with exposure period because of loss the cell fluids, also these results agree with [29] .

The inhibition of growth of studied alga in present study also happened when addition of different concentrations of lead, that's may be due to the lead effect on algae in log phase which causes reduction in cells division during this phase [15] and then effect on algae growth rate in general.

(2)Effect of heavy metals addition together

The interaction between heavy metals compounds in ecosystems or in cultures may be increase or decrease the metals toxicity and this interactions are appear in many different forms according to interacted metals or its concentrations and the effect of interaction may be as antagonism effect which means one of them decrease the toxic effect of the other metal or synergism effect which mean the toxic effects of interacted metals are increase in comparison with sum of effects , in other few state the effect is equilibrium which mean the effects of interacted metals is equal to sum of single effects [30].

The results of present study showed the antagonism effect of both metals when used concentrations (1 mg Cd/l +50 mg Pb/l) ; (0.5 mg Cd/l+40 mg Pb/l) and (0.1 mg Cd/l+30 mg Pb/l), the growth rates increased in comparison with effects of single metals , and similar results for doubling time, this antagonism effect occur as results for interaction of metals ions in its activity; or one of them is interacted in opposite form with the action of other [31], that's agree with [32].

Other studies showed ability of heavy metals on happens the antagonism and synergism effects for same metals; De-carralho *et al.,* [33] when mix Cd , Cu and Zn metals as the following (Cd + Zn ; Cd + Cu and Cu + Zn) were showed the two effects together. In other studies each of Cd and Pb when mix it together causes the states of synergism and antagonism effects according to exposure period [2], these differences may be due to the used sources of Cd and Pb in different studies.

(3)Accumulation of heavy metals

The heavy metals which removed by algae from ecosystem or culture are accumulate and store inside the algae cells as result for absorption and adsorption process.

For comparison between studied metals in present study as accumulation rates ,the study showed the accumulated amounts of lead were more than for cadmium, that's may be due to the lead has high intimacy for engagement to cell wall or to the mechanisms of algae to resistance of lead are weak than its for cadmium ; and Patterson [34] found the algae cells make on detoxification of lead inside the cells (internal detoxification) while the cells detoxification of Cupper and Cadmium by exclusion outside of cell .

In present study showed decrease of accumulation rate with increase added metal concentrations that's may be due to acute toxic effect of metal which more increase with increase of metal concentration and exposure period by its effect on metabolism process of algae like algae ability to energy production which necessary for resistance process of toxic effects ,or algae ability on production of vital metabolites which consider one of resistance means for toxic effect like metals accumulation , the differences of accumulated amounts of heavy metals in algae may be due to differences of metal type and concentration; algae species and exposure period, that's agree with [35].

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التأثير التضادي أو التآزري للمعادن الثقيلة (الكادميوم و الرصاص) على نمو الطحلب الأخضر *Chlorella vulgaris*

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هيئة التعليم التقني

درس التأثير التضادي أو التعاوني لمعدني الكادميوم والرصاص معا وبتراكيز مختلفة على نمو الطحلب الأخضر *Chlorella vulgaris* ،حيث جرى أولا دراسة تأثير المعدنين بصورة منفردة على نمو الطحلب ، اضيف الكادميوم بالتراكيز (0.1 ، 0.5 ، 1 ، 2 ، 3) ملغم / لتر للوسط الزرعي ، وكان التركيز 3 ملغم / لتر هو الاكثر سمية للطحلب اذ بلغت اقل قيمة لمحتوى الكلوروفيل في اليوم الثاني 1.9 مايكروغرام / 100 مل عند هذا التركيز المضاف مقارنة بمعاملة السيطرة (2.4 مايكروغرام / 100 مل) ، أما الرصاص فقد اضيف بالتراكيز التالية (30 ، 40 ، 50 ، 75 ، 100) ملغم / لتر وكان التركيز 100 ملغم / لتر هو الاكثر سمية ، اذ انخفض محتوى الكلوروفيل الى 1.6 مكغم/100 مل مقارنة بمعاملة السيطرة .

وفي تجارب أخرى تم دمج المعدنين سويةً بالتراكيز (Cd 0.1 + Pb 30 و Cd 0.5 + Pb 40 و Cd 1 + Pb 50) ملغم / لتر واضافتهما للوسط الزرعي فقد كان التركيز (Cd 0.1 + Pb 30) هو الاقل سمية حيث بلغت اعلى قيمة للوزن الجاف 63.3 ملغم / غم اما محتوى الكلوروفيل فقد بلغ 11.5 مكغم/100 مل. وقد اظهر كل من معدني الرصاص والكادميوم تأثير تضادي خلال فترة التعريض (168 ساعة) وانعكس هذا التأثير على معدلات النمو التي ارتفعت وانخفض زمن التضاعف.