Toxic and accumulation effects of Cadmium and Lead on *Microcystis aeruginosa* in the growth medium.

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Abstract

The present study illustrates the toxicity of some heavy metals (Cadmium and lead) individually or together in presence of plant nutrients (nitrogen and phosphorus) in Beijerinck medium on the growth of blue green alga *Microcystis aeruginosa* using the biomass (total number) as indicator , as well as, its ability to metal accumulation .

The effects of Cd or Pb on the alga growth were variable due to heavy metal nature, concentration and exposure time. Toxicity of both metals declined with increasing of nitrogen and phosphorus concentrations in the medium. Presence of phosphorus in the growth medium did increase the accumulation of Cd and Pb in the growing algal biomass. Thus, *M. aeruginosa* may be use as biological clearing agent.

Key words: Heavy metal, Cd, Pb, Toxicity, Accumulation, Algae.

Introduction

The shift from the agricultural pattern of life to the industrial life pattern, human water uses are somewhat undergoing changes. Of which plenty amounts of water served industrial purposes, which in turn results in industrial wastes. Such wastes, however, may create several environmental pollution problems. Heavy metals like Cadmium and Lead may be constituents of such wastes. Cd and Pb may directly affect the aquatic biota as well as aquatic environment, it could affect the metabolic pathway of the aquatic fauna and flora (Forestner, 1995).

Algae inhibiting waste water may metabolize or accumulate Cd or Pb elements and /or compounds available in such waste (Favero *et al.*,1996) . Coexisting of Cd or /and Pb with metabolic activities and /or accumulation may be of interfering potentials to algae membrane permeability (Grill *et al.*, 1987 and Ross&Kaye, 1994).

The nature of algae inhibiting the effect of heavy metal pollution can be visualized through a laboratory scale. The investigation tries to study the possibility of removing the heavy metals (Cd & Pb) by the test alga *M. aeruginosa* and the effect of culture media on metal toxicity to the studied alga.

Material and Methods

The test alga was *M. aeruginosa* Kuetz isolate (AUFRC NO.44) . This isolate was maintained on Bijerinck medium(Table 1), and used to obtain axenic culture (Patterson 1983). All experiments were persuaded under standard constant conditions of temperature, light, incubation and media.

Gruded concentrations of Cd as $CdCl_2.5H_2O$ (0.1, 0.5, 1, 3 mg/l) and Pb as $Pb(NO_3)_2$ (30, 40, 75, 100 mg/l) were separately or in combinations in the algal growth medium. Nitrogen (NH4NO$_3$) and phosphorus (K_2HPO_4) were added at graded concentration 0.1, 0.15, 0.3, 0.5 mg/l of N and 1, 3,5,10 mg/l of P.

Toxicity was assayed by the percent of growth inhibition as in the following formula

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$$\%Inhibition = \left[1 - \frac{XT}{XC}\right] * 100$$

Where, XT is the total algae cell numbers of test treatment while XC is the total algae cell numbers in control. Seven treatments were designed to match such toxicity, as; *M. aeruginosa* growth in culture media.

1- *M. aeruginosa* with Cd.
2- *M. aeruginosa* with Pb.
3- *M. aeruginosa* with Cd&Pb.
4- As in 2 with N, P and N-P mixtures.
5- As in 3 = = = = = =.
6- As in 4 = = = = = =.

The Cd and Pb accumulation in the algal biomass and their concentrations were done as given in APHA(1985).

Results

As the graded concentrations of cadmium and lead, the exposure time was increased in the growth medium, the growth inhibition percent of the tested alga was increased. Lag phase lasted for 48 hrs (fig.1). The exponential phase was not clear. The nature of the result is suggestive for linear correlation between Cd concentration and growth inhibition but it was of negative nature (r = -0.97, P 0.05) as given in table 2.

The growth inhibition percentages and exposure time of the alga growth in medium containing graded Pb concentration is increased whenever Pb concentration increase and exposure time increases. The exponential phase was rather unclear and the death phase was started at 120hrs of exposure (r = -0.99, P 0.05) table 3.

When Cd & Pb were mixed together and incorporated in the growth medium, both antagonistic and synergistic effects were noted on the alga growth.

The addition of phosphorus to growth media containing Cd and /or Pb was found to be of toxicity repairing effect (table 4, 5). Likewise, the addition of nitrogen to the growth media containing Cd, Pb or Cd-Pb was of toxicity repairing effect (table 6,7).

The tested alga accumulates Cd, Pb such accumulation were lowered as the nitrogen concentration and exposure times were increased.

The presence of phosphorus coexisted with Cd, Pb or Cd-Pb in the growth medium of the test alga, did increased the accumulation of the heavy metal.

Discussion

The highest concentration of Cd & Pb incorporated growth media could inhibit about 50 % of the algae growth (Tables 2-7). This could be attributed to either one or more of the followings;

1- Inhibition of photosynthesis and respiration through inhibition of CO_2 fixation or cation removal (Rai *et al.*, 1990),

2- Reduction of polyhedral bodies containing Ribulose-1-5-diphosphate carboxylase (Lanaras & Codd, 1982),

3-link to the active groups within cell membrane structures that are involved in the membrane permeability (Rai *et al.*, 1990),

4- Rearrangement of the enzyme membrane active sites (Vymazal, 1987),

5- Inhibition of Fe uptake (Harrison&Morel, 1983),

6- Fatty bodies, cell volume and thylakoids surface area reduction (Rai *et al.*, 1990).

Lead toxicity may be due to its link to SH group of active molecules inhibiting their functions or due to its link to phosphorus in the DNA molecules leading to genetic change. Finally, Pb may change thylakoids surface area which leads to inhibition of photosynthesis (Jackson *et al.*, 1991).

The Cd, Pb and/or Cd-Pb mixture incorporated with *M .aeruginosa* growth medium led to

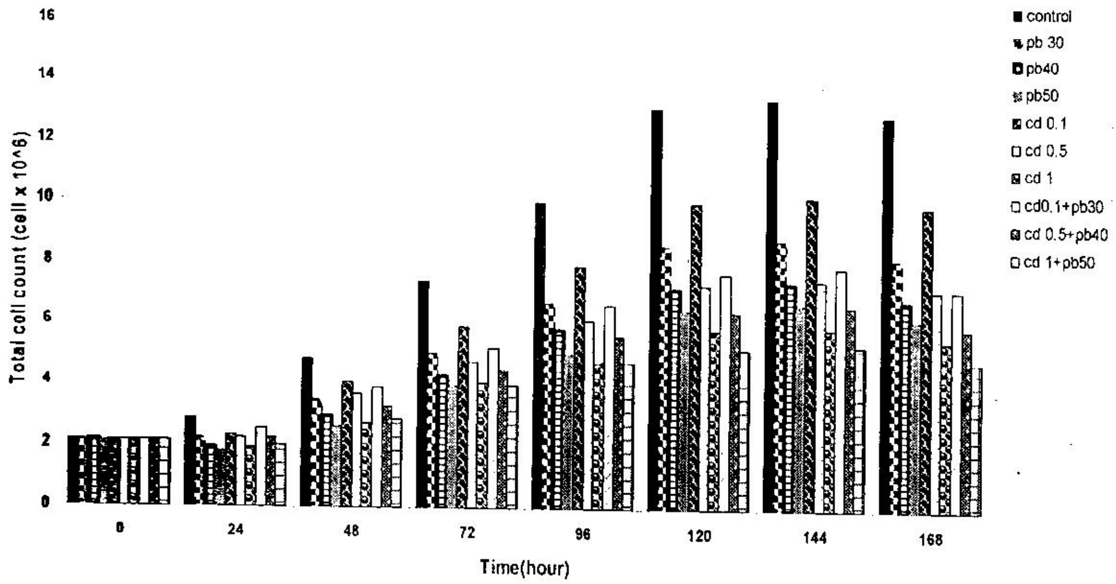

Fig (1) Variation in cell number of *Microcystis aeruginosa* after exposure to different concentration of Cd and Pb or together (mg/l).

Table (1): Composition of the Bijerinck medium (Stein, 1973).

Stock No.	Salts	Concentration g/l
1. use 100 ml/l	NH_4NO_3	1.5
	K_2HPO_4	0.2
	$MgSO_4$	0.2
	$CaCl_2.2H_2O$	0.0027
2. use 40 ml/l	KH_2PO_4	9.1
3. use 60 ml/l	K_2HPO_4	11.16
4. Micronutrients use 1ml/l	H_3BO_3	1g/100 ml
	$CuSO_4.5H_2O$	0.15g/100 ml
	EDTA	5g/100 ml
	$MnCl_2.4H_2O$	0.5g/100 ml
	$FeSO_4.7H_2O$	0.5g/100 ml
	$(NH_4)_6MO_7O_{24}.4H_2O$	0.15g.100 ml

growth inhibition (Tables 2&3). Such growth inhibition can be cute by N,P addition. Since N,P enhance the Cd, Pb accumulation in the tested alga (Tables 4-7), therefore, the alga can be of use in high nutrient water (N,P) for clearance of such heavy metals from contaminated water, this was also concluded by other studies (Huntsman & Sunda,1980; Fayed *et al.* 1983; Pollen&Travieso,1997; Bajguz,2000; Ross,2000; Forstenr,1995).

Table 2: The inhibition percent growth of alga *Microcystis aeruginosa*; exposure to 1 mg/l of cadmium and 50 mg/l of lead with different concentration of phosphorus during 120 hours.

Phosphorus concentration (mg/l)	Inhibition percent				
	Time (hour)				
	24	48	72	96	120
Control 0.02	21	29	32	43	53
0.1 (Cd)	18	27	31	40	52
0.15 (Cd)	15	25	30	38	49
0.3 (Cd)	12	22	26	35	38
0.5 (Cd)	5	19	21	30	33
Control 0.02	28	39	41	46	49
0.1 (Pb)	25	37	39	43	45
0.15 (Pb)	22	31	32	39	41
0.3 (Pb)	13	19	24	29	34
0.5 (Pb)	3	11	17	21	25

- Control: It mean natural concentration of phosphorus in Beijerinck medium.

Table (3): The inhibition percent growth of alga *Microcystis aeruginosa* exposure to 1 mg/l of cadmium and 50 mg/l of lead with different concentrations of nitrogen during 120 hour.

Nitrogen concentration (mg/l)		Inhibition percent Time (hour)				
		24	48	72	96	120
Control 0.12		21	29	32	43	53
1	Cd	19	24	28	35	41
3	Cd	18	20	24	32	37
5	Cd	21	25	31	39	47
10	Cd	24	34	37	45	51
Control 0.12		28	39	41	46	49
1	Pb	3	13	21	27	31
3	Pb	6	11	16	24	30
5	Pb	16	28	33	38	40
10	Pb	19	35	41	44	46

- Control: It mean natural concentration of nitrogen in Beijerinck medium

Table 4: The accumulated of Cd (mg/g dry weight) in *Microcystis aeruginosa*; after exposure to 1 mg Cd/l with different concentrations of phosphorus (Mean ± SD).

$P-PO_4$ (mg/l)	Accumulated Cd (mg/g(		
	24hrs.	72hrs.	168hrs.
0.1	0.937± 0.013 a	0.666± 0.01 b	0.217± 0.01 c
0.5	0.944± 0.001 a	0.886± 0.001 d	0.287± 0.02 e

- Means within each column of the same superscript letter are not significant differences (P< 0.05.(

Table (5): The accumulated of Pb (mg/g dry weight) in *Microcystis aeruginosa*; after exposure to 50 mg Pb/l with different concentrations of phosphorus (Mean ± SD).

$P-PO_4$ (mg/l)	Accumulated Pb (mg/g(		
	24hrs.	72hrs.	168hrs.
0.1	8.225± 0.007 a	4.945± 0.08 b	4.294± 0.01 c
0.5	15.75± 0.14 d	7.927± 0.11 e	4.413± 0.01 c

- Means within each column of the same superscript letter are not significant differences (P< 0.05)

Table6: The accumulated of Cd (mg/g dry weight) in *Microcystis aeruginosa*; after exposure to 1 mg Cd/l with different concentrations of nitrogen (Mean ± SD).

$N-NO_3$ (mg/l)	Accumulated Cd (mg/g(		
	24hrs.	72hrs.	168hrs.
1	0.815± 0.4 a	0.507± 0.4 a	0.427± 0.008 a
10	0.147± 0.01 b	0.079± 0.01 b	0.035± 0.07 b

- Means within each column of the same superscript letter are not significant differences (P< 0.05.(

Table7: The accumulated of Pb (mg/g dry weight) in *Microcystis aeruginosa*; after exposure to 50 mg Pb/l with different concentrations of phosphorus (Mean ± SD).

$N-NO_3$ (mg/l)	Accumulated Pb (mg/g(		
	24hrs.	72hrs.	168hrs.
1	6.216± 0.001 a	2.066± 0.01 b	1.675± 0.01 b
10	3.490± 0.005 c	2.114± 0.15 b	1.378± 0.01 b

- Means within each column of the same superscript letter are not significant differences (P< 0.05)

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التاثير السمي والتراكمي للكادميوم والرصاص على طحلب *Microcystis aeruginosa* في وسط النمو

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الخلاصة:

تضمنت الدراسة بيان سمية بعض المعادن الثقيلة (الكادميوم والرصاص) منفردة ومجتمعة وبوجود المغذيات النباتية (النتروجين والفسفور) في وسط بجيرنك (Beijerinck) على نمو الطحلب الاخضر المزرق *Microcystis aeruginosa* واستخدم الكتلة الحية (العدد الكلي) كمؤشر للدراسة اضافة الى قابلية الطحلب على تراكم المعدن. تاثير الرصاص والكادميوم على نمو الطحلب المدروس متغيرة بسبب طبيعة المعدن ووقت التعريض و التراكيز . انخفضت سمية المعادن بزيادة تراكيز الفسفور و النتروجين في الوسط الغذائي . لوحظ بان تراكيز الفسفور في الوسط الغذائي ساعد في زيادة من قابلية الطحلب لتراكم الكادميوم ، لذلك من الممكن استعمال الطحلب *Microcystis aeruginosa* لمعالجة المعادن الثقيلة في الانظمة المائية الغنية بالعناصر المغذية.