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DISTURBANCES IN THE SIZE OF VARIOUS METABOLIC POOLS OF SOME ISOLATED SOIL ALGAL SPECIES IN RESPONSE TO HEAVY METAL TOXICITY

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Abstract

The growth and some metabolic activities of the five species namely *Chlamydomonas reinhardtii* (Dang), *Chlorococcum humicola* (Nag), *Scenedesmus obliquus* (Turp) Kütz (green algae) and *Anabaena circinalis* (Rabh.) and *Wollea saccata* (Wollea Bornet and Flahault) (Blue-green algae) isolated from soil at Assiut, Egypt were investigated under various concentrations of Cd^{2+}, Ni^{2+} and Pb^{2+}. The growth of the *C. reinhardtii*, *C. humicola* and *S. obliquus* were inhibited by all used heavy metals. The highest inhibitory effect was exerted by Ni^{2+} or Cd^{2+}, while the lowest toxicity was exerted by Pb^{2+}. However, the growth was enhanced by low and medium concentrations of Pb^{2+}. The toxicity of heavy metals for *C. reinhardtii*, *C. humicola*, *S. obliquus* and *W. saccata* was as follows $Ni^{2+} > Cd^{2+} > Pb^{2+}$. In case of *A. circinalis* the toxicity of heavy metal was as follows $Cd^{2+} > Ni^{2+} > Pb^{2+}$. Soluble sugars, soluble protein, free amino acids and proline of all species, exhibited a high significant increase with all tested heavy metals supplemented. Insoluble carbohydrates, were generally lowered in various treatments, of heavy metals irrespective the test algae.

Key word: Green algae, Blue green algae, Cd^{2+}, Ni^{2+}, Pb^{2+}.

Introduction

Cadmium (Cd^{2+}) and Nickel (Ni^{2+}) are widely used in a variety of industrial processes, including plastic manufacturing, electroplating, and as well as in paints (**Siripornadulsil *et al.*, 2002**). In soils, Cd^{2+} is found in various forms and speciation (**Adriano, 1986**). Beginning with exchangeable phase where absorption of Cd^{2+} by electrostatic altercation to negatively charged exchange sites on clays, organic particles, and hydroxyl oxides occurred. Then absorption with oxides, hydroxides and hydrous oxides observed in reducible phase followed by carbonate phase; organic phase; lattice phase; sulfide phase and finally soluble

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phase which exists in soil solution in either the ionic or complexes forms (**Cook and Morrow, 1995; Yaron *et al.*, 1996**). The toxicity and accumulation of Cd^{2+} by algae have been reviewed recently (**Robidoux *et al.*, 2004; Andrade *et al.*, 2005; Iman *et al.*, 2008**). Cadmium inhibits cell division and biomass. Motility can be lethal for algae, the toxicity of Cd^{2+} is depended on both the organism and toxicity criteria employed although specific trend is senility are difficult to identify.

In soils all over the world the average concentration of nickel (Ni^{2+}) is probably 20 mg/kg (20 ppm), which obscures much variation between soil types (**McGrath, 1987**). A considerable variations of Ni^{2+} concentration may occurred due to the parent material as well as anthropogenic sources, including metal smelting, sewage sludge disposal, phosphate rock used as fertilizer and the mining (**McGrath, 1995**). **Rashed *et al.*** (**1995**) gave a range of 21 to 44 ppm as the Ni^{2+} content of alluvial soils in the Nile Delta, Egypt with an average of 32 ppm for total content and from 0.38 to 1.34 ppm with an average of 0.66 ppm for available form. In Assiut City the average concentration of Ni^{2+} was 0.22 ppm in unpolluted soils (**I*ssa et al.*, 2006**).

Lead (Pb^{2+}) is a well-known pervasive chemical and is known for its toxicity. The major environmental sources of metallic lead and its salts are paint, autoexhaust, food and water. The soil adjacent to highways had lead accumulation in the range of 128 to 700 ppm decreased with distance from traffic and with soil depth (**Kelemen and Csordas, 1994**). The situations of Pb^{2+} in some Egyptian soils are reported by **El-Molla** (**1980**). He found that, Pb^{2+} content varied from 71 to 226 ppm in the surface soils adjacent to Cairo-Alexandria highway. **El-Gharably** (**1993**) recorded that the Pb^{2+} contents of sewage sludge collected from different sites in Assiut City ranged from 35 to 38 ppm, and the unpolluted soils ranged from 0.08 to 0.15 ppm. Lead tends to accumulate to high extent in algae. Pb^{2+} was found to be sequestered in polyphosphate bodies of the blue-green algae (*Plectonema boryanum*) and in cell sectors with polyphosphate bodies of green algae C*hlorella saccharophila* and diatoms *Navicula incerta* and *Nitzschia closterum* (**Jensen *et al.*, 1982**). **Wehr and Whitton** (**1983**) reported the growth of some algae species (e.g. blue-green algae *Phormidium autumnale*, *Aphanocapsa* sp., *Pseudoanabaena catenata*, diatoms *Achnanthes minutissima*, *Navicula tantula*, *Calonies bacillum*, *Cymbella bipartita* or green algae *Mougeotia* sp. and *Hormidium rivulare*) in waters with elevated lead concentrations. **Say and Whitton** (**1982**) reported the growth of a blue-green alga *Schizothrix* sp. in close association with anglesite ($PbSO_4$), the concentration of Pb^{2+} in the sediment having been 110g.kg^{-1}.

In Egypt and other countries, irrigation with sewage wastewater, sedimentation of sludge material and deposition of air-born particulates on plants and soils are probably the most important source of soil and plant contamination with heavy metals, particularly near industrial zones. These pollutants affect on algal growth and metabolic activities. This study aimed to follow the growth as well as the metabolic activities of the three green algae (*C. reinhardtii*, *C. humicola* and *S.obliquus* and two cyanobacteria (*A. circinalis* and *W. saccata* isolated from soil of different Cd^{2+}, Ni^{2+}, Pb^{2+} concentrations.

Materials and Methods

Three species of green algae (*C.reinhardtii*, *C. humicola* and *S. obliquus*) and two blue-green algae *A. circinalis* and *W. saccata* were isolated from selected soils contaminated with heavy metals at Assiut Governorate (for details of the algae under testing and for description of these polluted soil see (**Issa *et al.*, 2006**). The effects of (0.05, 0.1, 0.2 mM expressed as low, medium, high concentrations of Cd^{2+}, Ni^{2+} and $Pb^{2\ +}$ on the algal growth as well as some metabolic activities were assessed.

All algal species were cultivated in batch culture treated with various concentrations of Cd^{2+}, Ni^{2}, Pb^{2} (For details see **Issa *et al.*, 2006**).

The isolated blue green algae were cultured using a modified blue green algal medium BG-11 **(Rippka and Herdman, 1993)**. While the isolated green algae were cultured using a Bold's Basal medium **(Bischoff and Bold, 1963)**. The addition of the heavy metals treatments did not exert a large change in the pH of the medium. All cultures started with a pH of 7.1 and remained unchanged for one week. 3300 Walt tungsten lamp were used for illumination at room temperature.

The growth rate and generation time of each tested algal species was grown with various concentrations of heavy metals were followed by daily measurements of absorbance at 750 nm as described by **Lefort-Tran *et al.*** (**1988**). Optical density was used as a parameter for algal growth .The growth rate μ (d^{-1}) was determined from the following formula:

$$\mu(d^{-1}) = \frac{\ln N_1 - \ln N_0}{t_1 - t_0}$$

Where

N_1 = Optical density at time t_1.

N_0 = Optical density at time t_0.

t_1 - t_0= The time elapsed in days between two determinations of optical density.

While the generation time G (doubling of optical density) can be calculated as follows:

$$G = \frac{\ln 2}{\mu}\ d$$

After 7 days, the algal cells were harvested for measuring the growth and some metabolic estimation, in the late of exponential phase or beginning of the stationary phase according to the algal growth curve. The cell numbers (in case of green algae) were determined by counting of cell number microscopically using 1 mm deep haemocytometer slide. For determination of dry weight, 10 ml of algal culture, after filtered through glass fiber filter paper, was dried for two hours in oven at 105° C. The data were given as $mg.ml^{-1}$ algal culture.

Pigments represented by chlorophyll a (*chl a*) and chlorophyll b (*chl b*) were estimated in green algal species, while chlorophyll (*chl a*) only was measured in blue green algal species by extracting in hot methanol at 70°C for 10 minutes (**Marker, 1972; Metzner *et al.*, 1965**). The photosynthetic activity was measured polargraphically as oxygen evolution using a Clark type electrode (O_2 Meter CG867, Germany). The data obtained were calculated as µmoles O_2/mg chlorophyll/hour. Respiration was measured in the dark as O_2-uptake in the same sample. The anthrone sulphuric acid method (**Fales, 1951; Schlegel, 1956 and Badour, 1959**) was used for the determination of all carbohydrate including polysaccharides. While soluble, insoluble and total proteins were measured according to **Lowry *et al.* (1951)**. Free amino acids were extracted from algal suspension and determined according to the method of **Lee and Takahashi (1966)**. Proline was determined according to the method of **Bates *et al.* (1973)**. The data was obtained by four independent experiments and measured as means ± standard error (SE)

Results and Discussion

Comparing the results to the control (no heavy metals added) the growth rates of the *C. reinhardtii*, *C. humicola*, *S. obliquus* and *W. sacca*ta were inhibited by all applied heavy metals.

The highest inhibitory effect was detected by Ni^{2+} and Cd^{2+} while the lowest toxicity was exerted by Pb^{2+} (Figs. 1, 2, 3, 4 and 5). Meanwhile, the growth rate of *A. circinalis* was enhanced by low and medium concentrations of Pb^{2+} (Fig. 4). The growth rate as well as minimum generation time was recorded for all tested species and control cultures. **Rachlin *et al.* (1983)** recorded 50% reduction of growth of *Navicula* by certain concentrations of Cd^{2+}, Co^{2+}, Pb^{2+} and Zn. While the growth rate of *Kirchneriella lunaris* was inhibited by Cd^{2+}, Mn^{2+}, Ni^{2+} and Co^{2+} **(Issa *et al.*, 1995)**. On the other side, **El-Enany and Issa (2000)** stated that the growth of *Nostoc linckia* and *Nostoc rivularis* were showed a significant stimulation in low waste treatments compared to control culture, not only for the number of cells but also, the time required to reach the exponential and the stationary phases.

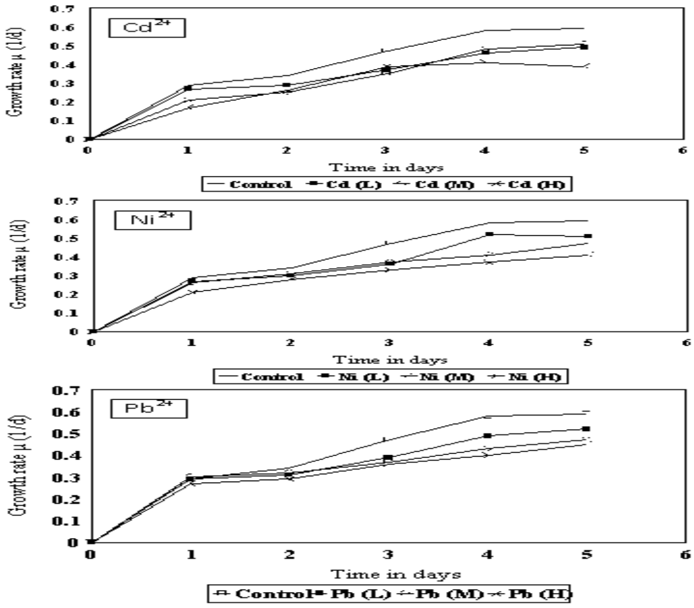

Figure (1): Growth rate of *Chlamydomonas reinhardtii* under various heavy metals concentrations. Values are means of three replicates; ES. is smaller than the symbol in all cases.

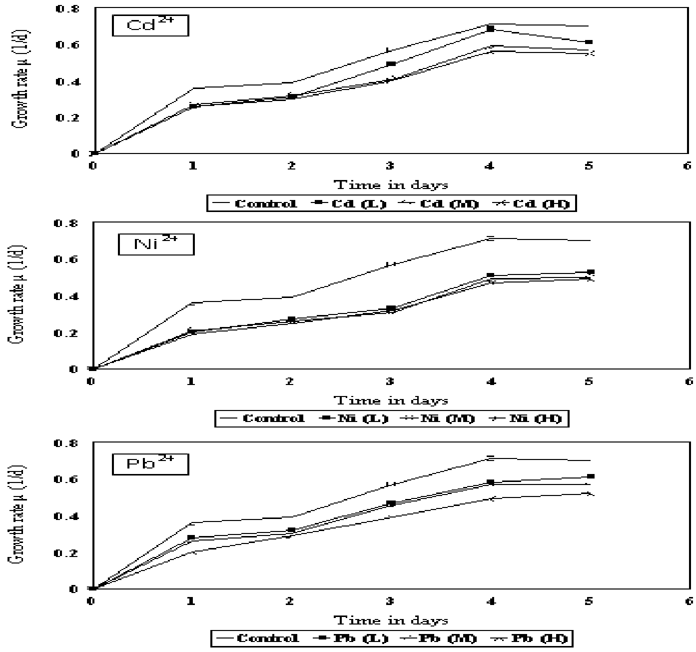

Figure (2): Growth rate of *Chlorococcum humicola* under various heavy metals concentrations. Values are means of three replicates; SE. is smaller than the symbol in all cases.

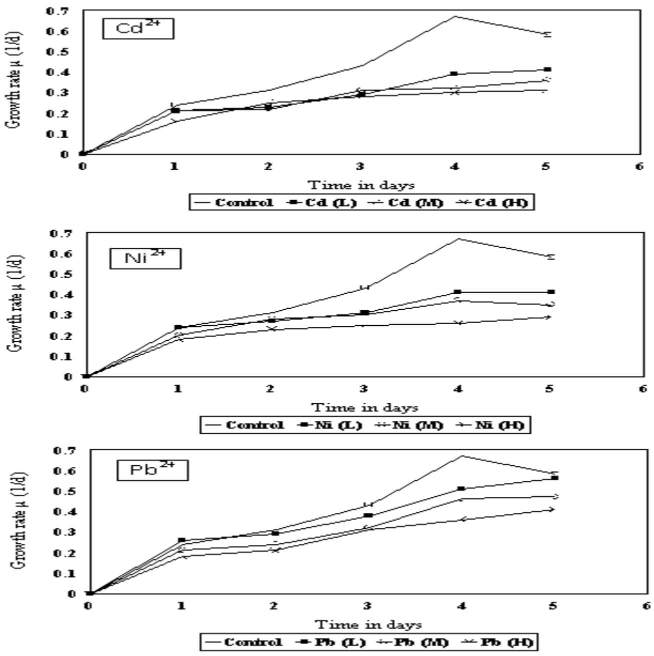

Figure (3): Growth rate of *Scenedesmus obliquus* under various heavy metals concentrations. Values are means of three replicates; s.e. is smaller than the symbol in all cases.

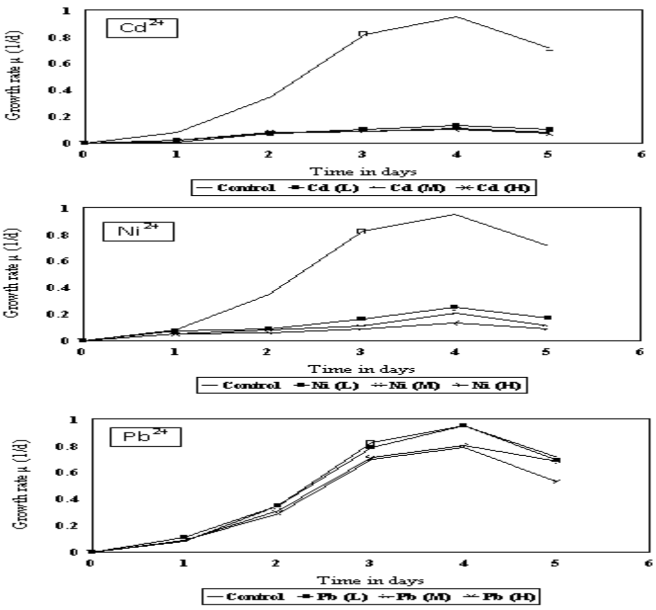

Figure (4): Growth rate of *Anabaena circinalis* under various heavy metals concentrations. Values are means of three replicates; s.e. is smaller than the symbol in all cases.

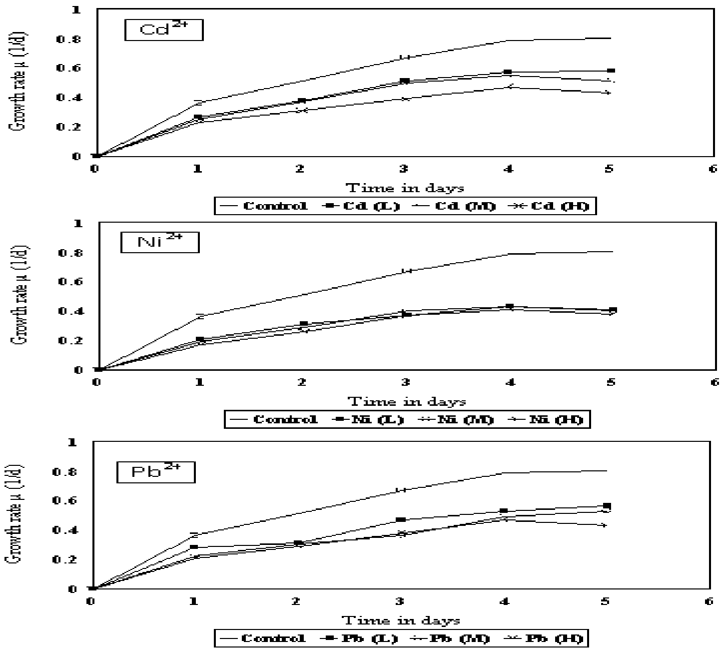

Figure (5): Growth rate of *Wollea saccata* under various heavy metals concentrations. Values are means of three replicates; SE is smaller than the symbol in all cases.

However, high concentration of wastes reduced growth of the two species tested.

In general, cell number, chlorophyll a, b and dry matter of *C. reinhardtii, C. humicola* and *S. obliquus* were markedly decreased with increasing heavy metals concentration (Table 1-3). However, Ni^{2+} and Cd^{2+} appear to have a serious effect on pigments. Also, the contents of chlorophyll a and dry matter of *A. circinalis* and *W. saccata* were generally decreased in Cd^{2+} and Ni^{2+} concentrations (Tables 4 and 5). In Pb^{2+} treated cultures, chlorophyll a of *W. saccata* was slightly increased and the dry matter was almost unchanged. The toxicity of heavy metals for *C. reinhardtii, C. humicola, S. obliquus* and *W. saccata* was as follows $Ni^{2+} > Cd^{2+} > Pb^{2+}$. In case of *A. circinalis* the toxicity of heavy metals was as follows $Cd^{2+} > Ni^{2+} > Pb^{2+}$. **Sabnis *et al.* (1969)** attributed that chlorophyll damage on the thylakoid membrane could be due to the affinity of heavy metals. **Issa *et al.* (1998)** stated that, the pigment fractions of *Kirchneriella lunaris* and *Scenedesmus obliquus* under various heavy metals were significantly decreased. Moreover, nickel and manganese were very toxic to pigment fractions. The reduction of chlorophyll may be due to sensitivity of the enzymes of chlorophyll biosynthesis towards heavy metals ions **(Abdel-Basset *et al.*, 1995).**

Photosynthetic O_2 evolution and respiratory oxygen uptake of the five species tested were also affected by these treatments. While the photosynthetic O_2 evolution was reduced proportionally to metal toxicity, and the respiratory oxygen uptake was enhanced by heavy metals applied. This results in accordance with **Mendoza-Cozatletal (2002).**

Generally, Cd^{2+} or Ni^{2+} have a serious effect on O_2 exchanges depending on the species tested. Green algae were highly tolerant to heavy metals than blue-green algae under these treatments (Tables 1-3). The toxic effects of Cd in *Euglena gracils* include inhibition of growth, motility, phototaxis and photosynthesis (**Mendoza-Cozatl *et al.*, 2002**). Cadmium is more toxic than zinc to the growth and photosynthetic O_2 evolution *of Anabaena variabalis* (**Attridge and Rowell, 1997; Axtell *et al.*, 2003**). **Takamura *et al.*** (**1989**) stated that, Cyanophyceae are sensitive to cupper, cadmium, zinc metals than other algae tested (green algae or diatoms) for photosynthetic activity, through the inhibition of photosystem II and/or reduction the four enzymes involved in the fixation of CO_2 for at least the first 2 days of the exponential growth.

As a result, the pool size of soluble sugars of the five species tested, exhibited a high significant increase by heavy metals (Cd^{2+}, Ni^{2+} and Pb^{2+}) supplemented. Insoluble carbohydrates were generally lowered in various treatments, irrespective (Tables1-5). The same results was obtained by **Adam (1995)** using the green algae *Kirchneriella lunaris* and Lead as heavy metals. **Hellebust (1965)** reported that *Phaeodactylum* secretes up to 7% of the total assimilation carbon, some of which are polysaccharides liberated from the cell surface, which in turn act as binding agents for lead. The level of storage carbohydrates was raised at low Cd^{2+} concentrations, while at high concentration the opposite was obtained **(Greger and Bertell, 1992).** Furthermore, the accumulation of saccharides exerts feedback inhibition of photosynthesis or even triggers gene expression resulting in lower activities of ribolose 1, 5 bisphosphate carboxylase/oxygenase **(Issa *et al.*, 2002).**

Similarly, the contents of soluble proteins in all five species tested were raised under heavy metals treated cultures (Tables 1-5). The elevation of soluble proteins at the expense of insoluble proteins generally indicates an inhibition in the growth efficiency by heavy metals supplemented. The toxicity of heavy metals was as follows $Cd^{2+} > Ni^{2+} > Pb^{2+}$. Total protein contents were mostly found to be generally lowered under conditions of stress (**Yupsanis *et al.*, 1994**). While soluble proteins were found to be raised when the plants were exposed to stress conditions (**Xu *et al.*, 1996**). Such elevation of soluble protein contents was mostly ascribed to a decline in the content of relatively higher molecular proteins **(Pelah *et al.*, 1997**). Algae detoxify heavy metals *via* metal-binding proteins of low molecular weight and high cysteine and metal content (**Zenk, 1996**). Furthermore, metallothionein proteins have been postulated to play a role in the detoxification of heavy metals (**Vitarella *et al.*, 1996 and El-Enanay and Issa, 2000**).

In parallel, free proline and free amino acids were significantly accumulated in the tested algal species by all heavy metals applied. The highest values of proline and free amino acids were recorded in *A. circinalis* and *W.*

Table (1): Response of *Chlamydomonas reinhardtii* Dang to heavy metals toxicity and their effects on growth as well as some metabolic activities.

Treatment		Cell No. Cell.ml^{-1} *10^{3}	μ (d^{-1})	G (d^{-1})	Dry wt. mg ml^{-1}	Chl. a μg ml^{-1}	Chl. b μg ml^{-1}	$O_2\uparrow$*	$O_2\downarrow$*	Carbohydrates S.C.	Carbohydrates Ins. C.	Carbohydrates T.C.	Proteins S.P.	Proteins Ins. P.	Proteins T.P.	A.A.	Proline
										mg.g dry wt $^{-1}$.							
Control		150 ±0.73	0.59	1.17	1.8 ±0.15	4.8 ±0.46	2.7 ±0.73	1.2 ±0.36	1.1 ±0.32	44 ±0.03	28 ±0.01	72 ±0.03	9 ±0.01	27 ±0.01	36 ±0.02	2.02 ±0.19	0.71 ±0.18
Cd^{2+}	L	1.25 ±0.27	0.49	1.41	1.7 ±0.07	2.6 ±0.57	1.8 ±0.20	1.7 ±0.61	1.4 ±0.40	49 ±0.03	6 ±0.02	55 ±0.01	14 ±0.01	18 ±0.01	32 ±0.03	2.99 ±0.04	0.69 ±0.14
	M	1.22 ±0.33	0.51	1.36	1.7 ±0.07	1.5 ±0.31	1.4 ±0.08	1.3 ±0.27	1.1 ±0.23	50 ±0.03	5 ±0.01	55 ±0.02	11 ±0.01	20 ±0.02	31 ±0.02	2.82 ±0.97	0.21 ±0.09
	H	22 ±0.65	0.41	1.69	1.6 ±0.13	1.1 ±0.03	1.3 ±0.12	1.8 ±0.03	1.7 ±0.02	36 ±0.05	4 ±0.01	40 ±0.04	8 ±0.00	12 ±0.01	20 ±0.01	2.11 ±0.47	0.19 ±0.08
Ni^{2+}	L	198 ±0.49	0.52	1.33	2.0 ±0.25	5.4 ±0.19	3.1 ±0.05	0.4 ±0.02	0.4 ±0.02	44 ±0.01	7 ±0.02	51 ±0.03	11 ±0.00	16 ±0.00	27 ±0.00	2.54 ±0.53	0.14 ±0.01
	M	133 ±0.28	0.47	1.47	1.7 ±0.13	2.0 ±0.15	1.5 ±0.14	1.0 ±0.01	0.9 ±0.01	59 ±0.00	10 ±0.01	69 ±0.01	12 ±0.00	15 ±0.01	27 ±0.01	3.65 ±0.21	0.28 ±0.01
	H	73 ±0.15	0.41	1.69	1.6 ±0.07	1.4 ±0.04	1.3 ±0.06	1.3 ±0.06	1.3 ±0.07	71 ±0.01	9 ±0.01	80 ±0.02	10 ±0.00	17 ±0.00	27 ±0.00	2.95 ±0.07	0.14 ±0.00
Pb^{2+}	L	140 ±0.18	0.52	1.33	1.7 ±0.06	4.9 ±0.72	3.0 ±0.33	0.8 ±0.41	0.8 ±0.42	73 ±0.06	22 ±0.03	95 ±0.08	19 ±0.01	18 ±0.02	37 ±0.03	2.28 ±0.38	0.13 ±0.19
	M	207 ±0.12	0.47	1.47	1.7 ±0.19	3.4 ±0.18	2.3 ±0.79	1.0 ±0.32	0.9 ±0.28	130 ±0.05	21 ±0.00	151 ±0.05	14 ±0.00	17 ±0.01	31 ±0.01	3.83 ±0.51	0.22 ±0.11
	H	152 ± 0.49	0.45	1.54	2.4 ±0.19	3.9 ±0.05	2.4 ±0.47	0.8 ±0.23	0.8 ±0.21	54 ±0.04	11 ±0.01	65 ±0.03	11 ±0.01	16 ±0.01	27 ±0.01	2.43 ±0.27	0.08 ±0.03

$O\uparrow$ (μmoles $O_2\uparrow$ mg chl. a h^{-1}); ** $O\downarrow$ (μmoles $O_2\downarrow$ mg chl. a h^{-1}), S.C = soluble carbohydrates, Ins. C. = insoluble carbohydrates, T.C. = total carbohydrates, S.P. = soluble proteins, Ins. P.= insoluble proteins, T.P.= Total proteins, A.A.= amino acids. L = Low, M= Medium, H= High.

Table (2): Response of *Chlorococcum humicola* Nag. to heavy metals toxicity and their effects on growth as well as some metabolic activities.

Treatment		Cell no Cell.ml^{-1} *10^{3}	µ (d^{-1})	G (d^{-1})	Dry wt. mg ml^{-1}	Chl. a µg ml^{-1}l	Chl. b µg ml^{-1}	O_2↑*	O_2↓**	Carbohydrates S.C.	Carbohydrates Ins. C.	Carbohydrates T.C.	Proteins S.P.	Proteins Ins. P.	Proteins T.P.	A.A.	Proline
										mg.g dry wt^{-1}.							
Control		95 ±0.96	0.71	0.98	0.4 ±0.02	2.5 ±0.23	2.1 ±0.01	2.6 ±0.11	1.9 ±0.12	21 ±0.00	90 ±0.02	111 ±0.03	25 ±0.00	23.5 ±0.00	48.5 ±0.00	5.3 ±0.18	2.5 ±0.20
Cd^{2+}	L	63 ±0.92	0.68	1.02	0.2 ±0.07	2.3 ±0.05	1.9 ±0.08	2.2 ±0.17	2.0 ±0.19	54 ±0.00	24.1 ±0.00	78.1 ±0.00	56 ±0.00	16 ±0.00	72 ±0.00	9.8 ±0.14	6.2 ±0.35
	M	47 ±0.67	0.59	1.17	0.2 ±0.04	2.1 ±0.68	2.3 ±0.23	1.9 ±0.34	1.8 ±0.33	44 ±0.01	92 ±0.01	136 ±0.02	48 ±0.01	13 ±0.00	61 ±0.01	10.6 ±0.43	4.2 ±0.35
	H	21 ±0.40	0.56	1.24	0.2 ±0.05	0.8 ±0.15	0.7 ±0.22	1.8 ±0.14	4.5 ±0.01	19 ±0.00	29 ±0.00	48 ±0.00	29 ±0.00	15 ±0.00	44 ±0.01	7.3 ±0.96	5.3 ±0.21
Ni^{2+}	L	80 ±0.97	0.53	1.31	0.6 ±0.54	2.1 ±0.68	1.8 ±0.46	2.1 ±0.66	2.0 ±0.68	19 ±0.00	16 ±0.05	35 ±0.05	12 ±0.01	2.8 ±0.00	14.8 ±0.01	3.7 ±0.34	1.7 ±0.61
	M	87 ±0.88	0.50	1.39	0.2 ±0.03	1.5 ±0.03	1.7 ±0.03	2.0 ±0.06	2.4 ±0.12	24 ±0.02	15 ±0.02	39 ±0.03	48 ±0.00	12 ±0.00	60 ±0.00	19.2 ±0.19	5.7 ±0.44
	H	88 ±0.60	0.49	1.41	0.3 ±0.04	1.8 ±0.01	1.0 ±0.42	2.0 ±0.19	2.5 ±0.22	38 ±0.00	53 ±0.01	91 ±0.01	32 ±0.00	8.7 ±0.00	40.7 ±0.00	9.3 ±0.44	3.1 ±0.27
Pb^{2+}	L	80 ±0.73	0.61	1.14	0.2 ±0.02	2.5 ±0.01	1.3 ±0.81	2.0 ±0.26	1.9 ±0.22	24 ±0.00	10.1 ±0.02	34.1 ±0.02	36 ±0.00	5.4 ±0.00	14.4 ±0.00	11.2 ±0.12	3.4 ±0.17
	M	82 ±0.40	0.57	1.22	0.2 ±0.01	1.9 ±0.03	2.1 ±0.04	2.1 ±0.05	1.9 ±0.01	39 ±0.01	19 ±0.06	58 ±0.05	46 ±0.00	5.5 ±0.00	51.5 ±0.00	14.4 ±0.12	4.0 ±0.18
	H	85 ±0.12	0.52	1.33	0.2 ±0.01	2.1 ±0.07	1.8 ±0.04	2.0 ±0.06	1.9 ±0.05	17 ±0.00	16 ±0.02	33 ±0.02	24 ±0.00	6.5 ±0.00	30.5 ±0.00	10.7 ±0.36	2.9 ±0.06

O↑ (µmoles O_2 ↑ mg chl. a h^{-1}) ; ** O↓ (µmoles O_2 ↓ mg chl. a h^{-1})S.C = soluble carbohydrates, Ins. C. = insoluble carbohydrates, T.C.= total carbohydrates, S.P.= soluble proteins, Ins. P.= insoluble proteins, T.P.= Total proteins, A.A.= amino acids. L = Low, M= Medium, H= High

Table (3): Response of *Scenedesmus obliquus* (Turp.)Kütz to heavy metals toxicity and their effects on growth as well as some metabolic activities.

Treatment		Cell no Cell.ml^{-1} *10^{3}	μ_x (d^{-1})	G (d^{-1})	Dry wt. mg ml^{-1}	Chl. a µg ml^{-1}	Chl. b µg ml^{-1}	$O_2\uparrow$*	$O_2\downarrow$**	Carbohydrates			Proteins			A.A.	Proline
										S.C.	Ins. C.	T.C.	S.P.	Ins. P.	T.P.		
										mg.g dry wt^{-1}.							
Control		197 ±0.03	0.67	1.03	1.3 ±0.14	4.4 ±0.17	2.3 ±0.12	1.6 ±0.04	0.6 ±0.03	19 ±0.06	92 ±0.04	111 ±0.02	10 ±0.00	26 ±0.00	36 ±0.00	3.7 ±0.11	1.02 ±0.01
Cd^{2+}	L	198 ±0.02	0.41	1.69	0.9 ±0.03	3.6 ±0.21	1.9 ±0.15	0.7 ±0.07	0.4 ±0.62	27 ±0.00	83 ±0.02	110 ±0.02	15 ±0.00	25 ±0.00	40 ±0.00	4.5 ±0.71	1.13 ±0.01
	M	48 ±0.02	0.36	1.93	0.8 ±0.02	2.5 ±0.01	1.1 ±0.26	0.7 ±0.13	0.7 ±0.12	35 ±0.00	59 ±0.03	94 ±0.03	13 ±0.00	18 ±0.00	31 ±0.01	4.5 ±0.26	1.62 ±0.03
	H	23 ±0.01	0.31	2.24	1.0 ±0.20	1.6 ±0.26	1.3 ±0.16	0.4 ±0.19	0.3 ±0.20	36 ±0.02	31 ±0.05	67 ±0.07	12 ±0.01	15 ±0.01	27 ±0.01	4.2 ±0.31	2.58 ±0.19
Ni^{2+}	L	220 ±0.02	0.41	1.69	1.3 ±0.12	4.9 ±0.03	3.1 ±0.28	0.4 ±0.01	0.4 ±0.00	18 ±0.01	67 ±0.00	85 ±0.01	10 ±0.00	21 ±0.01	31 ±0.01	3.2 ±0.25	1.39 ±0.02
	M	177 ±0.01	0.36	1.93	1.2 ±0.34	4.5 ±0.39	1.6 ±0.13	0.6 ±0.02	0.5 ±0.02	24 ±0.01	72 ±0.01	96 ±0.00	14 ±0.01	25 ±0.00	39 ±0.01	3.8 ±0.55	1.85 ±0.01
	H	78 ±0.02	0.31	2.24	1.8 ±0.36	4.7 ±0.14	1.2 ±0.89	0.5 ±0.02	0.5 ±0.03	23 ±0.01	41 ±0.02	64 ±0.03	8 ±0.00	14 ±0.01	20 ±0.01	2.4 ±0.75	0.98 ±0.01
Pb^{2+}	L	230 ±0.02	0.41	1.69	1.7 ±0.19	9.4 ±0.13	4.9 ±0.41	0.3 ±0.02	0.2 ±0.01	21 ±0.00	50 ±0.00	71 ±0.00	12 ±0.00	19 ±0.00	31 ±0.00	3.6 ±0.89	1.67 ±0.02
	M	185 ±0.00	0.47	1.47	1.4 ±0.26	9.8 ±0.03	5.0 ±0.38	0.3 ±0.02	0.2 ±0.01	19 ±0.00	71 ±0.00	90 ±0.00	10 ±0.00	27 ±0.00	37 ±0.00	3.3 ±0.15	1.32 ±0.00
	H	193 ±0.01	0.41	1.69	0.9 ±0.13	4.1 ±0.17	2.0 ±0.14	0.6 ±0.01	0.5 ±0.02	21 ±0.00	82 ±0.01	103 ±0.01	10 ±0.00	26 ±0.00	36 ±0.00	4.3 ±0.93	2.34 ±0.04

* O↑ (µmoles O_2 ↑ mg chl. a h^{-1}) ; ** O↓ (µmoles O_2 ↓ mg chl. a h^{-1})S.C = soluble carbohydrates, Ins. C. = insoluble carbohydrates, T.C.= total carbohydrates, S.P.= soluble proteins, Ins. P.= insoluble proteins, T.P.= Total proteins, A.A.= amino acids. L = Low, M= Medium, H= High.

Table (4): Response of *Anabaena circinalis* Rabh to heavy metals toxicity and their effects on growth as well as some metabolic activities.

Treatment		μ (d^{-1})	G (d^{-1})	Dry wt. mg ml^{-1}	Chl. a μg ml^{-1}	$O_2\uparrow$*	$O_2\downarrow$**	Carbohydrates			Proteins			A.A.	Proline
								S.C.	Ins. C.	T.C.	S.P.	Ins. P.	T.P.		
								mg.g dry wt $^{-1}$.							
Control		0.95	0.73	0.68 ±0.01	3.94 ±0.32	7.71 ±0.21	7.6 ±0.01	39 ±0.01	151 ±0.00	190 ±0.01	59 ±0.01	130 ±0.01	189 ±0.01	11.3 ±0.71	0.5 ±0.10
Cd^{2+}	L	0.13	5.3	0.17 ±0.01	1.32 ±0.35	12.1 ±0.05	11.6 ±0.06	108 ±0.06	152 ±0.08	260 ±0.13	84 ±0.07	108 ±0.05	112 ±0.12	27.9 ±0.50	3.0 ±0.27
	M	0.11	6.3	0.24 ±0.01	0.92 ±0.06	12.8 ±0.16	12.1 ±0.38	115 ±0.10	75 ±0.10	190 ±0.00	100 ±0.06	57 ±0.03	157 ±0.03	16 ±0.58	0.8 ±0.05
	H	0.10	6.9	0.68 ±0.01	0.84 ±0.04	12.8 ±0.45	12.1 ±0.62	38 ±0.08	34 ±0.14	72 ±0.06	31 ±0.03	18 ±0.00	49 ±0.03	15.0 ±0.25	0.3 ±0.04
Ni^{2+}	L	0.25	2.8	0.32 ±0.00	1.40 ±0.35	9.5 ±0.22	9.0 ±0.25	78 ±0.02	127 ±0.02	205 ±0.01	81 ±0.01	47 ±0.00	128 ±0.02	13.6 ±0.22	0.9 ±0.09
	M	0.21	3.3	0.38 ±0.01	1.67 ±0.11	8.1 ±0.28	7.8 ±0.33	52 ±0.01	117 ±0.09	169 ±0.09	61 ±0.01	42 ±0.00	103 ±0.01	11.5 ±0.64	0.9 ±0.03
	H	0.13	5.3	0.28 ±0.01	0.83 ±0.04	13.6 ±0.27	12.9 ±0.09	48 ±0.02	111 ±0.01	159 ±0.02	75 ±0.02	55 ±0.00	130 ±0.02	10.9 ±0.96	0.9 ±0.09
Pb^{2+}	L	0.95	0.73	0.75 ±0.00	4.65 ±0.57	6.4 ±0.86	6.0 ±0.75	59 ±0.01	181 ±0.09	240 ±0.12	102 ±0.02	72 ±0.03	174 ±0.04	13.3 ±0.67	1.1 ±0.22
	M	0.80	0.87	0.60 ±0.00	2.58 ±0.70	6.8 ±0.27	6.5 ±0.22	64 ±0.02	60 ±0.02	124 ±0.05	70 ±0.03	40 ±0.01	111 ±0.04	10.5 ±0.60	0.7 ±0.04
	H	0.79	0.88	1.01 ±0.01	3.97 ±0.16	6.0 ±0.40	5.7 ±0.38	46 ±0.05	38 ±0.03	84 ±0.02	51 ±0.03	47 ±0.03	98 ±0.07	8.1 ±0.89	0.5 ±0.03

* $O\uparrow$ (μmoles O_2 $\uparrow$ mg chl. a h^{-1}) ; ** $O\downarrow$ (μmoles O_2 $\downarrow$ mg chl. a h^{-1}),S.C = soluble carbohydrates, Ins. C. = insoluble carbohydrates, T.C.= total carbohydrates, S.P.= soluble proteins, Ins. P.= insoluble proteins, T.P.= Total proteins, A.A.= amino acids. L = Low, M= Medium, H= High.

Table (5): Response of *Wollea saccata* (Wollea) Bornet and Flahault to heavy metals toxicity and their effects on growth as well as some metabolic activities.

Treatment		μ (d^{-1})	G (d^{-1})	Dry wt. mg ml^{-1}	Chl. a $\mu g\ ml^{-1}$	$O_2\uparrow$*	$O_2\downarrow$**	Carbohydrates			Proteins			A.A.	Proline
								S.C.	Ins. C.	T.C.	S.P.	Ins. P.	T.P.		
								mg.g dry wt $^{-1}$..							
Control		0.80	0.87	0.9 ±0.28	0.8 ±0.07	5.8 ±0.71	5.6 ±0.62	5.0 ±0.00	58.3 ±0.01	63.3 ±0.01	64.8 ±0.19	34.3 ±0.02	99.1 ±0.20	9.2 ±0.62	2.9 ±0.26
Cd^{2+}	L	0.58	1.20	0.9 ±0.27	0.6 ±0.01	7.7 ±0.06	7.6 ±0.03	7.3 ±0.01	45.1 ±0.00	52.4 ±0.01	72.0 ±0.00	16.2 ±0.04	88.2 ±0.04	29.2 ±0.43	3.5 ±0.49
	M	0.55	1.26	1.0 ±0.04	0.6 ±0.04	7.2 ±0.72	7.1 ±0.68	8.1 ±0.03	11.6 ±0.08	19.7 ±0.05	83.5 ±0.15	12.5 ±0.03	96 ±0.12	32.2 ±0.25	3.2 ±0.42
	H	0.47	1.47	1.1 ±0.21	0.5 ±0.10	8.0 ±0.01	7.8 ±0.02	7.2 ±0.01	4.8 ±0.01	12.0 ±0.00	76.2 ±0.01	8.0 ±0.02	84.2 ±0.01	22.0 ±0.19	2.1 ±0.31
Ni^{2+}	L	0.43	1.61	1.0 ±0.02	0.5 ±0.03	8.8 ±0.05	8.6 ±0.00	5.8 ±0.01	43.2 ±0.07	49.0 ±0.08	67.1 ±0.01	10.2 ±0.01	77.3 ±0.01	29.4 ±0.33	1.8 ±0.25
	M	0.43	1.61	0.9 ±0.04	0.6 ±0.07	8.2 ±0.60	8.1 ±0.59	8.0 ±0.02	68.5 ±0.14	76.5 ±0.12	75.7 ±0.04	12.2 ±0.01	87.9 ±0.05	35.9 ±0.53	2.8 ±0.27
	H	0.41	1.69	0.6 ±0.01	0.5 ±0.07	8.8 ±0.36	8.6 ±0.35	10.7 ±0.01	76.2 ±0.03	86.9 ±0.04	140.4 ±0.44	18.4 ±0.05	158.9 ±0.49	52.4 ±0.15	5.6 ±0.41
Pb^{2+}	L	0.56	1.24	1.2 ±0.03	0.9 ±0.08	8.8 ±0.11	8.4 ±0.08	11.8 ±0.04	127 ±0.12	138.8 ±0.16	90.2 ±0.03	17.3 ±0.05	107.5 ±0.08	41.5 ±0.40	2.3 ±0.31
	M	0.53	1.31	1.2 ±0.08	0.9 ±0.12	10.6 ±0.53	10.4 ±0.56	10.9 ±0.02	111.6 ±0.26	122.5 ±0.25	84.3 ±0.06	14.8 ±0.01	99.1 ±0.07	36.8 ±0.62	1.6 ±0.12
	H	0.47	1.47	0.8 ±0.03	0.8 ±0.10	11.5 ±0.59	10.8 ±0.91	22.4 ±0.01	217 ±0.03	239.4 ±0.02	170.4 ±0.24	32.8 ±0.03	203.2 ±0.27	80.4 ±0.30	3.6 ±0.19

* $O\uparrow$ (µmoles O_2 $\uparrow$ mg chl. a h^{-1}) ; ** $O\downarrow$ (µmoles O_2 $\downarrow$ mg chl. a h^{-1}),S.C = soluble carbohydrates, Ins. C. = insoluble carbohydrates, T.C.= total carbohydrates, S.P.= soluble proteins, Ins. P.= insoluble proteins, T.P.= Total proteins, A.A.= amino acids. L = Low, M= Medium, H= High.

saccata and the lowest one in *S. obliquus* (Tables 1-5). Proline has been reported to be accumulated in tissues and/or organs of plant subjected to drought, salt, temperature, heavy metals stress, or infected by some pathogens in plants (**Nikolopoulos and Manetas, 1991; Alia Saradhi, 1992**). Proline inhibits metal-induced loss of potassium ions in *Chlorella vulgaris* and *Trebowxia erici* (chlorophyta) (**Backor *et al.* 2004**).

Wu *et al.* (**1995 and 1998)** mentioned that copper and cadmium treatment enhanced proline accumulation in green algae (*Chlorella* sp., *Pediastrum duplex*), the diatom (*Nitzschia palea*) and a Cyanobacterium (*Anacystis nidulans*). The relationship between proline level and metal accumulation revealed that the accumulation of free proline corresponds to the uptake of the metals (Cd^{2+}, Ni^{2+} and Mn^{2+}) by *Scenedesmus armatus* cells (**El-Enany and Issa, 2001**). Proline accumulation may play a role in heavy metal detoxification (**Costa and Morel, 1994**); it could be involved in metal chelation in cytoplasm (**Farago and Mullen, 1979**), especially in the case of metals with a preference for nitrogen or oxygen coordination over phytochelations (**Grill *et al.*, 1987**).

The overall conclusion of this work is that heavy metals exerted disturbances on the size of various metabolic pools of algae. The degree of disturbance depends upon the metal used and the algal species.

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الخلل في المسارات الايضية لبعض عزلات طحالب التربة كاستجابة لسمية العناصر الثقيلة

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أجرى هذا البحث لتوضيح الأثر السام للعناصر الثقيلة (الكادميوم والنيكل والرصاص) بتركيزات مختلفة (منخفض و متوسط و عالي) على النمو وبعض المسارات الأيضية لثلاثة أنواع من الطحالب الخضراء(*Chlamydomonas reinhardtii Dang., Chlorococcum humicola Nag.,* *Scenedesmus obliquus*(*Turp*)*Kütz*) ونوعين من الطحالب الخضراء المزرقة (*Anabaena circinalis Rabh* and *Wollea saccata (Wolle) Bornet and Flahault.*) وأسفر عن نتائج أمكن ايجازها فيما يلى:

أ)- تأثر بشدة كل من معدل النمو مسجلا إنخفاضا ملحوظا للطحالب فى جميع التركيزات المستخدمة ، وكان أكثر العناصر سمية هو النيكل والكادميوم بينما أقل العناصر سمية هو الرصاص. بينما سجل معدل النمو زيادة ملحوظة لطحلب *Anabaena circinalis* باستخدام التركيز الأدنى والمتوسط من الرصاص.

ب)- تناقص بشدة العدد الكلى وكلوروفيل أ ، ب وكذلك الوزن الجاف للطحالب الخضراء وذلك بزيادة تركيز العناصر الثقيلة المستخدمة وكان النيكل والكادميوم أكثر سمية على الأصباغ النباتية ، كما أظهر كلوروفيل (أ) والوزن الجاف لطحلبى *Anabaena circinalis, Wollae saccata* ناقص عند استخدام الكادميوم والنيكل وزاد كلوروفيل (أ) فى *Wollea saccata* زيادة طفيفة باستخدام عنصر الرصاص.

ج)- كان معدل السمية فى الطحالب الخضراء بالعناصر الثقيلة المستخدمة على النحو التالى: النيكل ثم الكادميوم ثم الرصاص ، بينما تغير فى *Anabaena circinalis* إلى الكادميوم ثم النيكل ثم الرصاص.

د)- تناقص معدل البناء الضوئى لجميع الطحالب بزيادة تركيز العنصر الثقيل المستخدم وازداد معدل التنفس فى جميع التركيزات المستخدمة لجميع أنواع الطحالب. وعموما كان للكادميوم والنيكل تأثير سلبى على عملية البناء الضوئى ، وكانت الطحالب الخضراء أكثر مقاومة من الطحالب الخضراء المزرقة.

هـ)- تراكمت السكريات والبروتينات الذائبة بزيادة العناصر الثقيلة على حساب السكريات والبروتينات غير الذائبة فى جميع الطحالب قيد الدراسة.

و)- حدثت زيادة ملحوظة لكل من الأحماض الأمينية الحرة والحمض الأمينى البرولين فى خلايا الطحالب المستخدمة وذلك تحت تأثير العناصر الثقيلية وسجلت أكثر زيادة فى الأحماض الأمينية والحمض الأمينى البرولين فى كل من *Anabaena circinalis, Wollea saccata* وأقل محتوى فى طحلب *Scenedesmus obliquus*.

واخيرا يجدر الاشارة الى ان العناصر الثقيلة الثلاثة المستخدمة احدثت تثبيطا فى النمو ومعظم المسارات الأيضية فى الطحالب قيد البحث ، ويعتمد هذا التأثير على نوع العنصر المستخدم وتركيزه وكذلك نوع الطحلب.