

## Impact of Different Concentrations of Zinc and Cadmium Chloride on Micro Algae *Chroococcus Minutus*

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**Abstract:** In the present investigation the effect of different concentrations of zinc and cadmium chloride ( $ZnCl_2$  and  $CdCl_2$ ) on one of the Cyanophyta "*Chroococcus minutus*" isolated from ponds in Sana'a Yemen was evaluated. The two heavy metals adversely affected cell division; however the protein and glucose contents of the cells were slightly affected. They caused destruction of chlorophyll a as the result obtained by measuring the optical density and the chlorophyll content. These observations were interpreted in terms of the action of metals on the reduction and oxidation steps in the biosynthesis pathway of pigments.

**Key words:** Cyanophyta, *Chroococcus* sp, chlorophyll, zinc chloride, cadmium chloride.

### INTRODUCTION

Recent attention has focused on the biological response to conditions of heavy metals stress in natural waters and cultivated soils. Algae are widely used as indicators of toxic substances, including metals in natural waters (Chiaudari and Vighi 1978; Bringmann and Kuhn 1978). Knauer *et al.* (1997) studied the growth of algae that indicate a high tolerance toward high cells may immobilize the metals intracellular. Toxic metals increased the lag phase of *Chlamydomonas variabilis* and lowered the growth rate of *Euglena gracilis* (Bonaly *et al.*, 1978). The study of algal-metal ion interactions is of particular importance, principally because the algae and other simple plants are the basic of food chain. The interest in the interactions between microorganisms and metal ions dissolved in aqueous is a long-standing one. With regard to the growth of algae subjected to heavy metal stress, some workers clarified this phenomenon (Rachlin and Farran, 1974; Kogan *et al.*, 1975; Rosko and Rachlin, 1977; Petriya, 1978 and De Filippis *et al.*, 1981).

The impact of chromium on heterotrophic bacteria and photosynthetic microorganisms by Viti and Givannetti (2001) showed that a chronic high concentration of chromium in soil affected both oxygenic photosynthetic microorganisms and heterotrophic bacteria, in the soil with low chromium levels, *Nostoc* dominated and possessed numerous hetrocysts.

This investigation is an endeavor to elucidate the stressing effects of various concentrations of zinc and cadmium upon the growth criteria of one organism that is common fresh water phytoplankton, this organism is *Chroococcus minutus* as a unicellular Cyanophyta in order to compare and contrast the tolerance to and role of these elements in algal growth. Therefore, this report will form the basis of more extensive and detailed work on the subject.

### MATERIALS AND METHODS

#### **Experimental Material:**

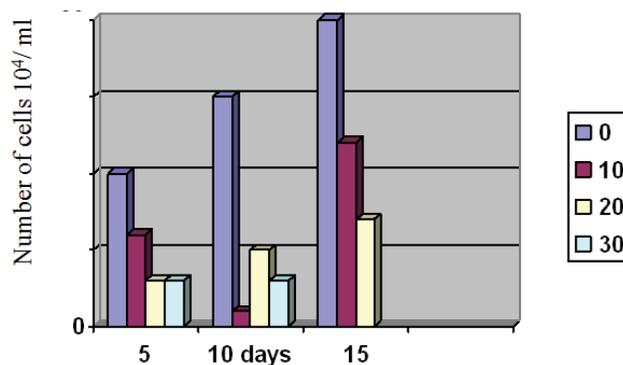
Cells of unicellular blue-green alga *Chroococcus minutus* Nageli 1849 (after Prescott 1962) was isolated from soil collected from Sana'a province. Isolation and purification was made by dilution and plating technique. One hundred ml of Alien and Agnomens medium (1955) were autoclaved in 250 ml Erlenmeyer glass flask, then inoculated with 10 ml of the algal cells  $5 \times 10^4$  cells / ml. Cultures were grown in temperature  $25 \pm 2$  °C in incubator provided by cool white fluorescent lamps set on 16:8-light and dark respectively photo-regime for 15 days. All cultures were shaken twice daily to prevent cells from clumping. Experiments were carried out in triplicate.

**Determination of Algal Growth Parameter:**

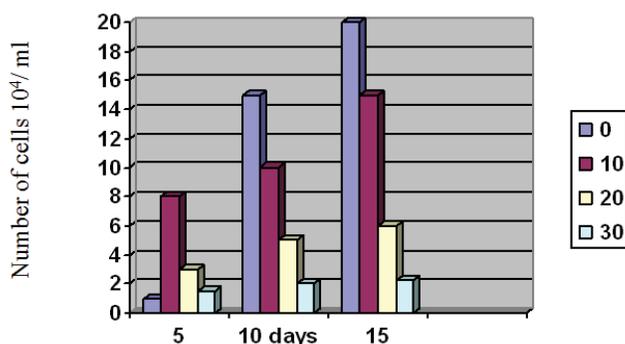
Cells were counted by 0.1 mm deep haemocytometer slide monitored the growth of cultures. Also the growth of cultures determined by measuring the optical density. (O.P) of cell suspension spectrophotometrically at 650 nm as described by Wetherell (1961). The change in dry weight production was determined by washing 5 ml from the culture with distilled water and incubated for 24 hours at 95 °C for drying, then weighted until constant weight. The chlorophyll a content was determined by taking 3 ml of culture by distilled water and the pellet was diluted by methanol (90%) and transferred to water bath at 55 °C for 20 minutes, then centrifuged (at 3000 rpm.), then take the supernatant which was colored and measure the chlorophyll a as described by Metzner *et al* (1965). The glucose content of algal cells measured as described by Dubois *et al* (1956). The total protein content of the cells was quantitatively determined as described by Lowery *et al.* (1951).

**RESULTS AND DISCUSSION**

The effects of zinc and cadmium on the unicellular Cyanophyta *Chroococcus minutus* indicate that each metal depresses the growth rate. In fig. 1 (a and b) the data shows the effects of two heavy metals by using different concentrations (10,20, and 30 ppm.) on *Chroococcus minutus* stimulate the growth by increasing in number of cells specially after 5 days in comparison by control; but after 10 and 15 days the growth rate decreased by decreasing the number of cells. When the dose of metal increased the number of cells decreased. These results in agreement with previous published data on other algae (Kamp-Nielsen, 1971; Nazzi, 1972; Rachlin and Farran 1974).



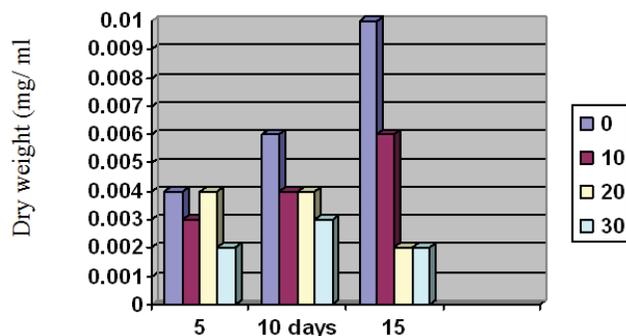
**Fig. 1a:** The effect of different concentrations of Cadmium chloride on growth of *chroococcus minutes* (cell number)



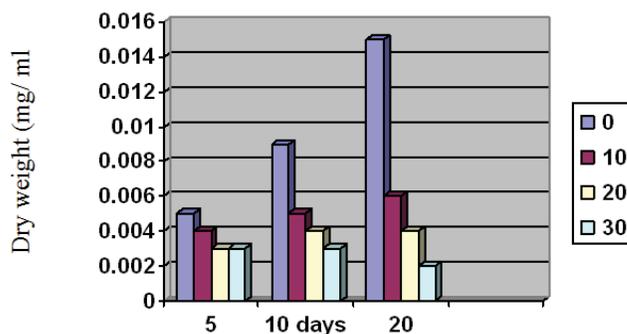
**Fig. 1b:** The effect of different concentrations of Zinc-Chloride on growth of *chroococcus minutes* (cell number)

The data in Fig.2 (a and b) showed the effect of two heavy metals (Zn and Cd) on *Chroococcus minutus* by determination of dry weight. Also the results indicated stimulation in growth rate after 5 days from initial incubation by increasing in dry weight but after 10 and 15 days the effect of metals inhibited the growth by

decreasing the dry weight, this indicated that there was relationship between the number of cell and the cell division and the dry weight. In Fig.3 (a and b) the growth rate of *Chroococcus minutus* under the effect of Zn and Cd was determined by measuring spectrophotometrically the optical density (O.D) at 650 nm of culture. The results indicated that there was high inhibitory effect at all doses used (10, 20 and 30 ppm).



**Fig. 2a:** The effect of different concentrations of Cadmium chloride on growth of *chroococcus minutes* (dry weight mg/ ml)

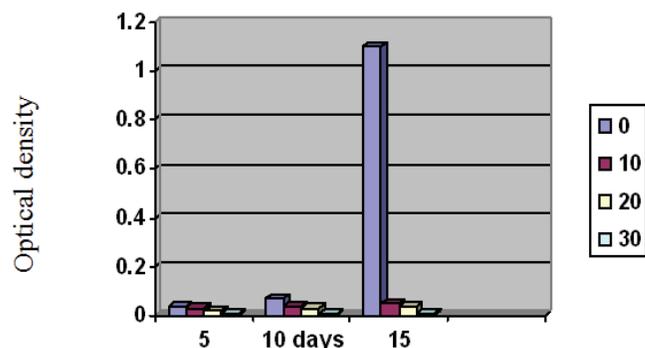


**Fig. 2b:** The effect of different concentrations of Zinc chloride on growth of *chroococcus minutes* (dry weight mg/ ml)

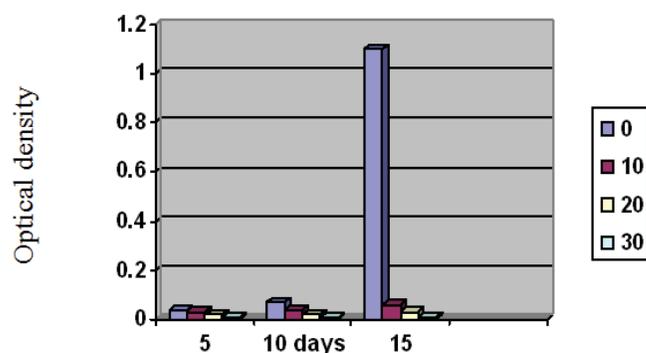
The results recorded similar to inhibitory effects of heavy metals (Zn ,Cd and others) on pigment accumulation (synthesis) noticed in investigations particularly at higher doses which were previously reported by some workers (Roskosk and Rachlin, 1977; and Defilippis *et al.*, 1981). The data in Table (1) showed the growth rate by measuring the synthesis of chlorophyll a of the organise *Chroococcus minutus*, after 5 days was relatively the same with the three doses used, but after 10 and 15 days the results obtained under the effect of Zn and Cd were more inhibitory with all doses used This means that in spite of the presence of cells, but has no effect on chlorophyll "a" synthesis in comparison with control. So the two heavy metals (Zn and Cd) caused an elevation in chlorophyll a synthesis and this result agree with general assumption that this measure can be used as indicator of stress, as observed by De Filippis and Pallaghy (1976) with *Chlorella* cells under heavy metals (Zn and Hg) stress condition.

The effect of (Zn and Cd) on *Chroococcus minutus* was obtained by measuring the glucose content which is relatively the same as control after 5 days but after 10 and 15 days, the heavy metals was more effective in decreasing the glucose content (Table 2) but the effect of Zinc was more than the effect of Cadmium with all doses used at the same period of treatment. This data is in agreement with the result obtained by Canterford *et al.* (1978) They found that the toxicity of Zn is more than Cd.

The data in Table (3) clearly showed the effect of two heavy metals (Zn and Cd) on the protein content in *Chroococcus minutus*, which decreased by increasing the dose of these metals. Also, the inhibitory effect of Zinc is more than Cadmium.



**Fig. 3a:** The effect of different concentrations of Cadmium chloride on growth of the *chroococcus minutus* (optical density at 650 nm)



**Fig. 3b:** The effect of different concentrations of Zinc chloride on growth of *chroococcus minute*(optical density at 650 nm)

**Table 1:** Effect of different concentrations of both Zinc and Cadmium chloride (ppm) on *Chroococcus minutus* (Chlorophyll "a" content  $\mu$ /ml.).

Treatment	Concentrations (ppm)	Days of treatment		
		5	10	15
ZnCl <sub>2</sub>	0	1.546	11.08	16.447
	10	0.564	0.358	0.375
	20	0.247	0.246	0.126
	30	0.247	0.232	0.025
CdCl <sub>2</sub>	0	1.546	11.08	16.447
	10	0.635	1.011	0.502
	20	0.435	0.494	0.243
	30	0.199	0.135	0.023

**Table 2:** Effect of different concentrations of both Zinc and Cadmiumchloride (ppm) on *Chroococcus minutus* ( $\mu$ g/ml, glucose).

Treatment	Concentrations (ppm)	Days of treatment		
		5	10	15
ZnCl <sub>2</sub>	0	192.6	313.7	386.2
	10	203.7	268.9	140.5
	20	192.6	221.3	132.3
	30	185.2	214.1	117.7
CdCl <sub>2</sub>	0	192.6	213.7	386.2
	10	210.2	320.3	192.6
	20	203.7	234.5	149.7
	30	203.7	205.1	116.4

**Table 3:** Effect of different concentrations of both Zinc and Cadmium chloride (ppm) on *Chroococcus minutus* (Total protein mg/ml.).

Treatment	Concentrations (ppm)	Days of treatment		
		5	10	15
ZnCl <sub>2</sub>	0	1.24	1.36	1.62
	10	0.49	0.21	0.19
	20	0.45	0.18	0.16
	30	0.37	0.15	0.12
CdCl <sub>2</sub>	0	1.36	1.24	1.62
	10	0.55	0.38	0.25
	20	0.39	0.24	0.18
	30	0.34	0.21	0.11

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