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EFFECTS OF PH ON TOXICITY OF CADMIUM, COBALT AND COPPER TO THE GREEN ALGA *SCENEDESMUS BIJUGA*

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Abstract

This study was conducted to elucidate the effects of pH on the toxicity of cadmium, cobalt and copper to the green alga *Scenedesmus bijuga*. The sublethal concentrations of Cd^{2+}, Co^{2+} and Cu^{2+} were obtained at 10^{-6}, 10^{-5}, 10^{-5} M, respectively. A standard initial inoculum of the tested alga was used to inoculate the culture flasks containing Kuhl's medium with different pH values (4, 6, 8, 10 and 12) supplied with the sublethal concentrations of each tested metal. The results revealed that the growth of *Scenedesmus bijuga* increased with increasing pH. However, the accumulation, the accumulation factor and the amount of free ionic forms of each metal decreased. Generally, it appears that the tested metals exert more toxic effect in acidic than in alkaline pH conditions.

Key Words: Algae; Heavy metals; pH; Toxicity

Introduction

The most important heavy metals from the point of view of water pollution are zinc, copper, lead, cadmium, mercury, nickel, cobalt and chromium (Vymazal 1995). Of these some metals (e.g. copper, zinc and cobalt) are essential trace elements to living organisms, but become toxic at higher concentrations. Other metal pollutants are non-essential and toxic even at relatively low concentrations (e.g. Pb, Hg, and Cd).

A variety of environmental factors are known to modify the toxicity of heavy metals on algae (Rai and Dey, 1980; Stokes, 1983; Vymazal, 1995; Khalil, 1997; Fathi *et al*., 2000; Pawlik, 2001; Pollumaa *et al*., 2001; Fathi, 2002, Winch *et al*., 2002; Siripornadulsil *et al*., 2002; Janssen *et al*., 2003; Fathi and Zaki, 2003). One of the most important of these factors concerns the determination of biological availability and the metals physiochemical state (Langston, 1990). Metal adsorption (Sequesterization) on to water borne particles or complexation with dissolved organics generally will reduce metal toxicity. However, in the natural environment it is often difficult or even impossible to characterize the form in which the metal exists (Lobban and Harrison, 1997).

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Both pH and redox potential can exert considerable effects on metal availabilities and ionization and thus the toxicity of heavy metals (Müller and Payer, 1979; Michnowiez and Weaks, 1984; Peterson *et al.*, 1984; Campbell and Stokes, 1985; Vymazal, 1990; Vymazal, 1995). Furthermore, metal accumulation by algae is influenced by a number of abiotic and biotic factors, one of these being pH (Vymazal, 1995). The objective of this study was to establish the response of the green alga *Scenedesmus bijuga* at different water pH valves to sublethal concentrations of Cd^{2+}, Co^{2+} and Cu^{2+}. These three previous heavy metals are known to be common in the Egyptian polluted waters (Fathi and Zaki, 2003) and accordingly were chosen for experimentation.

Material and Methods

Scenedesmus bijuga (Turp.) Lagerh., isolated from the Nile, was grown at a temperature of 27°C in Kuhl's medium (Kuhl, 1962), modified as follows: copper was eliminated as well as all known chelators (ferric citrate, citric acid, and Na-EDTA). The trace metal levels were reduced to 1/20th of the original amount proposed by Khul. Furthermore, 5 mM $NaHCO_3$ were added. All solutions were prepared using Millipore membrane filter 0.45μM (Schleicher & Schüll, Germany) filtered. All glassware was soaked for 24 h in 10-15% nitric acid, rinsed in distilled water, and air-dried before use.

Regarding the toxicity experiments, a standard cell number of the isolated alga was inoculated into 100-ml Kuhl's medium in 250-ml Erlenmeyer flasks. The cultures were supplied with various concentrations of each studied metal ranging from 10^{-3} to 10^{-9} M. The salts of cadmium and cobalt were supplied in the form of chlorides and copper as sulphate. At the end of the incubation period (7 days) the cultures were filtered and washed several times with metal-free medium; at least three replicates for each sample and controls were used.

Assay of the effects of pH on the toxicity of metals was carried out in 250 ml Erlenmeyer flasks. Khul medium adjusted to five different pH values (4, 6, 8, 10 and 12), supplied with the sublethal concentrations of cadmium, cobalt and copper (10^{-6} M, 10^{-5}M and 10^{-5}M, respectively). A metal- free medium was used as a control. These sublethal concentrations were suggested after a previous screening experiment (Fathi, unpublished). The pH was adjusted in each flask using an Extech 321990-pH meter. All assays were conducted using three replicates. A standard initial inoculum of the isolated alga was inoculated to the culture flasks. At the end of the incubation period, a 20-ml aliquot from each standing culture was filtered through a preweighed and dried 0.45 μm membrane filter (Schleicher & Schüll, Germany). The filters were again dried in an oven at 70°C for 12 h, placed in a desiccator for 3 h and weighed. Chlorophyll a was estimated in acetone extract according to Metzner *et al.*, (1965) and cell number was determined using a Hematocytometer chamber.

Metal accumulation by algal cells was measured after digestion of the washed and dried material. Digestion entailed 15 minutes in a boiling mixture of concentrated HNO_3 and HCl (1:1 V/V). The metal concentrations were then measured using a double beam Atomic Absorption Spectrophotometer (GBC 902 USA). Calculated values are the mean of triplicates, the standard deviation was less than 5% of these mean values. Results were tested by one-way analysis of variance (ANOVA). ANOVA effects and treatments differences were considered significant when $P< 0.05$.

Results and Discussion

Figure (1) shows cell number and Chlorophyll a content of *Scenedesmus bijuga* in a medium containing different concentrations of Cd^{2+}, Co^{2+} and Cu^{2+} (ranging from 10^{-3} to 10^{-9} M) after the incubation period. Results clearly show that the inhibitory and /or stimulatory effects of either of the used heavy metals depend on metal concentration. The higher doses of these metals strongly and adversely affected all the tested growth parameters. The total cell death occurred at concentrations 10^{-5} M of cadmium and at 10^{-4} M of cobalt and copper (lethal concentrations). The results also show that the inhibitory effect of cadmium to algal growth and Chlorophyll a content is more pronounced than for the other two tested metals. This indicates that all the tested metals had interfered with the metabolic pathways. Kahlil (1997) reported that the growth of *Phormidium fragile* decreased with increased concentration of mercury from 0.01 to 1.5 mg/ L. Fathi *et al*. (2000) reported that the higher doses of cobalt, mercury and vanadium strongly affected the growth criteria of *Scenedesmus bijuga* and *Anabaena spiroides.* The inhibitory effects of heavy metals on pigment accumulation, noticed in this investigation particularly at higher doses, may be attributed to inhibition of reductive steps in their biosynthetic pathway (De Filippis *et al*., 1981). Okamoto *et al.* (2001) reported that heavy metals are able to induce oxidative stress in chloroplasts of the unicellular alga *Gonyaulax*, particularly under acute conditions in addition to oxidative damage to proteins and lipids occurred in cells. The observed concentration-dependent reduction in Chlorophyll a, and cell count is in a good agreement with the findings of Hofner *et al.,* (1987), Rai *et al.,* (1991), Fathi *et al*., (2000), Fathi (2002) and Sponza (2002).

The data in figure (2) show that the dry weight, Chlorophyll a content and cell number were markedly enhanced with increasing pH towards the alkaline range, but slightly depressed at pH over than 8. Generally, one can see that in these experiments the toxicity of the tested metals decreased at the alkaline conditions. Campbell and Stokes (1985) divided metals into two groups: those for which a decrease in pH causes a decreased biological uptake (e.g. Cd, Cu, Zn), and those for which lower pH increases metal availability and therefore a decrease in a biological activity (e.g. Pb). Rai *et al*., (1981) pointed out that an increase in pH in the alkaline range has been found to increase the toxicity of heavy metals to natural populations of algae and to *Chlorella vulgaris* and *Hormidium rivulare* (in culture). However other relevant studies (Michnowiez and Weaks 1984; Fathi, 1995; Vymazal, 1995) indicated that heavy metals toxicity decreases in alkaline pH conditions. This observation is in good agreement with the data presented in this investigation.

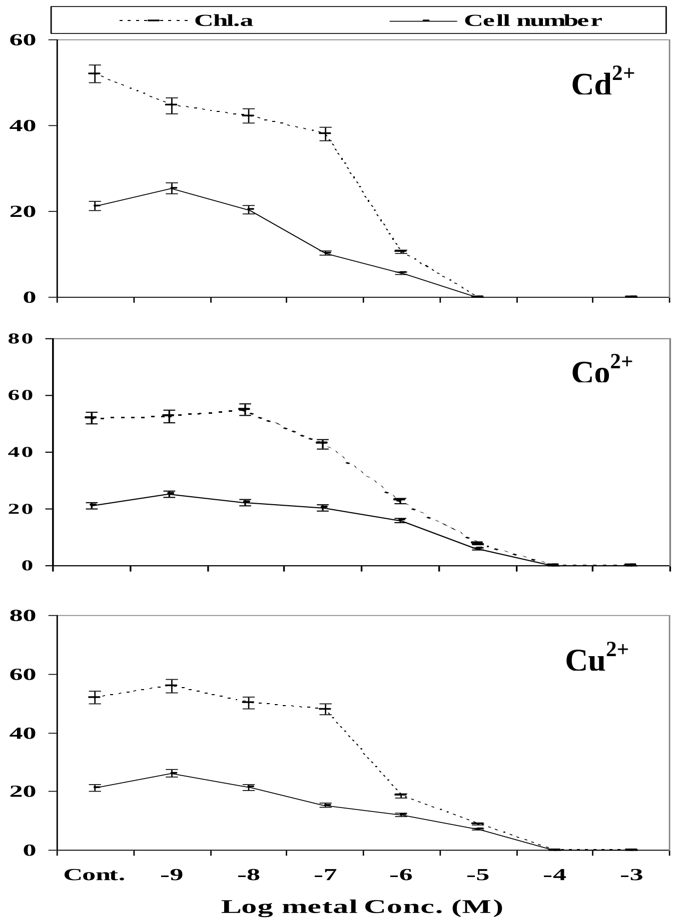

Figure (1): Effect of various concentrations of Cd^{2+}, Co^{2+} and Cu^{2+} on Chlorophyll a content (mgl^{-1}) and cell number (No. x 10^7 l^{-1}) of *Scenedesmus bijuga*. Vertical bars indicate SE, n=3.

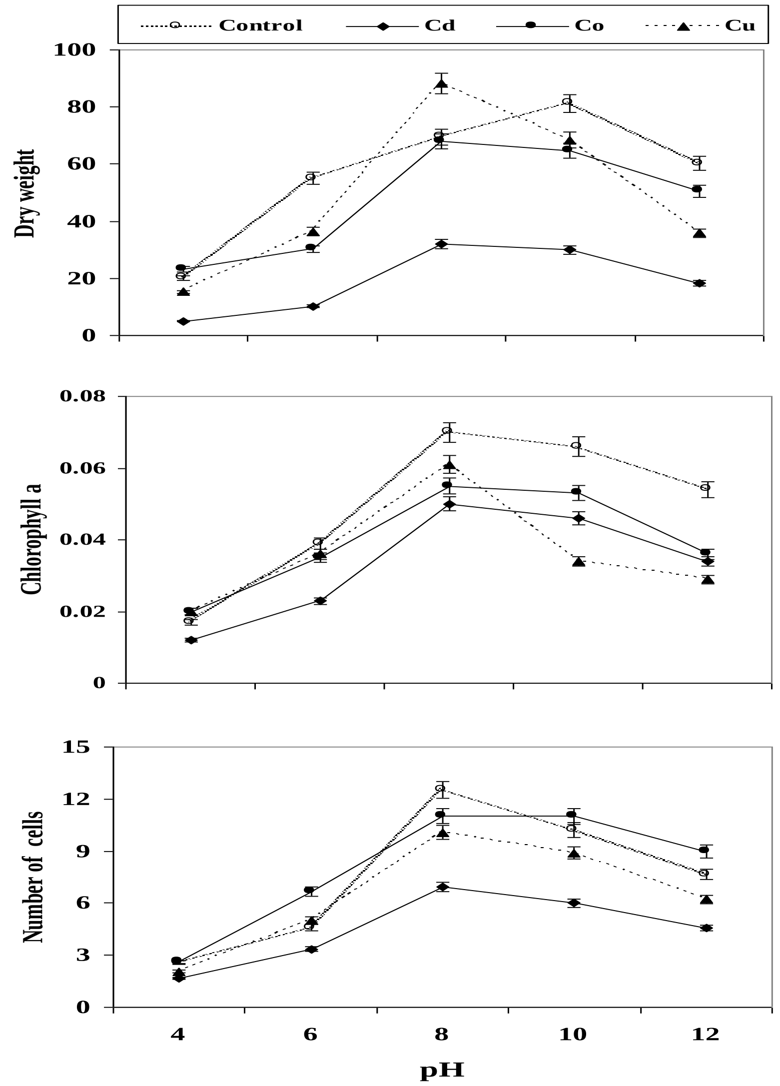

Figure (2): Dry weight (mgl^{-1}), Chlorophyll a content (µM) and cell number (No. x 10^7 l^{-1}) of *Scenedesmus bijuga* at different pH values in the presence of heavy metals at sublethal concentrations after 7 days incubation period. Vertical bars indicate SE, n=3.

Metal accumulation by algae is influenced by a number of abiotic (e.g. pH, chelating agents, redox potential, temperature, light) and biotic (e.g. cellular activity, algal biomass concentrations, extra cellular products) factors, which interact with the organisms and result in a complexity of relationships that determine the degree of metal accumulation (Lorch and Weber, 1985). Brooks and Rumsby (1965) defined the bioaccumulation factor as the ratio of concentration of an element in dry plant biomass and that in the water. The results of this investigation clearly show that the accumulation and bioaccumulation factors of the metals tested with *Scenedesmus bijuga* decreased with increasing pH values (Table 1). The accumulated metals by *Scenedesmus* are, in the order of the amount taken up: cobalt > copper > cadmium. Different organisms, however, have different sensitivities to the same metal, and the same organisms may be more or less damaged by different metals (Nakajima *et al*., 1981; Gadd, 1988; Reed and Gadd, 1990; Fathi and Falkner 1997; Fathi, 2002). It is also known that the uptake of an element from the surrounding medium is seldom exactly proportional to the amount of free ion present in the medium (Baudo, 1987). However, in this investigation the amount of accumulated metals decreased with increasing pH values, although the total metals concentrations were constant. This effect is attributed to the effect of pH and metal speciation and therefore in the available metal forms. Anderson and Morel (1982) demonstrated that the concentration of free ions controls the availability of metals.

Table 1. Effect of different pH values on the metal accumulation and bioaccumulation factor of *Scenedesmus bijuga* after 7 days incubation period.

Metal	Parameters / pH	4	6	8	10	12
Cd^{2+}	Metal accumulation (mg/g dry weight)	18.21	10.36	5.11	5.96	5.21
	Bioaccumulation factor	16.20	9.22	4.54	5.30	4.63
Co^{2+}	Metal accumulation (mg/g dry weight)	35.06	33.00	21.62	18.57	16.22
	Bioaccumulation factor	59.49	56.05	36.68	31.56	27.53
Cu^{2+}	Metal accumulation (mg/g dry weight)	29.62	25.56	15.31	12.62	14.00
	Bioaccumulation factor	46.62	40.22	24.09	19.86	22.03

From another point of view, evidence indicates that generally free ions are the most available metal species (Anderson and Morel 1982; Fathi, 1995; Fathi and Falkner, 1997). The concentration of a free metal ion in a heavy metal solution depends on the type and the concentration of anionic species, as well as on the existing pH value. On the other hand, dissolution of heavy metal ions can be expected to be in equilibrium with respective hydroxides or carbonates (Stumm and Morgan, 1981). The pH-dependence of the logarithmic heavy metal concentrations in equilibrium with the respective hydroxide or carbonate should describe a straight line (Fathi, 1995). According to Stumm and Morgan (1981) the solubility of heavy metals or the amount of free ionic forms of tested metals can be calculated for the investigated pH values using the following equations:-

(i) Assuming solubility with metal carbonates

$\text{Log}(M^{2+}) = \text{Log } K - \text{pH} - \text{Log}(HCO_3^-)$

(ii) Assuming solubility with metal hydroxides

$\text{Log } (M^{2+}) = \text{Log K} - 2 \text{ pH}$

Log K is the logarithmic the equilibrium constant.

Figure (3) shows the solubility (or free ionic forms) of tested heavy metals as carbonates or hydroxides under the investigation conditions as a function of pH at constant HCO_3^- (1 mM), in a Log (M^{2+}) - pH diagram. The data show that the amount of free ions of all tested metals decreased toward the alkaline range. Generally, it could be concluded that at low pH, metals exist as free cations, but at an alkaline pH, like that of Nile water, they precipitate as insoluble hydroxides or carbonates. Therefore the pH can have considerable effects on the availabilities and thus the toxicity and accumulation of heavy metals (Anderson and Morel, 1982; Peterson et al., 1984). Finally, it may be commented that the observations that the toxicity of cadmium, cobalt and copper to *Scenedesmus bijuga* decreases at increased pH toward into the alkaline range. It is suspected that this relationship may be of ecological importance in the control of pollution particularly acid pollution.

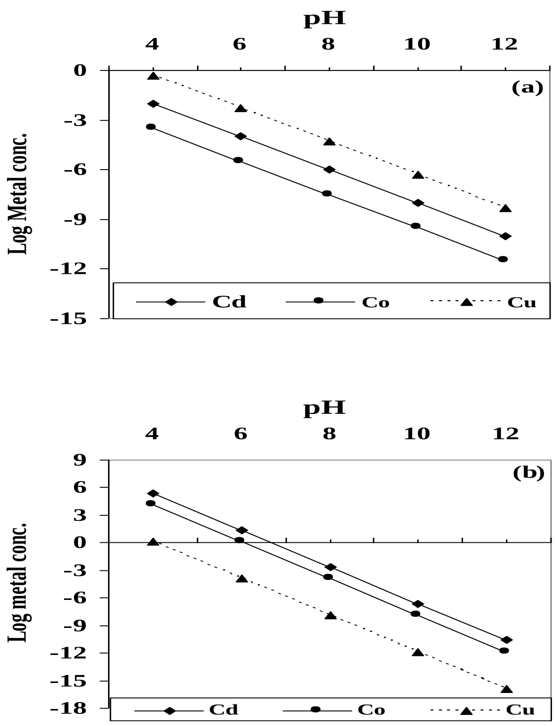

Figure (3): The solubility (or free ionic forms) of tested heavy metals as carbonates (a) and hydroxides (b) under the investigation conditions as a function of pH at constant HCO_3^- (1 mM).

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تأثير الرقم الهيدروجيني على سمية كل من الكادميوم والكوبالت والنحاس للطحلب الأخضر السيندسموز بوجوجيا

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تم في هذا البحث دراسة تأثير مستويات مختلفة من الرقم الهيدروجيني على سمية كل من الكادميوم والكوبالت والنحاس باستخدام الطحلب الأخضر سيندسموز بوجوجيا. وقد أظهرت النتائج أن التركيزات قبل المميتة لكل من الكادميوم والكوبالت والنحاس هي 10^{-5}، 10^{-6}، 10^{-6} موللر على التوالي.

وقد أظهرت النتائج أيضا أن مقاييس النمو المختلفة (الوزن الجاف، كلوروفيل أ و عدد الخلايا) قد زادت بزيادة الرقم الهيدروجيني ناحية الجهة القاعدية، بينما انخفضت نسبة التراكم ومعامل التراكم وكمية الأيونات الحرة للعناصر محل الدراسة. وعموما فان التأثير السام للعناصر محل الدراسة فد ذاد في الأوساط الحمضية وقل في الأوساط القاعدية.