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# Effects of pH on toxicity of cadmium, cobalt and copper to *Scenedesmus bijuga*

Adel A. Fathi [1] and Mohammed A. Al-Omair [2]

[1] *Biology Department, College of Science, King Faisal University, Saudi Arabia*
[2] *Chemistry Department, College of Science, King Faisal University, Saudi Arabia*

## Summary

This study was conducted to elucidate the effect of pH on the toxicity of cadmium, cobalt and copper to the green alga *Scenedesmus bijuga*. 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 metals tested exert more toxic effect in acidic than in alkaline pH conditions.

**Key words**: algae, *Scenedesmus bijuga*, 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). Some of these metals (e.g., Cu, Zn, Co) are essential trace elements for 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, Cd).

A variety of environmental factors is known to modify the toxicity of heavy metals to 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). Among them are biological availability and physio-chemical state of the metal (Langston, 1990). Metal adsorption (Sequesterization) by water-borne particles or complexation with dissolved organics will generally reduce metal toxicity. However, it is often difficult or even impossible to characterize the form in which the metal exists in the natural environment (Lobban and Harrison, 1997).

Both pH and redox potential can have considerable effects on metal availability and ionization and thus the toxicity of heavy metals (Muller and Payer, 1979; Michnowiez and Weaks, 1984; Peterson et al., 1984;

Campbell and Stokes, 1985; Vymazal, 1990, 1995). Furthermore, metal accumulation by algae is influenced by a number of abiotic and biotic factors, including pH (Vymazal, 1995). The objective of this study was to reveal the response of the green alga *Scenedesmus bijuga* at different water pH values to sublethal concentrations of $Cd^{2+}$, $Co^{2+}$ and $Cu^{2+}$. These three heavy metals are known to be common in Egyptian polluted waters (Fathi and Zaki, 2003).

## 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 were all known chelators (ferric citrate, citric acid, and Na-EDTA), and trace metal levels were reduced to 1/20th of the original amount proposed by Kuhl. Furthermore, 5 mM $NaHCO_3$ was added. All solutions were prepared using Millipore membrane filter 0.45 μM (Schleicher and Schüll, Germany). Before use, all glassware was soaked for 24h in 10-15% nitric acid, rinsed in distilled water and air-dried.

In toxicity experiments, a standard number of isolated algal cells was inoculated into 100-ml Kuhl's medium in 250-ml Erlenmeyer flasks. The cultures were supplied with various concentrations of each of the metals ranging from $10^{-3}$ to $10^{-9}$ M. The salts of cadmium and cobalt were supplied as 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. Kuhl medium, adjusted to five different pH values (4, 6, 8, 10 and 12), was 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 (Adel A. Fathi, unpublished). The pH was adjusted in each flask using a 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 and Schüll, Germany). The filters were again dried in an oven at 70°C for 12h, placed in a desiccator for 3h 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). 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 being 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 at $P < 0.05$.

## Results and Discussion

Table 1 shows the number of *Scenedesmus bijuga* and their Chlorophyll a content in a medium containing different concentrations of $Cd^{2+}$, $Co^{2+}$ and $Cu^{2+}$ (ranging from $10^{-3}$ to $10^{-9}$ M) after incubation. The results show clearly that the inhibitory and/or stimulatory effects of each of the heavy metals used depend

**Table 1**. Effect of $Cd^{2+}$, $Co^{2+}$ and $Cu^{2+}$ on the Chl a content ($mgl^{-1}$) and cell number (No. $\times 10^7$ $l^{-1}$) of *Scenedesmus bijuga* at various concentrations.

| Metal conc.(M) | Cd | | Co | | Cu | |
|---|---|---|---|---|---|---|
| | Chl a. Content ($mg\ l^{-1}$) | Cell number (No. $\times 10^7 l^{-1}$) | Chl a. Content ($mg\ l^{-1}$) | Cell number (No. $\times 10^7 l^{-1}$) | Chl a. Content ($mg\ l^{-1}$) | Cell number (No. $\times 10^7 l^{-1}$) |
| Cont. | 52.00 ± 2.35 | 21.2 ± 1.32 | 52.00 ± 2.35 | 21.2 ± 1.32 | 52.00 ± 2.35 | 21.2 ± 1.32 |
| $10^{-9}$ | 44.62 ± 2.00 | 25.32 ± 1.08 | 52.61 ± 1.06 | 25.21 ± 2.06 | 56.00 ± 3.00 | 26.23 ± 1.00 |
| $10^{-8}$ | 42.22 ± 1.86 | 20.34 ± 1.26 | 55.00 ± 2.00 | 22.32 ± 1.26 | 50.31 ± 3.00 | 21.41 ± 1.00 |
| $10^{-7}$ | 10.52 ± 0.98* | 10.26 ± 0.85* | 42.81 ± 1.66 | 20.32 ± 1.21 | 48.00 ± 2.61 | 15.32 ± 1.00 |
| $10^{-6}$ | 0.98 ± 0.00*** | 0.62 ± 0.35** | 22.92 ± 1.00* | 15.85 ± 1.20 | 18.61 ± 0.56* | 12.11 ± 0.53 |
| $10^{-5}$ | 0.00 | 0.00 | 1.60 ± 0.90** | 0.99 ± 0.05 | 0.82 ± 1.00*** | 0.21 ± 0.81** |
| $10^{-4}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $10^{-3}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *ANOVA* | *** | ** | *** | ** | ** | *** |

Means ± SD (n=3). Results of one-way ANOVA comparison of treatments to control indicate $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

**Table 2**. Dry weight (mg $l^{-1}$), Chl a content ( μM) and cell number (No. × $10^7$ $l^{-1}$) of *Scenedesmus bijuga* at different pH values in the presence of metals at sublethal concentrations after 7 days incubation period.

| pH values | Cd | | | Co | | | Cu | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dry weight (mg $l^{-1}$) | *Chl. a content* (μg M) | Cell number (No. × $10^7$ $l^{-1}$) | Dry weight (mg $l^{-1}$) | *Chl. a content* (μg M) | Cell number (No. × $10^7$ $l^{-1}$) | Dry weight (mg $l^{-1}$) | *Chl. a content* (μg M) | Cell number (No. × $10^7$ $l^{-1}$) |
| Control | 75.0 ± 1.15 | 0.08 ± 0.00 | 24.00 ± 0.00 | 75.0 ± 1.15 | 0.08 ± 0.00 | 24.00 ± 0.00 | 75.0 ± 1.15 | 0.08 ± 0.00 | 24.00 ± 0.00 |
| 4 | 5.0 ± 0.2 | 0.034 ± 0.00 | 1.68 ± 0.01 | 23.18 ± 0.15 | 0.036 ± 0.01 | 2.61 ± 0.12 | 15.21 ± 0.10 | 0.029 ± 0.00 | 2.06 ± 0.15 |
| 6 | 10.22 ± 0.15 | 0.046 ± 0.01 | 3.36 ± 0.10 | 30.26 ± 0.85 | 0.053 ± 0.02 | 6.66 ± 0.21 | 36.33 ± 0.20 | 0.034 ± 0.01 | 5.00 ± 0.20 |
| 8 | 32.11± 0.15* | 0.050 ± 0.01 | 6.92 ± 0.15* | 68.00 ± 1.54* | 0.055 ± 0.02 | 11.02 ± 0.20* | 88.26 ± 1.65** | 0.061 ± 0.01 | 10.06 ± 0.26* |
| 10 | 30.0 ± 0.21 | 0.023 ± 0.00 | 6.00 ± 0.12* | 64.61 ± 1.53** | 0.035 ± 0.00 | 11.00 ± 0.15 | 68.42 ± 1.62** | 0.036 ± 0.00* | 8.88 ± 0 30 |
| 12 | 18.21± 0.15 | 0.012 ± 0.00 | 4.75 ± 0.12 | 50.5 ± 1.50 | 0.020 ± 0.00 | 8.98 ± 0.20 | 35.8 ± 0.80 | 0.020 ± 0.00 | 6.21 ± 0.12 |
| *ANOVA* | ** | * | ** | ** | * | * | *** | *** | *** |

Means ± SD (n=3). Results of one-way ANOVA comparison of treatments to control indicate $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

on concentration. Higher doses of these metals affected strongly and adversely all the growth parameters tested. Total cell death occurred at $10^{-5}$ M of cadmium and at $10^{-4}$ M of cobalt and copper (lethal concentrations). The results also show that the inhibitory effect of cadmium on algal growth and Chlorophyll a content is more pronounced than that of the other two metals tested. This indicates that all the three metals had interfered with 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 higher doses of cobalt, mercury and vanadium affected strongly the growth parameters of *Scenedesmus bijuga* and *Anabaena spiroides*. The inhibitory effects of heavy metals on pigment accumulation, observed 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 can 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 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 Table 2 show that the dry weight, Chlorophyll a content and cell number were markedly enhanced with increasing pH towards the alkaline range, but slightly decreased at pH over 8. Generally, one can see that in these experiments the toxicity of the metals tested decreased under 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 increased availability and therefore decreased biological activity (e.g. Pb). Rai et al. (1981) pointed out that an increase in pH in the alkaline range increased the toxicity of heavy metals to natural populations of algae and to cultures of *Chlorella* and *Hormidium rivulare*. However, other relevant studies (Michnowiez and Weaks, 1984; Fathi, 1995; Vymazal, 1995) indicated that heavy metals toxicity decreased in alkaline pH conditions. The latter observation is in good agreement with the data of the present edinvestigation.

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. The degree of metal accumulation is a result of complex interactions between the factors and the organisms (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 the present investigation clearly show that the accumulation and bioaccumulation factors of the metals tested with *Scenedesmus bijuga* decreased with increasing pH (Table 3). The metals accumulated by *Scenedesmus* are, in decreasing order of the amount taken up: cobalt > copper > cadmium. Different organisms, however, have different sensitivities to the same metal, and the same organism 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 ions in the medium (Baudo, 1987). However, in our 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 availability of metals to algae.

From another point of view, free ions are generally the most available metal species (Anderson and Morel 1982; Fathi, 1995; Fathi and Falkner, 1997). 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 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 be 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 certain 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 equilibrium constant.

Table 4 shows the solubility (or free ionic forms) of the heavy metals tested as carbonates or hydroxides under the experimental 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 the metals decreased toward the alkaline range. In general, it could be concluded that at a low pH, metals exist as free cations, but at an alkaline pH, like that of the Nile water, they precipitate as insoluble hydroxides or carbonates. Therefore, pH can have considerable effects on the availabilit ies of heavy metals and thus on their toxicity (Anderson and Morel, 1982; Peterson et al., 1984). Finally, it may be said that the toxicity of cadmium, cobalt and copper to *Scenedesmus bijuga* decreases at increased pH towards the alkaline range. This relationship may be of ecological importance in the control of pollution, particularly acid pollution.

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**Table 3**. Effect of different pH values on the metal accumulation and bioaccumulation factor of *Scenedesmus bijuga* after 7 days growth 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 |

**Table 4**. 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).

| | Log. Cd conc. | | Log. Co conc. | | Log. Cu conc. | |
|---|---|---|---|---|---|---|
| pH values | Carbonate | Hydroxide | Carbonate | Hydroxide | Carbonate | Hydroxide |
| **4** | -2 | 5.4 | -3.5 | 4.1 | -0.3 | 0.1 |
| **6** | -4 | 1.4 | -5.5 | 0.1 | -2.3 | -3.9 |
| **8** | -6 | -2.6 | -7.5 | -3.9 | -4.3 | -7.9 |
| **10** | -8 | -6.6 | -9.5 | -7.9 | -6.3 | -11.9 |
| **12** | -10 | -10.6 | -11.5 | -11.9 | -8.3 | -15.9 |

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**Address for correspondence:** Adel A. Fathi. Biology Department, College of Science, King FaisalUniversity, Saudi Arabi, Al-Hufof, 31982; P.O. Box. 380, Saudi Arabia. E-mail: a.fathy@link.net

***Editorial responsibility: Andrew Goodkov***