Toxicity of Individual/Combined Effect of Zn^{+2}, Fe^{+2}, Cu^{+2} to *Synechocystis sp.* E35 Isolated from Kucukcekmece Lagoon, Istanbul

İstanbul Küçükçekmece Gölünden İzole Edilen *Synechocystis sp.* E35 üzerine Zn^{+2}, Fe^{+2} ve Cu^{+2} Metalerinin Tekli /Kombine Bileşiklerinin Toksisitesi

Research Article

Sevgi Demirel[1], Belma Aslim[2], Beyza Ustun[3], Ebru Sebnem Yilmaz[4*]
[1]Harran University, Department of Environmental Engineering, Sanliurfa, Turkey
[2]Gazi University, Molecular Biology Research Center, Golbasi, Ankara, Turkey
[3]Yildiz Technical University, Department of Environmental Engineering, Istanbul, Turkey
[4]Mustafa Kemal University, Faculty of Art and Science, Department of Biology, Hatay, Turkey

ABSTRACT

Iron, copper and zinc at toxic levels were found in water and sediment structure of Kucukcekmece Lake. In the research area, algae blooms have been observed dependent upon anthropogenic and abiotic factors at certain periods. Cyanobacteria species have been observed as dominant microorganism during algae bloom. The objective of the present study is to evaluate the toxic effects of various heavy metals on the *Synechocystis sp.* E35 isolated from Kucukcekmece Lagoon, Istanbul. Toxicity of Zn^{+2}, Fe^{+2}, Cu^{+2} and of metal mixture (Zn^{+2}-Cu^{+2}, Zn^{+2}-Fe^{+2}, Fe^{+2}-Cu^{+2} and Fe^{+2}-Cu^{+2}-Zn^{+2}) are investigated in the laboratory scale. The order of toxicity of the metals to the *Synechocystis sp.* E35 is $Cu^{+2} > Zn^{+2} > Fe^{+2}$ in growth media. The EC_{50} values of the Zn^{+2}, Fe^{+2}, Cu^{+2} on the inhibition of the growth of E35 isolate: are 6.94, 13.92 and 3.42 mg/l, respectively. Besides, combination of ternary metal systems showed stronger inhibitory effect on cells are 0.02 and 2.93 mg/l, respectively.

Key Words
Cyanobacteria, Kucukcekmece Lagoon, Heavy metal

ÖZET

Küçükçekmece gölünün su ve sediment yapısı toksik düzeyde demir,bakır ve çinko bulundurur. Araştırma alanında, belli dönemlerde antropojenik ve abiyotik faktörlere bağlı olarak yoğun alg üremeleri gözlenmiştir. Bunlar içerisinde siyanobakterilerin dominant grup olduğu tespit edilmiştir. Bu çalışmanın amacı, Küçükçekmece Gölünden izole edilen *Synechocystis sp.* E35 izolatı üzerine çeşitli ağır metallerin toksik etkisinin incelenmesidir. Laboratuar ölçeğinde, Zn^{+2}, Fe^{+2}, Cu^{+2} ve metal karışımlarının (Zn^{+2}-Cu^{+2}, Zn^{+2}-Fe^{+2}, Fe^{+2}-Cu^{+2} ve Fe^{+2}-Cu^{+2}-Zn^{+2}) etkisi araştırılmıştır. Tekli uygulamalarda metallerin toksisiteleri sırasıyla $Cu^{+2} > Zn^{+2} > Fe^{+2}$'dır. Metallerin EC_{50} değerleri ise sırasıyla 6.94, 13.92 ve 3.42 mg/l (Zn^{+2}, Fe^{+2}, Cu^{+2})'dir. Ayrıca , üçlü metal kombinasyon uygulamalarının da güçlü inhibitör etki gösterdiği tespit edilmiştir (EC_{10}= 0.02 mg/l ; EC_{50} =2.93 mg/l).

Anahtar Sözcükler
Siyanobakteri, Küçükçekmece Lagünü, Ağır metal

Article History: Received January 29, 2012; Revised March 28, 2012; Accepted May 3, 2012; Avaliable Online: June 19, 2012

Correspondence to: Ebru Şebnem Yılmaz, Mustafa Kemal University, Department of Biology, Hatay,Turkey
Tel: +90 326 245 58 36-16 25 Fax: +90 356 2455867 E-Mail: ebrusebnem@gmail.com

INTRODUCTION

In trace amounts, zinc, iron and copper are essential micronutrients that play an important role in many enzyme systems in living organisms [1]. At concentrations above those required for optimal growth, zinc, iron and copper have been shown to inhibit microbial growth [2]. Few detailed studies have examined the combined effects of zinc, iron and copper on cyanobacterium despite the fact that these elements are commonly present together in mining, industrial and domestic effluents.

A number of methods have been proposed for measuring metal toxicity in microbial systems. The more commonly used methods include the measurement of the respiratory rate, the assessment of various growth parameters, the measurement of cell viability via plate counting [3,4].

The acute toxicity of three metal ions, Zn, Fe and Cu, in single or mixture using *Synechocystis sp.* E35 isolated from Kucukcekmece Lagoon was investigated in this study.

Kucukcekmece Lake is one of the natural lagoon lakes in Turkey and is a habitat of various endemic species [5]. Iron, copper and zinc were found in the water and the sediment structure of Kucukcekmece Lake at toxic levels [6,7].

MATERIALS AND METHODS

Kucukcekmece Lake

Kucukcekmece Lake located in the European part of Istanbul in Turkey is a typical lagoon lake with a spoon shaped topography (Figure 1). It is at 15 km west of the city center. The deepest point of Kucukcekmece Lake is about 21 m. The surface area of the lake is approximately 17 km^2 and the water volume is 145 million m^3 at sea level [5].

Isolation of Microorganism

Synechocystis sp. E35 was isolated from Kucukcekmece Lake (İstanbul), Turkey. Sample was isolated by the method of Rippka et al. [8]. Isolate was identified according to cell division morphology [8] and grown in BG-11 medium: [$NaNO_3$, 15; K_2HPO_4, 0.4; $MgSO_4.7H_2O$, 0.75; $CaCl_2.2H_2O$, 0.36; citric acid, 0.06; iron(III) ammonium citrate, 0.06; Na_2-EDTA, 0.01; Na_2CO_3, 0.2 g L^{-1}, 1 mL; trace elements solution, (H_3BO_3, 61; $MnSO_4.H_2O$, 169; $ZnSO_4.7H_2O$, 287; $CuSO_4.5H_2O$, 2.5; $(NH_4)_6Mo_7O_{24}.4H_2O$, 12.5 mg L^{-1}) pH: 6.8] at 25 °C with light/dark cycle of 12/12 h by using an incubator shaker (MINITRON), for 30 days [9]. The

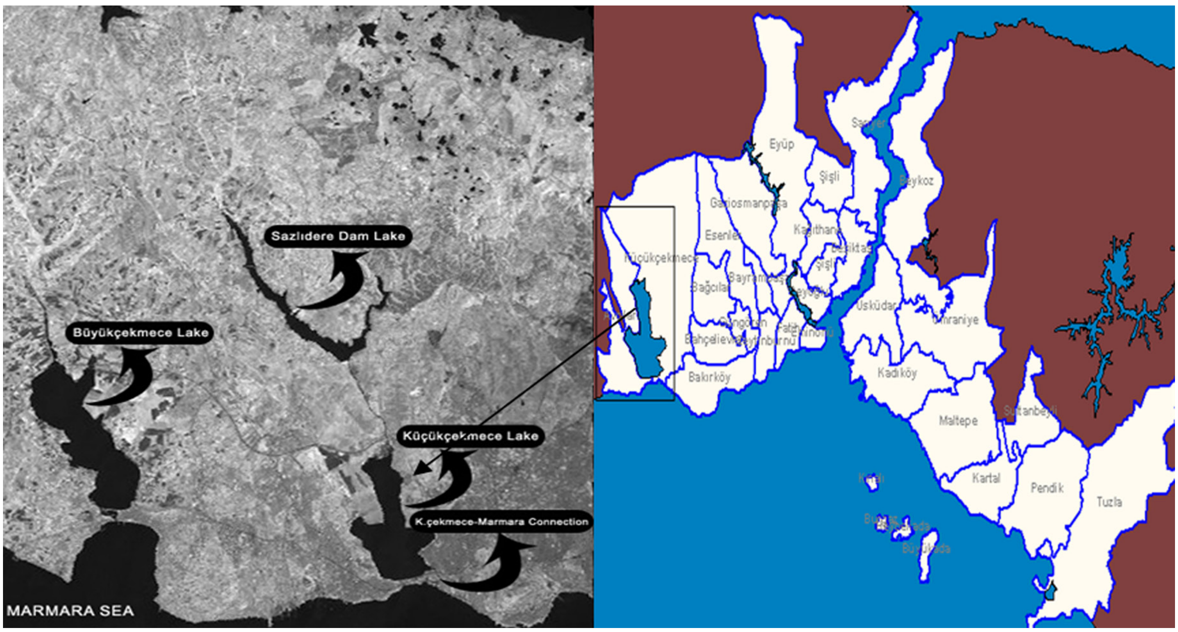

Figure 1. Location of Kucukcekmece Lagoon.

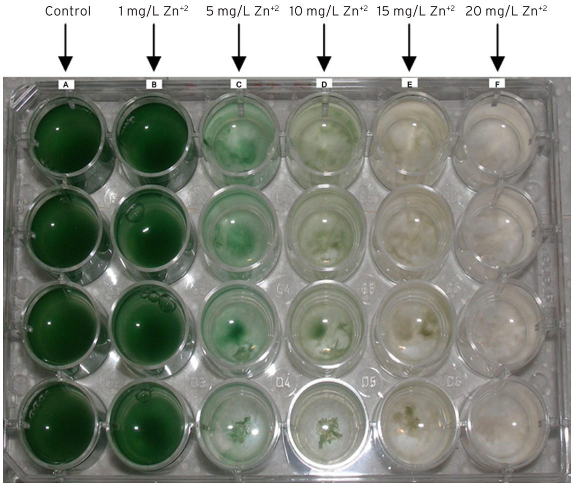

Figure 2. Experimental configuration of a 24 well microplate for toxicity testing (Zn^{+2} treatment, After 96 hours incubation period)

intensity of light during the light period was 3000 lux. Isolate was also identified as according to 16S rRNA by the method of Nubel et al. [10]. Isolation, identification and growth of the isolate were held in the Biotechnology Laboratory of Gazi University and also isolate is stored at the Culture Collection of Microalgae of Gazi University. Isolate code was entitled according to the culture collection of microalgae of Gazi University, Ankara.

Growth Inhibition

The toxicity tests were conducted on exponentially growing cells of *Synechocystis* sp. E35 that are exposed for 96 h in a 24-well microplate (sterile disposable) to different concentrations of metals under controlled experimental conditions of temperature and light (25 °C and 3000 lux) in an incubator shaker (Minitron). In such a compact microplate can be viewed as Figure 2.

Growth of *Synechocystis* sp. E35 was measured by chlorophyll-*a* 48 h intervals during 14 days [11]. The degree of growth inhibition by iron, zinc and copper in *Synechocystis* sp. E35 cells was calculated on the sixth day. *Synechocystis sp.* E35 is exposed five different metal concentrations (1, 5, 10, 15 and 20 mg/l for single metal systems and 1, 2, 3, 5 and 10 mg/l for binary and ternary metal systems) at incubation conditions (25 °C, pH 7, 100 rpm, 3000 lux light intensity). After an incubation period of 96 hours, cell growth for each metal concentration was measured using a spectrophotometer at 664 nm.

Effects on growth inhibition were expressed as the effective concentration giving 10% and 50% reduction (EC_{10} and EC_{50}) in cyanobacterial growth rate over 96 h compared with the controls by probit analysis [12].

RESULTS AND DISCUSSION

Effective concentrations of 96 hours (EC_{10} and EC_{50} values) for individual and mixture combinations of iron, zinc and copper for toxicity tests were showed in Table 1.

The results suggested that *Synechocystis* sp. E35 is more sensitive to copper, and indicated that the single toxicity of three metal elements to *Synechocystis* sp. E35 has such a sequence as Cu>Zn>Fe.

Based on the results of the study (shown in Table 1) it was found that the combined effects of all mixture trials were synergistic, namely, the toxicity of each metal ion was enhanced when coexisting with other heavy metals studied, while the enhancement is different for different mixtures.

Combination of iron, zinc and copper (ternary metal systems) appeared to have a stronger inhibitory effect on cell than that of a single and binary metal mixtures. Iron, zinc and copper had different effects on the growth of *Synechocystis* sp. E35 (Figures 3-5).

Compared to the control culture (lacking metals), the resistant isolate revealed higher chlorophyll-*a* content in the presence of 1 and 5 ppm Fe^{+2} concentrations (Figure 3). This suggested that availability of low concentrations of Fe^{+2} is essential for the growth of the *Synechocystis* sp. E35. However, the effects of higher concentrations of Fe^{+2} were detrimental and also inhibited the growth of the microorganism irreversibly.

An increase in Zn^{+2} concentrations caused a significant decrease in the chlorophyll-*a* content of *Synechocystis* sp. E35 (Figure 4).

Copper is an essential micronutrient for cyanobacteria because it is an integral component of plastocyanin in the photosynthetic electron-transport chain [13]. However, high doses of Cu^{+2} normally used for remaining unwanted cyanobacterial and algal growth in water reservoirs and drainage systems.

Synechocystis sp. E35 was affected negatively by the presence of copper (Figure 5). This result is in agreement with those of Surosz and Palinska who found chlorophyll-*a* concentration decreased with increased metal concentration [14].

CONCLUSIONS

Although some of metal elements are necessary for organisms, they are harmful to aquatic organisms if their concentrations exceed their critical level, which can be confirmed by this research work.

The results of this study clearly showed that the three metals (Fe, Zn and Cu) were more toxic when they were present in combination than when administered individually.

At present study, interactions between combinations of metals are not considered in water quality guidelines despite the fact that metal contaminants rarely occur alone. Our results showed that the toxicity of binary and ternary mixtures of iron, zinc and copper could not be predicted on the basis of the toxicity of the individual metals. Of major environmental concern are those interactions resulting in synergism, as observed in this study for mixtures of Fe, Zn and Cu.

Table 1. EC_{10} and EC_{50} values at 96 h

Metals	EC_{10} value mg/L	EC_{50} value mg/L
Fe^{+2}	11.52	13.92
Zn^{+2}	1.84	6.94
Cu^{+2}	0.25	3.42
$Fe^{+2}+Zn^{+2}$	1.22	7.03
$Fe^{+2}+Cu^{+2}$	0.36	3.70
$Zn^{+2}+Cu^{+2}$	0.20	3.12
$Fe^{+2}+Zn^{+2}+Cu^{+2}$	0.02	2.93

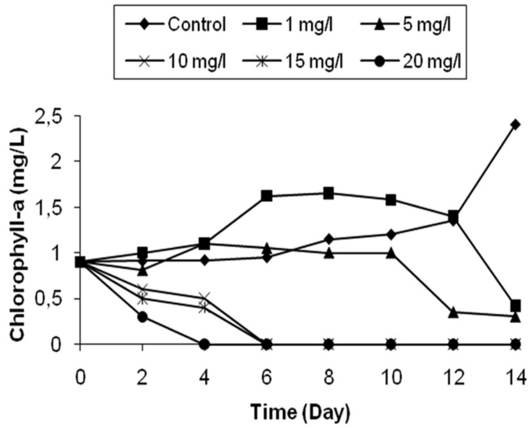

Figure 3. Growth inhibition of iron in Synechocystis sp. E35

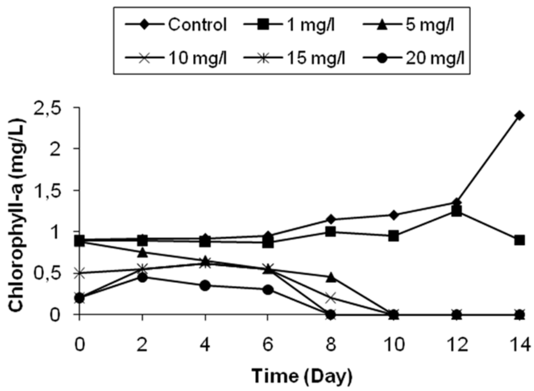

Figure 4. Growth inhibition of zinc in Synechocystis sp. E35

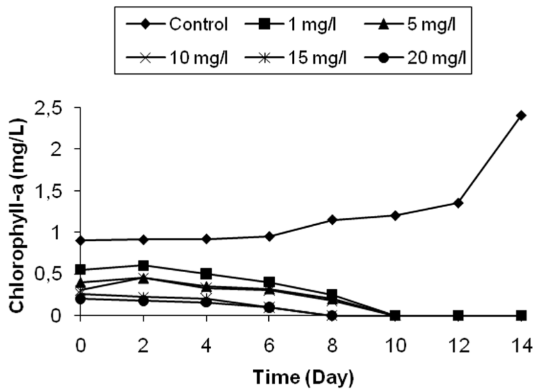

Figure 5. Growth inhibition of copper in Synechocystis sp. E35

Kucukcekmece lagoon which has various endemic species has been contaminated due to uncontrolled domestic and industrial discharges in last two decades. Internal and external pollutant sources should be taken under control for sustainability for the lagoon.

ACKNOWLEDGEMENTS

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK). Project Code: 105Y116.

REFERENCES

1. MG. Dominguez, LL. Maury, FJ. Florencio, JC. Reyes, A gene cluter involved in metal homeostasis in the cyanobacterium *Synechocystis* sp. strain PCC 6803, J Bacteriol., 182 (2000) 1507.
2. K. Hantke, Bacterial zinc uptake and regulators, Curr. Microbiol., 8 (2005) 196.
3. DJB. Dalzell, S. Alte, E. Aspichueta, A. Sota, J. Etxebarria, M. Gutierrez, CC. Hoffmann, D. Sales, U. Obst, N. Christofi, A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge, Chemosphere, 47 (2002) 535.
4. CD. Boswell, RE. Dick, LE. Macaskie, The effect of heavy metals and other environmental conditions on the anaerobic phosphate metabolism of *Acinetobacter johnsonii*. Microbiology, 145 (1999) 1711.
5. B. Ustun, N. Ince, G. Cansever, E. Ulger, B. Agcioglu, Z. Sapçı, S. Demirel, E. Okumus, The Development of Environmental Management Model in Küçükçekmece Basin, Joint Research and Development Project (GSRT–TUBITAK) Project No: 102Y011 (2005).
6. S. Demirel, ZS. Zengin, B. Ustun, Determination of Phytotoxicity Effect in Polluted Sediment: In Case "Kucukcekmece Lagoon and Sazlidere Creek", International Conference on Environmental Science and Technology, Houston, Texas, USA. Proceedings, 2 (2007) 201.
7. S. Demirel, Effect of physicochemical conditions on heavy metal biosorption in natural environments, PhD Thesis, Yıldız Technical University, Istanbul (2008).
8. RW. Castenholz, Phylum BX. Cyanobacteria. Oxygenic Photosynthetic Bacteria", In: Bergey's Manual of Systematic Bacteriology, Volume 1: The Archaea and the Deeply Branching and Phototropic Bacteria 2nd Ed., G. Garrity, DR. Boone, and RW. Castenholz, Springer–Verlag, New York, 474 (2001).
9. R. Rippka, J. Deruelles, J. Waterbury, M. Herdman, R. Stanier, Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria, J. Gen. Microbiol., 111 (1979) 1.
10. U. Nubel, F. Garcia–Pichel, G. Muyzer, PCR primers to amplify 16S rRNA genes from cyanobacteria, Appl. Environ. Microb., 63 (1997) 3327.
11. J. Myers, WA. Kratz, Relation Between Pigment Content And Photosynthetic characteristics in a Blue–Green Alga, J. Gen. Physiol., 39 (1955) 11.
12. DJ. Finney, Probit Analysis (rev. ed.), Cambridge Univ. Press, London,1963
13. E. Zak, B. Norling, R. Maitra, F. Huang, HB. Andersson Pakrasi, The initial steps of biogenesis of cyanobacterial photosystems occur in plasma membrane. Proceedings of the National Academy of Sciences, 98 (2001) 13443.
14. W. Surosz, KA. Palinska, Effects of Heavy–Metal Stress on Cyanobacterium Anabaena flos–aquae, Arch of Environ Con Tox., 48 (2004) 40.