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

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
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 km² and the water volume is 145 million m³ at sea level [5].

Isolation of Microorganism

Synechocystis sp. E35 was isolated from Kucukcekmece Lake (Istanbul), 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: [NaNO₃, 15; K₂HPO₄, 0.4; MgSO₄·7H₂O, 0.75; CaCl₂·2H₂O, 0.36; citric acid, 0.06; iron(III) ammonium citrate, 0.06; Na₂·EDTA, 0.01; Na₂CO₃, 0.2 g L⁻¹, 1 mL; trace elements solution, (H₃BO₃, 61; MnSO₄·H₂O, 169; ZnSO₄·7H₂O, 287; CuSO₄·5H₂O, 2.5; (NH₄)₆Mo₇O₂₄·4H₂O, 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.
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>
<thead>
<tr>
<th>Metals</th>
<th>EC$_{10}$ value mg/L</th>
<th>EC$_{50}$ value mg/L</th>
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<tr>
<td>Fe$^{2+}$</td>
<td>11.52</td>
<td>13.92</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>1.84</td>
<td>6.94</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>0.25</td>
<td>3.42</td>
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<td>7.03</td>
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<td>3.70</td>
</tr>
<tr>
<td>Zn$^{2+}$+Cu$^{2+}$</td>
<td>0.20</td>
<td>3.12</td>
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<tr>
<td>Fe$^{2+}$+Zn$^{2+}$+Cu$^{2+}$</td>
<td>0.02</td>
<td>2.93</td>
</tr>
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</table>

**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.

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REFERENCES


