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A case study on algal response to raw and treated effluents from an aluminum plating plant and a pharmaceutical plant

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Abstract

The algal growth responses to the effluents of an aluminum plating plant and to the wastewater from an analgesic/antiinflammatory-drug-producing pharmaceutical plant were investigated. Growth response of the marine alga *Dunaliella tertiolecta* was monitored by measuring the two response parameters optical density (OD_{640}) and in vitro chlorophyll fluorescence for a period of 14 days. Generally, the two response measurements gave similar results for all effluents but the raw effluents of the aluminum plating plant due to the composition of the wastewater. All wastes affected algal growth either by inhibition only or by stimulation at low concentrations and inhibition at high concentrations. Since pollutant tolerance of algae biased toxicity test results, acclimation of algae to the raw effluent of the aluminum plating plant was examined. Although the water quality parameters of treated effluent of both plants were in the permitted range reported by the Turkish Water Pollution Control Act, they inhibited growth at higher concentrations, implying that the two treatment plants were inefficient. Therefore, the importance of toxicity tests in wastewater discharge regulations was emphasized.

Keywords: Marine algae; Toxicity; Industrial effluents; Chlorophyll a; Growth inhibition; Area under the curve; Growth stimulation

1. Introduction

Effluent discharges from industrial facilities are complex, comprising many different components and varying continuously in quantity and quality. Conventional water quality parameters such as chemical oxygen demand and suspended solids may not detect toxic compounds present in a variety of industrial wastewaters and treated wastes, and chemical procedures alone cannot provide sufficient information on the potential harmful effects of chemicals on the aquatic environment. In some cases, the treated effluent does not exceed the discharge limits but the results of toxicity tests show potential toxicity (Lin et al., 1994). In fact, some chemicals are not totally eliminated because the conventional technology of treatment used in wastewater treatment plants appears to be insufficient for completely removing these specific compounds (Ternes, 1998). The toxic effects of unknown substances in complex mixtures or the possible synergistic effects among compounds in effluents can be detected only by toxicity testing.

In many developed countries, toxicity tests on industrial effluents are required to ensure that such discharges will not have adverse effects on the aquatic organisms in receiving water (Backers-Maessen, 1994; US EPA, 1984; NRA, 1993, 1994, 1995; Whitehouse and Dijk, 1996). In Turkey, however, environmental regulations on the pollution control of effluent discharges to receiving waters still rely on global physicochemical analysis. Only the fish toxicity test carried out with *Lepistes* sp., based on the toxicity dilution factor (TDF), is included in the Turkish Water Pollution and Control Regulation for some industrial effluents, but other toxicity tests, i.e., those using algae are not yet

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incorporated into the regulations (Turkish Water Pollution Control Regulation, 1992). Due to their role as primary producers in aquatic food webs, algae have high ecological relevance and thus great scientific importance. Additionally, animals do not respond to all wastes, whereas algae respond to all wastes by stimulation, inhibition, or both. For example, test assays on various effluents from municipalities and industries have demonstrated greater sensitivity to algae than to animals (Wanberg et al., 1995; Yen et al., 1996; Lewis et al., 1998; Walsh and Garnas, 1983). A large number of bioindicators and test organisms (mainly freshwater species) have been proposed in the past for the evaluation of ecotoxicity of anthropogenic compounds on aquatic environments. However, the proposal of seawater species as test organisms was scarce and this may be extremely important for the assessment of environmental impact in particular aquatic systems in some countries such as Turkey, which is surrounded on three sides by sea. We selected the unicellular marine algal species *Dunaliella tertiolecta* as a test organism for toxicity testing since it fulfills most of the criteria for a bioassay organism (Reish and Lemay, 1988) and has been proposed as a standard organism for seawater toxicity tests (APHA, 1998).

The present study was intended to determine the algal growth responses to the raw and treated effluents of an aluminum plating plant and a pharmaceutical plant. Since aluminum surface finishing has been a rapidly growing industry around the world for many decades and the presence of pharmaceuticals and their metabolites have been detected in wastewater and sewage treatment plants (STPs), discharge of these compounds to the environment is widespread (Daughton and Ternes, 1999; Daughton and Jones-Lepp, 2001; Kümmerer, 2001). Considering that the sensitivity of response variables (e.g., optical density and chlorophyll fluorescence) are different (Mayer et al., 1997) and that test duration is another important parameter for the assessment of toxicity to algae (Walsh et al., 1982), in this study we focused on comparing photometric and fluorometric measurements of the growth of algae exposed to the raw and treated effluents of an aluminum plating plant and an analgesic/antiinflammatory drug (i.e., naproxen)-producing pharmaceutical plant for a longer test duration, namely 14 days. We also focused on evaluating the treatment efficiency and emphasizing the incorporation of toxicity parameters into Turkish Environmental regulations to protect receiving waters. Finally, we also focused on studying the ability of *D. tertiolecta* to acclimate to a sample having the highest toxicity among the studied effluents, since acclimation influences the extrapolation of laboratory toxicity data to the natural environment.

2. Materials and methods

2.1. Test design

This study used the unicellular green algae *D. tertiolecta* supplied from the Marmara Scientific Research Center (Turkey). *D. tertiolecta* stock cultures were maintained in 500-mL Erlenmeyer flasks containing 100 mL natural seawater (S 24.7‰; conductivity 39 $\mu S\,cm^{-1}$; pH 8.4; NO_3^- 0.54 $mg\,L^{-1}$; PO_4^{3-} 0.58 $mg\,L^{-1}$; Al 0.64 $\mu g\,L^{-1}$) filtered thorough GF/C glass fiber filter (Whatman) and enriched by modified f/2 medium (Okay and Gaines, 1996). The tests were carried out in triplicate at $21 \pm 2\,°C$ under continuous illumination with two warm-light fluorescent lamps (40 W). Water quality parameters of raw and treated effluents from both plants were measured and are given in Table 1. For the TDF an aquarium with sufficient aeration, diluted wastewater, and fish (*Lepistes* sp.) were used. At the end of 24 h, the dilution in which all fish were alive was observed and accepted as the appropriate dilution ratio (Turkish Water Pollution Control Regulation, 1992). Physicochemical analyses were made according to standard methods (APHA, 1998). Before the toxicity experiment, raw and treated effluents were filtered through the sterile microfiber filter. Growth of alga was monitored by taking samples at regular intervals for 14 days.

During the experiments with nonacclimated algae, raw or treated effluents were introduced to the 500-mL Erlenmeyer flask containing growth medium ingredients and algae not previously exposed to raw or treated effluents. During the experiments with acclimated algae, algae previously exposed to 2.00% (v/v) raw effluents of the aluminum plating plant for 14 days were used.

Algae growth was evaluated both by optical density (OD_{640}) and by chlorophyll-*a* (Chl-*a*) measurements. Absorbance was determined with a Shimadzu UV-150.02 spectrophotometer at 640 nm. Chl-*a* was extracted and measured fluorometrically. For Chl-*a* analysis, a procedure given for acetone extraction (Method No. 10200H-3) as described in standard methods (APHA, 1998) was followed. Fluorescence was measured with a RF-4000 Model Shimadzu spectrofluorophotometer. For the acclimation experiments chlorophyll fluorescence was used instead of Chl-*a* concentration to follow the growth inhibition since 5.00% (v/v) of raw effluent Chl-*a* concentration was too low.

The most common parameter used in algal toxicity assays is the IC_{50}, i.e., the concentration of the tested substance that decreases the growth by 50%. IC_{50} values were determined using linear interpolation combined with bootstrapping (known as the IC_p method) as outlined in US EPA (1993) and Norberg-King (1993). The SC_{20} value (stimulatory concentration) describing