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Short communication

Chlorella test

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Abstract

Background and Purpose: *Chlorella test was performed for the first time. The interesting application of a newly proposed test in ecotoxicological research was explained. It was tested using the herbicide norflurazon as bleaching toxicant. The experimental object was green alga Chlorella kessleri Fott et Novak. [K&H, 1992].*

Materials and Methods: *Algae were treated on the surface of deep stock agar in tubes. Instead of dH_2O t the equivalent measure of aqueous solution of toxicant was used in the medium. This is the essence of the Chlorella test method.*

Results: *Already after a short period of two days, morphological changes were distinguished macroscopically. Bleaching of algae was present in particular tubes, as the result of the effect of the toxicant upon the algal growth and viability.*

Conclusions: *We propose Chlorella test as a toxicity test that provides easy detection of sublethal, lethal and NOEC. The use of this quick, simple, indicative, cheap and applicative test is proposed as a method for research on the preliminary effects of environmental pollutants upon green algae in culture.*

INTRODUCTION

As microalgae have a significant role as primary producers in aquatic ecosystems and are the basis of many food chains, tracing the effect of toxicants upon them is very important *(1, 2)*. *Chlorella* is a widely used unicellular model in ecotoxicological research, and also for photosynthesis study and regulation *(3)*. It is a suitable test-organism due to its small size, high reproduction rate and easy maintenance in culture *(4, 5)*. Little is known about the sensitivity of algae to herbicides and therefore this has been the subject of recent research *(1, 6)*. Norflurazon has a bleaching effect on newly developed chloroplasts, followed by a decrease in the photosynthesis and viability of the organism *(7–10)*.

MATERIAL AND METHODS

For our experiment alga *Chlorella kessleri* Fott et Novak. [K&H, 1992], strain LARG/1 was used. Green algae were maintained on the surface of sterile deep stock agar in tubes, according to Pratt R *(11)* (1941) and modified according to Horvatić J *et al. (5)* (2000) (medium: 2 g agar, 100 mg KNO_3, 1 ml $MgSO_4$ x $7H_2O$, 1 ml K_2HPO_4 and 0.1 ml $FeCl_3$, 100 ml dH_2O). Algae grew in tubes of 16 cm length and 15 mm

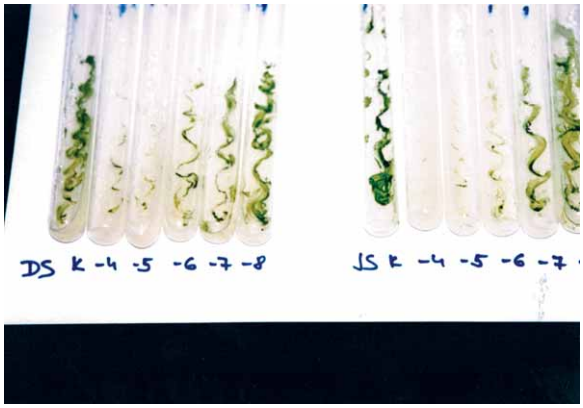

Figure 1. *Chlorella test. The effect of toxicant upon the algae* Chlorella *is defined by the level of bleaching in particular test tubes. K-control; –4, the highest concentration and the most obvious bleaching, –8, the lowest concentration with no bleaching at all (DS- low-light conditions, JS- high-light conditions; –4=2x10^{-4}, –5= 2x10^{-5}, –6=2x10^{-6}, –7=2x10^{-7}, –8=2x10^{-8} mol/L of aqueous solution of norflurazon).*

width in a clima-room in sterile conditions at 24 °C, by the constant light of 80 µmol/m^{2}s (fluorescent lamp Osram L36W/20, Cool/White/2850 lm Osram, Berlin, Germany), each containing the 5 ml of agar, inclined to 15°. After 14 days they were plated to the newly prepared deep agars in tubes by the platine needle in laminar. The content of one tube was divided and plated to deep agars into five new tubes. The length of each smear was 10 cm. Tubes were closed by cotton wool and transparent foil. By this optimization and standardization the constant quantity of clone cultures usable for our experiments was obtained. These defined terms were pre-conditions for the Chlorella test and were its constituent part. Organisms were treated with five concentrations of aqueous solution of norflurazon (SAN 9789, Sandoz Ltd., Basel, Switzerland; purity 80 %: 2×10^{-4}, 2×10^{-5}, 2×10^{-6}, 2×10^{-7} and 2×10^{-8} mol/L). Norflurazon was dissolved in aquarium water, 76 mg/L for the stock solution 2×10^{-4} mol/L. For the lower concentration, stock solution was diluted ten times in aquarium water. For the Chlorella test, exactly this (the same amount of) aqueous solution of norflurazon was put into the agar medium. Algae grew on this media under the same conditions as the control. Therefore, algae were treated on the deep stock agar in tubes so that instead of dH_2O the equivalent measure of aqueous solution of norflurazon of a particular concentration was put/used in the medium. This is the essence of the Chlorella test method.

RESULTS AND DISCUSSION

Morphological changes were possible to distinguish macroscopically after only two days, as bleaching of algae appeared in particular tubes. This was the result of chloroplast degradation and decreased viability of the cells. Initial bleaching, progress in bleaching and terminal bleaching by the end of the experiment (14–21 days) could be observed. The effect-range of a particular toxicant could be determined. Presence of green color, less intensity of green color or the absence of green color could be traced and remarked. We propose that Chlorella test method provides easy detection of sublethal, lethal and NOEC of primarily aquatic environmental pollutants in aqueous specimens. Also, the wider use of this method as a toxicity test was proposed, using toxicants with different mechanisms of action.

In this paper we proposed a quick, simple, indicative, cheap and applicative toxicity test for ecotoxicological research on the preliminary effect of xenobiotics upon green algae in culture. The principle of this method and remarkable changes are shown in Figure 1.

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