

The use of *Selenastrum capricornutum* growth potential as a measure of toxicity of a few selected compounds

JA van der Heever* and JU Grobbelaar

Department of Botany and Genetics, University of the Orange Free State, Bloemfontein 9300, South Africa

Abstract

Algal growth potential (AGP) assays were used to determine the possible toxicity of a few selected compounds. The conventional EC50 value was used to indicate toxicity. Four different parameters were used to determine algal growth rates in the presence of potentially toxic compounds, namely, cell numbers, dry mass, chlorophyll *a* measured fluorometrically and spectrophotometrically. Cu, Cd and atrazine were highly toxic, whilst Hg, phenol and gusathion showed little or no toxicity. The results showed that the time of exposure was very important, where on the one hand the algae adapted to the toxin rendering less toxic results, or long exposures indicating high toxicity where this was not apparent in the short exposures. The depletion of nutrients or the inability to distinguish between living and dead cells during long-term tests influenced the results, which made interpretations difficult. We recommend short-term tests and the selection of an appropriate growth parameter of which chlorophyll fluorescence gave promising results.

Introduction

In recent years, considerable effort has been devoted towards developing standardised procedures and guidelines for assessing the presence of toxic substances. Conducting algal growth potential (AGP) tests on a routine basis and working out standard procedures and guidelines are not merely a question of understanding algal growth phenomena. There are also questions of compromising between practical considerations (cost and simplicity) and scientific preferences. It is desirable that such compromises be made on sound scientific terms, which involves a thorough understanding of how algal test systems can be manipulated and how various experimental factors may influence the results (Nyholm and Källqvist, 1989). Test organisms for routine use should, first of all, be generally accepted laboratory organisms rather than species that are very sensitive or very abundant in nature. The widely used freshwater green alga *Selenastrum capricornutum* is such a species. Among the green algae, this species seems to have a "medium sensitivity" (Walsh and Merrill, 1984), and is easy to culture. It has a very characteristic shape, so that contamination of cultures is easily detected, and it is suitable for cell counting by means of electronic particle counters.

Various factors may influence the assessment of toxicity in water. The composition of the test medium has a significant effect on the growth rate. Changes in concentrations of nitrogen and phosphorus are important in affecting toxic limits but trace nutrients may also influence the results (Turbak et al., 1986; Adams and Dobbs, 1984). Little is known about how irradiance may interact with toxicity, but most green algae grow well in continuous irradiance (ISO, 1987; OECD, 1984; Miller et al., 1978; US EPA, 1982). Standardised tests are normally carried out at temperatures somewhat below the optimum for a particular test species (23 to 24°C for *S. capricornutum* [ISO, 1987; OECD, 1984; Miller et al., 1978]). Although it is relatively easy to control temperature, amongst the test flasks, it is more important to ensure that the temperature is uniform than to control the temperature at a fixed

level. Variations in the pH and pH might also influence toxicity (Nyholm and Källqvist, 1989). It was postulated that at pH 8, the lower amounts of H⁺ allowed divalent Cu²⁺ to bind to, and subsequently be accumulated in, the algal cells (Babich and Stotzky, 1980).

It has become clear in recent years that a general purpose standard toxicity test should preferably be of relatively short duration and restricted to the initial period of exponential growth, lasting 2 to 4 d (Walsh et al., 1982; Nyholm and Källqvist, 1989). Measuring final yields after 14 d incubations (Miller et al., 1978) is problematic. Even with considerable reductions in the growth rate the final yield will not be affected, because the toxicant-affected cultures may gradually "catch up" with the controls when nutrients become limiting, and in the duration of the test, toxicity can be lost due to various mechanisms and thus have little or no effect on the final yield (Walsh et al., 1982). Nyholm (1985) concluded, from a theoretical mathematical viewpoint, that some measure of the specific growth rate should be used as the response variable in algal growth inhibition tests, rather than the biomass at the end of the test because the specific growth rate is a function of the growth-rate limiting nutrient, or toxin concentration, when all other factors are in excess.

Inhibitory effects in algal growth tests may be expressed in several ways. Endpoints other than biomass or growth rate could be used, e.g. dry mass, cell numbers or chlorophyll *a* concentration. The objective of this study was to evaluate the toxicity of a few selected compounds, using the AGP of *Selenastrum capricornutum* as measured with different biomass indicators. Although controversy exists with regard to the choice of the response variable, the average growth rate (Nyholm, 1985), was chosen for the purpose of this study.

Material and methods

Unialgal cultures of *Selenastrum capricornutum* (CCAP 278/4) served as test organism and were obtained from the Culture Collection of Algae and Protozoa (CCAP), Natural Environment Research Council, Cambridge, UK and were grown in synthetic algal nutrient medium (SANM) (Miller et al., 1978). They were grown semi-continuously in a Conviron Model E7H (Controlled Environments, Winnipeg, Canada) growth cabinet at 23 ± 2°C.

* To whom all correspondence should be addressed.
(051) 401-2857; fax (051) 448-8772; e-mail plant@rs.uovs.ac.za
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