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

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Abstract

Algal growth potential (AGP) assays were used to determine the possible toxicity of a few selected compounds. The conventional EC_{50} 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 Cu^{2+} 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.

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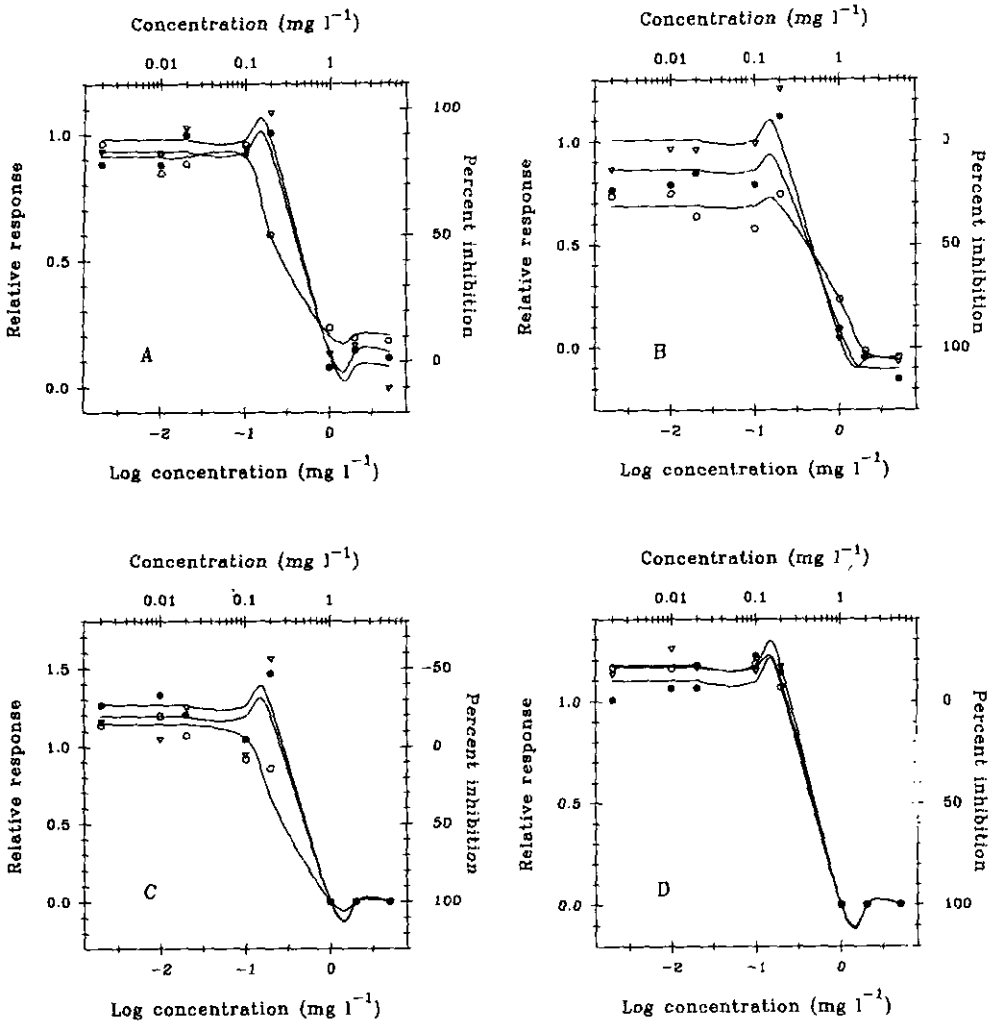

Figure 1
The relative growth response of Selenastrum capricornutum *to different concentrations of copper. Relative responses were obtained from changes in cell numbers (n·mℓ$^{-1}$) (A), dry mass (mg·ℓ$^{-1}$) (B), chlorophyll a content (mg·ℓ$^{-1}$), measured; fluorometrically (C) and spectrophotometrically (D) (○ = 24h, ● = 48h and ▽ = 72h)*

Continuous light was supplied at 300 µmol photon·m^{-2}·s^{-1} by cool white fluorescent tubes and incandescent lamps arranged alongside and above cultures. The medium was well buffered because the pH remained at 7.5 ± 0.5 even after continuously aerating with air which also kept the cells in suspension. Cells in the exponential growth phase (about 10^4 cells·mℓ$^{-1}$) were used in the experiments. The exponential growth phase was determined from daily cell counts with a Coulter Multisizer II counter (Coulter Electronics, UK) and the exponential growth phase was determined from graphical plots. Autoclaved Erlenmeyer flasks (125 mℓ) containing 50 mℓ medium were inoculated with the test organisms under sterile conditions to give a final concentration of approximately 10^4 cells·mℓ$^{-1}$.

Batch culture toxicity tests were conducted with the six reference toxicants. The toxicants were added aseptically to the cultures as 1 mℓ volumes from stock solutions to give the desired final concentrations (Table 1). The control flasks received 1 mℓ sterile distilled water instead of toxin. Immediately after the addition of a toxin, whilst stirring 10 mℓ of the cultures were withdrawn for analysis. Further samples were taken after 24, 48 and 72 h. The pH in the experimental flasks was maintained at 7.5 ± 0.5 for the duration of the test and with each substance tested.

For each flask growth rates were determined from increases in:

- dry mass
- cell numbers
- chlorophyll *a* content measured spectrophotometrically
- chlorophyll *a* content measured fluorometrically.

TABLE 1
TOXIC COMPOUNDS AND THEIR CONCENTRATIONS USED IN THE TESTS

Compound	Concentration (mg·ℓ$^{-1}$)
Cadmium	0.005, 0.01, 0.05, 0.1, 0.25, 0.5 and 1
Copper	0.002, 0.01. 0.02, 0.1, 0.2, 1, 2 and 5
Mercury	0.002, 0.005, 0.01, 0.02, 0.05 and 0.1
Atrazine (an organochloride)	0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1 and 5
Gusathion or **Azinphos-methyl** (an organophosphate)	0.00001, 0.0001, 0.001, 0.01, 0.1 and 1
Phenol	0.001, 0.01, 0.1, 1, 10 and 100

From the cell counts and volumes dry mass was calculated using a conversion factor of 3.6 x 10^{-7} (mg dry mass·ℓ$^{-1}$)/[MCV (µm^3) cells·mℓ$^{-1}$)] which was determined for *S. capricornutum* by Miller et al. (1978). Chlorophyll *a*, measurements were done according to Sartory (1982) and Sartory and Grobbelaar (1984).

The pigment extract was used for chlorophyll *a* fluorescence measurements using a Hitachi Model F-2000 fluorescence spectrophotometer (Hitachi, Ltd. Tokyo, Japan) against a standard curve of chlorophyll *a* (99% pure, Sigma Chemical Co.) at an

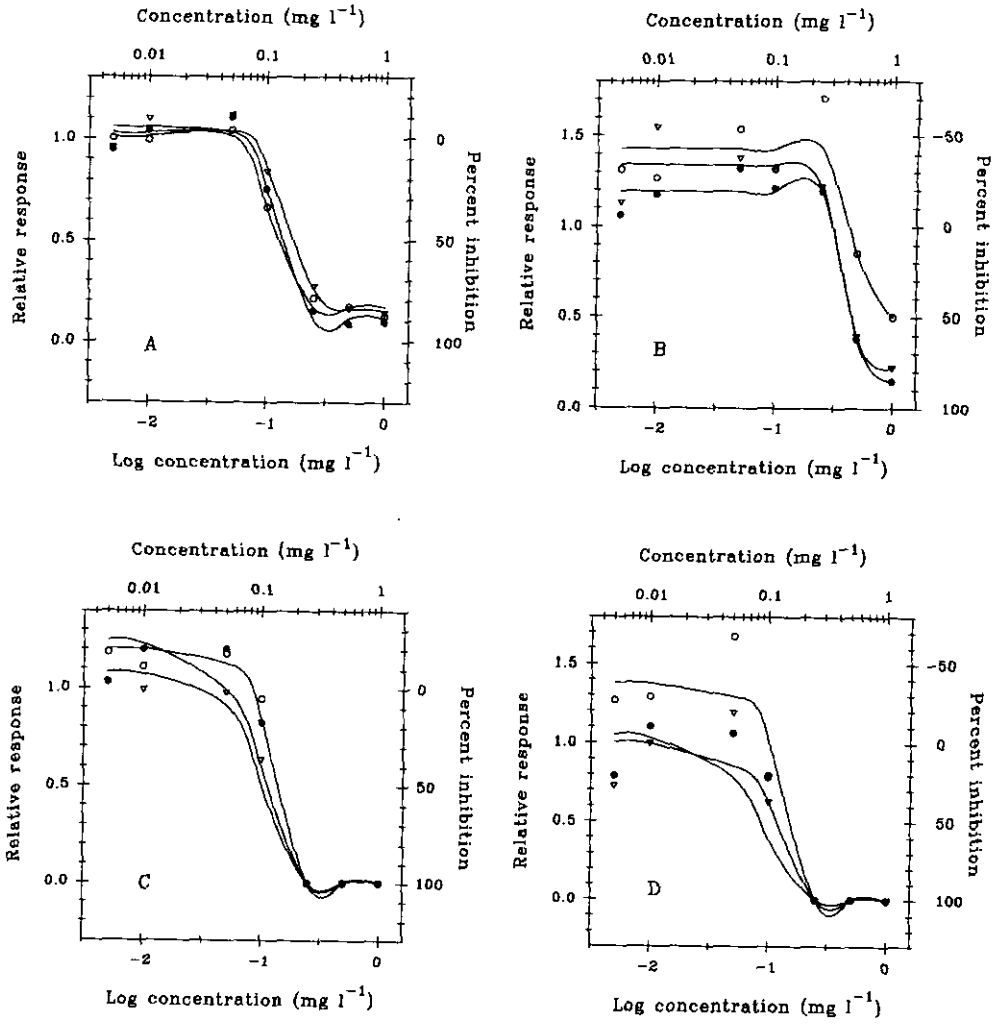

Figure 2
The relative growth response of Selenastrum capricornutum *to different concentrations of cadmium. Relative responses were obtained from changes in cell numbers (n·mt^{-1}) (A), dry mass (mg·t^{-1}) (B), chlorophyll a content (mg·t^{-1}), measured; fluorometrically (C) and spectrophotometrically (D) (○= 24h, ●= 48h and ▽= 72h)*

excitation wavelength of 430 nm and an emission wavelength of 663 nm.

The average growth rate was calculated as:

$$\mu_{av} = (\ln X_t - \ln X_o) / t \quad (1)$$

where X_o is the biomass at time zero and X_t represents the biomass of cultures at time t (Nyholm, 1985).

The average growth rate for each parameter (average of 2 to 3 replicates) was taken as the measured response, from which the relative response (p) was calculated:

$$p = r / r_o \quad (2)$$

where r = the measured response and r_o = the control response. The percentage inhibition (q) is then calculated from Eq. (1) as follows:

$$q = (1 - p) \times 100 \quad (3)$$

Dose-response curves were now obtained from non-linear least square fits of the data using a four-parameter logistic equation (Nyholm et al., 1992):

$$y = a / [1 + \exp^{b(z-c)}] + d \quad (4)$$

where:

z = log (concentration) (mg·t^{-1}) (more symmetric curves were obtained by using log *c* instead of *c*)
a = response range
b = slope coefficient
c = inflection point of curve
d = minimum response.

The EC (effective concentration) estimates are calculated from the non-linear least square fits using the regression curve inversely. With the logarithmic equation, estimating EC_{10} (concentration/dose causing a 10% inhibition) the inverse function is:

$$\log(EC_{10}) = \{\ln [(a / 0.9 - d) - 1] / b\} + c \quad (5)$$

For EC_{50} (50% inhibition):

$$\log(EC_{50}) = \{\ln [(a / 0.5 - d) - 1] / b\} + c \quad (6)$$

For EC_{90} (90% inhibition):

$$\log(EC_{90}) = \{\ln [(a / 0.1 - d) - 1] / b\} + c \quad (7)$$

Results

The relative response of *S. capricornutum* to various concentrations of copper is shown in Fig. 1. The results show that copper at a concentration of 1 mg·t^{-1} completely inhibited *S. capricornutum* growth as measured using all four growth indicators (Figs. 1 A,

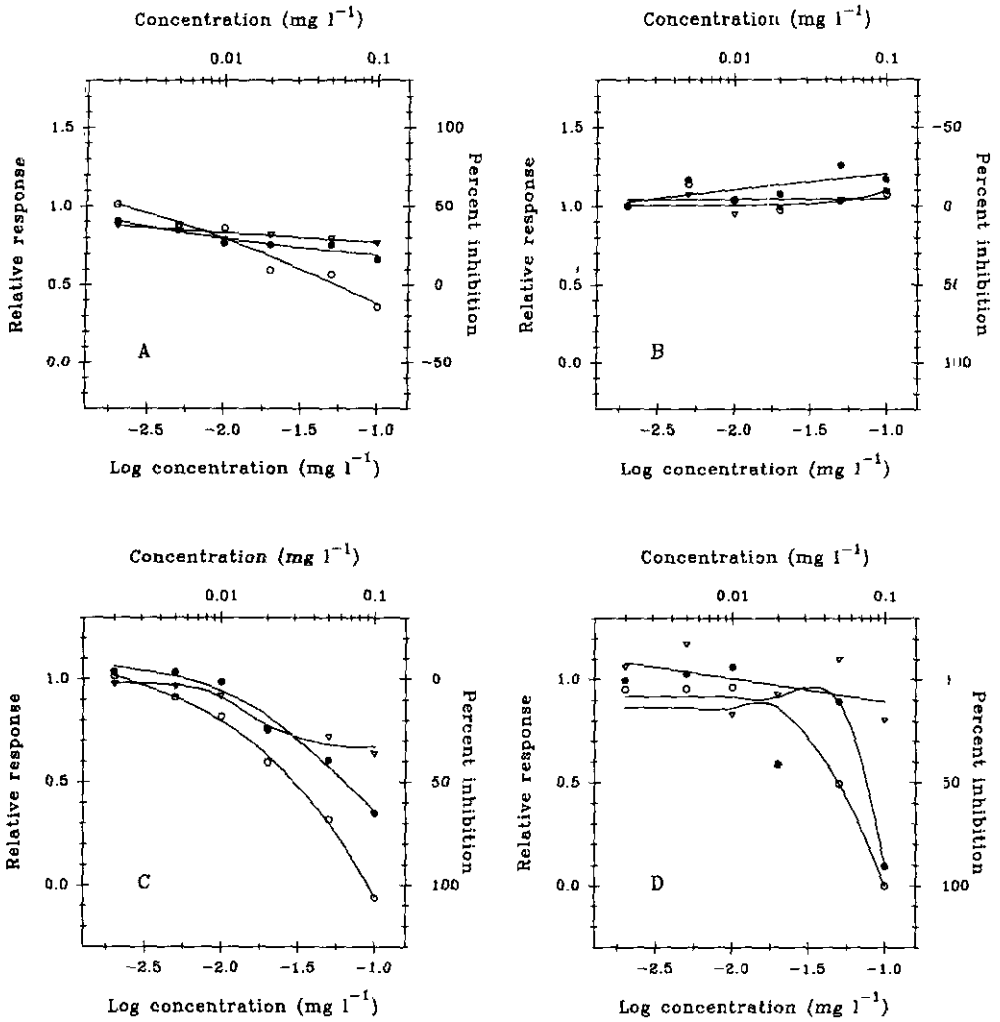

Figure 3
The relative growth response of Selenastrum capricornutum *to different concentrations of mercury. Relative responses were obtained from changes in cell numbers (n·ml^{-1}) (A), dry mass (mg·l^{-1}) (B), chlorophyll a content (mg·l^{-1}), measured; fluorometrically (C) and spectrophotometrically (D) (○= 24h, ●= 48h and ▽ = 72h)*

B, C and D). Copper slightly stimulated growth at concentrations of 0.002 to 0.2 mg·l^{-1} as indicated by the chlorophyll *a* content (Fig. 1 C and D). In terms of cell numbers (Fig. 1 A) some inhibition at a concentration of 0.2 mg·l^{-1} was seen after 24 h. The inhibitory responses after 24 h are turned into stimulatory responses after 48 and 72 h of exposure. The stimulation after 48 and 72 h at a concentration of 0.2 mg·l^{-1} was higher than the initial stimulation at concentrations below 0.2 mg·l^{-1}.

The relative response of *S. capricornutum* to various concentrations of cadmium is shown in Fig. 2. Enhancement of growth was observed at low concentrations of 0.005 and 0.01 mg·l^{-1} cadmium, especially in terms of dry mass (Fig. 2 B) and fluorometric chlorophyll *a* (Fig. 2 D) where the stimulation was nearly 50% (i.e. a relative response of 0.5) after 24 h of exposure. This stimulatory effect declined to that of the control after 48 and 72 h exposure (Fig. 2 D). *S. capricornutum* growth was increasingly inhibited with an increasing cadmium concentration between 0.05 and 1.0 mg·l^{-1}. With a tenfold increase (0.1 to 1.0 mg·l^{-1}) in the dosage the response varied from virtually no inhibition to 100% inhibition. Both copper and cadmium were highly toxic as illustrated by the zero responses in the graphs (Figs. 1 and 2).

The sensitivity of *S. capricornutum* to mercury is variable (Fig. 3) depending on the biomass parameter used. A relative response of 0.5 (50% inhibition) was obtained with cell number (Fig. 3 A) and chlorophyll *a* measurements (Fig. 3 C and D) after 24 h exposure to mercury. After 48 h exposure only chlorophyll *a* measurements showed a 50% inhibition and after 72 h exposure no EC_{50} could be determined in the concentration range of 0.002 to 0.1 mg Hg(II)·l^{-1}. The first observable (EC_{10}) inhibition of *S. capricornutum* growth occurred at between 0.002 and 0.005 mg·l^{-1} mercury for cell number (Fig. 3 A) and chlorophyll *a* (Fig. 3 C and D) measurements. No toxicity was observed when the growth rate was measured in terms of dry mass (Fig. 3 B).

The responses of *S. capricornutum* to various concentrations of atrazine are shown in Fig. 4. The results show that atrazine at low concentrations (0.001 to 0.1 mg·l^{-1}) stimulated algal growth as measured in terms of the chlorophyll *a* content (Fig. 4 C and D). The inhibition increased with an increase in the atrazine concentration of between 0.1 and 5 mg·l^{-1}, a range of almost 0 to almost 100% inhibition. It is also clear, from Figs. 4 C and D, that *S. capricornutum* was less inhibited by atrazine below 1 mg·l^{-1} over time and it seemed as though the algae recovered after an initial inhibition. This also applied to growth as measured in terms of cell numbers and dry mass (Figs. 4 A and B).

Dose-response curves for *S. capricornutum* exposed to different gusathion concentrations are shown in Fig. 5. It is clear from the results that growth was not affected by gusathion over the concentration range of 1 x 10^{-5} to 1 mg·l^{-1}.

Figure 6 shows the effect which phenol had on algal growth. Although no EC_{50} values were obtained in these experiments there exists some variance amongst the different parameters used for quantifying algal growth. With measurement of cell numbers (Fig. 6 A) a steady decline in response was observed with an increase in the concentration of the phenol. On the other hand no observable

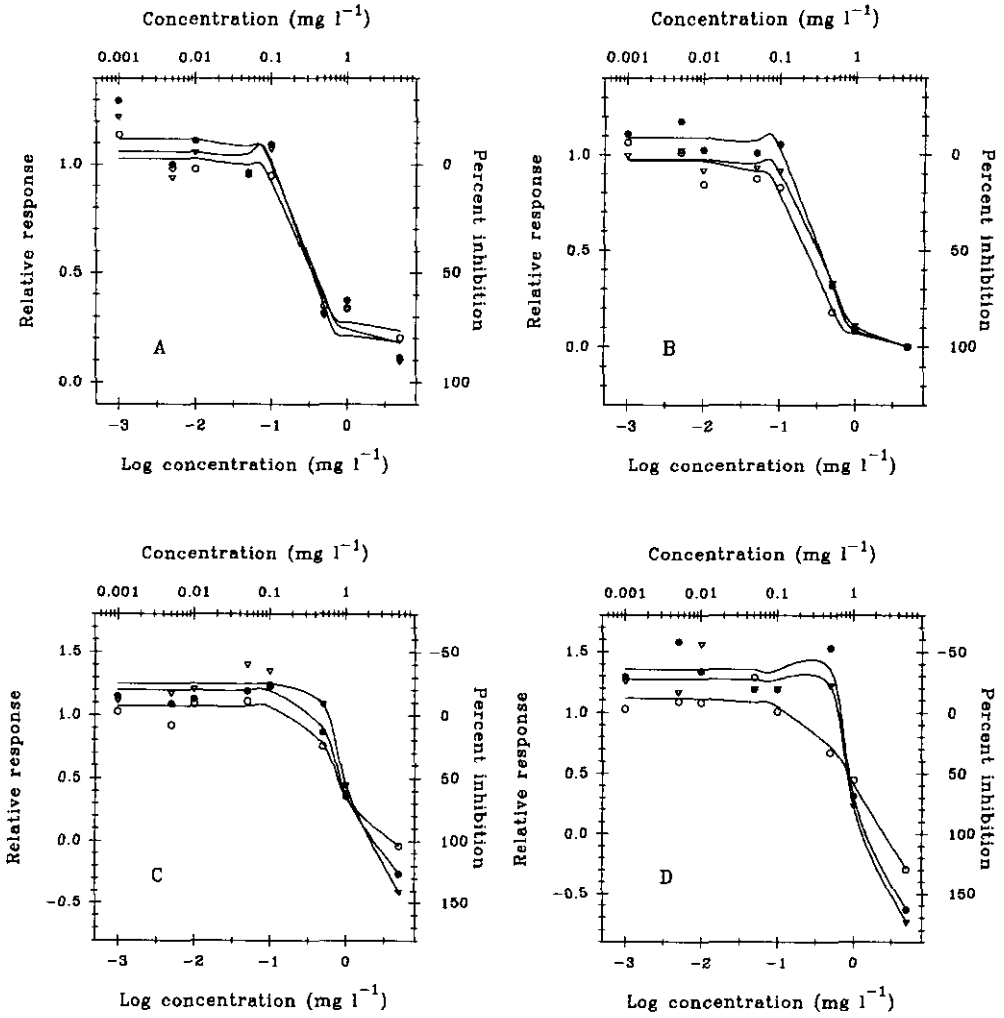

Figure 4
The relative growth response of Selenastrum capricornutum *to different concentrations of atrazine. Relative responses were obtained from changes in cell numbers ($n \cdot ml^{-1}$) (A), dry mass ($mg \cdot l^{-1}$) (B), chlorophyll a content ($mg \cdot l^{-1}$), measured; fluorometrically (C) and spectrophotometrically (D) (○= 24h, ●= 48h and ▽ = 72h)*

effect (less than 10% inhibition) could be seen in terms of dry mass (Fig. 6 B). An initial stimulation of growth by 15% was seen at low concentrations in terms of fluorometric chlorophyll*a* measurements (Fig. 6 C), after which growth was increasingly inhibited at higher phenol concentrations. Growth in terms of spectrophotometric chlorophyll *a* measurements (Fig. 6 D) showed a time dependence where after 24 h exposure no effect was observed, whilst a 40% inhibition was seen after 72 h exposure to 1 mg phenol·l^{-1}.

Discussion

Toxicity tests using algae as test organisms provide an important method of assessing the effects of elements in solution on biological systems. Since algae are at the base of most aquatic food webs, any factor affecting them will have consequences for entire systems. The results presented clearly show that the algae used (*S. capricornutum*) are sensitive to certain toxic substances and that the degree of toxicity depends on the particular substance, as well as the growth parameters used. The EC values are given in Table 2 for the various compounds tested.

Christensen and Nyholm (1984) reported EC values of 0.0263, 0.0485 and 0.0717 mg·l^{-1} for Cu (EC_{10}, EC_{50} and EC_{90} respectively) which is one order of magnitude lower than the values of 0.151, 0.227 and 0.290 mg·l^{-1} as determined in this study in terms of fluorometric chlorophyll *a* measurements after 24 h of exposure (Table 2). According to Wong (1989) both *S. capricornutum* and *Scenedesmus* sp. were unable to survive a Cu concentration above 90 µg·l^{-1} which is in the same order as the EC_{90} value reported by Christensen and Nyholm (1984). Our results suggest that the degree of copper toxicity decreases with time as indicated by all four growth parameters. This indicates an adaptation to copper. The differences were highly significant (P = 0.009) between 24 and 48 h exposure and significant (P = 0.05) between 48 and 72 h exposure. It is, therefore, imperative that the duration of copper toxicity tests, employing algae, be as short as possible to ensure maximum sensitivity.

Our results support reports that Cd is extremely toxic to freshwater algae. An EC_{50} value for Cd of 0.019 mg·l^{-1} was reported by Vocke et al. (1980) which is five times lower than the EC_{50} value of ± 0.1 mg Cd·l^{-1} as determined in our investigation for *S. capricornutum* growth in terms of chlorophyll *a* measurements (Table 2). Vocke et al. (1980) used a two-week exposure test, whilst ours was at maximum only after 72 h. It may well be that they could detect Cd concentrations as low as 0.019 mg·l^{-1}, but it is also possible that some or other factor became limiting in their assays. This caution is confirmed by Nyholm (1985) who pointed out that tests should be conducted over an exposure period of only 2 to 3 d to avoid the possible effect that depletion of nutrients might have on the end result.

Harriss et al. (1970), noted that the toxicity of mercurial compounds decreased with increasing cell concentrations. Our results show that algal growth was inhibited over time by Hg, as indicated by a decrease in cell numbers with time when compared to the control culture (Fig. 3 A). This increase in cell numbers could

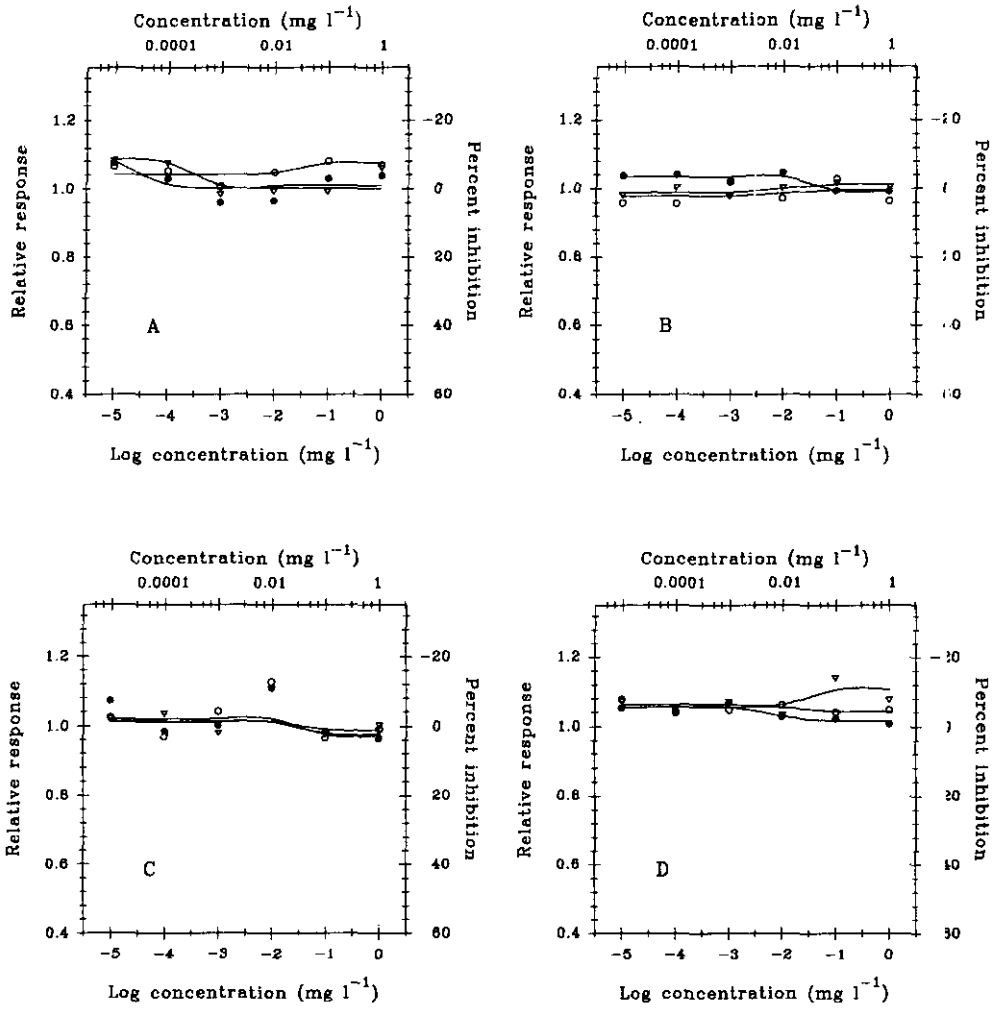

Figure 5
The relative growth response of Selenastrum capricornutum *to different concentrations of gusathion. Relative responses were obtained from changes in cell numbers (n mℓ^{-1}) (A), dry mass (mg·ℓ^{-1}) (B), chlorophyll a content (mg·ℓ^{-1}), measured; fluorometrically (C) and spectrophotometrically (D) (○= 24h, ●= 48h and ▽= 72h)*

possibly explain why EC_{50} and EC_{90} values could not be calculated for Hg after 72 h of exposure and why the EC_{10} values increased with time. The EC_{50} value for Hg, as derived from chlorophyll *a* fluorescence measurements, of 0.030 mg·ℓ^{-1} (Table 2) compares well with the 0.033 mg·ℓ^{-1} as reported by Vocke et al. (1980).

Atrazine generally inhibited algal biomass growth (measured as cell numbers, dry mass, and chlorophyll *a* content determined spectrophotometrically or fluorometrically). Growth occurred over all three days but the growth rate decreased over time as can be seen from EC values in Table 2. The lowest EC_{50} value (0.340 mg·ℓ^{-1}) was obtained from calculations using cell numbers. The EC_{50} values obtained with cell numbers and chlorophyll*a* determined spectrophotometrically increased between days 1 and 2 and decreased between days 2 and 3. Fluorometric chlorophyll *a* measurements showed a progressive increase in EC_{10} and EC_{50} values from day 1 to 3. The significance of the results obtained with atrazine is that although the EC_{10} and EC_{50} values fluctuated over time and amongst measurements the lowest values were obtained after 24 h growth. EC_{90} values, where they could be calculated (only chlorophyll *a* measurements), show the opposite with the lowest value after 72 h growth. It can thus be concluded that the effect of atrazine decreased over time up to a certain level (EC_{90}) which can then be considered as the "lethal" dose after which recovery was no longer possible. The algae started recovering from day 2, which is consistent with findings of researchers who exposed isolated algal species to atrazine in the laboratory (Walsh, 1972; Abou-Waly et al., 1991). Although Abou-Waly et al. (1991) did not calculate EC values from their chlorophyll*a* measurements, they reported atrazine values of 0.23 to 0.42 mg·ℓ^{-1}, which significantly reduced *S. capricornutum* growth. This agrees favourably with our EC_{10} values (i.e. first observable effect) of 0.28 to 0.38 mg·ℓ^{-1} calculated from chlorophyll*a* measurements after 24 h (Table 2). Although EC_{90} values could not be calculated, results obtained from cell number measurements were the most sensitive and EC_{10} and EC_{50} values were determined. The dry mass measurements failed to give any meaningful results (Table 2) and this method of determining algal growth should be considered inferior.

Gusathion does not show any effect on algal growth as determined from our experiments. This was also confirmed by Mohapatra and Mohanty (1992) when they found that dimethoate (an organophosphorus pesticide) at 10 and 25 mg·ℓ^{-1} did not show any change in the sigmoidal pattern of growth of *Chlorella vulgaris* grown for 10 d on nutrient agar plates where cell multiplication was observed with a microscope. However, they reported sublethal concentrations (LC_{50} which is equal to EC_{50}) of 28.5 mg·ℓ^{-1}. This does not mean that *S. capricornutum* would have responded in the same way if we had tested higher concentrations of gusathion because Kühn and Pattard (1990) reported EC_{50} values ranging from 1.1 mg·ℓ^{-1} to 2.8 mg·ℓ^{-1} with a 72 h test period where *Scenedesmus subspicatus* (batch cultures in Erlenmeyer flasks) growth rates were used when exposed to another

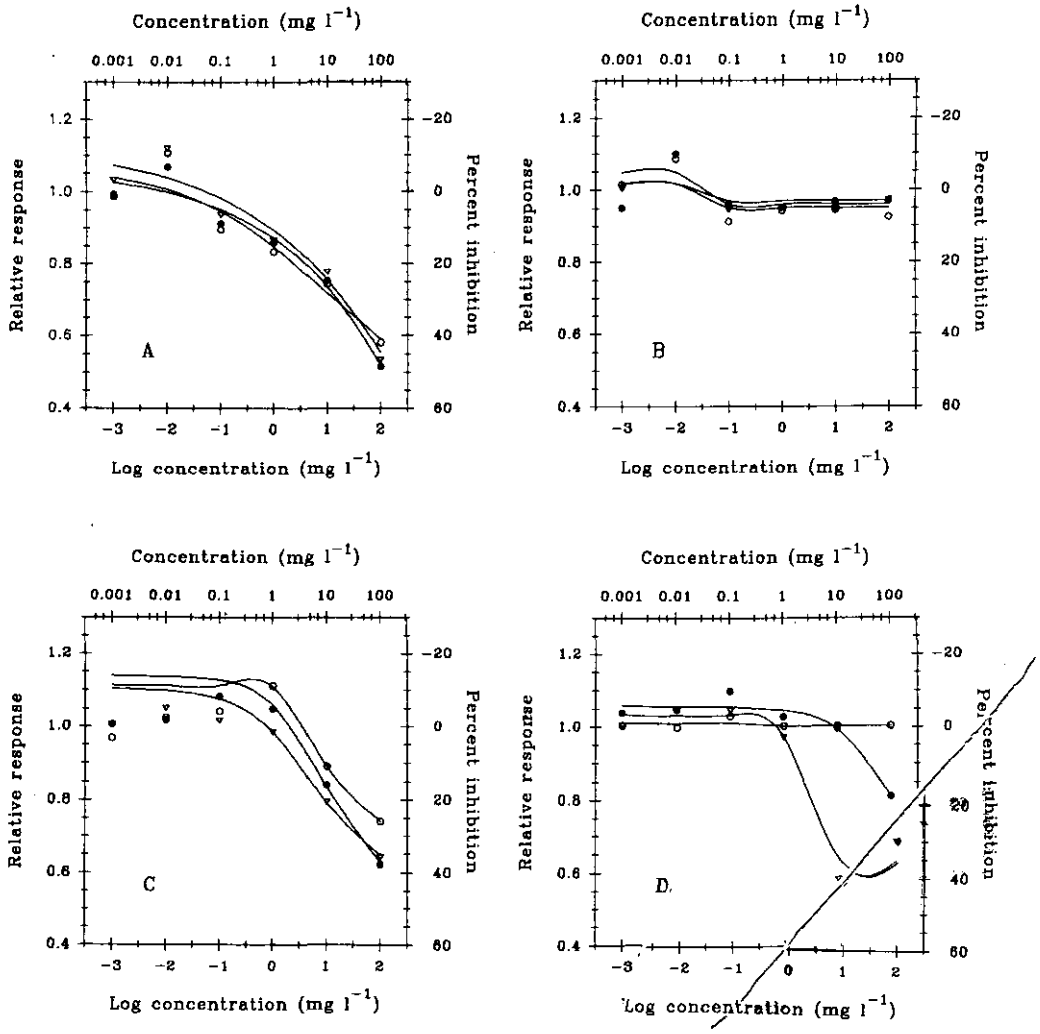

Figure 6
The relative growth response of Selenastrum capricornutum *to different concentrations of phenol. Relative responses were obtained from changes in cell numbers (n·mℓ$^{-1}$) (A), dry mass (mg·ℓ$^{-1}$) (B), chlorophyll a content (mg·ℓ$^{-1}$), measured; fluorometrically (C) and spectrophotometrically (D) (○= 24h, ●= 48h and ▽ = 72h)*

organophosphorus compound, namely phosphoric acid tributyl ester. In our tests the 1 mg·ℓ$^{-1}$ showed no effect.

Phenol volatility could have a marked influence on the results obtained from toxicity measurements. This effect can be offset, however, by providing appropriate modifications to seal individual flasks better and to improve experimental design. Thellen et al. (1989) reported an EC_{50} value of 69.7 mg·ℓ$^{-1}$ for phenol obtained with a microplate toxicity assay of over 96 h. None of the results obtained in this study were close to this value except for an EC_{10} of 42.55 mg·ℓ$^{-1}$ obtained after 48 h exposure in terms of chlorophyll *a* measured spectrophotometrically (Table 2). No EC_{50} values were obtained for phenol in the concentration range tested, which emphasises the fact that when working with a volatile substance, care should be taken to improve the experimental design. Our experiments did not allow for losses of volatile substances.

Because of the negative values of the growth rate, especially at high concentrations of the tested toxic compounds, after one day's exposure (Figs. 1, 2 and 4) and the ability of the algae to recover with time, results from such tests should be interpreted with caution. We are of the opinion that more meaningful results will be obtained when measurements are made every day for at least 3 d after exposure.

Comparing the four different growth parameters the following can be seen (See Table 2):

- Dry mass measurements were the least sensitive towards test compounds.
- The highest sensitivity for Cu was obtained after 24 h with fluorometric chlorophyll *a* measurements.
- Cd had an increasing effect over time as measured by all the parameters except cell numbers with the highest sensitivity obtained after 72 h from spectrophotometric chlorophyll *a* measurements.
- Hg had a decreasing effect over time but again cell numbers showed the opposite and the highest sensitivity was obtained with spectrophotometric chlorophyll *a* measurements after 24 h.
- Cell numbers were the most sensitive parameter for atrazine and phenol after 24 h.

Cell numbers failed to distinguish between living and dead cells and also gave contradictory results as far as Cd and Hg were concerned which render them an inappropriate parameter for the purpose of this experiment. There was no big difference between the two different chlorophyll *a* parameters although fluorometric measurements gave the higher sensitivity three out of five times (i.e. copper, atrazine and phenol). The fluorometric chlorophyll *a* measurements are, therefore, considered to give the better overall estimate of algal growth potential under toxicity stress, and we recommend its use as an algal growth parameter.

TABLE 2
SUMMARY OF THE RESULTS FROM ALGAL GROWTH POTENTIAL TOXICITY TESTS. EC-VALUES (IN $mg \cdot \ell^{-1}$) WERE CALCULATED FROM DOSE-RESPONSE CURVES. GROWTH RATES WERE DETERMINED FROM MEASUREMENTS OF DRY MASS, CELL NUMBERS, CHLOROPHYLL *A* CONCENTRATIONS DETERMINED SPECTROPHOTOMETRICALLY AND FLUOROMETRICALLY (NR = NO RESPONSE)

Compound	Time	Cell numbers			Dry mass			Chlorophyll *a*					
								Spectrophotometric			Fluorometric		
		EC10	EC50	EC90	EC10	EC50	EC90	EC10	EC50	EC90	EC10	EC50	EC90
Copper	24h	0.160	0.207	nr	nr	0.699	1.239	0.254	0.312	0.349	0.151	0.227	0.290
	48h	0.427	0.505	nr	0.603	0.940	nr	0.263	0.311	0.336	0.409	0.450	0.475
	72h	0.497	0.688	1.146	0.680	0.917	0.960	0.340	0.392	0.407	0.431	0.499	0.524
Cadmium	24h	0.092	0.104	nr	0.494	0.713	nr	0.116	0.161	0.200	0.093	0.139	0.173
	48h	0.093	0.109	nr	0.422	0.479	nr	0.039	0.126	0.199	0.063	0.111	0.154
	72h	0.093	0.164	nr	0.342	0.452	nr	0.033	0.097	0.135	0.053	0.101	0.136
Mercury	24h	0.005	0.053	nr	nr	nr	nr	0.002	0.050	0.052	0.005	0.030	0.076
	48h	0.002	nr	nr	nr	nr	nr	0.047	0.094	0.100	0.013	0.063	nr
	72h	0.001	nr	nr	nr	nr	nr	0.094	nr	nr	0.011	nr	nr
Atrazine	24h	0.110	0.340	nr	2.802	nr	nr	0.279	0.838	1.847	0.375	0.784	1.715
	48h	0.148	0.377	nr	nr	nr	nr	0.948	0.985	1.018	0.500	0.856	1.409
	72h	0.167	0.359	nr	nr	nr	nr	0.743	0.902	1.053	0.670	0.960	1.322
Gusathion	24h	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
	48h	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
	72h	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Phenol	24h	0.329	nr	nr	nr	nr	nr	nr	nr	nr	9.576	nr	nr
	48h	0.495	nr	nr	nr	nr	nr	42.55	nr	nr	5.946	nr	nr
	72h	0.969	nr	nr	nr	nr	nr	1.199	nr	nr	3.003	nr	nr

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