Photosynthetic and population growth response of the test alga Selenastrum capricornutum Printz to zinc, cadmium and suspended sediment elutriates

Pardos, Michel; Benninghoff, Christophe; Thomas, Richard Lynn

How to cite

PARDOS, Michel, BENNINGHOFF, Christophe, THOMAS, Richard Lynn. Photosynthetic and population growth response of the test alga Selenastrum capricornutum Printz to zinc, cadmium and suspended sediment elutriates. In: Journal of applied phycology, 1998, vol. 10, n° 2, p. 145–151. doi: 10.1023/A:1008043931094

This publication URL: https://archive-ouverte.unige.ch/unige:157560
Publication DOI: 10.1023/A:1008043931094

Journal of Applied Phycology **10:** 145–151, 1998.

Photosynthetic and population growth response of the test alga *Selenastrum capricornutum* Printz to zinc, cadmium and suspended sediment elutriates

Michel Pardos*, Christophe Benninghoff & Richard L. Thomas
Institute F.-A. Forel, University of Geneva, 10 Route de Suisse, CH-1290 Versoix, Switzerland

(*Author for correspondence, tel: +4122 9509214; fax: +4122 7551382; e-mail: michel.pardos@terre.unige.ch)

Received 29 January 1998; revised 19 March 1998; accepted 20 March 1998

Key words: ^{14}C, photosynthesis, population growth, *Selenastrum capricornutum*, suspended sediment elutriate, zinc, cadmium

Abstract

Short-term ^{14}C-fixation (4 h) *Selenastrum capricornutum* algal toxicity tests were conducted with Cd ($n = 8$), Zn ($n = 9$) and suspended sediment aqueous elutriates ($n = 28$) and the results were compared to those obtained in a 48 h population growth test. In order to provide more realistic experimental conditions, toxicity tests were carried out in prefiltered nutrient-spiked Lake Geneva water. The population growth inhibition test was significantly more sensitive than the ^{14}C-fixation test for Cd (median EC50-4h and EC50-48h values of 600 and 118 μg L^{-1}, respectively) whereas no significant difference was measured for Zn toxicity (median EC50-4h and EC50-48h values of 97 and 96 μg L^{-1}, respectively). With suspended sediment aqueous elutriates, the relative sensitivity of the two different end points is sample dependent, with ratios of the EC25 for the ^{14}C-fixation: population growth test ranging from < 0.26 to > 53.3. Elutriate toxicity shows no apparent relationship between the acute and chronic test, indicating that population growth inhibition cannot be derived directly or predicted from ^{14}C-fixation. Both tests with their specific advantages and limitations provide valuable complementary information to measure the impact of single toxicants or complex mixtures on aquatic plants.

Introduction

A variety of test methods are available to determine the phytotoxic effects of chemicals, effluents or sediments. The freshwater alga most frequently used is *Selenastrum capricornutum* Printz, recently renamed *Raphidocelis subcapitata* Korschikov. Laboratory toxicity studies undertaken with micro-algae have mostly focused on measuring end points resulting from chronic exposures of usually 3–4 days (Lewis, 1995).

Acute toxicity tests are seldom conducted with algae but photosynthetic activity has been a common parameter monitored in laboratory toxicity bioassays with cultured algae. The more common methods used for these analysis have been $^{14}CO_2$ uptake and oxygen evolution. The primary advantage of photosynthesis tests is their short duration, usually 2 to 4 h, but exposure times have also ranged from 0.5 to 24 h (Van der Heever & Grobbelaar, 1996; Hall et al., 1996). Generally, it appears that photosynthetic activity has been a less sensitive indicator than population growth measurements (3- to 4 days) of toxic effect. Such toxicity has been observed for cadmium, copper, diethyl phthalate, pentachlorophenol, atrazine, simazine, effluents (Versteeg, 1990) as well as anisole, phenol and sodium laurylsulphate (Nyholm & Damgaard, 1990) and oil shale process waters (Delistraty, 1986). However, comparisons of the sensitivity of the two test systems have not been made systematically for similar test conditions (medium, temperature, light, initial algal

biomass), in particular with complex environmental samples.

As part of a sediment toxicity assessment program of the Franco-Swiss International Commission for the Protection of the Waters of Lake Geneva (Pardos et al., 1994, 1996), the purpose of this work was to develop a short-term (4 h) test based on uptake and assimilation of radio-labeled carbon in *S. capricornutum* and to compare its sensitivity to the 48-h population growth inhibition test. Algae were exposed under similar test conditions to suspended sediment aqueous elutriates, Zn and Cd and the results of both types of end points are interpreted for sample toxicity comparison and laboratory to field extrapolation.

Material and methods

Suspended sediment collection and preparation

For the recovery of suspended sediment, large volume water samples were pumped in 1994 and 1995 from the river mouth of tributaries around Lake Geneva through three continuous-flow centrifuges operating in parallel (two Westphalia type KA2-06-175 and one Alfa-Laval Sedisamp system II). Flow rate for all centrifuges was maintained at 5–6 L min^{-1}, giving a total water throughput of 15–18 L min^{-1}. Since suspended solids were generally low in the rivers, water processing was carried out for $\sim$ 3 h, giving a total volume of some 2700–3240 L. The efficiency of particle separation in these centrifuges is known to be greater than 90% of the incoming suspended sediment (Ongley & Thomas, 1989; Santiago et al., 1990). Suspended sediments were collected at 28 locations in order to cover a wide range of known or suspected contamination by heavy metals and organic micropollutants. Median grain size values ranged from 7.2 to 43.3 μm and organic carbon content varied from 1.4 to 24.4% w/w (Pardos et al., 1996).

The suspended sediment samples were kept in the field in a cooler with ice-packs. On return to the laboratory ($<$ 24 h) subsamples for bioassays were kept at 4 °C before the extraction procedure ($<$ 24 h). Sediments were elutriated with Lake Geneva water taken from the surface to 5 m depth (station GE2) and prefiltered through a 0.45 μm pore size nitrocellulose filters. Filtered lake water characteristics were: pH 7.8 to 8.8; total alkalinity 1.6 to 1.8 mmol HCl L^{-1}; dissolved organic carbon 0.8 to 1.3 mg L^{-1}; Ca 40.1 to 43.5 mg L^{-1}; Mg 5.8 to 6.1 mg L^{-1}; Zn 2.3 to 21.0 μg L^{-1} and Cd $<$ 0.1 μg L^{-1}. The elutriation was carried out by mixing sediment to lake water in a solid to liquid volumetric ratio of 1:4, shaking by rotation for one hour, and filtering the supernatant at 0.45 μm following centrifugation (2000 $\times$ g, 15 min). Elutriates (pH range from 7.4 to 8.6) were prepared less than 24 h before the bioassay experiments.

Algal culture

Selenastrum capricornutum (Chlorophyceae) was cultured under axenic conditions in borosilicate glass flasks. The culture medium was the standard AAP media (U.S. EPA, 1989). Culture flasks were shaken continuously at 100 rpm and incubated at 24 $\pm$ 2 °C on a rotary shaker table under constant light (255 μmol photon m^{-2} s^{-1}).

An algal inoculum was prepared for each sample from fresh culture stocks sampled during the exponential growth phase by concentrating cells by centrifugation and resuspending them into an appropriate volume of nutrient-spiked lake water (see below). The final density was verified by cell counts.

Toxicity testing

In order to incorporate more realistic experimental conditions, samples were diluted using prefiltered (0.45 μm) Lake Geneva water with a control of lake water without elutriates. Exposure chambers were Falcon microplate (n°3047) containing 2 mL (population growth test) and 3 mL (photosynthetic activity) final exposure volume. Nutrients were added as a 10 μL volume of concentrated medium (211–311x AAP media) to all control and treatments wells before addition of algae. Inoculum (100 μL) was added to produce an initial cell density of 10^5 cells mL^{-1} (Ross et al., 1988). Five final serial dilutions of sediment elutriates were tested in duplicate or triplicate: 6.6, 19.9, 49.7, 76.2 and 96% or 2.4, 4.7, 9.5, 19.0, 37.9 and 75.8% elutriate depending on suspected toxicity. For Zn^2 ($ZnCl_2$) and Cd^2 ($CdCl_2$) toxicity analysis, serial dilutions from 40 to 470 μg Zn^2 L^{-1} and from 30 to 1650 μg Cd^2 L^{-1} were prepared for testing.

Total exposure time was 4 and 48 h and incubation conditions were 24 $\pm$ 2 °C, 255 μmol photon m^{-2} s^{-1} with no shaking. The decision to process sample incubation for 48 h and not 72 or 96 h as in the classical chronic toxicity tests was made following preliminary experiments demonstrating that precipitation of calcite in the presence of photosynthesizing algae oc-

curred in the nutrient-spiked lake water medium when incubation time was > 48 h.

For the 4 h test, all treatments were spiked with 1 μCi $NaH^{14}CO_3$ at the beginning of the incubation. At the end of the incubation, a 2 mL aliquot was acidified with 200 μL of HCl 0.1N to stop biological activity and sonicated for 45 min to remove un-assimilated inorganic ^{14}C in order to measure only the assimilated radioactive carbon. Furthermore, total available ^{14}C was measured on an additional 0.5 mL aliquot. Radioactivity was counted with a liquid scintillation counter (Canberra Packard TriCarb 1500). The amount of assimilated carbon was calculated from the ratio: [(assimilated ^{14}C/available ^{14}C) $\times$ available $^{12}C \times 1.06$], where the constant is a correction factor for the selective uptake of the isotope (Plumb & Lee, 1979). Total inorganic carbon in filtered lake water and in final filtered elutriates was measured to account for the background inorganic carbon content of the elutriates, which may exceed the inorganic carbon levels of the lake water. Inorganic carbon concentrations were measured by calculation using pH and alkalinity data (Stumm & Morgan, 1996).

For the 48-h test, cells were quantified with an electronic particle counter (Coulter Counter, TA model, 50 μm cell) at the end of the incubation. Depending on the cell density observed during the incubation, 0.5 to 1 mL of algal culture diluted in 25 mL of NaCl 1% was used for precise cell counting.

End point determinations and statistical analyses

The results of acute and chronic toxicity are reported as the EC25 or EC50 values (concentration that reduces photosynthesis/growth of 25 and 50% v/v, respectively) and their 95% confidence intervals. The lower the EC25 or EC50, the more toxic the aqueous extract. These end points are estimated using the linear interpolation method (U.S. EPA, 1994). Spearman rank-correlation analysis was done to underline possible links between acute and chronic toxicities in algae exposed to sediment elutriates. Mann-Whitney rank sum test was applied to check for differences between the 4 and 48 h tests for Zn and Cd. Significance for both statistical tests was set at $P < 0.05$. When end point values were undefined (i.e., > 96% v/v), they were arbitrarily set at the detection limit.

Results

The total biomass after 48-h incubation of control cultures (nutrient-spiked lake water) in population growth inhibition tests reached on average 2.46 10^6 cells mL^{-1} $\pm$12.3% ($n = 112$), corresponding to an increase from initial conditions (10^5 cells mL^{-1}) of a factor 24.6. Since the test duration is longer than the reproductive cycle of the organism and that the factor increase is > 16 (ISO, 1989), this bioassay is considered as representative of a chronic toxicity. In ^{14}C-assimilation tests, photosynthetic activity averaged 142.5 mgC m^{-3} h^{-1} $\pm$11.7% ($n = 99$). As a consequence, fluctuation in *S. capricornutum* population growth and photosynthetic activity was not of major concern during our study.

Zn and Cd tests

Median EC50 values ($n = 9$) for Zn to *S. capricornutum* in nutrient-spiked Lake Geneva water were 97 (25^{th} and 75^{th} percentile of 70 and 111 μg L^{-1}) and 96 μg L^{-1} (25^{th} and 75^{th} percentile of 90 and 103 μg L^{-1}) after 4 h and 48 h incubation, respectively (Figure 1). For Cd, median EC50 values ($n = 8$) were at 600 μg L^{-1} (25^{th} and 75^{th} percentile of 397 and 655 μg L^{-1}) for the short-term test and at 118 μg L^{-1} (25^{th} and 75^{th} percentile of 110 and 128 μg L^{-1}) for the chronic exposure (Figure 1). The growth inhibition test is significantly ($P < 0.001$) more sensitive than the ^{14}C assimilation for Cd whereas there is no statistically difference ($P = 0.930$) in median EC50 concentrations for Zn. The short- to long-term EC50 ratio is 1.0 and 5.1 for Zn and Cd, respectively.

Sediment elutriates

The calculated EC25 (% elutriate, v/v) for the 28 suspended sediment elutriates are reported in Table 1. When considering the 25% effect, 24 (85.7%) and 12 (42.9%) elutriates are inhibitory, respectively to photosynthetic and population growth response. EC25-4 h varied from 24.3 to > 96%, while EC25-48 h ranged from 1.8 to > 96%. Based on EC25 data, 10 samples are toxic for both photosynthetic and growth responses (case I, Table 1); 14 elutriates are only toxic in the acute test (case II, Table 1); 2 are only toxic in the chronic test (case III, Table 1); and 2 samples are non toxic for the two endpoints (case IV, Table 1).

EC25 photosynthetic to growth ratio calculated for the sediment elutriates investigated vary from < 0.26 to > 53.3. The ^{14}C-fixation test is more sensitive

Table 1. Comparison of the toxicity (EC25 values and 95% confidence intervals) of suspended sediment elutriates to the alga *S. capricornutum* as assessed by the 4 h ^{14}C-assimilation test and the 48 h population growth test. Case I: samples toxic after 4 and 48 h; Case II: samples only toxic after 4 h; Case III: samples only toxic after 48 h; Case IV: samples non toxic after 4 and 48 h

Case	Sample	^{14}C-Assimilation		Population Growth		EC25-4h/
		EC25-4h (% v/v)	95% C.I.	EC25-48h (% v/v)	95% C.I.	EC25-48h ratio
I	#1	24.3	18.0–30.6	21.4	20.9–21.4	1.14
	#2	24.7	21.9–27.1	24.5	17.6–33.5	1.01
	#3	28.9	24.5–34.3	11.4	11.0–11.7	2.54
	#4	29.8	24.1–35.5	73.9	73.0–74.7	0.40
	#5	32.4	23.3–38.3	3.8	3.2– 4.4	8.53
	#6	39.6	33.4–46.4	81.0	78.9–83.6	0.49
	#7	45.7	37.1–58.7	4.6	3.6–14.6	9.93
	#8	48.6	44.3–56.8	77.0	73.4–79.4	0.63
	#9	60.3	51.4–64.0	15.4	14.4–16.3	3.92
	#10	65.6	58.1–71.0	27.7	23.7–31.1	2.37
II	#11	24.8	23.9–25.9	> 96.0	NC	< 0.26
	#12	25.3	23.3–26.7	> 96.0	NC	< 0.26
	#13	25.5	16.3–39.3	> 96.0*	NC	< 0.26
	#14	32.0	29.8–33.8	> 96.0	NC	< 0.33
	#15	33.8	32.1–35.5	> 96.0	NC	< 0.35
	#16	34.3	32.2–35.9	> 96.0	NC	< 0.36
	#17	36.0	28.6–49.7	> 96.0	NC	< 0.38
	#18	43.3	36.7–48.8	> 96.0	NC	< 0.45
	#19	43.8	41.1–45.7	> 96.0	NC	< 0.46
	#20	48.3	42.8–57.5	> 96.0	NC	< 0.50
	#21	52.0	43.9–79.4	> 96.0*	NC	< 0.54
	#22	59.5	49.1–65.6	> 96.0	NC	< 0.62
	#23	60.5	55.3–66.4	> 96.0	NC	< 0.63
	#24	88.0	77.9–94.7	> 96.0	NC	< 0.92
III	#25	> 96.0	NC	13.6	9.5–16.0	> 7.06
	#26	> 96.0*	NC	1.8	1.5– 2.3	> 53.3
IV	#27	> 96.0	NC	> 96.0	NC	1.0
	#28	> 96.0	NC	> 96.0	NC	1.0

*: stimulation (> 20%) at the maximum elutriate concentration tested.
NC: non calculable.

in 60.7% of the cases than the chronic test, whereas 25% of the elutriates are more toxic in the population growth test (Figure 2). Spearman rank order correlations between EC25-4 h and EC25-48 h ($r_s = 0.00$, $P = 0.997$, $n = 28$) revealed no relationship between the two variables. In addition, if we consider only samples that are toxic for both end points parameters (Case I, Table 1), results are similar with again no strong relationship between photosynthetic activity and growth of *S. capricornutum* ($r_s = 0.152$, $P = 0.656$, $n = 10$).

Discussion

The results showed clearly that the relative sensitivity of the two procedures (^{14}C short-term uptake and population growth measurement) is sample dependent and the data obtained with suspended sediment elutriates (Table 1) demonstrated no apparent relationship between the two endpoints.

As analyses of photosynthesis by ^{14}C-fixation reflect an effect on a specific physiological process,

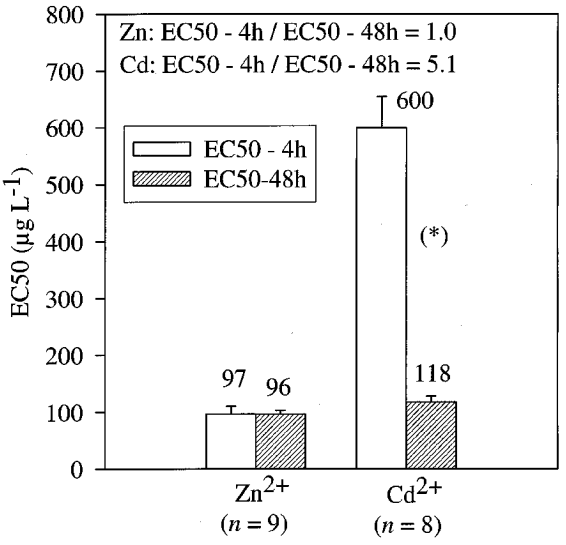

Figure 1. Comparison of the toxicity (median EC50 value and 75th percentile) of Zn and Cd to the alga *S. capricornutum* as assessed by the 4 h ^{14}C-assimilation test and the 48 h population growth test. *: statistically significant ($P < 0.001$).

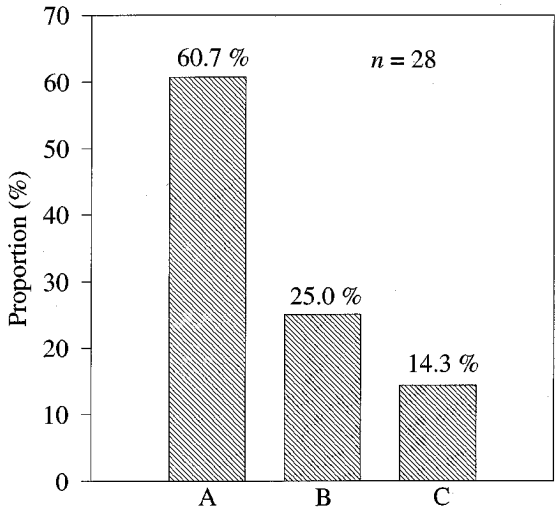

Figure 2. Relative sensitivity of the *S. capricornutum* ^{14}C-assimilation test and the 48 h population growth test based on EC25 values of suspended sediment elutriates. A: ^{14}C-assimilation test more sensitive; B: Population growth test more sensitive; C: Same test result (sample is non toxic or has no significant difference between EC25 values based on the 95% confidence intervals).

while measurements of growth reflect an integrated effect on all physiological processes, different scenarios are possible to explain these results. Compounds classified as non-specific physiological metabolic inhibitors (e.g., phenol) which indirectly affect photosynthesis and/or require more than 4 h to cause detectable toxicity will have high short- to long-term test ratios. Therefore, the use of a photosynthesis test does not make it possible in these cases to estimate the full toxic effect, as it is suggested with Cd toxicity results. In contrast, in long-term tests, toxicants have additional time and cellular sites to cause toxicity which could explain the high toxicities measured in some samples after 48 h (e.g., samples #5, 7 and 26, Table 1) and high short- to long-term EC25 ratios (Table 1). On the other hand, diminution of toxicity with increasing incubation time may indicate the presence of volatile contaminants, toxicant metabolism (Dijkman et al., 1997), speciation effects or may reflect the bonding of metals by intracellular phosphates rendering them less toxic (Wong et al., 1995).

The results of this study are in good agreement with other work in the sense that short- (^{14}C fixation, O_2 generation and esterase inhibition) to long-term ratios for algae have been reported to be compound specific and ranged from 0.02 to > 161 (Versteeg, 1990; Nyholm & Damgaard, 1990; Snell et al., 1996), encompassing the range in our results (Table 1). However, in view of results in the literature (Delistraty, 1986; Versteeg, 1990; Nyholm & Damgaard, 1990; Snell et al., 1996), the fact that the photosynthesis inhibition test was more sensitive for the majority of the samples than the population growth test (Figure 2) was surprising and apparently reflects the fact that elutriates from suspended sediments consists of many different kinds of chemical compound inhibiting photosynthesis. Furthermore, various effects including the synergistic, antagonistic, and additive effects of all the chemical, physical, and biological components present in complex mixtures can adversely affect the physiological and biochemical functions of the test organisms in various ways and in a more complex manner than single toxicant assays. In addition, these data confirm that chronic toxicity cannot be directly derived from acute toxicity testing with the same species (Garric et al., 1993) and that both short- and long-term algal tests provide valuable complementary information in monitoring programs when the purpose is to compare relative toxicity of one sediment with one another.

The significance of these effects when extrapolating the data given here to potential field events requires a through understanding of the ecosystem structure and function (Graney et al., 1995) as well as the identification of the causes of toxicity and a precise

knowledge of the limitations of the laboratory bioassay used. Drawbacks of the experimental protocol in chronic algal tests can complicate the interpretation of results and reduce possible extrapolation of data to environmental or field situations. For instance, changes in media pH due to rapid algal growth and in toxicants to algal cell ratio during chronic exposures are some of these well known limitations. In addition, the apparent toxicity of sample components can be affected by factors that are poorly understood such as hydrolysis, volatilization, biodegradation of sample constituents as well as bacterial growth in the elutriates, which are usually rich in dissolved organic carbon. The effect of these factors on the bioavailability and toxicity of environmental contaminants confounds chronic algal toxicity data interpretation and gives rise to criticism because of the unrealistic experimental conditions of the chronic tests (Lewis, 1995). These test limitations can be addressed by limiting exposure to a few hours as in the short-term (4 h) toxicity test presented here. On this basis alone, the photosynthetic procedure is preferable for determining a more realistic acute impact on the ecosystem and improving laboratory to field extrapolations, in particular if the method is used for measuring effects on natural phytoplankton assemblage such as in the study of Santiago et al. (1993). Nevertheless, long-term tests are still needed for environmental hazard assessment and development of semistatic chronic algal tests (Radetski et al., 1994) with more realistic designs could greatly improve field to laboratory extrapolations.

This study demonstrates that the short-term algal *S. capricornutum* biotest based on ^{14}C-fixation can be a useful tool to measure the impact of single toxicants or compounds released by suspended sediments on aquatic plants. However both types of test (acute or chronic) have their specific advantages depending on the purpose of testing and should be considered as complementary.

Acknowledgements

The authors are grateful to the Franco-Swiss International Commission for the Protection of the Waters of Lake Geneva (CIPEL) for their financial support to a sediment toxicity assessment program that allowed this work to be carried out.

References

Delistraty D (1986) Growth and photosynthetic response of a freshwater alga, *Selenastrum capricornutum*, to an oil shale by-product water. Bull. envir. Contam. Toxicol. 36: 114–121.

Dijkman NA, Van Vlaardingen PLA, Admiraal WA (1997) Biological variation in sensitivity to N-heterocyclic PAHs; Effects of acridine on seven species of micro-algae. Envir. Pollut. 95: 121–126.

Garric J, Vindimian E, Ferard JF (1993) Ecotoxicology and wastewaters: Some practical applications. Sci. Total Envir., Supplement (part 2): 1085–1103.

Graney RL, Giesy JP, Clark JR (1995) Field studies. In Rand GM (ed.), Fundamentals of Aquatic Toxicology: Effects, Environment Fate, and Risk Assessment, 2nd Edition. Taylor & Francis, Washington D.C., USA, 257–305.

Hall NE, Fairchild JF, La Point TW, Heine PR, Ruessler DS, Ingersoll CG (1996) Problems and recommendations in using algal toxicity testing to evaluate contaminated sediments. J. Great Lakes Res. 22: 545–556.

ISO (1989) Water Quality: Freshwater algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*. ISO 8692, International organization for standardization.

Lewis MA (1995) Algae and vascular plant tests. In Rand GM (ed.), Fundamentals of Aquatic Toxicology: Effects, Environment Fate, and Risk Assessment, 2nd Edition. Taylor & Francis, Washington D.C., USA, 135–169.

Nyholm N, Damgaard BM (1990) A comparison of the algal growth inhibition toxicity test method with the short-term ^{14}C-assimilation test. Chemosphere 21: 671–679.

Ongley ED, Thomas RL (1989) Dewatering suspended solids by continuous flow centrifugation: Practical considerations. Hydrological processes 3: 255–260.

Pardos M, Benninghoff C, Thomas RL, Dumas M (1994) Caractérisation écotoxicologique des affluents à leur embouchure. Rapp. Comm. Int. Prot. Eaux Léman contre pollut., Campagne 1993, Lausanne, Switzerland, 171–189.

Pardos M, Benninghoff C, Thomas RL, Dumas M (1996) Caractérisation écotoxicologique des affluents du Léman à leur embouchure et du bassin versant du Rhône aval: complément d'étude. Rapp. Comm. Int. Prot. Eaux Léman contre pollut., Campagne 1995, Lausanne, Switzerland, 219–237.

Plumb RH, Jr, Lee GF (1979) A comparison of C-14 incubation technique. Wat. Air Soil Pollut. 11: 289–299.

Radetski C, Ferard JF, Blaise C (1994) A semistatic microplate-based phytotoxicity test. Envir. Toxicol. Chem. 14: 299–302.

Ross P, Jarry V, Sloterdijk H (1988) A rapid bioassay using the green alga *Selenastrum capricornutum* to screen for toxicity in St. Lawrence River sediment elutriates. In Cairns J, Jr, Pratt JR (eds), Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals. ASTM STP 988. Philadelphia, American Society for Testing and Materials, 68–73.

Santiago S, Thomas RL, Larbaigt G, Rossel D, Echeverria MA, Tarradellas J, Loizeau JL, McCarthy L, Mayfield CI, Corvi C (1993) Comparative ecotoxicity of suspended sediment in the lower Rhone river using algal fractionation, Microtox® and *Daphnia magna* bioassays. Hydrobiologia 25: 231–244.

Santiago S, Thomas RL, Loizeau JL, Favarger PY, Vernet JP (1990) Further discussion on the intercomparison of the trace metal concentrations and particle size of fluvial sediment recovered from two centrifuge systems. Hydrological processes 4: 283–287.

Snell TW, Mitchell JL, Burbank SE (1996) Rapid toxicity assessment with microalgae using in vivo esterase inhibition. In

Ostrander GK (ed.), Techniques in Aquatic Toxicology. Lewis Publishers, Boca Raton, 13–22.

Stumm W, Morgan JJ (1996) Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters, 3^{rd} Edition. John Wiley & Sons. Inc., New York, NY, USA.

U.S. EPA (1994) Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms. Third Edition. EPA/600/4-91/002. Environmental monitoring systems laboratory, office of research and development, U.S. environmental protection agency, Cincinnati, Ohio.

U.S. EPA (1989) Algal (*Selenastrum capricornutum*) growth test. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Environmental Monitoring Systems Laboratory, Environmental Protection Agency, Cincinnati, Ohio.

Van der Heever JA, Grobbelaar JU (1996) Evaluation of a short-incubation-time small-volume radiocarbon-uptake algal toxicity test. J. appl. Phycol. 8: 65–71.

Versteeg DJ (1990) Comparison of short- and long-term toxicity test results for the green alga, *Selenastrum capricornutum*. In Wang W, Gorsuch JW, Lower WR (eds), Plants for Toxicity Assessment. ASTM STP 1091. Philadelphia, American Society for Testing and Materials, 40–48.

Wong SL, Wainwright JF, Pimenta J (1995) Quantification of total and metal toxicity in wastewater using algal bioassays. Aquat. Toxicol. 31: 57–75.