Accepted Manuscript

Title: Effects of spill-treating agents on growth kinetics of marine microalgae

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PII: S0304-3894(13)00488-3
DOI: <http://dx.doi.org/doi:10.1016/j.jhazmat.2013.07.010>
Reference: HAZMAT 15234

To appear in: *Journal of Hazardous Materials*

Received date: 8-2-2013
Revised date: 24-5-2013
Accepted date: 7-7-2013

Please cite this article as: D. Rial, M.A. Murado, A. Menduiña, P. Fuciños, P. González, J. Mirón, J.A. Vázquez, Effects of spill-treating agents on growth kinetics of marine microalgae, *Journal of Hazardous Materials* (2013), <http://dx.doi.org/10.1016/j.jhazmat.2013.07.010>

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13

13

14 **Abstract**

15

16 The effects of four spill-treating agents (STAs) (CytoSol, Finasol® OSR 51, Agma
 17 OSD 569 and OD4000) on the growth kinetics of three marine microalgae (*Isochrysis*
 18 *galbana*, *Chaetoceros gracilis*, *Phaeodactylum tricornutum*) were studied. Chlorophyll
 19 a concentration and optical density at 700 nm were assessed to describe the logistic
 20 growth of algae in batch cultures. The optical density data were initially analysed as
 21 described for standard algal growth inhibition tests and subsequently modelled by a
 22 bivariate model, as a function of time and dose, to assess the toxic effects on growth
 23 parameters. Increasing trends in EC_{50} and EC_{10} values with time were found with the
 24 standard approach. In 8 of the 11 tests, the lag phase (λ) or the time required to achieve
 25 half the maximum biomass (τ) was significantly dependent on the STA concentration. A
 26 global parameter ($EC_{50,\tau}$) was calculated to summarize the effects of STAs on growth
 27 parameters in the bivariate model. The ranking of sensitivity as $EC_{50,\tau}$ values was *I.*
 28 *galbana* > *C. gracilis* > *P. tricornutum*. For all species tested, the least toxic agent was
 29 Agma OSD 569, followed by CytoSol. The mathematical model allowed successful
 30 ecotoxicological evaluation of chemicals on microalgal growth.

31

32 **Keywords:** spill-treating agents; Weibull and logistic equations; algae growth kinetics;
 33 EC_{50} ; toxicological end-points

34

34

35

36 **1. Introduction**

37 The usual clean-up options for oiled rocky shores—manual removal, low or high
 38 pressure water washing—lose their effectiveness with weathering, and the efficiency of
 39 bioremediation methods decreases markedly after 6-8 months [1, 2]. Chemical cleaning
 40 may be a useful alternative for removing weathered slicks, although discharge limits
 41 should be set, depending on the receiving environment, to mitigate the impact of the
 42 cleaning operations [3]. Dispersants are spill-treating agents (STAs) that promote the
 43 formation of small oil droplets, thus reducing the impact on the coast, whereas surface-
 44 washing agents or beach cleaners act through a mechanism known as detergency and
 45 are more effective in removing oil from solid surfaces [4]. For instance, the surface-
 46 washing agent CytoSol demonstrated remarkable results in removing oil from the
 47 *Prestige* accumulated on the Galician coast [3].

48

49 The effects of chemical compounds (e.g. heavy metals, hydrocarbons, STAs) on living
 50 organisms is often assessed by extrapolating to the ecosystem short-term toxicity data
 51 for three species at three different trophic levels, primary producers and primary and
 52 secondary consumers [5]. The microalgal chronic toxicity test is simple, fast and cheap;
 53 microalgae are the commonest primary producers, and the sensitivity of the test is equal
 54 to or greater than that of toxicity tests in representatives of primary and secondary
 55 consumers (typically daphnia and fish) [6]. In the microalgal test, biomass is measured
 56 72-96 h after the start of the test, and inhibition of biomass production is determined as
 57 cell number, dry weight, chlorophyll content or average specific growth rate [7, 8]. This
 58 approach, however, does not allow comprehensive study of the growth curves and the

real effect of toxic agents on the kinetics that describe the different stages of microbial growth [9].

The logistic equation is a useful model of the set of microalgal growth phases in batch culture [10]. Some authors have found that the effect of a toxic agent on microalgal growth is manifested in the curve as maximum biomass, growth rate or lag phase [11]. Recently, a bivariate model was developed to evaluate toxic effects on growth kinetics by combining the logistic equation to describe microbial growth and a cumulative Weibull function of the dose-response relations of the parameters of the growth curve [9, 12]. This model allows a comprehensive description of inhibitory effects in the experimental domain of concentration and time.

The aim of this study was to assess the effects of four cleaning agents on microalgal growth kinetics. Three marine microalgae with wide distribution and high economic importance for their use in aquaculture were selected: the haptophyte *Isochrysis galbana*, the centric diatom *Chaetoceros gracilis* and the pennate diatom *Phaeodactylum tricornutum*. Two indirect measures of cell density, chlorophyll a concentration and optical density, were assessed to describe the logistic growth of algae in batch cultures. The optical density data were analysed by the approaches proposed in the standard microalgal test and in the bivariate model.

2. Material and methods

2.1. Spill-treating agents

Four STAs were selected by their ability to remove fuel adhering to granite rock [13]: CytoSol (CytoCulture International, Inc., Point Richmond, California, USA),

FINASOL® OSR 51 (Total Special Fluids, Paris, France), Agma OSD 569 (Agma plc, Northumberland, United Kingdom) and OD4000 (Innospec Ltd, Cheshire, United Kingdom). The products were kindly provided by the manufacturers or trade representatives.

2.2. Microalgal bioassay

Algal strains of *Isochrysis galbana* (05/0401) and *Chaetoceros gracilis* (07/0102) from the Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC) were kindly provided by Dr J.L. Garrido (Instituto de Investigaciones Marinas, IIM-CSIC). *Phaeodactylum tricornutum* strain CCMP 630 was provided by Dr J. Pintado (IIM-CSIC). Microalgal culture medium was formulated from 0.22 µm filtered seawater, autoclaved at 121 °C for 20 min, salinity adjusted to 30 ‰ and supplemented with f/2 medium (with sodium metasilicate for diatoms). Cultures were maintained in 250-ml flasks at 14 °C and 12:12 h light-dark cycle to minimize growth and staff work.

Cell density (cell/mL) was determined indirectly by optical density measurements ($OD_{\lambda = 700 \text{ nm}}$) using a regression line generated by taking measurements every 3-4 days [14]:

$$OD_{700} = b + m \cdot n \quad (1)$$

where n is the cell density (cell/mL), b is a constant and m the slope. b values were 0.043±0.024, 0.035±0.019 and 0.041±0.025 (mean±standard deviation); m values were $1.44 \times 10^{-7} \pm 5.51 \times 10^{-9}$, $1.72 \times 10^{-7} \pm 6.24 \times 10^{-9}$ and $9.08 \times 10^{-8} \pm 4.35 \times 10^{-9}$ and the coefficients

of determination were 0.96, 0.95 and 0.94 for *I. galbana*, *C. gracilis*, *P. tricornutum*, respectively.

110

Cultures were performed in the experiments under orbital shaking conditions (200 rpm, 20 °C, 24-h light, irradiance=162±18 $\mu\text{E m}^{-2}\text{s}^{-1}$). The inoculum of each organism consisted of cells from a 72-h culture centrifuged at 4000 rpm for 20 min. The optical density of the culture was adjusted to 0.05 before the start of the assay (4.9×10^4 , 8.8×10^4 and 9.4×10^4 cell/mL for *I. galbana*, *C. gracilis* and *P. tricornutum*, respectively). Ten μL of the STA diluted in acetone were added to each 30-mL culture tube with 10 mL of culture medium (maximum acetone concentration of 1 mL/L in the solvent control group) to obtain the final concentrations of STA shown in Table 1.

119

To avoid contamination, each tube was sampled two or three times, and five series of tubes were used per treatment. Each STA concentration and control without chemical was studied in triplicate for each experimental series. At regular intervals (every 12 and 24 h during the first 48 h and every 24 h thereafter), 2-mL samples were removed to measure optical density ($\lambda = 700 \text{ nm}$). The absorbance of the agent, measured previously, was subtracted from the value obtained in culture.

126

2.3. Pigment analysis

From each tube, 3 mL were taken at the selected times (every 36h during the first 72h and every 24 or 48h thereafter), the volume corresponding to the replicas were mixed, ~100 mg of magnesium carbonate were added, and the sample was centrifuged at 4000 rpm for 20 min. The algal pellet was extracted with 2 mL of 90% acetone for 24 h at 4

132 °C. The chlorophyll a concentration was estimated from the concentration in the extract
133 according to the equation of Jeffrey and Humphrey [15]:

134

$$135 \quad Chla = 11.85(OD_{664} - OD_{750}) - 1.54(OD_{647} - OD_{750}) - 0.08(OD_{630} - OD_{750}) \quad (2)$$

136

137 where *Chla* is the chlorophyll a concentration (mg/L), and OD_{664} , OD_{647} , OD_{630} , OD_{750}
138 are the optical densities at the respective wavelengths.

139

140 2.4. Growth models

141 2.4.1. Standard approach

142 Average specific growth rate was calculated from the optical density (absorbance at 700
143 nm) as described in the OECD guidelines [16]:

144

$$145 \quad \mu_{0-j} = \frac{\ln X_j - \ln X_0}{t_j} \quad (3)$$

146

147 where μ_{0-j} is the average specific growth rate from time 0 to j (h^{-1}), X_j is the biomass at
148 time j , X_0 is the biomass at the beginning of the test, and t_j is the time considered (h).

149 The percentage inhibition of the average specific growth rate for each treatment
150 replicate was calculated from the equation:

151

$$152 \quad I_r = \frac{\mu_c - \mu_t}{\mu_c} \times 100 \quad (4)$$

153

154 where I_r is the percentage inhibition, μ_c is the mean average specific growth rate in the
155 control, and μ_t is the average specific growth rate of the treatment replicate.

156

157 EC_{50} and EC_{10} were obtained by nonlinear fitting of the Weibull model to the observed
158 growth inhibition at the tested doses [3, 17]:

159

$$160 \quad I_r = I_{rm} \left\{ 1 - \exp \left[- \ln 2 \left(\frac{C}{EC_{50}} \right)^a \right] \right\} \quad (5)$$

161

$$162 \quad I_r = I_{rm} \left\{ 1 - \exp \left[\ln 0.9 \left(\frac{C}{EC_{10}} \right)^a \right] \right\} \quad (6)$$

163

164 where I_{rm} is the maximum percentage inhibition, C is the concentration of STA ($\mu\text{L/L}$),
165 EC_{50} is the concentration ($\mu\text{L/L}$) corresponding to semi-maximal percentage inhibition,
166 EC_{10} is the concentration ($\mu\text{L/L}$) equivalent to 10% of the maximum percentage
167 inhibition, and a is a shape parameter related to the maximum slope of the response.

168

169 2.4.2. Bivariate equation approach

170 The bivariate model is based on a reparameterised logistic equation to describe growth
171 combined with a Weibull function to describe concentration-dependent variations in
172 growth parameters [9, 12]:

173

$$174 \quad X = X_{m\bullet} \left\{ 1 + \exp \left[2 + \frac{4v_{m\bullet}}{X_{m\bullet}} (\lambda_\bullet - t) \right] \right\}^{-1} ; \text{ with:} \quad (7)$$

$$175 \quad X_{m\bullet} = X_m \left\{ 1 - K_x \left[1 - \exp \left(- \ln 2 \left(C/m_x \right)^{a_x} \right) \right] \right\}$$

$$176 \quad v_{m\bullet} = v_m \left\{ 1 - K_v \left[1 - \exp \left(- \ln 2 \left(C/m_v \right)^{a_v} \right) \right] \right\}$$

$$\lambda_{\bullet} = \lambda \left\{ 1 + K_{\lambda} \left[1 - \exp \left(- \ln 2 \left(C / m_{\lambda} \right)^{a_{\lambda}} \right) \right] \right\}$$

178

179 where X is the biomass measured as absorbance at 700 nm (absorbance units), X_m is the
180 maximum biomass (absorbance units), v_m is the maximum growth rate (absorbance
181 units/h), λ is the lag phase (h), C is the concentration of STA ($\mu\text{L/L}$), and X_m , v_m and λ
182 are the modified growth parameters. The value of each parameter in the logistic
183 equation of growth (X_m , v_m , λ) depends on the parameters of the Weibull equation
184 (e.g. for λ the parameters are K_{λ} , m_{λ} , a_{λ}) and variable C according to the expressions
185 cited above.

186

187 A further reparameterisation of the logistic equation was also used:

188

$$X = X_{m\bullet} \left\{ 1 + \exp \left[\frac{4v_{m\bullet}}{X_{m\bullet}} (\tau_{\bullet} - t) \right] \right\}^{-1} ; \tau_{\bullet} = \tau \left\{ 1 + K_{\tau} \left[1 - \exp \left(- \ln 2 \left(C / m_{\tau} \right)^{a_{\tau}} \right) \right] \right\} \quad (8)$$

189

190
191 where τ is the time required to achieve half the maximum biomass (h), and X_m and v_m
192 are the functions described in equation (7).

193

194 Equations (9) and (10) address the possibility that parameters λ and τ are better
195 described by linear or exponential functions of the concentration:

196

$$\theta_{\bullet} = \theta(1 + \alpha D) = \theta + \alpha_{\theta} C \quad (9)$$

198

$$\theta_{\bullet} = \theta \left\{ 1 + \left[\exp(\beta_{\theta} D) - 1 \right] \right\} = \theta \exp(\beta_{\theta} C) \quad (10)$$

199

200

201 where θ is the parameter considered (h), α_{θ} is the slope of the linear function (h L/ μ L)202 and β_{θ} is the slope of the exponential function (h L/ μ L).

203

204 The concentration that reduces the biomass to 50% of that produced by the control at
 205 time τ , $EC_{50,\tau}$ (μ L/L), was calculated in order to compare the global toxicity of STA on
 206 microalgae growth [9].

207

208 *2.5. Numerical methods*

209 Fitting procedures and parametric estimates from the experimental results were
 210 performed by minimising the sum of quadratic differences between observed and
 211 model-predicted values, with the nonlinear least-squares (quasi-Newton) method of the
 212 macro ‘Solver’ of the Microsoft Excel XP spreadsheet. Confidence intervals from the
 213 parametric estimates (Student t test) were determined with the freely available
 214 ‘SolverAid’ macro (<http://www.bowdoin.edu/~rdelevie/excellaneous/>).

215

216 **3. Results**217 *3.1. Chlorophyll a content*

218 Control cultures of *P. tricornutum* and *I. galbana* showed maximum chlorophyll a
 219 concentrations in the exponential or declining growth phase, whereas *C. gracilis*
 220 showed maximum values in the stationary phase (Figure 1). Maximum chlorophyll a
 221 concentrations were obtained in controls before τ (the time corresponding to half the
 222 maximal biomass obtained by individual fittings to optical density data) for *P.*
 223 *tricornutum* ($\tau \sim 104$ -122 min) and then *I. galbana* ($\tau \sim 58$ -69 min). For *I. galbana*, the
 224 maximum chlorophyll a content in controls was found in the exponential phase (0.21-

0.30 pg/cell) and minimum values in the stationary phase (0.11-0.16 pg/cell). The chlorophyll a concentration was higher for treatments with STAs than in controls in a relevant number of cases and even higher for longer times than τ , mainly in *C. gracilis* (Figure 1).

229

3.2. Growth analysis

Increasing EC_{50} and EC_{10} values were observed with time in *C. gracilis* (data not shown) and *I. galbana* (Figure 2). In many cases, the confidence intervals of EC_x corresponding to times of 24, 72 and > 120 h did not overlap (Figure 2), indicating significant differences among EC_x values.

235

Fitting optical density data into the bivariate model gave statistically significant parameters, adjusted R^2 values greater than 0.969 and good consistency of the model in all cases ($p < 0.001$) (Tables 2 and 3). Figure 3 shows the suitability of the model for describing broad toxic effects on microalgal growth, with a significant dose-dependence of the time parameter defined by the logistic growth equation (λ or τ) in 8 of the 11 tests. Equations 9 and 10 usefully described that effect on time parameters in five of the eight cases (Tables 2 and 3). Parameter X_m was also shown to be dose-dependent on five occasions. The only case in which v_m was statistically significantly modified by STA was observed in the combination OD4000 and *P. tricornutum* (Tables 2 and 3). The sensitivity of *P. tricornutum* to Finasol OSR 51 was almost null and is therefore not shown in Figure 3.

247

248 The ranking of sensitivity as $EC_{50,\tau}$ values was *I. galbana* > *C. gracilis* > *P. tricornutum*.
 249 For all species tested, the least toxic agent (higher $EC_{50,\tau}$) was Agma OSD 569,
 250 followed by CytoSol (Tables 2 and 3).

251

252 **4. Discussion**

253 The chlorophyll a concentration is a reliable indirect measure of cell density in some
 254 algal growth tests [16, 18], with a good correlation with biomass. A maximum
 255 chlorophyll a concentration was found for the controls of *I. galbana* and *P. tricornutum*
 256 in the exponential growth phase, defined from individual adjustments to optical density
 257 data. Several authors [19, 20] have reported that the cellular content of chlorophyll a
 258 generally decreases between exponential growth and the stationary phase and explained
 259 this drop by limitations in nutrients and light and cell ageing. This is consistent with the
 260 results for the cellular content of chlorophyll a in *I. galbana* in the present work.
 261 Variations in chlorophyll a content between the growth phases of a batch culture might
 262 explain the deviations in concentrations in a logistic pattern (Figure 1). Higher
 263 concentrations in the stationary phase were also observed in a significant proportion of
 264 treatments when compared with control production, consistent with the results obtained
 265 with the dispersant Seaklin-101NT on *Tetraselmis suecica* [21] and BP1100X on
 266 *Chlorella salina* [22]. Use of another direct or indirect measure of cell density (e.g.
 267 absorbance at 700 nm) would simplify the description of microalgal kinetics.

268

269 Microalgae toxicity tests are commonly based on measuring growth inhibition at the
 270 exponential phase for 3-4 days [16, 18]. The short duration of the test reduces
 271 experimental time and avoids any decrease in toxicity by a reduction in the growth rate
 272 in controls due to nutrient limitation, a decline in the intracellular concentration of the

273 toxic agent due to cell growth and extracellular concentration by adsorption,
 274 volatilization or detoxification [23]. The prediction of a constant value over time for
 275 EC_x calculated from the growth rate [24] is valid, however, only when the assumptions
 276 of exponential growth and no lag phase are fulfilled. For *I. galbana*, the tested agents
 277 affected the values only of λ and τ (Table 2), and the EC_x value increased significantly
 278 with time (Figure 2). Hence, choice of a single value of EC_x is arbitrary or meaningless.

279

280 Kooijman et al. [11] developed a bivariate model to describe the logistic growth of a
 281 microalgal population exposed to increasing concentrations of a toxic agent. Rial et al.
 282 [9] proposed the Weibull model to describe the effect of concentration on the kinetics of
 283 the logistic growth equation. In this study, two modifier equations, linear and
 284 exponential, were also considered for the temporary parameters of the growth equation
 285 (λ or τ) in cases in which an asymptote for these parameters is not found at high
 286 concentrations. An accurate description and predictive ability in the experimental
 287 domain were obtained by fitting the bivariate model to optical density data (Tables 2
 288 and 3). The parameters of the logistic equation that were most frequently affected were
 289 λ - τ , followed by the maximum biomass (X_m) and the maximum growth rate, which was
 290 affected in only one case. These results contrast with those of approaches in which
 291 effects on X_m or λ are not taken into account [16, 18]. A marked effect of a dispersant on
 292 the lag phase has been reported previously [21, 22]. The underlying mechanisms may be
 293 inhibition of cell division [25] or mortality of the inoculum at the beginning of the test,
 294 depending on the dispersant concentration [26]. As similar optical density values would
 295 be found for both mechanisms, it was impossible to determine which option is more
 296 plausible with our approach. Some authors have considered the inhibitory effect on
 297 maximum biomass (X_m) as an alternative to the average specific growth rate [10, 27]. In

298 this study, the effect on X_m was related to the toxic agent and alga studied (Tables 2 and
 299 3); and its use with that perspective would also lead to an underestimate of the toxicity.
 300 The $EC_{50,\tau}$ encompasses any effect on the logistic growth parameters in the time
 301 required to produce semi-maximum biomass in the control. The sensitivity of this
 302 parameter was comparable to or higher than the EC_{50} calculated from the average
 303 specific growth rate at 3-4 days for *I. galbana* (Table 2 and Figure 2).
 304
 305 Use of phylogenetically different species of microalgae has been recommended for
 306 assessing the toxicity of a dispersant because of high species-specific sensitivity [28].
 307 For the same STAs, variations of one order of magnitude in $EC_{50,\tau}$ values were obtained
 308 for the tested species (CytoSol, 21.8-369.0 $\mu\text{L/L}$; Agma OSD 569, 65.3->350 $\mu\text{L/L}$;
 309 OD4000, 18.7-115.8 $\mu\text{L/L}$; Finasol OSR 51, 15.3-50.9 $\mu\text{L/L}$). *I. galbana* was most
 310 sensitive to the tested agents ($EC_{50,\tau}$, 15.3-65.3 $\mu\text{L/L}$), with less sensitivity for diatoms
 311 (34.1-124.5 $\mu\text{L/L}$ for *C. gracilis*) and *P. tricornutum* (115.8-> 500 $\mu\text{L/L}$). An even
 312 wider range of EC_{50} values was shown for 12 dispersants tested on *Chlamydomonas*
 313 *reinhardtii* (1-700 $\mu\text{L/L}$) [29] and for six surfactants assayed on *Dunaliella tertiolecta*
 314 (20-3000 mg/L) [30].
 315
 316 Surfactants act by denaturing and binding protein in the cell wall and thus altering the
 317 permeability of the membrane to nutrients and chemicals [28]; therefore, the particular
 318 characteristics of the cell wall in each species condition the toxic response. The high
 319 sensitivity of *I. galbana* may be due to a lower surface:volume ratio and the existence of
 320 a siliceous cell wall in diatoms. Neither hypothesis is consistent with the results of other
 321 authors [31, 32], who found that *C. gracilis* was as or more sensitive than *I. galbana* to
 322 four groups of surfactants.

323

324 Some authors have suggested that the microalgal test is more useful for comparing
 325 toxicity than for assessing hazard in the field [11]. The sensitivity of microalgal species
 326 to a surfactant can vary by three orders of magnitude [28]; therefore, the behaviour of an
 327 exposed phytoplankton community cannot be predicted. Microcosm and mesocosm
 328 experiments are interesting alternatives for reducing uncertainty in this type of
 329 evaluation. Jung et al. [33] studied the combined effect of oil and dispersant on a
 330 phytoplankton community in a mesocosm and reported alteration of the dominant
 331 species and a decrease in phytoplankton abundance and chlorophyll a. These authors
 332 also found that the combination of dispersant and oil was more toxic to the planktonic
 333 ecosystem than oil, highlighting the need for a rigorous assessment of combined effects
 334 [17].

335

336 The increased bioavailability of hydrocarbons in the water column with use of
 337 dispersants may also affect trophic transfer [34]. Wolfe et al. [35-37] found that the
 338 uptake of naphthalene and phenanthrene by *I. galbana* was higher after treatment with
 339 Prudhoe Bay crude oil plus Corexit 9527 than with oil alone. These authors also
 340 concluded that inclusion of this microalga as a diet for *Brachionus plicatilis* was
 341 responsible for a significant percentage of the bioaccumulation observed in the water-
 342 accommodated fraction of oil or oil plus dispersant. This suggests that microalgae are
 343 key elements for assessing exposure and risk related to trophic transfer.

344

345 **5. Conclusions**

346 We propose a bivariate model for characterizing the effects of STAs on the kinetics of
 347 algal growth that allows accurate experimental data modelling, clear quantification of

348 toxicity and a rigorous comparison of the chemicals. The equation could be useful for
 349 ecotoxicological evaluations of the effects of contaminants on the growth of microalgae
 350 and microorganisms. These comparisons showed that *P. tricornutum* is the least
 351 sensitivity microalga, and Agma OSD 569 is the least toxic STA studied.

352

353 **Acknowledgements**

354 Diego Rial was awarded an Isabel Barreto contract, and we are grateful for this financial
 355 support (Dirección Xeral de Investigación, Desenvolvemento e Innovación; Xunta de
 356 Galicia). This study was funded by the Ministerio de Ciencia y Tecnología (CTM2010-
 357 18411 co-financed by FEDER funds from European Union). We also thanks to
 358 Elisabeth Heseltine for editing the manuscript.

359

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466 **TABLE CAPTIONS**

467

468 **Table 1.** Final concentrations of spill-treating agents (STAs) in culture media

469

470 **Table 2.** Parametric estimates and confidence intervals ($\alpha=0.05$) corresponding to
 471 models (7-10) applied to the effect of spill-treating agents (STAs) on the growth of *I.*
 472 *galbana* and *C. gracilis*. Time parameters (λ or τ) are modified in each case by the
 473 concentration-dependence functions indicated. *adj r*²: adjusted coefficient of multiple
 474 determinations. *p* value from Fisher F test ($\alpha = 0.05$).

475

476 **Table 3.** Parametric estimates and confidence intervals ($\alpha=0.05$) corresponding to
 477 model (8) applied to the effect of spill-treating agents (STAs) on the growth of *P.*
 478 *tricornutum*. Time parameters (λ or τ) are modified in each case by the concentration-
 479 dependence functions indicated. *adj r*²: adjusted coefficient of multiple determinations.
 480 *p* value from Fisher F test ($\alpha = 0.05$).

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483 **Table 1**

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STA	<i>C</i> (μL/L)		
	<i>I. galbana</i>	<i>C. gracilis</i>	<i>P. tricornutum</i>
CytoSol	0, 15, 30, 62.5,	0, 5, 10, 20, 35,	0, 30, 60, 125,
	100, 150, 250	50, 100, 110,	200, 300, 400,
		120, 140	500, 700
Agma OSD 569	0, 15, 30, 62.5,	0, 30, 60, 80,	0, 50, 100, 125,
	100, 150, 250	90, 100, 120,	150, 200, 250,
		140, 165, 210	350
OD4000	0, 0.5, 1 5, 10,	0, 5, 10, 20, 40,	0, 50, 75, 100,
	20, 50	60, 100	110, 125, 140,
			150, 200, 250
Finasol OSR 51	0, 6, 12, 25, 40,	0, 20, 50, 80,	
	60, 100	90, 100, 110,	
		120, 160, 200	

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Table 2

		<i>I. galbana</i>				<i>C. gracilis</i>			
Parameter		CytoSol	Agma	OD4000	Finasol	CytoSol	Agma	OD4000	Finasol
			OSD 569		OSR 51		OSD 569		OSR 51
		Eqs (7) and (10)	Eq (8)	Eqs (7) and (10)	Eq (8)	Eqs (7) and (10)	Eq (7)	Eqs (7) and (10)	Eqs. (7) and (9)
Growth model	X_m	1.217±0.057	1.343±0.037	1.268±0.029	1.310±0.039	1.948±0.044	2.056±0.058	2.036±0.091	1.863±0.061
	v_m	0.012±0.001	0.014±0.001	0.013±0.001	0.012±0.001	0.014±0.001	0.013±0.001	0.013±0.001	0.015±0.001
	$\lambda - \tau$	71.0±5.6	62.1±3.6	23.9±2.2	65.1±5.0	22.4±3.6	22.4±5.7	16.7±5.3	19.3±7.1
effect on X_m	K_x	-	-	-	-	-	0.946±0.033	-	1.000±0.736
	m_x	-	-	-	-	-	153.37±5.00	-	113.22±10.78
	a_x	-	-	-	-	-	25.01±10.30	-	13.02±7.59
effect on v_m	K_v	-	-	-	-	-	-	-	-
	m_v	-	-	-	-	-	-	-	-
	a_v	-	-	-	-	-	-	-	-
effect on λ or τ	$K_\lambda - K_\tau$	-	4.717±3.813	-	2.578±0.408	-	2.072±0.781	-	-
	$m_\lambda - m_\tau$	-	126.0±43.1	-	38.7±4.6	-	111.1±6.3	-	-
	$a_\lambda - a_\tau$	-	3.17±0.74	-	1.43±0.22	-	8.83±4.36	-	-
	$\alpha_\lambda - \alpha_\tau$	-	-	-	-	-	-	-	0.668±0.070
	$\beta_\lambda - \beta_\tau$	0.010±0.001	-	0.038±0.002	-	0.013±0.001	-	0.032±0.005	-
	$adj. r^2$	0.969	0.990	0.994	0.987	0.985	0.984	0.979	0.980
p -value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$EC_{50,\tau}$		21.8	65.3	18.7	15.3	74.6	124.5	34.1	50.9

Table 3

		<i>P. tricornutum</i>		
		CytoSol	Agma	OD4000
		OSD 569		
		Eq. (8)	Eq. (8)	Eq. (8)
Growth model	X_m	2.097±0.094	2.409±0.120	2.105±0.075
	v_m	0.011±0.001	0.012±0.001	0.010±0.001
	$\lambda - \tau$	97.4±7.1	112.5±5.9	104.7±5.0
effect on X_m	K_x	0.976±0.033	0.348±0.191	0.999±0.031
	m_x	355.5±10.7	224.1±78.1	117.4±2.7
	a_x	11.38±2.60	2.58±1.82	3.70±0.41
effect on v_m	K_v	-	-	1.000±0.034
	m_v	-	-	132.8±5.2
	a_v	-	-	11.76±4.85
effect on λ or τ	$K_\lambda - K_\tau$	-	-	-
	$m_\lambda - m_\tau$	-	-	-
	$a_\lambda - a_\tau$	-	-	-
	$\alpha_\lambda - \alpha_\tau$	-	-	-
	$\beta_\lambda - \beta_\tau$	-	-	-
	$adj. r^2$	0.969	0.975	0.983
		p -value	<0.001	<0.001
		$EC_{50,\tau}$	>350	115.8

FIGURE CAPTIONS

Figure 1. Kinetics of chlorophyll a production (mg/L) for spill-treating agents (STAs) (columns) and microalgal species (rows). Solid line: control. Dotted line: culture dosed with STA. Concentrations ($\mu\text{L/L}$) are noted by the symbols of the legend superimposed on each graph.

Figure 2. EC_x ($\mu\text{L/L}$) obtained for *I. galbana* vs. time for CytoSol (A), Agma OSD 569 (B), OD4000 (C), Finasol® OSR 51 (D). Symbols represent EC_{50} () and EC_{10} (). The error bars represent 95% confidence intervals.

Figure 3. Experimental data for growth kinetics and fit to Eq. 8 or 9 (surface response) for the tested microalgae (columns) and spill-treating agents (STAs) (rows). X: optical density at 700 nm; C: STA concentration ($\mu\text{L/L}$); t : time (h). The error bars are not shown, as these were less than 10% of the experimental mean value.

Figure 2

Figure 3

Highlights

- We studied the effect of four spill-treating agents on microalgal growth kinetics.
- The toxic effects were manifested on the logistic growth parameters.
- A reliable description of the growth kinetics was obtained by a bivariate model.
- The standard toxicological approach, although simpler, is more arbitrary.
- *I. galbana* was the most sensitivity microalga and Agma O.S.D. 569 the least toxic.