



**HAL**  
open science

## **Influence of bacteria on the response of microalgae to contaminant mixtures**

Eric Fouilland, Amandine Galès, Ines Beaugelin, Elodie Lanouguere, Olivier Pringault, Christophe Leboulanger

► **To cite this version:**

Eric Fouilland, Amandine Galès, Ines Beaugelin, Elodie Lanouguere, Olivier Pringault, et al.. Influence of bacteria on the response of microalgae to contaminant mixtures. *Chemosphere*, 2018, 211, pp.449-455. 10.1016/j.chemosphere.2018.07.161 . hal-02624252

**HAL Id: hal-02624252**

**<https://hal.inrae.fr/hal-02624252>**

Submitted on 26 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

# Accepted Manuscript

Influence of bacteria on the response of microalgae to contaminant mixtures

Eric Fouilland, Amandine Galès, Inès Beaugelin, Elodie Lanouguère, Olivier Pringault, Christophe Leboulanger



PII: S0045-6535(18)31426-7

DOI: 10.1016/j.chemosphere.2018.07.161

Reference: CHEM 21876

To appear in: *Chemosphere*

Received Date: 28 March 2018

Accepted Date: 27 July 2018

Please cite this article as: Eric Fouilland, Amandine Galès, Inès Beaugelin, Elodie Lanouguère, Olivier Pringault, Christophe Leboulanger, Influence of bacteria on the response of microalgae to contaminant mixtures, *Chemosphere* (2018), doi: 10.1016/j.chemosphere.2018.07.161

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Comment citer ce document :

Fouilland, E. (Auteur de correspondance), Galès, A., Beaugelin, I., Lanouguère, E., Pringault, O., Leboulanger, C. (2018). Influence of bacteria on the response of microalgae to contaminant mixtures. *Chemosphere*, 211, 449-455. , DOI : 10.1016/j.chemosphere.2018.07.161

## 1 Influence of bacteria on the response of microalgae to contaminant mixtures

2

3 Eric Fouilland<sup>a\*</sup>, Amandine Galès<sup>a,b</sup>, Inès Beaugelin<sup>a,c</sup>, Elodie Lanouguère<sup>a</sup>, Olivier Pringault<sup>a</sup>,4 Christophe Leboulanger<sup>a</sup>,5 <sup>a</sup>MARBEC, CNRS IRD IFREMER Univ Montpellier, Sète, France6 <sup>b</sup>INRA LBE, Narbonne, France7 <sup>c</sup>Present address: Institut de biosciences et biotechnologies, CEA Cadarache, St Paul Lez

8 Durance, France

9 \* Corresponding author: eric.fouilland@cnrs.fr

10

11

12 **Abstract**13 When microalgae are exposed to contaminants, the role of associated bacteria within  
14 the phycosphere, the microenvironment surrounding algal cells, remains largely unknown.15 The present study investigated the importance of algae-associated bacteria on the responses of  
16 microalgae growth to metallic and organic toxicant exposure. The effects of a polluted  
17 sediment elutriate, and of metal or pesticide mixtures at environmentally relevant18 concentrations ( $<10 \mu\text{g L}^{-1}$ ) were assessed on the growth of two microalgae strains: *Isochrysis*  
19 *galbana*, a prymnesiophyte, and *Thalassiosira delicatula*, a centric diatom. Both cultures20 were maintained as axenic or bacterized under similar conditions in batch cultures. In axenic  
21 conditions, the metal mixture addition at low concentrations alleviated limitation of growth by22 metals for *T. delicatula* relative to control, but inhibited *I. galbana* growth at highest  
23 concentration. In similar axenic conditions, both *T. delicatula* and *I. galbana* growth were24 negatively inhibited by pesticide mixture at concentrations as low as  $10 \text{ ng L}^{-1}$ . The bacterial  
25 diversities associated with the two microalgae strains were significantly different (Bray–

26 Curtis dissimilarity greater than 0.9) but their impact on microalgae growth was similar. The  
27 presence of bacteria reduced algal growth rate by *ca.* 50% compared to axenic cultures,  
28 whereas no significant effect of sediment elutriate, metal or pesticide mixtures was noticed on  
29 non-axenic algal growth rates. These results show that bacteria may have a negative effect on  
30 algal growth but can reduce pesticide toxicity or metal availability to algae.

31

32 *Keywords: microbial interactions; metallic and pesticide contaminants; sediments*

ACCEPTED MANUSCRIPT

33 **1. Introduction**

34

35 Microalgae as primary oxygen producers in aquatic ecosystems are of prime  
36 ecological importance, and represent the first trophic level in the aquatic food web (Azam and  
37 Malfatti, 2007; Field et al., 1998). The region surrounding individual algal cells, named the  
38 phycosphere, enriched in exuded organic molecules, is considered as an aquatic analogue of  
39 the rhizosphere where microorganisms interact with plants in the terrestrial ecosystem  
40 (Seymour et al., 2017). Within the phycosphere, microalgae interact with bacteria within a  
41 large range from symbiosis to parasitism, conferring advantages or disadvantages to both  
42 partners (Bell and Mitchell, 1972). The mechanisms of interactions between bacteria and  
43 phytoplankton are diverse and involve specific cellular processes and fine communication (e.g.  
44 quorum sensing) (Amin et al., 2012). Such mechanisms may result in antibacterial or  
45 algacide activities (Mu et al., 2007; Ribalet et al., 2008) or substrate competition as  
46 experimentally observed between manipulated consortium of microalgae and bacteria (Le  
47 Chevanton et al., 2013). On the other hand, the presence of bacteria could offer to microalgae  
48 a capacity for tolerance and adaptation to stressful conditions, such as chemical exposure.  
49 Indeed, the heterotrophic metabolism of highly diverse bacterial communities in the field and  
50 their ability to degrade, metabolize and immobilize a large number of organic and inorganic  
51 compounds (Bouwer and Zehnder, 1993; Bruins et al., 2000), make it possible to assign them  
52 an ecological role of protecting microalgae, particularly in polluted environments. It can also  
53 be hypothesized that microalgal growth may be further improved when the latter are  
54 associated with bacteria subjected to chronic contaminants that could develop greater  
55 tolerance capacities than naive bacteria and therefore allow microalgae to benefit from these  
56 bacterial capacities to cope with pollutants (Bauer et al., 2010).

57 Therefore, the main hypothesis tested in this study proposes that the presence of  
58 bacteria with degrading or immobilizing ability would reduce the sensitivity of microalgae to  
59 organic or metal contaminants, counterbalancing any potential bacterial algacide activity.  
60

61 In order to test this hypothesis, the present study focused on the effect of a sediment  
62 elutriate issued from the resuspension of polluted sediments on the growth of two microalgae  
63 strains commonly found in marine environments: *Isochrysis galbana*, a small prymnesiophyte,  
64 and *Thalassiosira delicatula*, a centric diatom. *Isochrysis galbana* is a well-known  
65 phytoplankton species, traditionally used in aquaculture and biotechnology due its capacity to  
66 produce large biomass (Williams and Laurens, 2010) whereas *Thalassiosira delicatula*  
67 represents a model for diatom study, belonging to a genus widely distributed throughout the  
68 world's oceans (Armbrurst et al., 2004). Both strains were growing either in axenic or non-  
69 axenic condition, i.e. associated with bacteria naturally selected during culture selection and  
70 maintenance processes. The growth of xenic and axenic strains were compared when exposed  
71 to the total (including native bacteria) or dissolved fraction of the resuspended sediment, or  
72 artificial mixtures containing either the main metallic or organic contaminants found in these  
73 sediments.

74

75

## 76 **2. Materials and Methods**

77

### 78 *2.1. Elutriate and contaminated artificial mixtures*

79

#### 80 *2.1.1. Elutriate and filtered elutriate preparation*

81 The elutriate was obtained by mixing seawater (3/4 by volume) and sediment (1/4)  
82 sampled in February 2015 from the Bizerte Lagoon, for 12 hours, followed by decantation for  
83 12 hours. The elutriate thus represented the supernatant obtained after decantation. It still  
84 contained unsettled particulate matter, resident bacteria and water-soluble contaminants. The  
85 filtered elutriate was obtained after filtration of the elutriate on a 0.2  $\mu\text{m}$  membrane, leading  
86 to a sterile mixture with only the dissolved fraction of chemical compounds. More details on  
87 sediment location and sampling can be found in (Pringault et al., 2016).

88

### 89 *2.1.2 Artificial mixture of contaminants*

90 Two types of artificial mixtures of contaminants were produced in the laboratory,  
91 using actual concentrations found after chemical analyses in the elutriate (HydroSciences  
92 Montpellier laboratory (HSM) for metals and by the Ecole des Mines d'Alès for pesticides,  
93 see (Pringault et al., 2016) for analytical set-up). One of these mixtures contained the organic  
94 contaminants, mostly pesticides resulting from the agricultural activity of the Bizerte area,  
95 whereas the other mixture contained the metallic fraction resulting from the industrial and  
96 harbour activities of the city.

97 The artificial metal mixture was prepared in Milli-Q water, containing 0.026  $\mu\text{g L}^{-1}$  of  
98 cadmium Cd, 0.068  $\mu\text{g L}^{-1}$  of copper Cu, 0.208  $\mu\text{g L}^{-1}$  of nickel Ni, 0.13  $\mu\text{g L}^{-1}$  of lead Pb,  
99 1.615  $\mu\text{g L}^{-1}$  of zinc Zn and 4.896  $\mu\text{g L}^{-1}$  of arsenic As.

100 The artificial pesticide mixture was prepared in Milli-Q water with a mixture of the  
101 pesticides measured in the elutriate and containing 7.5  $\text{ng L}^{-1}$  of DIA (deisopropylatrazine, an  
102 atrazine metabolite), 7.7  $\text{ng L}^{-1}$  of DCPU (N-3,4 dichlorophenylurea, a diuron degradation  
103 product), 8.8  $\text{ng L}^{-1}$  of diuron, 10.1  $\text{ng L}^{-1}$  of simazine and 12.7  $\text{ng L}^{-1}$  of alachlor. All the  
104 pesticides measured in sediment elutriate were herbicides or their degradation products. A

105 concentrated stock solution of both mixtures was performed in order to test a range of mixture  
106 concentrations.

107

## 108 2.2 *Microalgae cultures*

109 The microalgal strains studied were *Isochrysis Galbana* (CCAP 927) isolated from a  
110 marine fish pond in the British Isles and *Thalassiosira delicatula* (RCC 2560) isolated from  
111 the English Channel. They were grown in a F/2 + Si medium and subjected to light exposure  
112 according to a 16h/8h day/night cycle in an incubator maintained at 20 °C under neon lighting  
113 (Vossloh Schwabe ELXe 218.526), providing an average intensity of 100  $\mu\text{E m}^2 \text{s}^{-1}$   
114 (measured with a LI-COR®, Li-1400 equipped with a Walz US-SQS/L spherical micro  
115 sensor). The microalgae cultures were inoculated in fresh medium every 2 weeks to maintain  
116 active growth before the experiments.

117 Axenization of cultures was carried out according to the protocol of The Culture  
118 Collection of Algae and Protozoa (CCAP) C.N. Campbell  
119 (<https://www.ccap.ac.uk/knowledgebase.htm>) using a mixture of antibiotics (Cefotaxime 500  
120 mg L<sup>-1</sup>, Carbenicillin 50 mg L<sup>-1</sup>, Kanamycin 200 mg L<sup>-1</sup>, Augmentin 200 mg L<sup>-1</sup>) at  
121 concentrations of 0 to 10% and with contact times of 24 to 80 hours. The verification of the  
122 axenization was carried out by adding 4 ', 6'- $\beta$ -diamidino- $\alpha$ -2- $\alpha$ -phenylindole (DAPI),  
123 filtration and observation under epifluorescence microscopy with UV excitation at 360 nm ;  
124 spreading in a Petri dish on Marine Broth medium (15% agar) was also performed. The axeny  
125 of microalgae cultures was checked regularly, especially prior to the beginning of the toxicity  
126 experiments.

127

## 128 2.3 *Bacterial diversity analysis*



129 Bacterial 16S rDNA was extracted from 10mL of sample filtered on a 0.2  $\mu$ m  
130 membrane (PALL Supor® 200 PES), using the DNeasy PowerWater Kit (Qiagen) according  
131 to the manufacturer's instructions.

132 The V4-V5 region of the 16S rRNA gene was amplified over 30 amplification cycles at an  
133 annealing temperature of 65 °C, with the forward primer and the reverse primer (Table 1)  
134 with their respective linkers. The resulting products were purified and loaded onto the  
135 Illumina MiSeq cartridge for sequencing of paired 300 bp reads following manufacturer's  
136 instructions (v3 chemistry). Sequencing and library preparation were performed at the  
137 Genotoul Lifescience Network Genome and Transcriptome Core Facility in Toulouse, France  
138 (get.genotoul.fr).

139 A modified version of the standard operation procedure for MiSeq data (Kozich et al.,  
140 2013) in Mothur version 1.35.0 (Schloss et al., 2009) was used for alignment and as a  
141 taxonomic outline. Using Mothur, representative sequences of bacterial operational  
142 taxonomic units (OTUs) were identified at the 3% level.

143 An R script was used to perform a hierarchical clustering using R command hclust  
144 with the Bray–Curtis index

#### 146 2.4. *Experimental design and statistical analysis*

147 An experimental design has been carried out to study the growth of microalgae in the  
148 absence of bacteria (axenic microalgal strains) or in the presence of bacteria (xenic strains  
149 with culture associated bacteria) under i) a range of concentrations of a mixture of metals at  
150 concentrations found in elutriate x1, and at greater concentrations: x3,x10, x31; and ii) a  
151 range of concentrations of a mixture of pesticides at concentrations found in elutriate x1, and  
152 at greater concentrations: x10, x31, x97, x310; iii) the sediment elutriate containing bacteria;

153 iv) the filtered (0.2  $\mu\text{m}$ ) sediment elutriate; v) culture medium without contaminants (=   
154 positive control).

155 All the tests were carried out in 96-well microplates, 6 replicates were made for   
156 artificial mixture treatments, and 12 replicates for controls, elutriate and filtered elutriate   
157 treatments. Each well (300  $\mu\text{L}$ ) contained 10% of microalgae inoculum, 10% of artificial   
158 mixtures or elutriate, and 80% of culture media. Therefore, the concentrations of elutriate and   
159 all the artificial mixtures were diluted to 1/10. The microplates were covered with a Breathe-   
160 Easy® membrane allowing gas exchange and ensuring an absence of contamination   
161 throughout the follow-up of the growth which lasted ten days. This test was carried out for   
162 each of the strains (*Isochrysis galbana* and *Thalassiosira delicatula*) in axenic and non-axenic   
163 conditions. The growth of algal strains was monitored by measuring the optical density at 650   
164 nm every 24 h until reaching the plateau (i.e. stationary phase) on a Chameleon (Hidex,   
165 Finland) microplate reader. The wavelength of 650 nm was chosen according to previous   
166 studies (Ben Othman et al., 2012) corresponding to the absorption peak of chlorophyll   
167 pigments with a minimal contribution of bacterial cells to light attenuation. The maximum   
168 growth rates were obtained by fitting Verhulst growth curve (see (Fouilland et al., 2014)) to   
169 experimental data. An analysis of variance was used to determine the significance of the   
170 difference in growth rates between treatments and with or without axenization, followed by a   
171 Bonferroni post hoc test using SYSTAT 11 version. Significance threshold was set at  $p < 0.05$ .   
172

173 **3. Results and Discussion**

174

175 *3.1. Differential sensitivity of axenic microalgal strains to contaminants*

176 Axenisation of both algal strains was successfully maintained during the present study,  
177 as no bacterial cells were observed using epifluorescence microscope and culture techniques  
178 performed just before the experiments. A significant reduction in the growth rates of both  
179 axenic strains was observed when supplemented with the total elutriate, but not with the  
180 filtered elutriate (Fig. 1A). A reduction of light availability due to the presence of large  
181 particles in the total elutriate is unlikely as no difference in light absorbance (DO) was  
182 observed between control (culture media only) and after total elutriate addition (10% of total  
183 well volume) at the beginning of the experiment. This observation suggests that only the  
184 particulate fraction of the elutriate containing the bacterial community can affect the growth  
185 of both algal strains. The potential negative effect of bacteria presence in the elutriate on the  
186 algal growth is supported by the results obtained with xenic microalgae as discussed below.

187 When comparing the responses of the two axenic microalgae strains tested in the  
188 present study, *Thalassiosira delicatula* showed a higher sensitivity to metal and pesticide  
189 additions compared to *Isochrysis galbana*. Growth rates of *T. delicatula* increased by 40%  
190 when low concentrations of metals were added to their media corresponding to x1 and x3 of  
191 the concentrations measured in the elutriate (Fig. 1A). A reduction of 60% of *T. delicatula*  
192 growth rates was observed for all the pesticide concentrations tested except the one  
193 corresponding to the pesticide concentration measured in the elutriate (x1). The growth rates  
194 of *I. galbana* were more slightly modified and only at higher concentrations of the metal or  
195 pesticide mixture (Fig. 1A). These results suggest that the additions of the metal mixture at  
196 low concentrations relieved a growth limitation by metal ions for *T. delicatula* in culture  
197 rather than a growth inhibition, while the growth of *I. galbana* was significantly inhibited at

198 the highest concentration of the metal mixture tested (x31). Arsenic, lead, nickel and  
199 cadmium are not normally present in the F/2 culture media, amongst them only cadmium at  
200 low concentrations was found in the literature to induce growth enhancement in diatoms (Lee  
201 et al., 1995; Masmoudi et al., 2013). On the other hand, *T. delicatula* was negatively affected  
202 by the pesticide mixture in a magnitude greater than observed for *I. galbana* (Fig. 1A).  
203 Previous studies highlighted the interspecific difference in toxicity of various pesticides on  
204 marine algal growth rates (Staley et al., 2015; Walsh, 1972) at mg L<sup>-1</sup> scale, but no conclusive  
205 evidences were generally provided regarding the phylogenetic importance in the tolerance or  
206 sensitivity of microalgae species. Our results suggest that the diatom *T. delicatula* and the  
207 prymnesiophyte *I. galbana* can be negatively inhibited by exposure to a mixture of pesticides  
208 at concentrations as low as 10 ng L<sup>-1</sup>, the diatom being even more sensitive than the  
209 prymnesiophyte, confirming what has been observed in oceanic and coastal phytoplankton  
210 strains (Huertas et al., 2010).

211 Therefore, the lack of effect, or the positive effect, of metal and pesticide mixtures on  
212 microalgal growth at concentrations found in elutriate (x1) may explain why microalgae  
213 growth was not affected when supplemented with filtered elutriate containing both  
214 contaminants. This is also consistent with the absence of any toxic effect observed during a  
215 previous experiment performed using a filtered sediment elutriate from Bizerte Lagoon and  
216 added to a natural phytoplankton community (Ben Othman et al., 2017).

217

### 218 3.2. Bacteria affect algal growth rates and algal sensitivity to contaminants

219 For both microalgae strains studied here, the presence of bacteria did significantly  
220 reduce their growth rate by ca. 50%, from 0.8 to 0.4 d<sup>-1</sup> for *Isochrysis galbana* and from 0.9  
221 to 0.5 d<sup>-1</sup> for *Thalassiosira delicatula* (Table 1) in control conditions. This suggests that  
222 bacteria i) may act as competitors for nutrient resources and/or ii) may release toxic

223 compounds partially inhibiting, algal growth rates (Mayali and Azam, 2004). This would  
224 explain the reduction in the growth rate for axenic strains in the presence of total elutriate  
225 containing bacteria. Competition for nutrients is unlikely in the present study, as both bacteria  
226 and microalgae were growing in high macro- and micronutrient culture media and the  
227 maximal growth rates of microalgae are calculated using the first 2–3 days of the growing  
228 phase. The algal growth inhibition by the presence of bacteria seems not to be related to a  
229 specific bacterial diversity associated with the microalgae, as the bacterial community was  
230 rather different between the two strains (Bray–Curtis dissimilarity greater than 0.5), being  
231 dominated by the class of Flavobacteria for *Thalassiosira delicatula*, and the class of Alpha-  
232 and Betaproteobacteria for *Isochrysis galbana* (Fig. 2). This is further supported by the  
233 growth reduction of both axenic microalgae strains when supplemented with the elutriate  
234 containing a diverse bacterial community (Fig. 2). The additional presence of bacteria from  
235 elutriate in the xenic culture of both strains did not lead to an extra reduction in their growth  
236 (Fig. 1B) relative to the controls. We therefore suggest that bacteria growing with microalgae  
237 strains are probably opportunistic bacteria that benefit from the microalgae phycosphere but  
238 to the detriment of algal growth. This is supported by a recent study showing that all the  
239 bacteria isolated from a marine algal culture collection had a negative effect on the growth  
240 rate of *Dunaliella* sp. when experimentally associated with the axenic microalgae, even when  
241 these bacteria were initially isolated from *Dunaliella* cultures (Le Chevanton et al., 2013).

242 Interestingly, in contrast to the observations made under axenic algal growth  
243 conditions, in the presence of bacteria, neither microalgal strain was significantly affected by  
244 either the metal or pesticide mixture (Fig. 2B). This suggests that even if bacteria may have an  
245 algaecide effect, they can also reduce toxicity or metal availability to algae (e.g. pesticide  
246 degradation, metal immobilization). For example, *T. delicatula* under pesticide mixtures  
247 showed growth rates as low as 0.3 d<sup>-1</sup> without bacteria but always greater than 0.5 d<sup>-1</sup> with

248 bacteria (Table 2). It seems that the reduction of *ca.* 50% of algal growth rate with the  
249 presence of bacteria allowed microalgae to better resist pesticide contamination. A reduction  
250 in toxicity by bacteria may be suggested, although further investigation is required to clearly  
251 evidence their ability to degrade or immobilize these toxic compounds within the 2–3 days of  
252 the microalgae growing phase. The herbicides and degradation products included in the  
253 mixture assayed during this work are considered as being resistance to rapid biodegradation,  
254 and half-lives are often comprised of between a week and a few months. For example,  
255 atrazine and degradation products lasted more than a month in marine sediments (Smalling  
256 and Aelion, 2006), whereas simazine degradation was only observed with selected bacterial  
257 strain supplemented with carbon sources (Liu et al., 2018). Diuron and its metabolites  
258 exhibited half-lives of longer than five days in selected bacterial pure cultures (Villaverde et  
259 al., 2017), and it took more than 100 days to degrade alachlor in artificial wetlands (Elsayed  
260 et al., 2015). As a consequence, it appears unlikely that significant degradation of the  
261 pesticide mixture occurred within the duration of the present experiment.

262 We also suggest that the presence of bacteria would induce microalgae to dedicate  
263 parts of their resources to defense rather than growth, such as the release of molecules with a  
264 high adsorption capability allowing them to cope with all other stressing factors, including  
265 toxic compounds. Such a strategy can be seen as a growth–defense trade-off, similar to the  
266 phenomenon that was first observed in forestry studies of plant–insect interactions. This  
267 trade-off strategy is based on the assumption that plants possess a limited pool of resources  
268 that can be invested either in growth or in defense (Coley et al., 1985). A similar strategy was  
269 evidenced for microalgae associated with inducible defenses against predators (Zhu et al.,  
270 2016) but never suggested when in interaction with bacteria, as far as we know.

271

272 The present study clearly shows that interactions between bacteria and phytoplankton  
273 can influence the sensitivity of microalgae to toxic compounds and to metal availability.  
274 These interactions did not seem to be species dependent, as they were not related to the  
275 bacterial community composition or phytoplankton species. This suggests that the influence  
276 of bacteria on algal sensitivity to contaminants could be generalized to various species of  
277 microalgae and bacteria. Such results can be applied for bioremediation of toxic contaminants  
278 in heavily polluted environments or as wastewater final treatment. These purposes will benefit  
279 from innovative approaches such as the design and the use of artificially optimised microbial  
280 consortia to remediate toxic chemicals. Microalgae-bacteria consortia can favour the presence  
281 of cometabolism which is recognized as a successful bioremediation approach to biodegrade  
282 recalcitrant molecules (Hazen, 2010). But further studies are required to assess the  
283 degradation and/or immobilization of pesticide and metal compounds by bacteria  
284 communities usually observed within the phycosphere and to evidence any change in the  
285 metabolism and physiology of microalgae (e.g. release of scavenging molecules) when in the  
286 presence of bacteria. This study was performed under nutrient replete conditions but  
287 additional investigations would be necessary to explore any change in the bacteria-microalgae  
288 relationships when exposed to nutrient limitation in addition to contaminants. Such limitation  
289 may affect algal growth in a stronger extent than toxic compounds, further limiting the  
290 potential protective effect of bacteria.

291

## 292 **Acknowledgments**

293 We would like to thank the two reviewers for their constructive comments. This study  
294 was supported by the RISCO and PHYCOVER projects, which were funded by the French  
295 National Agency for Research (respectively, ANR-13-CESA-0001 and ANR-14-CE04-0011).

296 We should like to thank Chrystelle Bancon-Montigny (UMR 5559 HydroSciences



297 Montpellier) and Catherine Gonzalez (Ecole des Mines d'Alès, Alès), for providing the  
298 analyses and the artificial mixtures of pesticides and metals used in this study.

299

300

301

## 302 **References**

303 Amin, S.A., Parker, M.S., Armbrust, E.V., 2012. Interactions between diatoms and bacteria.

304 *Microbiol. Mol. Biol. Rev.* 76, 667–84. <https://doi.org/10.1128/MMBR.00007-12>

305 Armbrust, E.V., Berges, J.A., Bowler, C., Green, B.R., Martinez, D., Putnam, N.H., et al.

306 2004. The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and  
307 metabolism. *Science* 306, 79-86.

308 Azam, F., Malfatti, F., 2007. Microbial structuring of marine ecosystems. *Nat. Rev. Microbiol.*

309 5, 966–966. <https://doi.org/10.1038/nrmicro1798>

310 Bauer, N., Grossart, H., Hilt, S., 2010. Effects of bacterial communities on the sensitivity of  
311 the phytoplankton *Stephanodiscus minutulus* and *Desmodesmus armatus* to tannic acid.

312 *Aquat. Microb. Ecol.* 59, 295–306. <https://doi.org/10.3354/ame01402>

313 Bell, W., Mitchell, R., 1972. Chemotactic and growth responses of marine bacteria to algal  
314 extracellular products. *Biol. Bull.* 143, 265–277. <https://doi.org/10.2307/1540052>

315 Ben Othman, H., Leboulanger, C., Le Floc'h, E., Hadj Mabrouk, H., Sakka Hlaili, A., 2012.

316 Toxicity of benzo(a)anthracene and fluoranthene to marine phytoplankton in culture:  
317 does cell size really matter? *J. Hazardous Mater.* 243, 204-2011.

318 Ben Othman, H., Pringault, O., Louati, H., Sakka Hlaili, A., Leboulanger, C., 2017. Impact of  
319 contaminated sediment elutriate on coastal phytoplankton community (Thau lagoon,

320 Mediterranean Sea, France). *J. Exp. Mar. Bio. Ecol.* 486, 1–12.



- 321 <https://doi.org/10.1016/J.JEMBE.2016.09.006>
- 322 Bouwer, E.J., Zehnder, A.J.B., 1993. Bioremediation of organic compounds — putting  
323 microbial metabolism to work. *Trends Biotechnol.* 11, 360–367.
- 324 [https://doi.org/10.1016/0167-7799\(93\)90159-7](https://doi.org/10.1016/0167-7799(93)90159-7)
- 325 Bruins, M.R., Kapil, S., Oehme, F.W., 2000. Microbial Resistance to Metals in the  
326 Environment. *Ecotoxicol. Environ. Saf.* 45, 198–207.
- 327 <https://doi.org/10.1006/EESA.1999.1860>
- 328 Coley, P.D., Bryant, J.P., Chapin, F.S., 1985. Resource Availability and Plant Antiherbivore  
329 Defense. *Science (80- )*. 230, 895–899. <https://doi.org/10.1126/science.230.4728.895>
- 330 Elsayed, O.F., Maillard, E., Vuilleumier, S., Millet, M., Imfeld, G., 2015. Degradation of  
331 chloroacetanilide herbicides and bacterial community composition in lab-scale wetlands.  
332 *Sci. Total Environ.* 520, 222–231. <https://doi.org/10.1016/j.scitotenv.2015.03.061>
- 333 Field, C.B., Behrenfeld, M.J., Randerson, J.T., Falkowski, P., 1998. Primary production of the  
334 biosphere: integrating terrestrial and oceanic components. *Science* 281, 237–40.
- 335 <https://doi.org/10.1126/SCIENCE.281.5374.237>
- 336 Fouilland, E., Vasseur, C., Leboulanger, C., Le Floc'h, E., Carré, C., Marty, B., Steyer, J.-P.,  
337 Sialve, B., 2014. Coupling algal biomass production and anaerobic digestion: Production  
338 assessment of some native temperate and tropical microalgae. *Biomass and Bioenergy*  
339 70, 564–569. <https://doi.org/10.1016/J.BIOMBIOE.2014.08.027>
- 340 Hazen, T., 2010. Cometabolic Bioremediation. In: Timmis KN (Eds), *Handbook of*  
341 *Hydrocarbon and Lipid Microbiology*. Springer, Berlin. pp 2505-2514.
- 342 Huertas, I.E., Rouco, M., López-Rodas, V., Costas, E., 2010. Estimating the capability of  
343 different phytoplankton groups to adapt to contamination: herbicides will affect  
344 phytoplankton species differently. *New Phytol.* 188, 478–487.

- 345 <https://doi.org/10.1111/j.1469-8137.2010.03370.x>
- 346 Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013.
- 347 Development of a dual-index sequencing strategy and curation pipeline for analyzing
- 348 amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ.*
- 349 *Microbiol.* 79, 5112–20. <https://doi.org/10.1128/AEM.01043-13>
- 350 Le Chevanton, M., Garnier, M., Bougaran, G., Schreiber, N., Lukomska, E., Bérard, J.-B.,
- 351 Fouilland, E., Bernard, O., Cadoret, J.-P., 2013. Screening and selection of growth-
- 352 promoting bacteria for *Dunaliella* cultures. *Algal Res.* 2, 212–222.
- 353 <https://doi.org/10.1016/J.ALGAL.2013.05.003>
- 354 Lee, J.G., Roberts, S.B., Morel, F.M.M., 1995. Cadmium: A nutrient for the marine diatom
- 355 *Thalassiosira weissflogii*. *Limnol. Oceanogr.* 40, 1056–1063.
- 356 <https://doi.org/10.4319/lo.1995.40.6.1056>
- 357 Liu, J., Pan, D., Wu, X., Chen, H., Cao, H., Li, Q.X., Hua, R., 2018. Enhanced degradation of
- 358 prometryn and other s-triazine herbicides in pure cultures and wastewater by polyvinyl
- 359 alcohol-sodium alginate immobilized *Leucobacter* sp. JW-1. *Sci. Total Environ.* 615,
- 360 78–86. <https://doi.org/10.1016/j.scitotenv.2017.09.208>
- 361 Masmoudi, S., Nguyen-Deroche, N., Caruso, A., Ayadi, H., Morant-Manceau, A., Tremblin,
- 362 G., Martine, M., Schoefs, B., 2013. Cadmium, Copper, Sodium and Zinc Effects on
- 363 Diatoms: from Heaven to Hell — a Review. *Cryptogam. Algal.* 34, 185–225.
- 364 <https://doi.org/10.7872/crya.v34.iss2.2013.185>
- 365 Mayali, X., Azam, F., 2004. Algicidal Bacteria in the Sea and their Impact on Algal Blooms1.
- 366 *J. Eukaryot. Microbiol.* 51, 139–144. [https://doi.org/10.1111/j.1550-](https://doi.org/10.1111/j.1550-7408.2004.tb00538.x)
- 367 [7408.2004.tb00538.x](https://doi.org/10.1111/j.1550-7408.2004.tb00538.x)
- 368 Mu, R., Fan, Z., Pei, H., Yuan, X., Liu, S., Wang, X., 2007. Isolation and algae-lysing

- 369 characteristics of the algicidal bacterium B5. *J. Environ. Sci. (China)* 19, 1336–40.
- 370 Pringault, O., Lafabrie, C., Avezac, M., Bancon-Montigny, C., Carre, C., Chalghaf, M.,  
371 Delpoux, S., Duvivier, A., Elbaz-Poulichet, F., Gonzalez, C., Got, P., Leboulanger, C.,  
372 Spinelli, S., Sakka Hlaili, A., Bouvy, M., 2016. Consequences of contaminant mixture  
373 on the dynamics and functional diversity of bacterioplankton in a southwestern  
374 Mediterranean coastal ecosystem. *Chemosphere* 144, 1060–1073.  
375 <https://doi.org/10.1016/J.CHEMOSPHERE.2015.09.093>
- 376 Ribalet, F., Intertaglia, L., Lebaron, P., Casotti, R., 2008. Differential effect of three  
377 polyunsaturated aldehydes on marine bacterial isolates. *Aquat. Toxicol.* 86, 249–255.  
378 <https://doi.org/10.1016/J.AQUATOX.2007.11.005>
- 379 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B.,  
380 Lesniewski, R. a, Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B.,  
381 Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source,  
382 platform-independent, community-supported software for describing and comparing  
383 microbial communities. *Appl. Environ. Microbiol.* 75, 7537–41.  
384 <https://doi.org/10.1128/AEM.01541-09>
- 385 Seymour, J.R., Amin, S.A., Raina, J.B., Stocker, R., 2017. Zooming in on the phycosphere:  
386 The ecological interface for phytoplankton-bacteria relationships. *Nat. Microbiol.* 2.  
387 <https://doi.org/10.1038/nmicrobiol.2017.65>
- 388 Smalling, K.L., Aelion, C.M., 2006. Biological and chemical transformation of atrazine in  
389 coastal aquatic sediments. *Chemosphere* 62, 188–196.  
390 <https://doi.org/10.1016/j.chemosphere.2005.05.042>
- 391 Staley, Z.R., Harwood, V.J., Rohr, J.R., 2015. A synthesis of the effects of pesticides on  
392 microbial persistence in aquatic ecosystems. *Crit. Rev. Toxicol.* 45, 813–836.

- 393 <https://doi.org/10.3109/10408444.2015.1065471>
- 394 Villaverde, J., Rubio-Bellido, M., Merchán, F., Morillo, E., 2017. Bioremediation of diuron  
395 contaminated soils by a novel degrading microbial consortium. *J. Environ. Manage.* 188,  
396 379–386. <https://doi.org/10.1016/j.jenvman.2016.12.020>
- 397 Walsh, G., 1972. Effects of herbicides on photosynthesis and growth of marine unicellular  
398 algae. *Hyacinth Control J.*
- 399 Williams, P.J.le B., Laurens, L.M.L., 2010. Microalgae as biodiesel & biomass feedstocks:  
400 Review & analysis of the biochemistry, energetics & economics. *Energ. Environ. Sci.* 3,  
401 554-590.
- 402 Zhu, X., Wang, J., Chen, Q., Chen, G., Huang, Y., Yang, Z., 2016. Costs and trade-offs of  
403 grazer-induced defenses in *Scenedesmus* under deficient resource. *Sci. Rep.* 6, 22594.  
404 <https://doi.org/10.1038/srep22594>

406 Table 1. 16S rRNA gene sequencing primers

forward primer	5'-CTTCCCTACACGACGCTCTTCCGATCTGTGYCAGCMGCCGCGGTA-3'
reverse primer	5'-GGAGTTCAGACGTGTGCTCTTCCGATCTCCCGYCAATTCMTTTRAGT-3'

407

408

Comment citer ce document :

19

Fouilland, E. (Auteur de correspondance), Galès, A., Beaugelin, I., Lanouguère, E., Pringault, O., Leboulanger, C. (2018). Influence of bacteria on the response of microalgae to contaminant mixtures. *Chemosphere*, 211, 449-455. , DOI : 10.1016/j.chemosphere.2018.07.161

CHEMOSPHERE SHORT COMMUNICATION

409 Table 2. Growth rates (mean and standard error – SE) of axenic and xenic algal strains measured with or without (control) the addition of total  
 410 elutriate, filtered elutriate, mixture of metals and pesticides at concentrations from those found in elutriate (x1) to x31 for metals and x310 for  
 411 pesticides. Greyed values represent significant differences with Control ( $p < 0.05$ )

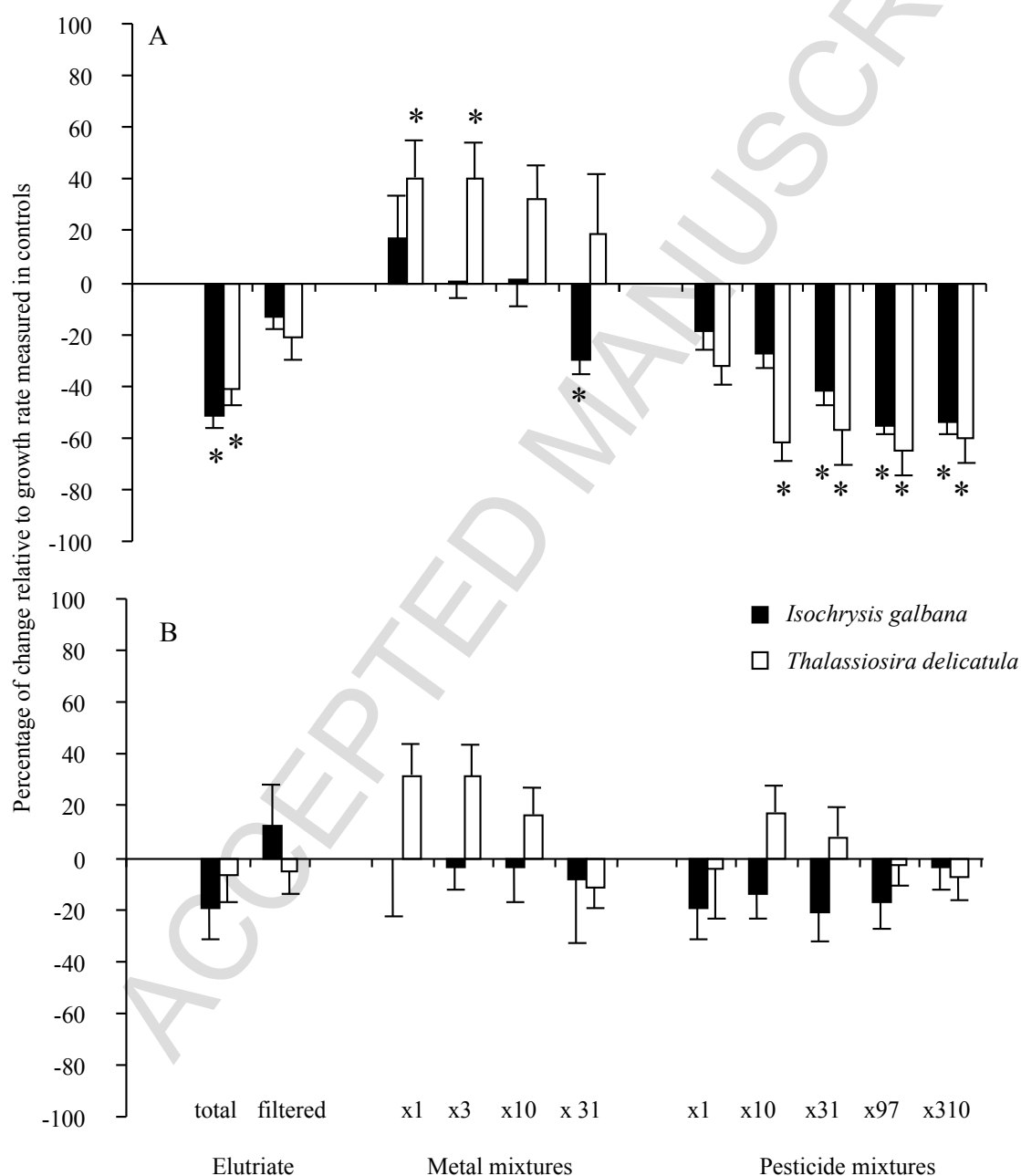
		<i>Isochrysis galbana</i>				<i>Thalassiosira delicatula</i>			
		axenic		xenic		axenic		xenic	
		mean	SE	mean	SE	mean	SE	mean	SE
	Control	0.83	0.04	0.38	0.03	0.92	0.09	0.54	0.05
	total elutriate	0.40	0.03	0.31	0.02	0.54	0.02	0.51	0.04
	filtered elutriate	0.72	0.03	0.43	0.02	0.72	0.03	0.51	0.02
	x1	0.98	0.13	0.38	0.04	1.28	0.05	0.71	0.02
	x3	0.84	0.04	0.37	0.01	1.28	0.06	0.71	0.02
metal mixture	x10	0.84	0.07	0.37	0.02	1.21	0.03	0.63	0.01
	x31	0.58	0.04	0.35	0.04	1.09	0.18	0.48	0.02
	x1	0.68	0.05	0.31	0.02	0.62	0.02	0.52	0.10
	x10	0.60	0.04	0.33	0.01	0.35	0.06	0.63	0.02
pesticide mixture	x31	0.49	0.05	0.30	0.02	0.39	0.12	0.58	0.03
	x97	0.37	0.03	0.32	0.02	0.32	0.08	0.53	0.01
	x310	0.38	0.03	0.37	0.01	0.36	0.08	0.50	0.02

412

413

Comment citer ce document :

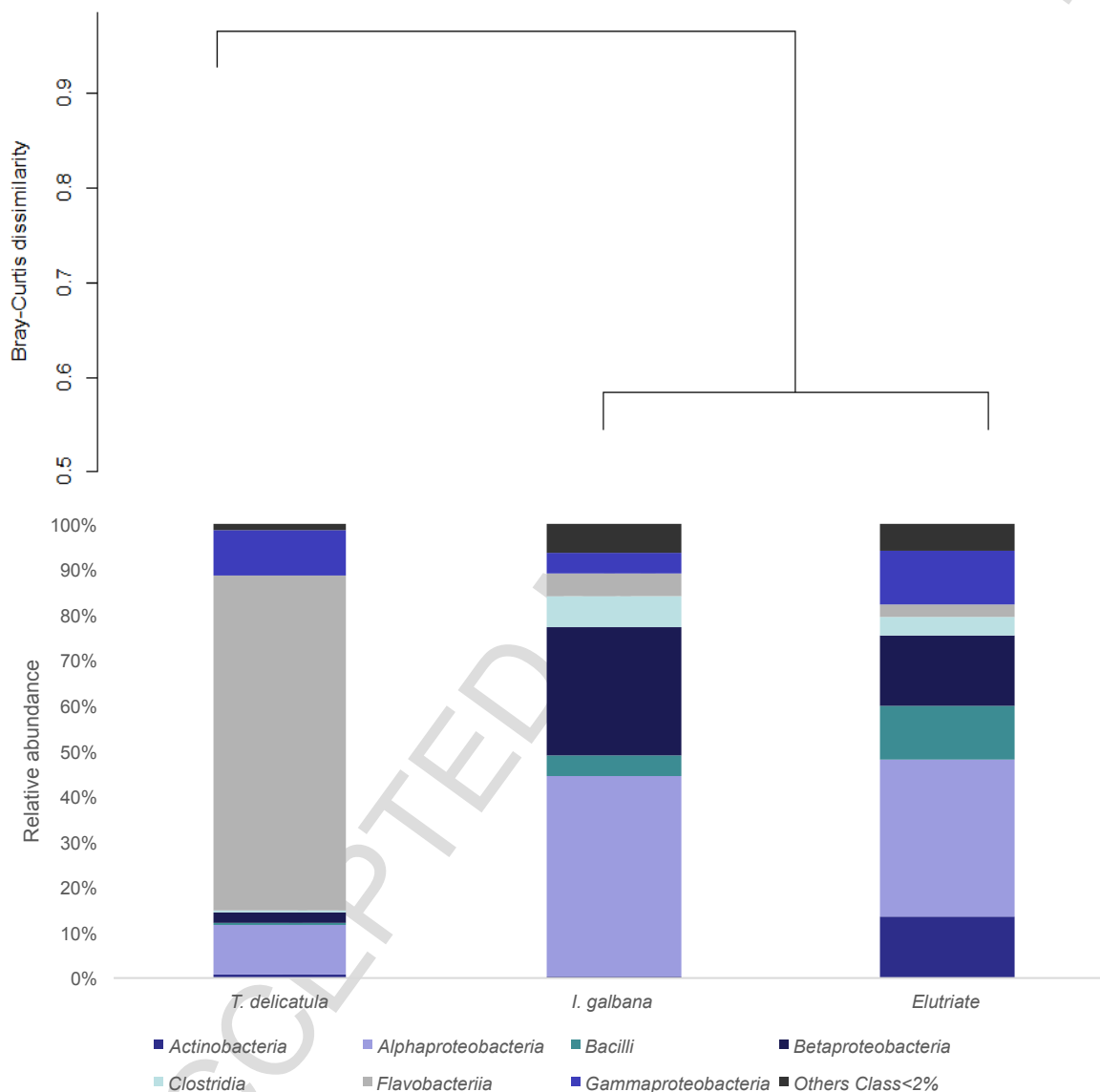
414 Figure 1. Change (mean and SE) in growth rates measured with elutriate, metal or pesticide  
 415 mixture additions relative to control (no addition) and expressed in percentage for *Isochrysis*  
 416 *galbana* and *Thalassiosira delicatula* under (A) axenic and (B) xenic culture conditions.  
 417 Positive and negative values (%) correspond to, respectively, an increase and a decrease in  
 418 growth rate relative to control. Asterisks denote a significant difference ( $p < 0.05$ ) between  
 419 treatments and control.



420

421

422 Figure 2. Bacterial diversity observed in xenic cultures of *Thalassiosira delicatula* and  
 423 *Isochrysis galbana* and in the sediment elutriate: the dendrogram shows the clustering of  
 424 bacterial communities found in cultures, based on Bray–Curtis similarities calculated at the  
 425 OTU level and bar plots show the relative abundance of the main bacterial classes observed.



426



## Highlights

- The presence of bacteria in the culture medium negatively affected microalgae growth
- The absence of bacteria resulted in short term microalgae response to metals and pesticides at low dose
- Metals and pesticides were not toxic to microalgae when growing with bacteria