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1 **Single toxicity of arsenic and combined trace metal exposure to a microalga**  
2 **of ecological and commercial interest: *Diacronema lutheri***

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8

9 **Highlights-**

- 10 1. Cell count significantly reduced in trace metal mixture than Arsenic (As V).  
11 2. Total As reduced in the single exposure of As V than in mixture on day 20.  
12 3. Bioaccumulation and bio-concentration factor of As V increased in mixed trace metal.  
13 4. Chl *a* content was lowest in mixed trace metal when compared to As V treatment.  
14 5. De-epoxidation ratio in the mixed trace metal was higher than in As V alone.

15

16 **Abstract-**

17 Eco-toxicological assays with species of economic interest such as *Diacronema lutheri* are  
18 essential for industries that produce aquaculture feed, natural food additives and also in drug  
19 developing industries. Our study involved the exposure of a single and combined toxicity of  
20 arsenic (As V) to *D. lutheri* for the entire algal growth phase and highlighted that a combined  
21 exposure of As V with other essential (Copper, Cu and Nickel, Ni) and non-essential  
22 (Cadmium, Cd; Lead, Pb) trace metals reduced significantly the cell number, chlorophyll *a*  
23 content, and also significantly increased the de-epoxidation ratio (DR) as a stress response  
24 when compared to the single toxicity of As V. Arsenic, as one of the ubiquitous trace metal  
25 and an active industrial effluent is reported to have an increased bio-concentration factor when  
26 in mixture with other trace metals in this study. In the combined exposure, the concentration

27 of total As bio-accumulated by *D. lutheri* was higher than in the single exposure. Hence,  
28 polluted areas with the prevalence of multiple contaminants along with the highly toxic trace  
29 metals like As can impose a greater risk to the exposed organisms that may get further bio-  
30 magnified in the food chain. Our study highlights the consequences and the response of *D.*  
31 *lutheri* in terms of contamination from single and multiple trace metals in order to obtain a  
32 safer biomass production for the growing need of natural derivatives.

33

34 **Keywords** – Arsenic, trace metals mixture, bioaccumulation, bioconcentration factor, de-  
35 epoxidation ratio, chlorophyll *a*.

36

## 37 **1. Introduction**

38 Trace metals are persistent and have proved to be toxic to surrounding organisms  
39 in all spheres of the ecosystem (Anandkumar et al., 2019; Rajaram et al., 2020; Das  
40 et al., 2020a; Yang et al., 2020). The scenario post-industrialization has posed a  
41 serious ultimatum to numerous species around the world with continuous exposure  
42 to harmful substances that are often disposed of in coastal areas (Johnson et al.,  
43 2003; Schwarzenbach et al., 2006; Webster et al., 2011). Several studies have  
44 reported the effects of such contaminants on marine organisms and the  
45 consequences on their growth, physiology, metabolism, morphology, etc.  
46 (Baumann et al., 2009; Diaz et al., 2012; Facey et al., 2019; Mamun et al., 2019a;  
47 Dayras et al., 2020). Some trace metals like copper (Cu), nickel (Ni), zinc (Zn) play  
48 essential roles in the metabolism of the organism while others like arsenic (As),  
49 cadmium (Cd), lead (Pb) have no such capacity (Zidour et al., 2019; Das et al.,  
50 2020a). The essential trace metals are required at certain concentrations in the  
51 organism which, when exceeded, can be toxic (Chen et al., 2018; Yang et al., 2020).

52 Amongst the non-essential trace metals, Arsenic is often considered as ubiquitous  
53 in nature posing a high risk of toxicity through its exposure (Hak et al., 2020; Huang  
54 et al., 2021). Further, As is considered as an extremely varied toxicant and a  
55 predominant carcinogen that persists in the entire food chain of the ecosystem  
56 existing in several organic and inorganic states (Sghaier et al., 2015; Mitra et al.,  
57 2017; Mamun et al., 2019 a, b; Hussain et al., 2021). An inorganic state of As;  
58 arsenate (As V) has been reported as the chief state that exists in an aerobic  
59 ecosystem and often gets absorbed into the body surface of aquatic organisms  
60 (Mitra et al., 2017; Mamun et al., 2019 a, b). In general, such toxic trace metals are  
61 often present in a combined complexity with other metals and thus individual effect  
62 on the organism depends on the bioavailability of each metal (Kadiene et al., 2019  
63 a, b; Zidour et al., 2019; Das et al., 2020a). Laboratory-based studies of single  
64 toxicity of a trace metal have however taken their place for few decades, but  
65 comparisons of single and combined toxicity are still in the way of making a  
66 profound understanding in eco-toxicology (Zidour et al., 2019; Das et al., 2020a).  
67 Previous studies have focused on the single toxicity of As V on marine organisms  
68 and its capacity of bio-accumulation and biotransformation whereas, a comparison  
69 of varied pathways of exposure (single and combined toxicity) on the first trophic  
70 level of the food chain has not been much explored (Upadhyay et al., 2018; Hak et  
71 al., 2020; Huang et al., 2021). In addition, the industrial wastes that are usually  
72 emitted into the adjacent water bodies, are often present as a cocktail of trace metals  
73 and other organic pollutants that gets exposed to the inhabiting organisms, which  
74 further substantiates the need of considering the effect of combined trace metal  
75 and/or other contaminants in ecotoxicology. Therefore, experimental approaches

76 comparing culture conditions (control) with that of single toxicity and combined  
77 toxicity of trace metals should be encouraged.

78 . To comprehend the effects of such contaminants, phytoplankton, being one of the  
79 primary producers of the aquatic sphere played a major role as a bio-indicator in  
80 assessing the threats prevailing in a particular environment and risk assessment  
81 assays (Salama et al., 2019; Rajaram et al., 2020; Yang et al., 2020). The  
82 bioaccumulation of trace metals in algae usually occurs by active or passive  
83 transports of ions into the cell membranes that act as hydrocolloids, affecting its  
84 growth by decelerating photosynthetic activity (Leal et al., 2016; Salama et al.,  
85 2019). Trace metals can often cause oxidative stress and increase the cellular  
86 reactive oxygen species (ROS) that can further cause alteration in enzymatic  
87 activity, pigment contents including protein degradation (Cheloni and Slaveykova  
88 et al., 2018; Salama et al., 2019). Apart from this, trace metals have also shown  
89 adverse effects on the morphology, and the coherence of cell membranes  
90 controlling the permeability of the cell wall of algal species (Mamun et al., 2019b;  
91 Costa et al., 2019; Salama et al., 2019). Marine micro/macroalgae have shown  
92 resilience to such toxicity and pathways to depurate these substances by  
93 compartmentalizing the toxins in specific cellular cavities like vacuoles followed  
94 by a release of the contaminants into the medium through efflux pump (Perales-  
95 Vela et al., 2006; Juang and Chang et al., 2016; Salama et al., 2019). They have also  
96 proved their adaptability to trace metals by the formation of metallothioneins and  
97 phytochelatins (Navarrete et al., 2019, Costa et al., 2019). In general, algal species  
98 are capable of bio-accumulating trace metals that often penetrate and get stored in  
99 their tissues and also poses a threat of bio-magnification of contaminants to higher  
100 trophic levels (Yin et al., 2011; Tao et al., 2012; Yang et al., 2020). Besides the risk

101 of biomagnification, studies have also highlighted the ability of micro/macroalgal  
102 species for bioremediation, reporting high removal efficiency of trace metals,  
103 secondary pollutants and hydrophobic compounds by some algal species like  
104 *Spirulina*, *Chlorella*, *Cladophora* and many others (Mahdavi et al., 2012; Jasrotia  
105 et al., 2014; Yang et al., 2015; Gao et al., 2016). Hence, studies on the effect of  
106 highly toxic trace metals like As and its combined toxicity with other trace metals  
107 can further benefit our understanding on the removal efficiency of such  
108 contaminants by microalgae near anthropogenic quarters. *Diacronema lutheri*  
109 (Droop) Bendif & Véron, 2011 is one such ecologically and economically important  
110 microalgae that is widely cultivated worldwide. Indeed, this microalga constitutes  
111 loads of phytosterols and is also extensively used as a feed in the aquaculture  
112 industry (Ahmed et al., 2015; Bernaerts et al., 2018; Huang et al., 2020). Such  
113 phytosterol rich organic sources are the natural alternatives that are often used as  
114 additives in food items for their ability to lower the levels of low-density lipoprotein  
115 (LDL) cholesterol and also beholds anti-inflammatory, anti-cancerous and anti-  
116 oxidative properties (Kim et al., 2008; Carmona et al., 2010; Ahmed et al., 2015).  
117 Therefore, toxicological assays implementing toxicity from varied sources and  
118 pathways involving economical species with medicinal and therapeutic benefits like  
119 *D. lutheri* should be encouraged. Out of the few studies conducted on the eco-  
120 toxicological aspect involving *D. lutheri*, some of them reported that when *D.*  
121 *lutheri* was exposed to dissolve organic contaminant originating from nearby  
122 industrial catchment areas in Scotland, showed significant inhibition of growth rate  
123 in nearly all the stations for higher concentrations of pesticides; that was useful in  
124 assessing the risk of that particular habitat (Emelogu et al., 2013; 2014). Therefore,  
125 the objective of our study was to monitor the effect of As V which is predominant

126 and shows high adsorption ability, as a single source of toxicant and a combined  
127 mixture of essential (Cu; Ni) and non-essential (Cd; Pb) trace metals to comprehend  
128 the bioaccumulation and bio-concentration of these metals when detected in a  
129 mixture. The further objective was to analyze the effect of the bioaccumulation of  
130 trace metals on the growth curve and the pigment contents of *D. lutheri* comparing  
131 it with the controlled culture conditions. The tangential aim of this study was to  
132 highlight the risks of bioaccumulation and bio-concentration of trace metals to  
133 optimize the growth and production of this economically beneficial algal species  
134 when used in medicines and the aquaculture industry as a feed for higher trophic  
135 communities. *Diacronema lutheri* is used as an aquaculture feed in our laboratory  
136 regularly for years, and the amount of trace metal used in this study corresponds to  
137 the lethal concentration of the bio-indicator copepod *Eurytemora affinis* (Kwok et  
138 al., 2015, Souissi and Souissi, 2021), with regards to enhancing our understanding  
139 on the consequences in case of a trace metal contamination in our culture systems  
140 emerging through the algal feed source. Such eco-toxicological assays with *D.*  
141 *lutheri* are scarce and can help to better understand the mechanism of environmental  
142 toxicity and the cocktail effect of contaminants in addition to the benefits in the  
143 commercial industries to improve the quality and quantity of biomasses that can be  
144 safely used as a natural alternative to drugs.

145

## 146 **2. Material and methods**

### 147 **2.1 Algal species- *Diacronema lutheri***

148 *Diacronema lutheri* from the Roscoff Culture Collection (RCC-1537), France was  
149 cultivated in artificial seawater (osmosed water with artificial salt, Coral salt-pro)  
150 in round bottom flasks of 6 L and was maintained in an incubator with controlled

151 physical parameters (12:12h light: dark in 18°C, salinity 33) (Arias et al., 2017;  
152 Gnouma et al., 2017, Dayras et al., 2020). The formulation of the culture was  
153 ensured by adding an adequate supply of nutrition through the Conway medium in  
154 every culture flask by following the same composition mentioned in Tlili et al.,  
155 2016.

156

## 157 **2.2 Exposure to trace metals: single and combined toxicity**

158 To understand the variability in growth, pigment concentration, and ability to bio-  
159 accumulate trace metals by *D. lutheri*, two different treatments with trace metal  
160 stress along with control were designed in triplicates. The trace metal stress applied  
161 to the algae was single toxicity of As V and a mixture of trace metals (As V + Cd +  
162 Cu + Ni + Pb). The metal concentrations used in this study corresponds to the 10%  
163 lethal concentration (LC50%) of each trace metal when exposed to the invertebrate  
164 aquatic model *Eurytemora affinis*, a bio-indicator, calanoid copepod which is often  
165 fed with *D. lutheri* in the mass cultures of our laboratory (Zidour et al., 2019;  
166 Kadiene et al., 2019b; Das et al., 2020a, Dayras et al., 2020). The LC 50% (96  
167 hours) for Cd, Ni, Cu, and Pb when exposed to *E. affinis* was used according to our  
168 previously reported studies (Cd - 108.5  $\mu\text{g.L}^{-1}$ , Cu - 33.5  $\mu\text{g.L}^{-1}$ , Ni - 125.5  $\mu\text{g.L}^{-1}$ ,  
169 Pb - 413.5  $\mu\text{g.L}^{-1}$ ) (Zidour et al., 2019; Das et al., 2020a). The lethal concentration  
170 used for As V (96 hours, 449.78  $\mu\text{g.L}^{-1}$ ) was determined in our laboratory following  
171 the same protocol as reported in Tlili et al., 2016 (supplementary data, Table 1S).  
172 The exposure for all the treatments was executed in 6 L sterilized flasks using  
173 artificial seawater, under controlled light and temperature conditions along with  
174 constant re-suspension for a symmetrical availability of nutrients, light, and  
175 temperature for all the algal cells (Tlili et al., 2016; Arias et al., 2017; Gnouma et



176 al., 2017, Dayras et al., 2020). The exposure started by adding sterilized algal  
177 culture at the log phase along with the Conway medium and vitamin (vitamin B1,  
178 vitamin B12) to the experimental flasks to anticipate a successive exponential  
179 growth. The composition of Conway medium and vitamin was the same as reported  
180 in Arias et al., 2017 and Tlili et al., 2016. The entire growth curve of *D. lutheri* with  
181 daily cell count was accounted for in our study from day 1 (T1) until day 23 (T23).

182

### 183 **2.3 Cell counting: Daily growth monitoring**

184 Cell counting was done every day from T1 to T23 for monitoring the daily growth  
185 of the algal cells in each treatment and replicate by using an inverted microscope at  
186 100-fold magnification (Olympus, IX 71, Tokyo, Japan) attached to a high storage  
187 capacity computer to preserve all photographs of the fields observed under  
188 microscope for quantification of cell numbers (Guillard and Sieracki 2005; Arias et  
189 al., 2017). A volume of 2 mL sample from each treatment was collected at a  
190 particular time daily in Eppendorf tubes and was fixed with 5% lugol solution in  
191 triplicates and then stored at 4°C before quantification (Iwasawa et al., 2009; Arias  
192 et al., 2017). A haemocytometer (Malassez cell slide, 0.1 mm depth, Japan) was  
193 used for observing the algal cells under the microscope and each field was  
194 photographed for counting the number of cells (Gnouma et al., 2017, Dayras et al.,  
195 2020). A software named ImageJ (<https://imagej.nih.gov/ij/>) was later used for  
196 counting the number of cells in each field from each sample and replicates.

197

### 198 **2.4 Analysis for trace metal concentration – ICP-MS**

199 The analysis for trace metals was executed with an inductive coupled plasma  
200 quadrupole mass spectrometer (ICP-MS) (ICP-MS 7900, Agilent Technologies,

201 United States) equipped with a standard introduction system [borosilicate Micro  
202 Mist concentric nebulizer ( $0.4 \text{ mL min}^{-1}$ ), quartz double pass spray chamber cooled  
203 at  $2^\circ \text{C}$ , quartz torch (2.5 mm ID) and nickel cones. The algal samples were  
204 collected at every phase of the entire growth curve of *D. lutheri*. The sub-sampling  
205 intervals for trace metal analysis were day 7 (T7, exponential phase), day 14 (T14,  
206 early stationary phase), day 17 (T17, late stationary phase), and day 20 (T20, lysis  
207 phase). The algal samples collected at the mentioned intervals were filtered on  
208 sterile membrane filters of  $0.45 \mu\text{m}$  from all the conditions and were dried for  
209 further pre-treatment. The pre-treatment of the filters comprised of acid digestion,  
210 where nitric acid and hydrochloric acid were mixed in the ratio of 1:3 and added to  
211 the Teflon tube containing the filter. These tubes were then put in a water bath of  
212  $100^\circ \text{C}$  for at least 4 hours placed inside a laminar flow cabinet. After the digestion,  
213 a final volume of 5 ml was adapted in each Teflon tube with ultrapure double  
214 distilled water (Milli Q). The samples were then transferred to sterilized Falcon  
215 tubes and were analyzed for trace metal concentration under ICP-MS. The trace  
216 metal concentration remaining in the residual seawater after filtering the algal  
217 biomass was also considered by collecting the filtrate (15 mL) directly into  
218 sterilized Falcon tubes and fixing it with ultrapure nitric acid ( $20 \mu\text{L}$ ). These tubes  
219 were kept in dark until further analysis under ICP-MS without any need of pre-  
220 treatment (Ouddane et al., 1990; Zidour et al., 2019; Kadiene et al., 2019a; Das et  
221 al., 2020a). In the entire process of trace metal analysis, all the quality control  
222 measures were implied by maintaining the optimum quality for all the standard  
223 solutions, and for trace metal identification a reference of multi-elemental standard  
224 was utilized (Ouddane et al., 1990). Certified Reference Materials (CRM) PACS-3  
225 and HISS-1 was used to validate the trace metal concentrations and the

226 mineralisation procedure constituted of  $\leq 10\%$  total analytical errors. The bio-  
227 concentration factor (BCF) of each trace metal in *D. lutheri* was calculated from the  
228 concentration of trace metal bio-accumulated in algae and the residual trace metal  
229 in the medium for each condition (Das et al., 2020a).

230

## 231 **2.5 Analysis for pigment concentration – HPLC**

232 Samples for pigment analysis were collected at all phases of the algal growth curve  
233 of *D. lutheri*, in the same manner as for trace metals, on day 7, day 14, day 17, and  
234 day 20. After filtration through GF/F Whatman filters, they were stored at  $-20^{\circ}\text{C}$  in  
235 the dark until extraction. Pigment extraction was performed by grinding the filters  
236 with methanol and 4 drops ( $30\mu\text{L}$ ) of methylene chloride, avoiding exposure to  
237 light. The extracts were further concentrated after filtration on  
238 polytetrafluoroethylene membrane filter of  $0.45\ \mu\text{m}$  by dry-evaporation under a  
239 nitrogen stream. Desalination of the extract was accomplished with a mixture  
240 (50:50 v/v) of distilled water and methylene chloride. Phase separation was  
241 followed to obtain the organic portion from each sample, which was then dry-  
242 evaporated under nitrogen gas. Each sample was dissolved in  $40\ \mu\text{L}$  of methanol  
243 before injection, from which a volume of  $20\ \mu\text{L}$  was injected in the high-  
244 performance liquid chromatography instrument (Shimadzu, Nexera XR; C18  
245 reverse-phase column, Allure, Restek). The separation was performed using a  
246 solvent delivery profile adapted from Arsalane et al. (1994). Along with the  
247 photosynthetic pigments, the photoprotective pigments were also studied to denote  
248 any possible stress response that triggered in the algal cells when exposed to trace  
249 metals from single and combined toxicity. The xanthophyll cycle has often proved  
250 to be a sensitive parameter to indicate metal stress and its role in the photoprotection

251 process underneath high-light has been reported in previous studies (Bertrand et al.,  
252 2001; Brunet et al., 2014; Yan et al., 2017). The reversible reaction between  
253 diadinoxanthin and diatoxanthin in the xanthophyll cycle often referred to as the  
254 de-epoxidation ratio (DR) was calculated by the following equation (1) (Brunet et  
255 al., 2014; Yan et al., 2017):

256

$$\text{Eq. (1)..... DR} = \frac{\text{Diatoxanthin}}{\text{Diadinoxanthin} + \text{Diatoxanthin}}$$

258

## 259 **2.6 Statistical analysis**

260 The daily cell count, chlorophyll *a* concentration, de-epoxidation ratio (DR),  
261 bioaccumulation of trace metals, bio-concentration factor in each treatment  
262 including the control were compared by using analysis of variance (ANOVA) at  
263  $p < 0.05$ , after meeting all the requirements of normality and homogeneity of  
264 variance. To test the normality and the homogeneity a Shapiro Wilk normality test  
265 and a Levene's Test for Homogeneity of Variance were carried out respectively. To  
266 compare the significance in each condition Tukey multiple comparisons of means  
267 (95% family-wise confidence level) was applied to the data. All the analyses were  
268 accomplished by using R studio (4.0.4).

269

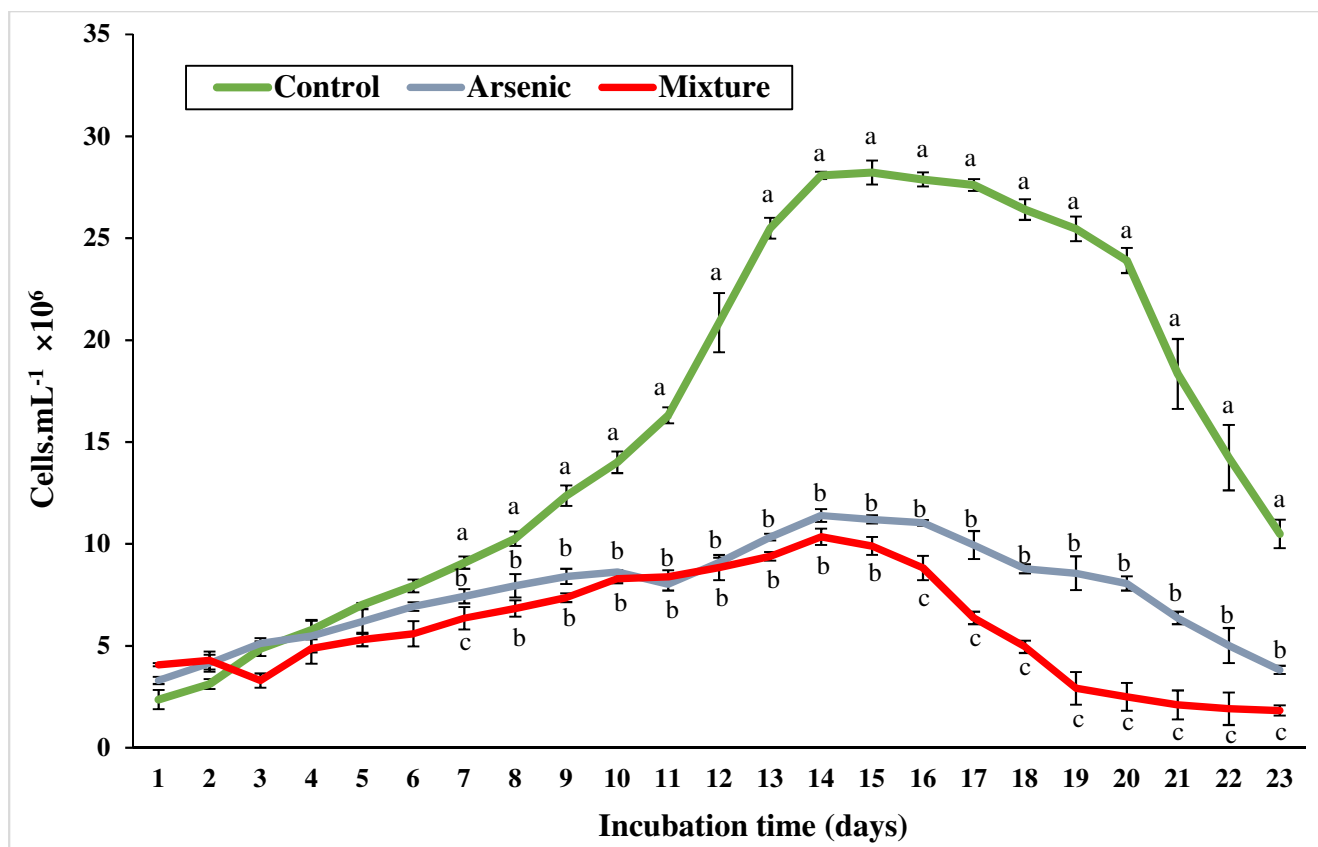
## 270 **3. Results**

### 271 **3.1 Daily growth**

272 Fig. 1 corresponds to the growth curve of *D. lutheri* from T1 to T23 (day 1- day  
273 23), in each treatment, namely control, As V (single exposure) and mixed trace  
274 metals (combined exposure). The phases of algal growth curve are well

275 distinguished in the control treatment with the highest concentration of cells  
276 reaching on day 15 with  $28 \times 10^6$  cells mL<sup>-1</sup>. The cell density in control increased  
277 from T7 to T15 and then reached a stationary phase from T16 to T18, followed by  
278 the lysis phase or death phase with decreasing number of cells until T23. The trace  
279 metal exposed treatments (As V and combined) showed significantly lower  
280 concentration of cells than control from T7 until T23. After 7 days of continuous  
281 exposure the combined trace metal mixture showed statistically the least  
282 concentration of cells when compared to As V exposure and control. The single  
283 exposure of As V and the trace metal mixture differed significantly only at T7 and  
284 thereafter from T16 to T23. However, the cell count in As V was significantly lower  
285 than control, but higher than combined exposure. In both the As V and combined  
286 trace metal exposure, the phases of algal growth were not well defined and showed  
287 high variability especially during the early to late stationary phase. The lysis phase  
288 for combined exposure started at T16 showing significantly lower no. of cells from  
289 As V exposure until T23. The single exposure to As V however showed higher no.  
290 of cells than the combined trace metal mainly in the exponential and lysis phase.  
291 The sub-lethal concentrations of single trace metal exposure (As V) and combined  
292 trace metal exposure (As + Cd + Cu + Ni + Pb) could reduce the biomass  
293 significantly throughout the algal growth curve.

294



295

296 **Fig. 1** Growth curves of control and trace metal exposed treatment from day 1 (T1) until day  
 297 23 (T23). The letters (a, b, c) signify statistical differences between each treatment, at  $p < 0.05$ .  
 298 The error bar shows the standard deviation between the three replicates in each treatment.

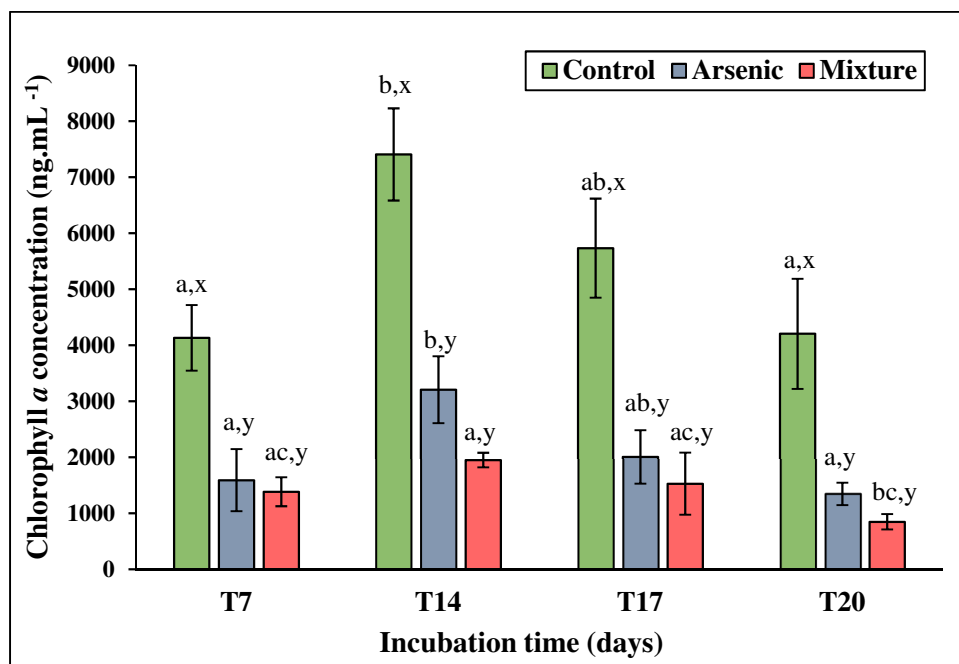
299

### 300 3.2 Pigment concentrations

#### 301 3.2.1 Chlorophyll *a* concentration

302 Fig. 2 shows the chlorophyll *a* (chl *a*) concentration in each treatment at specific  
 303 intervals in the entire growth curve of *D. lutheri*. The control treatment at each  
 304 interval had significantly higher amount of chl *a* content than the trace metal  
 305 exposed treatments. The highest chl *a* content was observed on day 14 (T14),  
 306 when the pigment value increased significantly compared to the exponential  
 307 phase at T7 in case of control and As V exposure, but however, the pigment  
 308 content did not significantly increase in the combined trace metal treatment. In

309 comparison between the trace metal exposed treatments, As V exposure showed  
 310 higher chl *a* concentration at each interval of growth phases, although not  
 311 significantly. In both the trace metal exposed treatments, the chl *a* content  
 312 decreased significantly at T20 gradually from T14. The chl *a* content and the  
 313 cell count of *D. lutheri* at specific intervals of the growth curve in all the  
 314 treatments including control showed a significant positive correlation, with a *p*-  
 315 value <0.01, marking the coherence of both the parameters in this study.



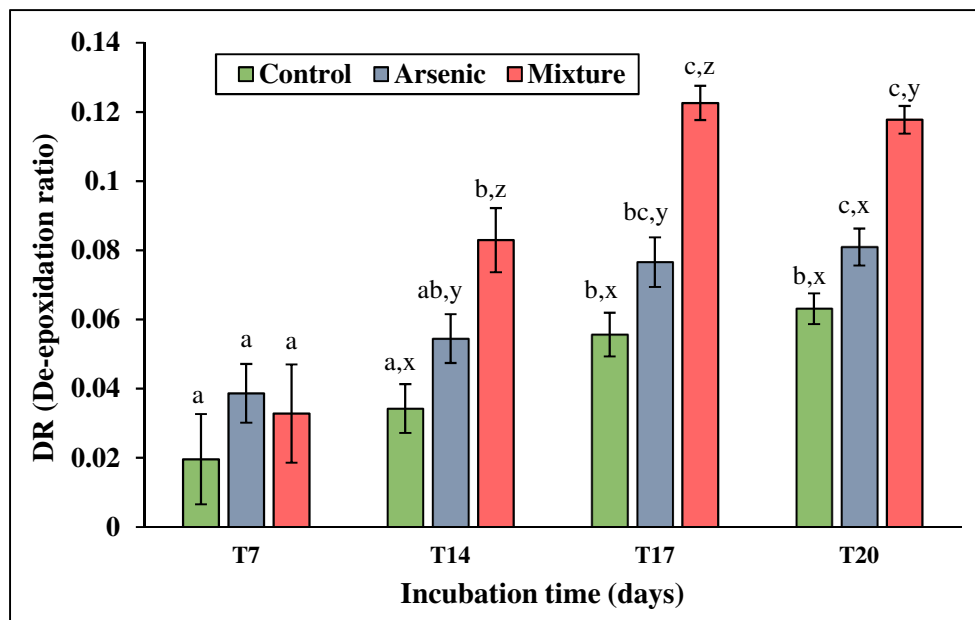
316  
 317 **Fig. 2.** Chlorophyll *a* concentration for each treatment at all phases of growth in *D. lutheri*.  
 318 Letters a, b, c shows significant differences between incubation times; x, y shows significant  
 319 differences between treatments, at *p* < 0.05. The error bar shows the standard deviation between  
 320 the three replicates in each treatment.

321

### 322 3.2.2 De-epoxidation ratio

323 Fig. 3 shows the de-epoxidation ratio for all the treatments at each incubation  
 324 time throughout the growth curve of *D. lutheri*. A significantly higher de-

325 epoxidation ratio was observed for the trace metal combined treatment at the  
 326 linear phases (T14, T17) and the lysis phase (T20). At the exponential phase,  
 327 the DR was not significantly different between the treatments control, As V  
 328 exposure and combined toxicity of trace metals (As V+ Cd + Cu + Ni + Pb). In  
 329 the single exposure of As V treatment, the de-epoxidation state was significantly  
 330 higher than in control in the linear phases (T14, T17) but not at the exponential  
 331 and the lysis phase. In comparison between the trace metal exposed treatments,  
 332 mixed trace metal showed significantly higher DR than the As V exposed  
 333 treatment at all the stages, except the exponential phase (T7).



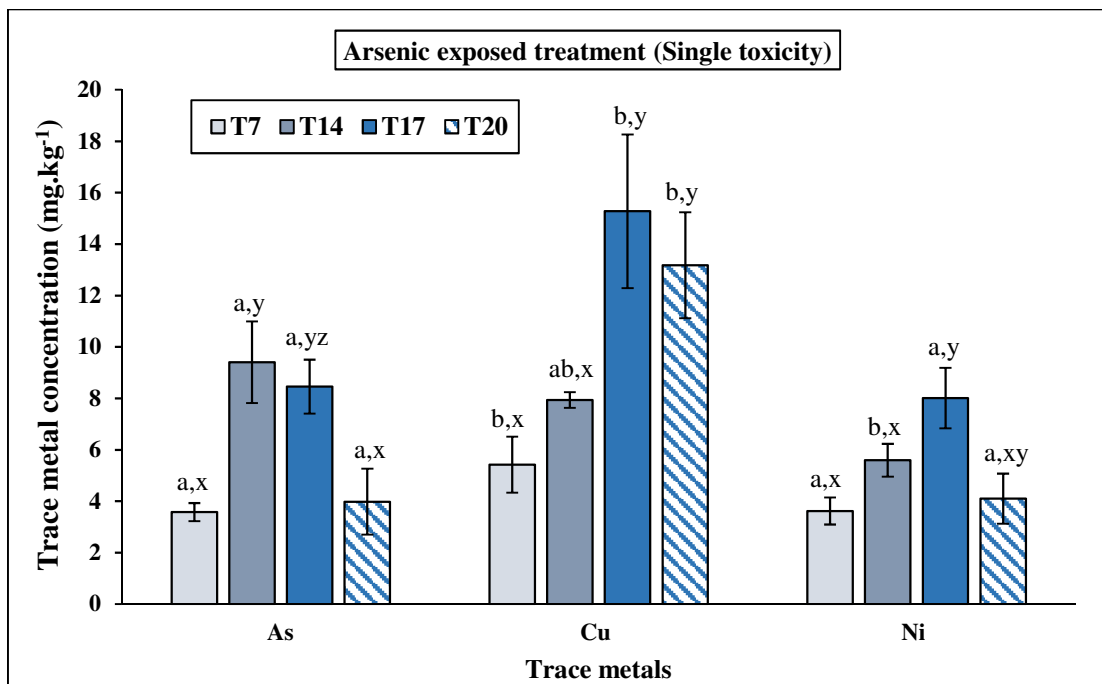
334  
 335 **Fig. 3.** De-epoxidation (DR) state for each treatment at all phases of growth in *D. lutheri*.  
 336 Letters a, b, c shows significant differences between incubation times; x, y, z shows significant  
 337 differences between treatments, at  $p < 0.05$ . The error bar shows the standard deviation between  
 338 the three replicates in each treatment.

### 339 3.3 Bioaccumulation of trace metals

#### 340 3.3.1 Single exposure – Arsenic



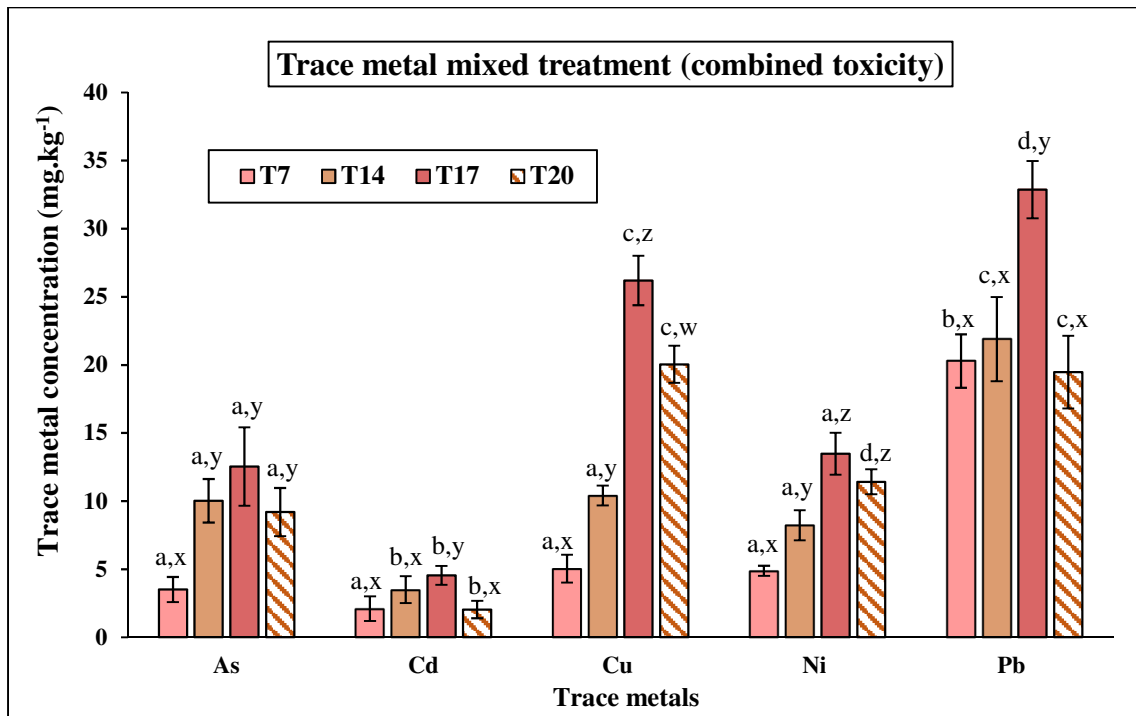
341 In the single exposure of As V, the essential trace metals (Cu and Ni) were also  
 342 detected in *D. lutheri* at the specific intervals (T7, T14, T17 and T20) as shown  
 343 in Fig. 4. The bioaccumulation of total As measured at each interval showed  
 344 significantly higher value at T14 when compared to the exponential phase (T7)  
 345 and lysis phase (T20). Moreover, at T20 we observed a significant decrease in  
 346 the total As concentration bio-accumulated in *D. lutheri*. In this treatment no  
 347 additional Cu and/ or Ni was added in the exposure beaker but was detected in  
 348 the trace metal analysis as they play essential roles in algal metabolism. The  
 349 highest bioaccumulation of Cu and Ni was found at T17 increasing significantly  
 350 from T7. However, at T20 both Cu and Ni did not show any significant decrease  
 351 of trace metal concentration compared to T17.



352 **Fig. 4.** Bio-accumulation of trace metals from the single toxicity exposed treatment of arsenic.  
 353 Letters a, b shows significant differences between trace metals; x, y, z shows significant  
 354 differences between incubation times within a single trace metal, at  $p < 0.05$ . The error bar  
 355 shows the standard deviation between the three replicates in each treatment.  
 356

357 **3.3.2 Combined exposure of trace metals (As + Cd+ Cu+ Ni+ Pb)**

358 Fig. 5 shows the bioaccumulation of all the trace metal combined in one  
359 exposure at specific incubation times in the entire growth curve of *D. lutheri*.  
360 The highest bioaccumulation was by Pb at T17 followed by Cu > Ni > As > Cd.  
361 All the exposed trace metals showed highest bioaccumulation at T17 correlating  
362 positively with the increase in cells concentration in the linear phase. However,  
363 at the lysis phase (T20) each combined metal showed a decrease in trace metal  
364 concentrations, out of which Cu and Pb showed a significant decrease. This  
365 finding was in contrast to the single exposure of As V treatment (Fig. 4) in  
366 which Cu did not show any significant reduction at T20 given the fact that this  
367 metal has essential metabolic functions. Inversely, when As V was exposed  
368 through a single exposure (Fig. 4) it showed a significant decrease in  
369 bioaccumulation at T20 but in combination with other trace metals no such  
370 reduction was noted (Fig. 5). The total As concentration in the single exposure  
371 was highest at T14 (Fig. 4), but when combined with other trace metals, the  
372 highest bioaccumulation was reached at T17 also denoting higher values of total  
373 As when in mixture with other trace metals.



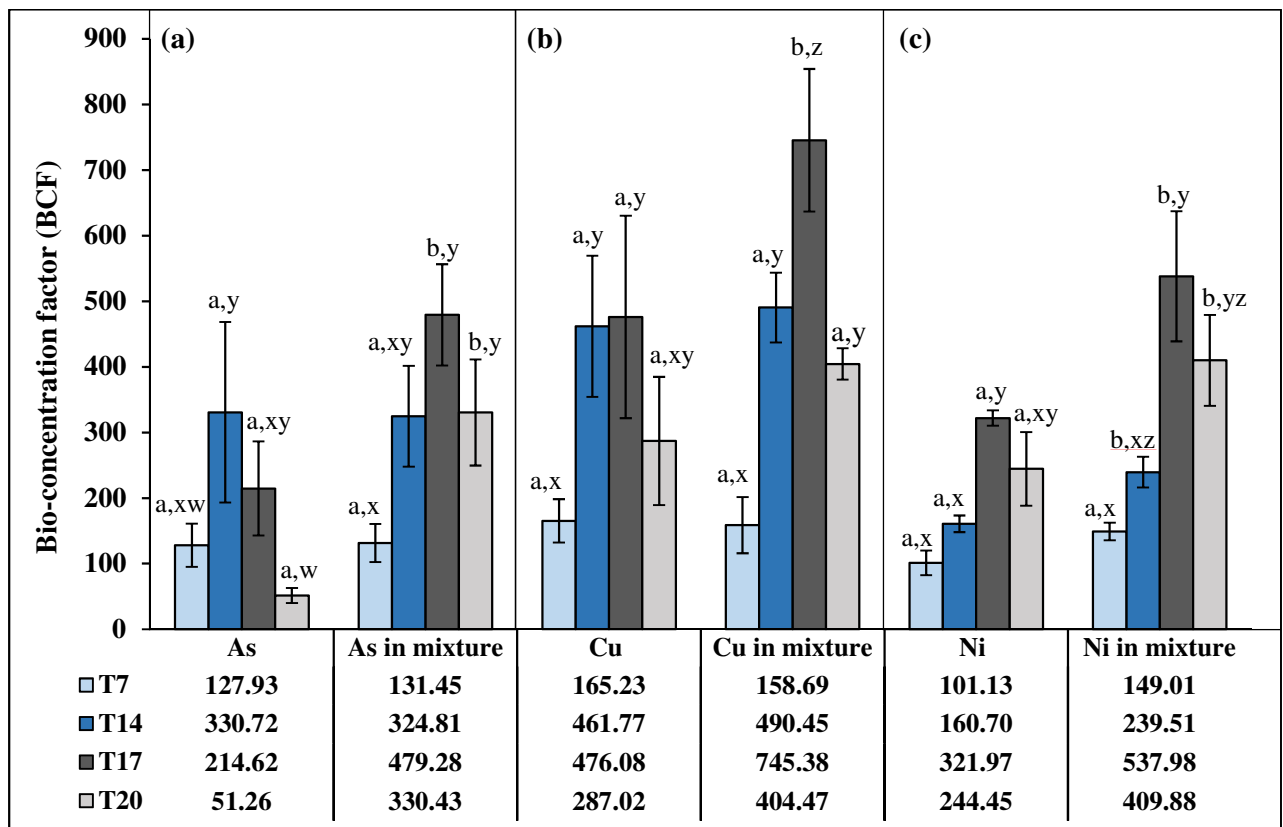
374

375 **Fig. 5.** Bio-accumulation of trace metals from mixture treatment. Letters a, b, c, d shows  
 376 significant differences between trace metals; w, x, y, z shows significant differences between  
 377 incubation times within a single trace metal, at  $p < 0.05$ . The error bar shows the standard  
 378 deviation between the three replicates in each treatment.

### 379 3.3.3 Bio-concentration (BCF) of trace metals

380 In the single exposure to As V treatment, only Cu and Ni were detected (without  
 381 any addition) marking their essential role in algal metabolism. However, no Cd  
 382 and Pb was detected in this treatment. Hence, Fig. 6a shows a comparison  
 383 between the BCF of As exposures (single and combined with other trace  
 384 metals), Fig. 6b, shows Cu exposure (naturally present and combined with other  
 385 trace metals) and Fig. 6c, shows Ni exposure (naturally present and combined  
 386 with other trace metals). In As V single exposure, the highest BCF was reached  
 387 at T14, increasing significantly from T7 and further reducing significantly at  
 388 T20. On the other hand, when As V was combined with other trace metals, the  
 389 highest BCF was noted at T17, significantly increasing from T7 but showing no

390 significant reduction of trace metal concentration at T20. When both exposures  
 391 (single and combined) were compared, a significant difference in BCF of total  
 392 As at T17 and T20 was noted. The Cu detected in the single exposure of As V  
 393 showed significantly higher BCF at T14 and T17 than T7 thereafter reducing at  
 394 T20 although not significantly. Further, the comparison between the naturally  
 395 detected Cu and the Cu added with other trace metals showed, a significantly  
 396 higher BCF of Cu at T17 in mixture along with a significant reduction at T20.  
 397 On the other hand, Ni when detected in the single exposure of As V showed  
 398 highest BCF at T17 increasing significantly from T7 and T14 but lacking any  
 399 reduction of trace metal concentration at T20. The comparison between Ni  
 400 naturally detected and when in mixture with other trace metals showed  
 401 significantly higher BCF in mixed trace metal exposure at T14, T17 and at T20  
 402 without any significant decrease in both the cases.



403

404 **Fig. 6 (a).** Comparison of the bio-concentration factor (BCF) of total As in single toxicity and  
405 in mixture, where, w, x, y, z denotes the significant differences between the incubation time in  
406 a single treatment and letters a, b denotes the significant differences between total As single  
407 and mixture treatment on a single day of exposure.

408 **(b and c).** Comparison of the bio-concentration factor of Cu and Ni detected in the single  
409 exposure of As V (due to their essential roles) and in trace metal mixture, where x, y, z denotes  
410 the significant differences between the incubation times in a single treatment and letters a, b  
411 denotes the significant differences between Cu single and mixture treatment and Ni single and  
412 mixture treatment on each day of exposure at  $p < 0.05$ . The error bar shows the standard  
413 deviation between the three replicates in each treatment.

## 414 **4. Discussion**

### 415 **4.1 Cell growth of *Diacronema lutheri***

416 Previous studies have reported that exposure to trace metals along with other  
417 contaminants such as organic pollutants and/or microplastics affected the growth  
418 rate of many algal species, in general (Emelogu et al., 2014; Chia et al., 2015;  
419 Bellingeri et al., 2019, Mamun et al., 2019 a, b; Navarrete et al., 2019; Wilson et  
420 al., 2019; Huang et al., 2021). However, these studies had varied exposure strategies  
421 ranging from combined toxicity of trace metals, microplastics, and organic  
422 pollutants, etc. coupled with several nutrient limiting exposures (Chia et al., 2014,  
423 2015; Navarrete et al, 2019; Huang et al., 2021). A recent study on single exposure  
424 of As and combined exposure of As and Cu on three algal species showed a lower  
425 growth rate of the algal cells in the combined exposure after 10 days (Huang et al.,  
426 2021). In this study, a similar observation was noted, where the algal cell density in  
427 the combined exposure of the essential (Cu, Ni) and non-essential (As, Cd, Pb) trace  
428 metals varied significantly after 7 days of exposure from the single toxicity of As

429 V and control. Furthermore, similar to our observation, other studies also reported  
430 the significant inhibition in the growth rate after the exponential stage and described  
431 it as a combined effect of the toxicity from trace metal and the nutrient limiting  
432 condition mainly at the end of each algal growth curve (Serra et al., 2010; Monteiro  
433 et al. 2011; Chia et al., 2013, 2014, 2015). Huang et al., 2021 also observed a time-  
434 dependent effect on the declining growth rate of the algal species irrespective of the  
435 concentration of As used in their study. In comparison between single toxicity and  
436 combined toxicity, previous studies on varied organisms have shown a decrease in  
437 the growth rate when exposed to a combination of trace metals, microplastics, and  
438 nano-plastics (Yan et al., 2015; Tang et al., 2015; Kim et al., 2017; Barboza et al.,  
439 2018; Huang et al., 2021). Synergistic toxicity of Cd, Cu, Pb, and Zn significantly  
440 affected the enzyme activities and chlorophyll *a* content in an estuarine plant  
441 (*Avicennia marina*) in comparison to their exposures, that posed no significant  
442 effects (Mac Farlane and Burchett, 2002; Yan et al., 2015). In addition to our study,  
443 a combination of trace metals like As, Cd, Cu has been reported to damage the  
444 membranes of cells disrupting the permeability and its capacity to uptake nutrients  
445 because of the trace metals binding to the sulfhydryl groups and preventing several  
446 enzymatic activities that are crucial for the metabolism, growth, physiological  
447 conditions and morphological characteristics (Chattopadhyay et al., 2002; Zheng et  
448 al., 2008; Hu and Zhou, 2010; Chia et al., 2014, Huang et al., 2021).

449

#### 450 **4.2 Pigment concentrations of *D. lutheri***

451 *Diacronema lutheri* is a haptophyte (pavlovophycean) consisting of flagellum at the  
452 hind-site with two flagella of unequal length. They contain pigments like  
453 fucoxanthin, carotenoids, type A pigments that includes chl *a*, *c1*, *c2*,  
454 chlorophyllides, pheophytine *a*, diadinoxanthin, diatoxanthin and  $\beta$ -carotene (Van

455 Lenning et al., 2003; Bendif et al., 2011; Kim et al., 2021). The pigment chl *a* has  
456 been an indicator to the trace metal stress in previous studies, which reported a  
457 decrease in its content when algal cells are often impaired by the trace metals, and  
458 as a result affecting its ability to assimilate CO<sub>2</sub>, hindering the process of  
459 photosynthesis and lowering the biomass in acute and chronic exposures (Qian et  
460 al., 2009; Chia et al., 2013; Liu et al., 2014; Li et al., 2018). Additionally, in a recent  
461 study, exposure to both the forms of arsenic (As III and As V) could induce a  
462 significant decrease in the chl *a* and *b* content in an aquatic plant, *Vallisneria natans*  
463 (Li et al., 2018). Similar to the previous findings, our study has shown a significant  
464 decrease in chl *a* content of *D. lutheri* in the trace metal exposed treatments when  
465 compared to control conditions. Studies have also reported similar observations on  
466 varied trace metals when exposed to large terrestrial flora, where soluble forms of  
467 trace metals having a high affinity towards the sulfhydryl group bind and affects  
468 the pigment contents and enzyme activities retarding the growth of the organism  
469 (Tomar et al., 2000; Ali et al., 2002; Li et al., 2018). However, when related to the  
470 number of cells, algal species like *Chlorella vulgaris*, *Scenedesmus obliquus*, some  
471 cyanobacterial and periphyton communities have shown higher contents of chl *a*  
472 per cell, along with high carbohydrate and lipid biomolecules in response to trace  
473 metal stress and limitation of nutrient (Soldo and Behra, 2000; Omar 2002, Okmen  
474 et al., 2011; Chia et al., 2015, Mamun et al., 2019a). Although a significant decrease  
475 in chl *a* content from control to trace metal exposed treatments was observed in our  
476 study, a comparison between the single exposure and combined exposure to trace  
477 metal showed no significant differences. On the other hand, the comparison of cell  
478 density in the single and combined trace metal exposures showed significant  
479 differences in the exponential phase and at the stationary phase. Such observations

480 where the cell growth was retarded and yet a higher content of chl *a* per cell was  
481 observed in toxic exposures have been illustrated in previous studies as a probable  
482 mechanism of increasing the chl *a* content followed by the synthesis of sugars,  
483 carbohydrates, and other biomolecules to compensate the declined growth of the  
484 algal cells (Okmen et al., 2011; Chia et al., 2013, 2015, Tang et al., 2015). Chia et  
485 al. (2013; 2015) further explained this strategy as a stress response observed in the  
486 algae *Chlorella vulgaris* when exposed to Cd in several nutrient limiting conditions.  
487 Our study reports similar observation in the case of combined toxicity of trace  
488 metals that included Cu and Ni (essential trace metals) in sub-lethal concentrations  
489 which presumably induced such stress responses as reported by previous studies  
490 (Davaranpanah et al., 2015; Wang et al., 2015; Ashraf et al., 2016; Hang et al., 2016;  
491 Huang et al., 2021). However, conclusively the synergistic effect of As, Cd, and Pb  
492 (non-essential elements) led to a reduced content of chl *a* when compared to  
493 individual exposure of As V, however not significantly. Chlorophyll *a* was however  
494 the most abundant pigment found in *D. lutheri* followed by fucoxanthin that ranged  
495 from 1950 ng.mL<sup>-1</sup> to 2100 ng.mL<sup>-1</sup> in control cells, 600 ng.mL<sup>-1</sup> to 400 ng.mL<sup>-1</sup>  
496 in single exposure of As and the lowest concentration found in the combined trace  
497 metal treatment ranging from 350 ng.mL<sup>-1</sup> to 200 ng.mL<sup>-1</sup>; all measured at the  
498 exponential phase. In addition, considerable amount of pheophytine *a* was observed  
499 with control showing the highest value of 550 ng.mL<sup>-1</sup> on an average, followed by  
500 single exposure of As (258 ng.mL<sup>-1</sup> on an average), and mixture of trace metals  
501 (137 ng.mL<sup>-1</sup> on an average) all measured at the exponential phase. The de-  
502 epoxidation ratio (DR), in previous studies, have been reported as a good indicator  
503 of the photo-oxidative stress response when high-light or any form of toxic  
504 substances are encountered by the algal cells (Eskling et al., 1997; Bertrand et al.,



2001; Ait Ali et al., 2008; Yan et al., 2017; Cabrita et al., 2018; Zsiros et al., 2020). Zsiros et al. (2020), showed that *Chlorella variabilis*, exhibited a higher DR value when the algal cells were exposed to the trace metal Chromium (Cr). Our study showed a similar finding, where significantly higher DR was observed in case of trace metal exposed treatments, consistently at all phases excepting the exponential phase (first 7 days of exposure) where algal cells perhaps could combat the toxic effects of trace metals with sufficient nutrient availability and increasing enzymatic activity induced by the toxic exposure (Okmen et al., 2011; Chia et al., 2013, 2015; Das et al., 2018, Mamun et al., 2019a). Although studies on the comparison of single and combined exposure of trace metals and its effect on the DR values are rare, in general, trace metal stress on organisms have shown to regulate the DR, through non-photochemical quenching and dissipation of excess energy in to protect the cell membranes from reactive oxygen species, indicating varied oxidative stress responses induced by sublethal concentrations of environmental toxicants, which was also observed in the present study (Demmig-Adams et al., 2014; Yan et al., 2017; Bethmann et al., 2019; Zsiros et al., 2020).

521

### 522 **4.3 Bioaccumulation of trace metals in *D. lutheri***

523 Trace metal accumulation by algae has been reported to cause considerable  
524 damages in cell structure, growth rate, pigment contents, enzyme activities, and  
525 several other metabolic functions in previous studies (Zhang et al., 2019; Mamun  
526 et al., 2019 a, b; Hussain et al., 2021; Huang et al., 2021). Studies considered the  
527 trace metal accumulated in the biomass/algal mass and also the residual trace metal  
528 concentration in the culture medium to emphasize the importance of studying bio-  
529 concentration factors (Baumann et al., 2009; Huang et al., 2021). Our study has  
530 collectively considered both the parameters to highlight the harmful effects caused

531 by the toxic exposures of As V and combined trace metals (As + Cd + Cu + Ni+  
532 Pb). In this study the bioaccumulation of all the trace metals in the combined metal  
533 exposed treatment increased significantly in the linear phase (Day 17), and the order  
534 of the bioaccumulation was  $Pb > Cu > Ni > As > Cd$ . This observation was in  
535 accordance with some previous studies that also observed Cu and Pb have the  
536 highest and Cd to have a lower concentration of bioaccumulation in varied species  
537 of macroalgae like *Ulva intestinalis*, *Cladophora rupestris*, *Chondrus crispus*, and  
538 microalgae like *Amphora coffaeiformis*, *Dunaliella salina*, *Euglena* sp. and many  
539 others (Yu and Wang, 2004; Baumann et al., 2009, Chiellini et al., 2020; Elleuch et  
540 al., 2021). In a recent investigation on phytoplankton blooms, the authors reported  
541 that Cu and Pb has a greater potential to be bioavailable from their particulate forms  
542 and thereby can get rapidly bio-accumulated by phytoplankton posing higher risks  
543 for bio-magnification whereas trace metals like Cd, Chromium (Cr), Cobalt (Co)  
544 has shown lower particle reactivity (Cabrita et al., 2020; IAEA report, 2004).  
545 Mamun et al. (2019a), in a study based on the toxicity of As V, reported a rapid  
546 accumulation of As by the macroalgae *Sargassum horneri* that resulted in  
547 significant differences of residual total As in the medium throughout the exposure  
548 period of 7 days. This observation was similar to our study on microalgae, as we  
549 observed the total As accumulation to significantly increase from T7 to T14 in the  
550 single exposure thereafter showing a trend of significant reduction at T20 (Mamun  
551 et al., 2019 a, b; Huang et al., 2021; Hussain et al., 2021). However, in the combined  
552 exposure of As V and other trace metals, the total As accumulation was highest at  
553 T17 increasing significantly from T7 with an increasing period of exposure but  
554 lacking any significant decline in the trace metal concentration at the lysis phase  
555 (T20). Although literature with such comparison is deficient, a recent study on the

556 single toxicity of As V and combined As V and Cu toxicity reported a similar  
557 observation (Huang et al., 2021). In addition to some previous studies, they reported  
558 the sharp increase in the total arsenic content of the algal species and towards the  
559 lysis phase a lower concentration in both the treatments (single and combined) but  
560 reporting a higher absorption rate and bioaccumulation of As from the combined  
561 exposure (Zheng et al., 2008; Dong et al., 2014; Wang et al., 2015; Hussain et al.,  
562 2021; Huang et al., 2021). In our study, we found a similar trend of higher  
563 bioaccumulation of total As in the case of combined exposure but, the absorption  
564 rate was slower in contrast to the above study, perhaps because of the comparative  
565 use of lower concentration of all the trace metal depicting an environmentally  
566 realistic condition. Moreover, the speciation of As throughout the exposure period  
567 in Huang et al., (2021), showed that As V can have enhanced detoxification when  
568 Cu was added and can be bio-transformed to a less toxic methylated state of As.  
569 However, in the current study, the combined exposure showed no significant  
570 reduction of trace metal at the lysis phase, perhaps because total As concentration  
571 was taken into account and the analysis for the speciation of the As species was  
572 lacking, which can be considered in the future. The present study followed the  
573 growth phases in batch mode comparing single toxicity of As V and combined  
574 toxicity in environmentally realistic conditions to comprehend the mechanisms  
575 developed by microalgae and possibly denote certain stress indicators of trace metal  
576 toxicity when exposed through varied pathways. Along with the As speciation, a  
577 detailed comprehension of how As can physiologically affect the photosynthetic  
578 machinery of *D. lutheri* can be considered in the future.

579

## 580 **5. Conclusion**

581 . In this study, we report that the effects caused by exposure of As V to *D. lutheri*  
582 can lead to varied responses when microalgae are exposed to single toxicity or when  
583 combined with other trace metals in a mixture (both essential and non-essential).  
584 The cell concentration in the combined trace metal exposure was significantly lower  
585 than in single exposure of As V after the stationary phase. The bioaccumulation and  
586 its bio-concentration factor of total As increased in *D. lutheri* when exposed along  
587 with a mixture of other trace metals. Our study also highlighted the loss of chl *a*  
588 pigment and the significantly higher value of DR as a stress response when As V is  
589 mixed with other trace metals. Hence, in aquatic areas where the trace metals are  
590 prevalent, the consequences of the contamination can be adverse depending on the  
591 exposure to multiple toxicants along with As V. Such studies can help in bio-  
592 remediation given the higher bio-concentration factor in the mixture for As, and  
593 also can reveal the risks of bio-magnification into higher trophic organisms. Eco-  
594 toxicological assays using *D. lutheri* are extremely rare and such observations can  
595 shed light on the resistance and/or vulnerability of this economically beneficial  
596 microalga species to enhance its production for safe and healthy therapeutic  
597 products. Detailed speciation of As in single toxicity and a mixture when exposed  
598 to *D. lutheri* or other economical algal species awaits, which can further improve  
599 the production and understanding of the varied responses for human benefits.

600

601

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## 617 **References-**

618

619 Ahmed, F., Zhou, W., Schenk, P. M., 2015. *Pavlova lutheri* is a high-level producer of  
620 phytosterols. *Algal Research*, 10, 210-217.  
621 <https://doi.org/10.1016/j.algal.2015.05.013>

622

623 Ait Ali, N. A., Juneau, P., Didur, O., Perreault, F., Popovic, R., 2008. Effect of dichromate on  
624 photosystem II activity in xanthophyll-deficient mutants of *Chlamydomonas reinhardtii*.  
625 *Photosynth. Res.* 95, 45–53.  
626 <https://doi.org/10.1007/s11120-007-9227-4>

627

628 Ali, M.B., Chun, H.S., Kim, B.K., Lee, C.B., 2002. Cadmium-induced changes in antioxidant  
629 enzyme activities in rice (*Oryza sativa* L. cv. Dongjin). *J. Plant Biol.* 45 (3), 134–140.  
630 <https://doi.org/10.1007/BF03030305>

631

632 Anandkumar, A., Nagarajan, R., Prabakaran, K., Bing, C.H., Rajaram, R., Li, J., Du, D., 2019.  
633 Bioaccumulation of trace metals in the coastal Borneo (Malaysia) and health risk assessment.  
634 *Mar. Pollut. Bull.* 145, 56–66.  
635 [https://doi.org/10.1016/j.marpolbul.2019.05.002\\*](https://doi.org/10.1016/j.marpolbul.2019.05.002*)

636

637 Arias, A.H., Souissi, A., Glippa, O., Roussin, M., Dumoulin, D., Net, S., Ouddane, B., Souissi,  
638 S., 2017. Removal and Biodegradation of Phenanthrene, Fluoranthene and Pyrene by the  
639 Marine Algae *Rhodomonas baltica* Enriched from North Atlantic Coasts. *Bull Environ*  
640 *Contam Toxicol* 98, 392–399.  
641 <https://doi.org/10.1007/s00128-016-1967-4>

642

643 Arsalane, W., Rousseau, B., Duval, J.C., 1994. Influence of the pool size of the xanthophyll  
644 cycle on the effects of light stress in a diatom-competition between photoprotection and  
645 photoinhibition. *Photochem Photobiol*, 60, 237-243.  
646 <https://doi.org/10.1111/j.1751-1097.1994.tb05097.x>  
647

648 Ashraf, M.Y., Roohi, M., Lqbal, Z., Ashraf, M., Öztürk, M., Salih, G., 2016. Cadmium (Cd)  
649 and lead (Pb) induced changes in growth, some biochemical attributes, and mineral;  
650 accumulation in two cultivars of mung bean [*Vigna radiata* (L.) Wilczek]. *Commun. Soil Sci.*  
651 *Plant Anal.* 47 (4), 405–413.  
652 10.1080/00103624.2015.1118117  
653

654 Barboza, L.G.A., Vieira, L.R., Guilhermino, L., 2018. Single and combined effects of  
655 microplastics and mercury on juveniles of the European seabass (*Dicentrarchus labrax*):  
656 changes in behavioural responses and reduction of swimming velocity and resistance time.  
657 *Environ. Pollut.* 236, 1014–1019.  
658 <https://doi.org/10.1016/j.envpol.2017.12.082>  
659

660 Baumann, H.A., Morrison, L., Stengel, D.B., 2009. Metal accumulation and toxicity measured  
661 by PAM—chlorophyll fluorescence in seven species of marine macroalgae. *Ecotoxicol.*  
662 *Environ. Saf.* 72, 1063–1075.  
663 <https://doi.org/10.1016/j.ecoenv.2008.10.010>.  
664

665 Bellingeri, A., Bergami, E., Grassi, G., Faleri, C., Redondo-Hasselerharm, P., Koelmans, A.  
666 A., Corsi, I., 2019. Combined effects of nanoplastics and copper on the freshwater alga  
667 *Raphidocelis subcapitata*. *Aquatic Toxicology*, 210, 179-187.  
668 <https://doi.org/10.1016/j.aquatox.2019.02.022>.  
669

670 Bendif, E.M., Probert, I., Hervé, A., Billard, C., Goux, D., Lelong, C., Cadoret, J.P, Véron, B.,  
671 2011. Integrative taxonomy of the Pavlovophyceae (Haptophyta): a reassessment. *Protist.*  
672 162,738–761, Crossref. PubMed.  
673 <http://dx.doi.org/10.1016%2Fj.protis.2011.11.006>  
674

675 Bernaerts, T.M.M., Gheysen, L., Kyomugasho, C., Jamsazzadeh Kermani, Z., Vandionant S.,  
676 Foubert, I., Hendrickx, M.E., Van Loey, A.M., 2018. Comparison of microalgal biomasses as  
677 functional food ingredients: Focus on the composition of cell wall related polysaccharides.  
678 *Algal Research*, 32, 150-161,  
679 <https://doi.org/10.1016/j.algal.2018.03.017>.  
680

681 Bertrand, M., Schoefs, B., Siffel P., Rohacek, K., Molnar, I., 2001. Cadmium inhibits  
682 epoxidation of diatoxanthin to diadinoxanthin in the xanthophyll cycle of the marine diatom  
683 *Phaeodactylum tricorutum* . *FEBS Letters*, 508 (1), 153-156.  
684 [https://doi.org/10.1016/S0014-5793\(01\)03050-2](https://doi.org/10.1016/S0014-5793(01)03050-2)  
685

686 Bethmann, S., Melzer, M., Schwarz, N. and Jahns, P., 2019. The zeaxanthin epoxidase is  
687 degraded along with the D1 protein during photoinhibition of photosystem II. *Plant Direct*, 3,  
688 <https://doi.org/10.1002/pld3.185>.  
689

690 Brunet, C., Chandrasekaran, R., Barra, L., Giovagnetti, V., Corato, F., Ruban, A. V., 2014.  
691 Spectral Radiation Dependent Photoprotective Mechanism in the Diatom *Pseudo-nitzschia*  
692 *multistriata*. *PLOS ONE* 9(1), e87015.

693 <https://doi.org/10.1371/journal.pone.0087015>  
694  
695 Cabrita, M.T., Duarte, B., Gameiro, C., Godinho, R.M., Caçador, I., 2018. Photochemical  
696 features and trace element substituted chlorophylls as early detection biomarkers of metal  
697 exposure in the model diatom *Phaeodactylum tricornutum*. Ecol. Indicat. 95, 1038-1052.  
698 <https://doi.org/10.1016/j.ecolind.2017.07.057>  
699  
700 Cabrita, M.T., Brito, P., Caçador, I., Duarte, B., 2020. Impacts of phytoplankton blooms on  
701 trace metal recycling and bioavailability during dredging events in the Sado estuary (Portugal).  
702 Marine Environmental Research, 153, 104837,  
703 <https://doi.org/10.1016/j.marenvres.2019.104837>.  
704  
705 Carmona, M.A., Jiménez, C., Jiménez-Sanchidrián, C., Peña, F., Ruiz, J.R., 2010. Isolation of  
706 sterols from sunflower oil deodorizer distillate, J. Food Eng. 101 (2), 210–213.  
707 <https://doi.org/10.1016/j.jfoodeng.2010.07.004>  
708  
709 Chattopadhyay, S., Bhaumik, S., Purkayastha, M., Basu, S., Chaudhuri, A.N., Gupta, S.D.,  
710 2002. Apoptosis and necrosis in developing brain cells due to arsenic toxicity and protection  
711 with antioxidants. Toxicol. Lett. 136, 65–76.  
712 [https://doi.org/10.1016/S0378-4274\(02\)00282-5](https://doi.org/10.1016/S0378-4274(02)00282-5)  
713  
714 Cheloni, G., Slaveykova, V.I., 2018. Combined Effects of Trace Metals and Light on  
715 Photosynthetic Microorganisms in Aquatic Environment. Environments , 5, 81.  
716 <https://doi.org/10.3390/environments5070081>  
717  
718  
719 Chen, L., Zhou, S., Shi, Y., Wang, C., Li, B., Li, Y., Wu, S., 2018. Heavy metals in food crops,  
720 soil, and water in the Lihe River watershed of the Taihu Region and their potential health risks  
721 when ingested. Sci. Total Environ. 615, 141-149.  
722 <https://doi.org/10.1016/j.scitotenv.2017.09.230>.  
723  
724 Chia, A.M., Lombardi, A.T., Melão, M.G.G., Parrish, C.C., 2013. Effects of cadmium and  
725 nitrogen on lipid composition of *Chlorella vulgaris* (Trebouxiophyceae).  
726 Eur. J. Phycol. 48, 1–11.  
727 <https://doi.org/10.1080/09670262.2012.750687>  
728  
729 Chia, A.M., Chimdirim, P.K., Japhet, W.S., 2014. Lead induced antioxidant response and  
730 phenotypic plasticity of *Scenedesmus quadricauda* (Turp.) de Brebisson under different  
731 nitrogen concentrations. J. Appl. Phycol., 27, 293–302  
732 <http://dx.doi.org/10.1007/s10811-014-0312-8>.  
733  
734 Chia, M.A., Lombardi, A.T., da Graça Gama Melão, M., Parrish, C.C., 2015. Combined  
735 nitrogen limitation and cadmium stress stimulate total carbohydrates, lipids, protein and amino  
736 acid accumulation in *Chlorella vulgaris* (Trebouxiophyceae). Aquatic Toxicology, 160, 87-95.  
737 <https://doi.org/10.1016/j.aquatox.2015.01.002>.  
738  
739 Chiellini, C., Guglielminetti, L., Pistelli, L., Ciurli, A., 2020. Screening of trace metal elements  
740 for pollution tolerance of freshwater and marine microalgal strains: Overview and perspectives.  
741 Algal Research, 45, 101751,  
742 <https://doi.org/10.1016/j.algal.2019.101751>.

743  
744 Costa, G.B., Ramlov, F., Koerich, G., Navarro, B.B., Cabral, D., Rodrigues, E.R.O., Ramos,  
745 B., Fadigas, S.D., Rörig, L.R., Maraschin, M., Horta, P.A., 2019. The effects of mining tailings  
746 in the physiology of benthic algae: Understanding the relation between mud's inductive  
747 acidification and the heavy metal's toxicity. *Environmental and Experimental Botany*, 167,  
748 103818.  
749 <https://doi.org/10.1016/j.envexpbot.2019.103818>.

750 Das, S., Tseng, L.C., Chou, C., Wang, L., Souissi, S., Hwang, J.S., 2018. Effects of cadmium  
751 exposure on antioxidant enzymes and histological changes in the mud shrimp *Austinoergia*  
752 *edulis* (Crustacea: Decapoda). *Environ. Sci. Pollut. Res. Int.* 26 (8), 7752-7762.  
753 <https://doi.org/10.1007/s11356-018-04113-x>.

754  
755 Das, S., Ouddane, B., Hwang, J.S., Souissi, S., 2020a. Intergenerational effects of resuspended  
756 sediment and trace metal mixtures on life cycle traits of a pelagic copepod. *Environ. Pollut.*  
757 267, 115460.  
758 <https://doi.org/10.1016/j.envpol.2020.115460>

759  
760 Das, S., Aria A.H., Cheng, J.O., Souissi, S., Hwang, J.S., Ko, F.C., 2020b. Occurrence and  
761 distribution of anthropogenic persistent organic pollutants in coastal sediments and mud  
762 shrimps from the wetland of central Taiwan, *PloS One*, 15, 1–17.  
763 <https://doi.org/10.1371/journal.pone.0227367>

764  
765 Davarpanah, E., Guilhermino, L., 2015. Single and combined effects of microplastics and  
766 copper on the population growth of the marine microalgae *Tetraselmis chuii*. *Estuar., Coast.*  
767 *Shelf Sci.* 167, 269–275.  
768 <https://doi.org/10.1016/j.ecss.2015.07.023>

769  
770 Dayras, P., Bialais, C., Ouddane, B., Lee, J.-S., Souissi, S., 2020. Effects of different routes of  
771 exposure to metals on bioaccumulation and population growth of the cyclopoid copepod  
772 *Paracyclops nana*. *Chemosphere* 248, 125926.  
773 <https://doi.org/10.1016/j.chemosphere.2020.125926>.

774  
775 Demmig-Adams, B., Stewart, J.J., Burch, T.A., Adams, W.W., 2014. Insights from Placing  
776 Photosynthetic Light Harvesting into Context. *The Journal of Physical Chemistry*  
777 *Letters* 5 (16), 2880-2889.  
778 <https://doi.org/10.1021/jz5010768>

779  
780 Díaz, O., Tapia, Y., Muñoz, O., Montoro, R., Velez, D., Almela, C., 2012. Total and  
781 inorganic arsenic concentrations in different species of economically important algae harvested  
782 from coastal zones of Chile. *Food Chem Toxicol.* 50 (3–4), 744–749.  
783 <https://doi.org/10.1016/j.fct.2011.11.024>

784  
785 Dong, X.X., Yan, C.Y., Wang, D.S., Zhao, P.H., Zhou, S.L., Wang, M., 2014. Removal and  
786 bioconcentration of Cu, Pb and Cd by combined aquatic plant purification system. *Environ.*  
787 *Eng.* 8, 1447–1453.  
<https://doi.org/10.1016/j.ecoenv.2021.112024>



788 Elleuch, J., Hmani, R., Drira, M., Michaud, P., Fendri, I., Abdelkafi, S., 2021. Potential of three  
789 local marine microalgae from Tunisian coasts for cadmium, lead and chromium removals. *Sci.*  
790 *Total Environ*, 799, 149464.  
791 <https://doi.org/10.1016/j.scitotenv.2021.149464>.  
792

793 Emelogu, E.S., Seiler, T.B., Pollard, P., Robinson, C.D., Webster, L., et al. 2014. Evaluations  
794 of combined zebrafish (*Danio rerio*) embryo and marine phytoplankton (*Diacronema lutheri*)  
795 toxicity of dissolved organic contaminants in the Ythan catchment, Scotland, UK. *Environ Sci*  
796 *Pollut Res* 21, 5537–5546.  
797 <https://doi.org/10.1007/s11356-013-2488>  
798

799 Emelogu E.S., Pollard, P., Dymond, P., Robinson, C.D., Webster, L., McKenzie, C., Dobson,  
800 J., Bresnan, E., Moffat, C. F., 2013. Occurrence and potential combined toxicity of dissolved  
801 organic contaminants in the Forth estuary and Firth of Forth, Scotland assessed using passive  
802 samplers and an algal toxicity test. *Sci Total Environ* 461–462, 230– 239.  
803 <https://doi.org/10.1016/j.scitotenv.2013.05.011>  
804

805 Eskling, M., Arvidsson, P.O., Akerlund, H.E., 1997. The xanthophyll cycle, its regulation and  
806 components. *Physiol. Plantarum* 100, 806–816.  
807 <https://doi.org/10.1111/j.1399-3054.1997.tb00007.x>  
808

809 Facey, J.A., Apte, S.C., Mitrovic, S.M., 2019. A Review of the Effect of Trace Metals on  
810 Freshwater Cyanobacterial Growth and Toxin Production. *Toxins*, 11, 643.  
811 <https://doi.org/10.3390/toxins11110643>  
812

813 Gao, F., Li, C., Yang, Z.H., Zeng, G.M., Mu, J., Liu, M., Cui, W., 2016. Removal of  
814 nutrients, organic matter, and metal from domestic secondary effluent through microalgae  
815 cultivation in a membrane photobioreactor. *J Chem Technol Biotechnol*, 91, 2713–2719.  
816 <https://doi.org/10.1002/jctb.4879>  
817

818 Gnouma, A., Sadovskaya, I., Souissi, A., Sebai, K., Medhioub, A., Grard, T. and Souissi, S.,  
819 2017. Changes in fatty acids profile, monosaccharide profile and protein content during batch  
820 growth of *Isochrysis galbana* (Tiso). *Aquac Res*, 48, 4982-4990.  
821 <https://doi.org/10.1111/are.13316>  
822

823 Guillard, R.R., Sieracki, M.S., 2005. Counting cells in cultures with the light microscope. In:  
824 Andersen RA (ed) *Algal culturing techniques*, 1st edn. Academic Press, 239–252.  
825

826 Hak, K., Ritchie, R., Dumme, V., 2020. Bioaccumulation and physiological responses of the  
827 Cocktail, *Ceratophyllum demersum* exposed to copper, zinc and in combination. *Ecotoxicol.*  
828 *Environ. Saf.* 189, 110049.  
829 <https://doi.org/10.1016/j.ecoenv.2019.110049>  
830

831 Hang, T.T., Ngo, D.V., Matsukawa, M., Maiko, O., Tatsuo, K., Kaori, O., Shinya, Y., 2016.  
832 Heavy metal biosorption from aqueous solutions by algae inhabiting rice paddies in Vietnam.  
833 *J. Environ. Chem. Eng.* 4, 2529–2535.  
834 <https://doi.org/10.1016/j.jece.2016.04.038>  
835

836 Hu, H., Zhou, Q., 2010. Regulation of inorganic carbon acquisition by nitrogen and phosphorus  
837 levels in the *Nannochloropsis* sp. *World J. Microbiol. Biotech.* 26, 957–961.

838 <https://doi.org/10.1007/s11274-009-0253-6>  
839  
840  
841 Huang, B., Mimouni, V., Lukomska, E., Morant-Manceau, A., Bougaran, G., 2020. Carbon  
842 Partitioning and Lipid Remodeling During Phosphorus and Nitrogen Starvation in the Marine  
843 Microalga *Diacronema lutheri* (Haptophyta). *J. Phycol.*, 56, 908–922.  
844 <https://doi.org/10.1111/jpy.12995>  
845  
846 Huang, Z., Chen, B., Zhang, J., Yang, C., Wang, J., Song, F., Li, S., 2021. Absorption and  
847 speciation of arsenic by microalgae under arsenic-copper Co-exposure, *Ecotoxicology and*  
848 *Environmental Safety*, 213, 112024,  
849 <https://doi.org/10.1016/j.ecoenv.2021.112024>.  
850  
851 Hussain M.M., Wang J., Bibi I., Shahid M., Niazi N.K., Iqbal J., Mian I.A., Shaheen, S.M.,  
852 Bashir S., Shah N.S., Hina K., Rinklebe J., 2021. Arsenic speciation and biotransformation  
853 pathways in the aquatic ecosystem: The significance of algae. *Journal of Hazardous Materials*,  
854 403, 124027,  
855 <https://doi.org/10.1016/j.jhazmat.2020.124027>.  
856  
857 IAEA, 2004. Sediment Distribution Coefficients and Concentration Factors for Biota in the  
858 Marine Environment. International Atomic Energy Agency Technical Report Series 422.  
859  
860 Iwasawa, K., Murata, A., Taguchi, S., 2009. Cell shrinkage of *Isochrysis galbana*  
861 (Prymnesiophyceae) during storage with preservatives. *Plankton Benthos Res* 4(3), 120–121.  
862 <https://doi.org/10.3800/pbr.4.120>  
863  
864 Jasrotia, S., Kansal, A., Kishore, V., 2014. Arsenic phyco-remediation by *Cladophora* algae  
865 and measurement of arsenic speciation and location of active absorption site using electron  
866 microscopy. *Microchem J*, 114, 197–202.  
867 <https://doi.org/10.1016/j.microc.2014.01.005>  
868  
869  
870 Johnson, D.B., 2003. Chemical and microbiological characteristics of mineral spoils and  
871 drainage waters at abandoned coal and metal mines. *Water, Air, Soil Pollut.*, 3 (1), 47–66.  
872 <https://doi.org/10.1023/A:1022107520836>  
873  
874 Juang, Y.J., Chang, J.S., 2016. Applications of microfluidics in microalgae biotechnology: a  
875 review. *Biotechnol J*, 3, 327–335  
876 <https://doi.org/10.1002/biot.201500278>  
  
877 Kadiene, E.U., Meng, P.-J., Hwang, J.-S., Souissi, S., 2019a. Acute and chronic toxicity of  
878 Cadmium on the copepod *Pseudodiaptomus annandalei*: a life history traits approach.  
879 *Chemosphere* 233, 396-404.  
880 <https://doi.org/10.1016/j.chemosphere.2019.05.220>.  
881 Kadiene, E.U., Ouddane, B., Hwang, J.S., Souissi, S., 2019b. Bioaccumulation of metals in  
882 calanoid copepods by oral intake. *Sci. Rep.* 9, 9492.  
883 <https://doi.org/10.1038/s41598-019-45987-2>.  
884

885 Kim, H.-J., Fan, X., Gabbi, C., Yakimchuk, K., Parini, P., Warner, M., 2008. Liver X receptor  
886  $\beta$  (LXR $\beta$ ): a link between  $\beta$ -sitosterol and amyotrophic lateral sclerosis–Parkinson's dementia.  
887 Proc. Natl. Acad. Sci. U. S. A. 105 (6), 2094–2099.  
888 <https://doi.org/10.1073/pnas.0711599105>  
889

890 Kim, D., Chae, Y., An, Y.-J., 2017. Mixture Toxicity of Nickel and Microplastics with  
891 Different Functional Groups on *Daphnia magna*. Environmental Science &  
892 Technology, 51 (21), 12852-12858.  
893 [10.1021/acs.est.7b03732](https://doi.org/10.1021/acs.est.7b03732)  
894

895 Kim, S.Y., Kim, S., Yang, E. C., 2021. The complete mitochondrial genome and phylogeny  
896 of *Diacronema viridis* (Pavloales, Pavlovophyceae), Mitochondrial DNA Part B, 6(6), 1702-  
897 1703.  
898 [10.1080/23802359.2021.1915713](https://doi.org/10.1080/23802359.2021.1915713)  
899

900 Kwaansa-Ansah, E.E., Nti, S.O., Opoku, F., 2019. Heavy metals concentration and human  
901 health risk assessment in seven commercial fish species from Asafo Market, Ghana. Food Sci  
902 Biotechnol 28,569–579.  
903 <https://doi.org/10.1007/s10068-018-0485-z>  
904

905 Kwok, K.W., Souissi, S., Dur, G., Won, E.J., Lee, J.S., 2015. Copepods as references species  
906 in estuarine and marine waters. In: AmiardTriquet C, Amiard JC, Mouneyrac C (eds) Aquatic  
907 ecotoxicology: advancing tools for dealing with emerging risks. London: Academic Press, p  
908 281 –308.  
909 <https://doi.org/10.1016/B978-0-12-800949-9.00012-7>  
910

911 Leal, P.P., Hurd, C.L., Sander, S.G., Armstrong, E., Roleda, M.Y., 2016. Copper  
912 ecotoxicology of marine algae: a methodological appraisal, Chemistry and  
913 Ecology, 32(8), 786-800.  
914 [10.1080/02757540.2016.1177520](https://doi.org/10.1080/02757540.2016.1177520)

915 Li, B., Gu, B., Yang, Z., Zhang, T., 2018. The role of submerged macrophytes in  
916 phytoremediation of arsenic from contaminated water: a case study on *Vallisneria natans*  
917 (Lour.) Hara. Ecotoxicol. Environ. Saf. 165, 224–231.  
918 <https://doi.org/10.1016/j.ecoenv.2018.09.023>  
919

920 Liu, J., Sun, Z., Lavoie, M., Fan, X., Bai, X., Qian, H., 2014. Ammonium reduces chromium  
921 toxicity in the freshwater alga *Chlorella vulgaris*. Appl. Microbiol. Biotechnol., 99, 3249–3258.  
922 [http://dx.doi.org/10.1007/s00253-014-6218-1](https://doi.org/10.1007/s00253-014-6218-1).  
923

924 Mac Farlane, G.R., Burchett, M.D., 2002. Toxicity, growth and accumulation relationships of  
925 copper, lead and zinc in the grey mangrove *Avicennia marina*. (Forsk.) Vierh. Mar. Environ.  
926 Res., 54, 65–84.  
927 [https://doi.org/10.1016/S0141-1136\(02\)00095-8](https://doi.org/10.1016/S0141-1136(02)00095-8)  
928

929 Mahdavi, H., Ulrich, A.C., Liu, Y., 2012. Metal removal from oil sands tailings pond water  
930 by indigenous micro-alga. Chemosphere, 89, 350–354.  
931 <https://doi.org/10.1016/j.chemosphere.2012.04.041>  
932  
933

934 Mamun, M.A.A., Omori, Y., Miki, O., Rahman, I.M., Mashio, A.S., Maki, T., Hasegawa, H.,  
935 2019a. Comparative biotransformation and detoxification potential of arsenic by three  
936 macroalgae species in seawater: evidence from laboratory culture studies. *Chemosphere*, 228,  
937 117-127.  
938 <https://doi.org/10.1016/j.chemosphere.2019.04.056>  
939

940 Mamun, M.A.A., Omori, Y., Papry, R.I. et al., 2019b. Bioaccumulation and biotransformation  
941 of arsenic by the brown macroalga *Sargassum patens* C. Agardh in seawater: effects of  
942 phosphate and iron ions. *J Appl Phycol*, 31, 2669–2685.  
943 <https://doi.org/10.1007/s10811-018-1721-x>  
944 Mitra, A., Chatterjee, S., Gupta, D.K., 2017. Uptake, transport, and remediation of arsenic by  
945 algae and higher Plants. In: Gupta, D.K., Chatterjee, S. (Eds.), *Arsenic Contamination in the*  
946 *Environment: the Issues and Solutions*. Springer International Publishing, Cham, pp. 145-169.  
947

948 Monteiro, C.M., Fonseca, S.C., Castro, P.M.L., Malcata, F.X., 2011. Toxicity of cadmium and  
949 zinc on two microalgae, *Scenedesmus obliquus* and *Desmodesmus pleiomorphus* from  
950 Northern Portugal. *J. Appl. Phycol.* 23, 97–103.  
951 <https://doi.org/10.1007/s10811-010-9542-6>  
952

953 Navarrete, A., González, A., Gómez, M., Contreras, R.A., Díaz, P., Lobos, G., Brown, M.T.,  
954 Sáez, C.A., Moenne, A., 2019. Copper excess detoxification is mediated by a coordinated and  
955 complementary induction of glutathione, phytochelatins and metallothioneins in the green  
956 seaweed *Ulva compressa*. *Plant Physiology and Biochemistry*, 135, 423-431,  
957 <https://doi.org/10.1016/j.plaphy.2018.11.019>.

958 Okmen, G., Bozanta, E., Ugur, A., Ceyhan, N., 2011. Zinc effect on chlorophyll a, total  
959 carbohydrate, total protein contents and biomass of cyanobacterial species. *J. Appl. Biol. Sci.*  
960 5, 67–73.  
961

962 Omar, H.H., 2002. Adsorption of zinc ions by *Scenedesmus obliquus* and *S. quadricauda* and  
963 its effect on growth and metabolism. *Biol. Plantarum* 45, 261–266.  
964 <https://doi.org/10.1023/A:1015157009150>

965 Ouddane, B., 1990. Comportement des elements majeurs et mineurs dans un milieu soumis à  
966 des gradients physico-chimiques marines : cas de l'estuaire de la Seine. PhD thesis. Université  
967 des Sciences et Technologies de Lille, Lille, France.  
968

969 Perales-Vela, H.V., Pena-Castro, J.M., Canizares-Villanueva, R.O., 2006. Heavy metal  
970 detoxification in eukaryotic microalgae. *Chemosphere*, 64(1) –10.  
971 <https://doi.org/10.1016/j.chemosphere.2005.11.024>

972 Qian, H., Li, J., Sun, L., Chen, W., Sheng, G.D., Liu, W., Fu, Z., 2009. Combined effect of  
973 copper and cadmium on *Chlorella vulgaris* growth and photosynthesis-related gene  
974 transcription. *Aquat Toxicol* 94, 56–61.  
975 <https://doi.org/10.1016/j.aquatox.2009.05.014>  
976

977 Rajaram, R., Rameshkumar, S., Anandkumar, A., 2020. Health risk assessment and potentiality  
978 of green seaweeds on bioaccumulation of trace elements along the Palk Bay coast, Southeastern  
979 India. *Mar Pollut Bull*, 154, 111069.  
980 <https://doi.org/10.1016/j.marpolbul.2020.111069>.

981  
982 Salama, ES., Roh, H.S., Dev, S., Khan, M.A., Abou-Shanab, R.A.I., Chang, S.W., Jeon, B.H.,  
983 2019. Algae as a green technology for heavy metals removal from various wastewater. *World*  
984 *J Microbiol Biotechnol* 35, 75.  
985 <https://doi.org/10.1007/s11274-019-2648-3>  
986  
987  
988 Serra, A., Guasch, H., Admiraal, W., Van Beusekom, S.A.M., Van der Geest, H.G., 2010.  
989 Influence of phosphorus on copper sensitivity of fluvial periphyton: the role of chemical,  
990 physiological and community-related factors. *Ecotoxicology*, 19, 770–780.  
991 <https://doi.org/10.1007/s10646-009-0454-7>  
992  
993 Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten,  
994 U., Wehrli, B., 2006. The challenge of micropollutants in aquatic systems. *Science*, 313 (5790),  
995 1072–1077.  
996 [10.1126/science.1127291](https://doi.org/10.1126/science.1127291)  
997  
998 Sghaier, D.B., Duarte, B., Bankaji, I., Caçador, I., Sleimi, N., 2015. Growth, chlorophyll  
999 fluorescence and mineral nutrition in the halophyte *Tamarix gallica* cultivated in combined  
1000 stress conditions: arsenic and NaCl. *J. Photochem. Photobiol. B Biol.* 149, 204–214.  
1001 <https://doi.org/10.1016/j.jphotobiol.2015.06.003>  
1002  
1003 Soldo, D., Behra, R., 2000. Long-term effects of copper on the structure of freshwater  
1004 periphyton communities and their tolerance to copper zinc, nickel and silver. *Aquat. Toxicol.*  
1005 47, 181–189.  
1006 [https://doi.org/10.1016/S0166-445X\(99\)00020-X](https://doi.org/10.1016/S0166-445X(99)00020-X)  
1007  
1008 Souissi, S., Souissi, A., 2021. Promotion of the Development of Sentinel Species in the Water  
1009 Column: Example Using Body Size and Fecundity of the Egg-Bearing Calanoid  
1010 Copepod *Eurytemora affinis*. *Water*, 13, 1442.  
1011 <https://doi.org/10.3390/w13111442>  
1012  
1013 Tang, Y., Tian, J., Li, S., Xue, C., Xue, Z., Yin, D., Yu, S., 2015. Combined effects of graphene  
1014 oxide and Cd on the photosynthetic capacity and survival of *Microcystis aeruginosa*. *Sci. Total*  
1015 *Environ.* 532, 154–161.  
1016 <https://doi.org/10.1016/j.scitotenv.2015.05.081>  
1017  
1018 Tao, Y., Yuan, Z., Wei, M., Xiaona, H., 2012. Characterization of heavy metals in water and  
1019 sediments in Taihu Lake, China. *Environ. Monit. Assess.* 184, 4367-4382.  
1020 <https://doi.org/10.1007/s10661-011-2270-9>.  
1021  
1022 Tlili, S., Ovaert, J., Souissi, A., Ouddane, B., Souissi, S., 2016. Acute toxicity, uptake and  
1023 accumulation kinetics of nickel in an invasive copepod species: *Pseudodiaptomus marinus*.  
1024 *Chemosphere* 144, 1729–1737.  
1025 <https://doi.org/10.1016/j.chemosphere.2015.10.057>  
1026  
1027 Tomar, M., Kaur, I., Neelu, Bhatnagar, A.K., 2000. Effect of enhanced lead in soil on growth  
1028 and development of *Vigna radiata* (Linn.) Wilczek. *Indian J. Plant Physiol.* 5 (1), 13–18.  
1029

1030 Upadhyay, M.K., Yadav, P., Shukla, A., Srivastava, S., 2018. Utilizing the potential of  
1031 microorganisms for managing arsenic contamination: a feasible and sustainable approach.  
1032 Front. Environ. Sci. 6  
1033 <https://doi.org/10.3389/fenvs.2018.00024>.  
1034

1035 Van Lenning, K., Latasa, M., Estrada, M., Sáez, A.G., Medlin, L., Probert, I., Véron, B.,  
1036 Young, J., 2003. Pigment signatures and phylogenetic relationships of the Pavlovophyceae  
1037 (Haptophyta), J Phycol. 39,379–389. Crossref.  
1038 <https://doi.org/10.1046/j.1529-8817.2003.02064.x>  
1039

1040 Wang, Y., Wang, S., Xu, P.P., Liu, C., Liu, M.S., Wang, Y.L., Wang, C.H., Zhang, C.H., Ge,  
1041 Y., 2015. Review of arsenic speciation, toxicity and metabolism in microalgae. Rev. Environ.  
1042 Sci. Biotechnol. 14, 427–451.  
1043 <https://doi.org/10.1007/s11157-015-9371-9>.  
1044

1045

1046 Webster R.E., A.P. Dean., Pittman, J.K., 2011. Cadmium Exposure and Phosphorus Limitation  
1047 Increases Metal Content in the Freshwater Alga *Chlamydomonas reinhardtii*. Environmental  
1048 Science & Technology, 45 (17), 7489-7496.  
1049 [10.1021/es200814c](https://doi.org/10.1021/es200814c)  
1050

1051 Wilson, W., Zhang, Q., Rickaby, R.E.M., 2019. Susceptibility of algae to Cr toxicity reveals  
1052 contrasting metal management strategies. Limnol. Oceanogr. 64, 2271–2282.  
1053 <http://dx.doi.org/10.1002/lno.11183>  
1054

1055 Yan, J., Xiujun, X., Guangce, W., 2017. Effects of the heavy metal cadmium on photosynthetic  
1056 activity and the xanthophyll cycle in *Phaeodactylum tricornutum*. J. Ocean. Limnol. 36, 2194–  
1057 2201.  
1058 <https://doi.org/10.1007/s00343-019-7160-y>  
1059

1060

1061 Yan, Z., Li, X., Chen, J., Tam, N.F.Y., 2015. Combined toxicity of cadmium and copper in  
1062 *Avicennia marina* seedlings and the regulation of exogenous jasmonic acid. Ecotoxicol.  
1063 Environ. Saf. 113, 124–132.  
1064 <https://doi.org/10.1016/j.ecoenv.2014.11.031>  
1065

1066 Yang, J., Cao, J., Xing, G., Yuan, H., 2015. Lipid production combined with biosorption and  
1067 bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae  
1068 *Chlorella minutissima* UTEX2341. Bioresour Technol, 175,537–544.  
1069 <https://doi.org/10.1016/j.biortech.2014.10.124>  
1070

1071 Yang, J., Holbach, A., Wilhelms, A., Krieg, J., Qin, Y., Zheng, B., Zou, H., Qin, B., Zhu, G.,  
1072 Wu, T., Norra, S., 2020. Identifying spatio-temporal dynamics of trace metals in shallow  
1073 eutrophic lakes on the basis of a case study in Lake Taihu, China. Environmental Pollution,  
1074 264, 114802.  
1075 <https://doi.org/10.1016/j.envpol.2020.114802>.  
1076

1077 Yin, H., Gao, Y., Fan, C., 2011. Distribution, sources and ecological risk assessment of heavy  
1078 metals in surface sediments from Lake Taihu, China. Environ. Res. Lett. 6, 044012  
1079 <https://doi.org/10.1088/1748-9326/6/4/044012>.

1080  
1081 Yu, R.Q., Wang, W.X., 2004. Biokinetics of cadmium, selenium, and zinc in fresh water alga  
1082 *Scenedesmus obliquus* under different phosphorus and nitrogen conditions.  
1083 <https://doi.org/10.1016/j.envpol.2003.11.013>

1084 Zhang, J., Zhang, M., Zhang, S., Xu, Q., Liu, X., Zhang, Z., 2019. Nutrient distribution and  
1085 structure affect the behavior and speciation of arsenic in coastal waters: a case study in  
1086 southwestern coast of the Laizhou Bay, China. *Mar. Pollut. Bull.* 146, 377–386.  
1087 <https://doi.org/10.1016/j.marpolbul.2019.06.085>

1088 Zheng, Y.Q., Dai, X.J., Wang, L., Xu, W.Z., He, Z.Y., Ma, M., 2008. Arsenate reduces copper  
1089 phytotoxicity in gametophytes of *Pteris vittata*. *J. Plant Physiol.* 165, 1906–1916.  
1090 <https://doi.org/10.1016/j.jplph.2008.04.008>.

1091 Zidour, M., Boubechiche, Z., Pan, Y.J., Bialais, C., Cudennec, B., Grard, T., Drider, D.,  
1092 Flahaut, C., Ouddane, B., Souissi, S., 2019. Population response of the estuarine copepod  
1093 *Eurytemora affinis* to its bioaccumulation of trace metals. *Chemosphere*  
1094 220, 505-513.  
1095 <https://doi.org/10.1016/j.chemosphere.2018.12.148>.

1096 Zsiros, O., Nagy, G., Patai, R., Solymosi, K., Gasser, U., Polgar, T.F., Garab, G., Kovacs, L.,  
1097 Horcsik, Z.T., 2020. Similarities and differences in the effects of toxic concentrations of  
1098 cadmium and chromium on the structure and functions of thylakoid membranes in *Chlorella*  
1099 *variabilis*. *Front. Plant Sci.* 11, 1006.  
1100 <https://doi.org/10.3389/fpls.2020.01006>  
1101  
1102  
1103  
1104

