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1 **Comparative study of Cu uptake and early transcriptome responses in the green microalga**

2 ***Chlamydomonas reinhardtii* and the macrophyte *Elodea nuttallii***

3

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5

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13

14 **Abstract**

15 Microalgae are widely used as representative primary producers in ecotoxicology, while
16 macrophytes are much less studied. Here we compared the bioavailability and cellular toxicity
17 pathways of 2 h-exposure to 10^{-6} mol·L⁻¹ Cu in the macrophyte *Elodea nuttallii* and the green
18 microalga *Chlamydomonas reinhardtii*.

19 Uptake rate was similar but faster in the algae than in the macrophyte, while RNA-Sequencing
20 revealed a similar number of regulated genes. Early-regulated genes were congruent with
21 expected adverse outcome pathways for Cu with Gene Ontology terms including gene
22 regulation, energy metabolism, transport, cell processes, stress, antioxidant metabolism and
23 development. However, the gene regulation level was higher in *E. nuttallii* than in *C. reinhardtii*
24 and several categories were more represented in the macrophyte than in the microalga.
25 Moreover, several categories including oxidative pentose phosphate pathway (OPP), nitrate
26 metabolism and metal handling were only found for *E. nuttallii*, whereas categories such as cell
27 motility, polyamine metabolism, mitochondrial electron transport and tricarboxylic acid cycle
28 (TCA) were unique to *C. reinhardtii*. These differences were attributed to morphological and
29 metabolic differences and highlighted dissimilarities between a sessile and a mobile species. Our
30 results highlight the efficiency of transcriptomics to assess early molecular responses in biota,
31 and the importance of studying more aquatic plants for a better understanding on the impact and
32 fate of environmental contaminants.

33

34 **Keywords:** copper; primary producers; speciation modelling; toxicokinetics; transcriptomics.

35

36 **Capsule:** Cu accumulation is faster in the algae, but greater transcriptome response occurred in
37 the macrophyte.

38

39 **Introduction**

40 Primary producers are key organisms of aquatic ecosystems: phytoplankton sustains the largest
41 ecosystem on the Earth, contributing to about half of the primary production on our planet
42 although accounting for less than 1% of photosynthetic biomass (Bañuelos et al., 1998).
43 Macrophytes, including plants dominate primary production in shallow waters including littorals,
44 rivers, marshes, ponds and lakes (Noges et al., 2010). In addition they are key elements of the
45 aquatic ecosystems by providing support, shelter, food and oxygen to many organisms including
46 epiphytes (Thomaz and Cunha, 2010). Studying primary producers' response to a variation of the
47 concentrations of vital and toxic trace metals is thus an important step to understand and estimate
48 their impact in aquatic ecosystems. Indeed, if primary producers are affected they will also
49 indirectly influence higher trophic levels in an ecosystem through food webs (Daam et al., 2009;
50 Fleeger et al., 2003). Often microalgae are hypothesized as representative primary producers
51 based on the assumption that all organisms respond to stresses similarly (Clemens, 2006).
52 Nevertheless, seldom comparisons, for example of plants with algae (Beauvais-Fluck et al.,
53 2018a; Paz et al., 2007) or mosses (Rother et al., 2006), revealed the existence of different stress
54 and tolerance mechanisms. In such a context, the precise mechanisms of cellular handling (and
55 toxicity) are to further elucidate to better understand the similarities and differences in various
56 primary producers and anticipate trace metals effect in the environment.

57 Copper (Cu) is an essential metal to all plants and animals. It participates in fundamental
58 physiological processes (e.g. photosynthetic electron transport, mitochondrial respiration) and is
59 a cofactor for many enzymes (e.g. superoxide dismutases, cytochrome c oxidases) (Castruita et
60 al., 2011). Due to its high reactivity, Cu concentration is tightly regulated inside cells by a
61 complex homeostasis network (Andres-Colas et al., 2006). This homeostasis network has been

62 studied in several model species and there are evidences of a high conservation throughout the
63 evolution (Burkhead et al., 2009; Page et al., 2009). However, when in excessive concentrations
64 Cu causes oxidative stress and photosynthesis inhibition due to adverse effects on the same
65 cellular processes where it is needed, such as enzyme activity and photosynthetic electron
66 transport (Monferran et al., 2009; Razinger et al., 2010; Upadhyay et al., 2011). Thus, Cu
67 concentrations, and its biological availability are important parameter for environmental quality
68 in natural environments. Elevated Cu concentrations in aquatic ecosystems are directly related to
69 human activities involving the production of industrial (e.g. pesticide use and agricultural run-
70 off, mine tailings) and domestic wastes (e.g. urbanization, automobile exhausts). Naturally
71 occurring concentrations of Cu range between 10^{-9} mol·L⁻¹ to 10^{-8} mol·L⁻¹ in freshwater systems,
72 but Cu can easily reach 10^{-6} M in locations receiving anthropogenic inputs such as freshwater
73 ecosystems close to vineyards or mining areas (Kupper and Andresen, 2016).

74 The present study aimed thus to compare Cu toxicokinetic and transcriptomic responses in two
75 aquatic primary producers: a macrophyte *Elodea nuttallii* (Planch.) St. John and a green
76 microalga *Chlamydomonas reinhardtii* P.A. Dangeard, respectively representing aquatic plants
77 and phytoplankton typically found in the benthic environment and the water column. In the
78 present study, we hypothesized that bioavailability and responses to toxic metals were similar in
79 a microalgae and a macrophyte exposed in similar experimental conditions. More in detail, we
80 compared cellular toxicity pathways of Cu in both organisms using transcriptomics
81 (RNAsequencing; RNAseq) and determining the uptake. This research will thus increase our
82 level of understanding of the functioning of key organisms, and eventually, the data produced
83 will provide a scientific base to conduct sound risk assessment of our freshwater ecosystems to
84 preserve their high socio-economic and environmental value.

85

86 **Material and methods**

87 *Labware*

88 All material was washed in 10% HNO₃ baths, thoroughly rinsed with ultrapure water (MilliQ
89 Direct system, Merck Millipore) and dried under a laminar flow hood. Material for culture and
90 experiments, including media, were additionally autoclaved (1 bar, 121 °C, 20 min) to avoid
91 microbial contamination.

92

93 *Exposure of algae and macrophytes*

94 Typical growth and exposure conditions were used for each species. *Chlamydomonas reinhardtii*
95 P.A. Dangeard (wild type strain CPCC11, Canadian Phycological Culture Centre) were grown
96 under axenic conditions in an incubator (Multitron Infors HT) at 20.2 ± 0.5 °C with a continuous
97 light cycle (3600 lux) and a rotary shaking (115 rpm). Cells were cultured in a 4× diluted Tris-
98 Acetate-Phosphate medium (TAP) (Rensing et al., 2008). At the mid-exponential growth phase
99 (62 h after inoculation), cells were harvested by centrifugation (10 min, 1300g), rinsed and re-
100 suspended in the exposure medium at a final density of $(8.1 \pm 1.1) \cdot 10^5$ cells·mL⁻¹.

101 Shoots of *Elodea nuttallii* (Planch.) St. John were collected in Lake Geneva, and a culture
102 established and maintained in microcosms at 20 ± 1 °C with a 16/8 h light/dark cycle (1000 lux
103 (Regier et al., 2013b). Exposures were initiated 5 h after the start of light on three 10 cm-long
104 shoots without roots.

105 Exposures were conducted in the laboratory under the same controlled conditions than during
106 culture. Both organisms were exposed in triplicates to nominal concentration of $1 \cdot 10^{-6}$ mol·L⁻¹
107 and $2 \cdot 10^{-6}$ mol·L⁻¹ Cu added as CuSO₄ (Sigma Aldrich) for *E. nuttallii* and *C. reinhardtii*

108 respectively, in an artificial medium ($8.2 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1} \text{ CaCl}_2$, $3.6 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1} \text{ MgSO}_4$, $2.8 \cdot 10^{-4}$
109 $\text{mol} \cdot \text{L}^{-1} \text{ NaHCO}_3$, $1.0 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1} \text{ KH}_2\text{PO}_4$ and $5.0 \cdot 10^{-6} \text{ mol} \cdot \text{L}^{-1} \text{ NH}_4\text{NO}_3$, pH 6.9 ± 0.1) during
110 10, 30 min, 1, 4 and 8 h. Organisms exposed in the absence of metal in the medium were used as
111 control. The choice of Cu exposure concentration corresponds to a sublethal concentration in
112 similar experimental conditions, e.g. resulting in the EC20 growth inhibition in *C. reinhardtii* for
113 24 h-long exposure (Cheloni et al., 2014) and is 4× lower than the concentration resulting in a
114 15% decrease of chlorophyll content in *E. nuttallii* after 2 h-long exposure (Regier et al., 2015).

115

116 *Cu uptake and modelling*

117 Cu uptake by microalgae and macrophyte was characterized by total and intracellular Cu (Cu_{int})
118 contents. Half of exposed organisms was rinsed with $10^{-3} \text{ mol} \cdot \text{L}^{-1}$
119 ethylene·diamine·tetraacetic·acid (EDTA; Sigma-Aldrich, Buchs, Switzerland) prepared in the
120 exposure medium, to rinse metal surface-adsorbed or loosely bound to the cell wall and
121 determine Cu_{int} . The other half of exposed organisms was rinsed with medium without Cu
122 (media-rinsed) and therefore represents the sum of adsorbed Cu (Cu_{ads}) and Cu_{int} .

123 EDTA-rinsed and media-rinsed samples were freeze-dried (Beta 1-8 K), digested with 65%
124 HNO_3 (Suprapur Merck KGaA) at $90 \text{ }^\circ\text{C}$ for 1 h and analyzed by inductively coupled plasma
125 mass spectrometry (ICP-MS; 7700x, Agilent Technologies). Concentration in media was
126 measured in acidified samples (0.5% v/v HNO_3 Suprapur) by ICP-MS. Cu concentration in
127 unspiked artificial medium was $2.3 \pm 0.3 \cdot 10^{-9} \text{ mol} \cdot \text{L}^{-1} \text{ Cu}$. Measured initial concentration in
128 spiked media were $1.09 \pm 0.20 \cdot 10^{-6} \text{ mol} \cdot \text{L}^{-1} \text{ Cu}$ ($1.85 \pm 0.47 \cdot 10^{-7} \text{ mol} \cdot \text{L}^{-1} \text{ Cu}^{2+}$) and $2.26 \pm$
129 $0.2 \cdot 10^{-6} \text{ mol} \cdot \text{L}^{-1} \text{ Cu}$ ($3.84 \pm 0.51 \cdot 10^{-7} \text{ mol} \cdot \text{L}^{-1} \text{ Cu}^{2+}$), for *E. nuttallii* and *C. reinhardtii*,
130 respectively.

131 The Cu uptake was modelled using a first-order mass transfer model following two-compartment
132 system and equations below (eq 1 and 2):

133

$$134 \quad C_t = C_0 + \frac{a}{k(1 - e^{-kt})} \quad (\text{eq 1})$$

$$135 \quad a = k_1 \times C_e \quad (\text{eq 2})$$

136

137 where C_t is the metal concentration in cells ($\mu\text{mol}_{\text{Cu}} \cdot \text{g}^{-1} \cdot \text{dw}$) at time t (hours), k is the elimination
138 rate constant (h^{-1}) and a is the uptake flux ($\mu\text{mol}_{\text{Cu}} \cdot \text{g}^{-1} \cdot \text{dw} \cdot \text{h}^{-1}$), k_1 is the uptake rate constant
139 ($\mu\text{mol}_{\text{Cu}} \cdot \text{g}^{-1} \cdot \text{dw} \cdot \text{h}^{-1}$), C_e is the bioavailable concentration in the medium ($\mu\text{mol}_{\text{Cu}} \cdot \text{g}^{-1} \cdot \text{dw}$), and C_0
140 is the constitutive metal concentration measured in cells at the beginning of the exposure
141 (Gimbert et al., 2008; Martins and Boaventura, 2002). Statistics (t tests) and plots were done in
142 SigmaPlot.

143

144 *RNA-sequencing (RNAseq) and quantification of differential gene expression*

145 Transcriptome response of *C. reinhardtii* and *E. nuttallii* exposed 2 h to Cu was assessed through
146 RNASeq (Illumina HiSeq 2500 System). Total RNA was extracted as previously described using
147 TRI Reagent (Sigma-Aldrich, Buchs, Switzerland), and libraries were prepared following
148 manufacturer's protocols (Beauvais-Fluck et al., 2016, 2017; Regier et al., 2016). For *C.*
149 *reinhardtii*, reads were aligned with TopHat2 (Kim et al., 2013) to the genome *Creinhardtii* 236
150 V.9.0 (Conesa et al., 2005). For *E. nuttallii*, reads were mapped using the Burrows-Wheeler
151 Alignment (BWA v.0.7.10) tool (Li and Durbin, 2010) on the *de novo* transcriptome available
152 for this organism entailing 181'663 contigs with an average length of 880 bp (Regier et al.,
153 2016). We selected contigs showing a minimum coverage of 20 raw counts in all samples,

154 resulting in 99'030 sequences analyzed for differential gene expression in CLC Main Workbench
155 (Version 7, CLC bio, QIAGEN, Denmark). 50% of contigs are covered by the reads of at least in
156 one sample.

157 For both organisms reads were counted using the Python package HTSeq (Anders et al., 2015).
158 Differential gene expression analysis was performed in the software CLC Main Workbench
159 (Version 7, CLC bio, QIAGEN, Denmark) based on normalized counts and EdgeR package
160 (Robinson et al., 2010). Significant differently expressed transcripts vs Control were defined
161 with a threshold of false discovery rate (FDR) <0.1%. Ontology term assignments were done
162 using MapMan (Table S1) (Thimm et al., 2004; Usadel et al., 2009). Data are available in the
163 Gene Expression Omnibus database (GSE65109).

164

165 **Results and discussion**

166 *Cu uptake by primary producers*

167 Accumulation of Cu was measured in media-rinsed and EDTA-rinsed *E. nuttallii* and *C.*
168 *reinhardtii* over time (Figure 1). The one-compartment model well fitted Cu accumulation in *E.*
169 *nuttallii* and *C. reinhardtii*. In both organisms significant and similar (*a*) and (*k*) were estimated
170 by the model normalized by the effective concentration in media (Table 1). Modelling further
171 allowed estimating that the steady state was approached in less than 2 h for *C. reinhardtii*.
172 Concentrations measured in media-rinsed *C. reinhardtii* (up to 40 $\mu\text{mol}\cdot\text{g}^{-1}$ dw) reached a plateau
173 in 2 h. Similar, fast uptake and plateau has been observed in *C. reinhardtii* exposed to increasing
174 concentrations of ^{65}Cu (Jamers et al., 2013). The cellular concentrations of *C. reinhardtii* were in
175 the same order of magnitude as in previous studies with *C. reinhardtii* and other green freshwater
176 algae (Stoiber et al., 2012). For comparison, media-rinsed *E. nuttallii* (8 $\mu\text{mol}\cdot\text{g}^{-1}$ dw at 8h)

177 showed no obvious evidence of a plateau. Moreover, after 2 h exposure *E. nuttallii* internalized
178 (EDTA-washed) $1.33 \pm 0.31 \mu\text{mol}\cdot\text{g}^{-1} \text{ dw}$ ($1.04 \pm 0.24 \mu\text{mol}\cdot\text{g}^{-1} \text{ dw}$ in control), while *C.*
179 *reinhardtii* internalized $4.69 \pm 0.18 \mu\text{mol}\cdot\text{g}^{-1} \text{ dw}$ ($1.44 \pm 0.06 \mu\text{mol}\cdot\text{g}^{-1} \text{ dw}$ in control). Data
180 showed that internalization is similar when normalized by the effective concentration in media,
181 differences reflecting exposure condition, but faster in the algae than in the macrophyte. This
182 difference can be attributed to the fact that the full surface of the unicellular algae is in contact
183 with the media, whereas in the macrophyte only the external layer of cells is directly exposed,
184 most certainly resulting in a gradient of metal concentrations between cells. Besides, the surface-
185 to-volume ratio is much higher in an unicellular organism and thus is expected to result in higher
186 uptake, that is not observed in our experimental conditions, but could also result in a faster
187 uptake (Lindemann et al., 2016). However, proportion of Cu accumulated in cell walls was
188 higher in *C. reinhardtii* than in *E. nuttallii*, suggesting that adsorption of Cu was predominant in
189 *C. reinhardtii* and/or EDTA-washing procedure was more efficient. Cell walls are known to play
190 a central role in plant and microalgal tolerance to metals: for example, 50% of Cu was
191 accumulated in the cell walls in *Cystoseira tamariscifolia* (Celis-Pla et al., 2018), 20% was
192 adsorbed (or EDTA-extractable) for *Chlorella kessleri* (Lamelas et al., 2009). In the charophyte,
193 *Nitellopsis obtusa* exposed 3 h to both Cu-nanoparticles or CuSO₄, the major part of Cu
194 accumulated in cell walls (Manusadzianas et al., 2017). Similarly, a previous study in *E. nuttallii*
195 measured an increased proportion over time of cadmium and mercury in cell walls,
196 concomitantly with an increased lignification of cell walls after 7 d exposure (Larras et al.,
197 2013).

198 Circadian clock and light are also known to be central for nutrient acquisition in plants because
199 nutrient demands of a plant change according to the time of day, e.g. to drive photosynthesis in

200 chloroplasts and daily rhythms in transpiration rates (Haydon et al., 2015). Light intensity and
201 spectral composition also affected Cu uptake to *C. reinhardtii* (Cheloni et al., 2014). Here the
202 differences in light cycle applied to the microalgae (continuous) and the macrophyte (16 h light)
203 might also affect uptake because here plants have a synchronized circadian rhythm, while
204 microalgae show an average of all circadian stages. In *Arabidopsis thaliana*, cytosolic Cu was
205 shown to increase during light period and decrease during dark period (Penarrubia et al., 2009).
206 It is unclear if this is also the case here, but if this is the case, the different light regimes used for
207 both species could also explain in part the higher Cu concentrations reached in the microalgae
208 than the macrophyte. However, in similar experimental conditions including light regime, *E.*
209 *nutallii* exposed to Hg and Cd showed a plateau around 48 h-long exposure (Larras et al., 2013),
210 supporting that metal uptake in this species takes longer to reach equilibrium than in *C.*
211 *reinhardtii*. Nonetheless, because experimental settings can affect uptake and typical
212 experimental conditions are in general different for microalgae and plants, future research should
213 test organisms in completely identical conditions to allow a detailed comparison of
214 bioaccumulation data.

215 *Transcriptomic response*

216 In total 1397 and 1258 genes were regulated by 2 h exposure to 10^{-6} mol·L⁻¹ Cu in *C. reinhardtii*
217 and *E. nuttallii* respectively. The similar number of regulated genes, used as a proxy of stress,
218 suggested that both *E. nuttallii* and *C. reinhardtii* faced a similar level of stress (Dranguet et al.,
219 2017). However, among those, 841 (67%) and 624 (44%) genes were upregulated, while 417 and
220 773 were down-regulated in *E. nuttallii* and *C. reinhardtii*, respectively (Table S2 and S3).
221 Besides, the level of gene regulation was higher in *E. nuttallii* (log₂FC_{range}= 17.9) than in *C.*
222 *reinhardtii* (log₂FC_{range}= 8.8), suggesting a higher impact of Cu in the macrophyte than the

223 microalgae (Figure 2), in line with similar observations made for Hg in controlled and in the
224 field exposure comparing the same species in identical conditions (Beauvais-Fluck et al., 2018a;
225 Dranguet et al., 2017).

226 Among the 20 most highly regulated genes, 13 and 14 had an unknown function in in *C.*
227 *reinhardtii* and *E. nuttallii*, respectively (Table 2). The 7 most highly regulated genes with
228 known function were homologous to genes involved in reduction-oxidation (RedOx)
229 metabolism, in gene regulation and nutrient transport, as well as multigenic families with
230 numerous biochemical functions which precise function is therefore difficult to establish based
231 on sequence homologies. More globally, in term of abundance of GO terms for genes
232 significantly regulated in both species, a predominant part of regulated genes had unknown
233 function (62% for *C. reinhardtii* and 33% for *E. nuttallii*; Figure 3) indicating considerable
234 potential for new discovery in the biology of Cu. In *C. reinhardtii*, the most abundant GO
235 categories regulated by Cu exposure were involved in “gene regulation” (44%, i.e. RNA, protein,
236 signaling) and “cell processes” (10%; i.e. cell organization and cell motility) suggesting an
237 adaptation of the cell metabolism and structure (Figure 3). Transport and photosynthesis both
238 represented 8% of regulated genes. In *E. nuttallii*, the most abundant GO categories regulated by
239 Cu exposure were involved in “gene regulation” (34%, i.e. RNA, protein, signaling) and
240 “transport” (10%). The GO category “stress” represented 8% of regulated genes, while “cell
241 processes” represented 6% of regulated genes. Other GO categories including hormone
242 metabolism, stress, RedOx metabolism and development were less than 5% of regulated genes.

243 Enriched pathway analysis revealed a response of both species to avoid stress (e.g. oxidative
244 stress) and effects on development/growth and nutrition with a significant modification of the
245 energy metabolism. Regulated genes were thus in line with expected adverse outcome pathways

246 for Cu, i.e. impact on photosynthesis, RedOx, growth and nutrition, although only 2 h exposure
247 was performed. This confirms the potential of transcriptomics to reveal early-responses at
248 environmental concentrations (Beauvais-Fluck et al., 2018b; Dranguet et al., 2017; Regier et al.,
249 2013a). Not surprisingly this short exposure resulted in few physiological endpoints significantly
250 different vs control (Table S4 and S5) (Jamers et al., 2013; Jiang et al., 2016). More in detail,
251 here photosynthesis efficiency is reduced in *C. reinhardtii* by 7% (Table S5) and class III
252 peroxidase activity (POD) is reduced 50× in *E. nuttallii* (Table S2). In the same line, another
253 study with *C. reinhardtii* revealed that exposure to a similar free ion concentration 10^{-7} mol·L⁻¹
254 Cu²⁺, induced Glutathione Peroxidase genes after 2 h and reduced growth after 24 h, although no
255 cellular impact was measured including membrane permeability, reactive oxygen species
256 production and lipid peroxidation (Cheloni et al., 2014).

257 A previous study showed that exposure of *E. nuttallii* to 10^{-6} mol·L⁻¹ Cu reduced superoxide
258 dismutases activity after 1 h and reduced root growth after 24 h, but had no significant effect on
259 chlorophyll content, photosynthesis efficiency and class III peroxidase activity (Regier et al.,
260 2015). Similar observation has been obtained with Cu toxicity in *C. reinhardtii*: exposure to
261 excess Cu induced ROS production and antioxidative response in *C. reinhardtii* (Cheloni et al.,
262 2019; Jamers et al., 2006; Jiang et al., 2016; Stoiber et al., 2013). In the same line, a study on the
263 rootless submerged shoots of *Ceratophyllum demersum* exposed 6 weeks to a range of
264 concentrations between 10^{-9} - 10^{-7} mol·L⁻¹ Cu, showed that nutrient uptake/distribution,
265 photosynthesis efficiency and chlorophyll content were affected by Cu (Thomas et al., 2013).
266 Nutrition is impacted because an excess Cu competes with the various essential metals according
267 to the Irving–William series and induces deficiency of essential ions (Mg²⁺, Zn²⁺, etc.) (Mosulen
268 et al., 2003) and impairment of metalloprotein functioning. However, although all toxic metals

269 might induce the same core stress related changes on genes, transcriptome analysis has resulted
270 in the identification of genes specific to each metal (Kovalchuk et al., 2005; Simon et al., 2008;
271 Weber et al., 2006). Nonetheless, data have rarely been compared between species exposed in
272 similar experimental settings (Beauvais-Fluck et al., 2018a; Dranguet et al., 2017).

273 Here, several categories were more represented in the macrophyte than in the microalga (Figure
274 2), including stress (abiotic), development, cell vesicle transport, hormone metabolism (abscisic
275 acid, ethylene, jasmonate), cell wall (cellulose, hemicellulose and pectin synthesis), secondary
276 metabolism (phenylpropanoid, wax, flavonoids), and transport (transport P- and V-ATPases,
277 Major Intrinsic Proteins, nitrate). The present data for *E. nuttallii* were in agreement with a
278 microarray analysis in roots of rice exposed 3 h to $5 \cdot 10^{-6}$ mol·L⁻¹ Cu, notably concerning
279 dysregulation of genes involved in vesicle transport, flavonoids metabolism and jasmonate (Lin
280 et al., 2013). Authors further showed by knockout of genes necessary for this vesicle transport
281 and exposure of roots to vesicle trafficking inhibitors, that Cu interacts with vesicle transport and
282 that this vesicle transport is essential for signaling via ROS for activating defenses (Lin et al.,
283 2013). Results of the present study allowed to propose a possible model of cellular mechanisms
284 involved in Cu detoxification and protection in *E. nuttallii*: Cu increases intracellular transport,
285 e.g. vesicle trafficking and ABC transport, and induces a flavonoid-mediated detoxification
286 pathway. In addition, the toxicity mechanisms such as JA biosynthesis and cellular component
287 biogenesis were regulated in response to Cu exposure. In comparison, the categories of OPP,
288 nitrate metabolism and metal handling were absent in Cu regulated genes in *C. reinhardtii*.
289 Conversely, cell motility, DNA, polyamine metabolism, mitochondrial electron transport, and
290 TCA categories were found in *C. reinhardtii*, while absent in *E. nuttallii*. Moreover, the level of
291 regulation of the categories found in common in both species was higher in *E. nuttallii* than in *C.*

292 *reinhardtii*. These differences certainly highlight the dissimilarities between basal and
293 background metabolism in two different species, as well as between a sessile and a mobile
294 organism (Dranguet et al., 2017). Besides, genome sequencing has revealed that *C. reinhardtii*
295 possesses numerous genes derived from the last plant-animal common ancestor that have been
296 lost in angiosperms, including transporters and the possibility of extensive metabolic flexibility
297 (Merchant et al., 2007). Taken together, our divergent observations on how an unicellular and a
298 multicellular organism take up and are impacted by Cu may imply that homeostasis networks are
299 more species-specific than generally thought.

300 We further found several differences at the level of subcategories. For example, in the
301 'Photosynthesis' category, genes of *C. reinhardtii* were mainly involved in the light reaction, in
302 particular photosystem I (PSI), while in *E. nuttallii* genes were involved both in PS I and PS II,
303 as well as photorespiration, suggesting that the photosynthesis was impacted more widely by Cu
304 toxicity in the macrophyte. Generally, Cu has been reported to impact more PS II than PS I in
305 plants. In PS II, the reaction center and LHC II by substitution of Mg²⁺ in its chlorophyll have
306 been shown to be targets of Cu toxicity (Kupper and Andresen, 2016; Kupper et al., 1996). In the
307 macrophyte *C. demersum* nanomolar concentrations of Cu affected the PS II reaction center
308 (Thomas et al., 2013). In this regard, our finding of Cu impact on PSI in *C. reinhardtii* is striking
309 and might point to structural differences between photosystems as well as background defense
310 pools such as metallothioneins, phytochelatins and redox enzymes in the studied species
311 (Castruita et al., 2011).

312

313 **Conclusion**

314 Overall, the exposure to 10^{-6} mol·L⁻¹ Cu resulted in different cellular toxicity pathways in a
315 microalga and a macrophyte. This fact together with the distinct exposure routes of the benthic
316 macrophyte and lentic microalgae suggest that similar Cu concentrations might affect differently
317 both species in the ecosystem. Nonetheless, because experimental settings are known to affect
318 responses and experimental conditions are in general different for microalgae and plants, special
319 attention has to be put in future research in testing organisms in completely identical conditions.
320 Defining ecological thresholds of adverse outcomes for environmental contaminants represents a
321 critical component of chemical assessment and management programs. Our data also call for
322 including more species of aquatic plants for determining ecological thresholds for environmental
323 contaminants, e.g. more tests using representative species of plants in the laboratory and *in situ*
324 will be necessary in future studies (Beauvais-Fluck et al., 2018a; Dranguet et al., 2017).
325 However, transcriptomics is confirmed as a useful tool to assess early responses at environmental
326 concentrations and is promising for contaminated sites.

327

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335

336 **REFERENCES**

337 Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq--a Python framework to work with high-
338 throughput sequencing data. *Bioinformatics* 31, 166-169.

339 Andres-Colas, N., Sancenon, V., Rodriguez-Navarro, S., Mayo, S., Thiele, D.J., Ecker, J.R.,
340 Puig, S., Penarrubia, L., 2006. The *Arabidopsis* heavy metal P-type ATPase HMA5 interacts
341 with metallochaperones and functions in copper detoxification of roots. *Plant J* 45, 225-236.

342 Bañuelos, G.S., Ajwa, H.A., Wu, L., Zambrzuski, S., 1998. Selenium accumulation by *Brassica*
343 *napus* grown in Se-laden soil from different depths of Kesterson reservoir. *J Soil Contam* 7(4),
344 481-496.

345 Beauvais-Fluck, R., Slaveykova, V., Cosio, C., 2018a. Molecular effects of inorganic and methyl
346 mercury in aquatic primary producers: comparing impact to a macrophyte and a green microalga
347 in controlled conditions. *Geosciences* 8.

348 Beauvais-Fluck, R., Slaveykova, V.I., Cosio, C., 2016. Transcriptomic and physiological
349 responses of the green microalga *Chlamydomonas reinhardtii* during short-term exposure to sub-
350 nanomolar methylmercury concentrations. *Environ Sci Technol* 320, 401-407.

351 Beauvais-Fluck, R., Slaveykova, V.I., Cosio, C., 2017. Cellular toxicity pathways of inorganic
352 and methyl mercury in the green microalga *Chlamydomonas reinhardtii*. *Sci Rep* 7, 017-08515.

353 Beauvais-Fluck, R., Slaveykova, V.I., Skyllberg, U., Cosio, C., 2018b. Molecular effects,
354 speciation, and competition of inorganic and methyl mercury in the aquatic plant *Elodea*
355 *nuttallii*. *Environ Sci Technol* 52, 8876-8884.

356 Burkhead, J.L., Reynolds, K.A.G., Abdel-Ghany, S.E., Cohu, C.M., Pilon, M., 2009. Copper
357 homeostasis. *New Phytol* 182, 799-816.

358 Castruita, M., Casero, D., Karpowicz, S.J., Kropat, J., Vieler, A., Hsieh, S.I., Yan, W.H., Cokus,
359 S., Loo, J.A., Benning, C., Pellegrini, M., Merchant, S.S., 2011. Systems biology approach in

360 *Chlamydomonas* reveals connections between copper nutrition and multiple metabolic steps.
361 Plant Cell 23, 1273-1292.

362 Celis-Pla, P.S.M., Brown, M.T., Santillan-Sarmiento, A., Korbee, N., Saez, C.A., Figueroa, F.L.,
363 2018. Ecophysiological and metabolic responses to interactive exposure to nutrients and copper
364 excess in the brown macroalga *Cystoseira tamariscifolia*. Mar Poll Bull 128, 214-222.

365 Cheloni, G., Cosio, C., Slaveykova, V.I., 2014. Role of light irradiation on copper effects to
366 *Chlamydomonas reinhardtii*: from antagonism to synergism. Aq Toxicol 155, 275-282.

367 Cheloni, G., Gagnaux, V., Slaveykova, V.I., 2019. Species-species interactions modulate copper
368 toxicity under different visible light conditions. Ecotox Environ Saf 170, 771-777.

369 Clemens, S., 2006. Toxic metal accumulation, responses to exposure and mechanisms of
370 tolerance in plants. Biochimie 88, 1707-1719.

371 Conesa, A., Gotz, S., Garcia-Gomez, J.M., Terol, J., Talon, M., Robles, M., 2005. Blast2GO: a
372 universal tool for annotation, visualization and analysis in functional genomics research.
373 Bioinformatics 21, 3674-3676.

374 Daam, M.A., Satapornvanit, K., Van den Brink, P.J., Nogueira, A.J., 2009. Direct and indirect
375 effects of the fungicide Carbendazim in tropical freshwater microcosms. Arch Environ Contam
376 Toxicol 58, 315-324.

377 Dranguet, P., Cosio, C., Le Faucheur, S., Beauvais-Fluck, R., Freiburghaus, A., Worms, I.A.M.,
378 Petit, B., Civic, N., Docquier, M., Slaveykova, V.I., 2017. Transcriptomic approach for
379 assessment of the impact on microalga and macrophyte of *in-situ* exposure in river sites
380 contaminated by chlor-alkali plant effluents. Water res 121, 86-94.

381 Fleeger, J.W., Carman, K.R., Nisbet, R.M., 2003. Indirect effects of contaminants in aquatic
382 ecosystems. Sci Tot Environ 317, 207-233.

383 Gimbert, F., Mench, M., Coeurdassier, M., Badot, P.M., de Vaufleury, A., 2008. Kinetic and
384 dynamic aspects of soil-plant-snail transfer of cadmium in the field. *Environ Poll* 152, 736-745.

385 Haydon, M.J., Roman, A., Arshad, W., 2015. Nutrient homeostasis within the plant circadian
386 network. *Front plant sci* 6, 299-299.

387 Jamers, A., Blust, R., De Coen, W., Griffin, J.L., Jones, O.A.H., 2013. Copper toxicity in the
388 microalga *Chlamydomonas reinhardtii*: an integrated approach. *Biometals* 26, 731-740.

389 Jamers, A., Van der Ven, K., Moens, L., Robbens, J., Potters, G., Guisez, Y., Blust, R., De Coen,
390 W., 2006. Effect of copper exposure on gene expression profiles in *Chlamydomonas reinhardtii*
391 based on microarray analysis. *Aq Toxicol* 80, 249-260.

392 Jiang, Y.G., Zhu, Y.L., Hu, Z.L., Lei, A.P., Wang, J.X., 2016. Towards elucidation of the toxic
393 mechanism of copper on the model green alga *Chlamydomonas reinhardtii*. *Ecotox* 25, 1417-
394 1425.

395 Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., Salzberg, S.L., 2013. TopHat2:
396 accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions.
397 *Genome Biol* 14.

398 Kovalchuk, I., Titov, V., Hohn, B., Kovalchuka, O., 2005. Transcriptome profiling reveals
399 similarities and differences in plant responses to cadmium and lead. *Mutat Res* 570, 149-161.

400 Kupper, H., Andresen, E., 2016. Mechanisms of metal toxicity in plants. *Metallomics* 8, 269-
401 285.

402 Kupper, H., Kupper, F.C., Spiller, M., 1996. Environmental relevance of heavy metal-substituted
403 chlorophylls using the example of water plants. *J Exp Bot* 47, 259-266.

404 Lamelas, C., Pinheiro, J.P., Slaveykova, V.I., 2009. Effect of humic acid on Cd(II), Cu(II), and
405 Pb(II) uptake by freshwater algae: kinetic and cell wall speciation considerations. Environ Sci
406 Technol 43, 730-735.

407 Larras, F., Regier, N., Planchon, S., Poté, J., Renaut, J., Cosio, C., 2013. Physiological and
408 proteomic changes suggest an important role of cell walls in the high tolerance to metals of
409 *Elodea nuttallii*. J Haz Mat 263, 575-583

410 Li, H., Durbin, R., 2010. Fast and accurate long-read alignment with Burrows–Wheeler
411 transform. Bioinformatics 26, 589-595.

412 Lin, C.Y., Trinh, N.N., Fu, S.F., Hsiung, Y.C., Chia, L.C., Lin, C.W., Huang, H.J., 2013.
413 Comparison of early transcriptome responses to copper and cadmium in rice roots. Plant Mol
414 Biol 81, 507-522.

415 Lindemann, C., Fiksen, O., Andersen, K.H., Aksnes, D.L., 2016. Scaling laws in phytoplankton
416 nutrient uptake affinity. Front Mar Sci 3.

417 Manusadzianas, L., Gylyte, B., Grigutyte, R., Karitonas, R., Sadauskas, K., Vitkus, R.,
418 Siliauskas, L., Vaiciuniene, J., 2017. Accumulation of copper in the cell compartments of
419 charophyte *Nitellopsis obtusa* after its exposure to copper oxide nanoparticle suspension.
420 Environ Sci Poll Res 24, 27653-27661.

421 Martins, R.J.E., Boaventura, R.A.R., 2002. Uptake and release of zinc by aquatic bryophytes
422 (*Fontinalis antipyretica* L. ex. Hedw.). Water res 36, 5005-5012.

423 Merchant, S.S., Prochnik, S.E., Vallon, O., Harris, E.H., Karpowicz, S.J., Witman, G.B., Terry,
424 A., Salamov, A., Fritz-Laylin, L.K., Marechal-Drouard, L., Marshall, W.F., Qu, L.-H., Nelson,
425 D.R., Sanderfoot, A.A., Spalding, M.H., Kapitonov, V.V., Ren, Q., Ferris, P., Lindquist, E.,
426 Shapiro, H., Lucas, S.M., Grimwood, J., Schmutz, J., Cardol, P., Cerutti, H., Chanfreau, G.,

427 Chen, C.-L., Cognat, V., Croft, M.T., Dent, R., Dutcher, S., Fernandez, E., Fukuzawa, H.,
428 Gonzalez-Ballester, D., Gonzalez-Halphen, D., Hallmann, A., Hanikenne, M., Hippler, M.,
429 Inwood, W., Jabbari, K., Kalanon, M., Kuras, R., Lefebvre, P.A., Lemaire, S.D., Lobanov, A.V.,
430 Lohr, M., Manuell, A., Meier, I., Mets, L., Mittag, M., Mittelmeier, T., Moroney, J.V., Moseley,
431 J., Napoli, C., Nedelcu, A.M., Niyogi, K., Novoselov, S.V., Paulsen, I.T., Pazour, G., Purton, S.,
432 Ral, J.-P., Riano-Pachon, D.M., Riekhof, W., Rymarquis, L., Schroda, M., Stern, D., Umen, J.,
433 Willows, R., Wilson, N., Zimmer, S.L., Allmer, J., Balk, J., Bisova, K., Chen, C.-J., Elias, M.,
434 Gendler, K., Hauser, C., Lamb, M.R., Ledford, H., Long, J.C., Minagawa, J., Page, M.D., Pan, J.,
435 Pootakham, W., Roje, S., Rose, A., Stahlberg, E., Terauchi, A.M., Yang, P., Ball, S., Bowler, C.,
436 Dieckmann, C.L., Gladyshev, V.N., Green, P., Jorgensen, R., Mayfield, S., Mueller-Roeber, B.,
437 Rajamani, S., Sayre, R.T., Brokstein, P., Dubchak, I., Goodstein, D., Hornick, L., Huang, Y.W.,
438 Jhaveri, J., Luo, Y., Martinez, D., Ngau, W.C.A., Otilar, B., Poliakov, A., Porter, A.,
439 Szajkowski, L., Werner, G., Zhou, K., Grigoriev, I.V., Rokhsar, D.S., Grossman, A.R., 2007.
440 The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. Science
441 (New York, N.Y.) 318, 245-250.

442 Monferran, M.V., Agudo, J.A.S., Pignata, M.L., Wunderlin, D.A., 2009. Copper-induced
443 response of physiological parameters and antioxidant enzymes in the aquatic macrophyte
444 *Potamogeton pusillus*. Environ Poll 157, 2570-2576.

445 Mosulen, S., Dominguez, M.J., Vigara, J., Vilchez, C., Guiraum, A., Vega, J.M., 2003. Metal
446 toxicity in *Chlamydomonas reinhardtii*. Effect on sulfate and nitrate assimilation. Biomol Eng
447 20, 199-203.

448 Noges, T., Luup, H., Feldmann, T., 2010. Primary production of aquatic macrophytes and their
449 epiphytes in two shallow lakes (Peipsi and Vrtsjarv) in Estonia. Aq Ecol 44, 83-92.

450 Page, M.D., Kropat, J., Hamel, P.P., Merchant, S.S., 2009. Two *Chlamydomonas* CTR copper
451 transporters with a novel Cys-Met motif are localized to the plasma membrane and function in
452 copper assimilation. *Plant Cell* 21, 928-943.

453 Paz, Y., Katz, A., Pick, U., 2007. Multicopper ferroxidase involved in iron binding to transferrins
454 in *Dunaliella salina* plasma membranes. *J Biol Chem* 282, 8658-8666.

455 Penarrubia, L., Andres-Colas, N., Moreno, J., Puig, S., 2009. Regulation of copper transport in
456 *Arabidopsis thaliana*: a biochemical oscillator? *J Biol Inorg Chem* 15, 29.

457 Razinger, J., Drinovec, L., Zrimec, A., 2010. Real-time visualization of oxidative stress in a
458 floating macrophyte *Lemna minor* L. exposed to cadmium, copper, menadione, and AAPH.
459 *Environ Toxicol* 25, 573-580.

460 Regier, N., Baerlocher, L., Munsterkotter, M., Farinelli, L., Cosio, C., 2013a. Analysis of the
461 *Elodea nuttallii* transcriptome in response to mercury and cadmium pollution: development of
462 sensitive tools for rapid ecotoxicological testing. *Environ Sci Technol* 47, 8825-8834.

463 Regier, N., Beauvais-Fluck, R., Slaveykova, V.I., Cosio, C., 2016. *Elodea nuttallii* exposure to
464 mercury exposure under enhanced ultraviolet radiation: Effects on bioaccumulation,
465 transcriptome, pigment content and oxidative stress. *Aq Toxicol* 180, 218-226.

466 Regier, N., Cosio, C., von Moos, N., Slaveykova, V.I., 2015. Effects of copper-oxide
467 nanoparticles, dissolved copper and ultraviolet radiation on copper bioaccumulation,
468 photosynthesis and oxidative stress in the aquatic macrophyte *Elodea nuttallii*. *Chemosphere*
469 128, 56-61.

470 Regier, N., Larras, F., Bravo, A.G., Ungereanu, V.G., Cosio, C., 2013b. Hg bioaccumulation in
471 the macrophyte *Elodea nuttallii* in the field and in microcosm: Hg in shoots accumulated from
472 the water might involve Cu transporters. *Chemosphere* 90, 595-602.

473 Rensing, S.A., Lang, D., Zimmer, A.D., Terry, A., Salamov, A., Shapiro, H., Nishiyama, T.,
474 Perroud, P.F., Lindquist, E.A., Kamisugi, Y., Tanahashi, T., Sakakibara, K., Fujita, T., Oishi, K.,
475 Shin-I, T., Kuroki, Y., Toyoda, A., Suzuki, Y., Hashimoto, S., Yamaguchi, K., Sugano, S.,
476 Kohara, Y., Fujiyama, A., Anterola, A., Aoki, S., Ashton, N., Barbazuk, W.B., Barker, E.,
477 Bennetzen, J.L., Blankenship, R., Cho, S.H., Dutcher, S.K., Estelle, M., Fawcett, J.A., Gundlach,
478 H., Hanada, K., Heyl, A., Hicks, K.A., Hughes, J., Lohr, M., Mayer, K., Melkozernov, A.,
479 Murata, T., Nelson, D.R., Pils, B., Prigge, M., Reiss, B., Renner, T., Rombauts, S., Rushton, P.J.,
480 Sanderfoot, A., Schween, G., Shiu, S.H., Stueber, K., Theodoulou, F.L., Tu, H., Van de Peer, Y.,
481 Verrier, P.J., Waters, E., Wood, A., Yang, L.X., Cove, D., Cuming, A.C., Hasebe, M., Lucas, S.,
482 Mishler, B.D., Reski, R., Grigoriev, I.V., Quatrano, R.S., Boore, J.L., 2008. The *Physcomitrella*
483 genome reveals evolutionary insights into the conquest of land by plants. *Science* 319, 64-69.
484 Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for
485 differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139-140.
486 Rother, M., Krauss, G.J., Grass, G., Wesenberg, D., 2006. Sulphate assimilation under Cd²⁺
487 stress in *Physcomitrella patens* - combined transcript, enzyme and metabolite profiling. *Plant*
488 *Cell Environ* 29, 1801-1811.
489 Simon, D.F., Descombes, P., Zerges, W., Wilkinson, K.J., 2008. Global expression profiling of
490 *Chlamydomonas reinhardtii* exposed to trace levels of free cadmium. *Environ Toxicol Chem* 27,
491 1668-1675.
492 Stoiber, T.L., Shafer, M.M., Armstrong, D.E., 2012. Relationships between surface-bound and
493 internalized copper and cadmium and toxicity in *Chlamydomonas reinhardtii*. *Environ Toxicol*
494 *Chem* 31, 324-335.

495 Stoiber, T.L., Shafer, M.M., Armstrong, D.E., 2013. Induction of reactive oxygen species in
496 *Chlamydomonas reinhardtii* in response to contrasting trace metal exposures. Environ Toxicol
497 28, 516-523.

498 Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Muller, L.A.,
499 Rhee, S.Y., Stitt, M., 2004. MAPMAN: a user-driven tool to display genomics data sets onto
500 diagrams of metabolic pathways and other biological processes. Plant J 37, 914-939.

501 Thomas, G., Stark, H.J., Wellenreuther, G., Dickinson, B.C., Kupper, H., 2013. Effects of
502 nanomolar copper on water plants-Comparison of biochemical and biophysical mechanisms of
503 deficiency and sublethal toxicity under environmentally relevant conditions. Aq Toxicol 140,
504 27-36.

505 Thomaz, S.M., Cunha, E.R.d., 2010. The role of macrophytes in habitat structuring in aquatic
506 ecosystems: methods of measurement, causes and consequences on animal assemblages'
507 composition and biodiversity. Acta Limnol Brasil 22, 218-236.

508 Upadhyay, R.K., Sharma, G.D., Panda, S.K., 2011. Responses of antioxidant metabolism and
509 defense mechanism of aquatic macrophyte, *Pistia stratiotes* L. to zinc treatment under copper
510 stress. Proceed Nat Acad Sci India B-Biol Sci 81, 422-427.

511 Usadel, B., Poree, F., Nagel, A., Lohse, M., Czedik-Eysenberg, A., Stitt, M., 2009. A guide to
512 using MapMan to visualize and compare Omics data in plants: a case study in the crop species,
513 Maize. Plant Cell Environ 32, 1211-1229.

514 Weber, M., Trampczynska, A., Clemens, S., 2006. Comparative transcriptome analysis of toxic
515 metal responses in *Arabidopsis thaliana* and the Cd²⁺-hypertolerant facultative metallophyte
516 *Arabidopsis halleri*. Plant Cell Environ 29, 950-963.

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518

519 **Table 1:** Modelled parameters of Cu uptake in ethylenediaminetetraacetic acid (EDTA)-rinsed *E.*
520 *nutallii* or *C. reinhardtii* exposed to 10^{-6} mol·L⁻¹ Cu. Uptake flux (*a*) and elimination rate
521 constant (*k*) of Cu were divided by the effective concentration of metal at beginning of the test to
522 allow inter species comparison and are thus presented as *a'* and *k'*.

	<i>C. reinhardtii</i>	<i>E. nutallii</i>
<i>a'</i> (L·g ⁻¹ ·h ⁻¹)	0.32 ± 0.05	0.74 ± 0.87
<i>k'</i> (μmol·L ⁻¹ ·h ⁻¹)	0.07 ± 0.04	0.06 ± 0.08
R ²	0.85	0.93

523

524 Table 2: List of the 10 most up-regulated and 10 most down-regulated genes in *C. reinhardtii* and *E.nuttallii* exposed 2 h to Cu.
525 Identification, differential expression analysis (log2FC, FDR) and GO annotation are shown (NA = not assigned; Table S2 and S3
526 show complete analysis).

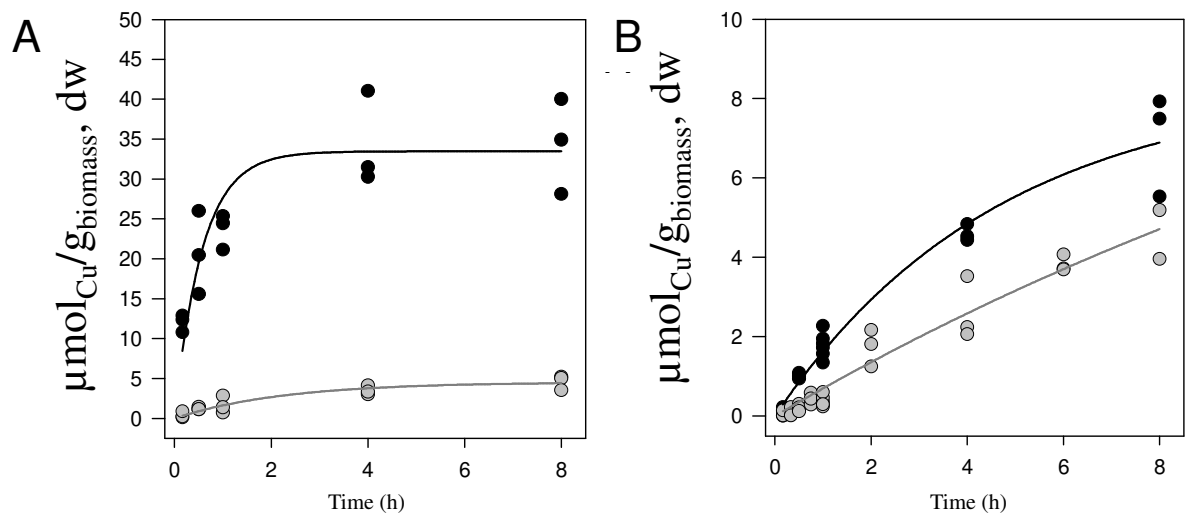
ID	log2FC	FDR	GO
<i>C. reinhardtii</i>			
g9712	5.64	6.05E-79	alpha/beta-Hydrolases superfamily protein
Cre07.g321800	4.63	1.68E-69	--NA--
Cre08.g360200	3.73	1.32E-09	solute:sodium symporters;urea transmembrane transporters
Cre12.g494600	3.55	3.89E-06	--NA--
Cre03.g173100	3.51	7.93E-83	--NA--
Cre02.g143900	3.22	2.01E-10	GDSL-like Lipase/Acylhydrolase superfamily protein
Cre12.g497550	2.90	1.05E-17	NAD(P)-binding Rossmann-fold superfamily protein
Cre10.g431050	2.88	4.50E-29	--NA--
Cre17.g737300	2.81	1.18E-69	--NA--
Cre12.g525450	-2.02	8.26E-23	--NA--
Cre13.g587350	-2.20	5.70E-17	--NA--
Cre12.g540100	-2.21	2.11E-12	--NA--
Cre06.g305100	-2.22	3.32E-18	--NA--
g5945	-2.22	2.34E-04	Histone superfamily protein
Cre12.g493100	-2.25	6.29E-07	--NA--
Cre16.g681350	-2.44	6.14E-06	--NA--
Cre16.g668850	-2.64	7.29E-44	--NA--
Cre16.g651050	-2.79	1.89E-10	Cytochrome c
Cre06.g253000	-3.12	7.92E-49	--NA--
<i>E. nuttallii</i>			
Locus_106988_Transcript_1_1_Confidence_1.000_Length_217	9.26	2.63E-12	---NA---
Locus_74420_Transcript_1_1_Confidence_1.000_Length_486	6.24	8.01E-67	---NA---
Locus_50101_Transcript_1_1_Confidence_1.000_Length_289	6.13	1.37E-10	probable e3 ubiquitin-protein ligase bah1-like 1

Locus_46396_Transcript_2_2_Confidence_0.750_Length_415	5.65	2.67E-08	---NA---
Locus_99585_Transcript_1_1_Confidence_1.000_Length_553	5.63	2.45E-34	---NA---
Locus_31732_Transcript_1_2_Confidence_0.667_Length_327	5.47	2.79E-10	---NA---
Locus_56031_Transcript_1_1_Confidence_1.000_Length_615	5.12	1.01E-36	---NA---
Locus_26963_Transcript_1_1_Confidence_1.000_Length_256	5.06	2.98E-13	---NA---
Locus_22115_Transcript_2_4_Confidence_0.375_Length_528	5.01	1.75E-06	Uncharacterized protein TCM_030494
Locus_25722_Transcript_4_7_Confidence_0.389_Length_809	4.91	1.84E-12	---NA---
Locus_5098_Transcript_3_4_Confidence_0.625_Length_746	-3.41	7.18E-05	octicosapeptide phox bem1p
Locus_155_Transcript_2_8_Confidence_0.474_Length_2350	-3.46	9.23E-29	nitrate reductase
Locus_6174_Transcript_8_8_Confidence_0.524_Length_1224	-3.52	1.18E-08	fe(2+) transport protein 1-like
Locus_6580_Transcript_4_11_Confidence_0.433_Length_1496	-3.58	4.77E-10	hpp family expressed
Locus_1727_Transcript_3_9_Confidence_0.450_Length_2249	-3.88	7.64E-12	uroporphyrinogen-iii c-methyltransferase-like
Locus_97468_Transcript_1_2_Confidence_0.667_Length_399	-4.40	9.24E-05	---NA---
Locus_97468_Transcript_2_2_Confidence_0.667_Length_399	-5.26	5.69E-11	---NA---
Locus_104442_Transcript_1_1_Confidence_1.000_Length_777	-5.40	1.72E-17	---NA---
Locus_7125_Transcript_1_3_Confidence_0.600_Length_453	-8.54	5.50E-08	---NA---
Locus_71727_Transcript_1_1_Confidence_1.000_Length_499	-8.65	6.76E-05	---NA---

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528

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530

531 **Figure 1:** Cu toxicokinetics in *C. reinhardtii* (A) and *E. nuttallii* (B) exposed to $10^{-6} \text{ mol}\cdot\text{L}^{-1}$ Cu.

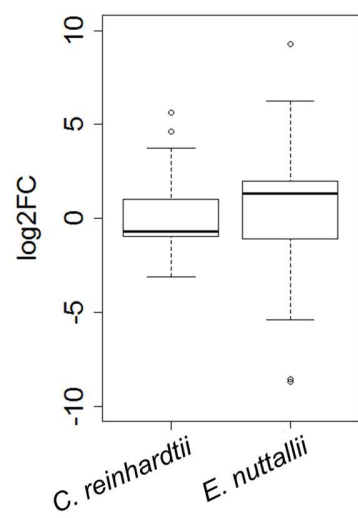
532 Organisms were ethylenediaminetetraacetic acid (EDTA)-rinsed (grey) or media-rinsed (black)

533 to differentiate between adsorbed and internalized metal.

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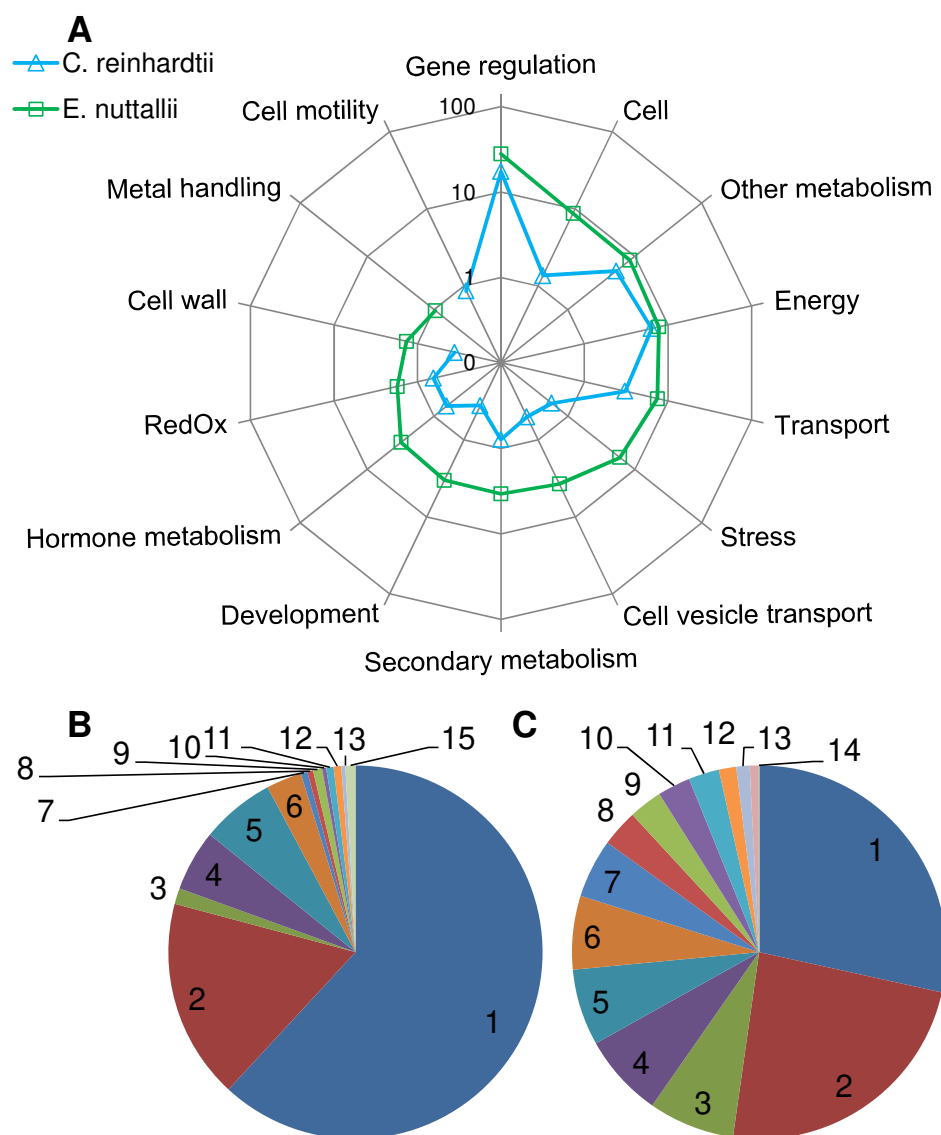
537

538 **Figure 2:** Fold-changes (log2FC) of significant regulated genes in *C. reinhardtii* and *E. nuttallii*

539 exposed 2 h to 10^{-6} mol·L⁻¹ Cu.

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541



542 **Figure 3:** Genes regulated in *C. reinhardtii* and *E. nuttallii* exposed 2 h to 10^{-6} mol·L⁻¹ Cu. The
 543 total number of significant dysregulated genes (DG) in gene ontology (GO) categories
 544 (MapMan) in *C. reinhardtii* (triangles) and *E. nuttallii* (square) exposed 2 h to Cu (A). The
 545 proportion (%) of main functional GO categories (MapMan) of dysregulated genes in *C.*
 546 *reinhartii* (B) and *E. nuttallii* (C) (GO categories legend is 1: Unknown, 2: Gene regulation, 3:
 547 Cell process, 4: Other metabolism, 5: Energy metabolism, 6: Transport, 7: Stress, 8: Cell vesicle

548 transport, 9: Secondary metabolism, 10: Development, 11: Hormone metabolism, 12: Oxidative
549 stress, 13: Cell wall, 14: Metal handling, 15: Cell Motility).