Differential Response of *Phaeodactylum tricornutum* and *Cylindrotheca fusiformis* to High Concentrations of Cu$^{2+}$ and Zn$^{2+}$

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Abstract: Diatoms can be used as biosensors to assess aquatic environment quality, because they are widely distributed in almost all aquatic environments and show varied sensitivities toward heavy metal ions. The marine planktonic diatoms *Phaeodactylum tricornutum* (*P. tricornutum*) and *Cylindrotheca fusiformis* (*C. fusiformis*) are typical representatives of planktonic diatoms and benthic diatoms, respectively. *C. fusiformis* is very sensitive to changes in the concentration of heavy metal ions, and can be used as an indicator of the quality of the sedimental environment, while *P. tricornutum* can tolerate higher concentrations of heavy metal ions. To explore the potential difference in responses to heavy metal ions between planktonic and benthic diatoms, we compared the transcriptome of *P. tricornutum* and *C. fusiformis* under Cu$^{2+}$ and Zn$^{2+}$ treatment. The results indicated that *P. tricornutum* has several genes involved in ion transmembrane transport and ion homeostasis, which are significantly downregulated under Cu$^{2+}$ and Zn$^{2+}$ treatment. However, this enrichment of ion transmembrane transport- and ion homeostasis-related genes was not observed in *C. fusiformis* under Cu$^{2+}$ and Zn$^{2+}$ treatment. Additionally, genes related to heavy metal ion stress response such as peroxiredoxin, peroxidase, catalase, glutathione metabolism, phytochelatin, oxidative stress and disulfide reductase, were upregulated in *P. tricornutum* under Cu$^{2+}$ and Zn$^{2+}$ treatment, whereas most of them were downregulated in *C. fusiformis* under Cu$^{2+}$ and Zn$^{2+}$ treatment. This difference in gene expression may be responsible for the difference in sensitivity to heavy metals between *P. tricornutum* and *C. fusiformis*.

Keywords: diatom; *Phaeodactylum tricornutum*; *Cylindrotheca fusiformis*; heavy metals; biological indicator

1. Introduction

The distribution and composition of biological communities are controlled or influenced by environmental variations such as disturbances, stressors, and biotic interactions and change in resources and hydraulic conditions [1]; therefore, such biological communities can be used as indicators of environmental conditions. Diatoms, for example, can be used as biosensors to assess aquatic environment quality, because diatoms are widely distributed in almost all aquatic environments [2], and different species of diatoms show varying sensitivities toward heavy metal ions [3]. Therefore, their species and distribution can be used as an indicator of the degree of heavy metal pollution in aquatic environments [4–7].
Since the relationship between diatoms and river pollution was revealed 70 years ago, the suitability of diatoms as bioassessment indicators for monitoring river quality has been demonstrated [8]. The sensitivity of diatoms to heavy metal ions is closely related to their response mechanisms [9]. Under high concentrations of heavy metal ions, diatoms tend to increase the synthesis of antioxidants or and metal chelators, maintain ion balance through transporters, and increase extracellular carbohydrate production [9]. Moreover, it is reported that motile diatoms can tolerate higher concentrations of heavy metal ions than non-motile diatoms [2], indicating that there might be differences in response mechanisms between planktonic and benthic diatoms.

The marine planktonic diatom *Phaeodactylum tricornutum* (*P. tricornutum*) is rich in polyunsaturated fatty acids, lipids, and fucoxanthin [10]. Therefore, it can be used as a food for aquaculture animals and as raw materials for biodiesel and health products [11,12]. Additionally, due to its clear genomic background [13], universal molecular toolbox [14], and stable transgene expression system [15,16], *P. tricornutum* is also considered as a model single-cell organism for studying physiology, evolution, and biochemistry in microalgae. *Cylindrotheca fusiformis* (*C. fusiformis*) is a benthic diatom with a weakly silicificated cell wall, and is rich in nutrients which can induce the attachment and metamorphosis of benthic animal seedlings; thus, it can be used as open bait for sea cucumbers, abalones, sea urchins, and other marine treasure seedlings [17,18]. *C. fusiformis* grows rapidly under aerated conditions, and sinks to the bottom quickly after stopping aerating, making it very easy to be collected. In addition, the suitable temperature for most diatoms ranges from 10 to 25 °C, whereas the optimum temperature for *C. fusiformis* is approximately 30 °C. This can ensure the supply of seedling bait in the high-temperature season.

Therefore, *P. tricornutum* and *C. fusiformis* are typical representatives of planktonic and benthic diatoms, respectively. A comparative analysis of *P. tricornutum* and *C. fusiformis* will help to understand the different response mechanisms of planktonic and benthic diatoms. It is reported that *C. fusiformis* is very sensitive to changes in the concentration of heavy metals, and can be used as an indicator of the quality of the sedimental environment, while *P. tricornutum* can tolerate higher concentrations of heavy metal ions [4,6,19]. We propose that this may be related to their varying response mechanisms.

In this study, we aimed to explore the potential differential responses to heavy metal ions between planktonic and benthic diatoms. We compared the growth of *P. tricornutum* and *C. fusiformis* under different Cu$^{2+}$ and Zn$^{2+}$ concentrations, and transcriptome analyses were conducted. Moreover, we explored the mechanisms by which *P. tricornutum* responds to heavy metal ions, and why *C. fusiformis* is more sensitive to heavy metal ions.

2. Materials and Methods

2.1. Cell Culture and Treatments

*P. tricornutum* and *C. fusiformis* were obtained from the Microalgae Culture Center at the Ocean University of China. For *P. tricornutum* and *C. fusiformis*, algal cells were cultured using sterilized artificial seawater supplemented with f/2 nutrients at 20 °C and with four times of f/2 nutrients (2f) at 25 °C, respectively [20]. All cultures were grown under a 12:12 darklight cycle under cool white fluorescent light (approximately 100 µmol m$^{-2}$ s$^{-1}$). Cell growth was detected by measuring the absorbance at 730 nm using a UV/visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan).

For treatment with high concentrations of Cu$^{2+}$ and Zn$^{2+}$, *P. tricornutum* and *C. fusiformis* cells were treated with Cu$^{2+}$ or Zn$^{2+}$ at final concentrations of 30 µM and 60 µM. Control cells were cultured in f/2 (for *P. tricornutum*) or 2f medium (for *C. fusiformis*). Each treatment was performed in triplicate in 250 mL flasks. Cell growth was detected on days 0, 1, 3, 5 and 7.

2.2. Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS) Analysis

For SEM-EDS analysis, *P. tricornutum* and *C. fusiformis* cells were treated with Cu$^{2+}$ at a final concentration of 5 µM (PTCu and CFCu) and Zn$^{2+}$ at a final concentration of 30 µM
Control cells were cultured in f/2 (for *P. tricornutum*, PTC) or 2f medium (for *C. fusiformis*, CFC). Each treatment was performed in triplicate in 2 L flasks. After 48 h, cell pellets were collected, further washed using distilled sea water, and centrifuged at 5000 × g for 4 min. The pellets were fixed with 2.5% glutaraldehyde (4 °C) overnight and sequentially dehydrated for 15 min each in 30%, 50%, 70%, 80%, 90%, 100% and 100% EtOH, followed by CO₂ critical point drying. Dried cells were placed on a conductive silicone rubber plate and treated with Gold sputtering, then viewed under the SEM (Hitachi’s TM4000 Plus, Hitachi Limited, Tokyo, Japan). EDS was performed with IXRF’s TM4-EDS.

2.3. Transcriptomic Analysis

For transcriptomic analysis, *P. tricornutum* and *C. fusiformis* cells were treated with Cu²⁺ at a final concentration of 5 µM (PTCu and CFCu) and Zn²⁺ at a final concentration of 30 µM (PTZn and CFZn). Control cells were cultured in f/2 (for *P. tricornutum*, PTC) or 2f medium (for *C. fusiformis*, CFC). Each treatment was performed in triplicate in 2 L flasks. After 48 h, cell pellets were collected, further washed using distilled sea water, and centrifuged at 5000 × g for 4 min. The pellets were frozen in liquid nitrogen and stored at −80 °C.

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. High-quality total RNA (OD260/280 = 1.8–2.2, OD260/230 ≥ 2.0, RIN ≥ 6.5, 28S:18S ≥ 1.0, >2 µg) was used to construct cDNA libraries for high-throughput RNA sequencing. Overall, 1 µg of total RNA was used to construct an RNA-seq transcriptome library, using the TruSeqTM RNA sample preparation Kit from Illumina (Illumina, San Diego, CA, USA) as per the manufacturer’s instructions. Furthermore, cDNA libraries were selected for cDNA target fragments of 200–300 base pairs in 2% low-range ultra-agarose, and further amplified using Phusion DNA polymerase (New England Biolabs (Beijing), Beijing, China). The amplified cDNA libraries were loaded into a NovaSeq 6000 sequencing system Illumina (Illumina, San Diego, CA, USA).

To generate clean reads, raw sequence reads were trimmed using SeqPrep (https://github.com/jstjohn/SeqPrep accessed on 5 October 2016), and the quality of the raw reads was controlled using Sickle (https://github.com/najoshi/sickle accessible on 15 March 2015) with default parameters. The clean reads were annotated according to Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Clusters of Orthologous Groups of proteins (COG), the NCBI non-redundant protein sequences database (NR), Swiss-Prot, and Pfam databases. The mapped reads were further normalized using the reads per kb per million methods for the identification of differentially expressed genes (DEGs). Abundant genes were quantified using RSEM (http://deweylab.biostat.wisc.edu/rsem/ accessed on 14 February 2020) [21]. Differential gene expression was determined using the “edgeR” package in R (http://www.bioconductor.org/packages/2.12/bioc/html/edgeR.html accessed on 9 May 2018), based on the following threshold parameters: log2 fold-change > 2 and p-value < 0.05 [22]. Functional annotation and enrichment analyses were performed and classified using the GO and KEGG databases.

3. Results

3.1. Effects of Cu²⁺ and Zn²⁺ on Growth of *P. tricornutum* and *C. fusiformis*

To investigate the effect of Cu²⁺, Zn²⁺ on *P. tricornutum* and *C. fusiformis*, we compared the growth rates of *P. tricornutum* and *C. fusiformis* at different concentrations (0, 30 and 60 µM Cu²⁺ and 0, 30 and 60 µM Zn²⁺). The results showed that 60 µM Cu²⁺ significantly decreased the growth of *P. tricornutum* (p-value < 0.05), while the influence of 30 µM Cu²⁺ was not significant (p-value > 0.05), although there was also a tendency to decrease compared with that of the control group (Figure 1a). Both 30 µM and 60 µM Cu²⁺ significantly decreased the growth of *C. fusiformis* (Figure 1b). Neither 30 nor 60 µM Zn²⁺ significantly influenced the growth of *P. tricornutum* (Figure 1a), while 60 µM Zn²⁺ decreased the growth of *C. fusiformis* on day 5 (Figure 1b). As both 30 µM and 60 µM Cu²⁺ significantly decreased the growth of *C. fusiformis*, the concentration of Cu²⁺ for the transcriptomic analysis was set...
to a lower level (5 μM). As 30 μM Zn$^{2+}$ did not significantly influence the growth of both *P. tricornutum* and *C. fusiformis*, the concentration of Zn$^{2+}$ for the transcriptomic analysis was set to 30 μM.

![Graph A](image1.png)

**Figure 1.** Growth of *P. tricornutum* (a) and *C. fusiformis* (b) under different Cu$^{2+}$ or Zn$^{2+}$ concentrations (0, 30 and 60 μM). Data points are the means of triplicates, and error bars represent the standard deviation.

### 3.2. Effects of Cu$^{2+}$ and Zn$^{2+}$ on Cell Morphology of *P. tricornutum* and *C. fusiformis*

To investigate the effect of Cu$^{2+}$ and Zn$^{2+}$ on cell morphology of *P. tricornutum* and *C. fusiformis*, we observed the cells with SEM. The results showed that both Cu$^{2+}$ and Zn$^{2+}$ did not significantly change the cell morphology of *P. tricornutum* (Figure 2a–c), while both Cu$^{2+}$ and Zn$^{2+}$ significantly changed the cell morphology of *C. fusiformis* (Figure 2d–f).
indicated that *P. tricornutum* was tolerant to Cu$^{2+}$ and Zn$^{2+}$, while *C. fusiformis* was more sensitive to Cu$^{2+}$ and Zn$^{2+}$.

Figure 2. Cell morphology of *P. tricornutum* (a–c) and *C. fusiformis* (d–f) under different Cu$^{2+}$ or Zn$^{2+}$ concentrations (control, 5 μM Cu$^{2+}$, and 30 μM Zn$^{2+}$) recorded on TM4000 Plus SEM. The scale represents 10 μm.

3.3. Accumulation of Cu$^{2+}$ and Zn$^{2+}$ on Biosilica Shell of *P. tricornutum* and *C. fusiformis*

To investigate the accumulation of Cu$^{2+}$ and Zn$^{2+}$ on the biosilica shell of *P. tricornutum* and *C. fusiformis*, EDS was conducted to analyze the concentration of Si, Cu and Zn on the cell surface of *P. tricornutum* and *C. fusiformis*. The results showed that in the control group (containing 0.04 μM Cu$^{2+}$ and 0.08 μM Zn$^{2+}$ in the medium) of *P. tricornutum*, the content of Cu and Zn was 16.72% and 13.76% (Figure 3a), respectively. In the Cu$^{2+}$ group (containing 5 μM Cu$^{2+}$ and 0.08 μM Zn$^{2+}$ in the medium) of *P. tricornutum*, the content of Cu and Zn was 18.70% and 13.75% (Figure 3b), respectively. In the Zn$^{2+}$ group (containing 0.04 μM Cu$^{2+}$ and 30 μM Zn$^{2+}$ in the medium) of *P. tricornutum* (Figure 3c), the content of Cu and Zn was 6.53% and 17.76%, respectively. While in the control group (containing 0.16 μM Cu$^{2+}$ and 0.32 μM Zn$^{2+}$ in the medium) of *C. fusiformis*, the content of Cu and Zn was 23.53% and 14.58% (Figure 3e), respectively. In the Zn$^{2+}$ group (containing 0.16 μM Cu$^{2+}$ and 30 μM Zn$^{2+}$ in the medium) of *C. fusiformis*, the content of Cu and Zn was 9.92% and 20.81% (Figure 3f), respectively. These results indicated that both *P. tricornutum* and *C. fusiformis* accumulated Cu and Zn on the cell surface.
To investigate the potential effect of Cu^{2+} and Zn^{2+} on gene transcription in *P. tricornutum*, we analyzed the transcriptome of *P. tricornutum* exposed to 5 µM Cu^{2+} (PTCu) and 30 µM Zn^{2+} (PTZn) for 48 h, with control (PTC) with no treatment of heavy metals. An average of 46,069,016 raw reads and 45,693,261 clean reads were generated from the total RNA extracted from *P. tricornutum*. A total of 98.39% of the clean read bases had a Q-value ≥ 20, and 94.99% of the clean read bases had a Q-value ≥ 30 (Table S1). De novo assembly generated 10,754 unigenes, including 10,167 known genes and 587 new genes. Figure 4 shows the length distribution of unigenes in *P. tricornutum*.

**Figure 3.** EDS analysis of *P. tricornutum* (a–c) and *C. fusiformis* (d–f) under different Cu^{2+} or Zn^{2+} concentrations (control, 5 µM Cu^{2+}, and 30 µM Zn^{2+}) recorded on TM4-EDS. The box in the figure indicates the area scanned by EDS. The data in the figure reflects the percentage of Si, Cu, and Zn elements.

**Figure 4.** Length distribution of transcripts in *P. tricornutum*. 

3.4. Effects of Cu^{2+} and Zn^{2+} on Gene Transcription in *P. tricornutum*

3.4.1. Annotation of *P. tricornutum* Transcriptome

To investigate the potential effect of Cu^{2+} and Zn^{2+} on gene transcription in *P. tricornutum*, we analyzed the transcriptome of *P. tricornutum* exposed to 5 µM Cu^{2+} (PTCu) and 30 µM Zn^{2+} (PTZn) for 48 h, with control (PTC) with no treatment of heavy metals. An average of 46,069,016 raw reads and 45,693,261 clean reads were generated from the total RNA extracted from *P. tricornutum*. A total of 98.39% of the clean read bases had a Q-value ≥ 20, and 94.99% of the clean read bases had a Q-value ≥ 30 (Table S1). De novo assembly generated 10,754 unigenes, including 10,167 known genes and 587 new genes. Figure 4 shows the length distribution of unigenes in *P. tricornutum*. 

**Figure 3.** Length distribution of transcripts in *P. tricornutum*. 

3.4. Effects of Cu^{2+} and Zn^{2+} on Gene Transcription in *P. tricornutum*
The acquired unigenes were annotated according to the GO, KEGG, COG, NR, Swiss-Prot, and Pfam databases. Of all the assembled unigenes, 82.28%, 46.02%, 72.75%, 99.19%, 56.1%, and 74.18% were annotated by GO, KEGG, COG, NR, Swiss-Prot, and Pfam, respectively (Figure 5, Table S2).

Figure 5. Functional annotation of unigenes in P. tricornutum.

3.4.2. Identification and Functional Enrichment Analysis of Different Express Genes (DEGs) in P. tricornutum upon Cu^{2+} Treatment

Transcriptome analysis of DEGs in P. tricornutum exposed to 5 μM Cu^{2+} was performed, using high-throughput RNA sequencing. A total of 2006 genes, including 1119 up- and 887 downregulated genes were detected to be significantly regulated (p < 0.05) under Cu^{2+} treatment, with a 2-fold change in abundance considered as the criterion of biologically significant difference (Table S3). DEGs were classified into three main functional categories of GO terms: molecular function (MF), biological process (BP), and cellular component (CC; Figure 6). The GO enrichment analysis for upregulated genes is shown in Figure 6a, in which only 20 annotation categories with the most significantly enriched DEPs are shown. For BP, DEGs were assigned to 13 subcategories involved in photosynthesis, carbon metabolism, and energy metabolism, with the three most abundant clusters being ‘protein-chromophore linkage’, ‘photosynthesis, light harvesting in photosystem I’, and ‘photosynthesis, light harvesting’. For CC, DEGs were classified into five subcategories involved in photosynthesis, ‘thylakoid membrane’, ‘chloroplast thylakoid membrane’, ‘plastid thylakoid membrane’, ‘photosynthetic membrane’, and ‘light-harvesting complex’. In the MF category, DEGs were divided into the two subcategories ‘chlorophyll-binding’ and ‘tetrapyrrole binding’. The GO enrichment analysis for downregulated genes is shown in Figure 6b, in which only 20 annotation categories with the most significantly enriched DEPs are shown. For BP, DEGs were assigned to 11 subcategories involved in metal ion homeostasis, cation homeostasis, and ion transport. For CC, DEGs were classified into four subcategories involved in the integral component of (plasma) membrane and intrinsic component of (plasma) membrane. In the MF category, the DEGs were divided into four subcategories involved in (inorganic) cation and inorganic molecular entity transmembrane transporter activity.

Overall, 19 DEGs involved in heavy metal ion stress response are listed in Table 1. These genes were mainly related to antioxidants such as peroxiredoxin, peroxidase, catalase, glutathione metabolism, phytochelatin, oxidative stress, and disulfide reductase. Most (14
out of 19) of these genes were upregulated, indicating their important roles in response to the high concentration of heavy metal ions.

Table 1. DEGs involved in heavy metal stress response in *P. tricornutum* under Cu$^{2+}$ treatment.

| Gene_id   | fc  | Regulate | nr                                      | Paths                                                                 |
|-----------|-----|----------|-----------------------------------------|----------------------------------------------------------------------|
| Pt04g03550| 5.7 | up       | XP_002181744.1 (predicted protein)       | map00480 (Glutathione metabolism); map00053 (Ascorbate and aldarate metabolism) |
| Pt05g02260| 2.1 | up       | XP_002186090.1 (catalase-peroxidase)    | map00360 (Phenylalanine metabolism); map00380 (Tryptophan metabolism) | Catalase-peroxidase
Table 1. DEGs involved in heavy metal stress response in *P. tricornutum* under Cu\(^{2+}\).

| Gene_id      | fc  | Regulate | nr                          | Paths                                                                 | Swissprot                      |
|--------------|-----|----------|-----------------------------|----------------------------------------------------------------------|--------------------------------|
| Pt04g03550   | 5.7 | up       | XP_002181744.1 (predicted protein) | map00480 (Glutathione metabolism); map00053 (Ascorbate and aldarate metabolism) | Probable L-ascorbate peroxidase |
| Pt05g02260   | 2.1 | up       | XP_002186090.1 (catalase-peroxidase) | (Phenylalanine metabolism); map00380 (Tryptophan metabolism) | Catalase-peroxidase |
| Pt08g02130   | 30.8| up       | XP_002179007.1 (predicted protein) | map00480 (Glutathione metabolism) | Probable cytosol aminopeptidase |
| Pt14g00980   | 0.3 | down     | XP_002181057.1 (predicted protein) | |                                  |
| Pt02g05550   | 3.3 | up       | XP_002177701.1 (predicted protein) | map00480 (Glutathione metabolism) |                                |
| Pt03g03150   | 0.4 | down     | XP_002185216.1 (glyoxalase) | map00620 (Pyruvate metabolism) | Hydroxyacylglutathione hydrolase |
| Pt02g03960   | 2.8 | up       | XP_00217790.1 (predicted protein) | map00620 (Pyruvate metabolism) |                                |
| Pt07g01050   | 0.4 | down     | XP_002185856.1 (predicted protein) | |                                |
| Pt15g02690   | 9.6 | up       | XP_002182163.1 (predicted protein) | map00480 (Glutathione metabolism) | Glutathione S-transferase DHAR2 |
| Pt12g00930   | 2.7 | up       | XP_002180005.1 (predicted protein) | map00480 (Glutathione metabolism) |                                |
| Pt01g09200   | 0.5 | down     | XP_002177254.1 (predicted protein, partial) | | Glutathione gamma-glutamylcysteinytransferase |
| Pt11g01900   | 10.3| up       | XP_002182079.1 (predicted protein) | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Phospholipid hydroperoxide glutathione peroxidase |
| Pt14g03650   | 2.1 | up       | XP_002180739.1 (glutathione peroxidase, partial) | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Phospholipid hydroperoxide glutathione peroxidase |
| Pt05g02470   | 2.2 | up       | XP_002186390.1 (predicted protein) | |                                |
| Pt11g01090   | 5.9 | up       | XP_002182079.1 (predicted protein) | |                                |
| Pt07g04170   | 5.1 | up       | XP_002176312.1 (peroxidase domain-containing protein) | | Putative heme-binding peroxidase |
| Pt11g03130   | 17.5| up       | XP_002181851.1 (predicted protein) | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Phospholipid hydroperoxide glutathione peroxidase |
| Pt14g03650   | 2.1 | up       | XP_002180739.1 (glutathione peroxidase, partial) | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Phospholipid hydroperoxide glutathione peroxidase |
| Pt21g01220   | 0.3 | down     | XP_002183862.1 (predicted protein) | |                                |

3.4.3. Effects of Zn\(^{2+}\) on Gene Transcription in *P. tricornutum*

Transcriptome analysis of differential gene expression in *P. tricornutum* exposed to 30 µM Zn\(^{2+}\) was performed, using high-throughput RNA sequencing. A total of 4043 genes, including 2184 up- and 1859 downregulated genes were detected to be significantly regulated (p < 0.05) under Zn\(^{2+}\) treatment (Table S4). The GO enrichment analysis for DEGs in *P. tricornutum* under Zn\(^{2+}\) treatment is shown in Figure 7, in which only 20 annotation categories with the most significantly enriched DEGs are shown. The GO enrichment for DEGs in *P. tricornutum* under Zn\(^{2+}\) treatment was similar to that under Cu\(^{2+}\) treatment, in which the upregulated genes were mainly involved in photosynthesis (Figure 7a), whereas
the downregulated genes were mainly involved in ion homeostasis, cation homeostasis, and ion transport (Figure 7b).

Figure 7. Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs) in *P. tricornutum* under Zn\(^{2+}\) treatment. (a) Upregulated genes in PTZn/PTC. (b) Downregulated genes in PTZn/PTC.

Overall, 24 DEGs involved in heavy metal ion stress response are listed in Table 2. These genes were mainly related to antioxidants such as peroxidase, catalase, peroxiredoxin, glutathione metabolism, phytochelatin biosynthetic process, oxidative stress, mutase superoxide dismutase, and disulfide reductase. Most (17 out of 24) of these genes were upregulated, indicating their important roles in response to the high concentration of heavy metal ions.
Table 2. DEGs involved in heavy metal stress response in *P. tricornutum* under Zn^{2+}.

| Gene_id      | fc    | Regulate | nr                           | Paths                                                                 | Swissprot                      |
|--------------|-------|----------|------------------------------|----------------------------------------------------------------------|--------------------------------|
| Pt04g03550   | 6.91  | up       | XP_002181744.1 (predicted protein) | map00480 (Glutathione metabolism); map00053 (Ascorbate and aldarate metabolism) | Probable L-ascorbate peroxidase 8 |
| Pt20g01650   | 2.55  | up       | XP_002182954.1 (catalase)     | map00630 (Glyoxylate and dicarboxylate metabolism); map00380 (Tryptophan metabolism); map04146 (Peroxisome) | Catalase                       |
| Pt13g01910   | 0.50  | down     | XP_002180867.1 (predicted protein) | map00480 (Glutathione metabolism) | Peroxiredoxin-6                |
| Pt23g00220   | 2.22  | up       | XP_002184868.1 (predicted protein) | map00630 (Glyoxylate and dicarboxylate metabolism); map00380 (Tryptophan metabolism); map04146 (Peroxisome) | Gpx-1                           |
| Pt10g01030   | 0.03  | down     | XP_002179508.1 (predicted protein) | map00480 (Glutathione metabolism) | Putative heme-binding peroxidase |
| Pt05g04280   | 9.78  | up       | XP_002186195.1 (UDF-glucose 6-dehydrogenase) | map00520 (Amino sugar and nucleotide sugar metabolism); map00040 (Pentose and glucuronate interconversions); map00053 (Ascorbate and aldarate metabolism) | GDP-mannose 3,5-epimerase        |
| Pt14g01270   | 0.24  | down     | XP_002180872.1 (l-ascorbate peroxidase, partial) | map00480 (Glutathione metabolism); map00053 (Ascorbate and aldarate metabolism) | Glutathione hydrolase-like YwrD proenzyme |
| Pt16g00880   | 6.11  | up       | XP_002179589.1 (nad-dependent epimerase/dehydratase) | map00460 (Cysaoamino acid metabolism); map00480 (Glutathione metabolism); map00430 (Taurine and hypotaurine metabolism) | Probable phospholipid hydroperoxide glutathione peroxidase |
| Pt08g03190   | 5.92  | up       | XP_002178726.1 (predicted protein) | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) |                                 |
| Pt04g01510   | 0.39  | down     | XP_002183098.1 (glutathione peroxidase domain-containing protein) | map00480 (Glutathione metabolism); map02010 (ABC transporters) |                                 |
| Pt02g03960   | 2.85  | up       | XP_002177790.1 (predicted protein) | map00480 (Glutathione metabolism); map02010 (ABC transporters) |                                 |
| Pt12g00930   | 4.58  | up       | XP_00218005.1 (predicted protein) | map00480 (Glutathione metabolism); map02010 (ABC transporters) |                                 |
| Pt21g02200   | 25.23 | up       | XP_002183815.1 (predicted protein) | map00480 (Glutathione metabolism); map02010 (ABC transporters) |                                 |
| Pt18g02190   | 6.06  | up       | XP_002185391.1 (predicted protein) |                                 |                                 |
| Pt05g02470   | 2.74  | up       | XP_002186390.1 (predicted protein) |                                 |                                 |
| Pt23g01150   | 8.11  | up       | XP_002184892.1 (predicted protein) |                                 |                                 |
| Pt08g02730   | 0.47  | down     | GAX19067.1 (hypothetical protein FisN_8Hh293 [Fistulifera solaris]) |                                 | ABC transporter G family member 1 |
Table 2. Cont.

| Gene_id     | fc  | Regulate | nr                                | Paths                              | Swissprot                      |
|-------------|-----|----------|-----------------------------------|------------------------------------|--------------------------------|
| Pt12g03160  | 0.48| down     | XP_002180322.1 (glutathione reductase) | map00480 (Glutathione metabolism)  | Glutathione reductase          |
|             |     |          | XP_002177253.1                     |                                    |                                |
| PtUn01s113  | 5.37| up       | XP_002180497.1 (mutase superoxide dismutase) | map04146 (Peroxisome)  | Superoxide dismutase           |
| Pt13g02930  | 12.70| up       | [Fe/Mn], partial                   | map04146 (Peroxisome)  | Superoxide dismutase           |
| Pt01g09190  | 7.47| up       | XP_002186201.1 (mutase superoxide dismutase) | map04146 (Peroxisome)  | Superoxide dismutase           |
| Pt05g04470  | 0.40| down     | XP_002176312.1 (5′-Nucleotidase or metallophosphoesterase) |                                    |                                |
| Pt07g04170  | 2.73| up       | (peroxidase domain-containing protein) | Putative heme-binding peroxidase |                                |
| Pt20g01220  | 2.27| up       | XP_002182845.1 (predicted protein) | map04146 (Peroxisome)  | Peroxiredoxin-2C              |

3.5. Effects of Cu^{2+} and Zn^{2+} on Gene Transcription in C. fusiformis

3.5.1. Annotation of C. fusiformis Transcriptome

To investigate the potential effect of Cu^{2+} and Zn^{2+} treatment on transcription in C. fusiformis, we analyzed the transcriptome of C. fusiformis exposed to 5 μM Cu^{2+} (CFCu) and 30 μM Zn^{2+} (CFZn) for 48 h, with no addition of heavy metal ions as the control (CFC). An average of 43,832,802 raw reads and 43,323,647 clean reads were generated from total RNA extracted from C. fusiformis. A total of 98.25% of the clean read bases had a Q-value ≥ 20, and 94.64% of the clean read bases had a Q-value ≥ 30 (Table S5). De novo assembly generated 26,146 unigenes. Figure 8 shows the length distribution of unigenes.

Figure 8. Length distribution of transcripts in C. fusiformis.
The acquired unigenes were annotated according to GO, KEGG, COG, NR, Swiss-Prot, and Pfam databases. Of all the assembled unigenes, 36.72%, 35.9%, 56.07%, 38.88%, 39.87%, and 55.78% were annotated by GO, KEGG, COG, NR, Swiss-Prot, and Pfam, respectively (Figure 9, Table S6).

Figure 9. Functional annotation of unigenes in C. fusiformis.

3.5.2. Effects of Cu\(^{2+}\) on Gene Transcription in C. fusiformis

Transcriptome analysis of differential gene expression in C. fusiformis exposed to 5 \(\mu\)M Cu\(^{2+}\) was performed, using high-throughput RNA sequencing. A total of 1133 genes, including 315 up- and 818 downregulated genes were detected to be significantly regulated (\(p < 0.05\)) under Cu\(^{2+}\) treatment (Table S7). The GO enrichment analysis for upregulated genes is shown in Figure 10a, in which only 20 annotation categories with the most significantly enriched DEPs are shown. For BP, DEGs were assigned to 17 subcategories involved in signal transduction, nucleotide biosynthetic, organophosphate biosynthetic, etc. For CC, DEGs were classified into 2 subcategories, the plasma membrane. In the MF category, the unigenes were divided into 2 subcategories, \(3',5'\)-cyclic-nucleotide phosphodiesterase activity, and cyclic-nucleotide phosphodiesterase activity. The GO enrichment analysis for downregulated genes is shown in Figure 10b. For BP, no DEGs were enriched. For CC, DEGs were classified into 2 subcategories as an intrinsic component of the membrane and integral component of the membrane. In the MF category, the unigenes were divided into 3 subcategories, phospholipid transporter, glutamyl-tRNA reductase, and lipase activities.

Overall, 8 DEGs involved in antioxidants are listed in Table 3, including 1 peroxiredoxin, 1 glutathione synthetase, 1 glutathione S-transferase, 1 glutathione peroxidase, 1 hydroxyacylglutathione hydrolase, 1 deaminated glutathione amidase, and 1 peroxinectin. In total, 7 out of 8 genes were downregulated, indicating considerable differences between C. fusiformis and P. tricornutum in response to the high concentration of heavy metal ions.
significantly enriched DEPs are shown. For BP, DEGs were assigned to 17 subcategories involved in signal transduction, nucleotide biosynthetic, organophosphate biosynthetic, etc. For CC, DEGs were classified into 1 subcategory, the plasma membrane. In the MF category, the unigenes were divided into 2 subcategories, 3ʹ,5ʹ-cyclic-nucleotide phosphodiesterase activity, and cyclic-nucleotide phosphodiesterase activity. The GO enrichment analysis for downregulated genes is shown in Figure 10b. For BP, no DEGs were enriched. For CC, DEGs were classified into 2 subcategories as an intrinsic component of the membrane and integral component of the membrane. In the MF category, the unigenes were divided into 3 subcategories, phospholipid transporter, glutamyl-tRNA reductase, and lipase activities.

Figure 10. Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs) in C. fusiformis under Cu²⁺ treatment. (a) Upregulated genes in CFCu/CFC. (b) Downregulated genes in CFCu/CFC.

Table 3. DEGs involved in heavy metal stress response in C. fusiformis under Cu²⁺.

| Gene_id              | nr_Description       | fc  | Regulate |Paths | Swissprot |
|----------------------|----------------------|-----|----------|------|-----------|
| TRINITY_DN14518_c0_g1| thioredoxin-like protein | 2.68| up       | map00940 (Phenylpropanoid biosynthesis)| 1-Cys peroxiredoxin A |
| TRINITY_DN1479_c0_g1| glutathione synthetase| 0.28| down     | map00270 (Cysteine and methionine metabolism); map00480 (Glutathione metabolism)| Glutathione synthetase |
| TRINITY_DN495_c0_g2 | hypothetical protein | 0.19| down     | map00480 (Glutathione metabolism); map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism)| Hydroperoxy fatty acid reductase gpx1 |
| TRINITY_DN7366_c1_g1| glutathione peroxidase| 0.43| down     | map00480 (Glutathione metabolism); map00480 (Glutathione metabolism)| Hydroxacylglutathione hydrolase |
| TRINITY_DN1758_c0_g1| hydroxacylglutathione hydrolase | 0.45| down     | map00620 (Pyruvate metabolism); map00790 (Folate biosynthesis)| 
| TRINITY_DN3215_c0_g1| hypothetical protein | 0.46| down     | map00270 (Cysteine and methionine metabolism); map00480 (Glutathione metabolism)| Glutamate–cysteine ligase catalytic subunit |
| TRINITY_DN2680_c0_g1| hypothetical protein | 0.49| down     | map00480 (Glutathione metabolism); map00480 (Glutathione metabolism)| 
| TRINITY_DN6304_c0_g1| hypothetical protein | 0.38| down     | | 

3.5.3. Effects of Zn²⁺ on gene transcription in C. fusiformis

Transcriptome analysis of differential gene expression in C. fusiformis exposed to 30 µM Zn²⁺ was performed using high-throughput RNA sequencing. A total of 1900 genes, including 854 up- and 1046 downregulated genes were detected to be significantly regulated (p < 0.05) under Zn²⁺ treatment (Table S8). The GO enrichment analysis for upregulated
genes is shown in Figure 11a. For BP, DEGs were enriched in 1 subcategory of cellular modified amino acid metabolic process. For CC, DEGs were classified into 3 subcategories, 3-oxoacyl-[acyl-carrier-protein] synthase activity, arginase activity, and cullin family protein binding. The GO enrichment analysis for downregulated genes is shown in Figure 11b, in which only 20 annotation categories with the most significantly enriched DEPs are shown. For BP, DEGs were assigned to 8 subcategories involved in the regulation of biological quality, homeostasis, posttranslational modification (amino acid modification), organelle assembly, response to topologically incorrect protein, etc. For CC, DEGs were classified into 3 subcategories as an intrinsic component of membrane, an integral component of membrane, and endoplasmic reticulum lumen. In the MF category, the unigenes were divided into 9 subcategories involved in catalytic activity, ATPase activity, tubulin (cytoskeletal protein, calcium ion, microtubule) binding, primary active transmembrane transporter activity, and protein kinase activity.

Figure 11. Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs) in C. fusiformis under Zn$^{2+}$ treatment. (a) Upregulated genes in CFZn/CFC. (b) Downregulated genes in CFZn/CFC.

Overall, 16 DEGs involved in antioxidants are listed in Table 4, including 1 thioredoxin-like protein, 1 glutathione synthetase, 4 glutathione S-transferase, peroxiredoxin, 1 glutathionyl-hydroquinone reductase, 1 glutathione peroxidase, 2 phytochelatin biosynthesis-related genes, 1 light-harvesting complex stress-related protein, 1 thyroid peroxidase, 1 oxidative stress-related Abc1-like protein, 1 catalase-peroxidase, 1 methionine sulfoxide reductase, and 1 peroxinectin. Half of them were downregulated, and the rest were upregulated, which is different from the result under Cu$^{2+}$ treatment.
Table 4. DEGs involved in heavy metal stress response in *C. fusiformis* under Zn$^{2+}$.

| Gene_id          | nr_Description                      | fc     | Significant | Regulate | Paths                                                                 | Swissprot                        |
|------------------|-------------------------------------|--------|-------------|----------|----------------------------------------------------------------------|----------------------------------|
| TRINITY_DN14518_c0_g1 | thioredoxin-like protein            | 3.17   | yes         | up       | map00940 (Phenylpropanoid biosynthesis)                              | 1-Cys peroxiredoxin A           |
| TRINITY_DN1479_c0_g1 | glutathione synthetase              | 0.33   | yes         | down     | map00270 (Cysteine and methionine metabolism); map00480 (Glutathione metabolism) | Glutathione synthetase           |
| TRINITY_DN1711_c0_g1 | hypothetical protein                | 2.81   | yes         | up       | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Glutathione S-transferase        |
| TRINITY_DN1711_c0_g2 | hypothetical protein                | 6.12   | yes         | up       | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Glutathione S-transferase 1      |
| TRINITY_DN2013_c0_g1 | glutathione-S-transferase           | 0.44   | yes         | down     | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Glutathione S-transferase        |
| TRINITY_DN327_c0_g2 | glutathione S-transferase           | 2.73   | yes         | up       | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Glutathione S-transferase 1      |
| TRINITY_DN6449_c0_g1 | hypothetical protein                | 2.06   | yes         | up       | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Glutathionyl-hydroquinone reductase |
| TRINITY_DN7366_c1_g1 | glutathione peroxidase              | 0.37   | yes         | down     | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | Hydroperoxy fatty acid reductase  |
| TRINITY_DN2338_c0_g3 | hypothetical protein                | 0.40   | yes         | down     | map00590 (Arachidonic acid metabolism); map00480 (Glutathione metabolism) | DEP domain-containing mTOR-interacting protein General L-amino acid-binding periplasmic protein Aap Light-harvesting complex stress-related protein Thyroid peroxidase Protein ACTIVITY OF BC1 COMPLEX KINASE 8 |
| TRINITY_DN3_c0_g4  | mercuric reductase                  | 0.43   | yes         | down     | map00196 (Photosynthesis—Antenna proteins)                           |                                  |
| TRINITY_DN17353_c0_g1 | LhcSR                              | 2.98   | yes         | up       | map00196 (Photosynthesis—Antenna proteins)                           |                                  |
| TRINITY_DN1775_c0_g1 | hypothetical protein                | 0.30   | yes         | down     | map00196 (Photosynthesis—Antenna proteins)                           |                                  |
| TRINITY_DN319_c0_g1  | oxidative stress-related Abc1-like protein | 2.10   | yes         | up       | map00196 (Photosynthesis—Antenna proteins)                           |                                  |
Table 4. Cont.

| Gene_id          | nr_Description          | fc  | Significant | Regulate | Paths                                                                 |
|------------------|-------------------------|-----|-------------|----------|-----------------------------------------------------------------------|
| TRINITY_DN3894_c1_g1 | catalase peroxidase     | 2.40| yes         | up       | map00940 (Phenylpropanoid biosynthesis); map00380 (Tryptophan metabolism); map00360 (Phenylalanine metabolism) |
| TRINITY_DN5279_c0_g2 | methionine sulfoxide reductase B | 0.45| yes         | down     | Peptide methionine sulfoxide reductase                                |
| TRINITY_DN6304_c0_g1 | hypothetical protein     | 0.39| yes         | down     | Peroxinectin A                                                       |

4. Discussion

Cu²⁺ and Zn²⁺ are crucial micronutrients for diatoms. When Cu²⁺ and Zn²⁺ are present in an adequate amount, diatoms exhibit a stronger fitness and grow faster. Cu²⁺ and Zn²⁺ are components of many enzymes in algae cells. Cu²⁺ is involved in the electron transport of photosynthesis by serving as a ligand of cytochrome oxidase and plastocyanin [23,24]. In addition, Cu²⁺ is a component of Cu-tyroninase and multicopper oxidase, which is involved in Fe-deficiency response [25,26]. Zn²⁺ is an important component for carbonic anhydrases, which are involved in CO₂ fixation, and zinc finger transcription factors, which are involved in gene transcription [27,28]. In addition, both Cu²⁺ and Zn²⁺ are important for Cu/Zn-SOD (superoxide dismutase) which is involved in anti-oxidation [29]. However, excess Cu²⁺ or Zn²⁺ will interfere with cellular physiology and biological processes, resulting in decreased cell growth and even death. Different types of cells have different types and amounts of enzymes, thus their demands for Cu²⁺ and Zn²⁺ are various. Meanwhile, as the shielding and permeation properties of cell membranes for heavy metal ions are different in various species, their tolerances to heavy metal ions are also various. In this study, the growth of both *P. tricornutum* and *C. fusiformis* was inhibited at 60 µM Cu²⁺, while 30 µM Cu²⁺ decreased the growth of *C. fusiformis*, yet did not have significant effect on the growth of *P. tricornutum* (Figure 1). Neither 30 nor 60 µM Zn²⁺ significantly influenced the growth of *P. tricornutum* (Figure 1a), while 60 µM Zn²⁺ decreased the growth of *C. fusiformis* on day five (Figure 1b). This indicated that *P. tricornutum* and *C. fusiformis* show different sensitivities to Cu²⁺ and Zn²⁺.

To explore the mechanism underlying the difference in susceptibility to heavy metals between *P. tricornutum* and *C. fusiformis*, transcriptomic analysis was conducted. Ion transport is reported to be a response mechanism to the high concentration of heavy metal ions. In this study, it has been shown that under high concentrations of both Cu²⁺ and Zn²⁺, most DEGs involved in photosynthesis were upregulated, indicating the effect of both Cu²⁺ and Zn²⁺ on photosynthesis in *P. tricornutum*. Meanwhile, most genes downregulated in *P. tricornutum* under Cu²⁺ treatment were involved in metal ion homeostasis and transmembrane ion transport. This indicated that ion homeostasis and transmembrane transport might be the main mechanisms for *P. tricornutum* to respond to high Cu²⁺ concentrations. Moreover, this enrichment of downregulated genes in metal ion transport was observed in *P. tricornutum* under Zn²⁺ treatment. However, the enrichment of downregulated genes in metal ion homeostasis-related genes did not occur under Zn²⁺ treatment, indicating a different response mechanism for Zn²⁺ to that for Cu²⁺.

Besides genes related to metal ion homeostasis and transmembrane ion transport, some other genes were previously reported to be involved in heavy metal stress response, including genes related to catalase, antioxidation, ascorbate metabolism, glutathione metabolism, phytochelatin, and oxidative stress [9]. These genes are listed in Tables 1–4. Most of these
genes were upregulated in *P. tricornutum* under both Cu\(^{2+}\) and Zn\(^{2+}\) treatments; however, only a few were upregulated in *C. fusiformis*, indicating that the response of *C. fusiformis* to heavy metal ion stress is different from that of *P. tricornutum*. Moreover, the enrichment of DEGs in ion homeostasis and transmembrane transport-related genes was not observed in *C. fusiformis* either. It is reported that *C. fusiformis* is sensitive to heavy metal ions [4,6], whereas *P. tricornutum* is more tolerant to Cu\(^{2+}\) stress [19]. We suspect that difference in gene expression might be one of the mechanism’s responses to the difference in susceptibility to heavy metals between *P. tricornutum* and *C. fusiformis*.

In addition, since the metal toxicity for cells is more related to intracellular metal bioaccumulation than to the metal concentration in water, and the fact that both *P. tricornutum* and *C. fusiformis* are widely considered biofilm-producing mixed diatoms [30–33], the role of metal ion management of the biofilm should be considered when exploring the mechanism underlying the difference in susceptibility to heavy metals between *P. tricornutum* and *C. fusiformis*. Using SEM and EDS analysis, we found that both *P. tricornutum* and *C. fusiformis* accumulated Cu\(^{2+}\) and Zn\(^{2+}\) onto the biosilica shell. In future work it would be informative to determine the intracellular concentrations of Cu\(^{2+}\) and Zn\(^{2+}\).

5. Conclusions

Transcriptome analysis of *P. tricornutum* and *C. fusiformis* under Cu\(^{2+}\) and Zn\(^{2+}\) treatments indicated that genes involved in metal ion homeostasis and transmembrane ion transport, and those related to catalase, antioxidation, ascorbate metabolism, glutathione metabolism, phytochelatin, and oxidative stress, might play important roles in the response of diatoms to heavy metal stress.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14203305/s1, Table S1: Clean data statistics for Phaeodactylum tricornutum (*P. tricornutum*) transcriptome; Table S2: Annotation statistics for *P. tricornutum* transcriptome; Table S3: Annotations for differentially expressed genes (DEGs) in *P. tricornutum* under Cu\(^{2+}\) treatment; Table S4: Annotations for DEGs in *P. tricornutum* under Zn\(^{2+}\) treatment; Table S5: Clean data statistics for Cylindrotheca fusiformis (*C. fusiformis*) transcriptome; Table S6: Annotation statistics for *C. fusiformis* transcriptome; Table S7: Annotations for DEGs in *C. fusiformis* under Cu\(^{2+}\) treatment; Table S8: Annotations for DEGs in *C. fusiformis* under Zn\(^{2+}\) treatment.

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References

1. Larned, S.T. A prospectus for periphyton: Recent and future ecological research. J. N. Am. Benthol. Soc. 2010, 29, 182–206. [CrossRef]
2. Butcher, R.W. Studies in the Ecology of Rivers: VII. The Algae of Organically Enriched Waters. J. Ecol. 1947, 35, 186–191. [CrossRef]
3. Tudesque, L.; Grenouillet, G.; Gevrey, M.; Khazaiea, K.; Brosse, S. Influence of small-scale gold mining on French Guiana streams: Are diatom assemblages valid disturbance sensors? Ecol. Indic. 2012, 14, 100–106. [CrossRef]
4. Sbihi, K.; Cherifi, O.; Bertrand, M. Toxicity and biosorption of chromium from aqueous solutions by the diatom Planodinium lanceolatum (Brebisson) Lange-Bertalot. Am. J. Sci. 2012, 3, 27–38. [CrossRef]
5. De Stefano, L.; Rottiroti, L.; De Stefano, M.; Lamberti, A.; Lettieri, S.; Setaro, A.; Maddalena, P. Marine diatoms as optical biosensors. Biosens. Bioelectron. 2009, 24, 1580–1584. [CrossRef]
6. Marie, M.; Kirsten, H.; Pamela, Q.; Negri, A.P. Additive toxicity of herbicide mixtures and comparative sensitivity of tropical benthic microalgae. Mar. Pollut. Bull. 2010, 60, 1978–1987.
7. Satoh, A.; Vudikaria, L.Q.; Kurano, N.; Miyachi, S. Evaluation of the sensitivity of marine microalgal strains to the heavy metals, Cu, As, Pb and Cd. Environ. Int. 2005, 31, 713–722. [CrossRef]
8. Rimet, F. Recent views on river pollution and diatoms. Hydrobiologia 2012, 683, 1–24. [CrossRef]
9. Masmoudi, S.; Nguyen-Deroche, N.; Caruso, A.; Ayadi, H.; Morant-Manceau, A.; Tremblin, G.; Bertrand, M.; Schoefs, B. Cadmium, copper, sodium and zinc effects on diatoms: From heaven to hell—A review. Cryptogam. Algol. 2013, 34, 185–225. [CrossRef]
10. Owens, T.G.; Wold, E.R. Light-Harvesting Function in the Diatom. Bioinformatics 2010, 26, 139–140. [CrossRef] [PubMed]
11. Hänsch, R.; Mende, R.R. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Cryptogam. Algol. 2012, 33, 27–38. [CrossRef]
12. Robinson, M.D.; McCarthy, D.J.; Smyth, G.K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010, 26, 139–140. [CrossRef] [PubMed]
13. Robinson, M.D.; McCarthy, D.J.; Smyth, G.K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010, 26, 139–140. [CrossRef] [PubMed]
14. Stukenberg, D.; Zauner, S.; Dell’Aquila, G.; Maier, U.G. Optimizing CRISPR/Cas9 for the Diatom Phaeodactylum tricornutum. Front. Plant Sci. 2018, 9, 740. [CrossRef]
15. Kawai, T.; Kaneko, M.; Hirota, T. Effects of temperature on the growth and photosynthesis of the diatom Phaeodactylum tricornutum. J. Phycol. 2006, 42, 1729–1743. [CrossRef]
16. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
17. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
18. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
19. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
20. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
21. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
22. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
23. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
24. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
25. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
26. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
27. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
28. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
29. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
30. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
31. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
32. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
33. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
34. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
35. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
36. Peers, G.; Price, N.M. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006, 441, 341–344. [CrossRef] [PubMed]
31. Willis, A.; Chiovitti, A.; Dugdale, T.M.; Wetherbee, R. Characterization of the extracellular matrix of *Phaeodactylum tricornutum* (Bacillariophyceae): Structure, composition, and adhesive characteristics. *J. Phycol.* **2013**, *49*, 937–949. [CrossRef]

32. Tong, C.Y.; Derek, C.J.C. The role of substrates towards marine diatom *Cylindrotheca fusiformis* adhesion and biofilm development. *J. Appl. Phycol* **2021**, *33*, 2845–2862. [CrossRef]

33. Tong, C.Y.; Derek, C.J.C. Biofilm formation of benthic diatoms on commercial polyvinylidene fluoride membrane. *Algal Res.* **2021**, *55*, 102260. [CrossRef]