Check for updates

RESEARCH ARTICLE

Comparison of cell size, chlorophyll fluorescence and cadmium (Cd^{2+}) bioaccumulation between wild-type and mutant strains of *Chlamydomonas reinhardtii* upon exposure to Cd^{2+} [version 1; peer review: 2 approved with reservations]

Ruby Ynalvez ¹, Hector Gonzalez-Cantu¹, Erick Vazquez-Cano²

¹Biology and Chemistry, Texas A&M International University, Laredo, Texas, 78041, USA

²Cell Biology, Harvard Medical School, Boston, MA, 02115, USA

V1 First published: 16 Aug 2021, 10:806
<https://doi.org/10.12688/f1000research.53997.1>
 Latest published: 25 Apr 2023, 10:806
<https://doi.org/10.12688/f1000research.53997.2>

Abstract

Background: Heavy metal contamination presents a constant threat to biological systems. Simultaneously, heavy metals have become one of the major contaminants in the aquatic ecosystems. In this regard, the investigation of heavy metal-tolerance genes in algae is relevant. *Chlamydomonas reinhardtii* is a unicellular green alga, and an excellent model organism used in heavy metal studies. In *C. reinhardtii*, a novel gene designated as *Cia7*, was hypothesized to play a role in heavy metal homeostasis due to CIA7's conserved cysteine-residue motif. This study compared two strains of *C. reinhardtii*, *cc4425*, the wild-type with the functional CIA7 protein and *cc5013*, the mutant strain with the disrupted *cia7* gene. The hypothesis was that the expression of *Cia7* contributes to an increased cadmium (Cd)-tolerance in *C. reinhardtii*. The Cd-tolerance would be described by physiological markers of microalgae health, and by intracellular accumulation of the metal.

Methods: The objectives of this study were (1) to compare chlorophyll fluorescence and cell size in *cc4425* and *cc5013* exposed to Cd^{2+} , and (2) to compare Cd^{2+} bioaccumulation in *cc4425* and *cc5013* strains in different growth media. Flow cytometry, and inductively coupled plasma optical emission spectrometry (ICP-OES) analysis were performed.

Results: There was no significant statistical difference in Cd^{2+} bioaccumulation between the two strains, *cc4425* and *cc5013*, regardless of growth media. However, a statistically significant difference in Cd^{2+} bioaccumulation ($p < 0.0001$) was determined between the media (with acetate and without acetate). The *cia7* mutant, *cc5013* was found to be more susceptible to a Cd^{2+} -induced decrease in chlorophyll fluorescence and had a reduced cell size compared to *cc4425*, the wild-type strain.

Open Peer Review

Approval Status ? ?

1

2

version 2

(revision)

25 Apr 2023

version 1

16 Aug 2021

?

[view](#)

?

[view](#)

1. **Jiangxin Wang** , Shenzhen University, Shenzhen, China
2. **Stefan Schmollinger** , University of California, Berkeley, Berkeley, USA

Any reports and responses or comments on the article can be found at the end of the article.

Conclusions: These observed differences between the strains suggest that CIA7's biological activity could play a direct or indirect role in increasing Cd tolerance in *C. reinhardtii*.

Keywords

cadmium tolerance, flow cytometry, ICP-OES analysis, DUF2256 domain, heavy metal, microalgae

Corresponding author: Ruby Ynalvez (rynalvez@tamiu.edu)

Author roles: **Ynalvez R:** Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Gonzalez-Cantu H:** Formal Analysis, Investigation, Methodology, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Vazquez-Cano E:** Investigation, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was funded by the Texas Higher Education Coordinating Board (THECB) - Norman Hackerman Advanced Research Program and the Office of Grants and Sponsored Research, Texas A&M International University. The funds were awarded to RA Ynalvez.

Copyright: © 2021 Ynalvez R *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Ynalvez R, Gonzalez-Cantu H and Vazquez-Cano E. **Comparison of cell size, chlorophyll fluorescence and cadmium (Cd²⁺) bioaccumulation between wild-type and mutant strains of *Chlamydomonas reinhardtii* upon exposure to Cd²⁺** [version 1; peer review: 2 approved with reservations] F1000Research 2021, 10:806 <https://doi.org/10.12688/f1000research.53997.1>

First published: 16 Aug 2021, 10:806 <https://doi.org/10.12688/f1000research.53997.1>

Introduction

Public health and environmental concerns from heavy metal contamination have increased in recent times. In fact, among all the pollutants, heavy metals have received particular attention due to their toxic nature, persistence in the environment, and bioaccumulative nature (Ali *et al.*, 2019; Al Osman *et al.*, 2019). Heavy metals are naturally occurring elements that have high atomic weights and densities at least five times greater than that of water. Arsenic, cadmium (Cd), chromium, lead, and mercury are among the priority metals of public health significance; this, in part, is due to their high degree of toxicity. These metals are systemic toxicants known to induce multiple organ damage, even at lower levels of exposure (Sevim *et al.*, 2020; Tchounwou *et al.*, 2012).

Heavy metals are known to be naturally occurring elements; however, anthropogenic activities introduce them in large quantities in the environment (Briffa *et al.*, 2020). The presence of heavy metals in nature can arise from environmental processes like volcanic emissions and rock weathering (Jan *et al.*, 2015; Tuzun *et al.*, 2005). On the other hand, anthropogenic activities include mercury (Hg) contamination from gold mining, lead (Pb) from its use as a gasoline additive, and Cd from fertilizers, batteries, and sewage water (Sevim *et al.*, 2020; Roberts, 2014). Human activities are contaminating the environment and its resources; these activities are discharging heavy metals at concentrations greater than what the environment can handle (Masindi and Muedi, 2018).

The final deposition points of heavy metal leachate are often aquatic environments, where microalgae are widely distributed and therefore exposed to these metals. Literature evidence suggests that toxic heavy metals may induce deleterious damage to the metabolism and physiology of microalgae (Xie *et al.*, 2019; Abu Bakar *et al.*, 2015). The damage that heavy metals exert at the cellular level is composite and targets different mechanisms. For example, intracellular heavy metal ions induce a variety of biochemical changes that affect the normal growth and metabolic activity of the cell system. These changes include blocking the functional groups of biomolecules, displacing essential metal ions, and modifying the native conformations of biomolecules (Priyadarshini *et al.*, 2019). Heavy metals are also known to interfere and inhibit protein activity. Proteins whose active domains possess sulfhydryl groups are especially susceptible to deleterious effects of heavy metals due to their affinity with them, resulting in irreversible binding (Fein *et al.*, 2019; Hua and Li, 2014). In addition, heavy metals can interfere with metalloproteins or metal-protein complexes by competing with an essential metal cofactor for an enzyme's binding site (Pinter and Stillman, 2015; Viehweger, 2014). A reported symptom of heavy metal toxicity in a cell is an increase in its oxidative stress, marked by an increase in reactive oxygen species (ROS) (Elbaz *et al.*, 2010; Jiang *et al.*, 2016). ROS, due to their oxidative character, damage cellular structures and macromolecules by interfering with non-covalent interactions.

To study the deleterious effects of heavy metals and their interactions within biological systems, eukaryotic microalgae have been deemed appropriate subjects (Leong and Chang, 2020; Miazek *et al.*, 2015). Microalgae have evolved resistance to heavy metal stress through their own metabolic pathways, which include metal immobilization by cell walls and extracellular polymeric substances (EPS), and transport through the cell membrane via transporters (Lu *et al.*, 2019; Liu *et al.*, 2016). It is this acclimatization that makes microalgae suitable candidates for heavy metal studies; they can survive relatively high metal concentrations, enabling the observations and analysis of heavy metal-induced effects.

Chlamydomonas reinhardtii is a unicellular green alga from the Chlorophyta lineage, and is the best-developed reference organism for studying algal biology, especially with respect to metal metabolism (Blaby-Haas and Merchant, 2012). *C. reinhardtii* has garnered scientific interest in the context of heavy metal toxicity due to its broad range of heavy metal resistance and tolerance (Zheng *et al.*, 2020; Ibuot *et al.*, 2018; Nowicka *et al.*, 2016). *C. reinhardtii* is also a potential candidate for phytoremediation, the process of utilizing plants and algae to bioremediate contaminated areas (Leong and Chang, 2020).

In *C. reinhardtii*, a primary line of defense against heavy metals is the cell wall. The cell wall displays a high affinity towards metallic cations, and cell wall-less mutants commonly exhibit greater sensitivity towards metals (McFie *et al.*, 1994; Collard and Matagne, 1990). Cell walls contain multiple negatively charged groups that form interactions with metal cations, thus sequestering metals and preventing metal uptake into the cytoplasm (Priyadarshini *et al.*, 2019; Samadani *et al.*, 2018; Hanikenne, 2003). Whereby, intracellularly, heavy metals are compartmentalized in vacuoles, allowing the cell to control the cytoplasmic metallic concentration, and neutralizing the heavy metal's toxic effect (Perales-Vela *et al.*, 2006). Mechanisms of heavy metal tolerance in *C. reinhardtii* also include transporters. For example, the mitochondrial half-size ABC (ATP-binding cassette) transporter is implicated in Cd-tolerance (Hanikenne *et al.*, 2005). Heavy metal-binding peptide synthesis has also been shown to be preferentially developed in microalgae and some fungi, thus granting these organisms a higher metal tolerance (Perales-Vela *et al.*, 2006).

C. reinhardtii showed various strategies to cope with Cd stress. Penen *et al.* (2017) previously reported that most Cd could diffuse in the whole cell, and complexed by thiol ligands. Thiol ligands were also shown to increase with cells' exposure

time to Cd, confirming thiol ligands' important role in Cd stress regulation. There is also pyrenoidal sequestration of Cd, leading to limited CO₂ fixation during Cd exposure (Penen *et al.*, 2019).

Metal tolerance studies including Cd in *C. reinhardtii* have been extensively reported in the literature. In reported studies of exogenous metallothionein gene expression in *C. reinhardtii*, Cd was reported as an inducer. The generation and characterization of Cd-resistant and hypersensitive *C. reinhardtii* mutants have also been reported in studies of heavy metal tolerance (Samadani *et al.*, 2018; Hanikenne, 2003; Hu *et al.*, 2001). In particular, the overexpression of metal transporters in *C. reinhardtii* for bioremediation is an attractive approach; it may allow both increased metal accumulation into the cell and increased metal tolerance, such as by transfer of a toxic metal out of the cytosol and into an internal compartment (Ibuot *et al.*, 2020; Ibuot *et al.*, 2017). The genetic manipulation of *C. reinhardtii* has been shown to be successful in creating stable, inherited phenotypes (Pollock *et al.*, 2017). Changes in the heavy metal biosorption capacity of *C. reinhardtii* have been reported in genetically modified strains (Ibuot *et al.*, 2020; Cheng *et al.*, 2019; Aksmann *et al.*, 2014; Lin *et al.*, 2013; Mayfield *et al.*, 2007; Shimogawara *et al.*, 1998).

A novel gene, *Cia7*, was isolated from an inorganic carbon-acquiring, and high carbon dioxide-requiring *C. reinhardtii* mutant, *cc5013* (Ynalvez *et al.*, 2008). The gene designated as *Cia7* was hypothesized to play a role in heavy metal tolerance or metal detoxification due to its translational product's amino acid sequence. Sequence alignment utilizing the U.S. National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST, RRID: SCR_004870) revealed homology with many uncharacterized proteins conserved in bacteria; proteins classified as belonging to a family of DUF2256 domain-containing proteins. Members of this family of hypothetical bacterial proteins have domains of unknown functions (DUF). Homologous proteins also included computer modeling predicted proteins, TRAP transporter small permease (92% identity) and an uncharacterized metal binding protein (72% identity). The protein sequence of CIA7 contained a conserved, repeated motif of four cysteine residues whose thiol functional group is hypothesized to chelate heavy metals (Ynalvez and Moroney, 2008). Based on this observation, the aim of this study was to investigate whether *Cia7* plays a potential role in metal tolerance in *C. reinhardtii*.

This study compared two strains of *C. reinhardtii*: *cc4425*, the wild-type with the functional CIA7 protein and *cc5013*, the strain that has a disruption in the *Cia7* gene due to a mutant *cia7*. It is hypothesized that CIA7, due to its conserved cysteine residue motif, could play a role in the molecular defense mechanism to attenuate heavy metal toxicity. More specifically, when subjected to metal (Cd²⁺) stress, *C. reinhardtii* *cc4425* cells that express a functional CIA7 would exhibit higher growth rate and chlorophyll fluorescence, but a decreased metal bioaccumulation when compared to the mutant *cc5013*. The objectives of this study were to compare cell size, chlorophyll fluorescence, and metal (Cd²⁺) bioaccumulation between the two strains *cc4425* (wild-type) and *cc5013* (mutant). Flow cytometry was used in this study. This technique rapidly analyzes single cells or particles as they flow past single or multiple lasers while suspended in a buffered salt-based solution (McKinnon, 2019). Flow cytometers are suitable for microalgae applications as the latter are often a collection of single cells, and microalgae possess endogenous pigments which autofluoresce, making immediate measurements possible, without the need for pre-treatment of cells (Jamers *et al.*, 2009). Flow cytometry analyses in metal studies using *C. reinhardtii* are relatively scarce. Therefore, this study will also provide a framework for future, related studies. In addition, the results of this study could provide a basis for the further investigation on the role of CIA7 in *C. reinhardtii*'s heavy metal tolerance or the further investigation on the potential use of *Cia7* expression as a biomarker for heavy metal contamination.

Methods

C. reinhardtii culture conditions

C. reinhardtii strains *cc4425* (wild-type) and *cc5013* (mutant *cia7*⁻) were obtained from the *Chlamydomonas* Resource Center in the University of Minnesota. Cells were maintained as stocks in 1.5% Tris-acetate-phosphate (TAP) agarose solid media, stored at room temperature and subcultured every seven days. Liquid cultures were initiated in 50 mL of TAP medium by using two loopfuls (4 mm spherical scraping) of cells for inoculation. Culture flasks were placed in an orbital shaker (VWR DS2-500-1) at 130 rpm under normal fluorescent room lighting until cultures reached optical density (OD₆₅₀) of 0.800 ± 0.050, measured using a Spectronic Genesys 8 spectrophotometer. This OD₆₅₀ of 0.800 has been calibrated to be equal to 1.0-3.0 × 10⁶ cells/mL, which is log phase in *C. reinhardtii*.

Flow cytometry analysis

Cells from both strains were cultured in liquid TAP to an OD₆₅₀ = 0.400 and subjected to their respective metal treatments (CdCl₂ at concentrations of 0 μM, 5 μM and 10 μM). Cd treatments in ¼X TAP amounted to a 50 mL final volume. Treatment flasks were prepared and cultured in replicates, one of them serving as the sampling culture flask, and the other as the supplemental culture flask. From the sampling culture flask, 900 μL of cells were taken for analysis, and the sampling flask was replenished with 900 μL of cells from the supplemental culture flask. The replenishment step was

done in order to maintain the same volume of cells in the sampling culture flask throughout the 96-hour period. Samples for flow cytometry analysis were diluted 5:1 in saline buffer. Three replicates with three trials were performed per concentration and per strain. Chlorophyll intensity level and cell size were measured using a Cytex DxP8 FACSCalibur Flow Cytometer acquiring 10,000 events. Chlorophyll intensity level was determined by utilizing laser BluFL3 (Cytex) with a wavelength filter 695/40 nm. Cell size was determined as a function of the forward scatter signal (FSC). Cell cultures *cc4425* and *cc5013* were treated with 0, 5, and 10 μM CdCl_2 at midlog phase ($\text{OD}_{650} = 0.400$). Samples were analyzed at 24-hour time points for a duration of 96 hours via flow cytometry.

An analysis of variance (ANOVA) associated with a $2 \times 3 \times 4$ factorial experiment in randomized complete block design was performed for data analysis. The factorial arrangement of treatments was the result of two levels of strain (*cc5013*, *cc4425*), three levels of Cd concentration (0 μM , 5 μM , and 10 μM), and four levels of time (1, 2, 3, and 4 days). To compare significant main and interaction effects, post-hoc tests in the form of least squares means comparison were performed using the PROC GLM tool of the SAS 9.4 (Statistical Analysis System, RRID:SCR_008567) statistical software. The usual levels of type-I error rates were used (i.e., * if $p < 0.05$, ** if $p < 0.01$ and *** if $p < 0.001$).

Inductively coupled plasma optical emission spectrometry (ICP-OES) analysis

C. reinhardtii strains were cultured in liquid media. Cells were kept under constant movement (130 rpm) in an orbital shaker until the cultures reached an OD_{650} of 0.800 ± 0.050 as determined by a Spectronic Genesys 8 spectrophotometer. An absorbance of $\text{OD}_{650} = 0.800$ was calibrated to be equal to $1.0\text{--}3.0 \times 10^6$ cells/mL (log phase in *C. reinhardtii*) (Lin *et al.*, 2013; Kirst *et al.*, 2012). Results from preliminary experiments established the sub-lethal Cd concentration of *cc4425* and *cc5013* at 10 μM . In this regard, cells were harvested and cultured in media treated with CdCl_2 at concentrations of 0 μM and 10 μM (0 ppb and 1830 ppb) to a final volume of 150 mL. Two different growth media were used: a minimal (Min) medium and a diluted, by a factor of $\frac{1}{4}$, TAP medium. Phosphorus has been reported as reducing the bioavailability of free metal ions due to its chelating character; therefore, the TAP medium was diluted (Nowicka *et al.*, 2016). Similarly, the Min medium was used to determine whether a difference existed in *C. reinhardtii*'s metal bioaccumulation between TAP and the acetate-deprived Min media.

Metal treatment flasks were washed with 1% HNO_3 to prevent metal cross-contamination between replicates. The treatment period was 4 days, after which cells reached the logarithmic phase of growth. On the 4th day, cells were equilibrated at $\text{OD}_{650} = 0.800$ (an absorbance at log phase) and harvested by centrifugation at 3,000 rpm for 10 minutes. The pellets were washed twice with millipore H_2O , then resuspended, followed by centrifugation at 3,000 rpm for 5 minutes to rinse pellets of extracellular metals. Washed pellets were desiccated at 100°C , and either frozen at -80°C or used for downstream analysis. Dry pellets were acidified with 5 mL of HNO_3 , incubated for 20 minutes, and digested in a MARS 6 microwave following the machine's internal protocol for plant material. Following digestion, samples were diluted 1:10, and analyzed for Cd, by ICP-OES (Agilent Varian 720-ES).

To analyze the data, an analysis of variance (ANOVA) associated with a $2 \times 2 \times 2$ factorial experiment in randomized complete block design was performed. The factorial arrangement of treatments was the result of two levels of strain (*cc5013*, *cc4425*), two levels of media ($\frac{1}{4}\text{X}$ TAP and Min), and two levels of Cd concentration (0 ppb and 1830 ppb). To compare significant main and interaction effects, a post-hoc test in the form of least squares means comparison was performed using the PROC GLM tool of the SAS 9.4 (Statistical Analysis System, RRID:SCR_008567) statistical software. To further explore the nature of a significant interaction between medium and concentration, a t-test comparing concentration levels was performed for each medium level. The usual levels of type-I error rates were used (i.e., * if $p < 0.05$, ** if $p < 0.01$ and *** if $p < 0.001$).

Results

Determination of cell size and chlorophyll content in *cc4425* and *cc5013*

A statistically significant difference in chlorophyll fluorescence was observed between *cc4425* and *cc5013* (6,127 and 3,858 counts respectively) at 10 μM Cd (Table 1, Figure 1). However, at the control treatment and at 5 μM treatment, *cc4425* and *cc5013* chlorophyll signals at BluFL3 were not statistically significantly different from each other (Figure 1). It is interesting to note that as the Cd concentration increased, chlorophyll intensity increased for wild-type strain *cc4425*, while chlorophyll intensity decreased for *cia7* mutant *cc5013* (Figure 1). This negative trend in chlorophyll intensity level might be indicative of heavy metal toxicity, to which *cc5013*, whose *Cia7* gene has been disrupted, might be more susceptible, including Cd susceptibility, compared to the wild-type *cc4425*.

Cell size was determined as a function of the forward scatter signal (FSC) (Figure 2). A similar empirical trend in the strains was observed in cell size as measured by the FSC. The effect of Cd on cell size was determined to be statistically significant between strains ($p < 0.0001$) at all concentrations (Table 2, Figure 2). The mutant, *cc5013* was observed to

Table 1. ANOVA results from a 2x3 factorial experiment with repeated measures for BluFL3 (695/40 nm) values.

Source	DF	SS	MS	Fc	p-value
Block	1	588031.5	588031.5	0.55	0.4660
Strain	1	15563995.5	15563995.5	14.62	0.0011
Concentration	2	3340620.4	1670310.2	1.57	0.2328
Strain * Concentration	2	9738520.2	4869260.1	4.57	0.0231
Days	3	13579951.9	4526650.6	4.25	0.0177
Strains * Days	3	16091012.8	5363671.0	5.04	0.0092
Days * Concentration	6	4322209.8	720368.3	0.68	0.6700
Strain * Days * Concentration	6	4299179.1	716529.8	0.67	0.6727

DF- degrees of freedom; SS- sum of squares; MS- mean squares; Fc- F computed; p-value- probability of Fc under null hypothesis.

Figure 1. Comparison of chlorophyll intensity signals as measured by BluFL3 (695/40 nm) at different CdCl₂ concentrations; *cc4425* (1) and *cc5013* (2). Data represents pooled data from 0-4 days, whereby sampling was done every 24 h. Error bars represent 95% confidence intervals. Statistical analyses of measurements are displayed in Table 1. **Figure 2.** Comparison of cell sizes as determined as a function of the forward scatter signal (FSC); *cc4425* (1) and *cc5013* (2). Data represents pooled data from 0-4 days, whereby sampling was done every 24 h. Error bars represent 95% confidence intervals. Statistical analyses of measurements are displayed in Table 2.

have greater cell size than wild-type *cc4425* especially under 0 μM Cd²⁺. Although not statistically significant ($p > 0.05$), *cc4425* showed an increase in cell size with increase in cadmium concentration, while *cc5013* showed a decrease in cell size (Figure 2).

Comparison of Cd²⁺ bioaccumulation between *cc4425* and *cc5013*

This bioaccumulation experiment aimed to investigate the interactions between the *in vivo* activity of CIA7 in differential Cd²⁺ concentrations (0 and 1830 ppb [10 μM]), in either a mixotrophic (1/4X TAP) or photoautotrophic (Min) growth

Table 2. ANOVA results from a 2 × 3 factorial experiment with repeated measures for FSC values.

Source	DF	SS	MS	Fc	p-value
Block	2	20329.0	10164.5	5.17	0.0103
Strain	1	579.1	579.1	0.29	0.5905
Concentration	1	60614.1	60614.1	30.82	<0.0001
Strain * Concentration	1	573.1	573.1	0.29	0.5925
Media	1	38596.8	38596.8	19.63	<0.0001
Strains * Media	1	527.9	527.9	0.27	0.6074
Media * Concentration	1	38480.4	38480.4	19.57	<0.0001
Strain * Media * Concentration	1	540.5	540.5	0.27	0.6032

DF - degrees of freedom; SS - sum of squares; MS - mean squares; Fc - F computed; p-value - probability of Fc under null hypothesis.

medium. Although the results are not indicative of a statistically significant correlation, the observations are nevertheless interesting. Due to the lack of *cc5013* characterization studies and CIA7's as-of-yet unknown function, it is difficult to predict what metabolic and physiological changes Cd²⁺ exposure could cause, and in which range. The factorial arrangement of treatments was the result of two levels of strain (*cc5013*, *cc4425*), two levels of medium type (¼X TAP and Min), and two levels of Cd concentration (0 and 1830 ppb). No significant statistical difference ($p < 0.5905$) was established between Cd²⁺ bioaccumulation in *cc4425* and *cc5013*, regardless of growth medium (Table 3). In ¼ X TAP, *cc4425*'s Cd²⁺ content was measured as 128.63 ppb, and in *cc5013* as 155.83 ppb. In Min, *cc4425*'s Cd²⁺ content was measured as 28.63 ppb, and *cc5013*'s as 29.14 ppb.

On the other hand, a statistically significant difference was determined in the media by concentration effect ($p < 0.0001$) (Table 4). There was a significant difference in cells' bioaccumulation of Cd²⁺ in 10 µM Cd (1830 ppb) treated ¼ X TAP (142.22 ppb) when compared to Cd²⁺ bioaccumulation in Min (28.88 ppb). On the other hand, as expected, there was no significant difference between ¼ X TAP and Min (14.53 ppb and 14.44 ppb respectively) at 0 ppb Cd (Table 4 and Figure 3). The main difference between the two media is the presence of acetate and phosphate in ¼ X TAP and their absence in Min. The pH of the two media were adjusted to pH 7.0. As previously mentioned, the TAP medium was diluted by a factor of ¼, as phosphorus has been reported as reducing the bioavailability of free metal ions due to its chelating character (Olanarin *et al.*, 2013).

Table 3. ANOVA associated with a 2 × 2 × 2 factorial experiment in randomized complete block design.

Source	DF	SS	MS	Fc	p-value
Block	1	73729933	73729933	0.94	0.3429
Strain	1	6833018155	6833018155	87.47	<0.0001
Concentration	2	200821693	100410847	1.29	0.2984
Strain * Concentration	2	917167779	458583890	5.87	0.0099
Days	3	1728984054	576328018	7.38	0.0016
Strains * Days	3	1614275755	538091918	6.89	0.0023
Days * Concentration	6	537732696	89622116	1.15	0.3722
Strain * Days * Concentration	6	510891929	85148655	1.09	0.4019

DF - degrees of freedom; SS - sum of squares; MS - mean squares; Fc - F computed; p-value - probability of Fc under null hypothesis.

Table 4. Results of the test comparing significant interaction between media and concentration.

Concentration	DF	SS	MS	Fc	p-value
¼X TAP versus Min at 0 ppb	1	0.04	0.04	0.00	0.9963
¼X TAP versus Min at 1830 ppb	1	77077.0	77077.0	39.19	<0.0001

DF - degrees of freedom; SS - sum of squares; MS - mean squares; Fc - F computed; p-value - probability of Fc under null hypothesis.

Figure 3. Comparison of least squares means exploring the significant interaction between medium and concentration. A t-test comparing concentration levels was performed for each media level. The “*” denotes a significant difference between two treatments.

Discussion

A statistically significant difference in chlorophyll fluorescence was observed between *cc4425* and *cc5013* (6,127 and 3,858 counts respectively) at 10 μM Cd (Figure 2). A possible mechanism behind the phenomenon reported in *cc5013* is a decrease in chlorophyll intensity level because of Cd substituting magnesium in chlorophyll, leading to the decrease in chlorophyll intensity (Grajek *et al.*, 2020; Rydzyński *et al.*, 2019; Kupper *et al.*, 1996). The apparent absence of this phenomenon in *cc4425* at the Cd concentrations used in this study might be, at least in part, contributed to by the biological activity of CIA7. This observed difference in chlorophyll fluorescence between the strains suggests that CIA7’s biological activity could play a direct or indirect role in increasing Cd tolerance in *C. reinhardtii*.

Although not statistically significant, *cc4425* showed an increase in cell size with an increase in Cd concentration, while *cc5013* showed a decrease in cell size. The trend observed in cell size in *cc4425* is partly supported by similar findings in Jamers *et al.* (2013), in which at 5 μM Cd²⁺ after 72 hours treatment, cells were bigger than the control group ($p < 0.05$). Furthermore, the trend reported in this study is likewise partly supported by Franklin *et al.*’s (2001) findings on the effect of Cu²⁺ in microalgae: *Selenastrum capricornutum* and *Chlorella sp.* In the same study, it was argued that increases in cell size in Cu²⁺ treatments were due to an increase in Na⁺ uptake, or the uncoupling of cell growth and cell division. However, whether Cd²⁺ causes the same effect is not yet known.

Cd bioaccumulation differences were analyzed in *C. reinhardtii* strains *cc4425* (wild-type) and *cc5013* (mutant). Intracellular metal accumulation has been reported as an indicator of metal tolerance (Collard and Matagne, 1994; Fernandez and Novillo 1995; Perez-Rama *et al.*, 2002; Jamers *et al.*, 2009). The mechanisms of metal tolerance in *C. reinhardtii* are dependent on metal concentration, availability of nutrients, as well as the general health of the cell (Igiri *et al.*, 2018; Roach *et al.*, 2013). Cd²⁺ bioaccumulation, for example, occurs once the concentration of the metal reaches critical levels sufficient to both saturate the binding capabilities of the cell wall, and hijack the transport proteins of essential metals with similar redox characteristics (i.e., Zn²⁺) (Brautigam *et al.*, 2011).

In this study, *cc4425* (wild-type) and *cc5013* (mutant strain *cia7*) were treated with Cd²⁺, 10 μM (1830 ppb) final concentration. Following a 96-hour exposure, the intracellular Cd²⁺ content was less than 10% of the total Cd²⁺ bioavailable. Due to similarities between this study and that by Fernandez and Novillo 1996, both including similar Cd²⁺ concentrations, prolonged exposure time and organism, it could be argued that the apparent lack of difference in terms of Cd²⁺ tolerance amongst *cc4425* and *cc5013* could be a result of the very well documented phytotoxic effects of Cd²⁺, reducing the uptake capabilities of both strains at 96 hours. A similar study on the metal accumulation in strains *cc4425* and *cc5013* was conducted by Vazquez *et al.* (2018) using Pb²⁺ in the form of Pb(NO₃)₂ at concentrations 0 μM and 150 μM , in which they reported no significant differences in the bioaccumulation of Pb²⁺ as measured analytically by ICP-OES. With both Cd²⁺ and Pb²⁺ being well-reported phytotoxic agents, it is possible that both of these metals exert irreversible photosynthetic damage after long-term exposures, and reduce the metal uptake capabilities. These could likely account for the non-significant difference in Cd²⁺ and Pb²⁺ bioaccumulation between the wild-type and mutant strains.

However, it was initially not expected that there would be a significant difference in cells’ bioaccumulation of Cd²⁺ caused by medium type. The presence of acetate and phosphate in the diluted TAP medium likely still contributed to a considerably higher buffering capacity compared to Min. Additionally, the pH affects the bioavailability of metals (Glaesener *et al.*, 2013; Olanarin *et al.*, 2013); for example, *C. reinhardtii* strains showed a higher uptake of Cd at pH

7 and higher Cd tolerance at pH 4 than pH 7 due to the exclusion of Cd at the cell wall surface, which was higher at pH 4 than pH 7 (Samadani *et al.*, 2018). Thus, the choice of medium in this kind of studies is critical and should be given a much careful consideration.

As a nutritional-rich growth media, the composition of TAP could potentially influence the cell response towards a toxicant (i.e., metals). The acetate present in TAP growth media could potentially shield *C. reinhardtii* from the phytotoxic effects of Cd, particularly photosynthesis impairment, as it could be integrated into the energy-yielding metabolic pathways of the cells. The findings of this study suggest that acetate supplementation triggers a variation in the response of *cc4425* and *cc5013* towards Cd stress. These findings are supported by Nagel *et al.*, (1995) who suggested that the biochemical or metabolic adaptations leading to an increase in Cd²⁺ tolerance resulted in heavy photosynthetic impairments, obligating the cell to upregulate carbohydrate metabolism as a means of energy, resulting in acetate overconsumption.

In evaluating biological parameters in *C. reinhardtii* treated with heavy metals, the growth medium's composition may display an effect in the cells' responses to heavy metals. Acetate has been described as capable of alleviating photosynthetic stress, with the cell incorporating it into its glyoxylate or citric acid cycle, while under photosynthesis impairment (Roach *et al.*, 2013). The previously reported recovery in growth and increase in reparation in the presence of acetate (Heifetz *et al.*, 2000; Jiang *et al.*, 2016) could have also contributed to the higher Cd bioaccumulation observed in ¼ X TAP.

Heavy metal contamination poses an environmental hazard, and the elucidation of mechanism of tolerance will be relevant in developing techniques and strategies for attenuating this threat. This protein, although of an unknown function, is likely to be significant because it is highly conserved in many organisms. Here we conclude that the results from the comparative analysis in this study indicate that *cc5013* (*cia7* mutant strain) is more sensitive to Cd²⁺ than the wild-type strain *cc4425*, which possesses a functional *Cia7* gene. Neither *cc4425* nor *cc5013* displayed a statistically significant difference in Cd²⁺ bioaccumulation at 10 µM (1830 ppb) CdCl₂.

However, a statistically significant interaction between Min media (absence of acetate and phosphate) and Cd²⁺ was observed, implying that a CIA7-mediated role in metal tolerance in *cc4425* could require a supplemental catabolite because of a diminished photosynthetic efficiency, as reported in the literature. Furthermore, the presence of a catabolite could potentially shield the detrimental effects of the metals, as observed in this study. It was established that *cc5013* was subjected to an increased photochemical quenching in the presence of Cd²⁺ as compared to *cc4425*. This could be attributed, at least partly, to Cd²⁺ substituting Mg²⁺ in the porphyrin group of chlorophylls. Cell size differences in the presence and absence of Cd were observed in both *cc501* and *cc4425*. The cell size differences could be attributed, at least partly, to an uncoupling of cell growth and cell division due to heavy metal stress.

For future studies, the determination and comparison of intracellular Cd²⁺ bioaccumulation between *cc4425* and *cc5013* assessed over a shorter period of time (6-12 h) is recommended. This assessment will likely provide a more dynamic analysis of the extent of metal accumulation with fewer generational gaps.

Data availability

Underlying data

Dryad: cell size, chlorophyll fluorescence and cadmium bioaccumulation between wild-type and mutant strains of *Chlamydomonas reinhardtii* upon exposure to cadmium, <https://doi.org/10.5061/dryad.m8pk0pb4> (Ynalvez *et al.*, 2021).

This project contains the following underlying data:

- ICP_OES_data_ynalvez_et al.csv (raw ICP-OES data)
- ODValues_ynalvez_et al.csv (raw OD values for each strain, Cd treatment and growth medium)
- raw_flowcytodata_chlorophyll_cellsize_ynalvez_et al.csv (raw forward scatter, flow cytometry and chlorophyll measurements)

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

References

- Abboud P, Wilkinson KJ: **Role of metal mixtures (Ca, Cu and Pb) on Cd bioaccumulation and phytochelatin production by *Chlamydomonas reinhardtii*.** *Environ. Pollut.* 2008; **179**: 33–38.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Abu Bakar L, Mat Amin N, Ahamad ZH: **Impact of mercury (II) nitrate on physiological and biochemical characteristics of selected marine algae of different classes.** *Procedia Environ. Sci.* 2015; **30**: 222–227.
[Publisher Full Text](#)
- Al Osman M, Yang F, Massey IF: **Exposure routes and health effects of heavy metals on children.** *Biometals.* 2019; **32**: 563–573.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ali H, Khan E, Ilahi I: **Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation.** *J Chem.* 2019;
[Publisher Full Text](#)
- Aksmann A, Pokora W, Bascik-Remisiewicz A, et al.: **Time-dependent changes in antioxidative enzyme expression and photosynthetic activity of *Chlamydomonas reinhardtii* cells under acute exposure to cadmium and anthracene.** *Ecotoxicol Environ Saf.* 2014; **110**: 31–40.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Blaby-Haas CE, Merchant SS: **The ins and outs of algal metal transport.** *Biochim. Biophys. Acta.* 2012; **1823**(9): 1531–1552.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Brautigam A, Schaumloffel D, Preud'Homme H, et al.: **Physiological characterization of cadmium-exposed *Chlamydomonas reinhardtii*.** *Plant Cell Environ.* 2011; **34**: 2071–2082.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Briffa J, Sinagra E, Blundell R: **Heavy metal pollution in the environment and their toxicological effects on humans.** *Heliyon.* 2020; **6**: 1–26.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Cheng SY, Show PL, Lau BF, et al.: **New prospects for modified algae in heavy metal adsorption.** *Trends Biotechnol.* 2019; **10**: 7–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Collard J, Matagne RF: **Cd²⁺ resistance in wild-type and mutant strains of *Chlamydomonas reinhardtii*.** *Environ. Exp. Bot.* 1994; **34**: 235–244.
[Publisher Full Text](#)
- Fernandez Leborans G, Novillo A: **Toxicity and bioaccumulation of cadmium in *Olisthodiscus luteus* (Raphidophyceae).** *Water Res.* 1996; **30**: 57–62.
[Publisher Full Text](#)
- Fein J, Yua Q, Namb J, et al.: **Bacterial cell envelope and extracellular sulfhydryl binding sites: Their roles in metal binding and bioavailability.** *Chem. Geol.* 2019; **521**: 28–38.
[Publisher Full Text](#)
- Elbaz E, Wei Y, Meng Q, et al.: **Mercury-induced oxidative stress and impact on antioxidant enzymes in *Chlamydomonas reinhardtii*.** *Ecotoxicology.* 2010; **19**: 1285–1293.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Franklin NM, Stauber JL, Lim RP, et al.: **Toxicity of metal mixtures to a tropical freshwater alga (*Chlorella* sp.): The effect of interactions between copper, cadmium, and zinc on metal cell binding and uptake.** *Environ. Toxicol. Chem.* 2002; **21**: 2414–2422.
[PubMed Abstract](#)
- Glaesener AG, Merchant SS, Blabby-Haas CE: **Iron economy in *Chlamydomonas reinhardtii*.** *Front. Plant Sci.* 2013 Sep 2; **4**: 337.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Grajek H, Rydzynski D, Piotrowicz-Cieślak A, et al.: **Cadmium ion-chlorophyll interaction – Examination of spectral properties and structure of the cadmium-chlorophyll complex and their relevance to photosynthesis inhibition.** *Chemosphere.* 2020; **261**: 127434.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hanikenne M: ***Chlamydomonas reinhardtii* as a eukaryotic photosynthetic model for studies of heavy metal homeostasis and tolerance.** *New Phytol.* 2003; **159**: 331–340.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hanikenne M, Motte P, Wu MCS, et al.: **A mitochondrial half-size ABC transporter is involved in cadmium tolerance in *Chlamydomonas reinhardtii*.** *Plant Cell Environ.* 2005; **28**: 863–873.
[Publisher Full Text](#)
- Heifetz PB, Forster B, Oxmond CB, et al.: **Effects of acetate on facultative autotrophy in *Chlamydomonas reinhardtii* assessed by photosynthetic measurements and stable isotope analyses.** *Plant Physiol.* 2000; **122**: 1439–1446.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hu S, Lau K, W K: **Cadmium sequestration in *Chlamydomonas reinhardtii*.** *Plant Sci.* 2001; **161**: 987–996.
[Publisher Full Text](#)
- Hua R, Li Z: **Sulfhydryl functionalized hydrogel with magnetism: Synthesis, characterization, and adsorption behavior study for heavy metal removal.** *Chem. Eng. J.* 2014; **249**: 189–200.
[Publisher Full Text](#)
- Ibuot A, Webster R, Williams L, et al.: **Increased metal tolerance and bioaccumulation of zinc and cadmium in *Chlamydomonas reinhardtii* expressing a AthMA4 C-terminal domain protein.** *Biotech. Bioeng.* 2020; **117**: 2996–3005.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ibuot A, Dean AP, McIntosh O, et al.: **Metal bioremediation by CrMTP4 over-expressing *Chlamydomonas reinhardtii* in comparison to natural wastewater-tolerant microalgae strains.** *Algal Res.* 2017; **24**(A): 89–96.
[Publisher Full Text](#)
- Igiri B, Okoduwa S, Gracido G, et al.: **Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review.** *J. Toxicol.* 2018; 2568038, 16 pages.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Jamers A, Blust R, De Coen W, et al.: **An omics based assessment of cadmium toxicity in the green alga *Chlamydomonas reinhardtii*.** *Aquat. Toxicol.* 2013; **126**: 355–364.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Jamers A, Lenjoy M, Deraedt P, et al.: **Flow cytometric analysis of the cadmium-exposed green alga *Chlamydomonas reinhardtii* (Chlorophyceae).** *Eur. J. Phycol.* 2009; **44**: 541–550.
[Publisher Full Text](#)
- Jan AT, Azam M, Siddiqui K, et al.: **Heavy metals and human health: Mechanistic insight into toxicity and counter defense system of antioxidants.** *Int. J. Mol. Sci.* 2015; **16**: 29592–29630.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Jiang Y, Zhu Y, Hu Z, et al.: **Towards elucidation of the toxic mechanisms of copper on the model green alga *Chlamydomonas reinhardtii*.** *Ecotoxicology.* 2016; **25**: 1417–1425.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kirst H, Garcia-Cerdan JG, Zurbruggen A, et al.: **Truncated photosystem chlorophyll antenna size in the green microalga *Chlamydomonas reinhardtii* upon deletion of the TLA3-CpSRP43 gene.** *Plant Physiol.* 2012; **4**: 2251–2260.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kupper H, Kupper F, Spiller M: **Environmental relevance of heavy metal-substituted chlorophylls using the example of water plants.** *J. Exp. Bot.* 1996; **47**: 259–266.
[Publisher Full Text](#)
- Lin YH, Pan KY, Hung CH, et al.: **Overexpression of ferredoxin, PETF, enhances tolerance to heat stress in *Chlamydomonas reinhardtii*.** *Int. J. Mol. Sci.* 2013; **10**: 20913–20929.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Liu L, Pohnert G, Wei D: **Extracellular metabolites from industrial microalgae and their biotechnological potential.** *Mar. Drugs* 2016; **14**: 191.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lu J, Ma Y, Xing G, et al.: **Revelation of microalgae's lipid production and resistance mechanism to ultra-high Cd stress by integrated transcriptome and physiochemical analyses.** *Environ. Pollut.* 2019; **250**: 186–195.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Leong Y, Changa J: **Bioremediation of heavy metals using microalgae: Recent advances and mechanisms.** *Bioresour. Technol.* 2020; **303**: 122886.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Masindi V, Muedi KL: **Environmental contamination by heavy metals.** *InTechOpen*; 2018.
[Publisher Full Text](#)
- Mayfield SP, Manuell AL, Chen S, et al.: ***Chlamydomonas reinhardtii* chloroplasts as protein factories.** *Curr. Opin. Biotech.* 2007; **18**: 126–133.
[PubMed Abstract](#) | [Publisher Full Text](#)
- McKinnon KM: **Flow Cytometry: An Overview.** *Curr. Protoc. Immunol.* 2019; **120**: 5.1.1–5.1.11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Miazek K, Iwanek W, Remacle C, et al.: **Effect of metals, metalloids and metallic nanoparticles on microalgae growth and industrial product biosynthesis: A review.** *Int. J. Mol. Sci.* 2015; **16**: 23929–23969.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Nowicka B, Plucinski B, Kuczynska P, et al.: **Physiological characterization of *Chlamydomonas reinhardtii* acclimated to chronic stress induced by Ag, Cd, Cr, Cu and Hg ions.** *Ecotoxicol. Environ. Saf.* 2016; **130**: 133–145.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Olaniran AO, Balgobind A, Pillay B: **Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies.** *Int. J. Mol. Sci.* 2013; **14**(5):

10197–10228.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Penen F, Isaure MP, Dobritsch D, et al.: **Pools of cadmium in *Chlamydomonas reinhardtii* revealed by chemical imaging and XAS spectroscopy.** *Metallomics*. 2017; **9**(7): 910–923.

[PubMed Abstract](#) | [Publisher Full Text](#)

Penen F, Isaure MP, Dobritsch D, et al.: **Pyrenoidal sequestration of cadmium impairs carbon dioxide fixation in a microalga.** *Plant Cell Environ*. 2019; **43**(2): 479–495.

[PubMed Abstract](#) | [Publisher Full Text](#)

Perales-Vela HV, Peña-Castro JM, Cañizares-Villanueva RO: **Heavy metal detoxification in eukaryotic microalgae.** *Chemosphere*. 2006; **64**(1): 1–10.

[PubMed Abstract](#) | [Publisher Full Text](#)

Perez-Rama M, Abalde Alonso J, Herrero Lopez C, et al.: **Cadmium removal by living cells of the marine microalga *Tetraselmis suecica*.** *Bioresour. Technol*. 2002; **84**: 265–270.

[PubMed Abstract](#) | [Publisher Full Text](#)

Pinter TB, Stillman MJ: **Kinetics of zinc and cadmium exchanges between metallothionein and carbonic anhydrase.** *Biochemistry-US*. 2015; **54**: 6284–6293.

[PubMed Abstract](#) | [Publisher Full Text](#)

Pollock SV, Mukherjee B, Bajsa-Hirschel J, et al.: **A robust protocol for efficient generation, and genomic characterization of insertional mutants of *Chlamydomonas reinhardtii*.** *Plant Methods*. 2017; **13**: 22.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Priyadarshini E, Priyadarshini S, Pradhan N: **Heavy metal resistance in algae and its application for metal nanoparticle synthesis.** *Appl. Microbiol. Biotechnol*. 2019; **103**: 3297–3316.

[PubMed Abstract](#) | [Publisher Full Text](#)

Roach T, Sedoud A, Krieger-Liszka A: **Acetate in mixotrophic growth medium affects photosystem II in *Chlamydomonas reinhardtii* and protects against photoinhibition.** *Biochim. Biophys. Acta*. 2013; **1827**: 1183–1190.

[PubMed Abstract](#) | [Publisher Full Text](#)

Rydziński D, Piotrowicz-Cieślak AI, Grajek H, et al.: **Chlorophyll degradation by tetracycline and cadmium in spinach (*Spinacia oleracea* L.) leaves.** *Int. J. Environ. Sci. Technol*. 2019; **16**(10): 6301–6314.

[Publisher Full Text](#)

Samadani M, Dewez D: **Cadmium accumulation and toxicity affect the extracytoplasmic polyphosphate level in *Chlamydomonas reinhardtii*.** *Ecotox. Environ. Saf*. 2018; **166**: 200–206.

[PubMed Abstract](#) | [Publisher Full Text](#)

Samadani M, Perreault F, Oukarroum A, et al.: **Effect of cadmium accumulation on green algae *Chlamydomonas reinhardtii* and acid-tolerant *Chlamydomonas* CPCC 121.** *Chemosphere*. 2018; **191**: 174–182.

[PubMed Abstract](#) | [Publisher Full Text](#)

Sevim C, Dogan E, Comakli S: **Cardiovascular disease and toxic metals.** *Curr. Opinion Toxicol*. 2020; **19**: 88–92.

[Publisher Full Text](#)

Shimogawara K, Fujiwara S, Grossman A, et al.: **High-efficiency transformation of *Chlamydomonas reinhardtii* by electroporation.** *Genetics*. 1998; **148**: 1821–1828.

[PubMed Abstract](#) | [Free Full Text](#)

Tchounwou PB, Yedjou CG, Patlolla AK, et al.: **Heavy Metals Toxicity and the Environment.** *NIH EXS*. 2012; **101**: 133–164.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Vazquez Cano E, Reyes V, Ynalvez RA: **Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis of lead bioaccumulation in *Chlamydomonas reinhardtii* and the role of Cia7 gene.** *The FASEB J*. 2018; **32**: 1_supplement, 665.5–665.5.

Viehweger K: **How plants cope with heavy metals.** *Bot. Stud*. 2014; **55**: 1–12.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Reference Source](#)

Xie M, Sun Y, Feng J, et al.: **Predicting the toxic effects of Cu and Cd on *Chlamydomonas reinhardtii* with a DEBtox model.** *Aquatic Toxicol*. 2019; **210**: 106–116.

[PubMed Abstract](#) | [Publisher Full Text](#)

Ynalvez RA, Moroney JV: **Identification and characterization of a novel inorganic carbon acquisition gene, CIA7, from an insertional mutant of *Chlamydomonas reinhardtii*.** *Funct. Plant Biol*. 2008; **35**: 373–381.

[PubMed Abstract](#) | [Publisher Full Text](#)

Ynalvez R, Gonzalez-Cantu H, Vazquez-Cano E: **Cell size, chlorophyll fluorescence and cadmium bioaccumulation between wild-type and mutant strains of *Chlamydomonas reinhardtii* upon exposure to cadmium.** *Dryad, Dataset*. 2021;

[Publisher Full Text](#)

Yu Z, Wei H, Hao R, et al.: **Physiological changes in *Chlamydomonas reinhardtii* after 1000 generations of selection of cadmium exposure at environmentally relevant concentrations.** *Environ. Sci. Process Impacts*. 2018; **20**(6): 923–933.

[PubMed Abstract](#) | [Publisher Full Text](#)

Zheng C, Aslam M, Liu X, et al.: **Impact of Pb on *Chlamydomonas reinhardtii* at Physiological and Transcriptional Levels.** *Front. Microbiol*. 2020; **11**: 1443.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 21 February 2022

<https://doi.org/10.5256/f1000research.57437.r121675>

© 2022 Schmollinger S. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Stefan Schmollinger

California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley, CA, USA

The article "Comparison of cell size, chlorophyll fluorescence and cadmium (Cd^{2+}) bioaccumulation between wild-type and mutant strains of *Chlamydomonas reinhardtii* upon exposure to Cd^{2+} " from Ynavez *et al.* analyses the effects of Cd on a *Chlamydomonas* wild-type strain and the *cia7* mutant. The authors find a reduction of Chl content, differences in cell size between Wt and mutant, but not to Cd treatment, and Cd accumulation in mixotrophically grown cells

In general, I would recommend indexing, but only after some revisions.

Comments:

Replication. The amount and kinds of replication should be clearly explained for each experiment, at least in the figure legend and in the methods. From the raw data that was deposited, I am assuming that there were either two separate experiments (replicate 1 and 2), where three individual, potentially technical, replicates for each condition were performed. Not completely clear from the methods for Figures 1 and 2, not at all for Figure 3.

The decision to combine the different days of Cd incubation I don't understand. Was there no information in the time series? For the ICP data, it looks like the Cd is accumulating between rep 1, 2, and 3. Were they collected on successive days and Cd slowly accumulated (both Wt and mutant show this tendency)?

ICP-OES data. The authors performed the analysis for both WT and *cia7*, but chose to only include one of the datasets in the figure, lacking error bars. This might be a consequence from point (2). Additionally, in the text, it is not clear which strain the authors refer to when discussing the results, or present in the figure. From the RawData I think there is no difference between the mutant and WT in terms of Cd accumulation, I do think the interpretation of the authors are correct that the only difference is between TAP and Min.

Figure quality: The figures could be easily improved by just modifying some of the standard, very basic Excel layout.

- Figures 1 and 2 should not be line graphs, but rather bar plots. You shall not connect what is indeed separate. From the description, I take it that the cultures in 0, 5, and 10 μM Cd are separate flasks, and were done in parallel.
- Y-axis tick labels are unnecessarily long in Figures 1 and 2, the description could be changed to arbitrary units (which is what they are), and a lot of zeros could be removed. The y-axis itself then could be labeled Chlorophyll fluorescence (a.u.) and Cell size (a.u.) in Fig 1 and 2, respectively. In Fig 3 just Cd^{2+} (ppb) is fine, the word “measured” is unnecessary.
- The x-axis label in Fig1 and 2 could be CdCl_2 (μM) instead of concentration (μM), which would give the reader the luxury to directly identify what concentration the authors are referring to. In Fig 3 the axis should also just be the Cd concentration, CdCl_2 (μM), the different media, distinguished by shades of grey, can be added as a legend, either somewhere in the graph or below.
- Same for the labels of the strains, instead of just 1 and 2, it could be wild-type (CC-4425) and *cia7* (CC-5013) in Fig 1 and 2.
- The white space in the graphs can be significantly reduced by choosing slightly wider bars, which can be done in Excel by reducing the gap width.

References for the minimal media, maybe also a citation for TAP (Harris 1989 for example), should be added to the methods

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I work on trace metal metabolism in *Chlamydomonas reinhardtii*, regularly

conducting elemental analysis of the alga on an Agilent 8900 ICP MS/MS system.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 19 January 2022

<https://doi.org/10.5256/f1000research.57437.r115295>

© 2022 Wang J. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Jiangxin Wang

Shenzhen Key Laboratory of Marine Bioresources and Eco-environmental Science, Shenzhen Engineering Laboratory for Marine Algal Biotechnology, Guangdong Provincial Key Laboratory for Plant Epigenetics, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, China

Heavy metals vs environments and algae is always an important field, and this ms compared cell size, chlorophyll fluorescence and cadmium (Cd^{2+}) bioaccumulation between wild-type and mutant strains of *Chlamydomonas reinhardtii* upon exposure to Cd ions. Experiments were well designed and statistical analyses were sufficient. However, the ms is rather descriptive, focusing on physiological/cellular levels. The more interesting points such as why and how this mutant is tolerant against Cd, or whether this mutant is also tolerant to other heavy metals. Also, the reviewer is interested to know why this mt has a small cell size even grown in the control medium.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Algae OMICS toxicology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research