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Oceane Hourtane, Patrice Gonzalez, Agnes Feurtet-Mazel, E. Kochoni, C. Fortin. Potential cellular targets of platinum in the freshwater microalgae *Chlamydomonas reinhardtii* and *Nitzschia palea* revealed by transcriptomics. *Ecotoxicology*, 2024, 10.1007/s10646-024-02746-y . hal-04546211

HAL Id: hal-04546211

<https://hal.science/hal-04546211>

Submitted on 15 Apr 2024

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Potential cellular targets of platinum in the freshwater microalgae *Chlamydomonas reinhardtii* and *Nitzschia palea* revealed by transcriptomics

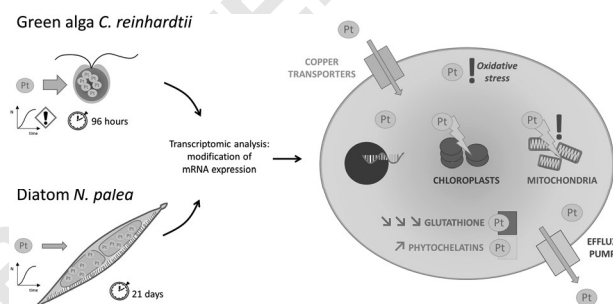
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Accepted: 27 February 2024
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Abstract

Platinum group element (PGE) levels have increased in natural aquatic environments in the last few decades, in particular as a consequence of the use of automobile catalytic converters on a global scale. Concentrations of Pt over tens of $\mu\text{g L}^{-1}$ have been observed in rivers and effluents. This raises questions regarding its possible impacts on aquatic ecosystems, as Pt natural background concentrations are extremely low to undetectable. Primary producers, such as microalgae, are of great ecological importance, as they are at the base of the food web. The purpose of this work was to better understand the impact of Pt on a cellular level for freshwater unicellular algae. Two species with different characteristics, a green alga *C. reinhardtii* and a diatom *N. palea*, were studied. The bioaccumulation of Pt as well as its effect on growth were quantified. Moreover, the induction or repression factors of 17 specific genes were determined and allowed for the determination of possible intracellular effects and pathways of Pt. Both species seemed to be experiencing copper deficiency as suggested by inductions of genes linked to copper transporters. This is an indication that Pt might be internalized through the Cu(I) metabolic pathway. Moreover, Pt could possibly be excreted using an efflux pump. Other highlights include a concentration-dependent negative impact of Pt on mitochondrial metabolism for *C. reinhardtii* which is not observed for *N. palea*. These findings allowed for a better understanding of some of the possible impacts of Pt on freshwater primary producers, and also lay the foundations for the investigation of pathways for Pt entry at the base of the aquatic trophic web.

Graphical Abstract



Keywords Platinum · Diatom · Unicellular green alga · Gene expression · Copper transporters

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Introduction

Catalytic converters were introduced in the mid-70s to decrease the emission of the main products of incomplete combustion of fossil fuels, which can pose a number of human health issues (Dey and Mehta 2020), in favor of

CO₂, H₂O and N₂. Since 1993, the most commonly used catalytic converter has been the three-way catalyst in which platinum and palladium are used to oxidize carbon monoxide and hydrocarbons while rhodium is used to reduce nitrogen oxides. Since its implementation, emissions of these compounds have been reduced by around 90% (Morton et al. 2001). Catalytic converters consist of a honeycomb-shaped cordierite (Al₃Mg₂AlSi₅O₁₈) support on which a porous γ -alumina inner coating serves as a support for 1–10 nm platinum group element (PGE) metal particles (Barefoot 1997; Ravindra et al. 2004). Over the vehicles' lifetime, abrasion of the catalyst's inner lining results in the emission of aluminum particles to which PGE nanocrystals are attached. This leads to the contamination of urban and suburban environments *via* materials that contain these metals, such as road dust (Ely et al. 2001). Thus, catalytic converters are a major source of local PGE contamination, particularly for soils along busy roads, and are of increasing concern globally. Indeed, PGE can be adsorbed onto fine particles and carried *via* wind, whereby deposition has been recorded in some of the least anthropized parts of the planet, such as Greenland (Barbante et al. 2001) and Antarctica (Soyol-Erdene et al. 2011). PGEs emitted by catalytic converters are mainly bound to aluminum oxides from the honeycomb structure of the converter, but also to other metal oxides (Palacios et al. 2000). According to Artelt et al. (2000), a fraction of 1% of the PGE emitted by catalytic converters is water-soluble, and around 10% of these compounds emitted in particulate and soluble form could be bioaccumulated (Paolucci et al. 2007). Moreover, in the case of Pt, hospital effluents also represent a significant source of contamination in aquatic environments, due to its use in anticancer treatments and its subsequent excretion by patients. Concentrations up to 145 $\mu\text{g Pt L}^{-1}$ have been reported in such effluents (Lenz et al. 2005). Nevertheless, maximum environmental concentrations are lower, with up to 35 $\mu\text{g Pt L}^{-1}$ measured in rivers (Odiyo et al. 2005). Thus, to assess the risk, bioaccumulation and/or toxicity of Pt have been studied in different aquatic organisms such as fish (Sures et al. 2005), crustaceans (Moldovan et al. 2001; Rauch and Morrison 1999; Zimmermann et al. 2017), aquatic plants (Diehl and Gagnon 2007), biofilms (Rauch et al. 2004) and microalgae (Hourtané et al. 2022). The present work focuses on microalgae and specifically on diatoms as primary producers of great ecological importance in running freshwaters. It aims to understand Pt potential cellular targets and pathways in this major first trophic level which could lead through the food web to possible Pt transfer to higher trophic levels and possibly disturbances in the structure of the ecosystem.

Q1–Q3

Molecular tools can be used to reveal subtle effects of contaminants at concentration levels which won't trigger a significant response when using standard toxicological

endpoints, such as growth. When organisms are exposed to sub-lethal concentrations of contaminants, gene expression can be altered to encode protein production and acclimatize to the new conditions. For example, significant differences in gene expression were observed for diatoms, although there were no observed variations in terms of growth (Kim Tiam et al. 2012; Osborn and Hook 2013). Studying gene expression by transcriptomic analyses also allows for the investigation of possible impacts on cellular functions. Metals, even at low concentrations, can induce an oxidative stress which affects the functioning of mitochondria and/or photosynthetic functions. Kim Tiam et al. (2012) showed that the expression of genes related to these two functions was altered in the presence of cadmium for the diatom *Eolimna minima*. Oxidative stress can induce the production of antioxidant defense enzymes such as superoxide dismutases (mitochondrial, chloroplasmic and cytoplasmic) and catalase (Kim Tiam et al. 2018), as well as glutathione peroxidase. The latter favors the oxidation of glutathione, a peptide, which helps regulate oxidative stress in the cell by scavenging hydrogen peroxide (Sies 1986). In addition to this role, glutathione can strongly bind certain metals and lead to their sequestration, and possibly their excretion (Glaeser et al. 1991), although this role is also played by more metal-specific peptides, such as phytochelatins. Phytochelatins are produced by plant species in response to exposure to certain metals (Callahan et al. 2006). They play a key role in the detoxification mechanisms of cells, allowing them to scavenge certain metals contributing to prevent the appearance of toxic effects (Callahan et al. 2006; Campbell and Hare 2009). As for metal internalization mechanisms and intracellular distribution, it has been shown that non-essential metals can use essential metal biological pathways, especially if they have similar chemical characteristics (Bridges and Zalups 2005). A wide diversity of more or less specific transmembrane transporter proteins are used for metal internalization by the cells and transport from one compartment to another as described for the alga *Chlamydomonas* in the work of Blaby-Haas and Merchant (2012). Studying the modification of the expression of genes related to the production of these transporters can lead to a better understanding of a metal's cellular pathway. In the case of platinum, thermodynamics suggest that it would be present in the oxidation state of Pt(II) within well-oxygenated solutions (Colombo et al. 2008), thus transport proteins specific to divalent ions such as Cu²⁺ and Zn²⁺ could be involved in its transport. Other types of more general transporters are also likely to play a role in Pt transport. For example, *nrampl* allows the internalization of the divalent ions Mn²⁺, Fe²⁺ and Co²⁺. In terms of chemical hardness, the thermodynamically stable Pt²⁺ ion is considered to be soft (class B) like Cu⁺ (Pearson 1993)

137 hinting that Copper transporters (CTR) may also play a role
138 in Pt internalization.

139 In this study we investigated the effects of Pt exposure
140 on microalgae, which are at the base of the food web and
141 the main contributors to primary production. Two species
142 with different characteristics were studied in order to
143 observe a wider range of the possible effects of Pt exposure
144 on primary producers : a unicellular green alga *Chlamy-*
145 *domonas reinhardtii*, for which Pt accumulation and toxic-
146 ity was previously studied (Hourtané et al. 2022), and a
147 periphytic diatom *Nitzschia palea*, for which no toxicity
148 data is available for Pt. The objectives were to compare
149 their responses over a growing period relevant to each
150 organism. In both cases, algae were harvested after expo-
151 sure to perform transcriptomic analyses and to investigate Pt
152 cellular effects and pathways allowing for a better under-
153 standing of its possible entryways in aquatic food webs.

154 Material and Methods

155 Reagent and solutions

156 Before use, labware was systematically rinsed three times
157 with deionised water, immersed in HNO₃ (ACS grade) 10%
158 (V/V) for 24 h, rinsed three times with deionised water and
159 then five times with ultrapure (MilliQ) water. To avoid
160 bacterial contamination of the cultures, labware was ster-
161 ilised at 121 °C and manipulations were carried out under a
162 laminar-flow hood. All solutions were prepared from
163 ultrapure (MilliQ) water, sterilised at 121 °C if needed and
164 stored at 4 °C.

165 Algal culture

166 Both freshwater algal species originated from the *Canadian*
167 *Phycological Culture Centre* (CPCC), University of
168 Waterloo, ON, Canada. The green alga *C. reinhardtii*
169 (CPCC#11) was grown in MHSM-1 medium (Modified
170 High Salt Medium 1) adjusted to pH = 6.00 ± 0.05 and the
171 diatom *N. palea* (CPCC#160) in modified CHU-10 medium
172 adjusted to pH = 7.00 ± 0.05. To ensure pH stability, a pH
173 buffer was used: 2-(N-morpholino)ethane sulfonic acid
174 (MES) at 10⁻² mol L⁻¹ in MHSM-1 and N-(2-Hydro-
175 xyethyl)piperazine-N'-(2-ethane sulfonic acid) (HEPES) at
176 4·10⁻³ mol L⁻¹ in modified CHU-10. The detailed compo-
177 sitions of both culture media are presented in the online
178 resource file.

179 The green alga *C. reinhardtii* cultures were axenic and
180 weekly inoculated in a fresh sterile nutrient medium,
181 whereas cultures were inoculated monthly for the diatom *N.*
182 *palea* due to its slower growth. Bacterial development in *N.*
183 *palea* cultures was observed after growth on agarose in a

Table 1 Experimental design for exposures to Pt

Exposure of alga		Green alga <i>C. reinhardtii</i>	Diatom <i>N. palea</i>
Exposure conditions	Material of Erlenmeyer	polycarbonate	Borosilicate glass
	Culture and exposure medium	MHSM-1	Modified CHU-10
	pH	6.00 ± 0.05	7.0 ± 0.7
	[Pt] (µg L ⁻¹)	0, 50 and 100	0, 10, 30, 40, 50, 75, 100
Studied parameters	Duration	96 h	21 d
	Metal bioaccumulation	96 h	21 d
	Growth: cell density	0, 6, 24, 48, 72, 96 h	0, 7, 14, 21 d
	Transcriptomic analyses	96 h	0, 7, 14, 21 d

184 petri dish. However, the bacterial presence was minimal as
185 no microscopic observation of the bacteria was possible
186 during its growth. In natural environments, periphytic dia-
187 toms like *N. palea* tend to agglomerate and develop mutual
188 beneficial relationships with other organisms, such as bac-
189 teria in biofilms. Thus, maintaining a culture of diatoms in
190 an axenic state is not environmentally relevant and can have
191 significant deleterious effects on the morphology and long
192 term growth of some diatom species (Windler et al. 2014).

193 Exposure experiments

194 A summary of exposure conditions and studied parameters
195 is shown in Table 1 to highlight the main differences
196 between the experiments for both species.

197 Exposure experiments were performed at concentrations
198 representative of contaminated ecosystems. There were
199 additional Pt exposure concentrations tested on the diatoms
200 compared to the green alga, due to the lack of previous
201 work on diatoms with Pt. There were also more sampling
202 times for transcriptomic analyses of diatoms in order to
203 observe the temporal evolution of gene expression
204 throughout their longer exposure. Experiments were carried
205 out in 100 mL culture medium in 250 mL polycarbonate (*C.*
206 *reinhardtii*) or glass (*N. palea*) Erlenmeyer flasks. The latter
207 was used for *N. palea*, because of its propensity to adhere to
208 plastic surfaces. Although glass is known to adsorb metals
209 more than polymer-based materials, the recovery of Pt was
210 reasonably good (93 ± 4% for polycarbonate flasks and
211 77 ± 4% for glass flasks) considering the exposure duration
212 (96 h vs 21 d, respectively). For the exposure of both spe-
213 cies of interest, Pt was added 72 h prior to inoculation and
214 added from a 1000 µg Pt mL⁻¹ standard solution of
215 (NH₄)₂Pt (IV) Cl₆ (10% HCl; Plasma CAL, SCP Science).

For each set of experiment, all tested conditions were conducted in triplicates. The studied parameters were population growth and gene expression modifications determined every 24 h for *C. reinhardtii* and every 7 d for *N. palea*. Total Pt concentrations in exposure media were verified at all sampling times including the beginning and end of the exposures, and Pt bioaccumulation was determined at the end of the exposure for *N. palea* (for *C. reinhardtii*, Pt uptake had already been characterized in the same experimental conditions; see Hourtané et al. (2022)). Exposure pH was monitored over time and a greater variability was observed for the diatom (7.0 ± 0.7) compared to the green alga (6.00 ± 0.05). All exposures were carried out under gentle and constant agitation (50–100 rpm) at room temperature and a continuous light of $63 \pm 24 \mu\text{E m}^{-2} \text{s}^{-1}$, allowing an asynchronous cellular division.

Algal growth measurements

Algal growth as a standard toxicity endpoint was followed during exposure experiments using a particle counter for cell enumeration (*Coulter Counter Z2* and *Z3*). For each replicate measurement, 1 mL was taken from the exposure media gently swirled beforehand and added to 9 mL of *Isoton II* solution (Beckman Coulter). For *N. palea*, an additional step was performed. Since this species is a periphytic diatom, the cells tend to naturally agglomerate. To disaggregate cell clumps as much as possible in order to facilitate cell enumeration, the culture was mechanically homogenized by repetitively pipetting in and out 5–10 mL of culture using a sterile pipette tip. Then, a 3 mL subsample was transferred to a vial which was immersed in an ultrasonic bath for 30 min, from which up to 1 mL was taken for cell enumeration.

Sample mineralization

N. palea cells were harvested by filtration using polysulfone filter holder units with 2 μm porosity polycarbonate filter membranes. Cells harvested on the filters were rinsed twice for 10 min using a $10^{-5} \text{ mol L}^{-1}$ EDTA solution to remove the adsorbed metal on the cell surface in order to quantify the intracellular Pt (Hassler et al. 2004). The filters were then folded in four, placed at the bottom of a tube (50 mL volumetric, Sarstedt), and then dried for 48 h at 70 °C. For mineralization, 2.5 mL of concentrated HCl (37% acid; Trace Metal grade; Fisher Chemicals) was added to digest the algae at room temperature (20 °C) for 48 h. Note that this technique allows only a partial digestion that leaves the silica frustules mostly intact. At the end of this time, the tube was filled up to 50 mL with ultrapure water to obtain a final HCl concentration of 5% (V/V). After analysis, the

bioaccumulation was expressed in μg of Pt per cell, using the known cell density and exposure volume. To control for the potential contribution of the filter membrane to the metal content in the mineralized samples, a second filter was occasionally added under the first one. These indicated that there was no overestimation of bioaccumulation due to metal adsorption on the filter membrane. The certified reference sample IAEA-450 (unicellular microalga, *Scenedesmus obliquus*) was mineralized using the same procedure and a mean Pt recovery of $80 \pm 9\%$ ($n = 8$) was obtained.

Platinum quantification

Prior to quantification, all samples were stored in 5% (V/V) HCl. Pt concentrations were measured for all of the mineralized samples using an ion-coupled plasma mass spectrometer (ICP-MS; Thermo Instrument, Xseries2). A Rh/Re 8 ppm standard was used to correct signal deviation and a series of quality controls ($[\text{Pt}] = 0.75$ and $25 \mu\text{g L}^{-1}$ prepared from S409 solution; C00-061-409, PlasmaCAL) were added every 12 to 15 samples. Aqua regia 5% (V/V) was used as a rinse solution between each sample. The detection limit by ICP-MS was always below $0.04 \mu\text{g L}^{-1}$ and the average recovery from standard addition was $83 \pm 14\%$ for all sample types combined. For experiments with *C. reinhardtii*, conditions were similar to the ones described in Hourtané et al. (2022) and for *N. palea*. Pt in the exposure media was quantified using graphite furnace atomic absorption spectroscopy (GFAAS; AA 240Z, Agilent Technologies) using a Pt hollow cathode lamp (Symalab, Agilent Technologies; $\lambda = 265.9 \text{ nm}$). The detection limit was $1.7 \mu\text{g L}^{-1}$ and quality control samples were run every 15 samples, with a recovery over the course of the analyses at $97 \pm 5\%$ ($n = 12$).

To verify that the two Pt quantification methods used were equivalent, a standard solution with a concentration of $35 \mu\text{g L}^{-1}$ was assayed three times by ICP-MS and then five times by GFAAS. The concentrations obtained were not statistically different (t-test; p -value = 0.10) confirming that the quantification of metal was equivalent with both methods. The main difference resides in the lower detection limit obtained with the ICP-MS.

Transcriptomic analyses

The response of both algae exposed to Pt was examined by determining the induction or repression factor of specific genes corresponding to known cellular functions: the synthesis of transporters involved in metal uptake, the photosystem and mitochondrial metabolisms, the responses to oxidative stress, the production of intracellular ligands such as phytochelatin, as well as a transporter that could be

Table 2 Targeted genes for *C. reinhardtii* (a) and *N. palea* (b) with their associated cellular functions

Targeted genes	Complete name	Associated cellular functions
<i>nramp1</i> ^b	Natural-resistance-associated macrophage protein 1	Metal transporter
<i>ctr2</i> ^{a,b}	Copper transporter 2	Copper transporters
<i>ctr3</i> ^a	Copper transporter 3	
<i>psaA</i> ^{a,b}	PsaA synthase protein	Photosynthesis. Photosystem 1; PS1
<i>d1</i> ^a	D1 protein	Photosynthesis. Photosystem 2; PS2
<i>sodFe</i> ^a	Chloroplasmic superoxide dismutase	Antioxidant defenses – chloroplasts
<i>coxI</i> ^{a,b}	Cytochrome C oxidase subunit I	Mitochondrial metabolism
<i>nad5</i> ^a	NADH Dehydrogenase subunit 5	
<i>12S</i> ^a	Mitochondrial ribosomal RNA 12S	
<i>sodMn</i> ^{a,b}	Mitochondrial superoxide dismutase	Antioxidant defenses - mitochondria
<i>cat</i> ^{a,b}	Catalase	Antioxidant defenses - cytoplasm
<i>sodCu</i> ^b	Cytoplasmic superoxide dismutase	
<i>fad</i> ^a	Flavin adenine dinucleotide synthase	
<i>gpx</i> ^a	Glutathione peroxidase 1	
<i>gst</i> ^{a,b}	Glutathione S-transferase	Detoxification
<i>pcs1</i> ^{a,b}	Phytochelatin synthase 1	
<i>mdr1</i> ^{a,b}	MultiDrug resistance	
<i>βact</i> ^a	β actin	Reference genes
<i>ef1α</i> ^a	Elongation factor 1 α	
<i>rpL13</i> ^{a,b}	Ribosomal protein L13	

involved in excretion mechanisms (Table 2). These genes were selected because their expressions were suspected to be modified by Pt. They correspond to important cellular functions that may be associated with a response to metal exposure (Blaby-Haas and Merchant 2012; Kim et al. 2017; Kim Tiam et al. 2012; Kim Tiam et al. 2018). Several diatom genes have already been studied in the literature (Kim Tiam et al. 2018; Moisset et al. 2015) and others were selected from the complete sequenced genome of *N. palea* which was obtained collaboratively. Tests have also been carried out with *C. reinhardtii*, whose complete genome has been sequenced and is available in databases (Blaby et al. 2014; Merchant et al. 2007). All selected genes could not be studied for both species due to either unavailability in the data banks or to the absence of functional primers. The reference genes made it possible to determine the relative gene expression differences for the targeted genes. Because they correspond to cellular functions linked to cellular integrity, their expression remains stable (Livak and Schmittgen 2001). The selected reference genes were *βact*, *ef1α* and *rpL13*. However, only the last one was used for *N. palea*, as it was the only one for which satisfactory amplifications for all experiments presented could be obtained.

RNA extraction

Total RNA extractions were performed using « SV Total RNA Isolation System » kits (Promega). Algal cells were

separated from the conservation solution (RNAlater, Invitrogen) by centrifugation or filtration. First, the cells were grinded in 1 mL of Trizol (TRI reagent solution, Invitrogen) with 0.10–0.11 mm diameter glass beads (B. Braun Biotech International) using a Fastprep (Biorad, 4 movements s⁻¹, 40 s). Then, 200 μL of chloroform (Fisher Chemicals) were added to perform deproteinization (Bienvenu et al. 1999). After mixing with a vortex for 10 s, a 5 min centrifugation (13,500 rpm or about 18,000 g, at room temperature) was used to separate the aqueous and organic phases. The aqueous phase, containing the nucleic acids (DNA and RNA), was collected and mixed with a volume of 75% ethanol before being deposited on an affinity column. The remaining hydrophilic proteins were rinsed off the column twice with a saline solution (RNA Wash Solution). Afterwards, a treatment with DNase I (1 U) was performed by incubating for 15 min at 37 °C, in order to break the DNA molecules. Once the treatments were completed, total RNAs were eluted with 30 μL of water (Nuclease Free) from the kit in tubes provided for this purpose. The total RNA thus obtained were stored at –80 °C until analysis or used right away to perform reverse transcription (RT).

A spectrophotometer (Biotek®) was used to estimate the purity of the extracted nucleic acids. Drops of 2 μL for each sample were placed on a specific plate (EPOCH) and the absorbances at 260 and 280 nm were simultaneously measured. A sample was considered sufficiently pure when the ratio of absorbance at 260 nm to absorbance at 280 nm was

368 greater than 1.8. The estimated quantity of RNA was also
 369 given by the instrument in $\text{ng } \mu\text{L}^{-1}$ and allows to verify that
 370 the extraction step was successful.

371 Reverse transcription

372 RT was performed using the “GoScript Reverse Tran-
 373 scription System” kit (Promega). This step allowed the
 374 transformation of the purified RNA into cDNA (com-
 375plementary DNA). For one sample, $1 \mu\text{L}$ of oligodT
 376 solution ($1 \mu\text{mol L}^{-1}$) was mixed with $1 \mu\text{L}$ of hexaprimer
 377 ($1 \mu\text{mol L}^{-1}$) and $10 \mu\text{L}$ of purified RNA (approximately
 378 $1 \mu\text{g}$). The mixture was then cycled in the thermal cyclor
 379 for 5 min at 70°C followed by a gradual decrease in
 380 temperature to 4°C to linearize the RNAs and allow the
 381 primers to bind. Then $1 \mu\text{L}$ of deoxyribonucleotide tri-
 382phosphate (dNTP) solution (10mmol L^{-1}), $4 \mu\text{L}$ of 5x
 383 activity buffer solution, $1.5 \mu\text{L}$ of MgCl_2 (25mmol L^{-1}),
 384 $1 \mu\text{L}$ of reverse transcriptase ($1 \text{U } \mu\text{L}^{-1}$) and $0.5 \mu\text{L}$ of
 385 ribonuclease inhibitor RNasin® solution were added. This
 386 mixture was put back into the thermocycler with the fol-
 387 lowing protocol: 5 min at 25°C , then 1 h at 42°C to
 388 perform the cDNA synthesis. Samples containing the
 389 synthesized cDNAs were stored at -20°C before per-
 390 forming qPCR (real-time quantitative polymerase chain
 391 reaction).

392 Amplifications

393 Real-time quantitative PCR was used to quantify gene
 394 expression. It was performed with a LightCycler 480
 395 (Roche), which allows real-time monitoring of cDNA
 396 amplification by adding the fluorescent intercalating agent
 397 SyberGreen to the reaction medium. The fluorescence
 398 detected at 530 nm was measured at the end of each cycle.
 399 The PCR reactions were carried out in 96-well microplates
 400 using the “GoTaq® qPCR Master Mix” kit (Promega)
 401 containing 2x buffer (activity buffer, *Taq* polymerase,
 402 MgCl_2 , dNTPs and SyberGreen). Beforehand, a mixture of
 403 the forward and reverse primers (see the online resource
 404 file) was produced for each gene ($2 \mu\text{M}$ of each primer). To
 405 each well, $13 \mu\text{L}$ of 2x diluted reagent mix ($10 \mu\text{L}$ 2x buffer
 406 + $3 \mu\text{L}$ water), $2 \mu\text{L}$ of primer mix and $5 \mu\text{L}$ of 1/10 diluted
 407 cDNA were added. For the amplification protocol, the
 408 plates were first heated to 95°C for 2 min to activate the
 409 *Taq* polymerase before performing 50 cycles of PCR
 410 (95°C , 15 s; 60°C , 1 min). At the end of the reaction, the
 411 quality of the amplifications was determined by performing
 412 melting curves at the end of the 50 cycles. These were
 413 carried out by continuous fluorescence measurements dur-
 414 ing a gradual rise in temperature of the device from 60°C to
 415 95°C . This allowed for a determination of the melting

temperature for the amplified DNA strands, which was
 specific to each gene.

Data curation following qPCR

LightCycler® 480 Software (version 1.5.1.62.SP3) was
 used to calculate the number of cycles (Ct) required for
 emitted fluorescence to significantly exceed the baseline
 level, allowing detection. It is the number n of cycles
 necessary to reach the beginning of the exponential phase
 of the PCR curve which depends on the number of initial
 copies of the target. The level of expression of each gene
 was determined relative to that of reference gene(s). The
 difference in output moments (ΔCt) between the average
 of the reference genes and the gene of interest was cal-
 culated according to the method described by (Livak and
 Schmittgen 2001).

$$\Delta\text{Ct} = \text{Ct}_{\text{targeted gene}} - \text{Ct}_{\text{reference gene}}$$

To determine the biological response to contamination,
 the different concentrations/exposure conditions were then
 compared to the corresponding controls. The induction
 factors (IF) were calculated according to the following
 equation:

$$\text{IF} = \frac{2^{-\Delta\text{Ct}_{\text{condition}}}}{2^{-\Delta\text{Ct}_{\text{control}}}}$$

Note also that platinum is known to interact with DNA
 molecules (Reedijk 2016), yet the potential impact on
 RNA extraction performance and therefore on the PCR
 results is unknown. Indeed, during the algal grinding step,
 intracellular Pt would be released into the medium, as well
 as proteins, DNA and RNA. With this possible issue in
 mind, the modification of gene expression was determined
 by qPCR, which compares the expression of a given gene
 to a reference gene, making it possible to limit the biases
 related to extraction performance. All the reference genes
 used for *C. reinhardtii* (βact , $\text{ef}1\alpha$ and *rpL13*) had similar
 exit times with no significant differences (one-way
 ANOVA) between the control and exposure conditions,
 showing that the presence of Pt did not seem to interfere
 with the extraction or PCR performance by binding to
 RNAs. Exit times of reference gene *rpL13*, used for *N.*
palea, were also similar for all Pt conditions. In conclu-
 sion, qPCR is preferred when working with Pt and
 reference genes βact , $\text{ef}1\alpha$ and *rpL13*.

Statistical analyses

The software R was used for all statistical analyses. Cell
 yields were compared between Pt concentration condi-
 tions at the same sampling time using t-tests. For the
 transcriptomic analyses, statistics were performed on the

464 $2^{-\Delta Ct}$ values. For each condition and targeted gene, nor-
 465 mality was first verified using a Shapiro test. Depending
 466 on the outcome, a parametric (one-way ANOVA) or non-
 467 parametric (Kruskal-Wallis) test was performed. The
 468 corresponding post-hoc tests were Tukey's test and
 469 Dunn's test, respectively. Several confidence levels were
 470 determined and correspond to the following p-values: *
 471 for p_{value} below 0.05, ** for p_{value} below 0.01, *** for
 472 p_{value} below 0.001.

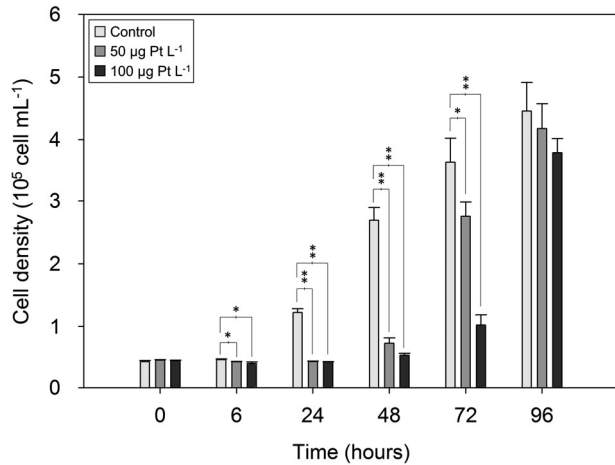


Fig. 1 Cell density of *C. reinhardtii* over time at 0, 50 and 100 µg Pt L⁻¹ in MHSM-1 medium at pH = 6.00 ± 0.05 (n = 3); asterisks represent statistical differences compared to the control

Results

Green alga response

Figure 1 and Table 3 respectively show growth curves and induction factors for *C. reinhardtii* during a 96 h exposure to Pt.

The growth of *C. reinhardtii* was delayed in the presence of Pt by approximately 2 to 3 days compared to control cells (Fig. 1), with significantly reduced growth for both Pt concentration conditions at 6, 24, 48 and 72 h.

In terms of gene expression (Table 3), there are a few repressions of significant importance observed for *d1* coding for protein D1 (photosystem II) and *gst* coding for glutathione S-transferase. This down regulation of *gst* is important, and there appears to be a concentration effect. Some not significant inductions are also observed, for *pcs1* at 50 µg Pt L⁻¹, *mdr1* at both exposure concentrations and *ctr2* and *ctr3* at 100 µg Pt L⁻¹.

Diatom response

Diatoms differ from green algae in several aspects, one of the most notable ones being their much slower growth. In this section, we report on Pt bioaccumulation (Fig. 2), cell growth (Fig. 3), and changes in *N. palea* gene expression over a 21 d exposure (Tables 4–6). Emphasis was placed on gene expression and its evolution during exposure.

In the diatoms collected after 21 d, internalization of Pt seems to increase linearly ($R^2 = 0.61$) with exposure

Table 3 Induction factors of targeted genes in *C. reinhardtii* after a 96 h exposure to sublethal concentrations of Pt (n = 3); inductions (IF > 2) are highlighted in orange and repressions (IF < 0.5) in green. Slashes indicate an absence of variation in gene expression: 0.5 < IF < 2; asterisks represent statistical differences compared to the control

Genes	Induction factors (IF) for each nominal [Pt] (µg L ⁻¹)	
	50	100
<i>ctr2</i>	/	3
<i>ctr3</i>	/	2
<i>psaA</i>	/	/
<i>d1</i>	0.4*	0.4*
<i>sodFe</i>	/	/
<i>cox1</i>	/	/
<i>nad5</i>	/	/
<i>12S</i>	/	/
<i>sodMn</i>	/	/
<i>cat</i>	/	/
<i>fud</i>	/	/
<i>gst</i>	0.003*	4.10 ⁻⁵ *
<i>pcs1</i>	2	/
<i>mdr1</i>	4	4

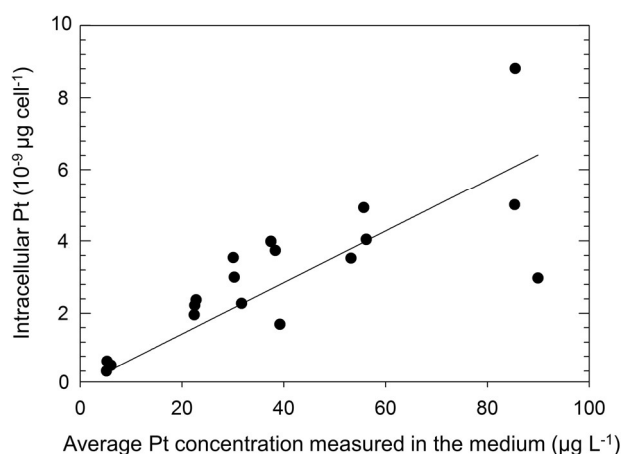


Fig. 2 Bioaccumulation of Pt in *N. palea* as a function of the mean measured exposure Pt concentrations in modified CHU-10 medium; pH = 7.0 ± 0.7 ($n = 3$)

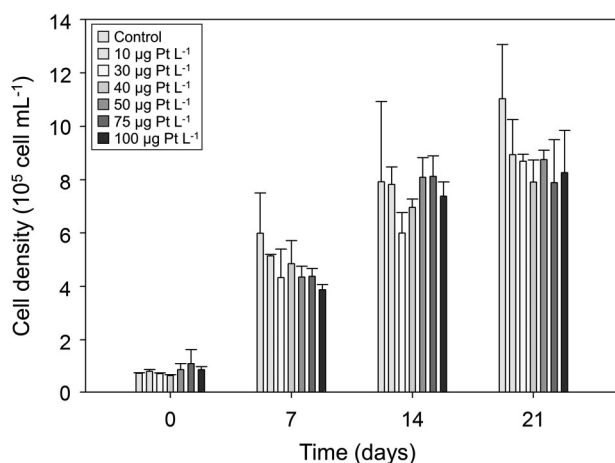


Fig. 3 Cell density over time for *N. palea* exposed to different Pt exposure concentrations in modified CHU-10 medium, pH = 7.0 ± 0.7 ($n = 3$)

concentration (Fig. 2). An apparent saturation seems to occur at the highest concentrations tested, with intracellular Pt reaching up to $5 \cdot 10^{-9} \mu\text{g cell}^{-1}$. However, a non-linear Michaelis-Menten model could not be fitted ($p > 0.05$).

No statistically significant effect on growth over time was observed throughout the Pt concentration range tested (Fig. 3) and no EC50 could be computed. The diatom *N. palea* seems to cope well with environmentally relevant Pt concentrations. Although, it should be noted that a high variability in cell enumeration due to the presence of diatom agglomerates may have contributed to conceal a significant toxic effect.

Looking at gene expression, Tables 4–6 show the IF of the genes studied in the diatom *N. palea* for the different sampling times, respectively 7, 14 and 21 days. First of all, these results show that there is a temporal evolution in *N. palea*'s response to Pt exposure. Effects are more important at 14 d with more gene expression modifications and also several significant inductions and repressions.

After 7 days, no effect on gene expression was observed at the lowest Pt concentration of $10 \mu\text{g Pt L}^{-1}$. For the medium ranged concentrations of $30\text{--}50 \mu\text{g Pt L}^{-1}$, there were several gene inductions observed, notably *cat* and *sodMn*, as well as *mdr1*. Induction of *mdr1* was only observed at $30 \mu\text{g Pt L}^{-1}$ and was significant. Above $75 \mu\text{g Pt L}^{-1}$, moderate (non-significant) repressions were observed in *psaA*, *pcs1*, *mdr1*, *gst* and *ctr2*.

After 14 d of exposure, responses were more pronounced, but followed a similar pattern with no modification of genetic expression at $10 \mu\text{g Pt L}^{-1}$, with inductions for the intermediate concentrations and repressions for the two highest concentrations. The main differences were the repressions of *cox1*, which were gradually increasing with exposure concentration starting at $40 \mu\text{g Pt L}^{-1}$ with IF from 0.5 to 0.1. The response was significantly different from the control at 50, 75 and $100 \mu\text{g Pt L}^{-1}$. For the other

Table 4 Induction factors obtained for *N. palea* after exposure for 7 days to Pt ($n = 3$); inductions (IF > 2) are highlighted in orange and repressions (IF < 0.5) in green. Slashes represent no observed variation in gene expression: $0.5 < \text{IF} < 2$, asterisks represent statistical differences compared to the control

Genes	Induction factors (IF) for each nominal [Pt] ($\mu\text{g L}^{-1}$)					
	10	30	40	50	75	100
<i>ctr2</i>	/	/	/	/	0.2	/
<i>nramp1</i>	/	/	/	/	/	/
<i>psaA</i>	/	/	/	/	0.4	/
<i>cox1</i>	/	/	/	/	/	/
<i>sodMn</i>	/	3	3	/	/	/
<i>cat</i>	/	3	4	2	/	/
<i>sodCu</i>	/	/	/	/	/	/
<i>gst</i>	/	/	/	0.4	0.5	/
<i>pcs1</i>	/	/	/	/	/	0.4
<i>mdr1</i>	/	2*	/	/	0.3	/

Table 5 Induction factor obtained for *N. palea* after exposure for 14 days to Pt ($n=3$); inductions (IF>2) are highlighted in orange and repressions (IF<0.5) in green. Slashes represent no observed variation in gene expression: $0.5 < \text{IF} < 2$, asterisks represent statistical differences compared to the control

Genes	Induction factors (IF) for each nominal [Pt] ($\mu\text{g L}^{-1}$)					
	10	30	40	50	75	100
<i>ctr2</i>	/	3	2	3	2	/
<i>nramp1</i>	/	/	/	/	/	0.2
<i>psaA</i>	/	/	/	/	0.3	0.1
<i>cox1</i>	/	/	0.5	0.4*	0.3*	0.1**
<i>sodMn</i>	/	2	3	2	/	0.3
<i>cat</i>	/	3	3*	3	/	0.5
<i>sodCu</i>	/	1.7**	/	/	/	/
<i>gst</i>	/	/	/	/	/	0.4*
<i>pcs1</i>	/	/	/	/	/	0.5
<i>mdr1</i>	/	2	4**	2	/	/

Table 6 Induction factor obtained for *N. palea* after exposure for 21 days to Pt ($n=3$); inductions (IF>2) are highlighted in orange and repressions (IF<0.5) in green. Slashes represent no observed variation in gene expression: $0.5 < \text{IF} < 2$, asterisks represent statistical differences compared to the control

Genes	Induction factors (IF) for each nominal [Pt] ($\mu\text{g L}^{-1}$)					
	10	30	40	50	75	100
<i>ctr2</i>	3	4	4	4	4	4
<i>nramp1</i>	/	/	/	/	/	/
<i>psaA</i>	/	/	/	/	/	0.4
<i>cox1</i>	/	/	/	/	/	0.4
<i>sodMn</i>	/	/	2	4	/	/
<i>cat</i>	/	2	/	4	3	3
<i>sodCu</i>	/	/	/	/	/	/
<i>gst</i>	0.5	0.5	0.4	0.5	/	0.5
<i>pcs1</i>	/	/	/	/	/	/
<i>mdr1</i>	/	/	/	/	/	/

534 repressions, there were some for *psaA* at both 75 and 100 $\mu\text{g Pt L}^{-1}$ and the rest were only observed at 100 $\mu\text{g Pt L}^{-1}$.
 535
 536 The genes involved were *nramp1*, *cat*, *sodMn* and *pcs1*, as
 537 well as *gst* with a significant down regulation. The genes for
 538 which inductions were determined at medium-range con-
 539 centrations were *ctr2* and *sodMn*, but also *sodCu*, *cat* and
 540 *mdr1*, the latter having, for certain Pt exposure concentra-
 541 tions, a significant increase in expression compared to the
 542 control.

543 At day 21, gene expression seems to be gradually
 544 returning to a normal level with no statistically significant
 545 differences observed with control cells. There were still
 546 some inductions of *ctr2* for all Pt exposure conditions, as
 547 well as *cat* and *sodMn* for some exposures. In terms of
 548 repressions, *gst* was down regulated for almost all
 549 conditions.

Discussion

550

Comparison between the green alga and the diatom

551

552 As shown in the *Results* section the growth of *C. reinhardtii*
 553 was significantly delayed in the presence of Pt, which is
 554 consistent with the known role of Pt as a cell division
 555 inhibitor (Jakupec et al., 2003). It is also in line with the
 556 known half maximal effective concentration of Pt for *C.*
 557 *reinhardtii* (EC₅₀ = 89 $\mu\text{g L}^{-1}$, 95% confidence interval of
 558 66–120 $\mu\text{g L}^{-1}$; Hourtané et al. (2022)). This extended lag
 559 phase could be due to an allocation of energy for defense
 560 and acclimation of the cell to the presence of Pt after which
 561 an exponential growth was observed. However, the diatom
 562 *N. palea* seemed to cope better with Pt than the green alga,
 563 as no significant inhibition of growth could be determined.
 564 Intracellular Pt reached quantities up to 5 $10^{-9} \mu\text{g cell}^{-1}$,

565 which is relatively similar to the maximum obtained for *C.*
566 *reinhardtii* after 96 h (Hourtané et al. 2022). When compar-
567 ing data for the same exposure concentration of 100 µg
568 Pt L⁻¹, intracellular content was approximately half as
569 much for the green alga.

570 To put these results into perspective, one must consider
571 the environmental context as well as similarities and dif-
572 ferences of the algae of interest in terms of their char-
573 acteristics and roles. Both species studied in this work are
574 unicellular algae, but rather far apart in terms of phylogeny.
575 They are indeed from different families: *Chlorophyta* for
576 the green alga *C. reinhardtii* (Merchant et al. 2007), while
577 *N. palea* is a diatom from the *Bacillariophyta* family
578 (Bagmet et al. 2020). They also have a number of mor-
579 phological differences such as size and shape: *C. reinhardtii*
580 is an ellipsoid of around 3 to 25 µm (Harris 2008), whereas
581 the diatom is a pennate species with length estimated
582 between 12 and 42 µm (Kociolek 2011). As described in the
583 results section, the diatom's growth is slower than that of
584 the green alga. Moreover, *N. palea* being a periphytic
585 organism, it tends to stick to glassware, or forms clumps,
586 while *C. reinhardtii* grows as a homogeneous suspension
587 when gently and regularly stirred. In terms of morphology
588 and physiology, the diatom cells are surrounded by a
589 siliceous frustule that they synthesize and they also secrete a
590 viscous mix of organic molecules that are regrouped under
591 the term "extracellular polymeric substances" (EPS)
592 (Hoagland et al. 1993). Both the frustule and the EPS matrix
593 can play a protective role for diatoms.

594 The maximum internalization levels of Pt seemed compar-
595 able for both species by the end of the growing period.
596 The diatom was however exposed for a much longer time,
597 suggesting the internalization fluxes may have been greater
598 for the green alga. To compare the corresponding fluxes,
599 internalization data for exposures to 100 µg Pt L⁻¹ were
600 used. The average cellular Pt contents (Pt_{cell}) were respec-
601 tively 1.4·10⁻¹⁷ and 2.9·10⁻¹⁷ mol Pt cell⁻¹ for *C. rein-*
602 *hardtii* and *N. palea* at the end their respective exposures,
603 96 h and 21 days. So, for the same exposure concentration,
604 the diatom accumulated approximately twice as much Pt.
605 Using the equation from the work of Lavoie et al. (2014), Pt
606 internalization fluxes V_{Pt} were estimated using the specific
607 algal growth rate µ and internalized Pt [Pt_{int}], [Pt_{int}] being
608 determined using Pt_{cell} and the algal cell surface S. The
609 respective surfaces were 7·10⁻¹¹ and 15·10⁻¹¹ m² for *C.*
610 *reinhardtii* and *N. palea*. The values were obtained by
611 approximating the green alga to a sphere of a diameter of
612 4.6 µm and the pennate diatom to a prism on elliptic base as
613 recommended in the work of (Hillebrand et al. 1999).

$$V_{Pt} = \mu [Pt_{int}] \quad [Pt_{int}] = \frac{Pt_{cell}}{S}$$

The calculated fluxes were of 15·10⁻⁸ for *C. reinhardtii*
and 8.5·10⁻⁸ mol m⁻² d⁻¹ for *N. palea*, so two-fold greater
for the green alga. This could potentially explain the greater
sensitivity of *C. reinhardtii* observed in the growth inhibi-
tion experiment.

These findings highlight that, if the presence of Pt at
realistic environmental concentrations can significantly
delay algal growth, its toxicity was weak enough that
growth could resume later on. Although, generally, diatoms
can be more sensitive than green algae, *N. palea* is con-
sidered relatively resistant to metals (Kim Tiam et al. 2018),
which was confirmed in this study for Pt. Its growth was not
affected in a monospecific culture. The conducted experi-
ments, although performed on only two algal species,
suggest a relatively low impact of Pt at environmentally
relevant concentrations on primary producers. Nevertheless,
it is worth noting that these organisms occupy a crucial
position at the base of the food web. As such they could
represent a significant entry point for Pt in the trophic chain.

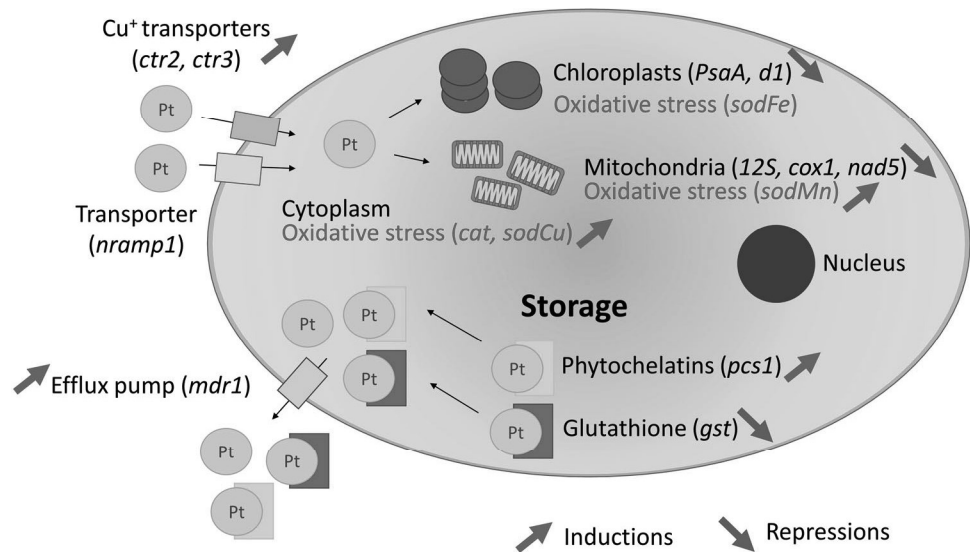
Moreover, subtle effects of Pt as well as possible cellular
pathways were determined using molecular tools. In terms
of transcriptomic analyses, as detailed in the following
section, both species seemed to have a rather similar reac-
tion to Pt exposure: a possible Cu deficiency suggesting Pt
internalization through copper transporters, a negative
impact on chloroplast metabolism as well as on antioxidant
defenses (possible reduction of the production of Glu-
tathione S-transferase), and a possible excretion by an efflux
pump. But there were still a few differences, for example
the possible impact on mitochondria only observed for *N.*
palea.

From transcriptomic results to possible cellular mechanisms

Transcriptomic analysis results need to be interpreted with
caution. The cellular modification of messenger RNA pro-
duction is the first step in producing specific proteins.
However, it does not constitute concrete proof of their
synthesis, but more so of an initiation of the process within
cells. Still, modification in genetic expression can provide
clues on cellular functions that might be affected, defense
mechanisms possibly activated as well as potential cellular
pathways for the metal of interest. With that in mind, Fig. 4
summarizes the different mechanisms investigated for Pt in
both species. The corresponding genes for which a mod-
ification of expression was studied using transcriptomic
analyses are also displayed.

With regards to metal internalization pathways, our
results suggest that Cu⁺ transporters likely constitute an
entryway for Pt into the cell. Non-essential metals have
been shown to often enter cells through the biological
pathways intended for essential metals (Sunda and

Fig. 4 Schematic representation of the genes of interest and the associated cellular functions and pathways studied in this work, as well as a summary of the results obtained by transcriptomic analyses; arrows represent the main expression modifications observed for each function for both studied species (*C. reinhardtii* and *N. palea*): upward orange arrows for inductions and downward green arrows for repressions



667 Huntsman 1998). Furthermore, Huang et al. (2014) showed
 668 that the *ctr2* gene, in addition to regulating Cu^+ ion
 669 exchange, plays a role in the sensitivity of mammalian cells
 670 to cisplatin. It is therefore possible that these transporters
 671 are involved in the internalization mechanisms of Pt. This
 672 use of specific proteins by Pt could ultimately lead to a Cu
 673 deficiency in the cell, since there could be competition
 674 between these two metals for internalization. The inductions
 675 of genes *ctr2* and/or *ctr3*, for both species studied in this
 676 work could be an indicator of the cells' potential deficiency
 677 in Cu. For *N. palea*, the temporal evolution seems to sug-
 678 gest this deficiency could be of increasing importance
 679 throughout the exposure. Therefore, such inductions sug-
 680 gest that Cu transporters could be one of Pt entryways into
 681 the cells. Inductions of *ctr2* also suggest that the cells seek
 682 to increase Cu uptake, which could lead to greater accu-
 683 mulation of Pt. Then, it follows that uptake of Pt could
 684 possibly be favored in environments with low available Cu
 685 such as humic waters as suggested by Hourtané et al.
 686 (2022), Cu having a much higher affinity for organic matter
 687 than Pt.

688 Once internalized, Pt could then accumulate in different
 689 parts of the cytoplasm, including within organelles of pri-
 690 mary importance such as the mitochondria or chloroplasts.
 691 Platinum is a soft (class B) metal with a high affinity for
 692 thiol groups. Thus, like silver, it could possibly also affect
 693 cellular processes in chloroplasts and mitochondria. Indeed,
 694 Ag^+ has been suggested to inhibit enzyme activity due to
 695 binding with thiol groups and subsequently affecting
 696 respiratory electron chain (mitochondria) and photo-
 697 synthetic electron chain (chloroplast) processes due to
 698 transport protein binding and its competitive substitution of
 699 Cu^+ in plastocyanin (Holt and Bard 2005; Yan and Chen
 700 2019). To assess the possible damage due to Pt presence in

these locations, the expressions of genes related to their
 respective metabolisms were determined.

Chloroplasts play a key role in energetic mechanisms and
 algal physiology, as they are the site of photosynthesis. The
 expression of genes coding for proteins of photosystem I:
psaA (both species), and photosystem II: *d1* (*C. reinhardtii*
 only) were studied in this work. Significant repressions of
d1 for *C. reinhardtii* and slight repressions of *psaA* in *N.*
palea suggest a possible impact of Pt on photosynthetic
 mechanisms. This could possibly be a consequence of an
 accumulation of Pt in the chloroplasts, as it was suggested
 for Pd in another green alga species *Raphidocelis sub-*
capitata (Vannini et al. 2011).

Mitochondria also play a critical role in different cellular
 processes, especially the energetic metabolism using an
 electron transport chain. Because of its importance, and the
 possible impact of metals on its functions, genes corre-
 sponding to mitochondrial metabolism were also studied in
 this work: *nad5* (*C. reinhardtii* only) and *cox1* (both species).
 No modification of their expression was observed for *C.*
reinhardtii, however, there were some significant repressions
 of *cox1* after 14 days of exposure for *N. palea*. Repressions
 were gradually more evident as Pt exposure concentrations
 increased from 40 to $100 \mu\text{g L}^{-1}$. The slight inductions of
 gene *sodMn*, the gene coding for mitochondrial superoxide
 dismutase, for *N. palea* from the middle range Pt exposure
 concentrations at all sampling times would appear to confirm
 this possible negative impact on mitochondrial metabolism.
 As such, this information could be consistent with metal
 accumulation in mitochondria. However, similar observations
 were not found for *C. reinhardtii*, possibly suggesting a dif-
 ferent effect mechanism. This particular species is known to
 produce many isoforms of phytochelatin to bind and detoxify
 class B metals (Lavoie et al. 2009). The role played by
 phytochelatin in metal sequestration is well known (Bukhari

736 et al. 2018; Callahan et al. 2006; Lee et al. 1996). The slight
737 overexpression of *pcs1* for *C. reinhardtii* suggests an onset of
738 a detoxification mechanism for the scavenging of Pt by
739 phytochelatins. However, it was not observed for *N. palea*,
740 and there was even a slight repression of gene *pcs1* deter-
741 mined at the highest exposure concentration of 100 µg Pt L⁻¹.

742 For *N. palea*, inductions of *cat* and *sodCu*, sometimes of
743 significant importance, suggest a possible activation of
744 oxidative stress defenses in the cytoplasm during exposure
745 to Pt. Glutathione is also involved in antioxidant defense,
746 minimizing the metal's intracellular effects *via* two main
747 mechanisms. The first is the reduction of reactive oxygen
748 species (ROS) by the oxidation of glutathione, this reaction
749 being favored by glutathione peroxidase (Nowicka 2022).
750 Another role of glutathione involves scavenging *via* metal
751 complexation. The sulfur atom in reduced glutathione has a
752 high affinity for soft metal cations, such as mercury, cad-
753 mium, silver and platinum (Pearson and Cowan 2021;
754 Wortelboer et al. 2008). Glutathione S-transferases (GSTs)
755 are catalysts for the conjugation of glutathione under its
756 reduced form to xenobiotics for the purpose of detoxifica-
757 tion (Nowicka 2022). For the corresponding gene studied
758 here: *gst*, there were strong repressions, always statistically
759 significant for *C. reinhardtii* and sometimes significant for
760 *N. palea* when exposed to Pt. These important repressions
761 of *gst* are counter intuitive as it would result in an increase
762 in oxidative threat to the cells. Nevertheless, it could be
763 coherent with the observations of Li et al. (2020) who
764 showed that *gst* is down regulated in Fe deficient dino-
765 flagellate *Fugacium kawagutii*. As metabolic pathways of
766 Fe and Cu are interlinked (Kochoni et al. 2022), these
767 repressions might be a symptom of Cu and/or Fe deficiency
768 of the cells, which would be consistent with the inductions
769 of *ctr2* and *ctr3* observed.

770 Regardless of the metal's form in the cytoplasm, it could
771 also be excreted by the cells. Although it has been sug-
772 gested by many authors, few concrete examples of metal
773 excretion by microalgae are listed in the literature. There is
774 the work of Lee et al. (1996), who demonstrated that the
775 excretion of Cd-phytochelatin complexes by the marine
776 diatom *T. weissflogii* in order to reduce intracellular Cd
777 concentrations. In this work, we studied the expression of
778 gene *mdr1* that codes for an ABC (ATP Binding Cassette)
779 transporter which plays a role in detoxification by active
780 efflux (Bard 2000). This type of membrane protein uses
781 available energy in the form of adenosine triphosphate
782 (ATP) to transport various metals (Achard et al., 2004;
783 Gonzalez et al. 2006; Kim et al. 2017), including cisplatin
784 (Wortelboer et al. 2008). Besides, the role of several ABC
785 transporters in the uptake of Pt across biological membranes
786 has been described for various mammal tissues in the
787 review of Sprowl et al. (2013). Some of these proteins
788 would also be involved in the detoxification of various

xenobiotics in species ranging from microorganisms to
789 humans including aquatic organisms and algae (Andolfo
790 et al. 2015; Ferreira et al. 2014; Shi et al. 2015). Indeed,
791 inductions of *mdr1* were observed for both species. In the
792 case of *N. palea*, statistically significant over expressions
793 were observed at 7 and 14 days. This overexpression of
794 *mdr1* suggests the possible use of this transporter as a Pt
795 detoxification mechanism. 796

797 Conclusion

798 In this work, two types of unicellular freshwater algae - a
799 fast-growing green alga, *C. reinhardtii*, and a slower
800 growing, sticky diatom species, *N. palea*, were exposed to
801 environmentally relevant concentrations of Pt. The diatom
802 appeared to be more resistant to Pt than the green alga. The
803 use of molecular tools allowed for the determination of
804 some possible effects and intracellular pathways of Pt. It
805 may be internalized by the cells *via* Cu⁺ transporters for
806 both primary producer species, which is consistent with
807 observations on mammalian cells from the literature. This
808 pathway could constitute an entryway for Pt into aquatic
809 food web, and there could be a greater risk associated with
810 Pt in waters with low available Cu. The results of the
811 transcriptomic analyses also highlighted the potential role of
812 phytochelatins for scavenging and detoxification of Pt for *C.*
813 *reinhardtii*, as well as a possible metal excretion using an
814 efflux pump for both species. The results on *N. palea* have
815 also shown the temporal evolution of gene expression and
816 the importance on having several sampling times, especially
817 when working with such a slow growing species. In this
818 specific case, 14 d was the exposure period for which the
819 most effects were determined in terms of the modification of
820 the expression of the genes selected. In the presence of Pt, it
821 would be interesting to assess possible impacts on the
822 frustule synthesis process and the regulation of cell division
823 and, in particular apoptosis (P53 protein). Furthermore, it
824 remains to be determined if these gene expression mod-
825 ifications actually lead to changes in the synthesis of the
826 corresponding protein using proteomic analyses. A better
827 understanding of Pt cellular management could provide
828 further insight into the effects of Pt on primary producers,
829 such organisms being of great ecological importance.

830 Data availability

831 All data used in this work are available at <https://doi.org/10.5683/SP3/I3GMF1> [This link will be activated once the
832 manuscript has been accepted for publication. In the
833 meantime, reviewers can access the data file through this
834 temporary private URL link: <https://borealisdata.ca/privat>
835

836 teurl.xhtml?token=58c8d48e-27a4-445d-ae4b-eacca
837 2694c03].

838 **Supplementary information** The online version contains supplement-
839 ary material available at <https://doi.org/10.1007/s10646-024-02746-y>.

840 **Acknowledgements** This work has been carried out with the financial
841 support of the Natural Sciences and Engineering Research Council of
842 Canada, Strategic project grant number STPGP 521467 – 18 entitled
843 “Terrestrial-aquatic mobility of technology critical elements in a
844 changing Canadian environment (TAMTeC) in collaboration with
845 Environment and Climate Change Canada (project number
846 GCXE17S011). O. Hourtané was supported by an EcotoQ scholarship
847 (FRQNT strategic network grant 309016). The authors would like to
848 thank the Functional Ecology & Environment Lab (LEFE UMR 5245
849 CNRS/INPT/UPS) of ENSAT (*Ecole Nationale Supérieure Agrono-*
850 *mique* de Toulouse), France, which sequenced the complete genome of
851 the diatom *N. palea* and allowed us to use it in collaboration. We
852 would also like to thank Pierre-Yves Gourves for the GFAAS analysis,
853 Julie Perreault for technical support in the use of ICP-MS, Vincent
854 Laderrière for performing mineralization tests on diatoms, Jean-Paul
855 Maalouf for his advice on statistical analyses and Scott Hepditch for
856 language assistance in the preparation of this manuscript.

857 **Author contributions** Claude Fortin, Patrice Gonzalez, and Agnès
858 Feurtet-Mazel contributed to the study conception and design as well
859 as supervision. Material preparation, data collection and analysis were
860 performed by Océane Hourtané and Emeric Kochoni. The first draft of
861 the manuscript was written by Océane Hourtané and all authors
862 commented on previous versions of the manuscript. All authors read
863 and approved the final manuscript.

864 Compliance with ethical standards

865 **Conflict of interest** The authors declare no competing interests.

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