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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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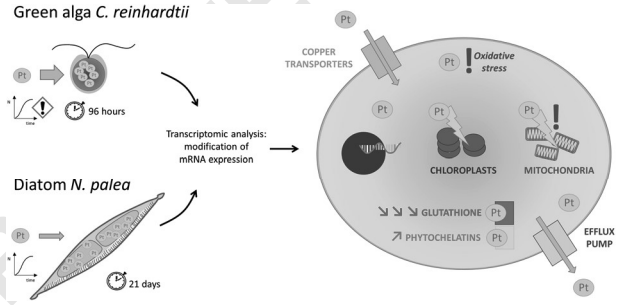
Abstract

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 μg 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

CO_2, H_2O and N_2.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 ($Al_3Mg_2AlSi_5O_{18}$) 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 µg Pt L^{-1} have been reported in such effluents (Lenz et al. 2005). Nevertheless, maximum environmental concentrations are lower, with up to 35 µg 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.

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, chloroplastic 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 Cu^{2+} and Zn^{2+} could be involved in its transport. Other types of more general transporters are also likely to play a role in Pt transport. For example, *nramp1* allows the internalization of the divalent ions Mn^{2+}, Fe^{2+} and Co^{2+}. In terms of chemical hardness, the thermodynamically stable Pt^{2+} ion is considered to be soft (class B) like Cu^{+} (Pearson 1993)

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

In this study we investigated the effects of Pt exposure on microalgae, which are at the base of the food web and the main contributors to primary production. Two species with different characteristics were studied in order to observe a wider range of the possible effects of Pt exposure on primary producers : a unicellular green alga *Chlamydomonas reinhardtii*, for which Pt accumulation and toxicity was previously studied (Hourtané et al. 2022), and a periphytic diatom *Nitzschia palea*, for which no toxicity data is available for Pt. The objectives were to compare their responses over a growing period relevant to each organism. In both cases, algae were harvested after exposure to perform transcriptomic analyses and to investigate Pt cellular effects and pathways allowing for a better understanding of its possible entryways in aquatic food webs.

Table 1 Experimental design for exposures to Pt

Exposure of alga		Green alga *C. reinhardtii*	Diatom *N. palea*
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
	Duration	96 h	21 d
Studied parameters	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

Material and Methods

Reagent and solutions

Before use, labware was systematically rinsed three times with deionised water, immersed in HNO_3 (ACS grade) 10% (V/V) for 24 h, rinsed three times with deionised water and then five times with ultrapure (MilliQ) water. To avoid bacterial contamination of the cultures, labware was sterilised at 121 °C and manipulations were carried out under a laminar-flow hood. All solutions were prepared from ultrapure (MilliQ) water, sterilised at 121 °C if needed and stored at 4 °C.

Algal culture

Both freshwater algal species originated from the *Canadian Phycological Culture Centre* (CPCC), University of Waterloo, ON, Canada. The green alga *C. reinhardtii* (CPCC#11) was grown in MHSM-1 medium (Modified High Salt Medium 1) adjusted to pH = 6.00 ± 0.05 and the diatom *N. palea* (CPCC#160) in modified CHU-10 medium adjusted to pH = 7.00 ± 0.05. To ensure pH stability, a pH buffer was used: 2-(N-morpholino)ethane sulfonic acid (MES) at 10^{-2} mol L^{-1} in MHSM-1 and N-(2-Hydroxyethyl)piperazine-N′-(2-ethane sulfonic acid) (HEPES) at $4{\cdot}10^{-3}$ mol L^{-1} in modified CHU-10. The detailed compositions of both culture media are presented in the online resource file.

The green alga *C. reinhardtii* cultures were axenic and weekly inoculated in a fresh sterile nutrient medium, whereas cultures were inoculated monthly for the diatom *N. palea* due to its slower growth. Bacterial development in *N. palea* cultures was observed after growth on agarose in a petri dish. However, the bacterial presence was minimal as no microscopic observation of the bacteria was possible during its growth. In natural environments, periphytic diatoms like *N. palea* tend to agglomerate and develop mutual beneficial relationships with other organisms, such as bacteria in biofilms. Thus, maintaining a culture of diatoms in an axenic state is not environmentally relevant and can have significant deleterious effects on the morphology and long term growth of some diatom species (Windler et al. 2014).

Exposure experiments

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

Exposure experiments were performed at concentrations representative of contaminated ecosystems. There were additional Pt exposure concentrations tested on the diatoms compared to the green alga, due to the lack of previous work on diatoms with Pt. There were also more sampling times for transcriptomic analyses of diatoms in order to observe the temporal evolution of gene expression throughout their longer exposure. Experiments were carried out in 100 mL culture medium in 250 mL polycarbonate (*C. reinhardtii*) or glass (*N. palea*) Erlenmeyer flasks. The latter was used for *N. palea*, because of its propensity to adhere to plastic surfaces. Although glass is known to adsorb metals more than polymer-based materials, the recovery of Pt was reasonably good (93 ± 4% for polycarbonate flasks and 77 ± 4% for glass flasks) considering the exposure duration (96 h vs 21 d, respectively). For the exposure of both species of interest, Pt was added 72 h prior to inoculation and added from a 1000 µg Pt mL^{-1} standard solution of $(NH_4)_2Pt$ (IV) Cl_6 (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 ± 24 µE m^{-2} 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} 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 ([Pt] = 0.75 and 25 µg 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 µg 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; $\lambda = 265.9$ nm). The detection limit was 1.7 µg 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 µg 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 phytochelatins, 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
nramp1[b]	Natural-resistance-associated macrophage protein 1	Metal transporter
ctr2[a.b]	Copper transporter 2	Copper transporters
ctr3[a]	Copper transporter 3	
psaA[a.b]	PsaA synthase protein	Photosynthesis. Photosystem 1; PS1
d1[a]	D1 protein	Photosynthesis. Photosystem 2; PS2
sodFe[a]	Chloroplasmic superoxide dismutase	Antioxidant defenses – chloroplasts
cox1[a.b]	Cytochrome C oxidase subunit I	Mitochondrial metabolism
nad5[a]	NADH Dehydrogenase subunit 5	
12S[a]	Mitochondrial ribosomal RNA 12S	
sodMn[a.b]	Mitochondrial superoxide dismutase	Antioxidant defenses - mitochondria
cat[a.b]	Catalase	Antioxidant defenses - cytoplasm
sodCu[b]	Cytoplasmic superoxide dismutase	
fad[a]	Flavin adenine dinucleotide synthase	
gpx[a]	Glutathione peroxidase 1	
gst[a.b]	Glutathione S-transferase	Detoxification
pcs1[a.b]	Phytochelatin synthase 1	
mdr1[a.b]	MiltiDrug resistance	
βact[a]	β actin	Reference genes
ef1α[a]	Elongation factor 1 α	
rpL13[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 (RNA*later*, 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

greater than 1.8. The estimated quantity of RNA was also given by the instrument in ng μL^{-1} and allows to verify that the extraction step was successful.

Reverse transcription

RT was performed using the "GoScript Reverse Transcription System" kit (Promega). This step allowed the transformation of the purified RNA into cDNA (complementary DNA). For one sample, 1 µL of oligodT solution (1 µmol L^{-1}) was mixed with 1 µL of hexaprimer (1 µmol L^{-1}) and 10 µL of purified RNA (approximately 1 µg). The mixture was then cycled in the thermal cycler for 5 min at 70 °C followed by a gradual decrease in temperature to 4 °C to linearize the RNAs and allow the primers to bind. Then 1 µL of deoxyribonucleotide triphosphate (dNTP) solution (10 mmol L^{-1}), 4 µL of 5x activity buffer solution, 1.5 µL of $MgCl_2$ (25 mmol L^{-1}), 1 µL of reverse transcriptase (1 U μL^{-1}) and 0.5 µL of ribonuclease inhibitor RNasin® solution were added. This mixture was put back into the thermocycler with the following protocol: 5 min at 25 °C, then 1 h at 42 °C to perform the cDNA synthesis. Samples containing the synthesized cDNAs were stored at −20 °C before performing qPCR (real-time quantitative polymerase chain reaction).

Amplifications

Real-time quantitative PCR was used to quantify gene expression. It was performed with a LightCycler 480 (Roche), which allows real-time monitoring of cDNA amplification by adding the fluorescent intercalating agent SyberGreen to the reaction medium. The fluorescence detected at 530 nm was measured at the end of each cycle. The PCR reactions were carried out in 96-well microplates using the "GoTaq® qPCR Master Mix" kit (Promega) containing 2x buffer (activity buffer, *Taq* polymerase, $MgCl_2$, dNTPs and SyberGreen). Beforehand, a mixture of the forward and reverse primers (see the online resource file) was produced for each gene (2 µM of each primer). To each well, 13 µL of 2x diluted reagent mix (10 µL 2x buffer + 3 µL water), 2 µL of primer mix and 5 µL of 1/10 diluted cDNA were added. For the amplification protocol, the plates were first heated to 95 °C for 2 min to activate the *Taq* polymerase before performing 50 cycles of PCR (95 °C, 15 s; 60 °C, 1 min). At the end of the reaction, the quality of the amplifications was determined by performing melting curves at the end of the 50 cycles. These were carried out by continuous fluorescence measurements during a gradual rise in temperature of the device from 60 °C to 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 calculated according to the method described by (Livak and Schmittgen 2001).

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

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:

$$\mathrm{IF} = \frac{2^{-\Delta Ct\,condition}}{2^{-\Delta Ct\,control}}$$

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, ef1α 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 conclusion, qPCR is preferred when working with Pt and reference genes βact, ef1α and *rpL13*.

Statistical analyses

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

$2^{-\Delta Ct}$ values. For each condition and targeted gene, normality was first verified using a Shapiro test. Depending on the outcome, a parametric (one-way ANOVA) or non-parametric (Kruskal-Wallis) test was performed. The corresponding post-hoc tests were Tukey's test and Dunn's test, respectively. Several confidence levels were determined and correspond to the following p-values: * for p_{value} below 0.05, ** for p_{value} below 0.01, *** for p_{value} below 0.001.

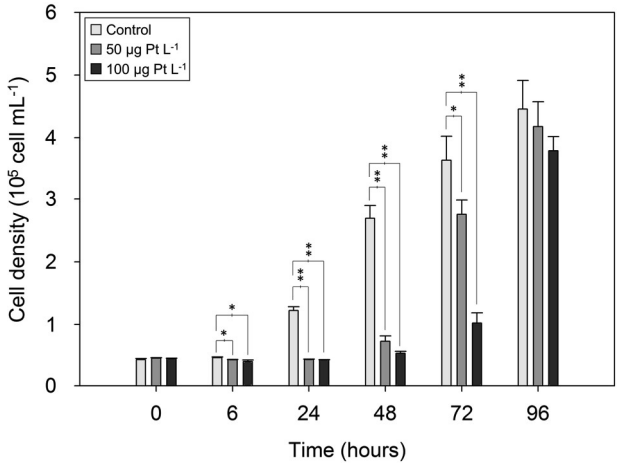

Fig. 1 Cell density of *C. reinhardtii* over time at 0, 50 and 100 µg Pt L^{-1} in MHSM-1 medium at pH = 6.00 ± 0.05 ($n = 3$); asterisks represent statistical differences compared to the control

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 significative 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 significative 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
ctr2	/	3
ctr3	/	2
psaA	/	/
d1	0.4*	0.4*
sodFe	/	/
cox1	/	/
nad5	/	/
12S	/	/
sodMn	/	/
cat	/	/
fad	/	/
gst	0.003*	4.10^{-5}*
pcs1	2	/
mdr1	4	4

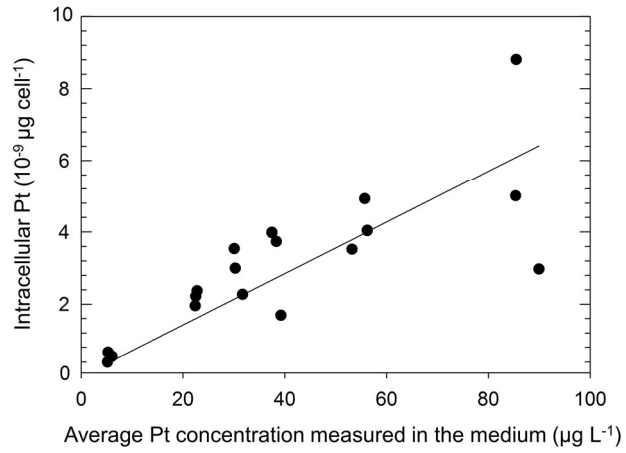

Fig. 2 Bioaccumulation of Pt in *N. palea* as a function of the mean measured exposure Pt concentrations in modified CHU-10 medium; pH = 7.0 ± 0.7 ($n = 3$)

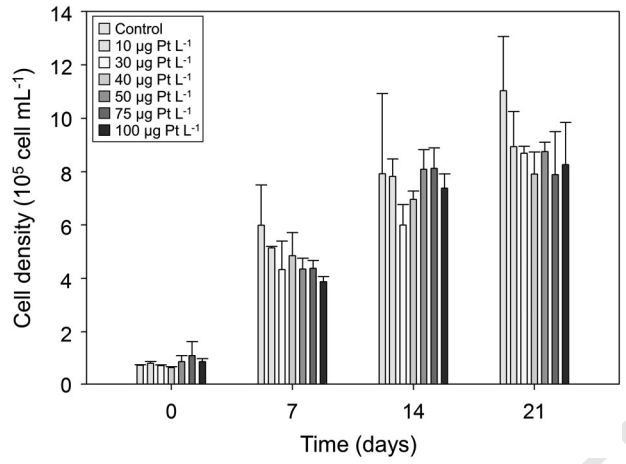

Fig. 3 Cell density over time for *N. palea* exposed to different Pt exposure concentrations in modified CHU-10 medium, pH = 7.0 ± 0.7 ($n = 3$)

concentration (Fig. 2). An apparent saturation seems to occur at the highest concentrations tested, with intracellular Pt reaching up to $5{\cdot}10^{-9}$ µg 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 significative inductions and repressions.

After 7 days, no effect on gene expression was observed at the lowest Pt concentration of 10 µg Pt L^{-1}. For the medium ranged concentrations of 30–50 µg 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 µg Pt L^{-1} and was significant. Above 75 µg 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 µg 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 µg Pt L^{-1} with IF from 0.5 to 0.1. The response was significantly different from the control at 50, 75 and 100 µg 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 < IF < 2, asterisks represent statistical differences compared to the control

Genes	Induction factors (IF) for each nominal [Pt] (µg L^{-1})					
	10	30	40	50	75	100
ctr2	/	/	/	/	0.2	/
nramp1	/	/	/	/	/	/
psaA	/	/	/	/	0.4	/
cox1	/	/	/	/	/	/
sodMn	/	3	3	/	/	/
cat	/	3	4	2	/	/
sodCu	/	/	/	/	/	/
gst	/	/	/	0.4	0.5	/
pcs1	/	/	/	/	/	0.4
mdr1	/	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 < IF < 2, asterisks represent statistical differences compared to the control

Genes	Induction factors (IF) for each nominal [Pt] (µg L^{-1})					
	10	30	40	50	75	100
ctr2	/	3	2	3	2	/
nramp1	/	/	/	/	/	0.2
psaA	/	/	/	/	0.3	0.1
cox1	/	/	0.5	0.4*	0.3*	0.1**
sodMn	/	2	3	2	/	0.3
cat	/	3	3*	3	/	0.5
sodCu	/	1.7**	/	/	/	/
gst	/	/	/	/	/	0.4*
pcs1	/	/	/	/	/	0.5
mdr1	/	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 < IF < 2, asterisks represent statistical differences compared to the control

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

repressions, there were some for *psaA* at both 75 and 100 µg Pt L^{-1} and the rest were only observed at 100 µg Pt L^{-1}. The genes involved were *nramp1*, *cat*, *sodMn* and *pcs1*, as well as *gst* with a significant down regulation. The genes for which inductions were determined at medium-range concentrations were *ctr2* and *sodMn*, but also *sodCu*, *cat* and *mdr1*, the latter having, for certain Pt exposure concentrations, a significant increase in expression compared to the control.

At day 21, gene expression seems to be gradually returning to a normal level with no statistically significant differences observed with control cells. There were still some inductions of *crt2* for all Pt exposure conditions, as well as *cat* and *sodMn* for some exposures. In terms of repressions, *gst* was down regulated for almost all conditions.

Discussion

Comparison between the green alga and the diatom

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

which is relatively similar to the maximum obtained for *C. reinhardtii* after 96 h (Hourtané et al. 2022). When comparing data for the same exposure concentration of 100 µg Pt L^{-1}, intracellular content was approximately half as much for the green alga.

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

The maximum internalization levels of Pt seemed comparable for both species by the end of the growing period. The diatom was however exposed for a much longer time, suggesting the internalization fluxes may have been greater for the green alga. To compare the corresponding fluxes, internalization data for exposures to 100 µg Pt L^{-1} were used. The average cellular Pt contents (Pt_{cell}) were respectively $1.4{\cdot}10^{-17}$ and $2.9{\cdot}10^{-17}$ mol Pt $cell^{-1}$ for *C. reinhardtii* and *N. palea* at the end their respective exposures, 96 h and 21 days. So, for the same exposure concentration, the diatom accumulated approximately twice as much Pt. Using the equation from the work of Lavoie et al. (2014), Pt internalization fluxes V_{Pt} were estimated using the specific algal growth rate µ and internalized Pt [Pt_{int}], [Pt_{int}] being determined using Pt_{cell} and the algal cell surface S. The respective surfaces were $7{\cdot}10^{-11}{\cdot}$and $15{\cdot}10^{-11}$ m^2 for *C. reinhardtii* and *N. palea*. The values were obtained by approximating the green alga to a sphere of a diameter of 4.6 µm and the pennate diatom to a prism on elliptic base as recommended in the work of (Hillebrand et al. 1999).

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

The calculated fluxes were of $15{\cdot}10^{-8}$ for *C. reinhardtii* and $8.5{\cdot}10^{-8}$ 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 inhibition 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 considered 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 experiments, 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 reaction 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 Glutathione 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 production 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 modification 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

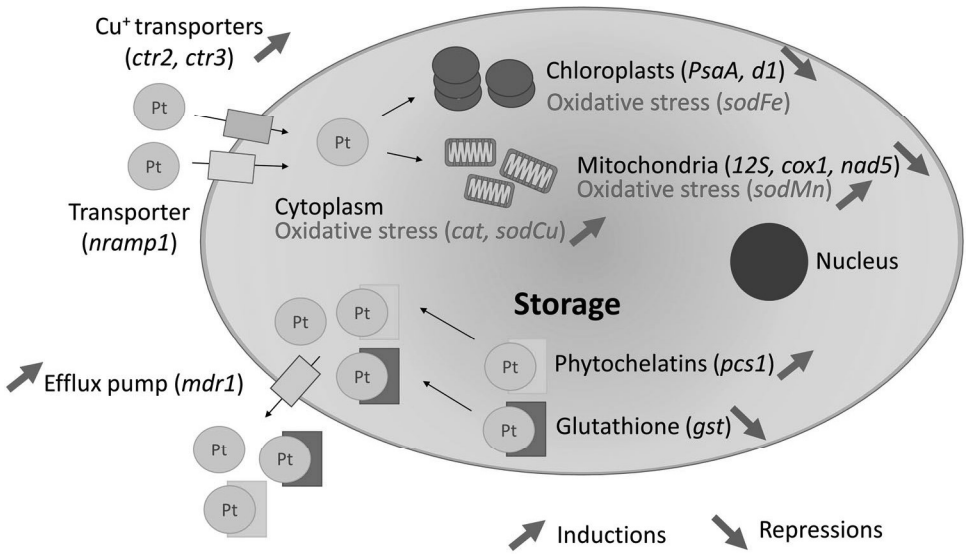

Fig. 4 Schematic representation of the genes of interest and the associated cellular functions and pathways studied in this work, as well as a summary of the results obtained by transcriptomic analyses; arrows represent the main expression modifications observed for each function for both studied species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

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

Once internalized, Pt could then accumulate in different parts of the cytoplasm, including within organelles of primary importance such as the mitochondria or chloroplasts. Platinum is a soft (class B) metal with a high affinity for thiol groups. Thus, like silver, it could possibly also affect cellular processes in chloroplasts and mitochondria. Indeed, Ag^{+} has been suggested to inhibit enzyme activity due to binding with thiol groups and subsequently affecting respiratory electron chain (mitochondria) and photosynthetic electron chain (chloroplast) processes due to transport protein binding and its competitive substitution of Cu^{+} in plastocyanin (Holt and Bard 2005; Yan and Chen 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. Significative 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 subcapitata* (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 corresponding 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 $\mu g\,L^{-1}$. 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 different effect mechanism. This particular species is known to produce many isoforms of phytochelatins to bind and detoxify class B metals (Lavoie et al. 2009). The role played by phytochelatins in metal sequestration is well known (Bukhari

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

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

Regardless of the metal's form in the cytoplasm, it could also be excreted by the cells. Although it has been suggested by many authors, few concrete examples of metal excretion by microalgae are listed in the literature. There is the work of Lee et al. (1996), who demonstrated that the excretion of Cd-phytochelatin complexes by the marine diatom *T. weissflogii* in order to reduce intracellular Cd concentrations. In this work, we studied the expression of gene *mdr1* that codes for an ABC (ATP Binding Cassette) transporter which plays a role in detoxification by active efflux (Bard 2000). This type of membrane protein uses available energy in the form of adenosine triphosphate (ATP) to transport various metals (Achard et al., 2004; Gonzalez et al. 2006; Kim et al. 2017), including cisplatin (Wortelboer et al. 2008). Besides, the role of several ABC transporters in the uptake of Pt across biological membranes has been described for various mammal tissues in the review of Sprowl et al. (2013). Some of these proteins would also be involved in the detoxification of various xenobiotics in species ranging from microorganisms to humans including aquatic organisms and algae (Andolfo et al. 2015; Ferreira et al. 2014; Shi et al. 2015). Indeed, inductions of *mdr1* were observed for both species. In the case of *N. palea*, statistically significant over expressions were observed at 7 and 14 days. This overexpression of *mdr1* suggests the possible use of this transporter as a Pt detoxification mechanism.

Conclusion

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

Data availability

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

teurl.xhtml?token=58c8d48e-27a4-445d-ae4b-eacca2694c03].

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

Achard M, Baudrimont M, Boudou A, Bourdineaud J (2004) Induction of a multixenobiotic resistance protein (MXR) in the Asiatic clam *Corbicula fluminea* after heavy metals exposure. Aqua Toxicol 67:347–357. https://doi.org/10.1016/j.aquatox.2004.01.014

Andolfo G, Ruocco M, Di Donato A, Frusciante L, Lorito M, Scala F, Ercolano MR (2015) Genetic variability and evolutionary diversification of membrane ABC transporters in plants. BMC Plant Biol 15:1–15. https://doi.org/10.1186/s12870-014-0323-2

Artelt, S, Levsen, K, König, H, Rosner, G (2000) Engine test bench experiments to determine platinum emissions from three-way catalytic converters. Anthropogenic platinum-group element emissions. Springer, pp. 33-44. https://doi.org/10.1007/978-3-642-59678-0_4.

Bagmet V, Abdullin SR, Mazina S, Nikulin AY, Nikulin VY, Gontcharov A (2020) Life cycle of *Nitzschia palea* (Kützing) W. Smith (Bacillariophyta). Russian J Dev Biol 51:106–114. https://doi.org/10.1134/S1062360420020022

Barbante C, Veysseyre A, Ferrari C, Van De Velde K, Morel C, Capodaglio G, Cescon P, Scarponi G, Boutron C (2001) Greenland snow evidence of large scale atmospheric contamination for platinum, palladium, and rhodium. Environ Sci Technol 35:835–839. https://doi.org/10.1021/es000146y

Bard SM (2000) Multixenobiotic resistance as a cellular defense mechanism in aquatic organisms. Aqua Toxicol. 48:357–389. https://doi.org/10.1016/S0166-445X(00)00088-6

Barefoot R (1997) Determination of platinum at trace levels in environmental and biological materials. Environ Sci Technol 31:309–314. https://doi.org/10.1021/es960712i

Bienvenu, T, Meunier, C, Bousquet, S, Chiron, S, Richard, L, Gautheret-Dejean, A, Rouselle, J, Feldmann, D (1999) Les techniques d'extraction de l'ADN à partir d'un échantillon sanguin. Vol. 57. Annales de Biologie Clinique, pp. 77-84

Blaby-Haas CE, Merchant SS (2012) The ins and outs of algal metal transport. Biochimica et Biophysica Acta 1823:1531–1552. https://doi.org/10.1016/j.bbamcr.2012.04.010

Blaby IK, Blaby-Haas CE, Tourasse N, Hom EF, Lopez D, Aksoy M, Grossman A, Umen J, Dutcher S, Porter M, King S, Witman GB, Stanke M, Harris EH, Goodstein D, Grimwood J, Schmutz J, Vallon O, Merchant SS, Prochnik S (2014) The Chlamydomonas genome project: a decade on. Trends Plants 19:672–680. https://doi.org/10.1016/j.tplants.2014.05.008

Bridges CC, Zalups RK (2005) Molecular and ionic mimicry and the transport of toxic metals. Toxicol Appl Pharmacol 204:274–308. https://doi.org/10.1016/j.taap.2004.09.007

Bukhari S, Tahir M, Akhter N, Anjum F, Anwar H, Mustafa G (2018) Phylogeny and comparative modeling of phytochelatin synthase from *Chlorella sp.* as an efficient bioagent for detoxification of heavy metals. J Biol Regulators Homeostatic Agents 32:1191–1197

Callahan DL, Baker AJ, Kolev SD, Wedd AG (2006) Metal ion ligands in hyperaccumulating plants. J Biol Inorg Chem 11:2–12. https://doi.org/10.1007/s00775-005-0056-7

Campbell PGC, Hare L (2009) Metal detoxification in freshwater animals. Roles of metallothioneins. Metal Ions Life Sci 5:239–277. https://doi.org/10.1515/9783110436273-014

Colombo C, Oates C, Monhemius A, Plant J (2008) Complexation of platinum, palladium and rhodium with inorganic ligands in the environment. Geochem: Explor, Environ, Anal 8:91–101. https://doi.org/10.1144/1467-7873/07-151

Dey S, Mehta N (2020) Automobile pollution control using catalysis. Resources, Environ Sustain 2:100006. https://doi.org/10.1016/j.resenv.2020.100006

Diehl DB, Gagnon ZE (2007) Interactions between essential nutrients with platinum group metals in submerged aquatic and emergent plants. Water, Air, Soil Poll 184:255–267. https://doi.org/10.1007/s11270-007-9414-0

Ely JC, Neal CR, Kulpa CF, Schneegurt MA, Seidler JA, Jain JC (2001) Implications of platinum-group element accumulation along US roads from catalytic-converter attrition. Environ Sci Technol 35:3816–3822. https://doi.org/10.1021/es001989s

Ferreira M, Costa J, Reis-Henriques MA (2014) ABC transporters in fish species: a review. Front Physiol 5:266. https://doi.org/10.3389/fphys.2014.00266

Glaeser H, Coblenz A, Kruczek R, Ruttke I, Ebert-Jung A, Wolf K (1991) Glutathione metabolism and heavy metal detoxification in Schizosaccharomyces pombe. Curr Genet 19:207–213. https://doi.org/10.1007/bf00336488

Gonzalez P, Baudrimont M, Boudou A, Bourdineaud J-P (2006) Comparative effects of direct cadmium contamination on gene expression in gills, liver, skeletal muscles and brain of the zebrafish (*Danio rerio*). Biometals 19:225–235. https://doi.org/10.1007/s10534-005-5670-x

Harris, EH (2008) The Chlamydomonas sourcebook: introduction to Chlamydomonas and its laboratory use. Academic Press.

Hassler CS, Slaveykova VI, Wilkinson KJ (2004) Discriminating between intra-and extracellular metals using chemical extractions. Limnol Oceanogr: Methods 2:237–247. https://doi.org/10.4319/lom.2004.2.237

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403–424. https://doi.org/10.1046/j.1529-8817.1999.3520403.x

Hoagland KD, Rosowski JR, Gretz MR, Roemer SC (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. J Phycol 29:537–566. https://doi.org/10.1111/j.0022-3646.1993.00537.x

Holt KB, Bard AJ (2005) Interaction of silver (I) ions with the respiratory chain of *Escherichia coli*: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag+. Biochemistry 44:13214–13223. https://doi.org/10.1021/bi0508542

Hourtané O, Rioux G, Campbell PG, Fortin C (2022) Algal bioaccumulation and toxicity of platinum are increased in the presence of humic acids. Environ Chem 19(4):144–155. https://doi.org/10.1071/EN22037

Huang CP, Fofana M, Chan J, Chang CJ, Howell SB (2014) Copper transporter 2 regulates intracellular copper and sensitivity to cisplatin. Metallomics 6:654–661. https://doi.org/10.1039/c3mt00331k

Jakupec MA, Galanski M, Keppler BK (2003) Tumour-inhibiting platinum complexes–state of the art and future perspectives. Rev Physiol, Biochem Pharmacol 146:1–54. https://doi.org/10.1007/s10254-002-0001-x

Kim H, Yim B, Kim J, Kim H, Lee Y-M (2017) Molecular characterization of ABC transporters in marine ciliate, *Euplotes crassus*: Identification and response to cadmium and benzo [a] pyrene. Marine Poll Bull 124:725–735. https://doi.org/10.1016/j.marpolbul.2017.01.046

Kim Tiam S, Feurtet-Mazel A, Delmas F, Mazzella N, Morin S, Daffe G, Gonzalez P (2012) Development of q-PCR approaches to assess water quality: effects of cadmium on gene expression of the diatom *Eolimna minima*. Water Res 46:934–942. https://doi.org/10.1016/j.watres.2011.11.005

Kim Tiam, S, Lavoie, I, Doose, C, Hamilton, PB, Fortin, C (2018) Morphological, physiological and molecular responses of *Nitzschia palea* under cadmium stress. Ecotoxicology. https://doi.org/10.1007/s10646-018-1945-1

Kochoni E, Doose C, Gonzalez P, Fortin C (2022) Role of iron in gene expression and in the modulation of copper uptake in a freshwater alga: Insights on Cu and Fe assimilation pathways. Environ Poll 305:119311. https://doi.org/10.1016/j.envpol.2022.119311

Kociolek, P (2011) *Nitzschia palea*. Diatoms of North America. https://diatoms.org/species/nitzschia_palea Accessed 21 August 2023

Lavoie M, Campbell PG, Fortin C (2014) Predicting cadmium accumulation and toxicity in a green alga in the presence of varying essential element concentrations using a biotic ligand model. Environ Sci Technol 48:1222–1229. https://doi.org/10.1021/es402630z

Lavoie M, Le Faucheur S, Fortin C, Campbell PG (2009) Cadmium detoxification strategies in two phytoplankton species: metal binding by newly synthesized thiolated peptides and metal sequestration in granules. Aqua Toxicol 92:65–75. https://doi.org/10.1016/j.aquatox.2008.12.007

Lee JG, Ahner BA, Morel FM (1996) Export of cadmium and phytochelatin by the marine diatom *Thalassiosira weissflogii*. Environ Sci Technol 30:1814–1821. https://doi.org/10.1021/es950331p

Lenz K, Hann S, Koellensperger G, Stefanka Z, Stingeder G, Weissenbacher N, Mahnik SN, Fuerhacker M (2005) Presence of cancerostatic platinum compounds in hospital wastewater and possible elimination by adsorption to activated sludge. Sci Tot Environ 345:141–152. https://doi.org/10.1016/j.scitotenv.2004.11.007

Li T, Lin X, Yu L, Lin S, Rodriguez IB, Ho T-Y (2020) RNA-seq profiling of *Fugacium kawagutii* reveals strong responses in metabolic processes and symbiosis potential to deficiencies of iron and other trace metals. Sci Tot Environ 705:135767. https://doi.org/10.1016/j.scitotenv.2019.135767

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Marechal-Drouard L, Marshall WF, Qu LH, Nelson DR, Sanderfoot AA, Spalding MH, Kapitonov VV, Ren Q, Ferris P, Lindquist E, Shapiro H, Lucas SM, Grimwood J, Schmutz J, Cardol P, Cerutti H, Chanfreau G, Chen CL, Cognat V, Croft MT, Dent R, Dutcher S, Fernandez E, Fukuzawa H, Gonzalez-Ballester D, Gonzalez-Halphen D, Hallmann A, Hanikenne M, Hippler M, Inwood W, Jabbari K, Kalanon M, Kuras R, Lefebvre PA, Lemaire SD, Lobanov AV, Lohr M, Manuell A, Meier I, Mets L, Mittag M, Mittelmeier T, Moroney JV, Moseley J, Napoli C, Nedelcu AM, Niyogi K, Novoselov SV, Paulsen IT, Pazour G, Purton S, Ral JP, Riano-Pachon DM, Riekhof W, Rymarquis L, Schroda M, Stern D, Umen J, Willows R, Wilson N, Zimmer SL, Allmer J, Balk J, Bisova K, Chen CJ, Elias M, Gendler K, Hauser C, Lamb MR, Ledford H, Long JC, Minagawa J, Page MD, Pan J, Pootakham W, Roje S, Rose A, Stahlberg E, Terauchi AM, Yang P, Ball S, Bowler C, Dieckmann CL, Gladyshev VN, Green P, Jorgensen R, Mayfield S, Mueller-Roeber B, Rajamani S, Sayre RT, Brokstein P, Dubchak I, Goodstein D, Hornick L, Huang YW, Jhaveri J, Luo Y, Martinez D, Ngau WC, Otillar B, Poliakov A, Porter A, Szajkowski L, Werner G, Zhou K, Grigoriev IV, Rokhsar DS, Grossman AR (2007) The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science 318:245–250. https://doi.org/10.1126/science.1143609

Moisset, S, Kim Tiam, S, Feurtet-Mazel, A, Morin, S, Delmas, F, Mazzella, N, Gonzalez, P (2015) Genetic and physiological responses of three freshwater diatoms to realistic diuron exposures. Environ Sci Poll Res 4046–4055. https://doi.org/10.1007/s11356-014-3523-2

Moldovan M, Rauch S, Gómez M, Antonia Palacios M, Morrison GM (2001) Bioaccumulation of palladium, platinum and rhodium from urban particulates and sediments by the freshwater isopod *Asellus aquaticus*. Water Res 35:4175–4183. https://doi.org/10.1016/S0043-1354(01)00136-1

Morton O, Puchelt H, Hernández E, Lounejeva E (2001) Traffic-related platinum group elements (PGE) in soils from Mexico City. J Geochem Explor 72:223–227. https://doi.org/10.1016/S0375-6742(01)00163-7

Nowicka B (2022) Heavy metal–induced stress in eukaryotic algae—mechanisms of heavy metal toxicity and tolerance with particular emphasis on oxidative stress in exposed cells and the role of antioxidant response. Environ Sci Poll Res 29:16860–16911. https://doi.org/10.1007/s11356-021-18419-w

Odiyo JO, Bapela HM, Mugwedi R, Chimuka L (2005) Metals in environmental media: A study of trace and platinum group metals in Thohoyandou, South Africa. Water SA 31:581-588. https://doi.org/10.4314/wsa.v31i4.5148

Osborn HL, Hook SE (2013) Using transcriptomic profiles in the diatom *Phaeodactylum tricornutum* to identify and prioritize stressors. Aqua Toxicol 138:12–25. https://doi.org/10.1016/j.aquatox.2013.04.002

Palacios MA, Gómez MM, Moldovan M, Morrison G, Rauch S, McLeod C, Ma R, Laserna J, Lucena P, Caroli S, Alimonti A, Petrucci F, Bocca B, Schramel P, Lustig S, Zischka M, Wass U, Stenbom B, Luna M, Saenz JC, Santamaría J, Torrens JM (2000) Platinum-group elements: quantification in collected exhaust

fumes and studies of catalyst surfaces. Sci Tot Environ 257:1–15. https://doi.org/10.1016/S0048-9697(00)00464-2

Paolucci C, Ponti J, Fabbri M, Breda D, Sabbioni E, Burastero S (2007) Platinum group elements enhance the allergic immune response by acting on dendritic cells. Allerg Immunol 143:225–236. https://doi.org/10.1159/000099466

Pearson, RG (1993) Chemical hardness—A historical introduction. Chemical hardness. Springer, pp. 1-10. https://doi.org/10.1007/BFb0036796

Pearson, SA, Cowan, J (2021) Glutathione-coordinated metal complexes as substrates for cellular transporters. Metallomics. 13. https://doi.org/10.1093/mtomcs/mfab015

Rauch S, Morrison GM (1999) Platinum uptake by the freshwater isopod *Asellus aquaticus* in urban rivers. Sci Tot Environ 235:261–268. https://doi.org/10.1016/S0048-9697(99)00203-X

Rauch S, Paulsson M, Wilewska M, Blanck H, Morrison GM (2004) Short-term toxicity and binding of platinum to freshwater periphyton communities. Arch Environ Contamination Toxicol 47:290–296. https://doi.org/10.1007/s00244-004-3197-8

Ravindra K, Bencs L, Van Grieken R (2004) Platinum group elements in the environment and their health risk. Sci Tot Environ 318:1–43. https://doi.org/10.1016/s0048-9697(03)00372-3

Reedijk J (2016) Metal-ion nucleic-acid interactions: A personal account. Inorganica Chimica Acta 452:268–272. https://doi.org/10.1016/j.ica.2015.12.011

Shi B, Huang Z, Xiang X, Huang M, Wang W-X, Ke C (2015) Transcriptome analysis of the key role of GAT2 gene in the hyper-accumulation of copper in the oyster *Crassostrea angulata*. Scientific Rep 5:17751. https://doi.org/10.1038/srep17751

Sies H (1986) Biochemistry of Oxidative Stress. Angewandte Chemie International Edition in English 25:1058–1071. https://doi.org/10.1002/anie.198610581

Soyol-Erdene TO, Huh Y, Hong S, Hur SD (2011) A 50-year record of platinum, iridium, and rhodium in Antarctic snow: volcanic and anthropogenic sources. Environ Sci Technol 45:5929–5935. https://doi.org/10.1021/es2005732

Sprowl JA, Ness RA, Sparreboom A (2013) Polymorphic transporters and platinum pharmacodynamics. Drug metabolism and pharmacokinetics 28:19–27. https://doi.org/10.2133/dmpk.DMPK-12-RV-073

Sunda WG, Huntsman SA (1998) Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems. Sci Tot Environ 219:165–181. https://doi.org/10.1016/S0048-9697(98)00226-5

Sures B, Thielen F, Baska F, Messerschmidt J, von Bohlen A (2005) The intestinal parasite Pomphorhynchus laevis as a sensitive accumulation indicator for the platinum group metals Pt, Pd, and Rh. Environ Res 98:83–88. https://doi.org/10.1016/j.envres.2004.05.010

Vannini C, Domingo G, Marsoni M, Fumagalli A, Terzaghi R, Labra M, De Mattia F, Onelli E, Bracale M (2011) Physiological and molecular effects associated with palladium treatment in *Pseudokirchneriella subcapitata*. Aqua Toxicol 102:104–113. https://doi.org/10.1016/j.aquatox.2011.01.002

Windler M, Bova D, Kryvenda A, Straile D, Gruber A, Kroth PG (2014) Influence of bacteria on cell size development and morphology of cultivated diatoms. Phycol Res 62:269–281. https://doi.org/10.1111/pre.12059

Wortelboer HM, Balvers MG, Usta M, van Bladeren PJ, Cnubben NH (2008) Glutathione-dependent interaction of heavy metal compounds with multidrug resistance proteins MRP1 and MRP2. Environ Toxicol Pharmacol 26:102–108. https://doi.org/10.1016/j.etap.2008.02.006

Yan A, Chen Z (2019) Impacts of silver nanoparticles on plants: a focus on the phytotoxicity and underlying mechanism. Int J Mol Sci 20:1003. https://doi.org/10.3390/ijms20051003

Zimmermann S, Wolff C, Sures B (2017) Toxicity of platinum, palladium and rhodium to *Daphnia magna* in single and binary metal exposure experiments. Environ Poll 224:368–376. https://doi.org/10.1016/j.envpol.2017.02.016

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