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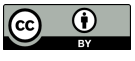
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Comparative analysis of species-specific dissolved gaseous mercury oxidation in phytoplankton cultures

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ABSTRACT

Phytoplankton species influence mercury cycling through bioaccumulation and Hg(II) reduction, however their contribution to oxidation of Hg(0) in aquatic systems remains largely overlooked. The present study aims at investigating the oxidation of Hg(0) by two phytoplankton species: the diatom *Cyclotella meneghiniana* and the green alga *Chlamydomonas reinhardtii*. The algae were exposed to gaseous Hg(0) at concentrations in the range of 6–12 μg m^{-3}, representative for contaminated environments, under various experimental conditions (open vs. closed systems, light vs dark, and alive vs dead cells).

The obtained results revealed, for a first time, that Hg(0) oxidation in *C. meneghiniana* cultures was light-dependent and limited to live cells, whereas *C. reinhardtii* maintained similar oxidation rates in both live and dead cells. *C. reinhardtii* cultures exhibited nearly tenfold higher Hg(0) oxidation efficiency than *C. meneghiniana*, demonstrating a strong species-dependent effect. Both species facilitated Hg(0) uptake from air into water, demonstrating a potential route for atmospheric Hg(0) to enter aquatic food webs. This novel evidence of phytoplankton-mediated Hg(0) oxidation highlights the importance of species identity and environmental conditions in governing Hg transformations and bioavailability. The results could have significant implications for understanding mercury bioaccumulation and toxicity in aquatic ecosystems. Further research is needed to clarify their contribution to Hg(0) oxidation in aquatic systems and to elucidate the underlying mechanisms driving the process.

1. Introduction

In aquatic environments, dissolved gaseous mercury (DGM, $Hg(0)_{aq}$), primarily composed of elemental mercury (Hg(0)), could represent between 1 % and 50 % of total mercury depending on environmental conditions and biological activity (Floreani et al., 2022). The exchange of Hg(0) between air and water surfaces plays a significant role in the global mercury cycle, regulating the flux of mercury between aquatic and atmospheric compartments (Floreani et al., 2022; Osterwalder et al., 2021). The concentrations of $Hg(0)_{aq}$ in aquatic systems are the result of several interconnected processes including: (i) partitioning of Hg(0) between air and water (Nerentorp Mastromonaco et al., 2017); (ii) reduction of Hg(II) by microorganism activity (Grégoire and Poulain, 2016; Kritee et al., 2007; Santos et al., 2025; Zhang et al., 2021) and photochemical reactions (Luo et al., 2020; Si et al., 2022; Poulain et al., 2004); (iii) oxidation of Hg(0) via photochemically generated reactive oxygen species (ROS) (Luo et al., 2020), abiotic dark oxidation (Zheng et al., 2019) and microbial oxidation (Colombo et al., 2013; Hu et al., 2013; Huang et al., 2020; Siciliano et al., 2002; Wang et al., 2016). Indeed, biological oxidation of $Hg(0)_{aq}$ has been documented in various microorganisms, including sulfate-reducing bacteria, such as *Desulfovibrio* spp. (Colombo et al., 2013; Hu et al., 2013; Huang et al., 2020; Siciliano et al., 2002; Wang et al., 2016), and iron-reducing bacteria (Colombo et al., 2013; Hu et al., 2013; Huang et al., 2019; Lu et al., 2016), as well as Hg-resistant yeasts, such as *Yarrowia* spp. (Oyetibo et al., 2019). Intracellular oxidation of Hg(0) in anaerobic bacteria has

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been linked to the presence of thiol-containing molecules (Wang et al., 2016). Some studies suggest that algal-associated bacteria may contribute to Hg(0) oxidation (Zhang et al., 2021).

A key uncertainty in the oxidation of $Hg(0)_{aq}$ in aquatic systems concerns the role of phytoplankton. While these primary producers are known to influence mercury cycling through bioaccumulation (Harding et al., 2018; Seelen et al., 2023), demethylation (Santos et al., 2025) and Hg(II) reduction (Liang et al., 2022; Santos et al., 2025), their potential to mediate Hg(0) oxidation is overlooked. Some studies suggest that algal exudates and photosynthetic activity may enhance Hg(0) oxidation, yet the extent to which these processes contribute relative to abiotic factors, such as photochemical or chemical oxidation remains unclear. Addressing this gap is critical for refining our understanding of Hg cycling in aquatic systems and improving predictive models of mercury fate. Very recently, in a pilot study, we have demonstrated for the first time, that phytoplankton species can significantly influence the partitioning of atmospheric Hg(0) with water by sequestering Hg (Santos et al., 2024a). While phytoplankton are well known for their ability to reduce Hg(II) to Hg(0), contributing to mercury evasion to the atmosphere, their capacity to oxidize Hg(0) back to Hg(II) (Liang et al., 2022; Santos et al., 2025), remains largely unexplored. Understanding this reverse transformation is essential, as it may counterbalance Hg(II) reduction and influence the overall direction and magnitude of mercury fluxes in aquatic environments.

The current study aims, therefore, to comparatively investigate the potential involvement of phytoplankton species in the oxidation of $Hg(0)_{aq}$ following exposure to gaseous Hg(0). To this end, the diatom *Cyclotella meneghiniana* and the green alga *Chlamydomonas reinhardtii*, representing two of the most abundant groups of phytoplankton were exposed to gaseous Hg(0) in different experimental settings (open vs. closed system, light vs dark, and live vs dead cells).

Based on our previous study (Santos et al., 2024a), we hypothesize that phytoplankton species can play a species-specific role in $Hg(0)_{aq}$ oxidation. This process may enhance the transfer of atmospheric Hg(0) to the aquatic system and promote the oxidation of $Hg(0)_{aq}$ to Hg(II). By examining both oxidation and the conditions under which it occurs, this study contributes to a more complete picture of the redox cycling of mercury mediated by phytoplankton.

2. Material and methods

2.1. Material cleaning and phytoplankton species culturing

All glassware was cleaned using hydrochloric acid 10 % (HCl proanalysis, Merck, Germany) baths for 1 h with sonication, rinsed with ultrapure water (Milli-Q Direct 8, Merck, Germany), and then pyrolyzed for 1–2 h at 550 °C. After pyrolysis, all material was autoclaved (30 min, 121 °C and 1 bar, LVSA 50/70, Zirbus Technology, Germany).

The diatom *Cyclotella meneghiniana* (strain CCAC 0036) was obtained from the Central Collection of Algal Cultures (CCAC, University of Duisburg-Essen, Germany), and the unicellular green alga *Chlamydomonas reinhardtii* (strain CPCC 11) was from the Canadian Phycological Culture Centre (CPCC, University of Waterloo, ON, Canada). These species represent two phylogenetically distinct freshwater organisms with different cell wall compositions: *C. reinhardtii* (Chlorophyceae, Chlorophyta), with a proteinaceous cell wall rich in hydroxyproline (Harris et al., 2009), and *C. meneghiniana* (Bacillariophyceae, Bacillariophyta), characterized by a silica-based frustule (Finkel, 2016). *C. reinhardtii* was chosen for its widespread use in bioaccumulation and ecotoxicity studies, while *C. meneghiniana* was selected for its role in carbon and silicon cycles (Ragueneau et al., 2006). Both species were selected due to their similar size and surface area, key for studying Hg(0) interactions (Millet et al., 2024).

C. reinhardtii and *C. meneghiniana* were grown at pH 7.0 ± 0.1 in four times diluted Tris-acetate-phosphate (TAP) (Harris, 2023) and synthetic freshwater (SFM) media (Santos et al., 2024b), respectively. Both species were cultivated in a specialized incubator at 20 °C with a continuous air purge of 150 mL min^{-1} under visible light with a light intensity of 100 μmol photons m^{-2} s^{-1} following a light:dark regime of 14:10 h. Air was supplied with an air pump and filtered through a capsule filter (0.2 μm Aervent®) and two gold traps to avoid biological and mercury contamination. Cultures were collected during exponential phase by gentle centrifugation (10 min, 20 °C and 1262×*g*), washed with fresh growth medium, and re-suspended in Hg-free growth medium to a final cell density of 1×10^6 cells mL^{-1} for exposure to gaseous Hg(0) as described below.

2.2. Oxidation assays

To investigate the oxidation of $Hg(0)_{aq}$ by the green alga *C. reinhardtii* and the diatom *C. meneghiniana* following exposure to gaseous Hg(0), two sets of experiments were conducted.

In experimental set 1, 50 mL of algae culture was prepared in 100 mL serum flasks, which were then closed and hermetically sealed (closed reactor – CR, Fig. S1a). The experimental strategy that has been applied in studies with other organisms (Colombo et al., 2013; Hu et al., 2013; Oyetibo et al., 2019) was followed. Algal cultures were exposed to a single spike of gaseous Hg(0) at concentrations of 6 μg m^{-3} and 12 μg m^{-3}, 1000 times higher than ambient levels (Wohlgemuth et al., 2020). However, Hg(0) concentrations fall within the range reported in contaminated sites, which varies between 0.03 and 240 μg m^{-3} (Gworek et al., 2017). To assess the effects of light and cellular viability on Hg(0) interactions, algae were also exposed to a constant Hg(0) concentration of 6 μg m^{-3} under light and dark conditions, using both live and dead cells. Dead cells were obtained by heating at 50 °C for 15–20 min, as previously described (Pickhardt and Fisher, 2007), then allowed to cool down before being transferred to each flask for further exposure. At the end of exposure, all flasks were purged simultaneously to minimize variability in the results. Purgeable Hg(0) was collected using Hg-free air as the carrier, directing it into analytical gold-coated sand traps positioned at the flask exits (see Fig. S1b). Samples of $Hg_{NP\text{-}system}$ (representing the entire system, comprising exposure media and algae), $Hg_{NP\text{-}diss}$ (dissolved fraction including exposure media and algal exudates), and $Hg_{NP\text{-}cell}$ (algae cells, separated through centrifugation at 4062×*g* for 10 min at 4 °C) were collected for total Hg (HgT) analysis. Abiotic controls were included in all experiments.

In experimental set 2 (Fig. S2), algae were exposed to gaseous Hg(0) in open-air reactors (OAR) comprising three 1 L borosilicate Erlenmeyers flasks containing 620 mL of algae in Hg-free growth medium. These were placed inside an algae incubator (Fitotron, model SGC120, Weiss Technik UK Ltd., Loughborough, UK) containing the contaminated atmosphere for 72 h (Fig. S2). The exposure concentration of Hg (0) were maintained at a constant level of ~6.0–7.7 μg m^{-3} as in the previous study (Santos et al., 2024a). No effect on algal growth was observed under the studied conditions, as the cell density of both species exposed to Hg(0) was comparable to that in the control treatments (Santos et al., 2024a). The level of gaseous Hg(0) was monitored using sorbent Anasorb® C300 (SKC), followed by analysis using CVAFS based on OSHA Method ID-140 (OSHA, 1991), and by ICPMS based on NIOSH Method 6009 (Eller and Cassinelli, 1994). Aliquots (5–50 mL into metal-free tubes) containing algae and exposure media were then collected at time points of 3, 6, 24, 48, and 72 h for HgT analysis.

Non-purgeable Hg (Hg_{NP}) was determined by purging each sample for 15 min with Hg-free air to remove purgeable Hg(0) (Oyetibo et al., 2019). Hg_{NP} is considered to represent oxidized Hg(II), as methylation has not been detected in these organisms. $Hg_{NP\text{-}system}$, $Hg_{NP\text{-}diss}$ and $Hg_{NP\text{-}cell}$ were collected for HgT analysis.

The purgeable-Hg (Hg(0)) was determined by calculating the difference in HgT concentration between the acidified whole-system sample ($Hg_{all\text{-}system}$) and the non-purgeable fraction of the whole-system sample ($Hg_{NP\text{-}system}$) following the approach of (Colombo et al., 2013, 2014; Oyetibo et al., 2019).

2.3. Dissolved gaseous Hg species analysis

The analysis of purgeable Hg (e.g. Hg(0)), assuming the absence of volatile dimethylmercury (DMHg) in the controlled system, was conducted using double amalgamation on gold-Atomic Fluorescence Spectrometry (DA/Au-AFS) (Bouchet et al., 2011). Briefly, gaseous Hg amalgamated on the gold-coated quartz sand traps was first desorbed at 600 °C under argon flow (ca. 70 mL min^{-1}) and subsequently pre-concentrated on a pure gold analytical trap. The determination of the Hg (0) amount is then achieved by AFS analysis after a second flash

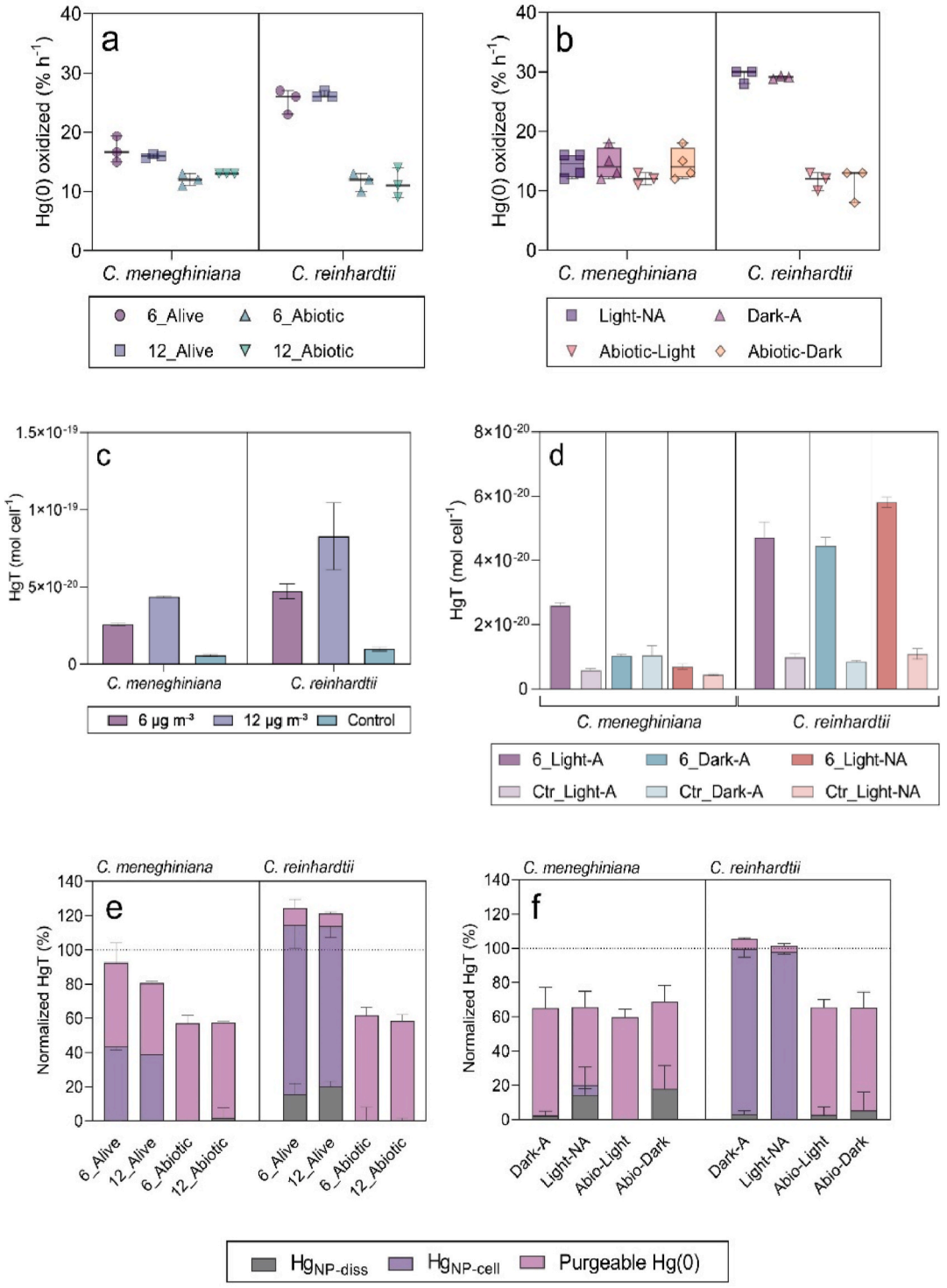


Fig. 1. Hg(0) oxidation and distribution among different fractions (purgeable Hg(0), and non-purgeable fractions: $Hg_{NP\text{-}cell}$ and $Hg_{NP\text{-}diss}$) after 3.5 h of CR incubations **(a)** Hg(0) oxidation rates in alive *C. meneghiniana* and *C. reinhardtii* under light conditions and respective abiotic controls. **(b)** Hg(0) oxidation rate after incubation with 6 µg m^{-3} for alive cells under dark (Dark-A) and dead cells (Light-NA) and their respective abiotic controls. (**c**) Cellular HgT content in *C. meneghiniana* and *C. reinhardtii* exposed to gaseous Hg(0) concentrations of 6 and 12 µg m^{-3} and respective controls (no injection of Hg(0)). (**d**) Cellular HgT content in the different conditions tested as present in (b). (**e**) Mercury distribution in different fractions (non-purgeable Hg: $Hg_{NP\text{-}cell}$ and $Hg_{NP\text{-}diss}$, and purgeable Hg: Hg(0)) for alive algae cells under light exposure at different Hg(0) exposures and abiotic controls, and (**f**) under varying conditions and exposure to 6 µg m^{-3} Hg(0).

desorption at 800 °C. The thermodesorption efficiency was controlled by carrying out two consecutive analyses of the gold-coated sand trap (Duval et al., 2023). Quantification of gaseous Hg was performed using an external calibration of the DA/Au-AFS device with a temperature-controlled Hg(0) source. The limit of detection (LOD) was 6 pg L^{-1}.

2.4. Cold vapor atomic fluorescence spectrometry (CVAFS)

Total mercury (HgT) concentration in the $Hg_{all\text{-}system}$, $Hg_{NP\text{-}system}$, $Hg_{NP\text{-}diss}$, and $Hg_{NP\text{-}cell}$ was measured using a MERX® Automated Total Mercury Analytical System (Brooks Rand Instruments, Seattle, WA, USA) according to the US EPA method 1631 (Environmental Protection Agency, U.S., 2002). $Hg_{NP\text{-}cell}$ fractions were previously digested for analysis as described elsewhere (Santos et al., 2022). The LOD and the limit of quantitation (LOQ) were 0.016 ng g^{-1} and 0.047 ng g^{-1}, respectively. The accuracy of the Hg measurements was verified by analyzing certified reference material of unicellular alga *Scenedesmus obliquus* (IAEA-450). The recovery efficiency was 90 ± 10 %.

2.5. Statistical analysis

Hg(0) oxidation rates and mercury distribution mass balances were calculated using Eqs. S1–S4. Graphical illustrations and statistical analyses were performed using GraphPad Prism 10 Software. The Shapiro-Wilk test was applied to assess data normality. When required, data were log-transformed (ln(Y)) to meet assumptions of normality and homoscedasticity before statistical analysis. One-way or two-way ANOVA, followed by Tukey's HSD post hoc test, was used to identify statistically significant differences between groups of an independent variable. For direct comparisons between two specific groups, independent t-tests were performed to assess mean differences. Significant differences between and within results from different exposure times were evaluated using post hoc multiple comparison tests.

3. Results

To explore potential $Hg(0)_{aq}$ oxidation in phytoplankton cultures, the experiments under light and dark conditions with alive and dead cells at short-term exposure of 3.5 h were performed. Determination of the non-purgeable, $Hg_{NP\text{-}system}$ in closed reactors, revealed that exposures to 6 and 12 μg m^{-3} of Hg(0) resulted in significant oxidation of $Hg(0)_{aq}$. Since no Hg(II) methylation has been observed for either phytoplankton species (Santos et al., 2025), the non-purgeable Hg in the medium can be considered to be oxidized form of Hg(II) (Colombo et al., 2014; Hu et al., 2013). Hg(0) oxidation rates of 23 ± 1 % h^{-1} to 25 ± 0.5 % h^{-1}, were observed for green alga *C. reinhardtii* under light exposure to 6 and 12 μg m^{-3} of Hg(0), respectively (Fig. 1a). These rates were significantly higher than those measured in control algae-free medium, which was 9 ± 1 % h^{-1}, highlighting the important role of *C. reinhardtii* in enhancing Hg(0) oxidation. Lower oxidation rates were observed in cultures of diatom *C. meneghiniana* in comparison to *C. reinhardtii*, showing the species specificity of the $Hg(0)_{aq}$ oxidation process. The oxidation rates in diatom cultures (15 ± 0.3 % h^{-1} at 12 μg m^{-3}) were slightly higher or comparable with those found in abiotic control under light conditions (13 ± 0.2 % h^{-1}), suggesting a more limited contribution of *C. meneghiniana* to Hg(0) oxidation. Furthermore, the oxidation rates obtained under dark conditions or with dead cells under the light conditions were comparable to those found in live cultures under light exposure for both diatom and green algae cells (Fig. 1b).

Significant Hg concentrations were found in algal cells, $Hg_{NP\text{-}cell}$ of both organisms exposed to gaseous Hg(0) compared to the control treatments in the absence of Hg(0) (Fig. 1c). The increase in the gaseous Hg(0) exposure concentration from 6 to 12 μg m^{-3} led to an increase of $Hg_{NP\text{-}cell}$, which was particularly pronounced for *C. reinhardtii* (Fig. 1c). Furthermore, no significant changes in the $Hg_{NP\text{-}cell}$ concentrations were observed for the exposure under dark conditions with alive and dead cells of *C. reinhardtii* (Fig. 1d). By contrast a significant decrease of the $Hg_{NP\text{-}cell}$ concentrations in dead cells exposure was observed, which was further lowered under dark exposure conditions for *C. meneghiniana*. These results indicate species-specific differences in cellular uptake, with *C. reinhardtii* exhibiting a light- and metabolism-independent uptake mechanism, while *C. meneghiniana* primarily relies on light-dependent pathways.

Consistently, comparison of the HgT in different fractions, $Hg_{NP\text{-}cell}$ and $Hg_{NP\text{-}diss}$, and purgeable-Hg: Hg(0)) obtained after Hg(0) exposure indicated that the majority of Hg in the system is accumulated in both algae (Fig. 1e). The percentage of $Hg_{NP\text{-}cell}$ and purgeable Hg(0) were comparable ($Hg_{NP\text{-}cell}$: 43 ± 2 % and 39 ± 0.2 %, and Hg(0): 49 ± 11 % and 41.6 ± 1.1 %, for exposure to 6 and 12 μg m^{-3} Hg(0), respectively) for *C. meneghiniana* (Fig. 1e). While for C. *reinhardtii*, $Hg_{NP\text{-}cell}$ presented 99 ± 14 % and 94 ± 7 % and Hg(0): 10 ± 5 % and 7 ± 0.8 %, respectively for exposure to 6 and 12 μg m^{-3} Hg(0). In the abiotic controls, Hg was predominantly detected as purgeable Hg(0), the non-oxidized form (Fig. 1e), with a minor concentration in the $Hg_{NP\text{-}diss}$, suggesting a minimal abiotic contribution to the oxidation in the phytoplankton cultures. Taken together, these results confirm that the oxidation of $Hg(0)_{aq}$ is predominantly a biotic process, which was particularly pronounced for *C. reinhardtii*.

To enhance environmental relevance, algae were further exposed to Hg(0) for an extended period of 72 h, a duration commonly used in algal ecotoxicity testing (OECD, 2011), within an open-air system. Exposure of phytoplankton cultures to gaseous Hg(0) resulted in a significant increase in the non-purgeable Hg concentration measured in the whole system ($Hg_{NP\text{-}system}$) for both green alga and diatom (Fig. 2). Despite the same exposure concentration of the gaseous Hg(0), the concentration of $Hg_{NP\text{-}system}$ was 4–6 times higher in the cultures of *C. reinhardtii* compared to those of *C. meneghiniana*. In addition for the cultures of both species, the concentration of $Hg_{NP\text{-}system}$ gradually increased over a period of 24–72 h (Fig. 2a–d). The concentrations of the dissolved non-purgeable Hg, $Hg_{NP\text{-}diss}$, in both cultures were much lower than those measured in the whole system and remained constant over time (Fig. 2a–d). The concentrations in the cellular non-purgeable $Hg_{NP\text{-}cell}$ were much higher in comparison with the $Hg_{NP\text{-}diss}$ and increased over the exposure time in both organisms.

The comparison of the percentages of purgeable Hg(0) and non-purgeable Hg in different fractions ($Hg_{NP\text{-}diss}$ and $Hg_{NP\text{-}cell}$) indicated that the majority of mercury is found in the $Hg_{NP\text{-}cell}$ along the sampling period (36–71 % in *C. meneghiniana* - Fig. 2b, and 59–91 % in *C. reinhardtii* - Fig. 2e). This percentage increased significantly over time for *C. reinhardtii*, whereas the increase was more modest for *C. meneghiniana*. This was demonstrated by the 10-fold greater $Hg_{NP\text{-}cell}$ concentration in cultures of *C. reinhardtii* (Fig. 2f) compared to *C. meneghiniana* (Fig. 2c) per cell after 72 h incubation. The concentration of $Hg(0)_{aq,\ ab}$ obtained in the algae-free media incubation (abiotic control, Fig. S3) was 6–52 times lower (over 24–72 h period) compared to incubations in the presence of cells, indicating the significant role of the phytoplankton species in both air/water partitioning and Hg(0) oxidation. $Hg(0)_{aq,ab}$ concentration initially increased until 6 h and then remained constant over 72 h suggesting that the partitioning of Hg(0) between air and water has reached equilibrium at 6 h. In addition, the purgeable Hg(0) concentrations in the assays with both organisms (Fig. 2a–d), closely align with the equilibrium concentration of $Hg(0)_{aq}$ in the water of 1.32 × 10^{-10} M estimated using the dimensionless Henry's law constant ($k_{H'}$, see SI) (Andersson et al., 2008).

4. Discussion

The results of the present study demonstrated for the first time, significant Hg(0) oxidation in algal cultures. For example, Hg(0) oxidation reached 58–60 % in *C. meneghiniana* cultures and 87.3–91.3 %

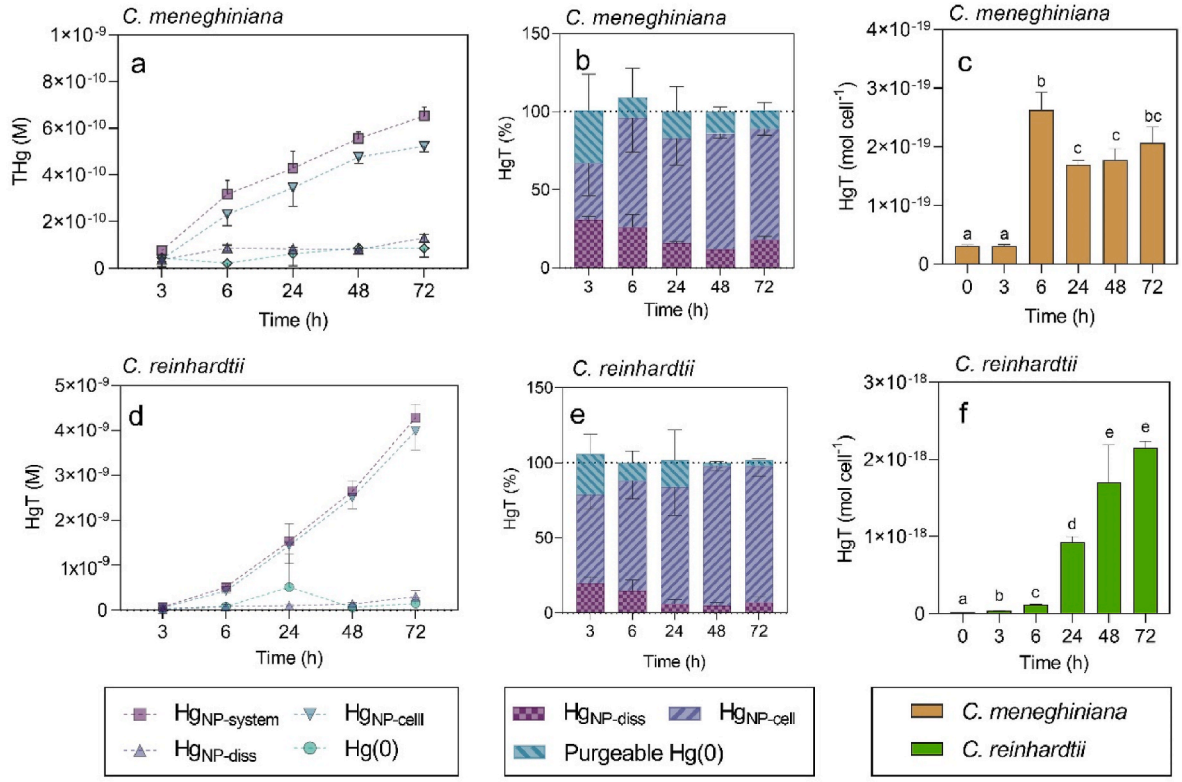


Fig. 2. Time course of purgeable Hg ($Hg(0)_{aq}$) and non-purgeable Hg (Hg_{NP}) concentrations in different fractions ($Hg_{NP\text{-}system}$, $Hg_{NP\text{-}diss}$, and $Hg_{NP\text{-}cell}$) of *C. meneghiniana* (**a**) and *C. reinhardtii* (**d**). The percentage distribution of HgT ($Hg_{NP\text{-}cell}$ and $Hg_{NP\text{-}diss}$) and Hg(0) mercury species in *C. meneghiniana* (**b**) and *C. reinhardtii* (**e**). The cellular total mercury content (mol $cell^{-1}$) in *C. meneghiniana* (**c**) and *C. reinhardtii* (**f**). Concentration of gaseous Hg(0): ~7.7 μg m^{-3}. Dots/bars and error bars represent the means and standard deviations, respectively (n = 3).

in *C. reinhardtii* cultures exposed to Hg(0) in open air reactors over a period of 72 h. These findings align with previous observations of Hg(0) oxidation by bacteria, specifically *D. desulfuricans* ND132 suspended in phosphate buffer, which produced 1.2 ± 0.1 ng of non-purgeable Hg (indicating ~70 % Hg(0) oxidation) after 2.5 h following the injection of 1.7 ng of Hg(0) (Colombo et al., 2013). However, it is important to understand that the concentration of Hg(0) used in this study are 10,000 times higher than in our current study which clearly shows the higher capacity of bacteria to oxidize Hg(0).

The proportion of cellular non-purgeable Hg, used as a measure for oxidized Hg(0), significantly increased over time, along with the decrease of dissolved non-purgeable Hg. This trend was accompanied by a decrease in purgeable Hg, which is used as an indicator of non-oxidized $Hg(0)_{aq}$. The accumulation and oxidation of $Hg(0)_{aq}$ in the cultures of these two representative phytoplankton species were significantly higher than in the abiotic controls, clearly confirming the important contribution of phytoplankton species to these processes.

A higher oxidation of $Hg(0)_{aq}$ to Hg(II) in cultures of *C. reinhardtii* was observed compared to *C. meneghiniana*, which is consistent with higher $Hg_{NP\text{-}cell}$ concentrations in green algae exposed to gaseous Hg(0) and support the assumption that the oxidation is biotically mediated. Higher cellular Hg concentrations observed in *C. reinhardtii* for the same Hg(0) exposure concentration align with a previous study, which showed that green algae cultures of *C. reinhardtii* and *Haematococcus pluvialis* exhibit a greater Hg accumulation capacity compared to diatoms *C. meneghiniana* and *Navicula pelliculosa* (Santos et al., 2024a). However, the results presented in that study were based on only two sampling points and did not differentiate between purgeable and non-purgeable mercury fractions. As a result, it was not possible to distinguish between gaseous and oxidized Hg(0) fractions, nor to estimate the accumulation rates (Santos et al., 2024a). The calculated zero-order kinetic accumulation rate constants were 7.6×10^{-12} M h^{-1} and 5.6×10^{-11} M h^{-1} for *C. meneghiniana* and *C. reinhardtii* exposed to Hg(0) in open air assays, revealing an accumulation rate approximately 8 times greater for green algae. Such difference in the accumulation rates by the two organisms exposed to Hg(0) could be explained by the higher surface area to volume ratio (S:V) for *C. reinhardtii* (0.86 ± 0.26 μm^{-1}), than the one for *C. meneghiniana* (0.65 ± 0.11 μm^{-1}) (Millet et al., 2024). The surface area to volume ratio is known to directly influence the exchange of gases with a higher ratio decreasing gas exchange efficiency (Harris and Theriot, 2018; Robischon, 2017). Furthermore, differences in cell wall/membrane permeability between algal species could also contribute to differences in $Hg_{NP\text{-}cell}$ concentrations. Specifically, *C. meneghiniania* and *C. reinhardtti* exhibit distinct cell wall structures and compositions. Diatom cell walls are mainly composed of silica structures enclosed in a membrane-bound compartment known as the silica deposition vesicle (Hildebrand et al., 2018). This silica is associated with proteins (Hecky et al., 1973; Tesson et al., 2017), long-chain polyamines (Kroger et al., 2000; Poulsen and Kroger, 2004), and polysaccharides (Gugi et al., 2015). In contrast, *C. reinhardtii* cell wall comprises five layer dense hydroxyproline-rich glycoproteins cross-linked with "14-3-3" proteins rich in serine (Ser) and threonine (Thr) (Poulhazan et al., 2024). Further investigation of Hg subcellular distribution in green algae and diatom cells following Hg(0) exposure could offer valuable mechanistic insights into cellular targets, analogous to findings reported for Hg(II) exposure by (Millet et al., 2024).

The greater accumulation and oxidation of Hg(0) in *C. reinhardtii* cultures compared to *C. meneghiniana* may also be linked to the latter's stronger ability to reduce Hg(II). Since oxidized Hg(II) concentrations are governed by the balance between oxidation and reduction, the enhanced reduction capacity of *C. meneghiniana* (Santos et al., 2025) could contribute to the observed differences. The reduction of Hg(II) to Hg(0) by various algal species is well documented, as recently reviewed (Li et al., 2024). Furthermore, previous studies have highlighted the

non-specific role of light in this transformation (Liang et al., 2022; Morelli et al., 2009; Wu and Wang, 2014; Zhang et al., 2021). The findings of the present study suggest that this process is particularly relevant for *C. meneghiniana*, which exhibited a light-dependent pattern of Hg(0) accumulation. Notably, significantly higher $Hg_{NP\text{-}cell}$ concentrations were observed in live algae exposed under light compared to dead algae exposure or those exposed under dark conditions, indicating the important role of light in the Hg accumulation and oxidation by diatoms.

The oxidation of $Hg(0)_{aq}$ in green alga *C. reinhardtti* cultures was comparable across the tested conditions. The similar outcomes between live cells under light and dark conditions could be attributed to 4 × diluted TAP medium used for *C. reinhardtii*, which contains acetate a carbon source, and supports a mixotrophic growth mode (Patel et al., 2019). However, similar results in heat-killed and alive cells further indicate that oxidation in green algal cultures was independent of cellular metabolism and light. These results suggest that, in *C. reinhardtii* cultures, the Hg(0) oxidation to Hg(II) is biologically rather than photochemically driven. Nevertheless, the present results do not allow to conclude whether Hg(0) is first oxidized to Hg(II), which in turn is taken up by the algae, or whether Hg(0) is taken up by algae and oxidized by an (intra)cellular process. Once partitioned between the air and aquatic environment, $Hg(0)_{aq}$ could adhere to the algal cell surface, where it may desorb back into the dissolved phase, oxidize to Hg(II), or penetrate the cell membrane. The possible mechanisms of Hg(0) entry in algae are unclear, but passive diffusion could be a plausible route. In green algae, the phospholipid bilayer of the cell membrane is known to facilitate the diffusion of gases at significant rates (Cooper and Hausman, 2000). Diatoms, in contrast, possess a porous silica cell wall, which may also permit gas exchange. Additionally, facilitated transport of gases, such as CO_2 and NH_3, has been linked to aquaporin channels in diatoms (Matsui et al., 2018). Another possibility is that $Hg(0)_{aq}$ is oxidized in the medium with the resulting Hg(II) being more readily adsorbed and internalized depending on its chemical speciation speciation (Dranguet et al., 2017; Le Faucheur et al., 2014; Lim et al., 2024).

The oxidation of Hg(0) can be driven by multiple cellular and extracellular processes. Photooxidation has been well-documented; however, under visible light, similar to the conditions used in this study, oxidation rates were reported to be significantly lower compared to those observed under combined visible and UV radiation (He et al., 2014; Lalonde et al., 2004). Additionally, chemical oxidation can occur in the presence of reactive oxidants, such as hydroxyl and chlorine radicals (Hines and Brezonik, 2004; Lin and Pehkonen, 1998). However, the significant oxidation of Hg(0) observed under dark exposure conditions, in particularly for green algae, ruled out photooxidation as the dominant process under the studied conditions. It can be further speculated that similar to the recently proposed demethylation pathways in green algae (Liang et al., 2023), dark cellular ROS, such as singlet oxygen, may play an important role in the oxidation of Hg(0) to Hg(II). Further experiments specifically targeting the roles of both cellular and extracellular ROS under light and dark conditions are needed to clarify their potential contribution to this process.

Furthermore, it has been suggested that analogous to bacterial Hg oxidation, phototrophic organisms could catalyze Hg(0) oxidation through membrane-bound thiols (Grégoire and Poulain, 2014). However, HPLC analysis of *C. reinhardtii* cell wall extracts detected methionine, an SH-containing amino acid residue, at an abundance of only 2 %, with no detectable cysteine residues (Poulhazan et al., 2024). This suggests that cell wall thiol-mediated oxidation is likely of minimal importance in this system. Similar to the anaerobic bacterium *Desulfovibrio desulfuricans* ND132, it can be assumed that Hg(0) oxidation is an intracellular process involving thiol-containing molecules that have yet to be identified (Wang et al., 2016). However, the experimental procedure used in this study did not allow for a distinction between adsorbed and intracellular Hg fractions, limiting our ability to determine the precise localization of oxidized Hg(II).

The time course of the changes of $Hg(0)_{aq}$ and comparison of the distribution of Hg between different fractions in the cell cultures ($Hg_{NP\text{-}diss}$, purgeable Hg, and $Hg_{NP\text{-}cell}$) upon Hg(0) exposure demonstrated the important role of the algae in the air-aquatic system partitioning. Algae could influence this equilibrium directly by sequestrating Hg or indirectly via producing extracellular polymeric substances (EPS) and thiol compounds (Gasco et al., 2024; Mangal et al., 2019) which could promote $Hg(0)_{aq}$ oxidation (Hu et al., 2013; Poulain et al., 2007; Zheng et al., 2013). In the case of *C. meneghiniana*, the results suggest a potential lack of involvement of EPS and thiol compounds in Hg(0) oxidation, as no significant differences were observed in dark exposure and exposure to dead cells and abiotic controls. In contrast, *C. reinhardtii* presented similar results across conditions, indicating the possible involvement of EPS and thiol compounds in these processes. The potential contribution of dissolved EPS and thiol compounds secreted by *C. reinhardtii* to the oxidation of Hg(0) was thus investigated, revealing no differences between HgT in the system in the presence of different EPS fraction and abiotic control after 24 and 48 h (Fig. S4). This finding is consistent with a previous study reporting that thiol compounds exhibited little or no reactivity with $Hg(0)_{aq}$ (Zheng et al., 2013).

Based on our findings, Hg(0) partitioning between air and phytoplankton cultures, Hg accumulation, and $Hg(0)_{aq}$ oxidation to Hg(II) are illustrated in Fig. 3. Phytoplankton species, such as green alga and diatom, can take up $Hg(0)_{aq}$ either directly or after $Hg(0)_{aq}$ oxidation to Hg(II), and thus favoring the partitioning of the atmospheric Hg(0) to the aquatic system and oxidation of $Hg(0)_{aq}$ to Hg(II). This study highlights the distinct biological roles of the diatom *C. meneghiniana* and the green alga *C. reinhardtii* in the oxidation of dissolved gaseous $Hg(0)_{aq}$. Mercury accumulation in *C. meneghiniana* was light-dependent, whereas *C. reinhardtii* showed similar results in both live and heat-killed cells when exposed to Hg(0). Green algae demonstrated a significantly higher efficiency in Hg(0) oxidation, nearly an order of magnitude greater than that of diatom *C. meneghiniana*, highlighting the algal species dependence on this process.

Our results reveal that phytoplankton species may play an important role in the partitioning of the Hg(0) from the air to the aquatic environment, favouring its dissolution and oxidation to Hg(II). These findings, obtained in well controlled laboratory conditions with specific phytoplankron species, if applicable at low atmospheric concentrations of Hg(0), could have profound implications for Hg cycling in water bodies as the conversion of $Hg(0)_{aq}$ to its oxidized Hg(II) determines its persistence and impact within aquatic ecosystems. Oxidation not only retains mercury within the system but also enhances its potential for bioaccumulation (Soto Cárdenas et al., 2018) and methylation (Colombo et al., 2013; Regnell and Watras, 2018), processes that significantly increase mercury toxicity. These transformations could contribute to the increase of total mercury concentrations in aquatic environments, influencing ecological and human health risks. In addition, the involvement of phytoplankton species in Hg(0) partitioning between air and water could help explain, at least in part, the higher atmospheric Hg(0) contribution to marine waters than the predicted (Jiskra et al., 2021). This research contributes to a deeper understanding of the role of phytoplankton in mercury cycling, suggesting that certain species may play an underappreciated role in the transformation and flux of mercury in aquatic ecosystems. Given the central role of phytoplankton in aquatic biogeochemical cycles and the potential for algal exudates or ROS to mediate Hg redox transformations, further research is needed to clarify their contribution to Hg(0) oxidation and underlying mechanisms.

5. Conclusions

This study aimed to explore the oxidation of $Hg(0)_{aq}$ in algal cultures, a largely understudied process that could have significant implications for aquatic mercury cycling. The results demonstrate that the diatom *C. meneghiniana* and the green alga *C. reinhardtii* play distinct roles in Hg

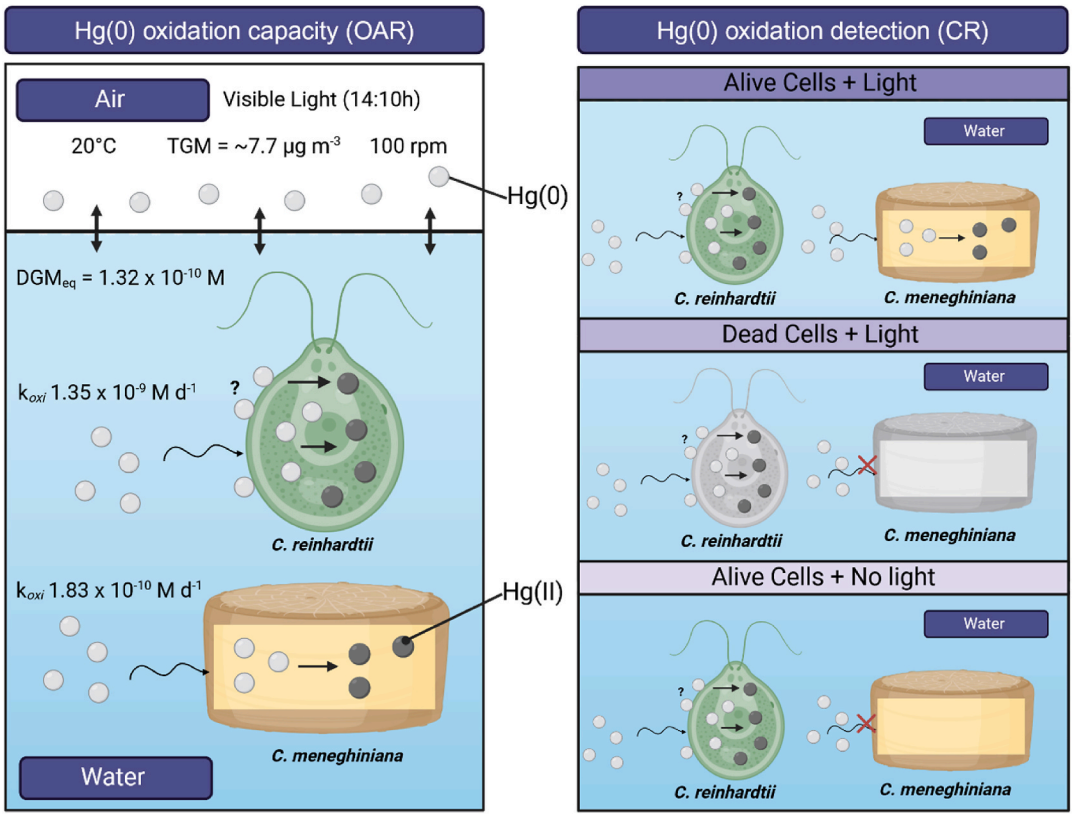


Fig. 3. Conceptual presentation of the exchange of Hg(0) between the air and water, the $Hg(0)_{aq}$ oxidation to Hg(II) and Hg uptake. Illustration created in BioRender. Santos et al. (2025) https://BioRender.com/z04t160.

(0) oxidation in aquatic systems. Experiments conducted at approximately 7.7 µg m^{-3} atmospheric Hg(0) in open reactors revealed accumulation rates of 7.6×10^{-12} M h^{-1} for *C. meneghiniana* and 5.6×10^{-11} M h^{-1} for *C. reinhardtii*. *C. reinhardtii* exhibited nearly 10 times higher Hg(0) oxidation efficiency compared to *C. meneghiniana*, highlighting the phytoplankton species-specific nature of Hg(0) oxidation. Moreover, Hg oxidation in *C. meneghiniana* was found to be light-dependent, while *C. reinhardtii* was able to oxidize Hg(0) under both light and dark conditions revealing light-independent oxidation. *C. reinhardtii* cultures retained its Hg(0) oxidation capacity in both live and heat-killed cell cultures, suggesting that the process may be mediated by cell-associated mechanisms independent of cellular activity. These findings emphasize that phytoplankton can influence the partitioning of Hg(0) between aquatic environments and the atmosphere, either by directly taking up $Hg(0)_{aq}$ or after its oxidation to Hg(II). Further studies are necessary to elucidate the specific mechanisms behind Hg(0) oxidation in different phytoplankton species and to assess their broader implications for mercury dynamics in contaminated environments.

CRediT authorship contribution statement

João P. Santos: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emmanuel Tessier:** Methodology. **Séverine Le Faucheur:** Writing – review & editing, Methodology. **David Amouroux:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Vera I. Slaveykova:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121764.

Data availability

Data will be made available on request.

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