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Interactions of thallium with marine phytoplankton

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Abstract

Thallium (Tl) is the most toxic metal for mammals, likely due to its chemical similarity to the bioessential potassium (K). The biogeochemical cycle of Tl is susceptible to perturbation from a number of anthropogenic activities. Tl accumulation in the food chain may cause chronic Tl poisoning from dietary intake for human beings. However, Tl accumulation in the marine biosphere has largely been overlooked because high concentrations of K are thought to inhibit any uptake of Tl by organisms. Here, for the first time, high accumulation of Tl in the cytosol by the ubiquitous and abundant marine phytoplankton *Emiliania huxleyi* was found, making it readily transferable to higher trophic levels. Dose-response and physiological data were presented from a range of 9 species of phytoplankton under a wide range of Tl concentrations (1 ng L^{-1} to 1 mg L^{-1}), spanning modern open ocean concentrations of 10–20 ng L^{-1}, and higher concentrations in the coastal and fresh waters. Of all phytoplankton studied here, the cyanobacteria *Synechococcus* and the haptophytes *Isochrysis galbana* and *Pavlova granifera* have the largest tolerance to Tl toxicity, while the chlorophyte *Micromonas pusilla* has the least tolerance to elevated Tl. Haptophytes, especially isochrysidales, accumulated significantly more Tl intracellularly (up to 300-fold higher) than chlorophytes, making them good candidates for Tl bioremediation studies. Potassium transporters from *E. huxleyi* are found to be different from all other phytoplankton in this study based on analyses of the proteome. Since Tl is well known to be taken into cells by mistake through potassium channels, the difference of K channels in *E. huxleyi* may be responsible for its highest accumulation of Tl. As one of the most widely distributed phytoplankton in the ocean, the bioaccumulation of Tl in *E. huxleyi* may transfer and concentrate through the marine food chain and have significant impacts on the Tl biogeochemical cycle in the modern ocean.

Keywords: Phytoplankton; Thallium; Bioaccumulation; Metallome; Biogeochemical cycle

1. INTRODUCTION

Thallium (Tl) can be released to the environment anthropogenically during combustion of fossil fuels, coal mining, processing and use in coal-fired power plants and other coal based industrial processes, and through refining processes of ores for other metals such as copper, gold, lead, uranium, and zinc (Karbowska, 2016; Peter and Viraraghavan, 2005; Schaub, 1996). Natural processes also mobilize Tl from bedrocks and ores and lead to enrichment of Tl in the aquatic system (Xiao et al., 2004). Concentrations of Tl in the aqueous environment are largely variable (Belzile and Chen, 2017; de Caritat and Reimann, 2017; Karbowska, 2016), from <1 ng L^{-1} in uncontaminated areas to more than 1100 μg L^{-1} in areas of contamination. Observations of Tl concentrations in seawater are still limited. Although the average concentration of Tl in most open ocean seawater was found to be quite low (~10–20 ng L^{-1}) (Böning et al., 2018; Matthews and Riley, 1970; Matthews and Riley, 1969; Nielsen et al., 2005; Owens et al., 2017), in coastal areas, the concentration can reach up to 10 μg L^{-1} (Chamsaz et al., 2009; Miyazaki and Tao, 1991). The concentrations in the coastal

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region are expected to be greater than open ocean seawater concentrations due to proximity to tributaries and non-point crustal sources (Liu et al., 2019c). Tl deposits or Tl-bearing minerals are widely distributed in many countries, and the risks of Tl contamination are suggested to increase especially in countries with economic growth (Liu et al., 2019b).

Even at sub microgram levels, Tl has been found to be toxic to aquatic organisms (Aoki et al., 2013; Borgmann et al., 1998; Rickwood et al., 2015; Turner and Furniss, 2012; Twiss et al., 2004), and once it enters and transfers along the aquatic food chain, it might become a risk to human beings with the potential to cause chronic poisoning through food consumption (Lin et al., 2001; Zitko et al., 1975; Zitko and Carson, 1975). Indeed, previous studies suggested that the bioaccumulation of thallium in lake fish might place human beings at risk even though the concentration of Tl in the water was low and comparable to that found in typical surface seawater (~14 ng L^{-1}) (Lin et al., 2001). Tl is the most toxic metal for mammals (the LD_{50} of Tl was lower than that of Hg, Cd, Pb, Cu, and Zn for rats (Zitko, 1975)), with a minimal lethal dose of 10 mg/kg being ascertained for human beings (Peter and Viraraghavan, 2005; Zitko, 1975). Although the chronic impacts for Tl exposure are still under research, a correlation between Tl content in the environment and cardiovascular disease has been established (Frattini, 2005), and the safe limit for daily Tl intake is 0.07 μg/kg body weight, or ~5 μg for a typical human being of 70 kg weight (Karbowska, 2016; "US EPA Drinking water criteria document for thallium," 1992). Therefore, understanding the behaviour of Tl in the environment and its accumulation in every component of the food chain is important (Liu et al., 2019a).

Most geochemical studies on Tl have focused on abiotic processes, such as volcanic degassing, weathering and riverine transport, and anthropogenic activities. A biological role in Tl cycles and the mass balance of Tl in the environment have seldom been considered; this may be due to the fact that Tl does not have a nutrient-like profile in the ocean (Belzile and Chen, 2017; Böning et al., 2018; Schlitzer et al., 2018). Similarly there is no known biological role for Tl although some early studies suggested that Tl might stimulate the growth of some microbes (Richards, 1932). Recent studies suggested that abiotic processes cannot fully explain the behaviour of Tl in the marine environment (Böning et al., 2018), and therefore the roles of biota should be considered.

Although the toxicity of Tl has been investigated (Couture et al., 2011), data on Tl accumulation by primary producers are still very sparse (Twining et al., 2003; Twiss et al., 2004), being particularly limited for marine phytoplankton (Turner and Furniss, 2012). Tl accumulation by primary producers was reported to be carrier-mediated (Kwan and Smith, 1991; Twiss et al., 2004), and mainly (90%) through potassium transport systems in the cell membrane (Avery et al., 1991; Brismar, 1998; Hassler et al., 2007), which explains the inhibition of Tl(I) uptake by K^+ in many fresh water organisms. The presence of Ca^{2+} was also shown to reduce Tl bioaccumulation in some taxa (Kwan and Smith, 1991), but had no effect on others (Hassler et al., 2007). With the high concentration of K and Ca in the ocean, marine phytoplankton employ different mechanisms (*e.g.* differences in membrane permeability) to control their intracellular K and Ca concentrations from fresh water organisms (Kilham and Hecky, 1988; Monteiro et al., 2016), which may affect Tl uptake in marine organisms.

This study focuses on the effect of Tl on various marine phytoplankton under a wide range of Tl concentrations. The dose-responses to Tl, change of metal homeostasis, accumulation and subcellular distribution of Tl, and the potassium transport systems (Gierth and Mäser, 2007) in these phytoplankton have been investigated. The results provide a fundamental framework to understand the bioaccumulation and toxicity of Tl in different lineages of marine phytoplankton, which further facilitate the understanding of the roles of primary producers in the marine Tl cycle and are a first component of the risk assessment of Tl accumulation in the marine food chain.

2. MATERIALS AND METHODS

2.1. Phytoplankton

The phytoplankton species studied here include cyanobacteria, chlorophytes, haptophytes, and diatoms. The detailed species and the sources are list in Table 1.

All marine species were cultured in Aquil media (Sunda et al., 2005) and the fresh water algae *Chlamydomonas reinhardtii* was cultured in HSMA media (Sueoka, 1960). All plasticware used in the experiments were cleaned by washing with 10% quartz distilled HCl and thorough rinsing with 18 MΩ cm water. The synthetic ocean water used for making Aquil media was cleaned by passing through a column filled with chelex 100 resin (Morel et al., 1979) to remove the metals. Residual Tl concentration in the growth media was anticipated to be low in the control group to which no Tl was added, but the detection limit of ICP-MS (10 ng L^{-1}) precluded quantification of this residual amount. It was assumed that the Tl concentration at the start of the experiments was substantially lower than the ICP-MS detection limit. Thus, the culture media were supplemented with different concentrations of Tl(I) in the experiments (in the form of $TlNO_3$, from 1 ng L^{-1} Tl to 1 mg L^{-1} Tl). Tl speciation in the medium was determined by using an equilibrium speciation model (Visual MINTEQ) and listed in Table 2. The stability constants were provided by the software, and all the compositions of the media were added into the calculation, including organic ligands such as EDTA. The pH was set to 8.1 for all the calculations. The cultures were grown at a constant temperature of 20 ± 1 °C and illuminated with 100 μmol photons m^{-2} s^{-1} on a light to dark cycle of 12:12 hours. The adapted growth rates for each species were monitored by measuring cell densities daily through a minimal of 3 divisions in each of the 3 generations of sub culturing, resulting in data from 9-15 divisions. The growth rates were computed from the linear form of the following equation using linear regression over the exponential phase:

Table 1
List of phytoplankton for Tl accumulation and toxicity study.

	Species	Strain name	Source
Cyanobacteria	*Synechococcus.* sp	PCC 8806	Pasteur Culture Collection
Chlorophyte	*C. reinhardtii*	CC-4414	Chlamydomonas Resource Center
	C. concordia	RCC 1	Roscoff Culture Collection
	M. pusila	RCC 834	Roscoff Culture Collection
Diatom	*T. pseudonana*	RCC 950	Roscoff Culture Collection
Haptophyte	*P. granifera*	RCC 1557	Roscoff Culture Collection
	I. galbana	RCC 1353	Roscoff Culture Collection
	E. huxleyi	RCC 174	Roscoff Culture Collection
	E. huxleyi (non-calcify)	RCC 1242	Roscoff Culture Collection
	E. huxleyi	OA 1	Field collection (56.6°N, 3.65°E)
		OA 4	Field collection (56.6°N, 3.65°E)
		OA 8	Field collection (45.7°N, 7.16°W)
		OA 15	Field collection (−40.26°N, 109.63°E)
		OA 16	Field collection (−38.31°N, 40.96°E)
		OA 23	Field collection (76.3°N, 2.92°W)

Table 2
Tl speciation in the Aquil medium at 100 ng L^{-1} Tl (0.5 nmol L^{-1} Tl).

Species	Concentration (M)	% of total concentration
Tl^+	2.4945×10^{-10}	49.889
TlBr (aq)	8.8593×10^{-13}	0.177
$TlBrCl^-$	3.6561×10^{-13}	0.073
TlCl (aq)	2.2554×10^{-10}	45.109
$TlEDTA^{3-}$	3.0615×10^{-16}	
TlF (aq)	5.6647×10^{-15}	
$TlHEDTA^{2-}$	8.3273×10^{-19}	
$TlNO_3$ (aq)	2.429×10^{-14}	
TlOH (aq)	1.0545×10^{-15}	
$TlSO_4^-$	2.3728×10^{-11}	4.746

$$N_2 = N_1 e^{\mu t}$$

where N_1 is the previous population size; N_2 is the current population size at a time t days after N_1; μ is the growth rate.

The population sizes were determined by using a TECAN Spark® Multimode plate reader to measure the optical density at 750 nm wavelength. The chlorophyll and phycocyanin fluorescence (Butterwick et al., 1982; Izydorczyk et al., 2005) are also monitored over the period of the experiments (Figs. S1, S2). A relationship between fluorescence and cell numbers was established for different phytoplankton in control groups (Fig. S3).

2.2. Photosystem II Fluorescence measurements

The maximum photochemical yield of photosystem II (F_v/F_m) and the functional absorption cross-section of photosystem II (σ_{PSII}) in different phytoplankton were measured during their exponential phase by using the Satlantic FIRe System fluorometer (Behrenfeld and Milligan, 2013).

2.3. Metallome analysis

The metallome analysis, including Cd, Tl, Pb, Ag, Y, Ge, Sn, Co, V, Ga, W, Ba, Br, I, Mo, Cu, Mn, Zr, Ni, Cr, Al, Sr, Zn, and Fe, largely followed our previous reported method in which multiple elements can be simultaneously measured in biologically derived samples by Inductively coupled plasma mass spectrometry (ICP-MS) (Zhang et al., 2018). Phytoplankton metallomes are reported as metal/P ratio, and P concentration was also measured by ICP-MS. The detection limits for different elements are in our previous report (Zhang et al., 2018) (supplementary materials Table S2). Sample preparation steps have been conducted under trace-metal-clean conditions and were undertaken in laminar flow hoods in the Clean Laboratory Suite at the Department of Earth Sciences, University of Oxford. In this study, cells were collected via centrifugation at 4700 rpm (4816 RCF) for 1 hour and washed two times with chelexed synthetic ocean water (SOW) and one time with Tris buffer (pH = 8.2) in order to remove the loosely bound metals on cell surfaces (Morel et al., 1979; Wilson et al., 2019). The pellets were then digested in quartz distilled HNO_3 (16 M) and 30% H_2O_2 (v/v) and the metal contents were measured using the ICP-MS (Zhang et al., 2018). For *C. reinhardtii*, Milli-Q water (18 M Ω cm, Merck Millipore, USA) was used instead of SOW. All centrifuge tubes (Metal-free, VWR, USA) and pipette-tips were immersed in 10% quartz distilled HCl before thorough rinsing with 18 M Ω cm water.

2.4. Subcellular distribution of Tl in *E. huxleyi*

The distribution of Tl in cells were determined in 6 *E. huxleyi* strains isolated from different areas of the ocean (Rickaby et al., 2016; Zhang et al., 2018), the strains are maintained and cultured in the biogeochemistry laboratory in the Department of Earth Sciences, University of Oxford. The cells were split into 3 procedurally defined cell fractions: (1) "Cytosol": proteins and some organelles within the cytoplasm of the cell. (2) "Cell Debris" (CD): the cell wall and surface membranes. (3) "Metal rich granules" (MRG): all insoluble material that was not broken down in the dissolution of the CD; this represents the NaOH-resistant fraction of the membranes. The different fractions were retrieved following procedures reported by

Wilson et al. (2019). Briefly, the cells were collected and placed on ice, and 1 mL of extraction buffer (20 mmol L^{-1} Tris, pH = 7.4) was added to each sample. The samples were disrupted by ultrasonication. Each sample was disrupted six times with 20 s bursts, at a pulse frequency of 0.4 s s^{-1}, with 20 s intervals between ultrasonications. The resulting suspension was then centrifuged for 30 mins at 13,200 rpm (16,000 RCF) and 4 °C. The supernatant containing the cytosol fraction, was collected for analysis. Then, 0.5 mL Milli-Q water was added to the pellet before boiling the samples for 2 mins, and then 0.5 mL of 1 mol L^{-1} NaOH was added into each sample and the samples were digested at 70 °C for 60 mins. The CD fraction can then be collected from the MRG via another centrifugation for 30 mins and collection of the resulting supernatant. Fractions were digested with in-house distilled HNO_3 and ultrapure H_2O_2. The respective solutions were then measured by ICP-MS (Zhang et al., 2018).

2.5. Potassium transporter system analysis

A novel orthogroup (groups of genes that are descended from a single gene in the last common ancestor of all the species being considered) inference algorithm (Orthofinder) (Emms and Kelly, 2015) was used for comparative analysis to identify homology relationships between published proteome sequences of various phytoplankton (Table S1). Potassium transporter systems of these phytoplankton were then identified by searching through the orthogroups for annotated sequences. The data are analysed using a singular value decomposition method (Alter et al., 2000).

2.6. Statistical analysis

All experiments were conducted in triplicates. All data are presented as mean ± standard deviation (SD) with $n \geq 3$. Significance levels were calculated using the student's t-test and values for $p \leq 0.05$ were accepted as statistically significant.

3. RESULTS

3.1. Different growth responses to Tl addition

Environmentally relevant concentrations of Tl (<100 ng L^{-1}) were not detrimental to growth in any strain (Fig. 1). The highest concentrations of Tl (1 mg L^{-1}) significantly inhibited the growth of the chlorophytes and the diatom ($p < 0.05$). However, the growth rates increased significantly at the highest Tl concentration (1 mg L^{-1}) in *Synechococcus. sp* (PCC 8806), *I. galbana,* and *P. granifera* (RCC1557).

For *E. huxleyi*, two strains were studied here, and slightly different responses were observed. The growth of the calcifying *E. huxleyi* (RCC174) was inhibited by Tl(I) when its concentration was over 1 µg L^{-1}. However, the non-calcifying *E. huxleyi* (RCC1242) had a much higher tolerance to Tl toxicity, and significant inhibition of growth was only observed in the highest concentration treatments (100 µg L^{-1} and 1 mg L^{-1}).

Of all species in this study, the chlorophyte *M. pusilla* had the least tolerance to Tl(I) toxicity. Although the growth rates remained unchanged for Tl(I) concentrations below 10 µg L^{-1}, the growth was fully inhibited in culture containing 100 µg L^{-1} and 1 mg L^{-1} Tl(I). The freshwater chlorophyte *Chlamydomonas reinhardtii* was more tolerant to Tl(I) than the marine chlorophyte *Chlamydomonas concordia*. Under 100 µg L^{-1} Tl, the growth in *C. Concordia* was significantly inhibited, and the relative growth rate dropped to 25%, whilst in *C. reinhardtii*, the growth rate was as high as in the control group. When Tl concentration increased to 1 mg L^{-1}, the growth rate only dropped slightly in *C. reinhardtii* ($\mu/\mu_{max} = 80\%$), while in *C. concordia*, μ/μ_{max} dropped to less than 20%.

3.2. Tl affects photochemical efficiency of photosystem II

For those species whose growth was stimulated by high levels of Tl, chlorophyll fluorescence analysis under different Tl^+ concentrations were done by SATLANTIC FIRe Fluorometer. The maximum yield of photochemistry of photosystem II (F_v/F_m) increased significantly in *Synechococcus* sp., *P. granifera*, and *I. galbana* under 1 mg L^{-1} Tl, and the functional absorption cross-section (σ_{PSII}) decreased significantly in *Synechococcus* sp., *I. galbana,* and *E. huxleyi* (Fig. 2). Environmentally relevant concentrations of Tl (<100 ng L^{-1} in seawater) do not have any significant impact on photosystem II in these species.

3.3. Accumulation of Tl in phytoplankton

Whole cell Tl/P (mmol/mol) in different species of phytoplankton in the control group and the group with 100 ng L^{-1} Tl were determined (Fig. 3). Under these concentrations, there was no accumulation of Tl in most species tested, except for both strains of *E. huxleyi*, in which much higher Tl/P ratios were found in both control and treatment groups. At 100 ng L^{-1} Tl concentration, *E. huxleyi* accumulated up to 0.07 mmol/mol (Tl/P), which can be converted to about 8 µg/g (Tl/biomass, wt/wt). Thus, Tl concentration in *E. huxleyi* was 80,000 times higher than the Tl concentration in the medium (*i.e.* the bioaccumulation factor of Tl in *E. huxleyi* was 80,000). Even in the control group of *E. huxleyi*, although the dissolved Tl concentration in the media was below detection, a significant amount of Tl was found accumulated in cells. Whole cell Tl/P in 100 ng L^{-1} groups were significantly higher than those in the control groups of *E. huxleyi* ($p < 0.01$). After the experiment, the growth media were measured again, and almost all of the Tl had been removed from those media used to culture *E. huxleyi*, suggesting significant biotic uptake (Table S3, Fig. 3). The decreased Tl concentration in Table S3 for *C. concordia* was attributed to cell surface adsorption of Tl, due to the fact that the washed cells did not have significant Tl accumulation detected in this study (Fig. 3).

3.4. Metallomes

To determine if environmentally relevant levels of Tl have any effect on phytoplankton element homeostasis,

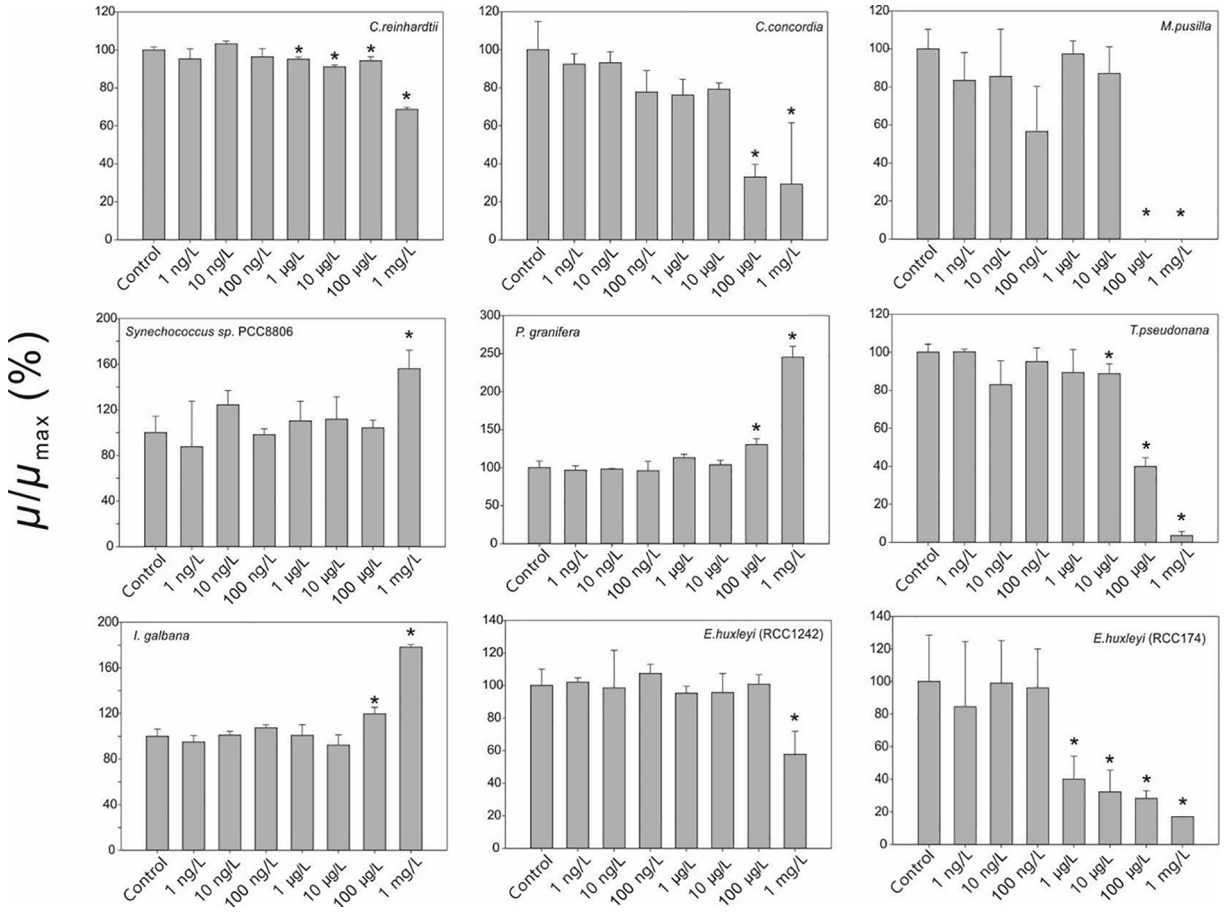

Fig. 1. Relative maximum growth rates of different algal species in groups with different Tl concentrations. μ is the specific growth rate calculated from exponential phase in each group, and μ_{max} is taken μ from the control group for each species. Data are presented as the average value calculated from three treatment replicates and the error bars are the standard deviation between the replicates (n = 3). *: $p < 0.05$ for comparison of treatment with control.

whole cell metallomes for various phytoplankton were also investigated in groups with 100 ng L^{-1} and the control group (Fig. 4). There were no significant changes of cellular concentrations for most trace elements measured in this study (Fig. 4). Compared with other phytoplankton species investigated in this study, the cellular content of many elements were significantly altered in *E. huxleyi* in the presence of Tl, which may be related to higher Tl accumulation in the species (Fig. 3). The chlorophyte *C. reinhardtii* exerted the tightest control over its elemental composition of all elements analysed in this study, compared to other phytoplankton investigated here. No observable difference in cellular Tl contents could be detected between the control group and the group supplemented with 100 ng L^{-1} in *Synechococcus* sp., *C. concordia*, *C. reinhardtii*, *P. granifera*, and *I. galbana*. In the Tl treated group, Ba and Sr content became elevated in *T. pseudonana* and *E. huxleyi*, while in *I. galbana*, Sr content decreased significantly. W concentration increased in the Tl treated group in *Synechococcus* sp. and *P. granifera*.

3.5. Subcellular distribution of Tl in *E. huxleyi*

After cellular uptake by *E. huxleyi*, Tl was mainly distributed intracellularly in the cytosol fraction (Fig. 5). This observation is confirmed in various strains collected from different areas of the ocean.

3.6. Potassium transporters in phytoplankton

In total 2398 potassium transport proteins/ion channels were identified in 27 phytoplankton based on their sequence similarities, and 12 orthogroups (types of potassium transporters) were found (Buchfink et al., 2014; Emms and Kelly, 2015). Identified potassium transporters are listed in Dataset S1. The numbers of K transporters in different orthogroups are listed in Dataset S2. A principle component analysis (PCA) based on singular value decomposition (SVD) was done on the types and numbers of potassium transporters in each species to define their potassium transport systems. Of the phytoplankton listed in Table 1, the

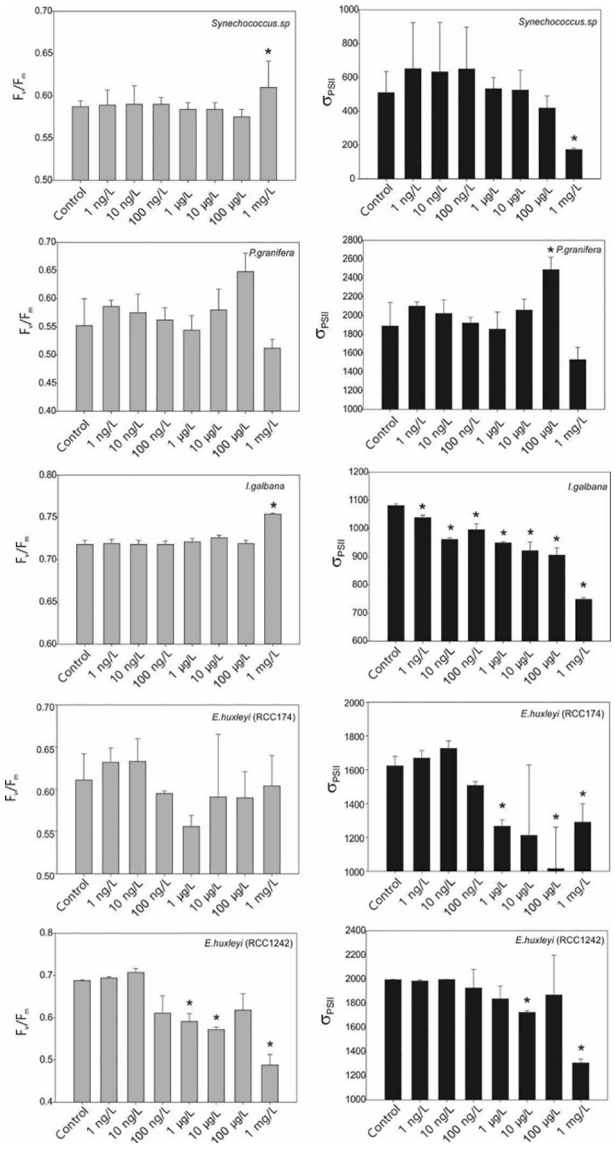

Fig. 2. Chlorophyll fluorescence analysis by SATLANTIC FIRe Fluorometer in groups with different Tl^{+} concentrations. Left panels: the maximum yield of photochemistry of photosystem II (F_v/F_m); right panels: the functional absorption cross-section of photosystem II (σ_{PSII}). Data are presented as the average value calculated from three biological replicates and the error bars are the standard deviation between the replicates (n = 3). *: $p < 0.05$ for comparison of treatment with control.

genomes of *C. reinhardtii*, *M. pusilla*, *T. pseudonana*, *E. huxleyi*, and *Synechococcus* are available and therefore are included in the analysis. The first two principle components clearly separate *E. huxleyi* from other phytoplankton (Fig. 6), and the difference in potassium transporter systems may be the reason why *E. huxleyi* accumulates much more Tl intracellularly than the other phytoplankton in this study.

4. DISCUSSION

4.1. Effects of Tl on phytoplankton growth and photosystem efficiency

Although the toxicity of Tl has been studied for decades, most previous studies focused on Tl concentrations that are much higher than environmental levels (Aoki et al., 2008, 2013; Rickwood et al., 2015; Ritchie and Larkum, 1998; Turner and Furniss, 2012; Twiss et al., 2004). The effects of low concentrations of Tl have seldom been considered, especially for marine species. This study focuses on the effects of both low and high concentrations of Tl, compared to oceanic concentrations, on the marine phytoplankton (from low ng L^{-1} up to 1 mg L^{-1} Tl(I)).

Although Tl is thought to be the most toxic metal for mammals, early studies investigated the toxicity of a range of different elements to phytoplankton and concluded that Tl was the least toxic to some phytoplankton, such as the marine diatom *D. Brightwellii* (the order of metal toxicity following $Hg^{2+} > Ag^{+} > Cu^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+} > Tl^{+}$) (Canterford and Canterford, 1980) and the marine bacterium *V. fischeri* (the order of metal toxicity following Be > Cu > Ag > Cr(VI) > Hg > Cd > Zn > Se > Cr(III) > Ni > Sb > Pb > As > Tl) (Hsieh et al., 2004). However, the toxicity of the metal may be affected by many parameters, such as the affinity to metal-chelating-agents (Pettit, 2006), the concentration of other nutrients (Twiss et al., 2004), and the species of phytoplankton (Couture et al., 2011). Indeed, in this study, large variations were found between different phytoplankton in response to different levels of Tl addition. In each Tl treatment, since other conditions were controlled to be the same for these different phytoplankton, the differences in responses may be related only to the species-specific Tl uptake ability of the phytoplankton. For example, in *C. concordia*, the Cu content slightly increased in the group with 100 ng L^{-1} Tl, which may be related to a greater expression of Cu-SOD in response to the toxicity (Galván-Arzate et al., 2005), but further work is required to confirm such a possibility within phytoplankton.

In this study, the marine cyanobacteria *Synechococcus* (PCC8806) had very high tolerance to Tl toxicity. The growth rates increased at highest Tl concentration and the lag phase was longer comparing to the control group. It has been suggested that cyanobacterium *Synechococcus* can actively extrude Tl^{+} after continuous exposure to the high concentration at 10 µmol L^{-1} (2 mg L^{-1}) (Ritchie and Larkum, 1998). During lag phase, it may have built resistance to Tl, potentially via extrusion, and then started to thrive. Indeed, no accumulation of Tl by *Synechococcus* was observed in this study (Fig. 3). However, the resistance to Tl cannot alone explain the higher growth rates observed at the highest concentration of Tl in this study. Similar trends were also found in the haptophytes *I. galbana* and *P. granifera*, whose growth rates increased under the higher levels of Tl exposure. The maximum yield of photochemistry of photosystem II (F_v/F_m) of

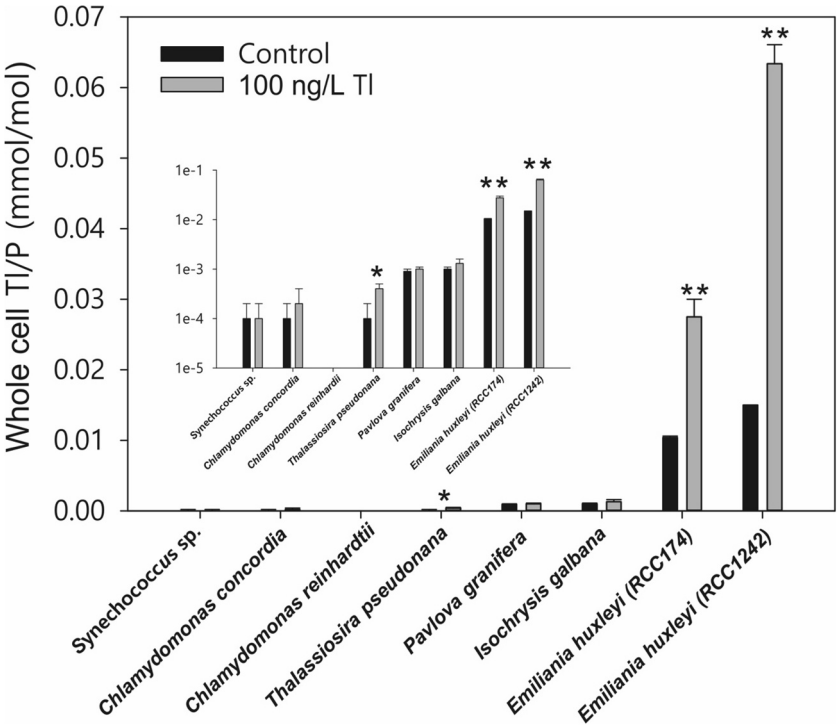

Fig. 3. Whole cell Tl/P (mmol/mol) in different species of phytoplankton. The control groups were cultured in Aquil media and the treatment groups were cultured in Aquil media with 100 ng L^{-1} (0.5 nmol L^{-1}) Tl(I). Whole cell Tl/P in 100 ng L^{-1} groups were significantly higher than that in control groups in *T. pseudonana* (*, $p < 0.05$) and *E. huxleyi* (**, $p < 0.01$). The data are presented using the log scale in the INSET.

these phytoplankton also increased in the group supplemented with a high level of Tl. However, the functional absorption cross-section of photosystem II (σ_{PSII}) generally decreased in those groups. In general, F_v/F_m and σ_{PSII} can be used to measure the stress caused to photosystem II (PSII) due to external factors, and lower F_v/F_m ratios are normally found under greater stress (Behrenfeld and Milligan, 2013). F_v/F_m ratios were also measured for a macroalga exposed to different concentrations of Tl as a measure of its toxicity, and the ratios decreased with increasing Tl concentrations (Turner and Furniss, 2012). However, in our study, higher F_v/F_m ratio was found in some phytoplankton associated with a high Tl concentrations and higher growth rate (Fig. 2). There are three possibilities to explain the findings here: 1) NO_3. Since we used $TlNO_3$ as the Tl(I) source, we have introduced about 5% more NO_3 into the growth media at highest Tl concentration group, and this might contribute to the increased growth rates observed in this study, but since growth rate increases were much greater than 5%, other factors may also contribute. 2) EDTA-binding metals. Although Tl is known to have a very poor binding affinity to EDTA, the addition of it might still affect the binding of EDTA to some other essential trace metals. It is possible that adding high concentrations of Tl in the growth medium increased the dissolution of other trace elements which stimulated the growth of phytoplankton. 3) The effect of Tl. Although Tl has long been considered as toxic, early studies indicated that it might stimulate the growth of some microorganisms such as the yeast *S. cerevisiae* (Richards, 1932), which raises the possibility that Tl acts as a nutrient for some organisms in different parts of the tree of life, an interesting avenue for future research. Any of these three possibilities may have been responsible for the increase of growth observed here, or it could be a combination of all three. Nonetheless, the same growth medium composition at the highest Tl concentration were used for all different marine species in this study, and it can be certain that the cyanobacteria *Synechococcus* and the haptophytes *I. galbana* and *P. granifera* had much higher Tl toxicity tolerance than the other marine phytoplankton investigated in this study.

4.2. Bioaccumulation of Tl and subcellular distribution

Bioaccumulation and subcellular distribution are important for the trophic transfer efficiency of one element or substance, normally represented by the assimilation efficiency (Wang and Zhang, 2013; Zhang et al., 2011). Although only limited data are available to describe Tl transfer along the food chain, the assimilation efficiencies (AEs) reported were high: 33–75% for fish (Lapointe et al., 2009), 40–50% for copepods (Twining and Fisher, 2004), and ~70% for an aquatic insect (Dumas and Hare, 2008). The AE (assimilation efficiency) values were found to be related to the prey type and Tl distribution in the prey (Lapointe et al., 2009). Elements in cell debris and metal-rich-granules associated with the membrane fraction are normally considered to be non-transferable across trophic levels.

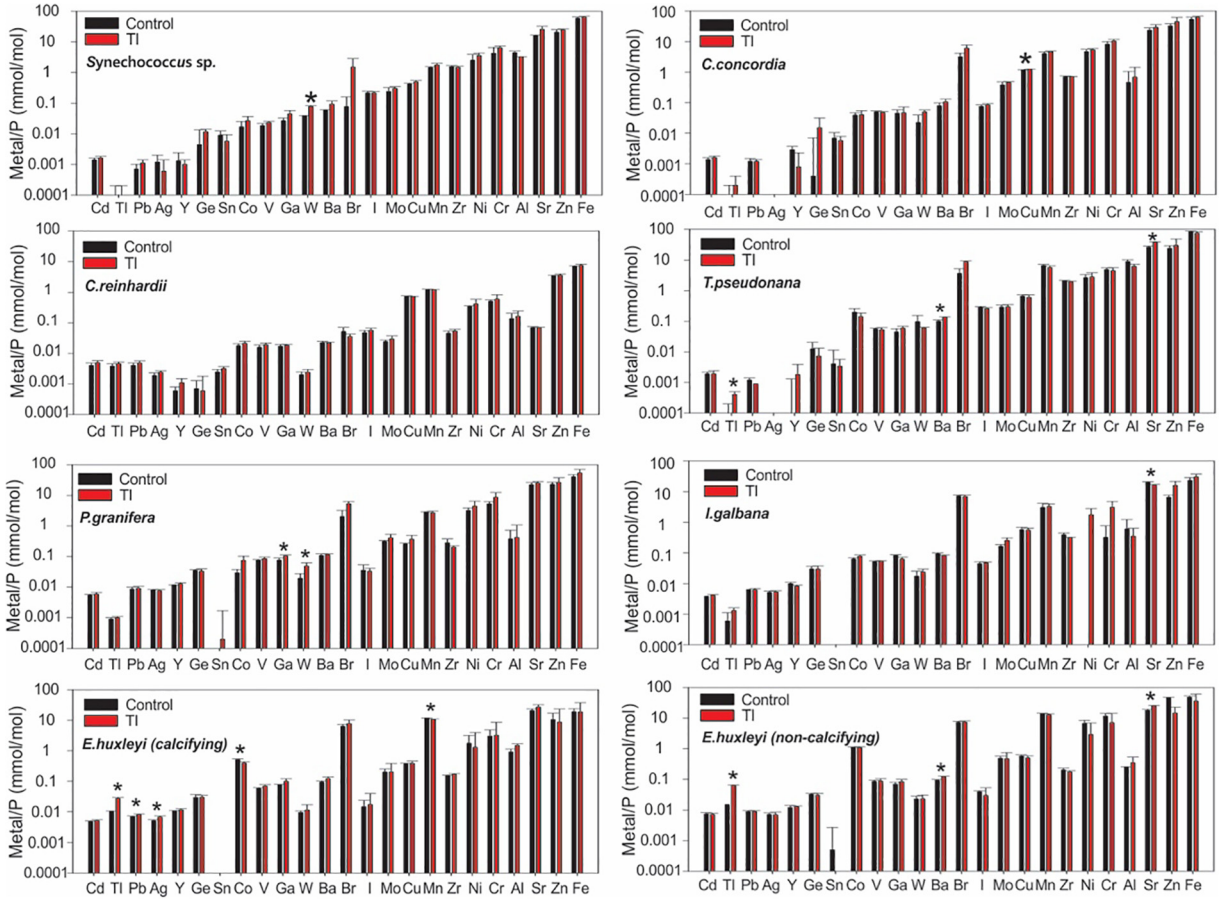

Fig. 4. Whole cell metallomes of various phytoplankton. Control group for every marine species contained only Aquil as growth medium, and for *C. reinhardtii* contained HSMA as growth media. Tl group contained growth media supplemented with 100 ng L^{-1} Tl(I). *: $p < 0.05$ for comparison of treatment with control.

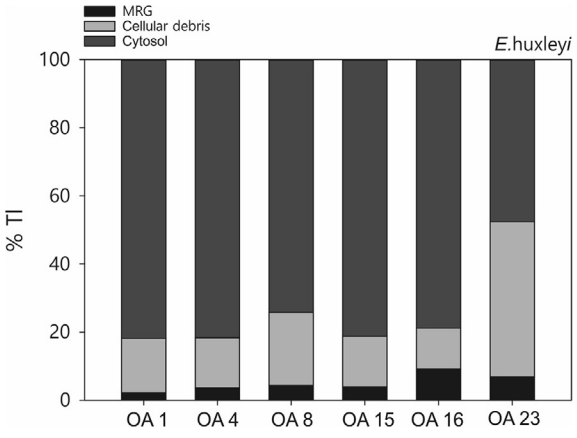

Fig. 5. Subcellular distribution of Tl in different *E. huxleyi* strains collected from different areas of the ocean. Largest amount of Tl was accumulated in the cytosol fraction. The metal-rich-granule (MRG) contained the least amount of Tl.

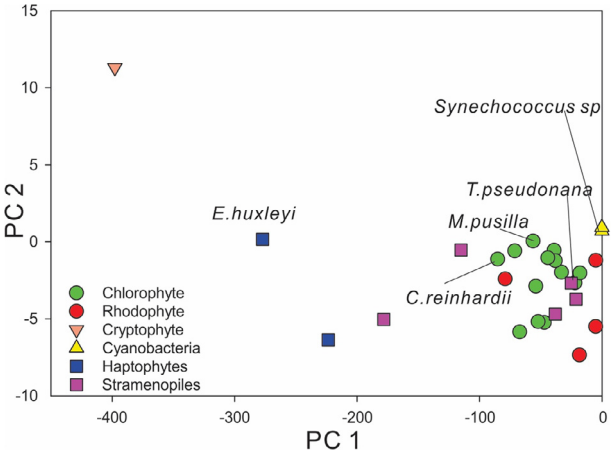

Fig. 6. Singular value decomposition analysis based on the potassium transporter sequences in various phytoplankton. *E. huxleyi* has a significantly different pattern of potassium transporters from other phytoplankton studied here.

In this study, Tl bioaccumulation was assessed in various species of phytoplankton under environmentally relevant concentrations (0 and 100 ng L^{-1} Tl), and the haptophyte *E. huxleyi* was found to be the strongest accumulator of Tl. Even when the Tl concentration in the growth media was extremely low (less than 1 ng L^{-1} Tl), elevated Tl concentrations were still detected in this

organism (Fig. 3) and its cellular concentration increased with the increase of Tl concentration in the media. The non-calcifying *E. huxleyi* accumulated more Tl than the calcifying *E. huxleyi* (Fig. 3). Tl/P in the phytoplankton studied here ranged between 10^{-5} mmol/mol to 0.07 mmol/mol at 0 to 100 ng L^{-1} Tl concentration. In an early field study, Tl in mixed phytoplankton from central Pacific were found to range from 0.016 to 0.79 μg Tl/g biomass (Flegal et al., 1986), which can be converted to about 10^{-4} to 0.006 mmol/mol Tl/P, taking 106C:16N:P:0.1Fe as the major elemental stoichiometry in phytoplankton, of comparable magnitude to our culture results.

The subcellular distribution of Tl in *E. huxleyi* was also investigated in this study. Although there were variations between the strains isolated from different areas (Rickaby et al., 2016; Zhang et al., 2018), and the Tl subcellular distribution was not entirely equivalent in every strain of *E. huxleyi* investigated, the trends are robust, with the cytosol fraction representing the largest pool of Tl, and the metal-rich-granules being the smallest, indicating that the MRG do not provide a defense or an intracellular store to buffer the cell against Tl toxicity in *E. huxleyi*. Localisation to the cytosol appears to be common across the tree of life. In the plant *Lemna minor*, Tl was mainly (85%) found to be in the cytosol fraction (Kwan and Smith, 1991). In animal cells, Tl in the cytosol ranged from 64% in the insect *Chironomus riparius* (Dumas and Hare, 2008), 45% in *Daphnia magna* (Lapointe et al., 2009), and 25–35% in the fish *Pimephales promelas* (Lapointe et al., 2009). Tl in the cytosol fraction is more readily assimilated in the gut of a consumer than those associated with cell membranes (Couture et al., 2011). This explains the high AE values found at the higher end of the food chain, and leads to more concern about the potential toxicity of Tl in the aquatic system which eventually may become dangerous to human beings (Peter and Viraraghavan, 2005; Queirolo et al., 2009; Xiao et al., 2004). In previous studies, Tl concentrations were determined to be approximately 1.5 mg/kg in lake trout from Lake Michigan where the average Tl concentration in water was 14 ng L^{-1} (very similar to modern ocean water) (Lin et al., 2001), and ~1 mg/kg in fish from Musa estuary (Fard et al., 2017). The safe limit for daily Tl intake is 5 μg for a 70 kg human being ("US EPA Drinking water criteria document for thallium," 1992), equating to consumption of only 3–4 g fish/day if the concentration of Tl in fish is 1.5 mg/kg. Even if fish consumption is on a weekly basis, Tl intake from *e.g.* a 100 g fillet of fish easily exceeds the safety level. Although there are limited thallium concentration data in fish available from the marine realm, considering the average Tl concentration of ~15 ng L^{-1} in seawater, and a wide distribution of *E. huxleyi* in world ocean, it is highly likely that marine fish might also accumulate Tl through both water and food (Lapointe et al., 2009). However, limited data are available to quantify the uptake and elimination rates of Tl(I) in marine fish from aqueous and dietary routes, and so it is hard to estimate the trophic transfer factors for Tl. Since Tl deposits and Tl-bearing minerals are widely distributed, and the risks of Tl contamination are suggested to increase in the future, especially in countries with economic growth (Liu et al., 2019b), further research is certainly required to understand Tl accumulation in higher trophic marine species that are consumed by humans and assess such a risk for human beings consuming seafood regularly. Our data show that at least some primary producers accumulate significant concentrations of Tl.

4.3. Insight from potassium transporters

Since Tl^+ and K^+ have the same ionic charge and a similar ionic radius, it has been proposed that Tl^+ can be taken up into the cell through K^+ channels and occupies certain K-binding sites in proteins (Tao et al., 2008; Villaverde and Verstraeten, 2003). The toxicity and accumulation of Tl in cells are related to the uptake mechanism. Ritchie and Larkum (1998) suggested that Tl^+ crossed the plasmalemma passively and the permeability of the plasmalemma to Tl^+ was higher than that of K^+ (Ritchie and Larkum, 1998). However, Kwan and Smith (1991) compared Tl uptake by *Lemna minor* in the light and dark and found that the uptake was reduced by 91% in the dark, suggesting that Tl uptake was not by passive diffusion (Kwan and Smith, 1991). A similar conclusion was drawn by Twiss et al. (2004) that uptake of Tl by *Chlorella* and *Stephanodiscus hantzschiii* requires an active metabolism (Twiss et al., 2004). Nevertheless, all those previous studies suggested that potassium transport systems played a major role in Tl uptake processes. Therefore, in this study, potassium transport systems were investigated by analysing the genome sequences of the phytoplankton investigated here, in order to obtain a fundamental framework within which to understand the different patterns of Tl uptake and accumulation across the phytoplankton.

Of all phytoplankton analysed in this study, cyanobacteria have the fewest potassium transporters identified, in total 10 shared between *Synechococcus WH8102* (Palenik et al., 2003) and *Prochlorococcus marinus MIT9313* (Rocap et al., 2003), and the sequences of a group of potassium transporters in cyanobacteria are distinctly different than other K^+ transporters found (Dataset S1, S2). The haptophyte and the cryophyte have significantly more inwardly rectifying potassium ion channels than other phyla of phytoplankton (Dataset S2). The green lineages generally have more than one group of high-affinity potassium transporters (Dataset S2). Singular value decomposition (SVD) was applied to analyse the data obtained in this study. The first 2 singular values capture 90% of the features of potassium transporters in phytoplankton, and therefore the 2 most principle components were selected to reconstruct the features in the 2-D plot (Fig. 6). The haptophyte *E. huxleyi* stands apart from the other phytoplankton investigated in our Tl accumulation study with distinct characteristics in their potassium transporter systems. This might be due to the fact that they have significantly more inwardly rectifying potassium ion channels than other phytoplankton, and these ion channels may also have a high affinity for Tl or be unable to discriminate between Tl and K. Although most green lineages have a group of high affinity potassium transporters, which might be able to exclude Tl due to their high selectivity for K, this group

of transporters was not found in the marine chlorophyte *M. pusilla*. *M. pusilla* is known to have a reduced genome (Worden et al., 2009), and the lack of high-affinity K transporters may explain its high vulnerability to Tl toxicity: the low-affinity K transporters may not be able to differentiate Tl from K.

The pattern of potassium transporters cannot explain the difference observed between the non-calcifying and calcifying *E. huxleyi*. One obvious possibility is that Tl transport is also associated with the role of Ca in this species, although the mechanism is not yet clear. We speculate that mistaken Tl uptake in *E. huxleyi* may also occur during the Ca transport process. Rapid uptake and transmembrane transport of Ca^{2+} is required by this organism to meet the demands of intracellular calcite precipitation, which lead to the formation of coccoliths in Golgi-derived vesicles and those coccoliths will eventually be secreted to the cell surface to form a protective coat (Thierstein and Young, 2004). A series of voltage-activated ion channels are present in coccolithophores for Ca transportation (Taylor, 2003). Some of the Ca transporters, such as two-pore Ca^{2+} channels (TPCs) and TRP (Transient Receptor Potential) channels, are shown to have weak or even non selectivity for Ca^{2+} and are also permeable to monovalent cations (Flockerzi, 2007; Peiter et al., 2005a, 2005b), so could be permeable to Tl^{+}. Therefore, Tl may have been brought into the *E. huxleyi* cells along with Ca. In the calcifying *E. huxleyi*, some Tl may have been transported into the vesicles and then expelled from the cell through secretion process of the coccoliths. However, in the non-calcifying *E. huxleyi*, this efflux pathway is not available, potentially explaining why Tl concentration is higher in the non-calcifying *E. huxleyi* than in the calcifying *E. huxleyi*.

Depending on the Ca transporters in the organism, i.e. their permeability to Tl, the impact of Ca on Tl uptake may be species dependent. This was observed in previous studies. Addition of Ca was reported to induce 40–45% reduction of Tl uptake in *Lemna minor* (Kwan and Smith, 1991). However, such trends were not observed in another Tl study with *Chlorella* (Hassler et al., 2007), where a 1000 fold change in Ca concentration had no significant impact on Tl accumulation.

4.4. Insights for the Tl biogeochemical cycle

In a previous review, Nielsen et al. (2017) calculated the mass balance of Tl isotopes in the oceans by estimating the source and sink fluxes (Nielsen et al., 2017). The marine Tl influx (~990 Mg/a) includes rivers, hydrothermal fluids, subaerial volcanism, mineral aerosols, and benthic fluxes from continental margins, and the output flux of Tl (~940 Mg/a) includes pelagic clays and altered ocean crust. Although Tl stable isotopes are not the focus of our study, we are interested in understanding the biological impact on the Tl cycle. From data collected in this study, *E. huxleyi* can accumulate about 1.6 μg Tl per g organic carbon produced under average seawater concentration of 15 ng L^{-1} Tl, assuming a C:P ratio of 106:1 (Redfield ratio). Coccolithophores contribute up to 10% of global ocean primary production (40–50 PgC/yr) and export about 0.1 PgC/yr of fixed organic carbon from the mixed layer into the deep sea sediment (Brown and Yoder, 1994; Godoi et al., 2009; Thierstein and Young, 2004; Van Der Wal et al., 1995). *E. huxleyi* is the most common coccolithophore in the world's ocean (Bernard et al., 2009; Feng et al., 2017). If we assume that *E. huxleyi* contributes to export 50% ±25% total fixed organic carbon produced by coccolithophores, then, in total, approximately 80 ± 40 Mg/a Tl can be fixed by *E. huxleyi* alone, which accounts for more than 5% of total marine Tl output fluxes. Since Tl is largely distributed in the cytosol fraction in the cell, and is highly soluble, it might have a high rate of, and therefore shallow, regeneration in the water column. Indeed, no significant surface water depletion in Tl has yet been resolved in the limited datasets available, suggesting limited export of Tl to depth in the oceans and likely to the sediments (Schlitzer et al., 2018). Moreover, this study focuses on Tl amendments to single cultures, while in natural environments there would be greater complexity in the phytoplankton community. It is difficult to estimate the export flux of Tl by phytoplankton from the surface ocean and to sediments without further research. However, the magnitude of the uptake flux suggests it could be an important component of the Tl cycle. Despite the low concentrations in cells and variations and uncertainties in the estimation in the global scale, the bioaccumulation of Tl from seawater should not be neglected when studying the Tl cycle in the marine environment, especially in areas where large coccolithophorid blooms occur. Mass balance shows that this Tl export has an analytically unresolvable impact on surface water Tl concentrations when averaged across the entire ocean. However, at times of high cell density and export, such as blooms, local depletions may occur. Similarly, if uptake of Tl into phytoplankton is associated with a significant isotopic fractionation, then this flux could be important for local Tl isotopic mass balance.

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DECLARATION OF COMPETING INTEREST

The authors claim no conflict of interests for this paper.

APPENDIX A. SUPPLEMENTARY MATERIAL

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