# Competition among trivalent elements (Al, Eu, Fe, Gd, Nd, Tm and Y) for uptake in algae and applicability of the Biotic Ligand Model

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## Abstract

Rare Earth Elements (REE) are essential in many new technologies. While anthropogenic dispersion of REE into the environment are expected in the future, their biogeochemical fate and interactions at biological interfaces are still largely unexplored. Due to their chemical nature (generally trivalent and hard metals), REE can potentially compete among themselves or with other ubiquitous trivalent metals for uptake sites at the surface of aquatic organisms. In the current study, the bioavailability and uptake of gadolinium (Gd) was assessed in the green alga, *Chlamydomonas reinhardtii*, while in the presence of various trivalent elements (Al, Eu, Fe, Nd, Tm and Y). In the absence of competitors, Gd uptake was well described by a Michaelis-Menten equation with an affinity constant ($K_{Gd}$) of $10^{7.1}$ and a maximum internalization flux ($J_{max}$) of 1.95 ± 0.09 × $10^{-2}$ amol·μm$^{-2}$·min$^{-1}$. Neither Al(III) nor Fe(III) had notable effects on Gd uptake in the conditions tested, however, Gd uptake was reduced with increasing concentrations of other REE. These had binding constants with uptake sites very similar to that of Gd ($K_{Nd,\ Y,\ Tm,\ Eu}$ = $10^{7.0}$). Our results suggest that the different REE likely share common transport sites and that the biotic ligand model (BLM) can be used to predict their uptake.

## Introduction

Rare earth elements (REE) comprise the lanthanide series, Y and Sc. Predominantly trivalent, these 15 elements coexist naturally and have similar physicochemical properties. Due to their catalytic and luminescent properties, and ideal magnetic behavior, REE have become strategic resources to a variety of recent applications, including military, electronics, clean energy and medicine (Goodenough et al. 2018; Gwenzi et al. 2018). They are also used in fertilizers, notably in China, due to their ability to promote crop growth and production (Pang et al. 2002). With increased usage in recent years, a subsequent increase in their anthropogenic input to aquatic environments is also expected (Kulaksiz and Bau 2013). Gadolinium (Gd) is a REE that has been widely used for several industrial and medical purposes (Ramalho et al. 2016) and was selected in the present study as a model REE. Every year, more than 200 tons of anthropogenic Gd are conveyed by rivers of densely populated and developed areas (Le Goff et al. 2019). As a result, Gd has been detected in a wide diversity of ecosystems and is recognized as an emergent contaminant in aquatic environments (Bau and Dulski 1996; Migaszewski and Galuszka 2016; Perrat et al. 2017; Rogowska et al. 2018).

Although anthropogenic inputs of REE are expected to increase in the future, few data are currently available on their biogeochemistry, and factors affecting their bioaccumulation are poorly understood. Indeed, the scarcity of data on REE interactions at biological interfaces limits the ability to predict their bioavailability and to assess their environmental risks and hazards. It is well accepted that the ecological risk of metals is related to their speciation and to the extent of ion competition for uptake sites during exposure. The uptake and toxicity of metals have often been interpreted in the context of the Biotic Ligand Model (BLM), which takes into consideration both competition and complexation using experimentally determined stability constants (Campbell and Fortin 2013).

While the BLM has been successfully used to predict the bioavailability of divalent metals toward aquatic organisms (Erickson 2013; Mebane et al. 2020), its applicability to the trivalent REE remains to be demonstrated. Indeed, only few studies recently related uptake and toxicity of REE to their free ion concentrations in the exposure solution, as expected based on the BLM (Aharchaou et al. 2020; Gong et al. 2020). Because the REE are characterized by low solubility in the presence of hydroxides (i.e., at circumneutral and alkaline pH) (El-Akl et al. 2015), carbonates (i.e., at high pH) and phosphates (ubiquitous in algal growth media), studying their bioavailability is challenging. Indeed, due to their low solubility in the presence of phosphate, REE precipitation has been observed in various studies and occurs in most artificial test media, especially algal growth media (Barry and Meehan 2000; Joonas et al. 2017; Lürling and Tolman 2010). In this study, we were able to circumvent this problem by using slightly acidic exposure conditions (pH 5) and using an organic source of phosphorus, allowing algae to grow without modifying the speciation of metals (De Schamphelaere et al. 2014).

Rare earth elements are generally trivalent and hard metals often found together in ore deposits. Thus, REE can potentially compete with each other or with other ubiquitous trivalent metals (e.g., Fe(III) and Al(III)) for binding at uptake sites of aquatic organisms. To test these potential interactions, a green alga, *Chlamydomonas reinhardtii*, was used to investigate the bioavailability and uptake of gadolinium (Gd) in the presence of increasing concentrations of different trivalent elements (Al, Eu, Fe, Nd, Tm and Y). Short-term (30 min) uptake experiments were conducted in carefully controlled and environmentally realistic conditions to verify whether the BLM could be used to predict the bioavailability and uptake of REE. We hypothesized that REE share a common uptake pathway, that REE internalization can be predicted by a one-site BLM and that the presence of ubiquitous trivalent metals such as Al and Fe inhibits Gd uptake.

## Materials and Methods

### *Preparation and optimization of cell culture conditions*

The unicellular green alga, *Chlamydomonas reinhardtii*, was selected for the present study because of its ease of culture, acidophilic character and well-known biology (Blaby-Haas and Merchant 2012; Harris 1989). Additionally, it is frequently used in uptake studies which provide a database of binding constants describing the uptake of different metals. Algae initially obtained from the Canadian Phycological Culture Center (University of Waterloo, Canada) were inoculated and grown in a sterile modified high salt-medium (MHSM-1; Supplementary Material, Table S1) at pH 5. These cultures were maintained in an environmental growth chamber (Conviron, CMP4030; Controlled environments) at a constant temperature (20.0 ± 0.2 °C; unless otherwise mentioned, uncertainty is defined as one standard deviation), illumination (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and with rotary agitation (100 rpm). Once a week, 2 mL aliquots of algal suspension were transferred into 100 mL of sterile culture medium for culture maintenance. To minimize the risk of REE precipitation, culture and exposure of algae were performed at a slightly acidic pH of 5.0 and phosphorus was added in culture medium under an organic form (β-glycerol-phosphate, 25 µM) (De Schamphelaere et al. 2014).

### *Experimental procedures*

All plastic and glassware were soaked for at least 24 h in 10% (v/v) $HNO_3$, thoroughly rinsed five times with deionized water and three times with ultrapure water, and dried under a laminar flow hood before use. Exposure solutions (Supplementary Material, Table S1) consisted of simplified culture media containing neither phosphate or pH buffers, nor trace metals. They were prepared and equilibrated 24 h before use, and the pH checked again and adjusted if necessary. Metal speciation and the concentrations of the free ions were calculated with MINEQL+ (version 5.0) based on total measured REE concentrations and using equilibrium constants from the National Institute of Standards and Technology (NIST) database appended with the values specific to REE as tabulated by Leguay et al. (2016).

Before each experiment, algal cells were inoculated to grow in the MHSM-1 medium to reach mid-exponential growth and then gently harvested on a 2-µm filter membrane (2 µm porosity, polycarbonate, Millipore) using a vacuum pressure of 10 cm Hg or less. Harvested cells were rinsed three times with 10 mL of sterile rinse solution (MHSM-R; Supplementary Material, Table S1). This rinsing step was performed to remove algal exudates likely present in the original algal culture. Size distribution, average surface area, and cellular density were immediately determined using an electronic particle counter (Multisizer TM 3 Coulter Counter with a 70 µm aperture; Beckman, USA) after appropriate dilution in Isoton III electrolyte isotonic solution (Beckman, USA; 100 µL of algae suspension with 9.9 mL isotonic solution). Cells were then resuspended into the desired exposure solutions to provide a cell population density of 50,000 $cells \cdot mL^{-1}$.

### *Uptake experiments*

The temporal evolution of Gd uptake was first studied at a low exposure concentration of 98.7 ± 2.3 nM for about 5, 20, 40 and 60 minutes to ensure that the uptake flux was constant over exposure duration. Subsequently, Gd uptake fluxes were determined after 30 min of exposure to six total Gd concentrations ranging from 0.1 nM to 10 µM. These short exposure durations were used to characterize Gd binding to the membrane transport systems, leading to metal accumulation prior to the onset of effects or other biological feedback. Short term exposures also minimize the effects of cell exudates that could affect the Gd speciation in solution and reduce changes in cell abundance or volume that would modify exposure parameters.

In competition tests, two sets of experiments were performed. In the first set, the effects of trivalent $Fe^{3+}$ and $Al^{3+}$ ions on Gd uptake were studied by exposing cells to six different Gd concentrations in the presence of two different concentrations of $Fe^{3+}$ or $Al^{3+}$. To do so, the algae were acclimated to a low iron growth medium (MHSM-LI; Supplementary Material, Table S1) in which the free $Fe^{3+}$ concentration was set to $5 \times 10^{-19}$ M for more than three growth cycles, and experimental media were prepared with varying free $Gd^{3+}$ concentrations from $1.0 \times 10^{-12}$ to $1.0 \times 10^{-7}$ M ($1.0 \times 10^{-10}$ to $1.0 \times 10^{-5}$ M total Gd; free $Gd^{3+}$ corresponded to 1% of total Gd). Exposures were performed in the presence of two different environmentally relevant concentrations of $Fe^{3+}$ or $Al^{3+}$; ($2.95 \times 10^{-18}$ or $2.95 \times 10^{-16}$ M $Fe^{3+}$ and $1.0 \times 10^{-7}$ or $1.0 \times 10^{-6}$ M $Al^{3+}$). To buffer free $Fe^{3+}$ and $Al^{3+}$ concentrations, nitrilotriacetic acid (NTA) was used and the free $NTA^{3-}$ concentration was kept constant ($2.0 \times 10^{-10}$ M) by adjusting the total NTA concentration. In the second set of competition experiments, the total concentration of Gd was set to $1 \times 10^{-7}$ M and the concentration of a second REE (Y, Nd, Eu and Tm) was increased from 0 to $1 \times 10^{-5}$ M.

The same initial cell density was used for all experiments and the cells were exposed to the desired conditions in triplicates for 30 min. Before each experiment, an aliquot (2 mL) of exposure media was retrieved before adding algae to determine actual Gd (and other REE in competition experiments) exposure concentrations. In all the experiments, gadolinium was spiked with radiolabeled $^{153}GdCl_3$ (Eckert & Ziegler Isotope Products, source number 1994-05-1, radionuclidic purity > 99%, initial specific activity of 62.6 $Ci \cdot g^{-1}$; 2.32 $TBq \cdot g^{-1}$). Concentrated analytical standards of Fe, Al, Y, Nd, Eu and Tm from SPC Science (1 $mg \cdot mL^{-1}$) were used to spike experimental media.

### *Determination of REE internalization fluxes and metal analysis*

After exposure, the cells were recovered on two 2-µm polycarbonate filter membranes; the bottom filter was used to determine background signal from metal adsorption. After two rinses with 10 mL of rinsing solution (MHSM-R; Supplementary Material, Table S1), cells were resuspended for 10 min in 10 mL of a rinsing solution supplemented with EDTA (1 mM) and then rinsed three times again with 10 mL of the rinsing solution. The fraction of Gd (or other REE) remaining associated with algal cells was operationally defined as the intracellular metal fraction. The $^{153}Gd$ radioactivity associated with the filter membranes was measured with a gamma counter (Counting window 26–167 keV; counting time of 300 sec; counting efficiency of 77%; Wallac Wizard2, PerkinElmer) to determine the amount of Gd accumulated in algae. Internalization fluxes (in $amol \cdot \mu m^{-2} \cdot min^{-1}$) were determined from the slope of the plot of accumulated Gd, normalized for cell surface area, as a function of time. In REE competition experiments, filters and algae were recovered after $^{153}Gd$ activity measurements and were then acid-digested in a mixture of 5 mL of trace metal grade nitric acid and 0.5 mL of hydrogen peroxide in 50 mL polypropylene centrifuge tubes. Solutions were left at 95 °C for 240 min, after which digests were totally clear. Aliquots of the filtrates obtained after harvesting algal cells were also sampled and acidified at 2% v/v with trace metal grade nitric acid to determine REE (i.e., Y, Nd, Eu and Tm) concentrations in the exposure solutions.

Cellular and dissolved REE (i.e., Nd, Y, Tm and Eu) concentrations in the different samples were measured using inductively coupled plasma mass spectrometry (XSeries 2; Thermo Scientific). The isotopes $^{103}Rh$ and $^{187}Re$ (and $^{93}Nb$ in the case of Y) were used as internal standards to correct for instrumental drift and possible matrix effects. Moreover, a blank and two quality control samples were passed every 10 to 20 samples. Four measurements were performed from each sample replicate, and the sampler was rinsed with 2% nitric acid for 30 s before performing the measurements of the next sample. A multi-element reference solution containing 1 $\mu g \cdot L^{-1}$ of Li, Be, Co, In, Ba, Ce and U was used as a standard reference solution for ICP-MS signal sensitivity and stability. For calibration, two multi-elemental solutions of several REE (Tm, Nd, Eu

and Y) were prepared with different concentrations between 1 and 300 nM. The calibration curve was validated with multi-element certified standards (C00-061-414; SPC Science); an average element recovery of 98 ± 2% was obtained. One reference material (*Lemna minor*, BCR-670) was used for quality control and digested at the same time as the algae and the filters. The average recoveries were 81 ± 4% for Nd, 79 ± 3% for Y and 84 ± 3% for Tm and 77 ± 4% for Eu (n = 3-6). For the control conditions (e.g., without REE), metal concentrations for both filters and filtrates were below the detection limit (0.3 nM).

### *Theoretical considerations*

For metal uptake to occur, the formation of a surface complex at a metal transport site ($R_{cell}$) must first take place. The metal is then translocated from the apical to the basolateral side of the membrane. The rate of metal uptake depends on the kinetics of this process, the number of transporter sites per cell, their affinity for the metal and the free metal's concentration in solution. Mathematically, this metal uptake rate ($J_{int}$, amol·μm$^{-2}$·min$^{-1}$) can be described by the Michaelis-Menten equation (Slaveykova and Wilkinson 2005):

$$J_{int} = \frac{J_{max}[M^{z+}]}{K_M + [M^{z+}]} \qquad \text{(Equation 1)}$$

Where [$M^{z+}$] (M) is the concentration of free ion, $J_{max}$ (amol·μm$^{-2}$·min$^{-1}$) is the maximum internalization flux, and $K_M$ (M) is the Michaelis-Menten constant (alternatively known as the half-saturation constant). Under equilibrium conditions (which is an assumption of the BLM), and in the absence of any metal uptake competitors, the affinity constant that describes the interaction between the metal and the biotic ligand is equivalent to $1/K_M$ ($K_{MRcell}$, M$^{-1}$).

Generally, metal ions with similar physicochemical properties can compete with a metal of interest for the transport site during the internalization process. Thus, the possible effects of competing cations on Gd uptake can be described by the following equation (François et al. 2007):

$$J_{int} = \frac{J_{max} \cdot K_{R_{cell}}^{Gd} \cdot [Gd^{3+}]}{1 + K_{R_{cell}}^{Gd} \cdot [Gd^{3+}] + K_{R_{cell}}^{Comp} \cdot [Comp^{z+}]} \qquad \text{(Equation 2)}$$

Where $K_{R_{cell}}^{Gd}$ and $K_{R_{cell}}^{Comp}$ are the affinity constants for Gd and the competitors with the transport sites, respectively; [$Gd^{3+}$] and [$Comp^{z+}$] are the concentrations of free Gd and competing ions in the exposure solution, respectively. Similarily, the uptake flux of the competing ions (i.e., Nd, Y, Eu and Tm) in the competition experiments can be described by the following equation:

$$J_{int} = \frac{J_{max} \cdot K_{R_{cell}}^{Comp} \cdot [Comp^{z+}]}{1 + K_{R_{cell}}^{Comp} \cdot [Comp^{z+}] + K_{R_{cell}}^{Gd} \cdot [Gd^{3+}]} \qquad \text{(Equation 3)}$$

## Results and Discussion

### *Gadolinium uptake over time*

Initial uptake experiments were performed to ensure that metal depletion in the exposure media and metal efflux were negligible. Indeed, intracellular Gd (Non EDTA-extractible) increased linearly with time (Figure 1), suggesting a constant internalization flux and negligible efflux of internalized Gd (at least over 1 h). This also signifies that an equilibrium was rapidly reached between Gd and binding sites at the algal cell surface, without any significant changes to the exposure medium (e.g., complexation of the Gd by algal exudates). The Gd internalization flux was

calculated from the slope (± one standard error) of the regression line (Figure 1) and was 1.34 ± 0.13 × $10^{-3}$ amol·µm$^{-2}$·min$^{-1}$ ($r^2$ = 0.93; p < 0.0001). This metal uptake flux is of the same order of magnitude at those found for Cd and Zn for an equivalent free metal exposure concentration also using *C. reinhardtii* (Lavoie et al. 2012).

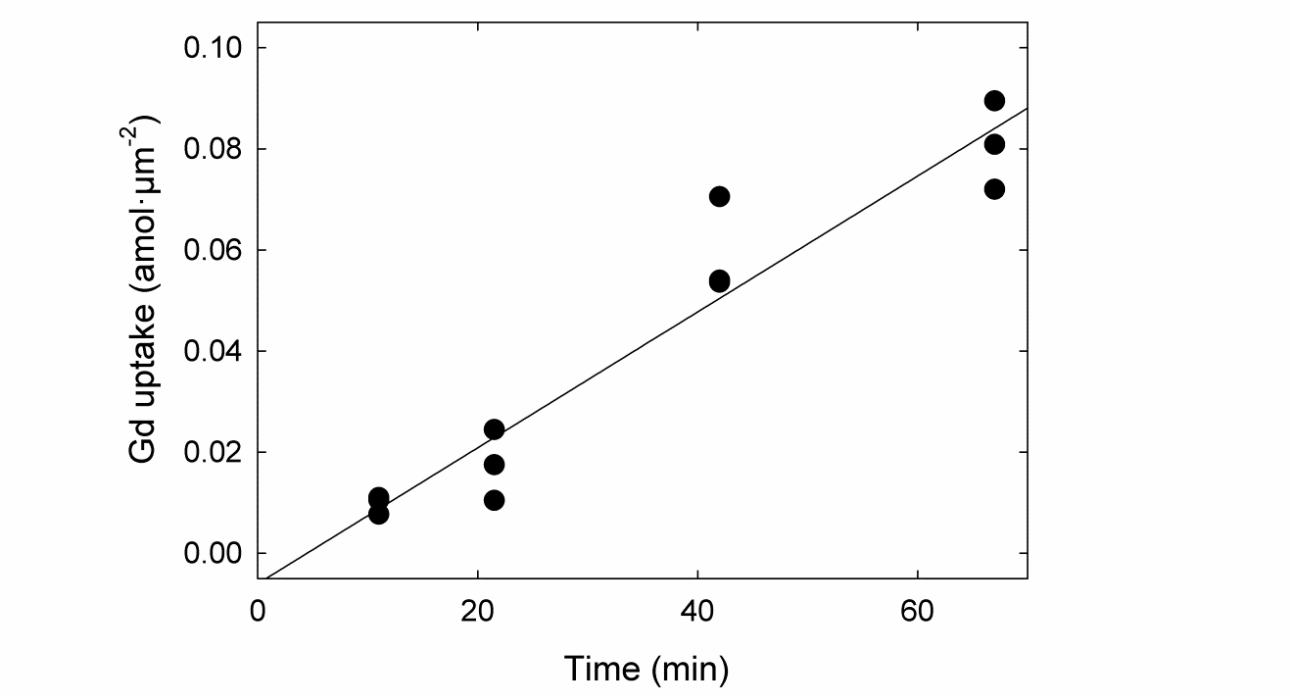


Figure 1: Evolution of intracellular Gd concentrations measured over 60 minutes of exposure to a total Gd concentration of $10^{-7}$ M.

Measurements of dissolved Gd concentrations in the exposure solutions corresponded to nominal concentrations and remained stable over time (Supplementary Material, Figure S1). The decrease in dissolved Gd was low (i.e., around 6%) and was observed from the first time point (5 min), and was likely due to adsorption onto the container walls and biological cell surfaces. Similar chemical behavior was reported in the literature for other REE (Tan et al. 2017; Yang and Wilkinson 2018). In the subsequent experiments, uptake fluxes were determined at a fixed exposure time of 30 min and the measured exposure concentrations were used in data analyses for all experiments.

### *Concentration dependence of Gadolinium uptake*

The internalization fluxes determined as a function of ambient $Gd^{3+}$ concentrations are shown in Figure 2. Typical Michaelis-Menten uptake kinetics were observed and a nonlinear regression was performed to fit the data to Equation 1. This suggests that a single dominant transport site is at play within the Gd concentration range tested. Other parameters obtained by fitting the data presented in Figure 2 ($r^2$ = 0.95; p < 0.0001) were the maximum internalization flux ($J_{max}$ = 1.95 ± 0.09 × $10^{-2}$ amol·µm$^{-2}$·min$^{-1}$) and an apparent Gd stability constant of $10^{7.1}$ ($10^{7.0}$ – $10^{7.2}$). This $J_{max}$ is within the range of values found in other studies performed with other REE with *C. reinhardtii* (e.g., $J_{max}$ Sm ≈ 9.00 × $10^{-3}$ amol·µm$^{-2}$·min$^{-1}$ (Tan et al. 2017); $J_{max}$ Nd ≈ 1.02 × $10^{-2}$ amol·µm$^{-2}$·min$^{-1}$ (Yang and Wilkinson 2018); $J_{max}$ Sc ≈ 3.02 × $10^{-2}$ amol·µm$^{-2}$·min$^{-1}$ (Crémazy et al.

2013); $J_{max}$ Eu ≈ 1.02 × $10^{-2}$ amol·μm$^{-2}$·min$^{-1}$ (Yang et al. 2014)). Similarly, the constant $K_{Gd}$ is very close to the values previously obtained for other REE with this alga under similar conditions (pH 6; $K_{Sm} = 10^{7.0}$ (Tan et al. 2017); $K_{Nd} = 10^{6.8}$ (Yang and Wilkinson 2018); $K_{Eu} = 10^{7.0}$ (Yang et al. 2014)). In Crémazy et al. (2013), Sc was found to have a stronger affinity to *C. reinhardtii* binding sites at pH 5 than Gd with a $K_{Sc}$ of $10^{8.5}$. Several publications have determined stability constants for some divalent metals using the same alga (e.g., $K_{Cu} = 10^{5.8}$; $K_{Pb} = 10^{5.9}$ (Chen et al. 2010); $K_{Ni} = 10^{5.1}$ (Worms et al. 2007); $K_{Cd} = 10^{6.0}$ (Kola and Wilkinson 2005)) that are lower than those observed for REE in this work, while maximum internalization fluxes for the same studies were 3 to 20 times higher than our value for Gd. It was however shown that Cd and Zn had multiple transporters, some of which had very high affinity and low capacity for these elements (Lavoie et al. 2012). In this study, we could only identify one single binding site over the range of REE concentrations tested. When we compare these values among REE, they seem to have comparable maximum internalization fluxes. This could suggest that they share a common uptake site. Finally, the similarity of stability constants among the lanthanide series implies that in a binary exposure at equimolar concentrations, about 50% reductions in uptake fluxes would be expected if uptake occurs over a single common transport site at concentrations close to saturation. This point will be examined in the next sections.

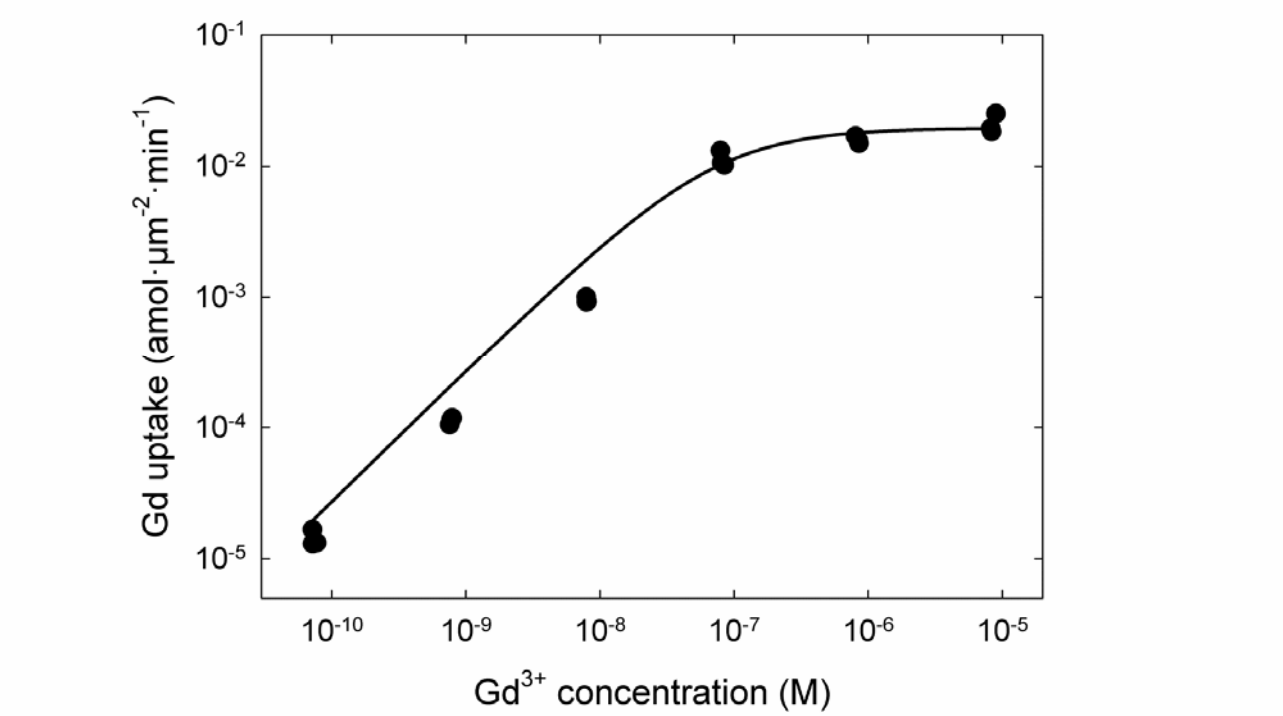


Figure 2: Internalization fluxes of Gd observed after 30 minutes of exposure, as a function of the free $Gd^{3+}$ concentration. The data was fitted through nonlinear regression to the Michaelis-Menten equation (n=3).

### *Potential competition of Iron and Aluminium with Gadolinium for uptake sites*

Considering that the REE are mainly trivalent and hard metals (i.e., there is preference to bind with oxygen-containing functional groups), we hypothesized that the ubiquitous, trivalent, hard elements, Fe(III) and Al(III) could modulate Gd uptake. However, for a range of total Gd exposure concentrations between 0.1 nM and 10 μM, a 100-fold increase in $Fe^{3+}$ concentration had no effect

on Gd uptake rates (Supplementary Material, Figure S2a). Similarly, increasing $Al^{3+}$ concentration 10 times in the exposure media had no effect on Gd uptake (Supplementary Material, Figure S2b). Thus, comparable uptake rates were obtained for similar Gd concentrations, regardless of the exposure $Fe^{3+}$ or $Al^{3+}$ concentrations. This indicates that the two trivalent elements present at concentrations typically observed in natural ecosystems (Lofts et al. 2008; Tipping 2005; Tipping et al. 2002) had no apparent effect on Gd uptake within algae. Despite the chemical similarity (i.e., they are hard metals with identical charges), these two elements have much smaller ionic radii compared to REE. Indeed, the REE-ionic radii in the trivalent form range from 0.86 to 1.03 Å (except for Sc with 0.75 Å), while Fe(III) and Al(III) have ionic radii of only 0.49 and 0.39 Å, respectively (Shannon 1976). Interestingly, $Mg^{2+}$ and $Ca^{2+}$ are two other hard metals with larger ionic radii (0.72 and 1.00 Å, respectively) and have been found to compete with different REE such as La and Ce, Sm, and Nd for uptake and accumulation in algae (Aharchaou et al. 2020; El-Akl et al. 2015; Tan et al. 2017; Yang and Wilkinson 2018). This therefore highlights the possible prevalence of the ionic radius over charge during competition. However, it should be noted that if the REE and the ubiquitous trivalent elements are sharing the same transport systems, and REE have a higher affinity for the transport system than those of $Al^{3+}$ or $Fe^{3+}$ (i.e., $K_{REE} > K_{Al}$ or $K_{Fe}$), no difference will be seen in the uptake curves (Supplementary Material, Figure S2). This is however counter intuitive since the charge:ionic radius ratio should result in greater affinity of Al and Fe over REE for all ligands. Therefore, we cannot exclude a common pathway, but we can state that within the environmental range of Al(III) and Fe(III) concentrations tested, there is no detectable competition. We thus anticipate that Al and Fe concentrations in the environment are unlikely to modify REE bioavailability.

### *Competition among REE for uptake*

Given that REE share common physicochemical properties and naturally occur together in mixtures in the environment (Voncken 2016), it is important to study their uptake in algae as mixtures of REE. Such exposures allow for verification of competition among elements at environmentally relevant concentrations, as they would occur in the field and cover light (Nd), medium (Eu) and heavy (Tm) REE as well as Y which is outside of the lanthanide series. Indeed, for the four studied competitors (i.e., Tm, Nd, Y and Eu), as their concentrations increased up to $10^{-5}$ M, a decrease in the Gd internalization flux was observed from equimolar concentrations and above (total Gd concentration was fixed at $10^{-7}$ M; free $Gd^{3+}$ corresponded to 88% of total Gd; Figure 3). At the highest concentration (i.e., $10^{-5}$ M) of each REE, the Gd uptake was reduced by more than 90% (i.e., 92% for Y and Eu, 94% for Tm and 98% for Nd). The presence of equimolar concentrations reduced Gd uptake by 13% in the case of Nd and Eu, and by 16% in the case of Y. Generally, Gd internalization fluxes were almost constant for concentrations < $10^{-7}$ M of the four competing REE (i.e., less than 6% of reduction, except for Nd with 18% of reduction at $10^{-8}$ M), while notable reductions in Gd uptake occurred once equimolar concentrations of the competing REE were reached, except for Tm. This observed reduction indicates that binding sites are getting close to saturation in these conditions. Surprisingly, equimolar concentrations ($10^{-7}$ M) of Tm and Gd resulted in an apparent 35% increase in Gd uptake. However, when Tm concentration reached $10^{-6}$ M, a 58% reduction in Gd uptake was observed. Such a result was unexpected but was confirmed by a second experiment showing identical results.

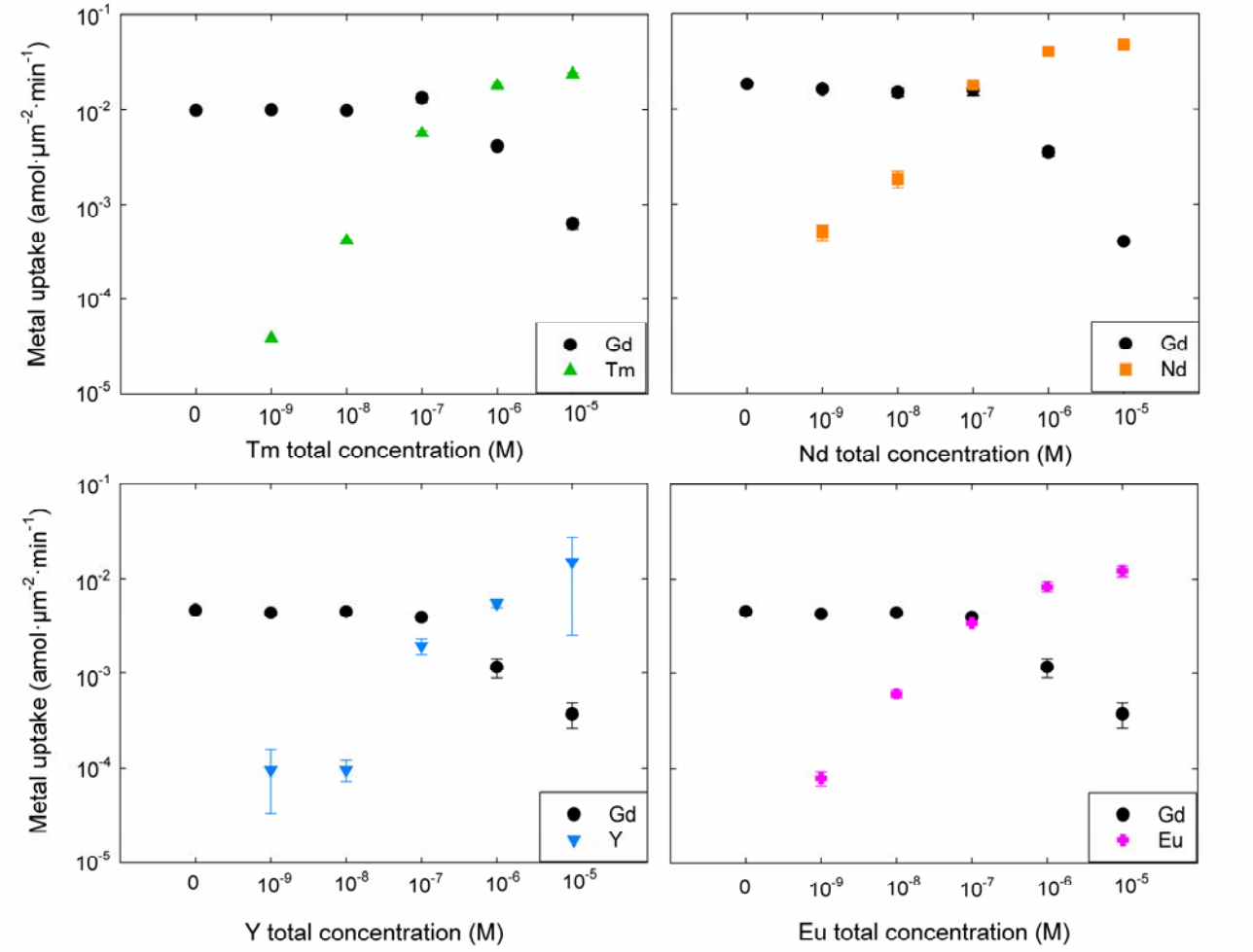


Figure 3: Uptake fluxes of Gd, Tm, Nd, Y and Eu in binary competition experiments. The total Gd concentration was fixed at $10^{-7}$ M while each REE concentration was individually increased from $10^{-9}$ to $10^{-5}$ M (n=3). Error bars represent standard deviations around the mean.

Overall, these reductions can be quantitatively explained by the competition of the studied elements ($Nd^{3+}$, $Y^{3+}$, $Tm^{3+}$ and $Eu^{3+}$) with $Gd^{3+}$ transport sites (Equation 2), with almost identical stability constants (Table 1). Indeed, Equation 2 assumes a common site of interaction, with stability constants $K_{Nd}$, $K_Y$, $K_{Tm}$ and $K_{Eu}$ corresponding to $10^{7.0}$ ($10^{6.8}$ – $10^{7.2}$), $10^{7.0}$ ($10^{6.9}$ – $10^{7.1}$), $10^{7.0}$ ($10^{6.7}$ – $10^{7.2}$) and $10^{7.0}$ ($10^{6.8}$ – $10^{7.2}$), respectively (Table 1). These constants were obtained by fitting the measured uptake fluxes of $Gd^{3+}$ for each competition experiment (i.e., in the presence of Nd, Y, Tm or Eu) with Equation 2 using a nonlinear regression. To do so, we used as a fixed parameter, the binding constant for $Gd^{3+}$ ($K_{Gd} = 10^{7.14}$; range of $10^{7.02}$ to $10^{7.24}$ based on standard deviations computed), obtained in the absence of any competitor. The maximum Gd internalization flux was not fixed as this parameter seemed to fluctuate from one experiment to another, varying between 0.80 and 4.81 × $10^{-2}$ amol·µm$^{-2}$·min$^{-1}$, indicating some biological variability in $J_{max}$ (Table 2). On the other hand, as can be seen in Table 1, the stability constants representing the interaction of each of the four studied REE with Gd uptake sites were identical and are close to those obtained in the absence of competition with overlapping uncertainty. However, it is important to note that the stability constant values reported in Table 1 correspond to the affinity of each element of the four REE for the Gd transport sites and do not necessarily correspond to the affinity

of their own transport sites. We could, nevertheless, determine the stability constant values for $Nd^{3+}$, $Y^{3+}$, $Tm^{3+}$ and $Eu^{3+}$ based on their own uptake kinetics (Figure 3). Indeed, the internalization fluxes (Figure 3) for all of the four elements were well described by the Michaelis-Menten equation (Equation 1) and the obtained set of stability constants and maximum internalization fluxes were summarized in Table 2. The stability constants of the four elements were relatively similar regardless of their estimation method (compare values from Tables 1 and 2), suggesting that the four elements probably share common transport sites. Moreover, these values and those of $J_{max}$ are very similar to previously published data, as mentioned earlier (Tan et al. 2017; Yang et al. 2014; Yang and Wilkinson 2018).

Table 1: Summary of the stability constants ($K_{REE}$ ± standard deviation) for the binding of Gd and the REE tested to the Gd biological uptake site.

| Cation | $K_{REE}$ ($\times 10^7$ $M^{-1}$) | log $K_{REE}$ [min-max] |
|---|---|---|
| **$Gd^{3+}$** | 1.39 ± 0.35 | 7.14 [7.02-7.24] |
| **$Nd^{3+}$** | 0.99 ± 0.42 | 7.00 [6.81-7.15] |
| **$Y^{3+}$** | 0.99 ± 0.21 | 7.00 [6.89-7.08] |
| **$Tm^{3+}$** | 0.98 ± 0.53 | 6.99 [6.65-7.18] |
| **$Eu^{3+}$** | 1.02 ± 0.38 | 7.01 [6.76-7.15] |

Table 2: Summary of the maximum internalization fluxes ($J_{max}$) and the stability constants ($K_{REE}$) for the binding of REE to their biological uptake sites (± standard deviation).

| Cation | $J_{max}$ ($\times 10^{-2}$ amol·µm$^{-2}$·min$^{-1}$) | $K_{REE}$ ($\times 10^7$ $M^{-1}$) | log $K_{REE}$ [min-max] |
|---|---|---|---|
| **$Nd^{3+}$** | 4.81 ± 0.07 | 0.63 ± 0.05 | 6.80 [6.76-6.83] |
| **$Y^{3+}$** | 0.80 ± 0.09 | 0.31 ± 0.14 | 6.49 [6.23-6.65] |
| **$Tm^{3+}$** | 2.42 ± 0.05 | 0.38 ± 0.04 | 6.58 [6.54-6.62] |
| **$Eu^{3+}$** | 1.20 ± 0.06 | 2.21 ± 0.49 | 7.35 [7.24-7.43] |

Our suggestion that REE share a common transport pathway is also coherent with results of toxicity studies that have found similar effects for several REE on algae. For example, Tai et al. (2010) have found that 14 REE have similar effects on the growth rates of the marine alga, *S. costatum*, with 96 h-EC50s around 29 µM. In the same study, only Y and Sc had significantly different toxicity with 96 h EC50s of 43 and 21 µM, respectively. Similarly, in a recent study, we have found that La and Ce had a very similar toxicity for *C. reinhardtii* (Aharchaou et al. 2020).

Considering the competitive effects of the four REE, the uptake fluxes of Gd were modeled using Equation 2 based on the assumption of a competitive reaction of each single REE at Gd uptake sites. Indeed, the $K_{Gd}$ from the single metal experiment and $J_{max,Gd}$ values obtained in the competition experiments with the four REE (Table 1) were used to model the data of the competition experiments. Overall, a good agreement was obtained between predicted and measured internalization fluxes (Supplementary Material, Figure S3), with most predictions lying within a two-fold deviation from the measured ones, except for Y where we observed an overestimation of Y uptake at higher concentrations. Similarly, based on a possible competitive reaction between the Gd and the four studied REE, the affinity constants summarized in Table 2 were used to calculate each one of the four REE uptake fluxes using Equation 3, and an excellent agreement was obtained between predicted and measured uptake fluxes (Figure 4). The good agreement between predicted and observed values of Gd fluxes indicates that a BLM-based model can be effectively used to predict REE uptake in mixtures.

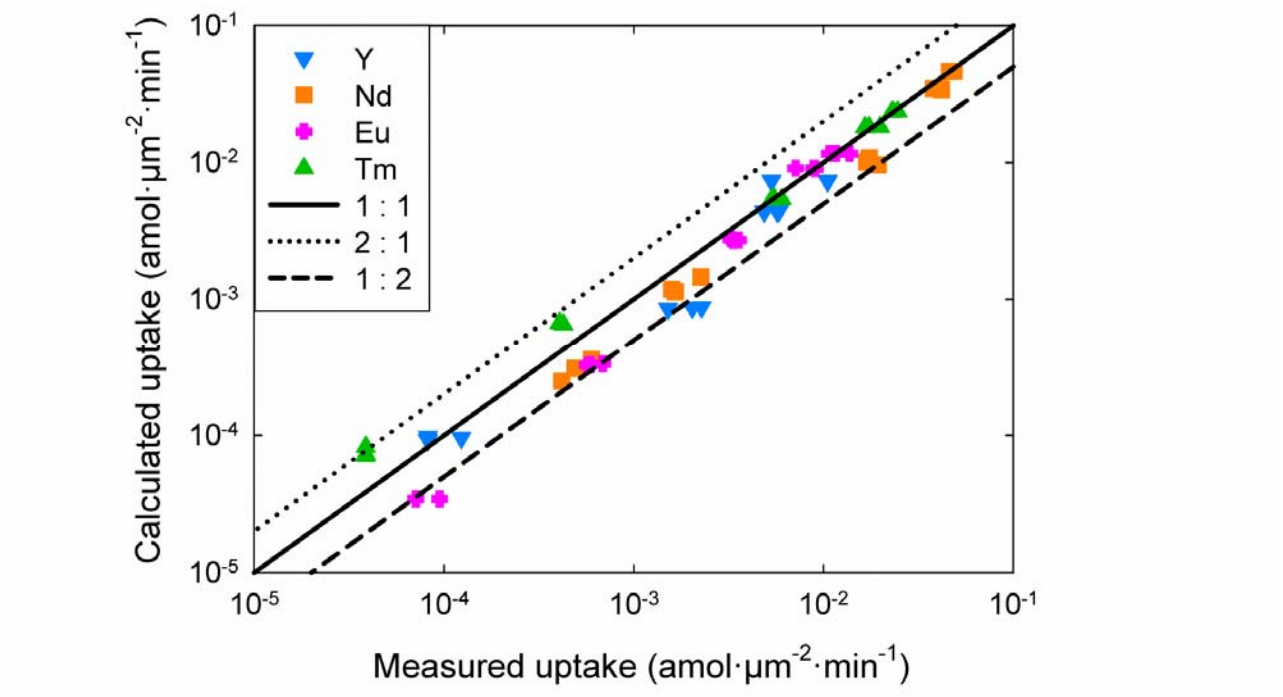


Figure 4: Internalization fluxes of Nd, Y, Eu and Tm, calculated using Equation 3, versus internalization fluxes determined experimentally. The 1:1 (solid), 1:2 and 2:1 (dashed) lines are drawn for comparison.

## Environmental implications

Although Fe, Al and Gd are all trivalent and hard metals (in oxic conditions), contrary to our initial hypothesis, no competition was observed for Gd uptake in *C. reinhardtii*. However, in the natural environment, these elements could indirectly influence REE bioavailability by competing for binding sites to natural organic matter (Marsac et al. 2013). The presence of relatively high Al and Fe concentrations could decrease the amount of REE bound to natural organic matter, and vice versa.

Given the similarity of the stability constants and the maximum internalization fluxes among the five studied REE, we believe that these elements are taken up by the same transporter. Additionally, since they commonly occur as a mixture in natural waters, competitive effects could mutually reduce their uptake in living organisms. Our results also confirmed that the conceptual framework of the BLM can be used for predicting competition among REE and their uptake. Whether this observed competition for uptake will also reduce REE long term effects (e.g. toxicity) remains to be demonstrated and calls for additional work in this area.

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## References

Aharchaou I, Beaubien C, Campbell PGC, Fortin C (2020) Lanthanum and cerium toxicity to the freshwater green alga *Chlorella fusca*: Applicability of the Biotic Ligand Model. Environ Toxicol Chem 39:996-1005 https://doi.org/10.1002/etc.4707

Barry MJ, Meehan BJ (2000) The acute and chronic toxicity of lanthanum to *Daphnia carinata*. Chemosphere 41:1669-1674 https://doi.org/10.1016/S0045-6535(00)00091-6

Bau M, Dulski P (1996) Anthropogenic origin of positive gadolinium anomalies in river waters. Earth Planet Sc Lett 143:245-255 https://doi.org/10.1016/0012-821x(96)00127-6

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

Campbell PGC, Fortin C (2013) Biotic Ligand Model. In: Férard J-F, Blaise C (eds) Encyclopedia of Aquatic Ecotoxicology. Springer Netherlands, Dordrecht, pp 237-246. https://doi.org/10.1007/978-94-007-5704-2

Chen ZZ, Zhu L, Wilkinson KJ (2010) Validation of the biotic ligand model in metal mixtures: Bioaccumulation of lead and copper. Environ Sci Technol 44:3580-3586 https://doi.org/10.1021/es1003457

Crémazy A, Campbell PGC, Fortin C (2013) The biotic ligand model can successfully predict the uptake of a trivalent ion by a unicellular alga below pH 6.50 but not above: Possible role of hydroxo-species. Environ Sci Technol 47:2408-2415 https://doi.org/10.1021/es3038388

De Schamphelaere KAC, Nys C, Janssen CR (2014) Toxicity of lead (Pb) to freshwater green algae: Development and validation of a bioavailability model and inter-species sensitivity comparison. Aquat Toxicol 155:348-359 https://doi.org/10.1016/j.aquatox.2014.07.008

El-Akl P, Smith S, Wilkinson KJ (2015) Linking the chemical speciation of cerium to its bioavailability in water for a freshwater alga. Environ Toxicol Chem 34:1711-1719 https://doi.org/10.1002/etc.2991

Erickson RJ (2013) The biotic ligand model approach for addressing effects of exposure water chemistry on aquatic toxicity of metals: Genesis and challenges. Environ Toxicol Chem 32:1212-1214 https://doi.org/10.1002/etc.2222

François L, Fortin C, Campbell PGC (2007) pH modulates transport rates of manganese and cadmium in the green alga *Chlamydomonas reinhardtii* through non-competitive

interactions: Implications for an algal BLM. Aquat Toxicol 84:123-132 https://doi.org/10.1016/j.aquatox.2007.02.019
Gong B, He E, Xia B, Ying R, Peijnenburg WJGM, Liu Y, Qiu H (2020) Bioavailability and phytotoxicity of rare earth metals to *Triticum aestivum* under various exposure scenarios. Ecotoxicol Environ Saf 205:111346 https://doi.org/10.1016/j.ecoenv.2020.111346
Goodenough KM, Wall F, Merriman D (2018) The rare earth elements: Demand, global resources, and challenges for resourcing future generations. Nat Resour Res 27:201-216 https://doi.org/10.1007/s11053-017-9336-5
Gwenzi W, Mangori L, Danha C, Chaukura N, Dunjana N, Sanganyado E (2018) Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. Sci Total Environ 636:299-313 https://doi.org/10.1016/j.scitotenv.2018.04.235
Harris EH (ed) (1989) The Chlamydomonas Sourcebook. Academic Press, Toronto
Joonas E, Aruoja V, Olli K, Syvertsen-Wiig G, Vija H, Kahru A (2017) Potency of (doped) rare earth oxide particles and their constituent metals to inhibit algal growth and induce direct toxic effects. Sci Total Environ 593:478-486 https://doi.org/10.1016/j.scitotenv.2017.03.184
Kola H, Wilkinson KJ (2005) Cadmium uptake by a green alga can be predicted by equilibrium modelling. Environ Sci Technol 39:3040-3047 https://doi.org/10.1021/es048655d
Kulaksiz S, Bau M (2013) Anthropogenic dissolved and colloid/nanoparticle-bound samarium, lanthanum and gadolinium in the Rhine River and the impending destruction of the natural rare earth element distribution in rivers. Earth Planet Sc Lett 362:43-50 https://doi.org/10.1016/j.epsl.2012.11.033
Lavoie M, Campbell PGC, Fortin C (2012) Extending the biotic ligand model to account for positive and negative feedback interactions between cadmium and zinc in a freshwater alga. Environ Sci Technol 46:12129-12136 https://doi.org/10.1021/Es302512r
Le Goff S, Barrat JA, Chauvaud L, Paulet YM, Gueguen B, Ben Salem D (2019) Compound-specific recording of gadolinium pollution in coastal waters by great scallops. Sci Rep 9 https://doi.org/10.1038/s41598-019-44539-y
Leguay S, Campbell PGC, Fortin C (2016) Determination of the free-ion concentration of rare earth elements by an ion-exchange technique: implementation, evaluation and limits. Environ Chem 13:478-488 https://doi.org/10.1071/en15136
Lofts S, Tipping E, Hamilton-Taylor J (2008) The chemical speciation of Fe(III) in freshwaters. Aquat Geochem 14:337-358 https://doi.org/10.1007/s10498-008-9040-5
Lürling M, Tolman Y (2010) Effects of lanthanum and lanthanum-modified clay on growth, survival and reproduction of *Daphnia magna*. Water Res 44:309-319 https://doi.org/10.1016/j.watres.2009.09.034
Marsac R, Davranche M, Gruau G, Dia A, Pédrot M, Le Coz-Bouhnik M, Briant N (2013) Effects of Fe competition on REE binding to humic acid: Origin of REE pattern variability in organic waters. Chem Geol 342:119-127 https://doi.org/10.1016/j.chemgeo.2013.01.020
Mebane CA, Chowdhury MJ, De Schamphelaere KAC, Lofts S, Paquin PR, Santore RC, Wood CM (2020) Metal bioavailability models: Current status, lessons learned, considerations for regulatory use, and the path forward. Environ Toxicol Chem 39:60-84 https://doi.org/10.1002/etc.4560
Migaszewski ZM, Galuszka A (2016) The use of gadolinium and europium concentrations as contaminant tracers in the Nida River watershed in south-central Poland. Geol Q 60:67-76 https://doi.org/10.7306/gq.1241
Pang X, Li D, Peng A (2002) Application of rare-earth elements in the agriculture of China and its environmental behavior in soil. Environ Sci Pollut Res Int 9:143-148 https://doi.org/10.1007/BF02987462

Perrat E, Parant M, Py JS, Rosin C, Cossu-Leguille C (2017) Bioaccumulation of gadolinium in freshwater bivalves. Environ Sci & Pollut Res 24:12405-12415 https://doi.org/10.1007/s11356-017-8869-9

Ramalho J, Semelka RC, Ramalho M, Nunes RH, AlObaidy M, Castillo M (2016) Gadolinium-based contrast agent accumulation and toxicity: An update. Am J Neuroradiol 37:1192-1198 https://doi.org/10.3174/ajnr.A4615

Rogowska J, Olkowska E, Ratajczyk W, Wolska L (2018) Gadolinium as a new emerging contaminant of aquatic environments. Environ Toxicol Chem 37:1523-1534 https://doi.org/10.1002/etc.4116

Shannon RD (1976) Revised effective ionic radii and systematic studies of interatomic distances in halides and chaleogenides. Acta Cryst 32:751 - 767 https://doi.org/10.1107/S0567739476001551

Slaveykova VI, Wilkinson KJ (2005) Predicting the bioavailability of metals and metal complexes: Critical review of the biotic ligand model. Environ Chem 2:9-24 https://doi.org/10.1071/En04076

Tai PD, Zhao Q, Su D, Li PJ, Stagnitti F (2010) Biological toxicity of lanthanide elements on algae. Chemosphere 80:1031-1035 https://doi.org/DOI 10.1016/j.chemosphere.2010.05.030

Tan Q-G, Yang G, Wilkinson KJ (2017) Biotic ligand model explains the effects of competition but not complexation for Sm biouptake by *Chlamydomonas reinhardtii*. Chemosphere 168:426-434 https://doi.org/10.1016/j.chemosphere.2016.10.051

Tipping E (2005) Modelling Al competition for heavy metal binding by dissolved organic matter in soil and surface waters of acid and neutral pH. Geoderma 127:293-304 https://doi.org/10.1016/j.geoderma.2004.12.003

Tipping E, Rey-Castro C, Bryan SE, Hamilton-Taylor J (2002) Al(III) and Fe(III) binding by humic substances in freshwaters, and implications for trace metal speciation. Geochim Cosmochim Acta 66:3211-3224 https://doi.org/10.1016/S0016-7037(02)00930-4

Voncken JHL (2016) The Rare Earth Elements—A Special Group of Metals. In: The Rare Earth Elements: An Introduction. SpringerBriefs in Earth Sciences. Springer International Publishing, Cham, pp 1-13. https://doi.org/10.1007/978-3-319-26809-5_1

Worms IAM, Parthasarathy N, Wilkinson KJ (2007) Ni uptake by a green alga. 1. Validation of equilibrium models for complexation effects. Environ Sci Technol 41:4258-4263 https://doi.org/10.1021/es0630339

Yang G, Tan QG, Zhu L, Wilkinson KJ (2014) The role of complexation and competition in the biouptake of europium by a unicellular alga. Environ Toxicol Chem 33:2609-2615 https://doi.org/10.1002/etc.2722

Yang G, Wilkinson KJ (2018) Biouptake of a rare earth metal (Nd) by *Chlamydomonas reinhardtii* - Bioavailability of small organic complexes and role of hardness ions. Environ Pollut 243:263-269 https://doi.org/10.1016/j.envpol.2018.08.066