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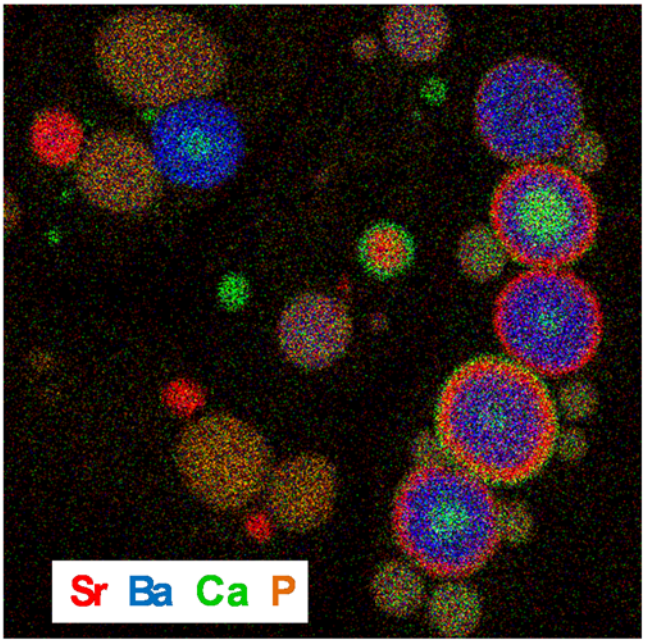
TOC/ABSTRACT ART



ABSTRACT

The uptakes of Ca, Sr and Ba by two cyanobacterial strains, *Cyanothece sp.* PCC7425 and *Gloeomargarita lithophora*, both forming intracellular carbonates, were investigated in laboratory cultures. In the culture medium BG-11 amended with 250 µM of Ca and 50 or 250 µM of Sr and Ba, *G. lithophora* accumulated first Ba, then Sr and finally Ca. Strontium and barium were completely accumulated by *G. lithophora* cells at rates between 0.02 and 0.10 fmol.h^{-1}.$cell^{-1}$ and down to extracellular concentrations below the detection limits of ICP-AES. Accumulation of Sr and Ba did not affect the growth rate of the strain. This sequential accumulation occurred mostly intracellularly within polyphosphate and carbonate granules and resulted in the formation of core-shell structures in carbonates. In contrast, *Cyanothece sp.* PCC7425 showed neither a preferential accumulation of heavier alkaline earth metals nor

core-shell structures in the carbonates. This indicated that fractionation between alkaline earth metals was not inherent to intracellularly calcifying cyanobacteria but was likely a genetically-based trait of *G. lithophora*. Overall, the capability of *G. lithophora* to sequester preferentially Sr and Ba at high rates may be of considerable interest for designing new remediation strategies and better understanding the geochemical cycles of these elements.

INTRODUCTION

Alkaline earth metals are lithophile elements with diverse abundances in the Earth. Strontium (Sr) has an average concentration of 316 ppm in the upper continental crust and 8.1 mg/L in oceans.[1,2] It is cycled by living organisms with diverse impacts on their biology. Sr is incorporated by plants, usually at the same rate as calcium (Ca), and can, at least in some cases, replace Ca to promote plant growth.[3,4] At a low concentration, Sr stimulates bone formation and can be useful against osteoporosis.[5,6] But at greater doses, Sr inhibits bone growth and can cause osteomalacia.[7] Strontium 89 and 90 are major polluting nuclear fission products in environments contaminated by nuclear weapons tests and nuclear reactors accidents such as the ones of Fukushima or Chernobyl.[8,9] Several (bio-)remediation strategies have therefore been developed to mitigate Sr dispersion and diverse (micro)organisms with unique bioremediation capabilities have been screened.[10-12] Barium (Ba), has also received particular attention regarding its toxicity, in particular in the context of sludge deposits formed during oil production, which can contain high Ba concentrations.[13,14] For example, Ba in its ionic form blocks Na-K pumps in cell membranes causing muscular paralysis in animals.[15,16]

Strontium and Ba have also been used as tracers for various (bio)geochemical processes. For example, Sr/Ca and Ba/Ca ratios have been suggested as good indicators for trophic level in

foodwebs in terrestrial environments.[17] Ba/Ca in otoliths has been used to infer where fish have lived.[18] The Ba/Ca ratio in foraminifers and other biomineralized carbonates has been used to trace past river discharge in the ocean.[19] Finally, in oceans, pelagic barite ($BaSO_4$), formed in the water column, records changes in ocean productivity.[20-22] Yet, the mechanisms of precipitation of pelagic barite and the microorganisms mediating this precipitation process are not known.[23] Recent studies exploring the possibility to use Ba stable isotopes for deciphering the global Ba cycle call for further efforts to identify the biological processes affecting this element.[21,22] Overall, there is a crucial need to better understand the biogeochemical cycle of Sr and Ba in order to strengthen the use of these proxies on one hand and design remediation strategies on the other hand.[24]

Several organisms able to trap Sr and/or Ba have been discovered, including some bacteria, micro-algae and plants.[11,25–35] Different mechanisms of sequestration may be involved. First, Sr^{2+} and Ba^{2+} may be adsorbed on the cell surface.[25] Some organisms such as the algae *Scenedesmus spinosus* and *Oedogonium* sp. Nak 1001, the cyanobacteria *Oscillatoria homogenea* and *Stigonema ocellatum* NIES-2131 and the aquatic plant *Egeria densa* We2 show a particularly high affinity for Sr, assigned to both sorption at the surface of the organisms (biosorption) and sequestration within the cells (bioaccumulation). (Fukuda, 2014; Dabbagh, 2007; Liu, 2014)[11,26,27] The argument for sorption has been based on more or less complex modeling of the metal sequestration data;[27] in simple cases, fixed amounts were fitted with a Langmuir isotherm.[26] However, some of these results should be considered with caution, since deciphering the amount of earth alkaline element uptake and adsorption by microorganisms is sometime difficult. For example, Sternberg et al.[36] showed that the apparent uptake of Ba by diatoms in laboratory cultures was actually due to sorption on Fe-oxyhydroxides formed in the cultures and not uptake by the cells.

In other cases, alkaline earth metals are coprecipitated in minerals formed by organisms or adsorbed on the surfaces of these minerals. For example, the micro-alga *Chlorella vulgaris* and the bacteria *Sporosarcina pasteurii* (ATC 11 859) and *Halomonas* sp. induce extracellular precipitation of strontianite ($SrCO_3$).[28,30,31] In all these cases, the efficiency of Sr and/or Ba sequestration depends on the chemical conditions prevailing in the extracellular solution, including pH or the saturation of the solution with concerned mineral phases. These phases tend to incorporate Ca in addition to Sr and Ba, depending on their concentrations in the extracellular solution and the relative solubilities of Ca-, Sr- and Ba-mineral phases.

In contrast, some organisms accumulate Sr or Ba mostly intracellularly. The efficiency of the accumulation may depend less on the chemical conditions in the extracellular solution but more on the physiological status of the cells.[37] For example, the yeast *Saccharomyces cerevisiae* traps Sr^{2+} within its vacuole, with Sr uptake involving similar molecular routes as those for Ca^{2+} transport.[38] Interestingly, it has been shown that some organisms accumulate different alkaline earth metals in proportions that differ from those in the external solution. For example, Sr/Ca and Ba/Ca ratios measured in the algae *Bryopsis maxima* were significantly higher than in the extracellular solution.[34] Last but not least, the desmid *Closterium moniliferum* forms barite ($BaSO_4$) and celestite ($SrSO_4$) within its vacuole by concentrating preferentially Sr and Ba over Ca.[35] This was explained by the lower solubilities of barite and celestite compared to calcium sulfate dihydrate. Radiolarians,[39] green algae of the genus *Chara*,[40] the ciliate genus *Loxodes*[41] and xenophyophores (a group of foraminifers) are additional examples of organisms accumulating Sr and/or Ba as sulfates.[42] This selectivity is of high interest since it may offer strategies to remediate more efficiently Sr and/or Ba from Ca-rich effluents. Moreover, some of these microorganisms have been suggested to drive the formation of pelagic barite in oceans, although their abundance may be too low.[23]

Interestingly, most of the organisms studied so far showing this selectivity upon alkaline earth metal uptake are eukaryotes. Therefore, the recently discovered cyanobacterium *Gloeomargarita lithophora* may provide an original model in this context.[43] Indeed, *G. lithophora* cells form intracellular Ca-, Sr- and Ba-carbonate granules in solutions containing a relatively high Ca concentration of 50 µM and low Ba and Sr concentrations of 0.001 and 0.013 µM, respectively. This preferential uptake of Sr and Ba over Ca by *G. lithophora* was suggested based on electron microscopy observations of a highly diverse microbial assemblage.[43] *G. lithophora* can accumulate a large amount of alkaline earth metal up to several fmoles per cell.[44] However, no detailed study has been performed yet on the uptake kinetics of Sr and Ba by isolated *G. lithophora* cells in a controlled culture medium.

The origin of this differential fractionation of alkaline earth metals remains enigmatic for *G. lithophora*. *In vitro* abiotic syntheses of carbonates similar to the granules observed in cyanobacteria showed no differential fractionation of Ca, Sr and Ba between the solution and the mineral phase.[45] This suggested that biological processes rather than physicochemical parameters, such as solubility differences between alkaline earth carbonates and/or different precipitation kinetics are responsible for this selectivity between alkaline earth metals in *G lithophora* cells. However, it is not known whether this selectivity is intrinsically related to the capability of *G. lithophora* to form intracellular carbonates. Interestingly, some other cyanobacterial species, phylogenetically distant from *G. lithophora* but forming intracellular carbonates,[46] were recently discovered but their capability to selectively sequester Sr and Ba over Ca has not been tested yet. If they showed such a capability, they may play a yet overlooked role in Ba and Sr geochemical cycles since they have been found in many locations worldwide and diverse continental environments.[47]

Here, we followed the time-evolution of the uptake of Ca, Sr and Ba by cells of *G. lithophora* and *Cyanothece sp*. PCC 7425 cultured in BG-11 amended with Sr and Ba. Both cyanobacterial strains form intracellular carbonate granules. We quantified and compared the uptakes of Ba, Sr and Ca by the two cyanobacterial strains and assessed their cellular distributions.

EXPERIMENTAL

Cyanobacterial strains and culture conditions

Two cyanobacterial strains were tested for Sr and Ba uptake: 1) *Gloeomargarita lithophora* C7 enriched and described by Couradeau et al.[43] and Benzerara et al.[46]; 2) the axenic strain *Cyanothece* sp. PCC 7425 was obtained from the Pasteur Collection of Cyanobacteria.[48]

Both strains were cultured in BG-11 medium at 30 °C under continuous light (5-10 µmol.m².s^{-1}).[48] BG-11 medium contained 17.65 mM of $NaNO_3$, 0.18 mM of K_2HPO_4, 0.3 mM of $MgSO_4$, 0.25 mM of $CaCl_2$, 0.03 mM of citric acid, 0.03 mM of ferric ammonium citrate, 0.003 mM EDTA, 0.38 mM of Na_2CO_3, and trace minerals.[48] Strontium and barium were added to BG-11 as chloride salts (Sigma Aldrich) at final concentrations of 50 or 250 µM. Sulfates may affect the solubility of Sr and Ba. However, this did not seem to play a significant role in their bioavailability here since: 1) measured dissolved Sr and Ba concentrations at t=0 (i.e., when cultures were inoculated) were approximately similar to added ones; 2) Sr and Ba were clearly incorporated by cyanobacteria as shown by microscopy analyses and 3) the changes with time of dissolved Sr and Ba concentrations were very different between strains suggesting that concentrations of Sr and Ba were not buffered by the precipitation of earth alkaline sulfates in the culture medium. Evaporation was compensated by daily addition of sterile milli-Q water. The optical density (OD) of the suspensions was measured at 730 nm. The precision on

OD measurements was 0.005. The relationships between OD and cell density were estimated as 9 x10^7 and 3 x10^7 cell.mL^{-1}.OD $unit^{-1}$ for *G. lithophora* and *Cyanothece* sp., respectively. The relation between OD and cell dry mass was 3.65 x10^{-4} g.OD $unit^{-1}$ for 1 mL of *G. lithophora* culture.

For bulk chemical analyses, 500 µL of culture were centrifuged at 5,000 g for 10 min. The pH of the supernatant was systematically measured. The precision on pH measurement was 0.01. Time variations of the pH in non-inoculated sterile controls were up to 0.1 pH units. Supernatants were then filtered at 0.22 µm and acidified by addition of 2 µL of HNO_3 (70 %) in order to measure dissolved Ca, Sr and Ba concentrations. For scanning transmission electron microscopy (STEM) observations, cell pellets were washed three times with milli-Q water before suspension in 500 µL of milli-Q water and deposition of 3 µL on carbon-coated 200-mesh copper grids.

ICP-AES measurements

Concentrations of dissolved Ca, Sr, Ba were measured using a Thermo Scientific™ iCAP™ 6200 ICP emission spectrometer equipped with a cetac ASX-520 autosampler. Ten milliliters of 2 % HNO_3 were added to 300 µL of acidified supernatants. Taking into account dilutions of the samples before ICP-AES measurements, detection limits for Ca, Sr and Ba concentrations were 0.37, 0.05 and 0.07 µM, respectively.

Uptake rates normalized by the number of cells were determined for each time step as followed: $$\frac{[X^{2+}]_{t-1}-[X^{2+}]_t}{average\ cell\ density\ between\ t_{-1}\ and\ t} \Big/ t - t_{-1}$$

Where X denotes one chemical element, t and t-1 are two consecutive sampling times.

Scanning transmission electron microscopy (STEM) and energy dispersive x-ray spectrometry (EDXS) analyses

STEM analyses were performed in the high angle annular dark field (HAADF) mode using a JEOL 2100F operating at 200 kV and equipped with a field emission gun and a JEOL EDXS detector. Semi-quantitative analyses of EDXS spectra were processed using the JEOL Analysis Station software following Li et al. procedure.[44] This was based on the use of K-factors. From this, the atomic percentage of selected element was assessed (here Ca, Mg, Sr and Ba in the carbonates; Ca, Mg, Sr, Ba, K and P in polyphosphates).

RESULTS AND DISCUSSION

Kinetics of Ca, Sr and Ba uptakes by *G. lithophora* and *Cyanothece sp*.

G. lithophora cells grew with comparable generation times (92 ± 8 h vs 108 ± 2 h) and up to similar OD values (~0.6) and extracellular pH (~9.3) in BG-11 amended with 50 μM Sr and Ba as in BG-11 without Sr and Ba (Figure S1 ; Table S1).

Extracellular concentrations of dissolved Ca, Sr and Ba measured immediately after their addition to BG-11 were 236 ± 8.8, 45 ± 0.8 and 42 ± 0.4 μM, respectively (Figure 1). The slight differences with expected concentrations (250 μM, 50 μM and 50 μM, respectively) may be due to dilution errors and/or some sulfate precipitation. The uptake of alkaline earth metals by *G. lithophora* cells occurred in three consecutive stages. During the first stage, within 175 h, the concentration of dissolved Ba decreased down to the detection limit (0.07 μM). During this stage, the concentration of Ca decreased by about 10 % only, while the Sr concentration remained constant. During the second stage, the dissolved Ba concentration remained below the detection limit, while the Sr concentration decreased down to the detection limit (0.05 μM) in 50 h. During this stage, the concentration of dissolved Ca remained constant. Finally, in a last stage, the Sr and Ba concentrations remained below detection limits, while

200 the concentration of dissolved Ca decreased down to 43 µM (this value was reached after
201 384 h of total incubation time). The durations of the different stages varied by 8-31 % between
202 replicates but they always followed the same order (Figure S2).
203 The uptake rates of alkaline earth metals ranged between 0.02 and 0.10 $fmol.h^{-1}.cell^{-1}$
204 (Figure S3). Taking replicates into account, average uptake rates were estimated to
205 0.058 ± 0.015 $fmol.h^{-1}.cell^{-1}$ for Ba, 0.049 ± 0.021 $fmol.h^{-1}.cell^{-1}$ for Sr and
206 0.026 ± 0.010 $fmol.h^{-1}.cell^{-1}$ of Ca. Uptake rates were therefore in the same order of
207 magnitude for the different alkaline earth metals, with a slight decrease over the duration of
208 the culture.
209 In *Cyanothece* sp. PCC 7425 cultures with Sr and Ba at 50 µM, the optical density increased up
210 to 1 in 350 h, while the pH increased and plateaued at around 9 after 250 hours (Figure S4).
211 The growth in cultures with Sr and Ba was slightly faster than in cultures without Sr and Ba
212 (~63 h vs 82 h; Figure S5).
213 The different stages of Ba, Sr and Ca uptake evidenced for *G. lithophora* were not observed
214 for *Cyanothece sp*. (Figure 2). In contrast, the concentration of dissolved Ca decreased first.
215 The concentrations of dissolved Sr and Ba only decreased in a later stage, when dissolved Ca
216 concentration had fallen down to about 50 µM, i.e. similar to initial concentrations of Sr and
217 Ba.
218 Overall, only *G. lithophora* showed preferential uptakes of Ba over Sr and Sr over Ca. This
219 selectivity for alkaline earth metals was therefore not related to intracellular carbonate
220 formation. Based on *in vitro* syntheses, Cam et al.[45] showed that the precipitation of
221 amorphous carbonates alone could not fractionate significantly Sr, Ba and Ca and suggested
222 that some other mechanisms must be operating in *G. lithophora*. The present study supports
223 this hypothesis and reveals that the molecular machinery allowing selective fractionation of

alkaline earth metals exists in *G. lithophora* but not *Cyanothece sp*. Interestingly, this process of alkaline earth metals sequestration by carbonates in solutions undersaturated with Sr and Ba carbonates was observed in Lake Leman in relation with the picoplankton.[49] Whether microorganisms with capabilities similar to those of *G. lithophora* occur in Lake Leman will have to be determined in the future.

Cellular distribution of Ca, Sr and Ba in *G. lithophora* and *Cyanothece* sp.

The evolution of the chemical composition of intracellular carbonates was assessed by STEM-HAADF. At 75 h, i.e., during Ba uptake, *G. lithophora* cells had divided once on average (Figure 1). The majority of the carbonate granules in the cells (55 % out of 31 observed granules) showed a bright outer shell around a dark core (Figure 3). Accordingly, EDXS analyses indicated that the core was rich in Ca and the brighter outer shell was rich in Ba. The thickness of the Ba-shell measured 80 ± 34 nm (11 analyzed particles). Considering the relative thicknesses of the shells, the diameters of the cores and the Ca/Ba ratios measured on the cores (Figure 3), EDXS measurements were consistent with pure Ca-carbonate cores covered by Ba-carbonate outer shells. Moreover, in the same cells, some carbonate granules (25 % over 31 counted granules) contained only Ba with no Ca-rich cores, while other granules (20 %) contained only Ca with no outer Ba-carbonate shell. In accordance with bulk chemical analyses of the solutions, mixed Ca- and Ba-containing carbonate granules were interpreted as resulting from the growth of Ba-carbonates during the first 75 hours around Ca-carbonate cores. Ca-carbonate cores formed in the pre-culture grown in Ba- and Sr-deprived BG-11. In the meantime, some new Ba-carbonate granules nucleated within the cells and some pre-existing Ca-carbonate granules did not grow further. Two concurrent nucleation pathways occur for barium carbonates: one forming particles *de novo* by homogeneous (in solution) or

heterogeneous (on organic template) nucleation and the other one by heterogeneous nucleation on the surface of preexisting calcium carbonates. The heterogeneous nucleation is followed by a two-step growth: very rapid 2-D growth on the underlying particle resulting in complete coverage, followed by slower growth perpendicular to the surface.

Cells contained a volume of 0.064 ± 0.043 $\mu m^3.cell^{-1}$ of both Ca- and Ba-carbonates on average. After 75 h of cell growth, there were 4.57×10^6 $cell.mL^{-1}$ in the cultures, accounting for a total uptake of 12.75×10^{-9} $mol.mL^{-1}$ of Ba, i.e., 2.8 $fmol.cell^{-1}$ of Ba. If all this Ba was trapped as carbonates only, this would represent a total volume of 0.151 μm^3 per cell based on a density of 4.3 $g.cm^{-3}$ for amorphous Ba-carbonate.[50] This is significantly more than the volume of carbonates observed by STEM, suggesting that only a fraction of Ba (~1/3) was contained in carbonate granules.

G. lithophora cells also contained phosphorus-rich granules (Figure 3), which were identified as polyphosphates (PolyP) by Li et al.[44] The preferential uptake of Ba over Sr and Ca was not restricted to carbonate granules only since in addition to Mg, Ca and K, PolyP granules contained Ba in cells sampled during the Ba uptake stage (Figure 3; Figure S6). Ba content ranged between 30 and 70 % (expressed as an atomic proportion over Mg+Ca+Sr+Ba) in PolyP. EDXS maps showed that Ba concentration was high in carbonate and PolyP granules (Figure 3) but below detection limits in the rest of the cells (Figure 3). Therefore, most of Ba was accumulated in PolyP and carbonate granules with possibly some minor amount complexed by proteins.

At 244 h, i.e., during the Sr uptake stage (Figure S2), Sr-carbonates were detected within the cells (Figure 3). Some particles showed a Ca-rich core and two outer superimposed shells, a Ba-rich intermediate shell and a Sr-rich external shell. Some carbonate granules containing mostly Sr, with minor amounts of Ca and Ba (Figure 3), were also observed. Polyphosphates

granules were homogeneous in composition and contained mostly Mg, K, Sr and Ba, sometimes with small, almost undetectable amounts of Ca. Their compositions were variable but most of them showed an increased proportion of Sr (15% on average; Figure S6).

Finally, at 310 h, during the Ca uptake stage, granules showed a Ca-rich core and three successive shells rich in Ba, Sr and Ca, respectively, from the center to the periphery (Figure 3). Other types of granules were observed but always with the same order for the shells, e.g., Ca-shells over Sr-cores; Ca-cores only; Sr-shells over Ba-shells. Despite some variability in chemical composition from cell to cell, polyphosphate granules contained higher concentrations of Ca than during the Sr uptake stage (27% vs. 6% during the Sr uptake stage; Figure S6).

Overall, we observed in *G. lithophora* cells 1) newly nucleated carbonate granules containing only one alkaline earth metal, named "simple", and 2) "composite" carbonate granules with outer shells containing Ba, Sr or Ca. "Simple" granules had a mean diameter of 215 ± 91 nm with a normal distribution (Figure S7), very similar to that observed for Ca-carbonate granules (217 ± 64 nm) in *G. lithophora* cultured in BG-11 with no Sr and Ba.[44] In contrast, "composite" granules showed larger diameters, i.e. 415 ± 115 nm, and measured up to 650 nm in diameter (Figure S7).

For a comparison, *Cyanothece sp.* cells were also observed by STEM-HAADF at 244 h, i.e. at a stage when they were mostly taking up Ca, and at 310 h, i.e. during the Ca, Ba and Sr uptake stage (Figure 4; Figure S8). In agreement with bulk chemical measurements, intracellular carbonates within these cells mostly contained Ca (82.2 ± 6.2 % atomic proportion of Ca over Mg+Ca+Sr+Ba) with several percent of Sr and Ba (on average 7.8 and 7.3 % at 310 h, of Sr and Ba respectively). However, in contrast to *G. lithophora, Cyanothece* sp. cells showed chemically homogeneous carbonate granules with no core-shell structure. Polyphosphates

296 also mostly contained Ca with only a few percent of Sr and Ba at 244h (3% of Sr and Ba) and a
297 slightly higher content in Ba and Sr at 310 h (11 and 5% respectively; Figure S8).
298
299 **Origin of selectivity for alkaline earth metals in *G. lithophora***
300 In this study, both *G. lithophora* and *Cyanothece sp.* strains incorporated alkaline earth metals
301 massively when cultured in BG-11 amended with Sr and Ba. However, only *Gloeomargarita*
302 *lithophora* achieved a sequential and preferential uptake of Ba over Sr and Sr over Ca. This
303 was detected by marked changes in the chemical composition of the extracellular solution as
304 well as in the chemical composition of the intracellular carbonate and polyphosphate
305 granules.
306 A preferential uptake of Sr and/or Ba over Ca has been observed previously in the desmid
307 green algae *Closterium moniliferum*.[35] The algae concentrate Ba and Sr in their vacuoles as Ba-
308 and Sr-sulfate crystals. Based on the large solubility differences of Ca-, Sr- and Ba-sulfates,
309 Krejci et al.[35] suggested that the preferential uptake of Sr and Ba over Ca was due to an
310 elemental fractionation induced by sulfate precipitation within the cells. In contrast, several
311 observations strongly argue against a fractionation of alkaline earth metals by mineral
312 precipitation in *G. lithophora* and favor the involvement of another biochemical process:
313 1) Cam et al.[45] showed that the solution/solid fractionations were very similar for Sr, Ba and
314 Ca for *in vitro* precipitation of amorphous carbonates; 2) no Sr-, Ba and/or Ca-sulfate phase
315 precursor to the carbonates, which could induce some distinct fractionation between Sr, Ba
316 and Ca, was observed within *G. lithophora*; 3) the composition of PolyP followed the same
317 succession of uptake stages as the carbonates; 4) *Cyanothece* sp. did not fractionate Sr, Ba
318 and Ca distinctly, although it also formed intracellular carbonates.

Unfortunately, no biochemical process explaining such a selectivity between alkaline earth elements is presently known. All known biomolecules have either a similar affinity for Ca and Sr and a lower affinity for Ba, or a high affinity for Ca and lower affinities for Sr and Ba.[52-56] Future work based for example on genetics and comparative genomics between strains incorporating alkaline earth metals selectively and others not able of this selective uptake may provide some clues about the biochemical origin of this selectivity.

Implications for bioremediation and the geochemical cycles of Ba and Sr

The masses of intracellular Ba, Ca and Sr in *G. lithophora* cells were estimated as a function of time and normalized to the total cell dry mass (Figure S9). For Ba, there was first an increase when uptake was higher than "dilution" by cell division, then a plateau and finally a decrease when no Ba was left in the extracellular solution but cell division was continuing. Baryum mass reached a maximum of 16 % of the total cell dry mass. Similar evolutions were observed for Sr and Ca with a maximum of 7 % for the mass of Sr over the total cell weight.

Cultures were also conducted in BG-11 containing higher initial Sr and Ba concentrations (245 µM). The pH and the OD increased similarly to what was observed in cultures with 50 µM of Sr and Ba with a slightly longer generation time (Figure S10). Similarly to what was observed in the presence of 50 µM of Sr and Ba, three uptake stages were detected (Figure S11). The concentration of dissolved Ba decreased first, and this step was followed by decreases of the concentrations of dissolved Sr and finally of dissolved Ca. In 650 h, cells took 245 µM of Ba, 245 µM of Sr and 245 µM of Ca. Under these conditions barium and strontium masses reached up to a maximum of 22 % and 8 % of total cell dry mass, respectively (Figure 5).

Altogether, these results show that *G. lithophora* can strongly accumulate Ba and Sr while staying viable and showing high selective affinity for these alkaline earth metals over Ca.

Moreover, this selective uptake seems to operate even at low Sr/Ca or Ba/Ca ratios in the extracellular solution since Ba and Sr are taken up preferentially down to their detection limits (0.07 and 0.05 µM, respectively, corresponding to a molar ratio between 2.0 and 2.9 $x10^{-4}$ with respect to Ca).

Several micro-organisms have been studied for their capability to store Sr or Ba with the ultimate scope of designing bioremediation strategies (Table 1). In the present study, only minimum uptake capabilities were determined for *G. lithophora* since our cultures exhausted the amount of Sr and Ba provided, without any evidence that they had reached their ultimate storage capacity. *G. lithophora* showed unique uptake capabilities per cell. For example, a cell of the desmid *Closterium moniliferum* had greater Sr uptake capability than a cell of *G. lithophora* (140 vs. 35 $fmol.cell^{-1}$) but when normalized to the cell mass, *G. lithophora* showed a much higher uptake (75 mg/g vs 2 mg/g).

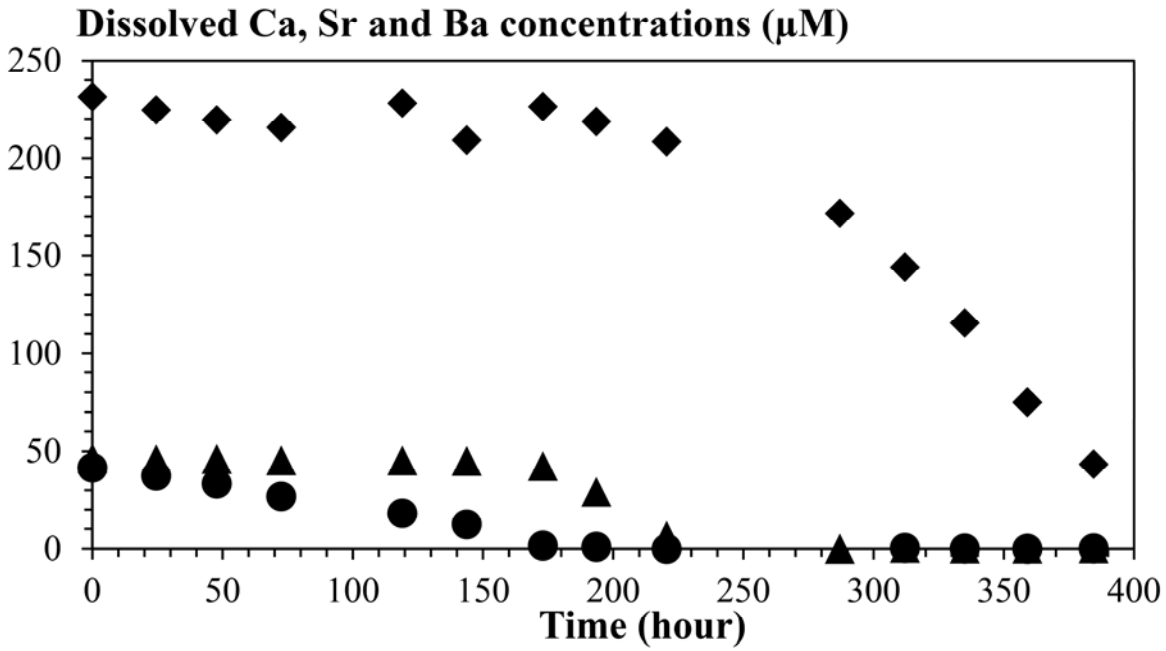
Fukuda et al. (2014)[14] screened 188 different strains of macro- and micro-organisms from aquatic environments in search for good candidates to bioremediate radionuclide contaminations, including isotopes of strontium. Cyanobacteria showed particularly high uptake capabilities (Fukuda, 2014).[14] They suggested that this may be due to some particular properties of their cell walls without further proof. Cyanobacteria forming intracellular carbonates, including but not restricted to *G. lithophora*, meet the different criteria mentioned by Fukuda et al.[11] as good candidates for bioremediation. Moreover, they have been isolated from different places around the world and can grow at varied temperatures.[46] Some are thermophilic, some mesophilic; some live in high altitude lakes, while others live in soils. Among intracellularly calcifying cyanobacteria, *Gloeomargarita lithophora* is particularly interesting: it preferentially sequesters Ba and Sr over Ca, which offers a unique solution to the general problem of remediating these pollutants in aqueous environments with naturally

high Ca concentrations. However, if cells are used directly in bioremediation strategies, several parameters will have to be determined in the future to fully assess the potential of *G. lithophora* such as its maximum uptake capability for Sr and its resistance to radioactivity from ^{89}Sr or ^{90}Sr. Alternatively, it will be important to understand the precise molecular mechanisms involved in the preferential uptake of Sr and Ba vs Ca which may inspire the design of new cell-free remediation strategies, complementing those already existing and using non-biological materials, such as synthetic chelators or ion exchangers.[10]

In parallel, the present study has implications for the geochemical cycles of Sr and Ba. First, it shows that some cyanobacteria concentrate significantly Sr and Ba in connection with the formation of carbonates and not sulfates as usually considered. With the aim of explaining the formation of pelagic barite in oceans, Gonzalez-Munoz et al.[57] showed that several heterotrophic marine bacterial strains could induce the precipitation of barite. However, they needed to culture them at relatively high Ba concentrations (2 mM). The existence of primary producers accumulating Ba significantly, such as *G. lithophora* may provide such a rich source of Ba. Moreover, Peek and Clementz[17] compiled numerous Sr/Ca and Ba/Ca measurements on biological samples. They suggested that most of the variations of these ratios were accounted by (abiotic) variations in geochemical sources of alkaline earth metals in terrestrial environments, while high accumulation of Sr and Ba by microorganisms tended to control Sr/Ca and Ba/Ca ratios at the base of marine foodwebs. Here, we show that microorganisms such as *G. lithophora* may play a similar role in continental environments. As a conclusion, the biochemical process evidenced in *G. lithophora* in the present study might have an important impact on the geochemical cycles of Sr and Ba if it is widespread. This will need to be assessed by future studies.

391 FIGURES

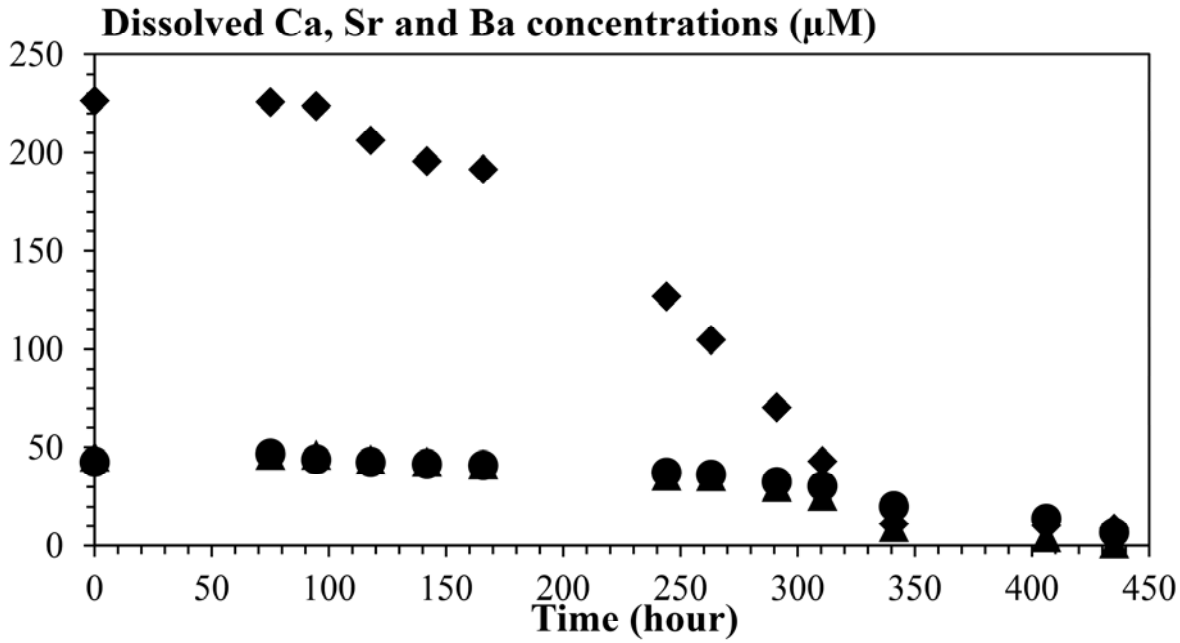
392



393

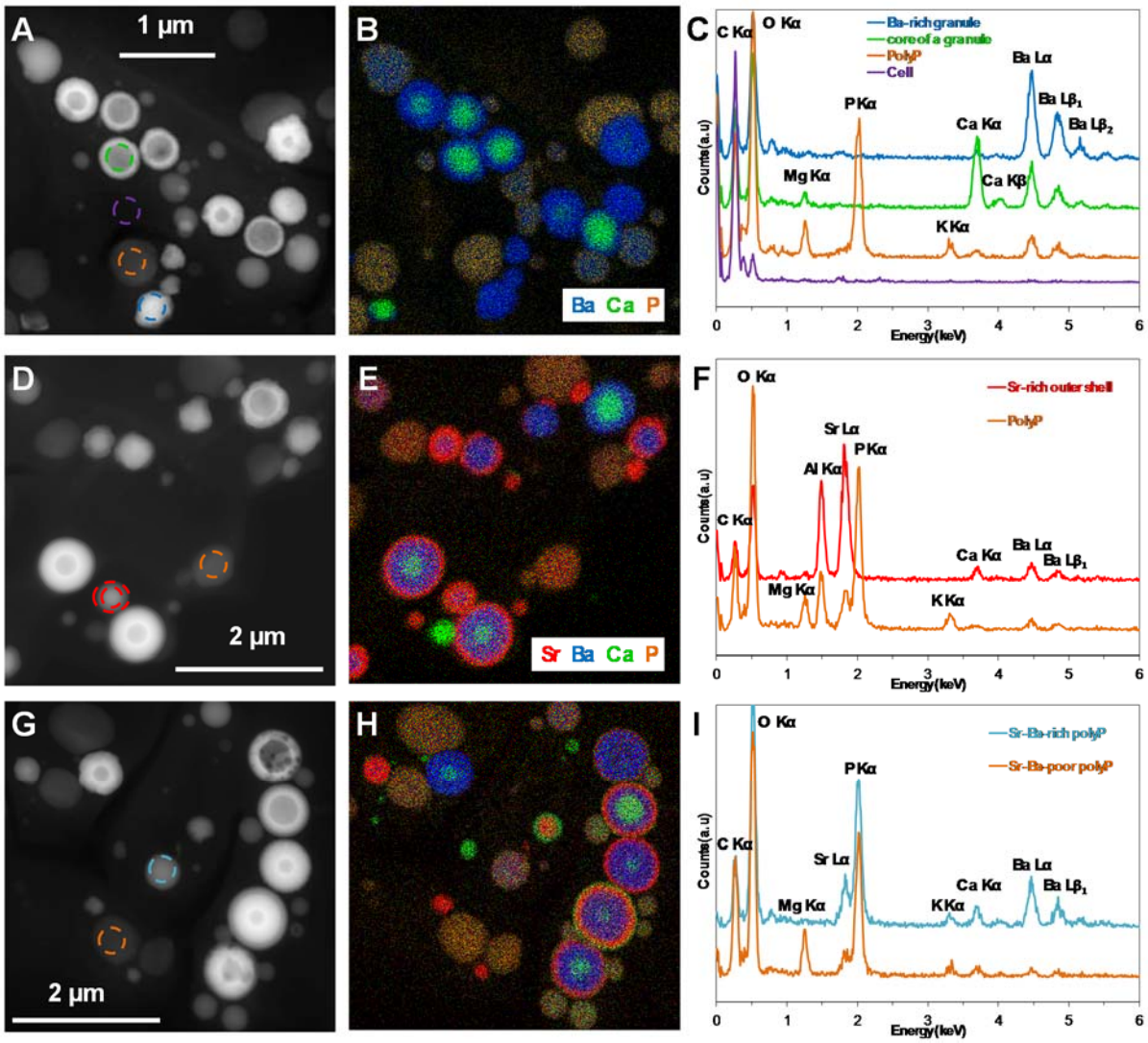
394 **Figure 1.** Time evolution of the concentrations of dissolved Ca (diamonds), Sr (triangles) and
395 Ba (circles) concentrations in a culture of *Gloeomargarita lithophora* inoculated in
396 BG-11.Error bars are smaller than symbols.

397



398

399 **Figure 2.** Time evolution of the concentrations of dissolved Ca (diamonds), Sr (triangles) and
400 Ba (circles) concentrations in a culture of *Cyanothece* sp. inoculated in BG-11. Analytical
401 standard deviations are smaller than the symbol sizes.

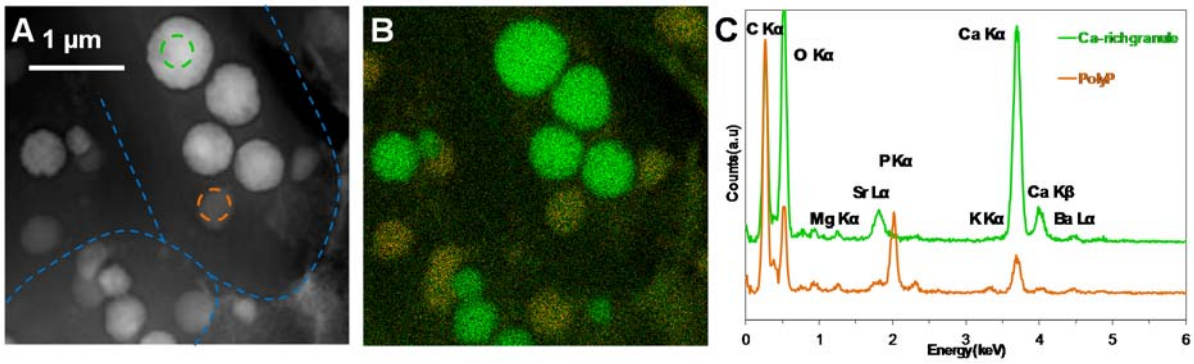


402

403 **Figure 3.** STEM and EDXS analyses of *Gloeomargarita lithophora* cells collected after 75 h
404 of culture during the Ba uptake stage (A, B and C), during the Sr uptake stage (D, E and F) and
405 during theca uptake stage (G, H and I). (A, D and G) STEM-HAADF Image of cells showing
406 layered carbonate granules. Colored circles show the different areas analyzed by EDXS. (B, E
407 and H) Corresponding EDXS map of calcium (green), strontium (red), barium (blue) and
408 phosphorus (orange). (C) EDXS spectra of the Ba-rich granule; a core of a carbonate granule;
409 a PolyP granule and a cellular area comprising no particle shown respectively in A by the blue;
410 green; orange and purple circles. (F) EDXS spectra of a Sr-rich outer shell of a granule and a
411 PolyP particle shown respectively by the red and the orange circles in D. Al comes from the

412 sample holder.(I) EDX spectra of the PolyP granules shown by the orange and blue circles in
413 G.

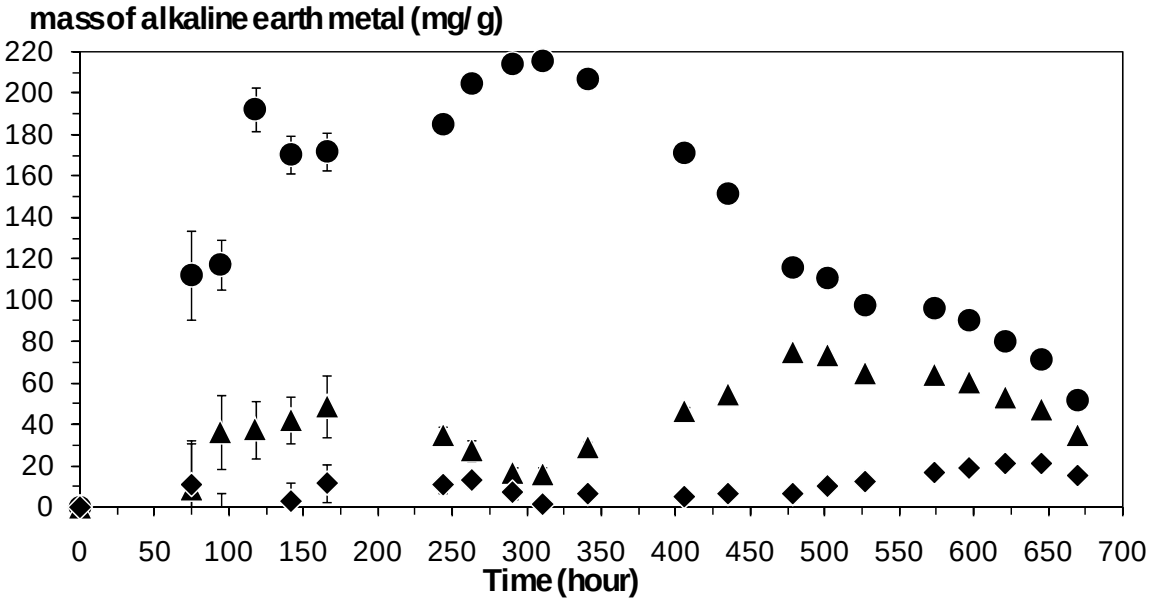
414



415

416 **Figure 4.** STEM analyses of two *Cyanothece* sp. cells cultured for 310 h in BG-11 medium
417 with 50 µM of strontium and 50 µM of barium. (A) STEM image in the HAADF mode. Circles
418 indicate the carbonate and PolyP granules on which EDXS spectra shown in C were measured.
419 (B) Corresponding EDXS map of calcium (green) and phosphorus (orange). (C) EDX spectra
420 of a carbonate granule and of a PolyP granule, respectively shown by the green and the orange
421 circles in A.

422



423

Figure 5. Time evolution of the mass of bioaccumulated Ba (circles), Sr (triangles) and Ca (diamonds) per total dry mass of the cells (mg/g) for a culture of *Gloeomargarita lithophora* in BG-11 medium with 245 µM of strontium and 245 µM of barium. When not visible, analytical errors bars are smaller than the size of the symbols.

TABLES

Type of organism / material	Name	Maximum uptake rate	Maximum uptake		Maximum remediation		Associated mechanism	References
		(fmol/h/cell)	(mg/g of dry matter)	(fmol/cell)	(%)	(µM)		
Strontium								
Non organic material	Layered metal sulfides				99.4	756		13
Aquatic plant	*E. densa* We2		0.0009		33.9		Adsorption	14
Algae	*B. maxima*		15.9				Intracellular accumulation	31
Algae	*S. spinosus*		2.5		76	787	Adsorption	23
Yeast	*S. cerevisiae*	1.2	20	0.315	85		Intracellular accumulation and adsorption	30,36
Desmid	*C. moniliferum*	1.7	2*	140			Intracellular sulfate	32,59
Micro-algae	*O.* sp. Nak 1001		0.0029		36.3		Adsorption	14
Bacterium	*H.* sp. SR4				80		Extracellular calcite	26
Bacterium	*B. pasteurii ATCC* 11859				36	40	Extracellular calcite	25
Bacterium	*S. pasteurii* ATCC 11859				59		Extracellular calcite	27
Cyanobacterium	*S. ocellatum* NIES 2131		0.0219		41.3		Adsorption	14
Cyanobacterium	*N. carneum*		0.00225		97.8		Adsorption	21
Cyanobacterium	*G. lithophora*	0.079	75	35	100	250	Intracellular amorphous carbonates	
Barium								
Algae	*B. maxima*		3.79				Intracellular accumulation	31
Desmid	*C. moniliferum*		46*	2112			Intracellular sulfate	60
Cyanobacterium	*G. lithophora*		220	64	100	250	Intracellular amorphous carbonates	

*calculated using a cell volume of 125,600 µm^3 and Kreger and Boeré (1969).[58]

Table 1. Remediation capabilities of selected materials and organisms.

ASSOCIATED CONTENT

Supporting Information

Data on culture replicates, composition of polyphosphate inclusions, size distributions of carbonate inclusions, data on cultures of *G. lithophora* with 250 μM of Sr and Ba are shown as supporting information (11 figures and 1 Table). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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