



HAL
open science

Sequestration of Radionuclides Radium-226 and Strontium-90 by Cyanobacteria Forming Intracellular Calcium Carbonates

Neha Mehta, Karim Benzerara, Benjamin Kocar, Virginie Chapon

► **To cite this version:**

Neha Mehta, Karim Benzerara, Benjamin Kocar, Virginie Chapon. Sequestration of Radionuclides Radium-226 and Strontium-90 by Cyanobacteria Forming Intracellular Calcium Carbonates. *Environmental Science and Technology*, 2019, 53 (21), pp.12639-12647. 10.1021/acs.est.9b03982 . cea-02374665

HAL Id: cea-02374665

<https://cea.hal.science/cea-02374665>

Submitted on 11 Dec 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Sequestration of radionuclides Radium-226 and Strontium-90 by 2 cyanobacteria forming intracellular calcium carbonates

3 Neha Mehta¹, Karim Benzerara^{2*}, Benjamin D. Kocar^{1,3*}, Virginie Chapon⁴

4 ^{1,3} Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge,
5 Massachusetts 02139, United States

6 ² Sorbonne Université, Muséum National d'Histoire Naturelle, UMR CNRS 7590, Institut de Minéralogie, de
7 Physique des Matériaux et de Cosmochimie, IMPMC, 75005 Paris, France

8 ³ Exponent, Inc. 1055 E. Colorado Blvd, Suite 500. Pasadena, California 91106, United States.

9 ⁴ CEA, CNRS, Aix-Marseille Université, UMR 7265 Biosciences and Biotechnologies Institute of Aix-
10 Marseille, 13108 Saint-Paul-lez-Durance, France.

11 *Corresponding Authors

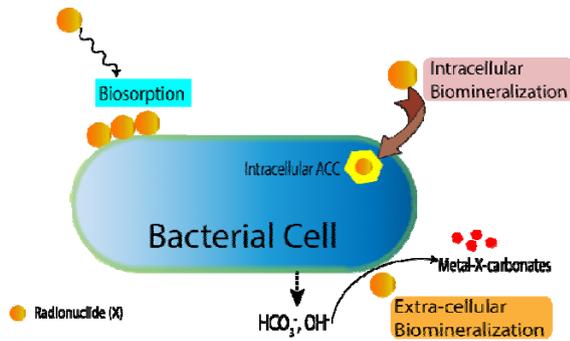
12 Email: bkocar@exponent.com; karim.benzerara@sorbonne-universite.fr

13

14 **ABSTRACT:** ²²⁶Ra is a naturally occurring radionuclide with a half-life of 1600 y. In
15 contrast, ⁹⁰Sr is a radionuclide of sole anthropogenic origin, produced by nuclear fission
16 reactions and has a half-life of 29 y; each of these radionuclides poses potential threats to
17 human and ecosystem health. Here, the cyanobacterium *G. lithophora*, capable of forming
18 intracellular amorphous calcium carbonate inclusions, was investigated for its ability to
19 uptake ²²⁶Ra and ⁹⁰Sr. In BG-11 medium, *G. lithophora* accumulated 3.9 μg g⁻¹ ²²⁶Ra within
20 144 h and 47.9 ng g⁻¹ ⁹⁰Sr within 1 h, corresponding to ~99% removal of trace radionuclides.
21 The presence of high concentration Ca²⁺ in the background media solution did not inhibit
22 ⁹⁰Sr and ²²⁶Ra uptake by *G. lithophora*. In contrast, dead biomass of *G. lithophora*
23 accumulated 0.8 μg g⁻¹ ²²⁶Ra and 8.87 ng g⁻¹ ⁹⁰Sr. Moreover, *Synechocystis*, a non-
24 biomineralizing cyanobacteria removed only 14% and 25% of ²²⁶Ra and ⁹⁰Sr, respectively.
25 This suggested that sequestration of ⁹⁰Sr and ²²⁶Ra was not intrinsic to all cyanobacteria but
26 was likely a specific biological trait of *G. lithophora* related to the formation of intracellular
27 amorphous Ca-carbonates. The unique ability of *G. lithophora* to uptake ⁹⁰Sr and ²²⁶Ra at

28 high rates makes it an attractive candidate for further studies involving bioremediation of
29 these radionuclides.

30



31

32 **TOC image**

33

34

35 **1. Introduction**

36 Cyanobacteria are a phylogenetically and ecologically diverse group of
37 photosynthetic bacteria, playing a vital role in the global cycling of numerous elements such
38 as carbon (C), calcium (Ca) and phosphorus (P)¹. In particular, their impact on the global
39 carbon cycle is of significant interest as they sequester atmospheric CO₂ into organic carbon
40 and biogenic calcium carbonates (CaCO₃) through calcification². In cyanobacteria, this
41 process has long been considered as extracellular and non-biologically controlled. However,
42 this paradigm was challenged by the recent discovery of several species of cyanobacteria
43 forming intracellular amorphous calcium carbonate (ACC) inclusions in very diverse
44 environments.³ Couradeau *et al.* first described this intracellular calcification in the
45 cyanobacterium *Gloeomargarita lithophora*, isolated from an alkaline freshwater lake
46 Alchichica microbialite, Mexico⁴. These ACC inclusions measured several hundreds of
47 nanometers in diameter, were poorly crystalline and were composed of CO₃ and Ca-Mg-Sr-

48 Ba, with Ba/Ca and Sr/Ca atomic ratios in inclusions higher by factors of 1370 and 90
49 respectively relative to the solutions in which cells grew^{3,4}. Recently, it was confirmed that
50 cyanobacteria capable of forming intracellular ACC contained a much higher content of
51 alkaline earth elements (AEE) than all other cyanobacteria⁵. In addition to intracellular ACC
52 inclusions, *G. lithophora* also forms intracellular polyphosphate inclusions (PolyP),
53 polymers of orthophosphate which may serve as reserves of P and/or energy for cells⁶.
54 Using stable isotopes, Cam *et al.* showed that *G. lithophora* preferentially accumulated Ba
55 over Sr and finally Ca within intracellular PolyP and ACC. Currently, the origin of such a
56 surprising selectivity remains unclear. The proven ability of *G. lithophora* to selectively
57 sequester Sr over Ca within intracellular inclusions provides an intriguing microbial
58 framework to examine if this ability extends to radioactive AEE such as radium (²²⁶Ra) and
59 radio-strontium (⁹⁰Sr). In the environment, ⁹⁰Sr and ²²⁶Ra are present at trace concentrations,
60 along with excess dissolved Ca. Whether the observed selective accumulation of AEE is
61 extended to ⁹⁰Sr and ²²⁶Ra at such low concentrations and in the presence of high
62 concentrations of Ca remains to be explored.

63 Radium-226 is a naturally occurring radioactive alkaline earth element (AEE) with a
64 half-life of 1600 y. It is one of the dominant soluble radionuclides present in groundwater⁷.
65 In the United States, ²²⁶Ra activity in drinking water is regulated at 0.2 Bq L⁻¹⁸. Potential
66 exposure to Ra could originate from the disposal and management of large volumes of Ra-
67 bearing waste streams associated with uranium mining, phosphate mining and milling
68 operations, coal mining and power plants, and conventional and unconventional oil and gas
69 extraction⁹. The Ra content of these waste streams can range from 100-1200 Bq L⁻¹, which
70 far exceeds the regulatory limit of 2.2 Bq L⁻¹ Ra in effluent waste stream¹⁰. During disposal

71 and storage of relatively large volumes of these streams, leaching of Ra (and other toxic
72 elements) could result in contamination of terrestrial and subsurface aquatic
73 environments^{11,12}.

74 In contrast, radio-Strontium (⁹⁰Sr) is a radionuclide of sole anthropogenic origin
75 produced by nuclear fission reactions. With its long half-life of 29 years, ⁹⁰Sr persists
76 sufficiently long in the environment to warrant health concerns. The main sources of ⁹⁰Sr in
77 the environment are the atmospheric tests of nuclear weapons conducted from 1945 to
78 1980's and nuclear accidents, such as Fukushima and Chernobyl^{13,14}. Measured ⁹⁰Sr specific
79 activity in the surface waters adjacent to the Fukushima Daiichi Nuclear Power Plant varied
80 between 0.2 - 400 kBq m⁻³, which were about 4 orders of magnitude greater than the pre-
81 accident level (1 Bq m⁻³)¹⁵. Strontium is very soluble, highly mobile and is bioavailable in the
82 environment. Its soluble form, Sr²⁺ can be transferred from contaminated soils and water into
83 living organisms through the food chain. A chemical analog of Ca²⁺, ⁹⁰Sr²⁺ concentrates in
84 bone tissues in the human body, increasing the probability of bone cancer after
85 accumulation and prolonged exposure¹⁶.

86 Several physiochemical strategies have been developed for decontamination of ²²⁶Ra
87 and ⁹⁰Sr from effluent waste streams. However, in many cases their application is limited
88 due to economic constraints¹⁷. In addition, the presence of high concentrations of Ca and
89 other co-contaminants often present within waste streams limits the selective removal of
90 ²²⁶Ra and ⁹⁰Sr using the traditional schemes^{18,19}. On the other hand, bioremediation
91 strategies are gaining significant interest owing to their relative cost-effectiveness and eco-
92 friendliness²⁰⁻²². Various types of organisms including fungi, algae, cyanobacteria, and
93 phytoplankton have been reported to retain ²²⁶Ra and ⁹⁰Sr^{9,23}. Different pathways of

94 sequestration can be involved. ^{226}Ra and ^{90}Sr can adhere to the surface of living and dead
95 cells. Ligands (carboxyl, amine, hydroxyl, phosphate, and sulfhydryl groups) present in the
96 cell membrane of both Gram-positive and Gram-negative bacteria can bind positively
97 charged metals through adsorption²⁴. Some examples of such microorganisms adsorbing
98 ^{226}Ra include dead microbial biomass of *Pseudomonas aeruginosa*, *Nostoc carneum*, *Nostoc*
99 *insulare*, *Oscillatoria geminana*, *Spirulina laxissima*, *Pseudomonas fluorescens*,
100 *Streptomyces nives*, and mixed bacterial cultures from activated wastewater sludges^{23,25}.
101 The dead biomass of bacterial strains isolated from areas with a high level of natural
102 radiation, such as *Citrobacter freundii*, *Chromobacterium*, *Chryseobacterium*, and
103 *Corynebacterium* also have been shown to adsorb ^{226}Ra ²⁶. In the case of ^{90}Sr , studies have
104 often been performed using stable Sr isotopes to examine the ^{90}Sr remediation potential of
105 an organism. Some organisms such as the algae *Scenedesmus spinosus* and *Oedogonium* sp.
106 *Nak 1001*, the cyanobacteria *Oscillatoria homogenea* and *Stigonema ocellatum* NIES-2131,
107 and the aquatic plant *Egeria densa* We2 have been reported to show high stable-Sr
108 adsorption^{27,28}. In addition to adsorption, many types of microorganisms may accumulate
109 ^{226}Ra and ^{90}Sr within extracellularly or intracellularly precipitated biogenic minerals. The
110 formation of biominerals occurs in response to localized changes in cellular or extracellular
111 microenvironments. To our knowledge, there is no known micro-organism which has been
112 reported to biomineralize ^{226}Ra . Several organisms have been screened for their ability to
113 (co)-precipitate Sr. For example, the microalga *Chlorella vulgaris*, soil bacterium
114 *Sporosarcina pasteurii* and the bacterium *Halomonas* sp. induce co-precipitation of
115 strontianite (SrCO_3) within extracellularly precipitated CaCO_3 mineral phase; the desmid
116 *Closterium moniliferum* forms celestite (SrSO_4) within its vacuole by concentrating

117 preferentially Sr and Ba over Ca²⁹⁻³². More recently, Cam *et al.* showed stable-Sr is
118 selectively accumulated within intracellular ACC and PolyP inclusions in the
119 cyanobacterium *G. lithophora*³³. Owing to selectivity for Sr compared to Ca, *G. lithophora*
120 forms carbonate with higher Sr content in comparison to other bacteria forming extracellular
121 carbonates. This means that the mass of the decontamination by products will therefore be
122 lower which may offer a cost advantage. Some of the non-photosynthetic bacteria which are
123 known to precipitate CaCO₃ efficiently, exhibit lower Sr removal efficiency in comparison
124 to *G. lithophora*. For example, *S. pasteurii* removes upto 59% of Sr from the solution
125 whereas *G. lithophora* removes 100% of Sr from the solution^{32,33}.

126 Despite screening of several organisms for removal of ⁹⁰Sr and ²²⁶Ra, these studies
127 have not demonstrated their potential for use in bioremediation. Indeed, research on ⁹⁰Sr
128 bioremediation has mostly utilized stable-Sr isotope as an analog of ⁹⁰Sr and at relatively
129 high concentrations. Owing to the radiotoxicity of ⁹⁰Sr, it is unclear whether the results
130 demonstrated using stable Sr isotopes would be replicated to the same extent in presence of
131 ⁹⁰Sr. Further, ²²⁶Ra and ⁹⁰Sr removed from solution through adsorption to biomass is prone
132 to rapid desorption upon changes in aqueous chemistry. Moreover, biomass is not selective
133 and other divalent cations (e.g. Ca, Ba), which are often present at relatively high
134 concentrations in ²²⁶Ra and/or ⁹⁰Sr contaminated aqueous environments, may outcompete
135 ²²⁶Ra and/or ⁹⁰Sr for binding sites, resulting in low removal efficiency^{22,24}. Thus,
136 effectiveness of ²²⁶Ra and ⁹⁰Sr bioremediation strategies will be dictated by (micro)-
137 organisms capability to selectively remove the radionuclide at trace concentration in the
138 presence of competing elements while also tolerating ionizing radiation.

139 Here, the role of the cyanobacterium *G. lithophora* forming intracellular biominerals
140 on sequestration of ^{226}Ra and ^{90}Sr at trace concentrations is evaluated. We selected *G.*
141 *lithophora* to evaluate as a potential bioremediation tool for removing ^{90}Sr and ^{226}Ra from
142 solution for several reasons. Because of the photoautotrophic lifestyle of cyanobacteria, *G.*
143 *lithophora* may exhibit tolerance to substantial levels of ionizing radiation³⁴. Moreover,
144 unlike other (micro)organisms, *G. lithophora* has been shown to preferentially sequester Sr
145 over Ca within intracellular ACC and PolyP inclusions, despite their chemical similarity.
146 These characteristics make *G. lithophora* a promising candidate for bioremediation of ^{226}Ra
147 and ^{90}Sr . Proving the ability of *G. lithophora* to sequester ^{226}Ra and ^{90}Sr through a novel
148 (co)-precipitation pathway will illustrate its promise for use in bioremediation efforts; also,
149 owing to its widespread ecological distribution³⁵, its cultivability and the availability of its
150 genome sequence³⁶, *G. lithophora* serves as an ideal model organism for examining
151 underlying biochemical processes responsible for bioaccumulation of radioactive AEE in
152 microbes. Accordingly, the goals of the current study are 1) to investigate and quantify ^{226}Ra
153 and ^{90}Sr uptake by *G. lithophora* using laboratory batch incubations, and 2) to identify
154 retention mechanism of ^{226}Ra and ^{90}Sr by *G. lithophora*.

155 2. EXPERIMENTAL SECTION:

156 ***Incubations and Culture Conditions***

157 The cyanobacterial strain *G. lithophora* was obtained from Institut de Minéralogie, de
158 Physique des Matériaux et de Cosmochimie, IMPMC, Paris, France and cultured in the BG-
159 11 medium at 30° C under continuous light ($5\text{-}10\ \mu\text{mol m}^2\ \text{s}^{-1}$) as described by Moreira *et al.*
160 ³⁶. The BG-11 medium contained $17.6 \times 10^{-3}\ \text{M NaNO}_3$, $0.23 \times 10^{-3}\ \text{M K}_2\text{HPO}_4$, $0.3 \times 10^{-3}\ \text{M}$
161 MgSO_4 , $2.5 \times 10^{-4}\ \text{M CaCl}_2$, $3.1 \times 10^{-5}\ \text{M}$ citric acid, $2 \times 10^{-5}\ \text{M}$ ferric ammonium citrate, 3×10^{-6}
162 M ethylenediaminetetraacetic acid (EDTA), $5 \times 10^{-4}\ \text{M NaHCO}_3$, and trace metals³⁷. The

163 optical density (OD) of the suspensions was measured at 730 nm; based on the previous
164 work by Cam *et al.*³³, the relationships between OD, cell density and cell dry mass of *G.*
165 *lithophora*, were estimated as 9×10^7 cells. mL⁻¹ OD unit⁻¹ and 3.65×10^{-4} g of dry mass.
166 OD unit⁻¹. mL⁻¹.

167 ***²²⁶Ra uptake by G. lithophora***

168 ²²⁶Ra uptake was measured during the growth stage of *G. lithophora* cultures, which
169 were started at low cell density (OD_{t=0}=0.039± 0.01). Pre-cultures used for inoculation were
170 centrifuged at 5000 g for 15 min and the cell pellets were re-suspended in BG-11 amended
171 with ²²⁶Ra and total Ca as chloride salts at concentrations of 7.5×10^{-10} M (*i.e.* 6153 Bq L⁻¹
172 ²²⁶Ra) and 6.32×10^{-4} M, respectively and buffered at pH 8 using HEPES buffer. Triplicate
173 incubations were performed. Three types of controls were included in the experimental
174 design: 1) an abiotic control consisting of BG-11 medium amended with 7.5×10^{-10} M of
175 ²²⁶Ra and 6.32×10^{-4} M of Ca²⁺. 2) Inactivated cells control consisted of *G. lithophora* cells
176 cultured in BG-11, killed by gamma irradiation (⁶⁰Co source, 55 h) and re-suspended at an
177 OD of 1 in fresh BG-11 medium with 5.2×10^{-10} M (*i.e.* 5600 Bq L⁻¹) of ²²⁶Ra and 2.5×10^{-4}
178 M of Ca²⁺. This control was used to quantify ²²⁶Ra uptake by dead *G. lithophora* biomass
179 and adsorption of ²²⁶Ra to walls of the culture vessel. 3) Living cells of *Synechocystis* sp.
180 PCC 6803 (obtained from Pasteur Collection of Cyanobacteria) cultured in BG-11 and
181 amended with 5.2×10^{-10} M (*i.e.* 5600 Bq L⁻¹) of ²²⁶Ra and 2.5×10^{-4} M of Ca²⁺. This
182 species has been shown to be unable to biomineralize intracellular ACC³. The OD of
183 *Synechocystis* sp. PCC 6803 cultures at the start of experiment was equal to 0.02 ± 0.002 .

184 Dissolved Ca²⁺ concentration and residual ²²⁶Ra activity in solution were measured in
185 all solutions as a function of time. For dissolved Ca²⁺ measurements, 100 µl of the culture

186 were mixed with 2 ml of standard BG-11 medium and were filtered on 0.22 μm PES syringe
187 filters. Filtrates were acidified with 3 mL of 3 % HNO_3 . Dissolved Ca^{2+} concentrations were
188 measured using an Agilent Inductively Coupled Optical Emission Spectrometer (ICP-OES).
189 Concentration of Ca^{2+} was also measured in the reagent blank (2 mL BG-11 + 3 mL 3 %
190 HNO_3) to eliminate background signal.

191 For measurement of residual ^{226}Ra in solution, 500 μl of the culture were mixed with
192 2 ml of standard BG-11 medium and were filtered on 0.22 μm PES syringe filters. Filtrates
193 were acidified with 3% HNO_3 and counted using a Canberra High Purity Germanium
194 Detector (HPGe) Gamma Spectrometer. Energy and efficiency calibration of gamma
195 spectroscopy was performed using a mixed multi-nuclide Eckert & Ziegler™ aqueous
196 gamma standard. ^{226}Ra was directly measured from its gamma peak at 186.5 keV.

197 ***⁹⁰Sr uptake by G. lithophora***

198 ^{90}Sr uptake by *G. lithophora* was studied following a different strategy. In this case,
199 cell suspensions of *G. lithophora* at high cell density were used. The high cell density of the
200 culture is expected to provide a faster uptake of ^{90}Sr . Cells previously grown in standard
201 BG-11 to an OD of 1 were centrifuged at 8500 g for 10 min and the cell pellets were re-
202 suspended in standard BG-11 at an OD of 0.5 (*i.e.* 0.1825 g L^{-1}). The suspensions were then
203 amended with 8.8×10^{-11} M of ^{90}Sr (*i.e.* 4×10^4 Bq L^{-1}). Three types of controls were
204 included in the experiment: 1) an abiotic control consisting of BG-11 medium amended with
205 8.8×10^{-11} M of ^{90}Sr . 2) Inactivated cells control consisting in *G. lithophora* cells grown in
206 BG-11 medium, freeze-dried and killed by autoclaving (121 °C in 20 min). Dried biomass
207 was suspended at 0.2 g L^{-1} in BG-11 amended with 8.8×10^{-11} M of ^{90}Sr . The protocol of
208 cell inactivation was different in this case than for experiments with ^{226}Ra . However, both

209 approaches were similarly efficient at providing dead cell controls which eventually show
210 low sorption capabilities of ^{226}Ra and ^{90}Sr as detailed later in the manuscript 3) Living cells
211 of *Synechocystis* sp. PCC 6803 grown in BG-11, collected by centrifugation at 8500 g for 10
212 min, re-suspended in fresh BG-11 at an OD of 0.5 and amended with 8.8×10^{-11} M of ^{90}Sr .

213 Triplicate incubations were performed for all experimental treatments. Total ^{90}Sr and
214 residual ^{90}Sr in solution were measured in all solutions after 0.5, 24 and 48 h incubation in
215 the light. Temperature was maintained at 30°C within a MINITRON incubator (INFORS)
216 with shaking at 100 rpm. For residual ^{90}Sr quantification, 1 mL of cell suspension was
217 centrifuged at 8500 g for 10 min and the supernatant was filtered on a 0.22 μm PVDF
218 syringe filter. The volume of the filtered supernatant was measured as 930 ± 2 μL . Two
219 milliliters of scintillating solution (Ultima GoldTM, PerkinElmer) were added to the filtrates
220 before counting on a Tri-Carb® 3100TR low activity liquid scintillation analyzer
221 (PerkinElmer). For total ^{90}Sr content quantification, 930 μL of cell suspension were mixed
222 with 2 mL scintillating solution and counted. ^{90}Sr standard solutions were prepared with the
223 ^{90}Sr stock solution (LEA, Pierrelatte, France), diluted in BG-11 medium and counted as
224 described for the other samples. The counts per second (CPS) values were corrected from
225 the background activity, measured with a control made of 930 μL of BG-11 medium and 2
226 mL scintillation solution. The concentration of ^{90}Sr (Bq L^{-1}) in the samples was derived
227 from the standards.

228 ***Calculation of bioaccumulation of ^{90}Sr and ^{226}Ra***

229 The mass of ^{90}Sr and ^{226}Ra accumulated by the cells at a time t and normalized by the cell
230 mass was determined as follows:

231 For ^{226}Ra :

$$\frac{(X_0 - X_t)\alpha}{\text{cell mass accumulated between 0 and } t}$$

233 and for ^{90}Sr :

$$\frac{(X_0 - X_t)\alpha}{\text{cell mass at } t}$$

235 where X denotes radionuclide activity (Bq L^{-1}), α is the factor to convert activity of a
236 radionuclide to mass units and t is the sampling time. The value of α for ^{226}Ra and ^{90}Sr is
237 equal to 1.2×10^{-13} (mol Bq^{-1}) and 2.2×10^{-15} (mol Bq^{-1}).

238

239 3. RESULTS AND DISCUSSION

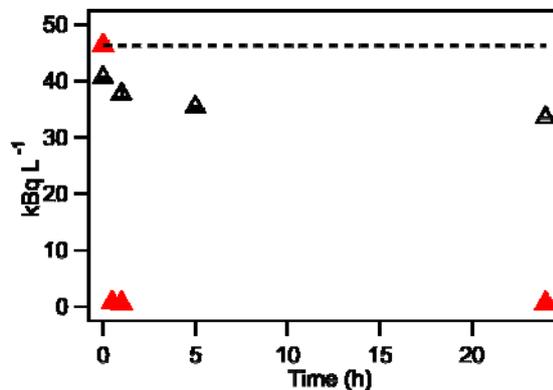


Figure 1: Time evolution of residual ^{90}Sr activity in the solution during exposure of *G. lithophora* cells to 8.8×10^{-11} M ^{90}Sr (\blacktriangle) and in the abiotic control (\triangle), both in BG-11. The dash lines denote the initial total activity of ^{90}Sr added to the cultures. When not visible, error bars are smaller than the symbols.

240 Kinetics of ^{90}Sr uptake by *G. lithophora*

241 In order to demonstrate the uptake of radionuclides by *G. lithophora*, we first
242 monitored uptake of ^{90}Sr at high cell density to avoid any potential toxic effects on its
243 growth by ^{90}Sr . The residual ^{90}Sr activity in solution during exposure of *G. lithophora* cells

244 to $8.8 \times 10^{-11} \text{ M } ^{90}\text{Sr}$ is shown in Figure 1. ^{90}Sr was rapidly removed from the solution in the
245 presence of *G. lithophora*: the residual ^{90}Sr activity in the solution decreased from $46.243 \pm$
246 0.604 kBq L^{-1} to $0.753 \pm 0.129 \text{ kBq L}^{-1}$ within 1 h, corresponding to the sequestration of 98
247 % of the radionuclide by the cells. The presence of divalent ions (e.g. Ca^{2+} , Mg^{2+}) in
248 solution did not inhibit ^{90}Sr uptake by *G. lithophora* cells, consistent with results obtained
249 with stable Sr^{33} . Following the observed rapid decline of the residual ^{90}Sr activity in the
250 solution, the rate of ^{90}Sr uptake by *G. lithophora* decreased. At the end of incubation (48 h),
251 the residual ^{90}Sr activity reached 0.608 kBq L^{-1} , corresponding to 99 % of radionuclide
252 removal by the cells. Here, removal of ^{90}Sr by *G. lithophora* occurred from solutions
253 containing a high concentration of Ca, with an initial $\text{Ca}/^{90}\text{Sr}$ molar ratio of 2.7×10^6 . Thus *G.*
254 *lithophora* can efficiently sequester ^{90}Sr at trace concentrations despite the presence of a
255 large excess of competing cations. Within abiotic controls, only 12% of ^{90}Sr was removed
256 from solution within 5 hours, and ^{90}Sr activity remained constant thereafter for the duration
257 of the experiment.

258 **Kinetics of ^{226}Ra uptake by *G. lithophora***

259

260 Based on the promising uptake of ^{90}Sr exhibited by *G. lithophora* cells, the next step

261 was to investigate whether the uptake of radiotoxic AEE could also occur during the growth

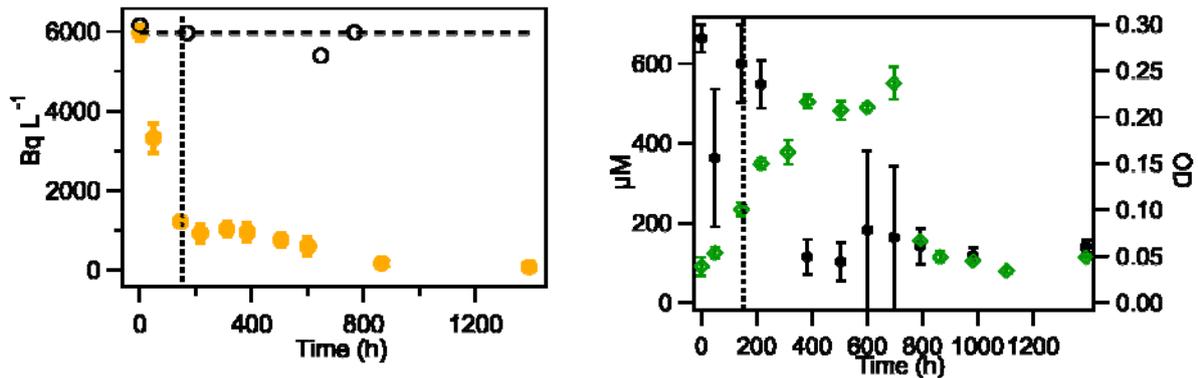


Figure 2: (Left) Time evolution of residual ^{226}Ra activity in the solution during growth of *G. lithophora* with $8 \times 10^{-10} \text{ M } ^{226}\text{Ra}$ (●) and in abiotic control (○), both in BG-11. Error bars denote the standard deviations of triplicate measurements. The horizontal dash line indicates the activity of ^{226}Ra added to the BG-11 media used for growing *G. lithophora*. (Right) Time evolution of dissolved Ca^{2+} (●) concentration and OD (◇) of cell suspension during growth of *G. lithophora* in BG-11 amended with $8 \times 10^{-10} \text{ M } ^{226}\text{Ra}$. When not visible, error bars are smaller than the symbols, except for the abiotic control, for which triplicate measurements were not performed. The vertical dash line indicates the beginning of the second stage of ^{226}Ra uptake.

262 stage of *G. lithophora*. For this purpose, we measured ^{226}Ra uptake upon the growth of *G.*

263 *lithophora* cells starting at a low cell density.

264 The uptake of ^{226}Ra by *G. lithophora* was examined within low-cell density cultures

265 for reasons described above, and occurred in two stages (Figure 2, left). In the first stage,

266 ^{226}Ra was rapidly removed from solution, with ^{226}Ra activity decreasing from 5964 ± 192.2

267 Bq L^{-1} to $1227.68 \pm 85.5 \text{ Bq L}^{-1}$ in 144 h. This removal was accompanied by a slight

268 decrease in dissolved Ca^{2+} (Figure 2, right). In the second stage, the rate of removal of ^{226}Ra

269 from the solution slowed down and residual ^{226}Ra activity in the solution further decreased

270 to 82 Bq L^{-1} in 1392 h. The initial period of this 2nd stage was marked with an appreciable

271 decrease in dissolved Ca^{2+} in comparison to the 1st stage. After this marked decrease,
272 dissolved Ca^{2+} remained constant.

273 *G. lithophora* showed active growth during 792 h as shown by OD measurements
274 (Figure 2, right). During this period, OD reached a value of ~ 0.3 , then a sharp decline in OD
275 was observed. Concomitantly, extracellular Ca^{2+} concentration remained constant and ^{226}Ra
276 in the soluble fraction decreased. The cause of this sharp decline in OD is unclear, but
277 possible reasons are presented later in the discussion (See Maximum uptake of ^{226}Ra and
278 ^{90}Sr in *G. lithophora*). At the end of incubation (1392 h), 99 ± 5 % of initial ^{226}Ra was
279 removed from the solution.

280 **Retention mechanisms of ^{226}Ra and ^{90}Sr in *G. lithophora***

281 Here, we systematically tested different microbial processes to explain mechanism of
 282 ^{226}Ra and ^{90}Sr sequestration by *G. lithophora*. First, to distinguish adsorption to dead cells from
 283 other mechanisms, we compared uptake of ^{226}Ra and ^{90}Sr between living and dead cells of *G.*
 284 *lithophora*. The average uptake of ^{226}Ra in inactivated cell control, composed of dead cells of *G.*
 285 *lithophora*, was equal to $0.8 \pm 0.1 \mu\text{g}$ (g of dry mass) $^{-1}$; in contrast the average ^{226}Ra uptake by
 286 active *G. lithophora* cells reached $3.9 \pm 0.9 \mu\text{g}$ (g of dry mass) $^{-1}$ (Table 1 Table 1). Likewise, the

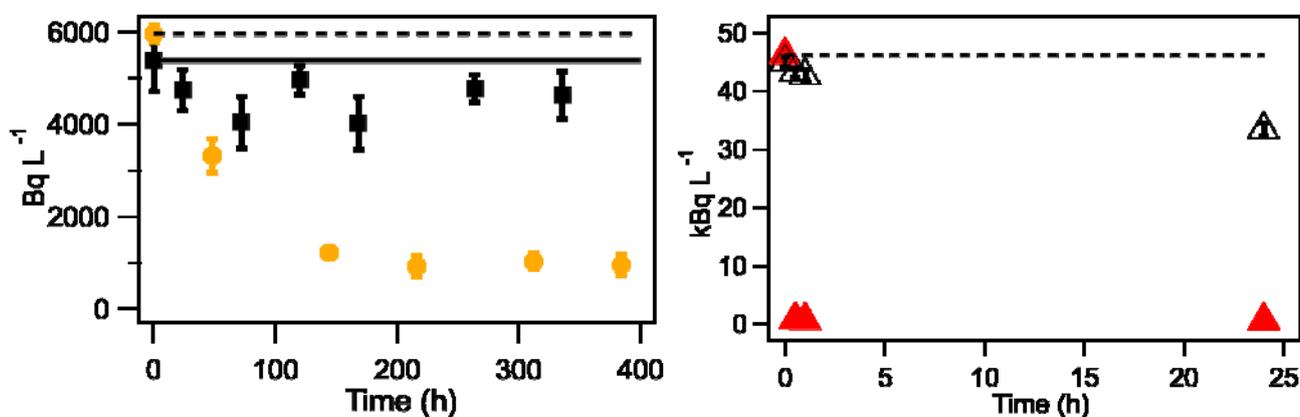


Figure 3: (Left) Time evolution of residual ^{226}Ra activity in the solution during growth of *G. lithophora* (●) and *Synechocystis* PCC 6803 (■) in BG-11. (Right) Time evolution of residual ^{90}Sr activity in high cell density suspensions (OD=0.5) of *G. lithophora* (▲) and *Synechocystis* PCC 6803 (Δ), both suspended in BG-11. The activity of ^{226}Ra added to BG-11 media is shown as dash line for *G. lithophora* cultures, and solid line for *Synechocystis* cultures. The activity of ^{90}Sr added to BG-11 media is shown as dash line for *G. lithophora* and *Synechocystis* cultures. Error bar shows $\pm\sigma$.

287 average uptake of ^{90}Sr by dead *G. lithophora* cells was equal to $8.87 \pm 0.2 \text{ ng}$ (g of dry mass) $^{-1}$;
 288 in contrast the average ^{90}Sr uptake rate by active cells of *G. lithophora* measured $47.97 \pm 1.5 \text{ ng}$
 289 (g of dry mass) $^{-1}$ (Table 1). Second, the relationship between biomineralization and
 290 sequestration of ^{226}Ra and ^{90}Sr was tested by comparing uptake of ^{226}Ra and ^{90}Sr by living cells
 291 of the non-biomineralizing *Synechocystis* and living cells of *G. lithophora*. The OD of
 292 *Synechocystis* during the incubation period is shown in Figure S1. Living cells of the non-
 293 biomineralizing *Synechocystis* retained $14 \pm 2 \%$ of ^{226}Ra in 336 h, while *G. lithophora* retained

294 83 ± 14 % of ^{226}Ra over the same duration (Figure 3). Similarly, incubation over 24 h of the non-
 295 biomineralizing *Synechocystis* at relatively high cell density (OD=0.5) retained 26 ± 1 % of ^{90}Sr ,
 296 while *G. lithophora* retained 99.5 ± 10 % of the initial ^{90}Sr (Figure 3). While there could be
 297 differences in the cell surface composition between both strains, we argue that these differences
 298 are likely not substantial enough to induce 6 times more ^{226}Ra and 4 times more ^{90}Sr retention in
 299 *G. lithophora* than in *Synechocystis* PCC 6803. Altogether, these results suggest specific cellular
 300 processes are responsible for ^{226}Ra and ^{90}Sr retention by *G. lithophora*.

301
 302
 303
 304
 305
 306

Table 1: Comparison of ^{226}Ra and ^{90}Sr uptake per unit biomass in different incubations

Sample ID	^{226}Ra Retained* $\mu\text{g (g of dry mass)}^{-1}$	^{90}Sr retained** $\text{ng (g of dry mass)}^{-1}$
Live <i>G. lithophora</i> cells	3.9 ± 0.9	47.9 ± 1.5
Dead <i>G. lithophora</i> cells	0.8 ± 0.1	8.87 ± 0.2

* mass uptake is average uptake during the first stage of Ra retention. The error bar shows $\pm\sigma$
 ** mass uptake is average uptake when cells were exposed to 8.8×10^{-11} M of ^{90}Sr for 1 h.

307 Microbial processes of ^{226}Ra and ^{90}Sr sequestration can broadly be categorized into
 308 three groups: extracellular biomineralization, adsorption, and intracellular
 309 biomineralization²⁴. Microbially induced extracellular biomineralization requires localized
 310 supersaturation of the solution with respect to Ca-carbonate phases. This results in
 311 precipitation of carbonate solids and ^{226}Ra and ^{90}Sr may co-precipitate within these

312 extracellular solids and be removed from the solution. Using Visual Minteq³⁸, we calculated
313 the saturation state of the solution with respect to Ca-carbonate phases to verify if this
314 mechanism was operational. Visual MINTEQ predicts that the experimental culture media
315 amended with ²²⁶Ra and ⁹⁰Sr remained undersaturated with respect to Ca-carbonate phase(s)
316 (Figure S2- S3). Moreover, the state of solution undersaturation with respect to Ca-
317 carbonate solids is consistent with the observation that dissolved ²²⁶Ra and ⁹⁰Sr activity
318 remained close to their initial activities in the abiotic control (Figure 1-2). Therefore,
319 extracellular precipitates of Ca-carbonate phases could not be a plausible sink of ²²⁶Ra and
320 ⁹⁰Sr in our study.

321 One of the physicochemical processes that has been used widely for sequestering low
322 levels of contaminants from aqueous solutions is adsorption. Here, based on Figure 3 and
323 Table 1, adsorption of ²²⁶Ra and ⁹⁰Sr at walls of culture flasks, at the cell surface and dead *G.*
324 *lithophora* biomass, removed 22% ²²⁶Ra and 18% ⁹⁰Sr from solution, suggesting that
325 adsorption alone cannot reproduce the degree of ²²⁶Ra and ⁹⁰Sr retained by *G. lithophora*.
326 The dissolved concentration of Ca²⁺ in ²²⁶Ra uptake experiments by active cells of *G.*
327 *lithophora* was higher than the concentration of dissolved Ca²⁺ in the inactivated cell control
328 and the *Synechocystis* cultures. Because of competing effects for adsorption between Ca and
329 Ra, this implies that the degree of ²²⁶Ra adsorption measured in the inactivated cell and by
330 *Synechocystis* controls was relatively overestimated compared to that in the presence of
331 active *G. lithophora* cells will likely be less in presence of excess Ca²⁺ furnished in ²²⁶Ra
332 uptake experiments (Figure 2). Moreover, the cell normalized rate of uptake measured for
333 ⁹⁰Sr is consistent with those measured by Cam et al. for stable Sr isotope and shown to be
334 associated with intracellular uptake rather than adsorption³³. These observations taken

335 altogether suggests that mechanism(s) other than adsorption are involved in ^{226}Ra and ^{90}Sr
336 uptake in *G. lithophora*.

337 Previously, TEM analyses of *G. lithophora* cells cultured in BG-11, amended with
338 high initial concentrations of stable Ba and Sr isotopes showed that most of the Sr and Ba
339 were sequestered within intracellular carbonates and to a lesser extent in intracellular
340 polyphosphates. Unfortunately, TEM analysis was not possible here to assess ^{226}Ra and ^{90}Sr
341 distribution within the cell because their concentrations were below the detection limits of
342 EDXS³⁹. Nevertheless, considering ^{226}Ra and ^{90}Sr chemical affinities and similarity to Ca
343 and Ba, we propose that incorporation of ^{226}Ra and ^{90}Sr into intracellular carbonate (and
344 polyphosphate) inclusions is likely the primary mechanisms for their sequestration in *G.*
345 *lithophora*. It remains a mystery why *G. lithophora* would sequester radiotoxic ^{226}Ra and
346 ^{90}Sr elements within the cell. Unlike Ca, which is essential as a co-factor for cyanobacteria
347 in the photosystem II complex⁴⁰, there is no documented biological function of ^{226}Ra , ^{90}Sr or
348 Ba. Some studies have argued that trace amounts of non-essential elements can be
349 assimilated together with their corresponding essential analogues²⁴. In fact, non-selective
350 transport of Sr^{2+} through cation channels have already been acknowledged by other
351 studies⁴¹. Hence, ^{226}Ra and ^{90}Sr could be “mistaken” for its chemical analog Ca, an essential
352 element, thereby resulting in their favorable uptake in *G. lithophora*. Alternatively, while
353 this is not ascertained by the present experiments, selective uptake of ^{226}Ra and ^{90}Sr by *G.*
354 *lithophora* should may also be considered because of two reasons: (1) Cam *et al.* showed
355 that *G. lithophora* selectively uptakes stable Sr over Ca^{33} ; (2) Uptake of ^{226}Ra and ^{90}Sr by *G.*
356 *lithophora* was not inhibited by Ca^{2+} , present at approximately 6 orders of magnitude higher
357 concentration than ^{226}Ra and ^{90}Sr . These observations favor involvement of another

358 biochemical process at play in *G. lithophora*. Typically, ion selectivity between similar ions
359 such Sr, Ra, Ba, Ca requires an ion transport pathway to have some specific binding sites
360 over at least part of its length. Future studies characterizing the affinity and specificity of
361 various transporters involved in uptake of AEE in *G. lithophora* along with measurement of
362 ^{90}Sr and ^{226}Ra uptake by *G. lithophora* at varying Ca: $^{90}\text{Sr}/^{226}\text{Ra}$ ratio may shed some light
363 on molecular processes involved in uptake of ^{90}Sr and ^{226}Ra by *G. lithophora*.

364 **Maximum uptake of ^{226}Ra and ^{90}Sr by *G. lithophora***

365 Understanding the maximum uptake capacity of ^{226}Ra and ^{90}Sr by *G. lithophora* is an
366 important criterion for its assessment as a potential bioremediation strategy. The maximum
367 radionuclide uptake capacity by a microorganism is an elusive concept as a zero net uptake
368 rate doesn't always signal maximum uptake capacity. For example, net uptake may appear
369 to cease when the amount of radionuclide added to the solution is exhausted before the cells
370 reach their maximum uptake capacity. In the case of ^{226}Ra , the total ^{226}Ra accumulated in *G.*
371 *lithophora* over the total duration of incubation equals to $133 \text{ kBq (g of dry mass)}^{-1}$ (Figure 2
372 and Table1). The OD decline after 792 h may possibly indicate cell lysis and/or cell
373 aggregation, settling and/or attachment of cells to the flasks. Visual inspection of the
374 cultures after 792 h did reveal some aggregation and settling of the cells (data no shown).
375 The decrease in the uptake of ^{226}Ra during the 2nd stage may signal that the cells have
376 reached maximum ^{226}Ra uptake capacity. To test whether this is the case, cultures of *G.*
377 *lithophora* were grown in BG-11 containing ^{226}Ra at a concentration 25 times higher than
378 the experiment here. The high ^{226}Ra concentration experiments showed that over 30 days
379 70% of ^{226}Ra was removed and $962 \text{ kBq (g of biomass)}^{-1}$ ^{226}Ra sequestered by *G. lithophora*
380 (Figure S4). This implies that the decrease in ^{226}Ra uptake rate during 2nd stage is not

381 because cells reached maximum uptake capacity but for some other reasons yet to be
382 discovered. Similar to ^{226}Ra , total ^{90}Sr accumulated by *G. lithophora* in 24 h was equal to
383 250 kBq (g of biomass)⁻¹(Figure 1 and Table1). To test whether this amount of ^{90}Sr
384 sequestered was equal to maximum uptake capacity, culture of *G. lithophora* at high cell
385 density were successively spiked with ^{90}Sr and uptake of ^{90}Sr measured in the supernatant.
386 The multiple spike experiments showed that 924 kBq (g of biomass)⁻¹ was incorporated
387 within *G. lithophora* over 24 hr (Figure S5). After 24 h, no further ^{90}Sr accumulation was
388 measured as the cells exhausted the amount of ^{90}Sr provided. Together, these observations
389 suggest that in the present study, measured ^{226}Ra and ^{90}Sr accumulation by *G. lithophora* is
390 a lower estimate of its maximum uptake capacity.

391 **Environmental Implications and Bioremediation**

392 Both uptake capacity and selectivity play an important feature for assessment of an
393 organism's effectiveness for ^{90}Sr and ^{226}Ra remediation. Tables 2 and 3 compare ^{90}Sr and
394 ^{226}Ra uptake capacity of *G. lithophora* with other organisms known to uptake ^{90}Sr or ^{226}Ra .
395 For the screened bioremediation candidates, uptake capacities of ^{90}Sr and ^{226}Ra are reported
396 assuming ideal conditions, *i.e.* in absence of Ca and other divalent ions. Such conditions are
397 not representative of environments contaminated with ^{90}Sr and ^{226}Ra , which contain excess
398 Ca and/or other divalent ions. Presence of Ca and other divalent ions in the solution may
399 compromise the uptake of ^{226}Ra and ^{90}Sr . For example, 40% of ^{90}Sr was removed by
400 *Vetiveria zizanoids* from solution containing Ca/ ^{90}Sr molar ratio of 3.6×10^6 , while 94%
401 removal was achieved in absence of Ca⁴². In another example, *Calotropis gigantea*
402 exhibited high accumulation of ^{90}Sr (104 ng g⁻¹) but from a solution containing only ^{90}Sr ⁴³.

403 Whether the high ^{90}Sr removal efficiency of *C. gigantea* is maintained within an
 404 environmentally relevant matrix remains unknown. In the case of ^{226}Ra , the uptake exhibited
 405 by dead biomass was achieved under conditions where ^{226}Ra was the only AEE present and
 406 therefore unlikely to be replicated in the environment to the same extent. Therefore, the
 407 current bioremediation strategies listed in Table 2-3 are inadequate in efficiently achieving
 408 selective removal of ^{226}Ra and ^{90}Sr in the presence of Ca.

409 **Table 2:** Comparison of ^{226}Ra uptake by *G. lithophora* with previously reported experimental data on ^{226}Ra
 410 uptake by organisms.

	Radium Uptake
Dead Biomass	($\mu\text{g g}^{-1}$)
<i>P. fluorescens</i> ⁴⁴	0.02
<i>Streptomyces nives</i> ⁴⁴	0.58
Activated sludge ⁴⁴	0.075
Activated sludge ⁴⁵	0.04
<i>N. carneum</i> ²⁵	1.3
<i>N. insulare</i> ²⁵	1.2
<i>O. geminata</i> ²⁵	1.35
<i>S. laxissima</i> ²⁵	1.3
<i>Serratia</i> sp. ZF03 ⁴⁵	1.03
<i>P. chrysogenum</i> ⁴⁶	0.076
<i>G. emersonii</i> ⁴⁷	1.86
Live Biomass	
<i>D. linearis</i> ⁴⁸	4.5
<i>G. lithophora</i> (this study)*	26.1

* based on *G. lithophora* grown in elevated ^{226}Ra activity in a preliminary experiment (Figure S4)

411

412 On the other hand, *G. lithophora* exhibits highest uptake of ^{90}Sr and ^{226}Ra among all
 413 the active and dead microorganisms. Since OD values in experiments using ^{90}Sr were
 414 measured under the same conditions and setup as in Cam et al³⁶, their conversion to cell
 415 numbers and cell mass are expected to be correct. Alternatively, we note that quantity of
 416 ^{226}Ra sequestered per unit biomass of *G. lithophora* cells might be approximate estimations

417 only because presence of ^{226}Ra during growth of *G. lithophora* may induce physiological
418 changes and alter the previously reported relationship between OD, cell density, and cell
419 mass³⁶. Unlike previously reported uptake studies, *G. lithophora* uptakes ^{90}Sr and ^{226}Ra in
420 presence of excess Ca- initial molar ratio of $\text{Ca}/^{90}\text{Sr}$ and $\text{Ca}/^{226}\text{Ra}$ of the cultures equal
421 2.3×10^6 and 1.2×10^6 respectively. Interestingly, ^{226}Ra accumulation by *G. lithophora*'s
422 overwhelmingly exceeds ^{226}Ra uptake capacity of *Dicranopteris linearis*, identified as a
423 ^{226}Ra hyper-accumulator⁴⁸. The remarkable ability of *G. lithophora* to retain high activities
424 of ^{226}Ra and ^{90}Sr in comparison with other organisms makes it an attractive candidate for
425 bioremediation applications in decontamination of low salinity radioactive wastewater
426 streams. Such a strategy could be envisioned as an *ex-situ* treatment, integrated (e.g. as a
427 bioreactor) within an existing wastewater treatment plant. Compared to *G. lithophora*, other
428 $^{90}\text{Sr}/^{226}\text{Ra}$ bioremediation candidates are mostly plants (Table 2-3). There are practical
429 considerations of using cyanobacterium *G. lithophora* vs. plants for bioremediation, such as
430 harvesting of biomass, growth times, duration of decontamination and treatment of
431 radioactive biomass. A complete development of *G. lithophora* based bioremediation would
432 require determining these aspects in future studies.

433 **Table 3:** Comparison of Sr uptake in *G. lithophora* with previously reported experimental data on ^{90}Sr uptake by
434 organisms

Organism	Uptake capacity (ng g ⁻¹)	% removal
<i>V. zizanioides</i> (0mM Ca) ⁴²	-	94%
<i>V. zizanioides</i> (40 mM Ca) ⁴²	-	40%
<i>Paspalum notatum</i> ^{49**}	0.14	20%
<i>Sorghum halense</i> ^{49**}	0.14	32%
<i>Panicum virginatum</i> ^{49**}	0.15	23%
<i>Amaranthus retroflexus</i> ⁵⁰	0.000077	5%
<i>Calotropis gigantea</i> ^{43*}	103.37	97%
<i>G. lithophora</i> (this study) ⁺	177	99%

* Uptake achieved in 168 h

** Concentration in plant tissue after 8 weeks of harvest

+ Based on multi-spike experiment (Figure S5)

435 Another aspect of bioremediation of radionuclides in the environment is the ability of
436 microorganism to survive in the presence of ionizing radiation. The intracellular uptake of
437 radiotoxic ^{226}Ra and ^{90}Sr could potentially result in oxidative damage in DNA, proteins and
438 lipids due to the generation of reactive oxygen species. Cyanobacteria have been detected in
439 radioactive areas surrounding Chernobyl nuclear reactor⁵¹, uranium mining operations⁵² and
440 in vicinity of hydrothermal spring caves⁵³, suggesting that they may have some degree of
441 resistance to ionizing radiation. However, little is known about *G. lithophora*'s
442 radiotolerance and response to oxidative stress and future experiments characterizing the
443 viability of *G. lithophora* cells when exposed to elevated radiation could provide some
444 insights in this regard.

445 *G. lithophora* sequesters ^{226}Ra and ^{90}Sr within intracellular ACC inclusions at very
446 low extracellular Ra/Ca concentration ratios, offering a unique solution to the general
447 problem of remediating ^{226}Ra and ^{90}Sr in aqueous environments with naturally high Ca
448 concentrations. Further studies investigating the stability of intracellular inclusions in
449 environmentally relevant matrices and optimization of various process parameters such as
450 maximum ^{226}Ra and ^{90}Sr uptake capacity, and tolerance to other metalloids present in the
451 waste streams will be needed to ensure successful development of *G. lithophora* based
452 bioremediation approach for ^{90}Sr and ^{226}Ra .

453 4. ASSOCIATED CONTENT

454 This manuscript is accompanied by Supporting Information. An extra document contains 5
455 figures. Data on OD of cultures in absence of radionuclide, saturation indices of Ca and Sr
456 solids in the solution, ^{226}Ra uptake by *G. lithophora* from BG-11 containing 210 kBq L^{-1}
457 ^{226}Ra , and data on uptake of ^{90}Sr by *G. lithophora* during successive spike experiment.

458 5. ACKNOWLEDGMENTS

459 We would like to thank Timothy McClure (MIT) for his assistance with ICP-OES,
460 Ryan Samz and Mitchell S Galanek, MIT Environmental Health and Safety, for supplying
461 ²²⁶Ra stock solutions, radiation protection equipment and safety expertise. We thank Fériel
462 Skouri-Panet and Margot Coutaud (IMPIC) for assistance in the Sr experiments. The
463 authors declare no competing financial interest.

464

465

466

467 **REFERENCE**

- 468 (1) Hutchins, D.; Mulholland, M.; Fu, F. Nutrient Cycles and Marine Microbes in a CO₂-
469 Enriched Ocean. *Oceanography* **2009**, 22 (4), 128–145.
- 470 (2) Riding, R. Cyanobacterial Calcification, Carbon Dioxide Concentrating Mechanisms,
471 and Proterozoic-Cambrian Changes in Atmospheric Composition. *Geobiology* **2006**, 4
472 (4), 299–316.
- 473 (3) Benzerara, K.; Skouri-Panet, F.; Li, J.; Ferard, C.; Gugger, M.; Laurent, T.;
474 Couradeau, E.; Ragon, M.; Cosmidis, J.; Menguy, N.; Margaret-Oliver, I.; Tavera, R.;
475 Lopez-Garcia, P.; Moreira, D. Intracellular Ca-Carbonate Biomineralization Is
476 Widespread in Cyanobacteria. *Proc. Natl. Acad. Sci.* **2014**, 111 (30).
- 477 (4) Couradeau, E.; Benzerara, K.; Gérard, E.; Moreira, D.; Bernard, S.; Brown, G. E. J.;
478 López-García, P. An Early-Branching Microbialite Cyanobacterium Forms
479 Intracellular Carbonates. *Science*. **2012**, 336, 459–462.
- 480 (5) De Wever, A.; Benzerara, K.; Gugger, M.; Coutaud, M.; Caumes, G.; Poinot, M.;
481 Skouri, F.; Thierry, P.; Elodie, L. Evidence of High Ca Uptake by Cyanobacteria
482 Forming Intracellular CaCO₃ and Impact on Their Growth. *Geobiology* **2019**, 1–15.
- 483 (6) Li, J.; Margaret Oliver, I.; Cam, N.; Boudier, T.; Blondeau, M.; Leroy, E.; Cosmidis,

- 484 J.; Skouri-Panet, F.; Guigner, J.-M.; Férard, C.; Poinso, M.; Moreira, D.; Lopez-
485 Garcia, P.; Cassier-Chauvat, C.; Chauvat, F.; Benzerara, K. Biomineralization
486 Patterns of Intracellular Carbonatogenesis in Cyanobacteria: Molecular Hypotheses.
487 *Minerals* **2016**, *6* (1), 10.
- 488 (7) Szabo, Z.; dePaul, V. T.; Fischer, J. M.; Kraemer, T. F.; Jacobsen, E. Occurrence and
489 Geochemistry of Radium in Water from Principal Drinking-Water Aquifer Systems of
490 the United States. *Appl. Geochemistry* **2012**, *27* (3), 729–752.
- 491 (8) United States Environmental Protection Agency. *Radionuclides Rule – Drinking*
492 *Water Requirements for States and Public Water Systems – US EPA*.
- 493 (9) International Atomic Energy Agency (IAEA). The Environmental Behaviour of
494 Radium: Revised Edition. *Tech. Reports Ser. No. 476* **2014**, No. 476, 44–51.
- 495 (10) United States Nuclear Regulatory Commission. NRC: 10 CFR Part 20 - Appendix B -
496 Radionuclide Table - Cesium-137. 2017.
- 497 (11) Warner, N. R.; Cidney, C. A.; Jackson, R. B.; Vengosh, A. Impacts of Shale Gas
498 Wastewater Disposal on Water Quality in Western PA. *Environ. Sci. Technol.* **2013**,
499 1–14.
- 500 (12) Chellam, S.; Clifford, D. A. Physical-Chemical Treatment of Groundwater
501 Contaminated by Leachate from Surface Disposal of Uranium Tailings. *J. Environ.*
502 *Eng.* **2002**, *128* (10), 942–952.
- 503 (13) Casacuberta, N.; Masqué, P.; Garcia-Orellana, J.; Garcia-Tenorio, R.; Buesseler, K.
504 O. ⁹⁰Sr and ⁸⁹Sr in Seawater off Japan as a Consequence of the Fukushima Dai-Ichi
505 Nuclear Accident. *Biogeosciences* **2013**, *10* (6), 3649–3659.
- 506 (14) Vakulovsky, S. M.; Nikitin, A. I.; Chumichev, V. B.; Katrich, I. Y.; Voitsekhovich,
507 O. A.; Medinets, V. I.; Pisarev, V. V.; Bovkum, L. A.; Khersonsky, E. S. Cesium-137
508 and Strontium-90 Contamination of Water Bodies in the Areas Affected by Releases
509 from the Chernobyl Nuclear Power Plant Accident: An Overview. *J. Environ.*
510 *Radioact.* **1994**, *23* (2), 103–122.
- 511 (15) Povinec, P. P.; Hirose, K.; Aoyama, M. Radiostrontium in the Western North Pacific:

- 512 Characteristics, Behavior, and the Fukushima Impact. *Environ. Sci. Technol.* **2012**, *46*
513 (18), 10356–10363.
- 514 (16) Idaho National Engineering Laboratory, I. Selected Radionuclides Important to Low-
515 Level Radioactive Waste Management. **1996**, No. November, 1–413.
- 516 (17) Pagnanelli, F.; Papini, M. P.; Toro, L.; Trifoni, M.; Vegliò, F. Biosorption of Metal
517 Ions on *Arthrobacter* Sp.: Biomass Characterization and Biosorption Modeling.
518 *Environ. Sci. Technol.* **2000**, *34* (13), 2773–2778.
- 519 (18) Volesky, B. Detoxification of Metal-Bearing Effluents: Biosorption for the next
520 Century. *Hydrometallurgy* **2001**, *59* (2–3), 203–216.
- 521 (19) Brower, J. B.; Ryan, R. L.; Pazirandeh, M. Comparison of Ion-Exchange Resins and
522 Biosorbents for the Removal of Heavy Metals from Plating Factory Wastewater.
523 **1997**, *31* (10), 2910–2914.
- 524 (20) Lloyd, J. R.; Renshaw, J. C. Bioremediation of Radioactive Waste: Radionuclide-
525 Microbe Interactions in Laboratory and Field-Scale Studies. *Curr. Opin. Biotechnol.*
526 **2005**, *16*, 254–260.
- 527 (21) Gadd, G. M. Metals, Minerals and Microbes: Geomicrobiology and Bioremediation.
528 *Microbiology* **2010**, *156* (3), 609–643.
- 529 (22) Ruggiero, C. E.; Boukhalfa, H.; Forsythe, J. H.; Lack, J. G.; Hersman, L. E.; Neu, M.
530 P. Actinide and Metal Toxicity to Prospective Bioremediation Bacteria. *Environ.*
531 *Microbiol.* **2005**, *7* (1), 88–97.
- 532 (23) Tsezos, M.; Keller, D. M. Adsorption of Radium-226 by Biological Origin
533 Absorbents. *Biotechnol. Bioeng.* **1983**, *25* (1), 201–215.
- 534 (24) Newsome, L.; Morris, K.; Lloyd, J. R. The Biogeochemistry and Bioremediation of
535 Uranium and Other Priority Radionuclides. *Chem. Geol.* **2014**, *363*, 164–184.
- 536 (25) Pohl, P.; Schimmack, W. Adsorption of Radionuclides (¹³⁴Cs, ⁸⁵Sr, ²²⁶Ra, ²⁴¹Am)
537 by Extracted Biomasses of Cyanobacteria (*Nostoc Carneum*, *N. Insulare*, *Oscillatoria*
538 *Geminata* and *Spirulina Laxis-Sima*) and Phaeophyceae (*Laminaria Digitata* and *L.*

- 539 Japonica; Waste Products from Alginat. *J. Appl. Phycol.* **2006**, *18* (2), 135–143.
- 540 (26) Satvatmanesh, D.; Siavoshi, F.; Beitollahi, M. M.; Amidi, J.; Fallahian, N.
541 Biosorption of ²²⁶Ra in High Level Natural Radiation Areas of Ramsar, Iran. *J.*
542 *Radioanal. Nucl. Chem.* **2003**, *258* (3), 483–486.
- 543 (27) Liu, M.; Dong, F.; Kang, W.; Sun, S.; Wei, H.; Zhang, W.; Nie, X.; Guo, Y.; Huang,
544 T.; Liu, Y. Biosorption of Strontium from Simulated Nuclear Wastewater by
545 *Scenedesmus Spinosus* under Culture Conditions: Adsorption and Bioaccumulation
546 Processes and Models. *Int. J. Environ. Res. Public Health* **2014**, *11* (6), 6099–6118.
- 547 (28) Fukuda, S. ya; Iwamoto, K.; Atsumi, M.; Yokoyama, A.; Nakayama, T.; Ishida, K.
548 ichiro; Inouye, I.; Shiraiwa, Y. Global Searches for Microalgae and Aquatic Plants
549 That Can Eliminate Radioactive Cesium, Iodine and Strontium from the Radio-
550 Polluted Aquatic Environment: A Bioremediation Strategy. *J. Plant Res.* **2014**, *127*
551 (1), 79–89.
- 552 (29) Krejci, M. R.; Finney, L.; Vogt, S.; Joester, D. Selective Sequestration of Strontium in
553 Desmid Green Algae by Biogenic Co-Precipitation with Barite. *ChemSusChem* **2011**,
554 *4* (4), 470–473.
- 555 (30) Lee, S. Y.; Jung, K. H.; Lee, J. E.; Lee, K. A.; Lee, S. H.; Lee, J. Y.; Lee, J. K.; Jeong,
556 J. T.; Lee, S. Y. Photosynthetic Biomineralization of Radioactive Sr via Microalgal
557 CO₂absorption. *Bioresour. Technol.* **2014**, *172*, 449–452.
- 558 (31) Achal, V.; Pan, X.; Zhang, D. Bioremediation of Strontium (Sr) Contaminated
559 Aquifer Quartz Sand Based on Carbonate Precipitation Induced by Sr Resistant
560 *Halomonas* Sp. *Chemosphere* **2012**, *89* (6), 764–768.
- 561 (32) Lauchnor, E. G.; Schultz, L. N.; Bugni, S.; Mitchell, A. C.; Cunningham, A. B.;
562 Gerlach, R. Bacterially Induced Calcium Carbonate Precipitation and Strontium
563 Coprecipitation in a Porous Media Flow System. *Environ. Sci. Technol.* **2013**, *47* (3),
564 1557–1564.
- 565 (33) Cam, N.; Benzerara, K.; Georgelin, T.; Jaber, M.; Lambert, J. F.; Poinso, M.; Skouri-
566 Panet, F.; Cordier, L. Selective Uptake of Alkaline Earth Metals by Cyanobacteria

- 567 Forming Intracellular Carbonates. *Environ. Sci. Technol.* **2016**, *50* (21), 11654–
568 11662.
- 569 (34) Bailey, S.; Grossman, A. Photoprotection in Cyanobacteria: Regulation of Light
570 Harvesting. *Photochem. Photobiol.* **2008**, *84* (6), 1410–1420.
- 571 (35) Ragon, M.; Benzerara, K.; Moreira, D.; Tavera, R.; Lopez-Garcia, P. 16S rDNA-
572 Based Analysis Reveals Cosmopolitan Occurrence but Limited Diversity of Two
573 Cyanobacterial Lineages with Contrasted Patterns of Intracellular Carbonate
574 Mineralization. *Front. Microbiol.* **2014**, *5*, 1–11.
- 575 (36) Moreira, D.; Tavera, R.; Benzerara, K.; Skouri-Panet, F.; Couradeau, E.; Gérard, E.;
576 Fonta, C. L.; Novelo, E.; Zivanovic, Y.; López-García, P. Description of
577 *Gloeomargarita Lithophora* Gen. Nov., Sp. Nov., a Thylakoid-Bearing, Basal-
578 Branching Cyanobacterium with Intracellular Carbonates, and Proposal for
579 *Gloeomargaritales* Ord. Nov. *Int. J. Syst. Evol. Microbiol.* **2017**, *67* (3), 653–658.
- 580 (37) Stanier, R. Y.; Deruelles, J.; Rippka, R.; Herdman, M.; Waterbury, J. B. Generic
581 Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria.
582 *Microbiology* **1979**, *111* (1), 1–61.
- 583 (38) Gustafsson, J. P. Visual MINTEQ 3.0 User Guide. *Dep. of L. Water Resour. eng* **2012**,
584 1–73.
- 585 (39) Blondeau, M.; Benzerara, K.; Ferard, C.; Guigner, J. M.; Poinot, M.; Coutaud, M.;
586 Tharaud, M.; Cordier, L.; Skouri-Panet, F. Impact of the Cyanobacterium
587 *Gloeomargarita Lithophora* on the Geochemical Cycles of Sr and Ba. *Chem. Geol.*
588 **2018**, *483*, 88–97.
- 589 (40) Debus, R. J. The Manganese and Calcium Ions of Photosynthetic Oxygen Evolution.
590 *Biochem. Biophys. Acta* **1992**, *1102* (3), 269–352.
- 591 (41) Gupta, D. K.; Schulz, W.; Steinhauser, G.; Walther, C. Radiostrontium Transport in
592 Plants and Phytoremediation. *Environ. Sci. Pollut. Res.* **2018**, *25* (30), 29996–30008.
- 593 (42) Singh, S.; Eapen, S.; Thorat, V.; Kaushik, C. P.; Raj, K.; D'souza, S.
594 Phytoremediation of ¹³⁷cesium and ⁹⁰strontium from Solutions and Low-Level

- 595 Nuclear Waste by *Vetiveria Zizanoides*. *Exotoxicology Enviornmental Saf.* **2008**, *69*,
596 306–311.
- 597 (43) Eapen, S.; Singh, S.; Thorat, V.; Kaushik, C. P.; Raj, K.; D’Souza, S. F.
598 Phytoremediation of Radiostrontium (⁹⁰ Sr) and Radiocesium (¹³⁷ Cs) Using Giant
599 Milky Weed (*Calotropis Gigantea* R.Br.) Plants. *Chemosphere* **2006**, *65* (11), 2071–
600 2073.
- 601 (44) Tsezos, M.; Keller, D. M. Adsorption of Radium-226 by Biological Origin
602 Absorbents. *Biotechnol. Bioeng.* **1983**, *25*, 201–215.
- 603 (45) Zakeri, F.; Noghabi, K. A.; Sadeghizadeh, M.; Kardan, M. R.; Masoomi, F.;
604 Farshidpour, M. R.; Atarilar, A. *Serratia* Sp. ZF03: An Efficient Radium Biosorbent
605 Isolated from Hot-Spring Waters in High Background Radiation Areas. *Bioresour.*
606 *Technol.* **2010**, *101* (23), 9163–9170.
- 607 (46) IAEA. Various. *Treat. Liq. Effl. from uranium mines mills* **2004**, No. October, 246 p.
- 608 (47) Heidari, F.; Riahi, H.; Aghamiri, M. R.; Shariatmadari, Z.; Zakeri, F. Isolation of an
609 Efficient Biosorbent of Radionuclides (²²⁶Ra,²³⁸U): Green Algae from High-
610 Background Radiation Areas in Iran. *J. Appl. Phycol.* **2017**, *29* (6), 2887–2898.
- 611 (48) Chao, J. H.; Chuang, C. Y. Accumulation of Radium in Relation to Some Chemical
612 Analogues in *Dicranopteris Linearis*. *Appl. Radiat. Isot.* **2011**, *69* (1), 261–267.
- 613 (49) Entry, J. A.; Watrud, L. S.; Reeves, M. Influence of Organic Amendments on the
614 Accumulation of ¹³⁷Cs and ⁹⁰Sr from Contaminated Soil by Three Grass Species.
615 *Water. Air. Soil Pollut.* **2001**, *126* (3–4), 385–398.
- 616 (50) Fuhrmann, M.; Lasat, M. M.; Ebbs, S. D.; Kochian, L. V.; Cornish, J. Uptake of
617 Cesium-137 and Strontium-90 from Contaminated Soil by Three Plant Species;
618 Application to Phytoremediation. *J. Environ. Qual.* **2017**, *31* (3), 904.
- 619 (51) Ragon, M.; Restoux, G.; Moreira, D.; Møller, A. P.; López-García, P. Sunlight-
620 Exposed Biofilm Microbial Communities Are Naturally Resistant to Chernobyl
621 Ionizing-Radiation Levels. *PLoS One* **2011**, *6* (7).

- 622 (52) Lusa, M.; Knuutinen, J.; Lindgren, M.; Virkanen, J.; Bomberg, M. Microbial
623 Communities in a Former Pilot-Scale Uranium Mine in Eastern Finland – Association
624 with Radium Immobilization. *Sci. Total Environ.* **2019**, *686*, 619–640.
- 625 (53) Enyedi, N. T.; Anda, D.; Borsodi, A. K.; Szabó, A.; Pál, S. E.; Óvári, M.; Márialigeti,
626 K.; Kovács-Bodor, P.; Mádl-Szőnyi, J.; Makk, J. Radioactive Environment Adapted
627 Bacterial Communities Constituting the Biofilms of Hydrothermal Spring Caves
628 (Budapest, Hungary). *J. Environ. Radioact.* **2019**, *203* (March), 8–17.
- 629

1 Sequestration of radionuclides Radium-226 and Strontium-90 by
2 cyanobacteria forming intracellular calcium carbonates

3 Neha Mehta¹, Karim Benzerara², Benjamin D. Kocar^{1,3*}, Virginie Chapon⁴

4 ^{1,3} Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge,
5 Massachusetts 02139, United States

6 ² Sorbonne Université, Muséum National d'Histoire Naturelle, UMR CNRS 7590, Institut de Minéralogie, de
7 Physique des Matériaux et de Cosmochimie, IMPMC, 75005 Paris, France

8 ³ Exponent, Inc. 1055 E. Colorado Blvd, Suite 500. Pasadena, California 91106, United States.

9 ⁴ CEA, CNRS, Aix-Marseille Université, UMR 7265 Biosciences and Biotechnologies Institute of Aix-
10 Marseille, 13108 Saint-Paul-lez-Durance, France.

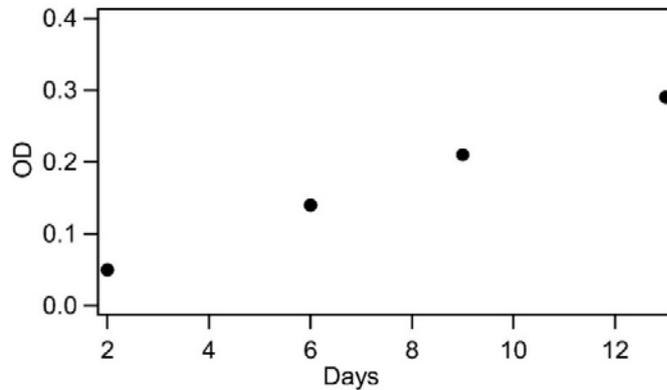
11 *Corresponding Author

12

13 4 Pages; 5 figures

14 Figure S1: Time evolution of OD (circles) of *Synechocystis* PCC 6803 cultures in BG-11 in triplicate.

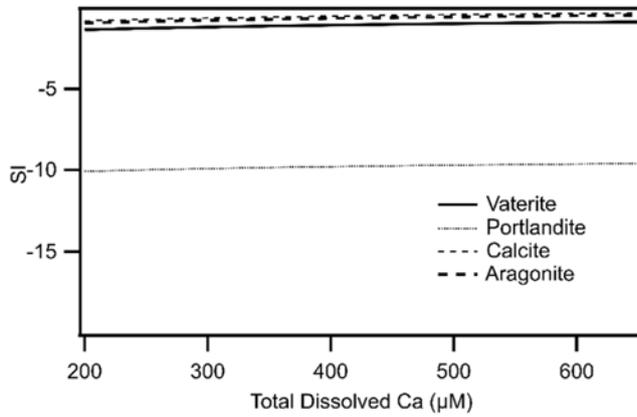
15



16 **Saturation Indices calculation:**

17 Visual MINTEQ (3.0) software package was used to calculate saturation indices of the culture
18 medium with several possible Ca and Sr solids based on the bulk chemical analyses. The
19 cultures were assumed to be in free exchange with the atmosphere with a partial CO₂ pressures
20 of 3.5 atm. Using multi-sweep analysis, saturation indices were calculated for all Ca-
21 carbonate phases reported in the Visual MINTEQ database as a function of dissolved Ca
22 concentration at fixed pH of 8, for cultures amended with ²²⁶Ra. In contrast, saturation indices
23 for all Ca and Sr solids reported in Visual MINTEQ database were calculated as a function of
24 pH and fixed dissolved Ca²⁺ for cultures amended with ⁹⁰Sr.

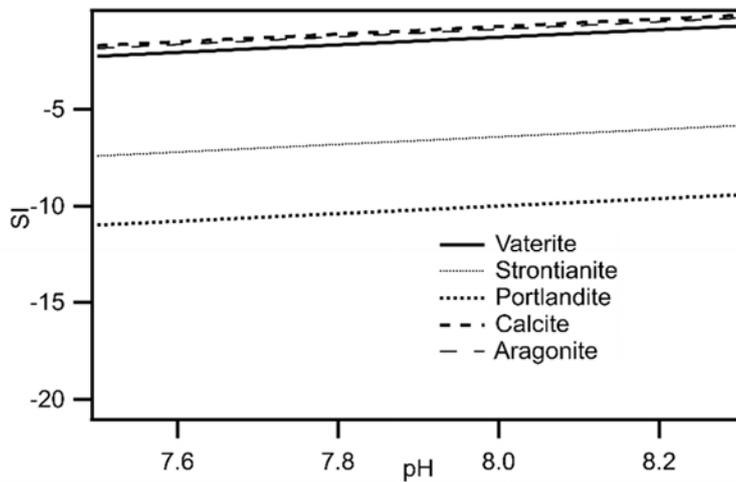
25 Figure S2: The saturation indices of the solution with Ca-carbonate solids that may possibly
26 remove ^{226}Ra are plotted as a function of dissolved total Ca concentration (at fixed pH=8)



27

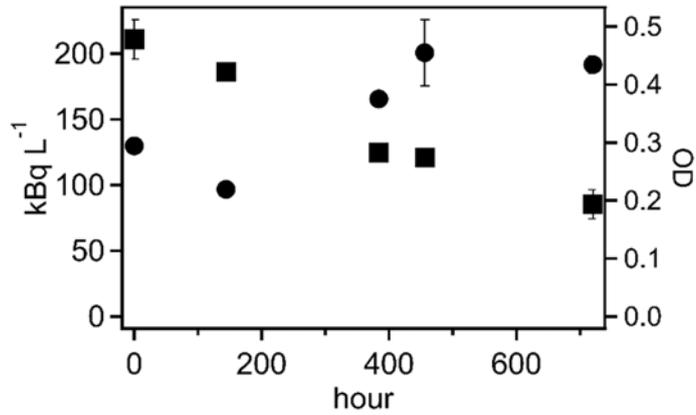
28

29 Figure S3: The saturation indices of the solution with Ca and Sr carbonate solids that may
30 possibly remove ^{90}Sr from solution, are plotted as a function of solution pH (at fixed total
31 dissolved $\text{Ca}^{2+}=250 \mu\text{M}$).



32

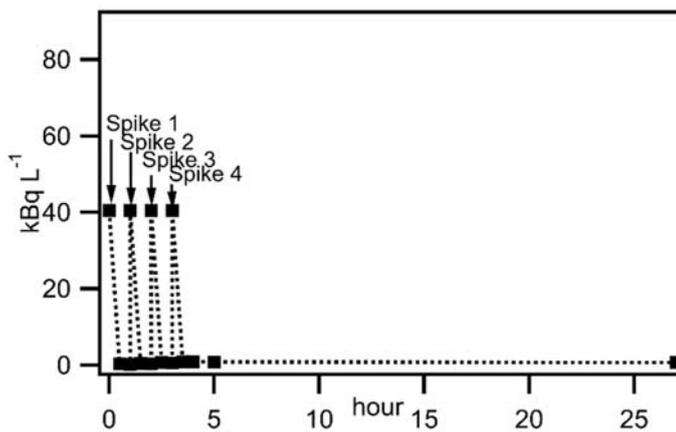
33 Figure S4: Cell grown in BG-11 amended with $210 \text{ kBq L}^{-1} \text{ }^{226}\text{Ra}$, and $6.32 \times 10^{-4} \text{ M Ca}$. The squares
34 denote residual ^{226}Ra activity in the solution and circles denote OD of the cell suspension.



35

36

37 Figure S5: *G. lithophora* cells suspended at OD=0.5 in BG-11 amended with ^{90}Sr . The BG-
38 11 was successively spiked with ^{90}Sr to measure maximum uptake of ^{90}Sr by *G. lithophora*.
39 The total amount of ^{90}Sr uptake measured 160 kBq L^{-1} (40 kBq L^{-1} per spike), which
40 considering an OD of 0.5 equals to $924 \text{ kBq (g of biomass)}^{-1}$



41

42

43