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# Strontium-90 pollution can be bioremediated with the green microalga *Tetraselmis chui*†

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Strontium-90 ($^{90}$Sr) is an artificial radioisotope produced by nuclear fission, with a relatively long half-life of 29 years. This radionuclide is released into the environment in the event of a nuclear incident, posing a serious risk to human and ecosystem health. There is a need to develop new efficient methods for the remediation of $^{90}$Sr, as current techniques for its removal have significant technical limitations and involve high energy and economic costs. Recently, several species of green microalgae within the class Chlorodendrophyceae have been found to form intracellular mineral inclusions of amorphous calcium carbonate (ACC), which can be highly enriched in natural (non-radiogenic) Sr. As bioremediation techniques are an attractive option to address radioactive pollution, we investigated the capacity of the unicellular alga *Tetraselmis chui* (class Chlorodendrophyceae) to sequester $^{90}$Sr. The $^{90}$Sr uptake capacity of *T. chui* cells was assessed in laboratory cultures by monitoring the time course of radioactivity in the culture medium using liquid scintillation counting (LSC). *T. chui* was shown to effectively sequester $^{90}$Sr, reducing the initial radioactivity of the culture medium by up to 50%. Thus, this study demonstrates the potential of the microalga *T. chui* to be used as a bioremediation agent against $^{90}$Sr pollution.

### Environmental significance

This study addresses the urgent issue of strontium-90 pollution, a hazardous radioactive isotope with a relatively long half-life. Human activities, such as nuclear weapons testing and nuclear accidents, have released this radionuclide into the environment, posing significant risks to human and ecosystem health. Current disposal methods are limited and costly. Our research introduces a promising solution using the green microalga *Tetraselmis chui* as an effective bioremediation agent against strontium-90 pollution, offering a sustainable and potentially more efficient approach to mitigate its harmful impacts on the environment and safeguarding the well-being of our ecosystems.

## Introduction

Radioactive contamination of ecosystems is a major environmental problem that poses a serious risk to wildlife and human health.[1] The presence of radioisotopes in the environment can be of natural origin (*e.g.*, weathering of U- or Th-rich minerals) or due to human activities such as mining, nuclear reprocessing activities, detonation of nuclear weapons, and nuclear accidents.[2,3] The radionuclide strontium-90 ($^{90}$Sr) is one of the most hazardous anthropogenic isotopes, with a relatively long half-life ($T_{1/2}$) of 28.91 years (compared to the human lifespan and to other well-known harmful radionuclides such as iodine-131 ($^{131}$I) ($T_{1/2} = 8$ days)).[2] Strontium-90, through $\beta^-$ decay ($Q^- = 546$ keV), is transformed into yttrium-90 ($^{90}$Y) ($T_{1/2} = 64.05$ hours), which in turn decays by $\beta^-$ radiation ($Q^- = 2280$ keV) into stable zirconium-90 ($^{90}$Zr).

Produced by nuclear fission, $^{90}$Sr can be released into the environment and bioaccumulate as it easily moves up the food chain.[4] Nuclear disasters such as Kyshtym (1957), Chernobyl (1986), and Fukushima (2011), as well as past nuclear weapons tests (1945–1980), represent the main source of $^{90}$Sr contamination.[5] Due to its high mobility, hazardous concentrations of this radionuclide have been detected in soils, sediments, waterbodies, fauna, and flora.[6,7] Human exposure to $^{90}$Sr occurs mainly through the ingestion of contaminated food and water.[5] Due to its biochemical similarity to Ca, $^{90}$Sr accumulates in the bone

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marrow, leading to chronic suppression of the immune function, hematopoietic bone marrow destruction, leukemia, and cancer.[8]

Traditional physicochemical techniques to remove radionuclides include processes such as precipitation, coagulation, ion exchange with resins, solvent extraction, selective adsorption, nanofiltration, reverse osmosis, and electrokinetic treatment.[5,9,10] However, these methods have technical limitations and high economic and energy costs, which makes the development of new effective bioremediation techniques very attractive.[11–14]

The microalgae *Scenedesmus spinosus* and *Closterium moniliferum* have been suggested as potential $^{90}$Sr bioremediation agents due to their high capacity to remove stable Sr from their living environment. *S. spinosus* selectively adsorbs Sr on the cell wall, while *C. moniliferum* sequesters Sr by intracellular precipitation of $(Ba,Sr)SO_4$ crystals.[15,16] Recently, many species of green microalgae of the class of Chlorodendrophyceae have been shown to form intracellular inclusions of amorphous calcium carbonate (ACC), called micropearls, that can contain high concentrations of natural Sr.[17] This suggests their possible use for $^{90}$Sr bioremediation purposes. The use of these microalgae would be of particular interest because they live in very diverse aquatic environments and some species such as *Tetraselmis suecica* and *Tetraselmis chui* are already massively cultivated as aquaculture feed.[18–21]

A recent study with laboratory cultures showed a high Sr removal from the culture medium by *T. chui* cells.[22] This high Sr uptake was related to the intracellular formation of micropearls, which supports the idea of considering micropearl-forming microalgae as potential bioremediation agents targeting $^{90}$Sr contamination. However, there is a lack of evidence proving the suitability of *T. chui* and other micropearl-forming species for the development of new bioremediation techniques for the removal of $^{90}$Sr. In particular, the ability of these microorganisms to bioaccumulate this radionuclide and to tolerate ionizing radiation has never been studied and is therefore the subject of this study.

The experiments presented here were conducted with laboratory cultures of *T. chui* amended with different $^{90}$Sr concentrations ranging from $3.1 \times 10^{-12}$ to $2.7 \times 10^{-10}$ M (=1.4 to 124.2 Bq mL$^{-1}$). These values fell within the higher range of $^{90}$Sr concentrations measured in the past in aquatic environments after major nuclear incidents.[6,23–26] The assessment of the $^{90}$Sr uptake capacity of *T. chui* cells was performed by following the time variation of the radioactivity of the culture medium by liquid scintillation counting (LSC).

## Material and methods

### Strains and culture conditions

The strains *Tetraselmis chui* (SAG 8-6) and *Tetraselmis marina* (202.80) were obtained from the Algal Culture Collection of the University of Göttingen, Germany. The algal growth medium[27,28] was prepared with sterilized (autoclaved) seawater from the Adriatic Sea (Lido di Jesolo, northeastern Italy). Salinity was adjusted to 20‰ before adding the algal nutrient solution (1000× concentrated). The natural Sr and Ca concentrations measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in the culture medium were $9 \times 10^{-5}$ and $1.1 \times 10^{-2}$ M, respectively.

*T. chui* was inoculated at a cell density of $2 \times 10^5$ cells per mL$^{-1}$ in 250 mL Erlenmeyer flasks containing 100 mL of algal medium amended with different concentrations of $^{90}SrCl_2$: $3.1 \times 10^{-12}$, $6.2 \times 10^{-11}$, and $2.7 \times 10^{-10}$ M (1.4, 28.2, and 124.2 Bq mL$^{-1}$, respectively). These culture conditions were selected on the basis of the highest $^{90}$Sr concentrations measured in the past in various aquatic environments contaminated with this radionuclide (Table 1). Cultures were established in triplicate and placed for 14-15 days at room temperature on an orbital shaker at 110 rpm and continuous light at 1500 lux intensity. Three types of controls were included in the experimental design based on a similar study performed with cyanobacteria.[29] For all $^{90}$Sr concentrations tested, non-inoculated culture media were used as abiotic controls. As an inactivated control, *T. chui* cells pre-killed by autoclaving were resuspended at a cell concentration of $2 \times 10^5$ cells per mL in culture media containing $3.1 \times 10^{-12}$ and $2.7 \times 10^{-10}$ M $^{90}$Sr. Finally, *T. marina* (SAG 202.80) cultures with an initial cell concentration of $1 \times 10^5$ cells per mL, amended with $3.1 \times 10^{-12}$ M $^{90}$Sr, were used as biotic controls as this species does not form micropearls and, therefore, is not supposed to bioaccumulate high concentrations of Sr. The controls were placed in the culture conditions cited above for *T. chui* cultures (*i.e.*, placed for 14–15 days at room temperature on an orbital shaker at 110 rpm and continuous light at 1500 lux intensity).

### Sampling

Two-mL samples were collected from the cultures and controls every two to four days. One mL was used to follow the growth of the algal cultures, while the remaining volume (1 mL) was used to measure the radioactivity of the culture media and, in some cases, for Scanning Electron Microscopy (SEM) observations.

### Monitoring of algal growth

The cell concentration of the cultures over time was estimated by measuring their optical density at 750 nm ($OD_{750}$) (Thermo Scientific Genesys 30 spectrophotometer). Prior to the measurements, a correlation between $OD_{750}$ and cell density of the *T. chui* cultures was established by performing cell counting in a Neubauer chamber (ESI Fig. S1†).

The cell concentration (cells per mL) was calculated as:

$$\text{Cell concentration} = OD_{750} \times 3.21 \times 10^6 \quad (1)$$

The biomass dry weight (DW) was estimated from $OD_{750}$ after obtaining the corresponding correlation. DW (g L$^{-1}$) was calculated as:

$$DW = OD_{750} \times 7.704 \quad (2)$$

Table 1 Strontium-90 concentrations measured in aquatic environments after major nuclear incidents

| Event | Year of the event | Measured $^{90}$Sr | | Contaminated body of water or groundwater | Year of measurement | Reference |
|---|---|---|---|---|---|---|
| | | (M) | (Bq mL$^{-1}$) | | | |
| Nuclear waste discharge in Techa River (Russia) | 1950–1958 | $6 \times 10^{-11}$ | 27 | Techa River | 1951 | 23 |
| Nuclear waste discharge Lake Karachay and Kishthym disaster (Russia) | 1950–1958 | $2 \times 10^{-11}$ | 8.8 | Aquifer in the vicinity of Lake Karachay | 1994 | 6 |
| | | $1.6 \times 10^{-8}$ | $7 \times 10^{4}$ | Lake Karachay | 1993 | 6 |
| Chernobyl disaster (Ukraine) | 1986 | $6.7 \times 10^{-15}$ to $2.2 \times 10^{-13}$ | $3 \times 10^{-3}$ to 0.1 | Borschi wetlands | 2000 | 24 |
| Fukushima Daiichi disaster (Japan) | 2011 | $2.2 \times 10^{-16}$ to $2.2 \times 10^{-12}$ | $1 \times 10^{-4}$ to 1 | Pacific Ocean near Fukushima Power Plant | 2011–2012 | 25<br>26 |

### Monitoring of strontium-90 uptake by *T. chui*

Strontium-90 uptake by *T. chui* was followed by measuring the radioactivity of the culture medium over time in *T. chui* cultures using liquid scintillation counting (LSC).

**Sample preparation.** One-mL samples of *T. chui* cultures were centrifuged in 1.5 mL Eppendorf tubes at 5000 rpm for 5 min. This centrifugation procedure was effective in obtaining well-defined cell pellets and clear supernatants, indicating a successful separation of the cells from the culture medium. Five hundred μL of the supernatants were accurately weighed using a high precision scale (AX504, Mettler Toledo) and mixed with 15 mL of scintillating solution (Ultima Gold AB, PerkinElmer) in 20 mL liquid scintillation vials. The same procedure was followed for the control cultures.

Samples from cultures amended with $3.1 \times 10^{-12}$ M $^{90}$Sr (1.4 Bq mL$^{-1}$) were measured for two hours using LSC (Packard 2900TR), while cultures amended with $6.2 \times 10^{-11}$ and $2.7 \times 10^{-10}$ M $^{90}$Sr (28.2, and 124.2 Bq mL$^{-1}$, respectively) were measured for one hour. Controls were measured in the same way.

**Strontium-90 and yttrium-90 detection efficiencies calibration.** The efficiency of $^{3}$H detection using LSC as a function of the spectral index of external standard (tSIE) was already known. However, the detection efficiencies of $^{90}$Sr and $^{90}$Y as a function of tSIE had to be calculated. For this purpose, the freeware program CN2003 (ref. 30) was used to simulate the detection efficiencies of $^{90}$Sr and $^{90}$Y as a function of $^{3}$H efficiency. These results, together with the tSIE as a function of $^{3}$H efficiency, were then used to calculate the LSC detection efficiencies of $^{90}$Sr and $^{90}$Y as a function of tSIE and to apply a fourth-degree polynomial fit for each element.

**Estimation of strontium-90 and yttrium-90 activities in the growth medium.** As mentioned above, $^{90}$Sr decays by $\beta^{-}$ radiation into $^{90}$Y, which in turn undergoes $\beta^{-}$ decay into stable $^{90}$Zr. Thus, the total activity in the culture media was the result of both $^{90}$Sr and $^{90}$Y activities. The total photon count obtained from the culture media using LSC was therefore the sum of the $^{90}$Sr and $^{90}$Y activities multiplied by their respective detection efficiencies as expressed in eqn (3).

$$\mathrm{cps}_{\mathrm{tot}}(t) = A^{^{90}\mathrm{Sr}}(t) \times \varepsilon^{^{90}\mathrm{Sr}} + A^{^{90}\mathrm{Y}}(t) \times \varepsilon^{^{90}\mathrm{Y}} \tag{3}$$

where $\mathrm{cps}_{\mathrm{tot}}(t)$ is the total number of photon counts per second obtained by liquid scintillation counting at time $t$, $A^{^{90}\mathrm{Sr}}(t)$ and $A^{^{90}\mathrm{Y}}(t)$ (Bq mL$^{-1}$) are the activities at time $t$ of $^{90}$Sr and $^{90}$Y, respectively, and $\varepsilon^{^{90}\mathrm{Sr}}$ and $\varepsilon^{^{90}\mathrm{Y}}$ their respective detection efficiencies.

Based on calculations using the nuclear science portal NUCLEONICA,[31] we considered that the secular equilibrium between $^{90}$Sr and $^{90}$Y was reached at the start of the experiments ($t = 0$) in the cultures, as more than 17 days elapsed between the production of $^{90}$Sr and the start of the experiments (ESI Fig. S2†). Under this equilibrium condition:

$$A_0^{^{90}\mathrm{Y}} = A_0^{^{90}\mathrm{Sr}} \tag{4}$$

In turn, the activity of a radio element is known to be expressed as eqn (5).

$$A^{\mathrm{X}}(t) = -\frac{\mathrm{d}N^{\mathrm{X}}}{\mathrm{d}t} = N^{\mathrm{X}}(t) \times \lambda^{\mathrm{X}} \tag{5}$$

where $A^{\mathrm{X}}(t)$ (Bq mL$^{-1}$) is the activity of the radioisotope X at time $t$, $N^{\mathrm{X}}(t)$ the number of atoms (atom per mL) of this radionuclide at time $t$ (s), and $\lambda^{\mathrm{X}}$ (s$^{-1}$) its decay constant. Therefore, combination of eqn (3)–(5) allowed to obtain the initial number of $^{90}$Sr atoms ($N_0^{^{90}\mathrm{Sr}}$).

The Bateman equations account for the number of atoms of a parent and daughter (in this case, $^{90}$Sr and $^{90}$Y) as defined in:

$$\frac{\mathrm{d}N^{^{90}\mathrm{Sr}}(t)}{\mathrm{d}t} = -\lambda^{^{90}\mathrm{Sr}} \times N^{^{90}\mathrm{Sr}}(t) \tag{6}$$

$$\frac{\mathrm{d}N^{^{90}\mathrm{Y}}(t)}{\mathrm{d}t} = \lambda^{^{90}\mathrm{Sr}} \times N^{^{90}\mathrm{Sr}}(t) - \lambda^{^{90}\mathrm{Y}} \times N^{^{90}\mathrm{Y}}(t) \tag{7}$$

Here, we assumed that (i) only $^{90}$Sr is taken up by the microalgae and (ii) $^{90}$Y produced inside the cells is not released into the culture medium (to date, no transmembrane transport of Y has been documented in the green microalgae class Chlorodendrophyceae). Hence, the decrease in radioactivity observed in the culture medium is mainly due to $^{90}$Sr removal by the microalgae, which occurs at a much higher rate than the natural decay rate of this radioelement. Therefore, eqn (6) was adapted to calculate the number of $^{90}$Sr atoms ($N^{^{90}\mathrm{Sr}}$) as a function of time as follows:

$$N^{^{90}\mathrm{Sr}}(t) = N_0^{^{90}\mathrm{Sr}} \times \mathrm{e}^{-\alpha^{^{90}\mathrm{Sr}} t} \tag{8}$$

where $\alpha$ ($\mathrm{s}^{-1}$) is the reduction coefficient of $^{90}\mathrm{Sr}$, mainly due to the microalgal uptake of this radioisotope and, to a lesser extent, to the natural decay of $^{90}\mathrm{Sr}$.

By solving the differential eqn (7), the number of $^{90}\mathrm{Y}$ atoms ($N^{^{90}\mathrm{Y}}$) was calculated as a function of time as:

$$N^{^{90}\mathrm{Y}}(t) = N_0^{^{90}\mathrm{Sr}}\left(\frac{\lambda^{^{90}\mathrm{Sr}}}{\lambda^{^{90}\mathrm{Y}}} - \frac{\lambda^{^{90}\mathrm{Sr}}}{\lambda^{^{90}\mathrm{Y}} - \alpha} \times \mathrm{e}^{-\lambda^{^{90}\mathrm{Y}} \times t} + \frac{\lambda^{^{90}\mathrm{Sr}}}{\lambda^{^{90}\mathrm{Y}} - \alpha} \times \mathrm{e}^{-\alpha \times t}\right) \tag{9}$$

After obtaining $N^{^{90}\mathrm{Sr}}(t)$ and $N^{^{90}\mathrm{Y}}(t)$ values, activities for each element were calculated by applying eqn (5).

**Data correction due to evaporation.** The effect of evaporation on the total activity of the culture medium was calculated by measuring the total activity of non-inoculated controls over time. For all $^{90}\mathrm{Sr}$ concentrations tested, the increase in radioactivity due to evaporation of the culture medium was calculated and subtracted from the data set.

**Strontium-90 uptake rate estimation.** Strontium-90 uptake rate (UR) was calculated using a formula applied in previous studies:[22,32]

$$\mathrm{UR}_j = \frac{\dfrac{[\mathrm{X}]_i - [\mathrm{X}]_j}{\overline{\mathrm{CD}}_{ij}}}{t_j - t_i} \tag{10}$$

where $t$ is time (h), $i$ and $j$ are two successive measuring times, $\overline{\mathrm{CD}}$ is the average cell density from time $i$ to $j$, and [X] the concentration of the element X (fmol $\mathrm{mL}^{-1}$).

### Scanning electron microscopy (SEM) observation and energy-dispersive X-ray spectroscopy (EDXS)

Samples for SEM observation were prepared by gently filtering 20 µL of *T. chui* cultures with polycarbonate membranes (Whatman® Nuclepore™) of 1 µm pore size. The membranes were dried at room temperature and placed on aluminum (Al) stubs using a double-sided conductive carbon tape. They were then coated with a 10 nm gold (Au) layer by sputter coating under low vacuum. Imaging and EDXS measurements were performed with a JEOL JSM 7001F Scanning Electron Microscope equipped with an EDXS detector (EX-943000S4L1Q; JEOL). Images were obtained using backscattered electrons. EDXS measurements of micropearls were performed with an accelerating voltage of 15 kV, a beam current of 7 nA, and an acquisition time of 30 s.

## Results and discussion

### Strontium-90 removal and its effect on *T. chui* cell growth

To study the $^{90}\mathrm{Sr}$ uptake by *T. chui* cells, we measured the radioactivity over time of the growth medium of *T. chui* cultures and controls amended with $3.1 \times 10^{-12}$, $6.2 \times 10^{-11}$, and $2.7 \times 10^{-10}$ M $^{90}\mathrm{Sr}$ (1.4, 28.2, and 124.2 Bq $\mathrm{mL}^{-1}$, respectively) (Fig. 1A–C). We also followed the growth of the cultures to determine the influence of cell density on $^{90}\mathrm{Sr}$ removal and, reciprocally, to understand the impact of radioactivity on culture*s* growth (Fig. 1D–F).

As mentioned above, the effect of evaporation (*i.e.*, the increase in radioactivity over time) was corrected for non-inoculated controls. While radioactivity decreased over time in *T. chui* cultures in all three growing conditions (Fig. 1A–C), it remained stable in *T. marina* and *T. chui* dead cell cultures (Fig. 2A, 3 and ESI Fig. S4†).

The total activity measured in *T. chui* cultures amended with $3.1 \times 10^{-12}$ M $^{90}\mathrm{Sr}$ (1.4 Bq $\mathrm{mL}^{-1}$) decreased by half after 15 days of growth (from 2.8 to 1.5 ± 0.7 Bq $\mathrm{mL}^{-1}$), reaching a final estimated $^{90}\mathrm{Sr}$ concentration of $1.7 \times 10^{-12}$ M $^{90}\mathrm{Sr}$ (0.77 Bq $\mathrm{mL}^{-1}$) (Fig. 1A). Cell density was $8.2 \times 10^5 \pm 1.3 \times 10^5$ cell per mL after 15 days of growth (Fig. 1D) and variation between the three replicates was minimal, showing comparable $^{90}\mathrm{Sr}$ removal and culture growth dynamics (ESI Fig. S3†). The $^{90}\mathrm{Sr}$ removal rate was estimated to be maximal the fourth day of growth, with a calculated value of $5.6 \times 10^{-7} \pm 1 \times 10^{-7}$ fmol per cell per day (Fig. 1G). Thereafter, the rate of $^{90}\mathrm{Sr}$ removal progressively decreased on the following days. The average concentration of $^{90}\mathrm{Sr}$ in the dry biomass was $5.5 \times 10^{-2} \pm 3.3 \times 10^{-3}$ ng $\mathrm{g}^{-1}$.

The total radioactivity of *T. chui* cultures with an initial $^{90}\mathrm{Sr}$ concentration of $6.2 \times 10^{-11}$ M (28.2 Bq $\mathrm{mL}^{-1}$) also decreased by 50% after 15 days of growth (from 56 to 27.6 ± 7.3 Bq $\mathrm{mL}^{-1}$), showing a final estimated $^{90}\mathrm{Sr}$ concentration of $2.8 \times 10^{-11}$ M (12.8 Bq $\mathrm{L}^{-1}$) (Fig. 1B). Although the cell concentration after 15 days of growth ($7.2 \times 10^5 \pm 1.8 \times 10^5$ cells per mL) was not significantly lower than in the previous case (Fig. 1E), the variation between replicates was higher than in the previously tested condition. Both cell growth and $^{90}\mathrm{Sr}$ removal were significantly higher in replicate 3 compared to replicates 1 and 2 (ESI Fig. S5†), but $^{90}\mathrm{Sr}$ removal rates were comparable between replicates. The highest $^{90}\mathrm{Sr}$ removal rate was estimated at $5.4 \times 10^{-5} \pm 6.5 \times 10^{-6}$ fmol per cell per day after one day of growth, and also decreased with time (Fig. 1H). The mean concentration of $^{90}\mathrm{Sr}$ in *T. chui* cells was 1.8 ± 0.3 ng per (g dry biomass).

Finally, the total activity measured in *T. chui* cultures amended with $2.7 \times 10^{-10}$ M $^{90}\mathrm{Sr}$ (124.2 Bq $\mathrm{mL}^{-1}$) decreased by 30% after 14 days of growth, from 244.8 to 170.8 ± 51.2 Bq $\mathrm{mL}^{-1}$ (Fig. 1C). The final $^{90}\mathrm{Sr}$ concentration was estimated at $1.95 \times 10^{-10}$ M (89.6 Bq $\mathrm{mL}^{-1}$). In this case, the variation between replicates was the highest observed, especially from the eighth day of growth onwards. After 14 days of growth, the cell density was $5.1 \times 10^5 \pm 2.7 \times 10^5$ cell per mL (Fig. 1F). Although culture growth was lower than that observed in the two previous cases, this difference was not significant due to the large variation between replicates. In fact, replicate 3 had a much higher growth and $^{90}\mathrm{Sr}$ removal rate than replicates 1 and 2 (ESI Fig. S6†). The highest calculated $^{90}\mathrm{Sr}$ removal rate was $5.6 \times 10^{-5} \pm 1.5 \times 10^{-5}$ fmol per cell per day and was observed during the first two days of growth (Fig. 1I). The average concentration of $^{90}\mathrm{Sr}$ in the dry biomass was 6 ± 1.5 ng $\mathrm{g}^{-1}$.

For all $^{90}\mathrm{Sr}$ concentrations tested, the Pearson correlation coefficient showed a strong negative correlation ($p < 0.0001$) between *T. chui* cell density and total radioactivity of the cultures, confirming that $^{90}\mathrm{Sr}$ removal is directly dependent on *T. chui* cell concentration in the cultures (Fig. 1J–L). *T. chui* cultures amended

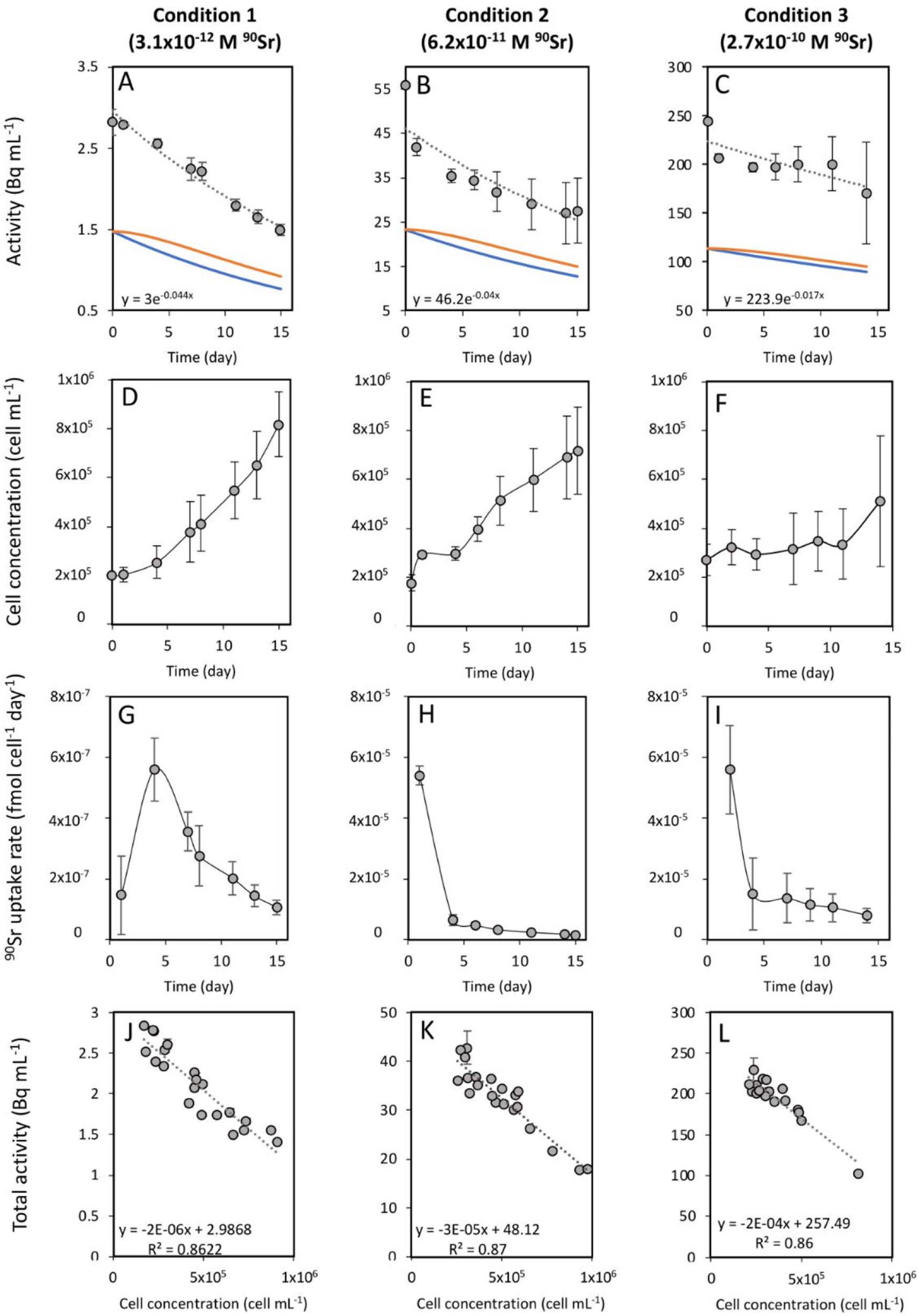

**Fig. 1** (A–C) Time evolution of the activity measured in the growth medium of *T. chui* cultures amended with (A) $3.1 \times 10^{-12}$, (B) $6.2 \times 10^{-11}$, and (C) $2.7 \times 10^{-10}$ M $^{90}$Sr. Grey circles correspond to the total activity of the culture medium. The dashed line represents the calculated exponential reduction fit of the total activity. The corresponding equation appears at the bottom left of each graph. The orange line corresponds to the estimated $^{90}$Y activity and the blue line to the estimated $^{90}$Sr activity. (D–F) Time evolution of the cell concentration of *T. chui* cultures amended with (D) $3.1 \times 10^{-12}$, (E) $6.2 \times 10^{-11}$, and (F) $2.7 \times 10^{-10}$ M $^{90}$Sr. (G–I) Variation over time of the $^{90}$Sr uptake rate by *T. chui* in cultures amended with (G) $3.1 \times 10^{-12}$, (H) $6.2 \times 10^{-11}$, and (I) $2.7 \times 10^{-10}$ M $^{90}$Sr. (J–L) Total activities measured in the growth medium of *T. chui* cultures as a function of their cell concentration (values of the three culture replicates are represented). Cultures were amended with (J) $3.1 \times 10^{-12}$, (K) $6.2 \times 10^{-11}$, and (L) $2.7 \times 10^{-10}$ M $^{90}$Sr. The dashed line corresponds to the estimated linear fit with its respective equation represented in the bottom left area of each graph. The error bars represent the standard deviations ($n = 3$).

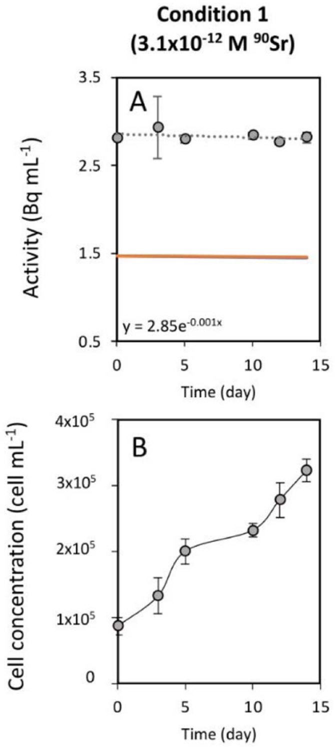

Fig. 2 (A) Time evolution of the activity measured in the growth medium of *T. marina* cultures amended with $3.1 \times 10^{-12}$ M $^{90}$Sr. Grey circles correspond to the total activity of the culture medium. The dashed line represents the calculated exponential reduction fit of the total activity. Its associated equation is expressed on the bottom left area of the graph. The orange line corresponds to the estimated $^{90}$Y activity and the blue line to the estimated $^{90}$Sr activity. In this case, both lines are superposed. (B) Time evolution of the cell concentration of *T. marina* cultures amended with $3.1 \times 10^{-12}$ M $^{90}$Sr. The error bars represent the standard deviations ($n = 3$).

with $2.7 \times 10^{-10}$ M $^{90}$Sr showed the most pronounced decrease in radioactivity as a function of cell density. In fact, the results show that the overall coefficients of radioactivity reduction are proportional to the initial concentration of $^{90}$Sr in the cultures (ESI Fig. S7†). Therefore, higher concentrations of $^{90}$Sr in the culture medium result in higher absorption rates by *T. chui* cells and higher estimated concentrations of $^{90}$Sr in their dry biomass. Further research is needed to determine the maximum $^{90}$Sr uptake capacity of *T. chui*.

### Strontium-90 accumulation mechanism in *T. chui*

Previous studies showed that the uptake of stable Sr by *T. chui* was related to the formation of micropearls, incorporating this alkaline earth metal into these mineral inclusions.[22] In this study, we show that *T. chui* also sequesters radioactive $^{90}$Sr from a seawater culture medium. Furthermore, we observe that, unlike living *T. chui* cells, *T. marina* (a non-micropearl forming species) and dead *T. chui* cells do not sequester $^{90}$Sr. This finding indicates that, as expected, the removal of $^{90}$Sr by *T. chui* must be due to its active uptake for micropearl formation. This suggests that other micropearl-forming species within the class Chlorodendrophyceae may also sequester $^{90}$Sr.

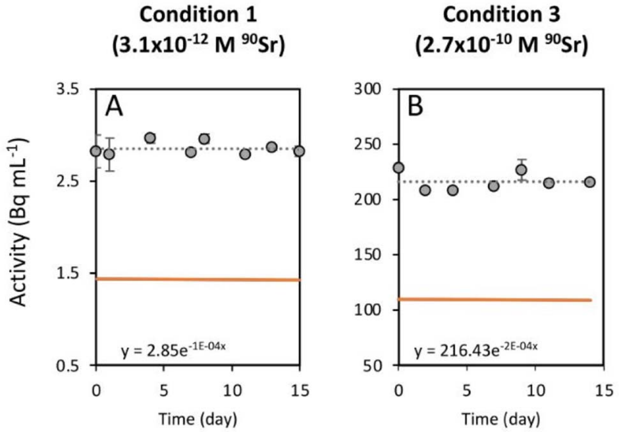

Fig. 3 Time evolution of the activity measured in a growth medium where dead *T. chui* cells were resuspended. The growth medium was amended with (A) $3.1 \times 10^{-12}$ M and (B) $2.7 \times 10^{-10}$ M $^{90}$Sr. Grey circles correspond to the total activity of the culture medium. The dashed line represents the calculated exponential reduction fit of the total activity. The corresponding equation is shown on the bottom left area of each graph. The orange line corresponds to the estimated $^{90}$Y activity and the blue line to the estimated $^{90}$Sr activity. In both cases lines are superposed. The error bars represent the standard deviations ($n = 3$).

### Effect of radioactivity in *T. chui* cell growth variability

The greater variation in growth observed between culture replicates with higher $^{90}$Sr concentrations, and especially with the highest concentration tested $^{90}$Sr ($2.7 \times 10^{-10}$ M = 124.2 Bq mL$^{-1}$), could indicate that, at these concentrations, $\beta^-$ radiation can alter the viability of microalgal cells. It is known that $^{90}$Sr and $^{90}$Y can affect plants, triggering growth abnormalities, as well as decreasing grain yield and seed viability[33,34] but, unfortunately, the effect of radiation on microalgae has hardly been studied in the past. A study in which the green microalga *Chlorella vulgaris* was cultured with 200 and 2000 Bq mL$^{-1}$ $^{90}$Sr showed that its growth was not altered at these concentrations.[35] However, in that study, most of the $^{90}$Sr did not penetrate the cells but was deposited on the cell wall, forming carbonates. In a study following the uptake of $^{90}$Sr by the cyanobacterium *Gloeomargarita lithophora* (which forms similar Sr-rich intracellular ACC inclusions), a possible toxic effect on the growth of cultures with $8.8 \times 10^{-11}$ M (39.7 Bq mL$^{-1}$) $^{90}$Sr was alluded to.[29] It is conceivable that intracellular accumulation of $^{90}$Sr in micropearls could cause cell damage due to the delivery of higher doses of $\beta^-$ radiation into the cells. It is important to note, however, that the lowest concentrations of $^{90}$Sr tested in this study are those that best correspond to actual contamination levels in aquatic environments (except in cases of extreme contamination such as that observed in Lake Karachay, Russia) (Table 1). Overall, the fact that some replicate cultures of *T. chui* grew even with high concentrations of $^{90}$Sr in the medium is a promising result, as it demonstrates that this species can accommodate high levels of ionising radiation.

Finally, the greater variability between replicates observed in this study compared to a previous study with non-radioactive Sr (reference) may not be entirely due to radioactivity but to suboptimal culture conditions. Indeed, the use

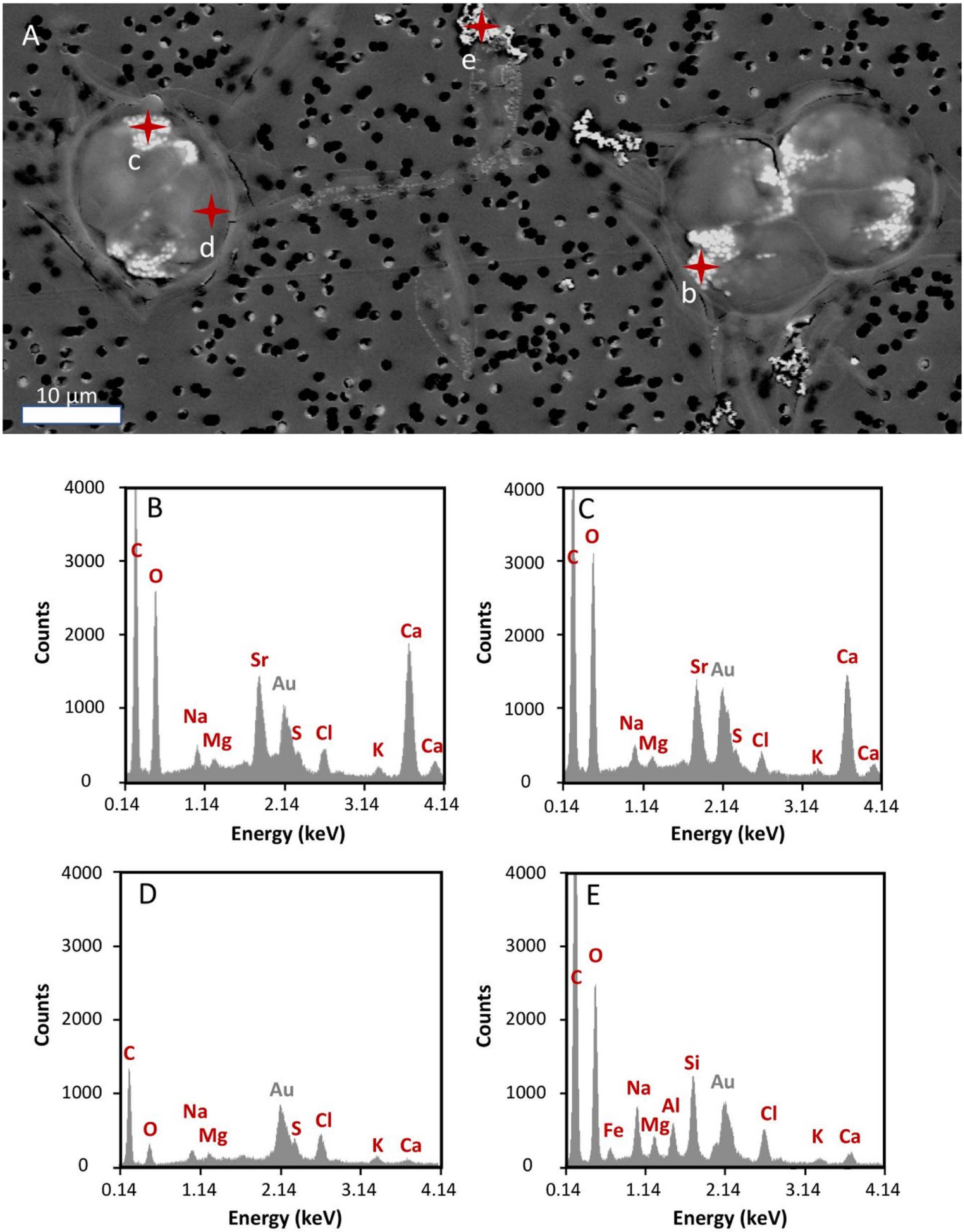

Fig. 4 (A) SEM image of *T. chui* cells grown for four days in a culture medium amended with $3.1 \times 10^{-12}$ M $^{90}$Sr. Three dividing cells are observed. Micropearls appear as bright white inclusions (<1 µm) in the anterior part of each daughter cell. Black dots correspond to the pores of the membrane used to prepare the sample. Red marks correspond to the EDXS analysis points. (B and C) EDXS spectra of *T. chui* micropearls (analysis points b and c, respectively). (D) EDXS spectrum of a cell area free of micropearls (analysis point d). (E) EDXS spectrum of a sea-salt particle present in the culture medium (analysis point e).

of a radioactive isotope made it necessary to work in a laboratory adapted to the handling of radioactive materials but where conditions were not optimal for microalgae cultivation.

### Effect of radioactivity in *T. chui* cell morphology and Sr accumulation

To prevent instrument contamination, we only analysed cell samples obtained from cultures with the lowest $^{90}$Sr concentration tested in this study (*i.e.*, $3.1 \times 10^{-12}$ M $^{90}$Sr). *T. chui* cells grown for four days in cultures amended with this $^{90}$Sr concentration were observed using SEM in order to detect any possible abnormality in cells morphology and in the process of micropearl formation due to $\beta^-$ radiation (Fig. 4). At this $^{90}$Sr concentration, the cells did not show any alteration and micropearls were present. In fact, even several cells were observed in the dividing stage. The size and shape of the micropearls corresponded to that observed in previous studies.[22] By performing EDXS analysis on the micropearls, we obtained their Sr/Ca mol% ratios (Fig. 4 and ESI Fig. S8†). The mean value was $0.32 \pm 0.09$ ($n = 18$, from 14 different cells). This value is comparable with the one obtained from *T. chui* cultures amended with stable Sr ($0.42 \pm 0.1$),[22] knowing that a strict comparison is precluded because of slightly different initial concentration in the culture media ($9 \times 10^{-5}$ M (stable) Sr and $1.1 \times 10^{-2}$ M Ca here, and $1 \times 10^{-4}$ M (stable) Sr, $2.5 \times 10^{-3}$ M Ca in the previous study).

### Is *T. chui* a plausible strontium-90 bioremediation agent?

At all $^{90}$Sr concentrations tested in this study, *T. chui* cultures reaching a cell density of approximately $1.5 \times 10^6$ cells per mL would be sufficient to remove more than 95% of the initially dissolved $^{90}$Sr (Fig. 1J and K). Since the present study demonstrates that $^{90}$Sr removal is directly related to the concentration of *T. chui* cells in the cultures and it has been shown that *T. chui* cell density can reach values above $3.2 \times 10^6$ cells per mL when culture conditions are optimized,[36] one can expect to obtain higher $^{90}$Sr removal in the cultures if cell growth is improved.

The use of species within the class Chlorodendrophyceae for the development of new $^{90}$Sr decontamination techniques has many clear advantages compared to the use of other organisms suggested for this purpose in the past, such as plants,[37,38] cyanobacteria,[29,39] and bacteria,[40,41] namely:

- The possibility of using organisms adapted to very diverse aquatic environments: Sr uptake by species of Chlorodendrophyceae is associated with their ability to form micropearls,[22] this ability being shared by species living in very different habitats, including hypersaline water, seawater, brackish water and freshwater.[17,20,42]
- *T. chui* and other species such as *Tetraselmis suecica* also have high growth performances over a wide range of salinities and light intensities, which further increases their field of application.[21,36,43,44]
- These species can be highly resistant to bacteria and cyanobacteria contamination, which is an essential requirement for these organisms to be used for the treatment of non-sterile water.[45–47]
- An advantage of using microalgae over other types of organisms is their rapid growth and the low energy cost associated with their production, as natural sunlight can be used as the main source of energy. Microalgae of the genus *Tetraselmis* are already largely produced in mass culture systems for aquaculture purposes, as well as for biodiesel production, $CO_2$ mitigation, and wastewater treatment.[48–51] Currently, several outdoor infrastructures (photobioreactors) for large-scale production of *Tetraselmis* (including *T. chui*) already exist and could serve as a basis for the future development of ex-situ $^{90}$Sr decontamination systems -based on *Tetraselmis*.[51,52]

## Conclusions

This study shows for the first time the ability of *Tetraselmis chui* to sequester radioactive $^{90}$Sr from a culture medium mainly composed of seawater, this capacity being related to the intracellular formation of micropearls. Strontium-90 removal depends on *T. chui* cell density in the cultures, as well as on the initial $^{90}$Sr concentrations in the medium. Although culture growth is not affected at $^{90}$Sr concentrations of $3.1 \times 10^{-12}$ M (1.4 Bq L$^{-1}$), we see a heterogeneous effect on *T. chui* cultures containing higher $^{90}$Sr concentrations that may be related to high levels of ionizing radiation.

Overall, our results clearly support the possibility of achieving complete $^{90}$Sr removal if *T. chui* growth is optimized. These results are highly relevant as *T. chui* is a robust species, easy to grow on a large scale and, therefore, particularly attractive for the development of new bioremediation techniques. Moreover, since $^{90}$Sr uptake is related to micropearl formation, it is likely that other micropearl-forming species could be used for the same purpose.

## Author contributions

I. Segovia-Campos (conceptualization, data curation, formal analysis, investigation, methodology, resources, visualization, validation, writing – original draft, writing – review and editing); A. Kanellakopolulos (conceptualization, data curation, formal analysis, investigation, methodology, resources, visualization, validation); I. J. Barrozo (data curation, formal analysis, investigation, methodology, validation); E. Fock-Chin-Ming (data curation, formal analysis, investigation); M. Filella (conceptualization, funding acquisition, methodology, supervision, validation, writing – original draft, writing – review and editing); A. Baxarias Fontaine (conceptualization, data curation, formal analysis, investigation, methodology, resources); S. Pallada (methodology, resources, supervision), G. Triscone (methodology, resources, supervision), K. Perron (methodology, resources, supervision); D. Ariztegui (conceptualization, funding acquisition, supervision, validation, writing – original draft, writing – review and editing).

## Conflicts of interest

There are no conflicts to declare.

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