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## Investigating the interaction of uranium(VI) with diatoms and their bacterial community: A microscopic and spectroscopic study

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### ABSTRACT

Diatoms and bacteria play a vital role in investigating the ecological effects of heavy metals in the environment. Despite separate studies on metal interactions with diatoms and bacteria, there is a significant gap in research regarding heavy metal interactions within a diatom-bacterium system, which closely mirrors natural conditions. In this study, we aim to address this gap by examining the interaction of uranium(VI) (U(VI)) with *Achnanthesidium saprophilum* freshwater diatoms and their natural bacterial community, primarily consisting of four successfully isolated bacterial strains (*Acidovorax facilis*, *Agrobacterium fabrum*, *Brevundimonas mediterranea*, and *Pseudomonas peli*) from the diatom culture. Uranium (U) bio-association experiments were performed both on the xenic *A. saprophilum* culture and on the four bacterial isolates. Scanning electron microscopy and transmission electron microscopy coupled with spectrum imaging analysis based on energy-dispersive X-ray spectroscopy revealed a clear co-localization of U and phosphorus both on the surface and inside *A. saprophilum* diatoms and the associated bacterial cells. Time-resolved laser-induced fluorescence spectroscopy with parallel factor analysis identified similar U(VI) binding motifs both on *A. saprophilum* diatoms and the four bacterial isolates. This is the first work providing valuable microscopic and spectroscopic data on U localization and speciation within a diatom-bacterium system, demonstrating the contribution of the co-occurring bacteria to the overall interaction with U, a factor non-negligible for future modeling and assessment of radiological effects on living microorganisms.

### 1. Introduction

Diatoms and bacteria, co-existing in abundance in any living space on Earth, are key bioindicators for assessing the ecological health in waters and wetland sediments that are contaminated by heavy metals (Custodio et al., 2022; Li et al., 2020; Pandey et al., 2014, 2017; Saxena

et al., 2021), or radionuclides (such as uranium (U)) (Herlory et al., 2013; Mondani et al., 2011; Sutcliffe et al., 2017; Xiao et al., 2019).

Various studies have focused on the possible bacteria-diatoms interactions (Amin et al., 2015, 2012), among which the symbiotic interactions have received increasing attention and have been reported since the last century (Grossart, 1999). The phycosphere is an important

**Abbreviations:** ASV, amplicon sequence variant; CRTD, Center for Regenerative Therapies Dresden; DM, diatom medium; DNA, deoxyribonucleic acid; DOM, dissolved organic matter; EDX, energy-dispersive X-ray spectroscopy; EPS, extracellular polymeric substances; HAADF-STEM, high-angle annular dark-field scanning transmission electron microscopy; HEPES, 4–2-hydroxyethyl-1-piperazineethanesulfonic acid; LM, lysogeny broth medium; PARAFAC, parallel factor analysis; PCR, polymerase chain reaction; PFA, paraformaldehyde; rRNA, ribosomal ribonucleic acid; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TRLFS, time-resolved laser-induced fluorescence spectroscopy; U, uranium; U(VI), uranium in the hexavalent oxidation state; v/v, volume percentage.

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notion for interactions between diatoms and bacteria in aquatic systems. It is defined as the surrounding microenvironment of an algal cell, where the growth of bacteria within this region is stimulated by the extracellular products released by the algal cell (Bell and Mitchell, 1972). There exist various interactions between heterotrophic bacteria and diatoms within the phycosphere, such as nutrient exchange, signal transduction and gene transfer (Amin et al., 2012; Kouzuma and Watanabe, 2015). In fact, the interaction between diatoms and bacteria may lead to the secretion of extracellular polymeric substances (EPS), resulting in cell aggregations and further biofilm formation (Bruckner et al., 2011; Gärdes et al., 2011; Windler et al., 2015). Moreover, diatoms can produce dissolved organic matter (DOM) as carbon source for the bacteria living in symbiosis within the phycosphere (Daly et al., 2021).

The mechanisms of the interaction of heavy metals with bacteria and diatoms have been well investigated over the past decades, including cell surface adsorption, metabolic uptake, biomineralization (induction of metal precipitation), and biotransformation (microbial oxidation and reduction of metal species) (Gadd, 2004, 1990; Gerber et al., 2016; Haferburg and Kothe, 2007; Kiran Marella et al., 2020; Ledin, 2000; Xiao et al., 2023). Besides, some studies reported the positive effect of the symbiotic interaction between diatoms and bacteria on the tolerance of these microbes towards heavy metal toxicity (Thomas and Robinson, 1987; You et al., 2021).

Currently, to the best of our knowledge, there has been little research focused on the molecular-level interaction between metal ions and diatoms that are symbiotically associated with bacteria (Pokrovsky et al., 2008). This raises a crucial question: what is the role of these bacteria in the comprehensive retention of metal ions within the phycosphere in a bacterial-diatom complex system?

This question is at the heart of this investigation, with a specific focus on uranium (U) as the metal of interest. U is a naturally occurring radioelement that can be found in soils, waters, and sediments. The chemical toxicity and radiotoxicity of U may pose a threat to the ecological health, especially when U enters the food chain (Carvalho et al., 2023). More specifically, we are focusing on a xenic *Achnanthes saprophilum* (*A. saprophilum*) system interacting with hexavalent U (U(VI)), where an interaction has been recently demonstrated through adsorption and internalization processes (He, 2023). In this study, our objective is to go a step ahead and identify the role of bacteria in these processes in the xenic *A. saprophilum* culture.

Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and spectrum imaging analysis based on energy-dispersive X-ray spectroscopy (EDX) were applied to localize U in the U(VI)-exposed xenic *A. saprophilum* culture. Moreover, U(VI) speciation in the exposed diatom culture was studied by time-resolved laser-induced fluorescence spectroscopy (TRLFS) coupled with parallel factor (PARAFAC) analysis (Drobot et al., 2015) and compared to that in U(VI)-exposed single-strain bacterial cultures isolated from the xenic diatom culture. The contribution of the isolated bacterial strains to the overall U interaction with the xenic culture of *A. saprophilum* is discussed.

## 2. Materials and methods

### 2.1. Diatom cultivation and taxonomy identification

The *A. saprophilum* diatoms were originated from water samples collected from Lac d'Aydat, a fresh water lake located in Puy-de-Dôme, France (45.663996°N 2.984505°E). The collected sample was purified using flow cytometry sorting (BD FACSAria™ Fusion Flow Cytometer, BD Biosciences) to get a single diatom species culture, which was cultivated in Diatom Medium (DM, medium proposed by Culture Collection of Algae and Protozoa) using culture flasks (Falcon, Fisher Scientific). The cultures were incubated at 20 °C (Lovibond Tintometer TC 135 S) with a day:night cycle of 12 h:12 h and with a constant horizontal agitation of 50 rpm (Unimax 1010, Heidolph). Every 15 days,

the diatom cells were transferred into fresh DM to maintain continuous diatom growth. Note that it was not possible to obtain an axenic culture of diatoms using flow cytometry, and bacteria originated from the lake are present in the diatom culture.

To verify the purity and taxonomy of the diatom culture, 18S rRNA genes amplicon sequencing using the next-generation sequencing approach was applied. The detailed description on the utilized protocol (from DNA extraction to diatom taxonomy assessment) is given in the [supplementary information](#). The taxonomy assignment of the diatoms was further supported by morphology examinations using SEM.

### 2.2. Bacterial diversity and isolation from diatom culture

To assess the bacterial diversity in the xenic diatom culture, amplicon sequencing targeting the V4 region of the 16S rRNA genes was applied (Walters et al., 2016), and the generated amplicon sequence variants (ASVs) from DADA2 (version 1.26) were subjected to the Silva SSU database 138.1 release (Quast et al., 2012) to obtain the taxonomy of the entire bacterial community. The detailed information, including the polymerase chain reaction (PCR) conditions and primers sets for the 16S rRNA genes amplification, together with the bioinformatic analyses, is described in the [supplementary information](#).

Moreover, to evaluate the potential contribution of the bacteria present in the diatom culture to the overall interaction with U, an attempt was made to select and isolate cultivable bacterial genera using a culture-dependent approach. Firstly, 10 µL of diluted diatom culture were spread on an agar plate comprising DM or 10 % lysogeny broth (LB) medium (see [Table S1](#) in the [supplementary information](#) for medium composition). Secondly, after several days of incubation at 25–29 °C, the formation of the bacterial colonies was observed on both types of agar plates, and colonies displaying different morphologies were selected and inoculated onto new agar plates. Finally, the streak plate technique was performed several times to obtain single bacterial colonies. The purified bacterial colonies were transferred into sterile Erlenmeyer flasks comprising liquid DM or 10 % LB medium and incubated on a shaker (KS 501 digital, IKA) at 95 rpm at 25 °C to obtain pure bacterial cultures. To identify the taxonomy of these bacterial isolates, nearly the full length of the 16S rRNA gene of each bacterial isolate was sequenced (detailed information is provided in the [supplementary information](#)).

### 2.3. U bio-association experiment

U bio-association experiments were performed both on the xenic diatom culture and on the single-strain bacterial isolate cultures (including the three most abundant bacterial strains in the xenic diatom culture) in a 2 mM NaNO<sub>3</sub> (>99 %, Sigma Aldrich) solution buffered with 3 mM of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, >99.5 %, Duchefa) at pH 7.0, with an exposure to 2 µM of uranyl (VI) nitrate for 45 min at room temperature. Note that the diatom sample was exposed to a higher U(VI) concentration of 10 µM for TEM-based investigations to obtain a better EDX-based spectrum imaging analysis quality. At the end of the experiment, the samples were centrifuged (2600×g, 10 min for diatoms and 10000×g, 5 min for bacteria; centrifuge 5804R, Eppendorf) and the resulting cell pellets were washed with ultrapure water.

### 2.4. Scanning electron microscopy, transmission electron microscopy and energy-dispersive X-ray spectroscopy

To prepare the SEM specimens, one drop of the U-free diatom suspension was transferred on a SEM pin stub followed by the addition of 2.5 % glutaraldehyde (25 %, SERVA Electrophoresis GmbH) solution prepared in 0.1 M phosphate buffer (10.107 g of Na<sub>2</sub>HPO<sub>4</sub>·7 H<sub>2</sub>O (≥98 %, ACS reagent, Merck) and 1.697 g of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (≥98 %, ACS reagent, Merck) in 500 mL of ultrapure water). The specimens were

consecutively dehydrated with a series of aqueous ethanol ( $\geq 99.8\%$ , HPLC grade, Fisher Scientific) solutions of increasing ethanol concentrations (20 %, 40 %, 60 %, 80 %, and 98 % v/v) followed by the addition of 1,1,1,3,3,3-hexamethyldisilazane (Carl Roth GmbH + Co. KG) at the end. The specimens were gold-coated with a sputter coater (Sputter Coater 108auto, Cressington) at 30 mA for 40 s to obtain an electrically conducting cap layer. The diatom specimens were analyzed with a scanning electron microscope (EVO 50, Carl Zeiss AG) operated at an accelerating voltage of 15.0 kV.

To prepare TEM specimens, diatom cells resulted from the U bio-association experiments were washed with Milli-Q water and transferred into fixing solution (pH 6.9) made of 4 % paraformaldehyde (PFA, pure; Carl Roth) and 0.1 M HEPES. Prior to the TEM-based analyses, thin sections of diatom cells were prepared at the Center for Regenerative Therapies Dresden (CRTD). The detailed preparation protocol is presented in Table S5 in the supplementary information. Bright-field TEM micrographs of diatom thin sections were recorded using an image-C<sub>s</sub>-corrected Titan 80–300 microscope (FEI) operated at an accelerating voltage of 300 kV. Selected areas of interest were further analyzed by high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) and spectrum imaging analysis based on energy-dispersive X-ray spectroscopy (EDX) with a Talos F200X microscope equipped with a high-brightness X-FEG electron source and a Super-X EDX detector system (FEI) using an accelerating voltage of 200 kV.

### 2.5. Time-resolved laser-induced fluorescence spectroscopy

TRLFS measurements were performed on the diatoms and bacterial isolate samples originated from the U bio-association experiment. The washed cells were transferred into disposable plastic cuvettes (half-micro, Rotilabo XK26.1, Carl Roth), frozen in liquid nitrogen, and stored at  $-80\text{ }^{\circ}\text{C}$  prior to the TRLFS measurement. The detailed information on the laser system as well as the measuring conditions are described in the work of Rajabi et al., (2021). Parallel factor (PARAFAC) analysis was done on the obtained TRLFS spectra using the MATLAB 6.0 software (The Mathworks Corporation) (Drobot et al., 2015).

### 2.6. Data availability

The raw sequences of the 18S and 16S amplicons generated from next-generation sequencing were deposited on NCBI under the accession numbers SAMN37801304 and SAMN37801305, respectively. The 16S sequences of four bacterial isolates were deposited to NCBI with the following accession numbers: *A. facilis* DM\_C\_1 (OR668907), *A. fabrum* LB\_B\_1 (OR668909), *B. mediterranea* LB\_T\_2\_1 (OR668908) and *P. peli* LB\_C\_1 (OR668910).

## 3. Results and discussion

### 3.1. Diatom purity and bacterial diversity

The 18S amplicon sequencing shows that only four ASVs ( $\sim 400$  bp) were generated from the diatom culture, with a relative abundance of

**Table 1**  
18S amplicon sequence variants analyses for diatom culture.

ASVs	Read counts	Relative abundance (%)	Taxonomy	18S sequence identity (%)
ASV_1	32387	87.5	<i>Achnantheidium saprophilum</i>	100.00
ASV_2	3594	9.7	<i>Achnantheidium saprophilum</i>	99.75
ASV_3	905	2.4	<i>Achnantheidium saprophilum</i>	99.75
ASV_4	120	0.3	<i>Achnantheidium saprophilum</i>	99.51

87.5 % for the most dominant ASV (Table 1). Based on the NCBI Nucleotide BLAST results, the taxonomy of the diatoms in culture is associated to the genus *Achnantheidium*. Moreover, ASVs displays high identity to partial 18S rRNA genes of *A. saprophilum* (GenBank: MN602031.1). The primer set used (i.e., D512f and D978r, targeting the V4 region of the 18S rRNA genes) has been tested with 123 diatom taxa with various genetic distance (uncorrected *p*-distance), providing sufficient taxa resolution at species level (Zimmermann et al., 2011). In addition, the morphology of the diatoms in culture was examined using SEM and compared with several species reported (Hlúbíková et al., 2011). The morphological characteristics of the diatoms in culture are globally in line with those of *A. saprophilum* (He, 2023). However, some changes in length of the diatom's valves as well as the number of striae were observed compared to the type material, which might be due to the applied culture conditions. Consequently, the diatom species in the xenic culture is very likely *A. saprophilum* with high purity.

Moreover, the bacterial community in the *A. saprophilum* diatom culture displays a low diversity, comprising only 14 sequences of ASVs (Table 2). Note that a high abundance of chloroplast sequence originating from *A. saprophilum* was filtered out and is not shown in the table. This diatom-associated bacterial community is dominated by the genera *Pseudomonas*, *Acidovorax*, *Brevundimonas* and *Flavobacterium* with relative abundances of 33.10 %, 21.82 %, 17.38 % and 12.55 %, respectively. Other minority genera (abundance  $<10\%$ ) include *Limnobacter*, *Tabrizicola*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, and uncultured bacteria.

### 3.2. Isolation and identification of bacteria living in the diatom culture

Bacterial colonies were observed on both types of agar plates at initial incubation but the quantity of colonies on the DM agar plates was lesser. Moreover, the formation of single colonies on the DM plates during the purification step was barely observed, and almost no growth was observed when these colonies were inoculated in liquid DM. On the contrary, single colonies with different morphologies were clearly formed on agar plates comprising 10 % LB medium, among which four representative colonies (based on the colony morphology) were selected, isolated, and cultivated in 10 % LB liquid medium accordingly.

The taxonomy of these four bacterial isolates was identified based on the nearly full length of the 16S rRNA genes and the sequences show very high identity to *Acidovorax facilis* CCUG 2113 (99.6 %), *Agrobacterium fabrum* C58 (100 %), *Brevundimonas mediterranea* V4.BO.18 (100 %), and *Pseudomonas peli* R-20805 T (100 %). Thus, our bacterial isolates are designated as *A. facilis* DM\_C\_1, *A. fabrum* LB\_B\_1, *B. mediterranea* LB\_T\_2\_1 and *P. peli* LB\_C\_1. Furthermore, the presence of these bacterial genera are congruent with the bacterial community identified using the 16S amplicon sequencing, noticing that in the SILVA 16S database, the genus *Agrobacterium* is classified as the combined affiliation *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (Lu and Salzberg, 2020).

In liquid culture, the four bacterial isolates grew only in 10 % LB medium, which contains, in contrast to DM, organic carbon sources. This suggests that the isolated bacterial strains are organotrophs. As reported in the literature, the dissolved organic matter (DOM) released by diatoms appears to attract bacteria towards the phycosphere via chemotaxis mechanism, resulting in bacterial colonization within the phycosphere. In turn, bacteria may also be beneficial to diatoms by providing micronutrients, such as nitrogen, vitamin B12 and iron (Amin et al., 2012). Therefore, the four isolated bacterial strains seem to rely on the DOM released by the diatoms in the xenic *A. saprophilum* culture. Given that the studied diatom culture was originated from a natural environment, it is likely that a symbiosis mechanism occurs in the xenic culture of *A. saprophilum* between the diatoms and the co-occurring bacterial community. In the literature, it has been demonstrated that the *A. fabrum* C58 isolate is able to synthesize siderophores in response to the iron deficiency (Rondon et al., 2004; Vinnik et al., 2021).



**Table 2**  
16S amplicon sequence variants analyses for bacterial community.

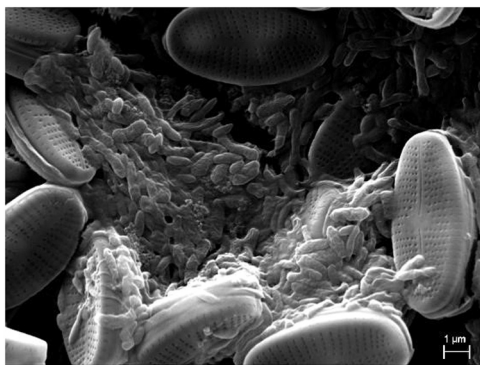
ASVs	Kingdom	Phylum	Class	Order	Family	Genus	Read counts	Relative abundance (%)
ASV_1	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	39616	33.10
ASV_2	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	<i>Acidovorax</i>	26117	21.82
ASV_3	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Brevundimonas</i>	20806	17.38
ASV_4	Bacteria	Bacteroidota	Bacteroidia	Bacteroidiales	Flavobacteriaceae	<i>Flavobacterium</i>	15021	12.55
ASV_5	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	<i>Limnobacter</i>	10044	8.39
ASV_6	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	<i>Tabrizicola</i>	3170	2.65
ASV_7	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	<i>Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium</i>	1538	1.28
ASV_8	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria	1410	1.18
ASV_9	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Phenylbacterium</i>	1259	1.05
ASV_10	Bacteria	Actinobacteriota	Actinobacteria	Propionibacteriales	Nocardioidaceae	<i>Aeromicrobium</i>	691	0.58
ASV_11	Bacteria	Uncultured bacteria	Uncultured bacteria	Uncultured bacteria	Uncultured bacteria	Uncultured bacteria	8	0.01
ASV_12	Bacteria	Uncultured bacteria	Uncultured bacteria	Uncultured bacteria	Uncultured bacteria	Uncultured bacteria	5	0.00
ASV_13	Bacteria	Uncultured bacteria	Uncultured bacteria	Uncultured bacteria	Uncultured bacteria	Uncultured bacteria	5	0.00
ASV_14	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	3	0.00

Moreover, *A. facilis* was reported to be actively involved in the aggregation of diatoms that provided in turn a suitable microenvironment for the former (Knoll et al., 2001).

### 3.3. Visualization of bacterium-diatom communities and localization of uranium

The presence of bacteria in diatom specimens was verified via SEM observation. Fig. 1 shows bacterial clusters on and between diatoms. In this work, it is reasonable to assume that the xenic diatom samples used in all the experiments are always accompanied by associated bacteria. Nevertheless, it is not possible for the moment to estimate the bacterial content present in the diatom culture.

TEM-based analyses were conducted on an *A. saprophilum* diatom sample exposed to 10  $\mu\text{M}$  U. Apart from the association of U with the diatoms, attention was also drawn on the interaction of U with the bacteria associated to the diatoms. Fig. 2 displays a bright-field TEM micrograph of a thin section of a U-exposed diatom sample along with the corresponding EDX-based element distribution maps for the highlighted regions. The bright-field TEM micrograph (Fig. 2a) indicates that in addition to diatoms, rod-shaped bacteria are present on the diatom surface and in suspension within the thin section. The EDX-based analyses reveal, besides others, the distribution of several elements of interest, such as silicon (Si), osmium (Os), phosphorus (P), and U. The Si distribution map in Fig. 2b clearly differentiates the diatom cell and the bacterium by illustrating the detailed structure of the diatom's unique silica frustule. The structural perforations across the frustule, which are



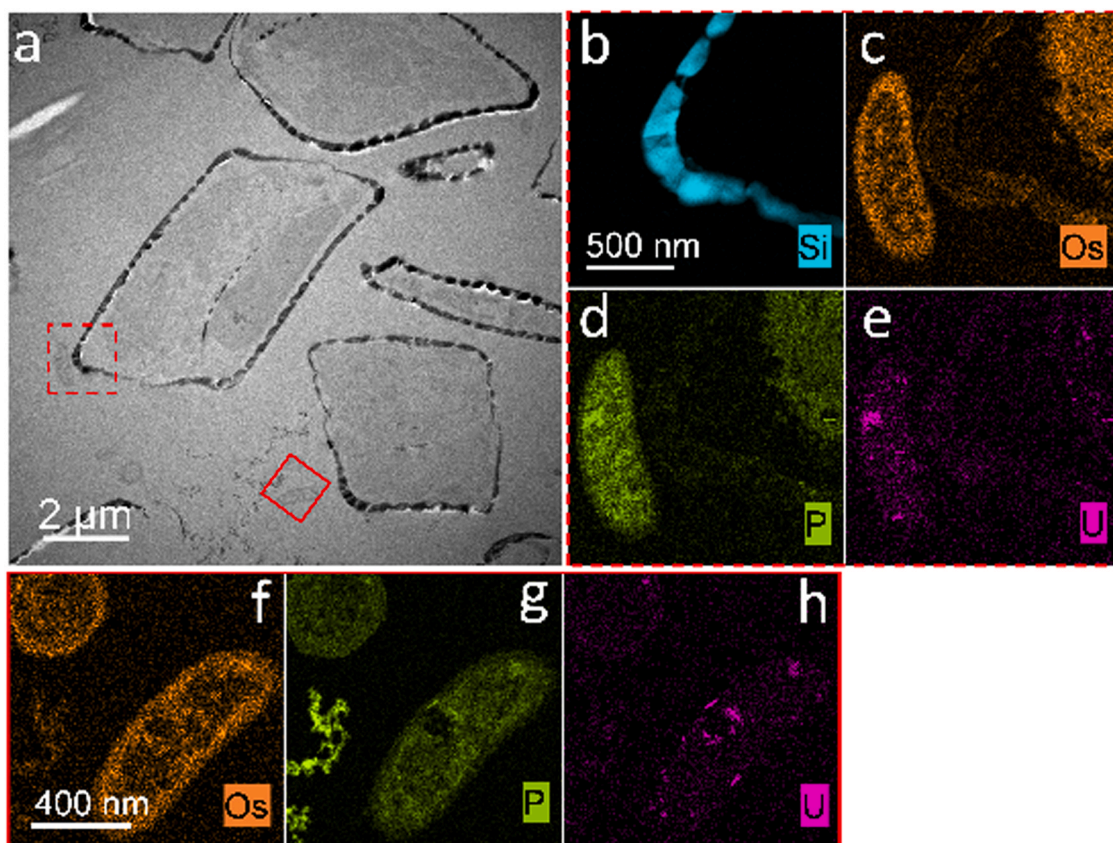
**Fig. 1.** SEM micrograph of a xenic *Achnanthisidium saprophilum* diatom culture specimen. The occurrence of bacterial clusters on the surface of the diatom cells is well illustrated.

called punctae, are also perfectly shown in the Si distribution map. Os originating from staining during the TEM specimen preparation process indicates the cell membrane structure of both diatom and bacterial cells. Comparing Fig. 2b and 2c, a slightly orange-colored structure is observed in the area, where the Si of the frustule of the diatom can be seen in Fig. 2b. This corresponds to the cell membrane of the diatom. The structure to the left represents a bacterium: in this area, no Si signal is observed. The Os signal shown in Fig. 2f can be assigned to a bacterial cell. Moreover, the P distribution maps (Fig. 2d, g) depict the location of polyphosphate components and other phosphate compounds in the cells. Interestingly, the EDX-based element distribution analyses suggest the presence of U both inside the diatom and bacterium cell (Fig. 2e, h), with a co-localization of U and P. This might indicate the interaction of U with phosphate/phosphoryl functional groups, which may originate, for example, from the lipopolysaccharides, and which have already been reported in previous studies for various microorganisms (Kelly et al., 2002; Krawczyk-Bärsch et al., 2018; Llorens et al., 2012; Lütke et al., 2012). Note that in the literature, U biosorption on the cell surface and U intracellular accumulation (cytoplasmic polyphosphate inclusion, in polyphosphate granules) were reported in the case of *A. facilis* bacteria via TEM-based analyses (Gerber et al., 2016).

### 3.4. Uranium(VI) speciation in diatoms and single-strain bacterial isolates

TRLFS measurements were performed on the U-loaded diatom and bacterial isolate samples. Fig. 3a shows the obtained luminescence spectra. At first glance, the U(VI) luminescence spectrum of the diatom sample differs from that of the bacterial isolates. Moreover, the U(VI) luminescence spectra of the *A. facilis*, *A. fabrum* and *B. mediterranea* isolates show similarities: there should be one or some common U(VI) species present in these samples. Interestingly, the U(VI) luminescence spectrum of the *P. peli* sample has a particular form, which seems to combine the spectral characteristic of both diatom and other bacterial isolate samples. To better evaluate these results, PARAFAC analyses were performed on these spectra.

Interestingly, the PARAFAC analysis suggests the presence of two detectable U(VI) species (spectra shown in Fig. 3b middle and below) in all the four bacterial isolate samples of *P. peli*, *B. mediterranea*, *A. fabrum* and *A. facilis*. The distribution of these two identified U(VI) species in the bacterial isolate samples is given in Fig. S1 in the supplementary information. However, in the case of the diatom sample, only one U(VI) species was identified under the applied experimental conditions. When comparing the obtained data, one common U(VI) species, i.e., the U(VI) species 1, can be found in both the diatom and the four bacterial isolate



**Fig. 2.** Bright-field TEM micrograph of a thin section of a diatom sample exposed to 10  $\mu\text{M}$  uranyl(VI) nitrate (a) with the presence of bacteria on the surface of the diatom frustule (highlighted area with dashed line) and in suspension (highlighted area with solid line). Corresponding EDX-based element mapping for the areas highlighted in (a) (area with dashed line: b-e; area with solid line: f-g) for silicon (Si), osmium (Os), phosphorus (P), and uranium (U).

samples, as illustrated in Fig. 3b (top and center).

To identify the nature of these U(VI) species, the obtained spectra were compared with reference spectra (Table 3). By comparing the well-resolved emission bands (494, 516, 540, 565 nm) of U(VI) species 1 that is similar to the U(VI) species found in the diatom sample with values cited in the literature, similarities to corresponding spectra of U(VI) carboxylate and phosphate compounds are found (cf. Table 3, Bernhard et al., 1996; Günther et al., 2008; Koban et al., 2004; Lütke et al., 2012; Panak et al., 2000). Thus, a predominant interaction of U(VI) with carboxyl and/or phosphate functional groups of the diatoms and bacteria is strongly suggested. Moreover, U(VI) species 2, which is only found in the bacterial isolates, exhibits similar emission bands to various reference spectra reported in the literature (cf. Table 3), e.g., spectra of a U-peptidoglycan species (Krawczyk-Bärsch et al., 2022), U bioassociated to *Idiomarina loihiensis* (Morcillo et al., 2014), or *A. facilis* (Krawczyk-Bärsch et al., 2018). In the work of Krawczyk-Bärsch et al., 2022, the peptidoglycan in the cell wall of a *Magnetospirillum* bacterial strain was reported as main ligand for U(VI) binding, whereby the binding occurs predominantly through carboxyl groups. At neutral conditions, Morcillo et al., 2014 pointed out that U(VI) is precipitated by an *Idiomarina* strain as uranyl phosphate phases. Krawczyk-Bärsch et al., 2018 reported that U(VI) is mainly bound to phosphoryl and carboxyl groups of the lipopolysaccharide and peptidoglycan of *A. facilis* cells. The contribution of carboxylate and phosphoryl groups to the binding of Cd was also reported in different freshwater diatoms (Pokrovsky et al., 2008). By comparison with these reference spectra and the conclusions drawn from them in the literature, we can assume the possible presence of different binding motifs of U(VI) in the bacterial isolates, most probably carboxyl and/or phosphoryl functional groups. Nevertheless, a clear differentiation between interaction of U(VI) with phosphoryl

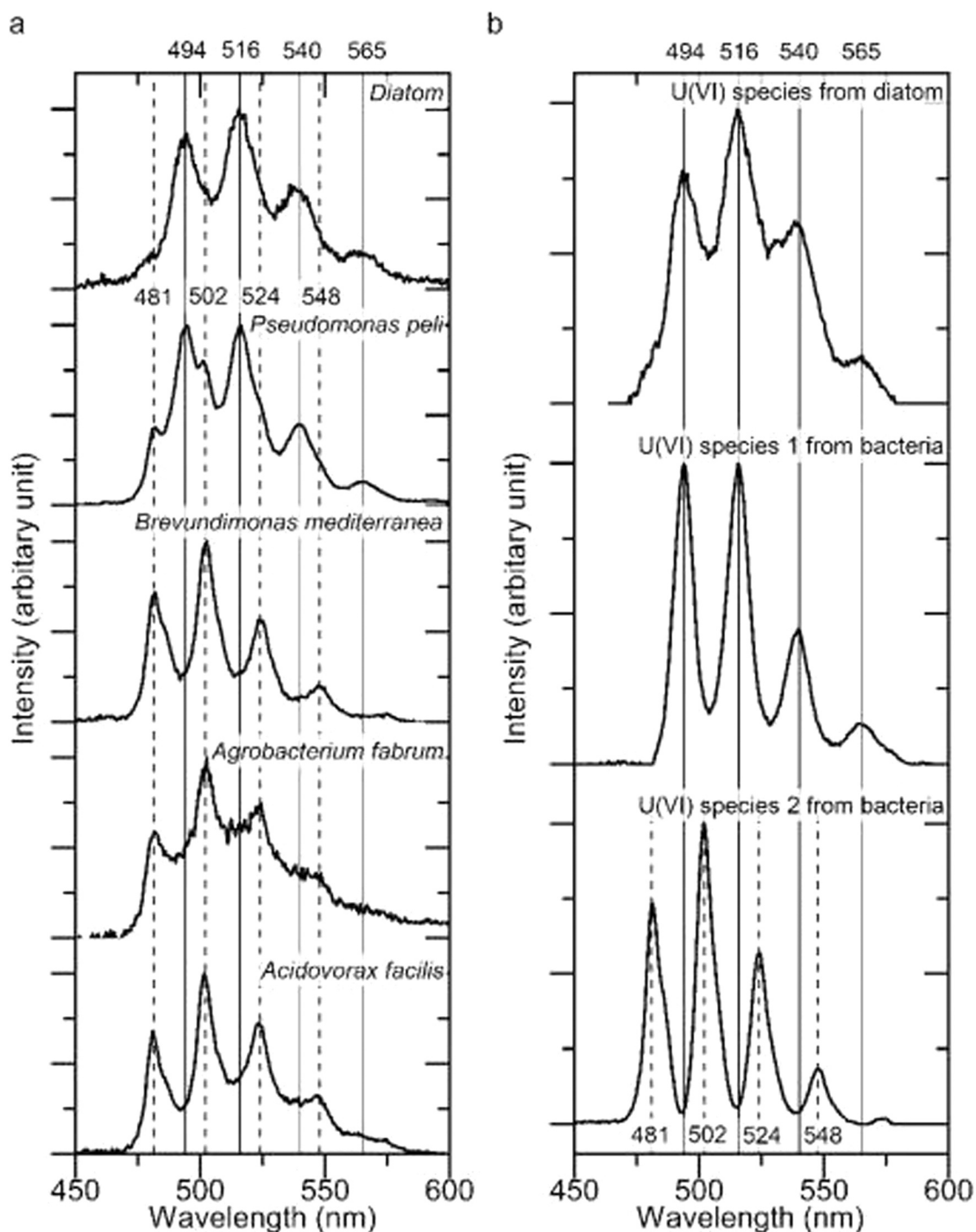
and/or carboxyl functional groups is not possible. An interaction with phosphoryl groups is nevertheless very likely, given that this interaction is supported by imaging showing the co-localization of U and P.

#### 4. Conclusion

Based on the microscopy and TRLFS results and even though only qualitative statements are possible at the moment, it can be asserted that bacteria should be taken into account when evaluating the interaction between U(VI) and the xenic diatom culture. This finding is even more interesting given that some of the isolated bacteria (e.g., *A. facilis* and *P. peli*) exhibit resistance towards heavy metals (e.g., U and chromium, respectively) (Augustynowicz et al., 2021; Gerber et al., 2016). It prompts the question of whether the bacterial community present in the diatom culture affects also the resistance of diatoms towards U. Furthermore, it is also important to know whether and which exudates are released from the diatoms. These may influence the bacterial community but also the U(VI) speciation. Eventually, the potential effects of U on the microbial metabolism have to be understood as these may also affect the diatoms as well as the behavior of U in the environment. Thus, further studies with an axenic *Achnantheidium saprophilum* diatom culture as well as with single bacterial strains would help to assess the effects of both bacteria and diatoms on the U sequestration in more detail.

#### CRediT authorship contribution statement

**Sindy Kluge:** Investigation. **Thorsten Stumpf:** Writing – review & editing, Funding acquisition. **Sean Ting-Shyang Wei:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Olivier Péron:** Writing – review & editing, Supervision. **Yihua He:**



**Fig. 3.** a) Luminescence spectra (prior to the PARAFAC analysis) of the diatom and single-strain bacteria (*P. peli*, *B. mediterranea*, *A. fabrum*, *A. facilis*) samples exposed to 2  $\mu\text{M}$  of uranyl(VI) nitrate (in 2 mM  $\text{NaNO}_3$ , 3 mM HEPES, pH 7.0); b) Comparison of the luminescence spectra of the U(VI) species found in the diatom sample (b, top) and in the bacterial isolates (b, center and below) that were obtained as result of the PARAFAC analysis. Note that two different U(VI) species were detected in the bacterial isolate samples (*P. peli*, *B. mediterranea*, *A. fabrum*, *A. facilis*), between which one common U(VI) species (U(VI) species 1) is also detected in the xenic diatom sample.

Writing – original draft, Methodology, Investigation, Conceptualization. **Vincent Breton:** Writing – review & editing. **Aude Beauger:** Writing – review & editing. **Clarisse Mallet:** Writing – review & editing. **Johannes Raff:** Writing – review & editing. **Robin Steudtner:** Writing – review & editing, Writing – original draft, Formal analysis. **René Hübner:** Writing – review & editing, Visualization, Investigation. **Vladyslav Sushko:** Writing – review & editing, Writing – original draft, Visualization, Investigation. **Gilles Montavon:** Writing – review & editing,

Supervision, Funding acquisition, Conceptualization. **Katrin Fleming:** Writing – review & editing, Investigation. **Susanne Sachs:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial



**Table 3**

Luminescence emission bands of U(VI) species identified in the *A. saprophilum* diatom sample and in the *A. facilis*, *A. fabrum*, *B. mediterranea*, *P. peli* isolate samples exposed to 2 μM of U(VI) in comparison to selected reference data.

Samples	Luminescence emission bands (nm)				Ref.		
U(VI) species 1 from bacterial isolates (similar to those observed in the diatom sample)	494	516	540	565	This work		
UO <sub>2</sub> (H <sub>2</sub> PO <sub>4</sub> ) <sup>+</sup> /UO <sub>2</sub> (HPO <sub>4</sub> ) (pH 3)	494	517	541	565	(Panak et al., 2000)		
UO <sub>2</sub> H <sub>3</sub> PO <sub>4</sub>	494	517	541	565	(Bernhard et al., 1996)		
Uranyl(VI) fructose 6-phosphate	478.9	497.1	519.0	543.3	568.9	598	(Koban et al., 2004)
UO <sub>2</sub> [CH <sub>3</sub> (CO <sub>2</sub> ) <sub>2</sub> ]	494	515	540	564			(Lütke et al., 2012)
UO <sub>2</sub> (glycine) <sub>2</sub> <sup>+</sup>	478.7	495.3	516.7	540.6	565.0	594.4	(Günther et al., 2008)
U(VI) species 2 from bacterial isolates	481	502	524	548			This work
<i>Magnetospirillum magneticum</i> AMB-1	479.9	500.3	522.0	546.5	572.9		(Krawczyk-Bärsch et al., 2022)
U-peptidoglycan species 3							
<i>Idiomarina loihiensis</i> (pH 7, solid)		502	522.6	545.9			(Morcillo et al., 2014)
<i>A. facilis</i> (pH 5.0)	480.7	499.7	520.1	545.1			(Krawczyk-Bärsch et al., 2018)

interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data Availability

Data will be made available on request.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2024.116893](https://doi.org/10.1016/j.ecoenv.2024.116893).

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