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Effects of thorium on bacterial, microalgal and micromeiofaunal community structures in a periphytic biofilm

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ABSTRACT

Few ecotoxicity studies are available on thorium (Th) which hinders the ability to evaluate its ecotoxicological risk. Its release in the environment is often associated with the extraction of rare earth elements and uranium, as well as the field applications of phosphate fertilizers. This study investigates the effects of Th on microbial communities of periphytic biofilms. Ceramic plates were left to colonize for one month in the laboratory with a biofilm sampled from Cap Rouge river (QC, Canada). Plates were randomly placed in channels containing culture media representing three different conditions: a control condition (C0; background Th concentrations of 0.004 ± 0.002 nM), a low Th concentration condition (C1; 0.18 ± 0.09 nM Th) and a moderately high Th condition (C10; 8.7 ± 3.4 nM) for up to 4 weeks. The presence of Th modified the diatom community by changing its taxonomic structure, reducing diversity and increasing cell density. The taxonomic structure of the bacterial community, followed by 16S metabarcoding analysis, was affected with a significant decrease in *Pseudanabaena* and *Shingopyxis* genera in the two Th exposed conditions. No direct toxic effect of Th was observed on counted micromeiofauna but the changes in diatom and bacterial communities could explain the higher number of individual diatoms and micromeiofauna observed in Th-exposed conditions. This work shows that low concentrations of Th can modify biofilm structure, which, in turn, could disturb its ecologically key functions.

1. Introduction

The development of new technologies has significantly increased the demand for rare earth elements. Canada has a strong mining potential, notably for rare earth elements and uranium, for which the country is the second largest world producer with 6938 tonnes in 2019 (NEA/IAEA, 2019). Thorium (Th) is a natural, radioactive, tetravalent, metallic element, often associated with the waste of rare earth elements and uranium mining extraction and is also found in phosphate fertilizers (Registry Agency for Toxic Substances and Disease, 1990). Thorium is used in the aeronautic, electronic and metallurgic industries, notably for its resistance to high temperature. It is also used in petrochemistry during cracking (Mernagh and Miezitis, 2008), and has good potential for future use as a nuclear power combustible (Loiseaux et al., 2002). Release of Th into the environment is of concern, however, as the environmental risks associated with Th are still not well understood, particularly in aquatic ecosystems. In natural freshwaters, Th has been found at concentrations between 0.003 and 700 μg L^{-1} (0.01 nM and 3 μM) (Casartelli and Miekeley, 2003; Correa et al., 2009; Godoy and Godoy, 2006; Lauria and Godoy, 2002; Ramli et al., 2005; Zhang et al., 2004). Concentrations of up to 300 μg L^{-1} (1.3 μM) of Th were measured in lakes located next to tin mines and between 800 and 1400 μg L^{-1} (3.4 and 6.0 μM) in the drainage water of uranium and iron mines in southern Brazil (Veado et al., 2006; Yusof et al., 2001). In freshwater, dissolved Th is mostly found in the hydroxide form $Th(OH)_4$. Its low solubility appears to reduce its mobility between the compartments of aquatic ecosystems and its mobility is influenced by associations with particles, colloids and dissolved organic matter (Choppin and Wong, 1998; Degueldre and Kline, 2007; Gascoyne, 1982; Hummel et al., 2002; Orlandini et al., 1990; Sanchez and Rodriguez-Alvarez, 1999). Literature on Th toxicity on freshwater organisms is scarce. For the cladoceran *Daphnia magna*, 24 h and 48 h effective concentrations 50% (EC_{50}s; 95% confidence interval) of Th on mortality were 7.3 (5.8–9.2) and 4.7 (3.2–6.6) μM, respectively (Ma et al., 2016). In another study, the growth of the green alga, *Monoraphidium* sp., was negatively impacted at a concentration of 43 μM of Th or higher and *Scenedesmus* sp. grew 16%

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and 26% less than controls at Th concentrations of 43 and 110 μM, respectively (de Queiroz et al., 2012). The EC_{50} for *Chlorella vulgaris*, another green alga, was reported at 15 μM (Evseeva et al., 2010). Recently, Peng et al. (2017) investigated the effect of Th speciation on the green alga, *Chorella pyrenoidosa*. The 96 h EC_{50} based on growth inhibition of this alga was 10 μM and nano-sized Th precipitates were observed inside the cells by transmission electron microscopy (Peng et al., 2017). Therefore, there is a need to better understand the potential of Th toxicity on aquatic organisms and to improve the risk assessment linked to this actinide.

Bioindicators are organisms used as indicators of the health of an ecosystem in which they live. They are widely used for ecotoxicological risk assessment in freshwater ecosystems (Li et al., 2010). Recent work on biofilm has demonstrated its potential as a bioindicator for freshwater contamination (Coste et al., 2009; Lavoie et al., 2012; Romaní et al., 2016). First, the internalization and capacity to retain metallic elements by benthic microorganisms facilitates the detection of ultra-trace elements (Behra et al., 2002; Holding et al., 2003). Second, periphytic microorganisms, such as diatom and bacterial communities, are currently used for freshwater monitoring, including metal impact assessments (Lavoie et al., 2019; Morin et al., 2012; Prygiel et al., 1999). Finally, periphytic biofilms have a key role in the functioning of aquatic ecosystems, where disturbances can lead to important perturbations of ecosystem services (Romaní et al., 2016). Biofilms host a diverse ecosystem comprising of bacteria, fungi, algae and micromeiofauna. While the majority of studies conducted on biofilms focus on autotrophic and bacterial taxa, grazing microbes are rarely considered (Neury-Ormanni et al., 2016). However, micromeiofauna communities play a key role in the biofilm food web and their disturbance can affect the global biological structure and its ecological function (Madoni and Zangrossi, 2009; Pratt and Cairns, 1985). In a previous study, we showed that zirconium (Zr), another tetravalent metallic element, affected ciliate and rotifer populations at low concentrations (0.5 ± 0.3 and 2.9 ± 0.3 nM respectively) (Doose et al., 2019). In this work, the effects of Th on the microbial communities of a periphytic biofilm were investigated with both microscopy and biomolecular approaches. Because Th solubility is low, the concentrations tested in this work were selected to avoid exceeding its solubility limit and to represent the range found in environmental freshwater systems. To our knowledge, no previous investigation was performed on the effects of Th on freshwater periphytic communities. The present study thus brings new understanding of the potential hazards of Th for freshwater ecosystems and contributes to improving risk assessment of mining activities that release Th into aquatic ecosystems.

2. Materials and methods

2.1. Experimental setup

To colonize periphyton, ceramic slides of 23 cm^2 were submerged for one month in a large channel inoculated with a periphyton suspension sampled from the Cap-Rouge river (Quebec, Canada). The Cap-Rouge River is 23.5 km long and has a catchment area of 82 km^2 and is located at the convergence of the three main geological provinces of Quebec: the Canadian Shield, the St. Lawrence Lowlands and the Appalachians. There are three major land uses in the watershed, with a forested area in the headwater, an agricultural zone in its mid course and finally an urbanized area in the downstream section including one major highway. The biofilm was collected in the lower section of the river (sampling location geographical coordinates: 46°45'48.8"N 71°21'24.0"W). The light flux was 80 μmol photon cm^{-2} s^{-1} and the photoperiod was 14 h:10 h for day and night, respectively. The slides were then randomly distributed into 12 aquaria containing a synthetic culture medium (Dauta) (Dauta, 1982). One week prior to Th exposure, three sets of quadruplicate, independent experimental channels (dimensions: 100 × 20 × 10 cm, 6 L) were filled with different exposure media to pre-equilibrate the metal with the adsorption sites of walls, tubes and reservoirs. At the beginning of the experiment, 288 slides were randomly transferred from the colonisation channel to the 12 experimental channels with targeted Th concentrations of 0 (C0), 1 (C1) and 10 nM (C10). The Th solution used to spike the exposure medium was an ICP standard solution (1000 ppm Th in 10% HNO_3, SCP science, Baie-d'Urfé, Canada). Following 1, 2 and 4 weeks of exposure, eight slides per channel were randomly sampled to make two composite samples per channel by pooling four slides. With four channels per condition, eight replicates were used for each exposure condition (C0, C1 and C10). Exposure solutions were renewed weekly and the temperature and pH were monitored before and after water renewal (with a mean ± standard deviation of 18.3 ± 0.3 °C for temperature and 8.1 ± 0.4 for pH). Total concentrations of Th, orthophosphates and nitrates were monitored once a week throughout the 4 weeks of exposure, before and after media renewal. Thorium concentrations were determined by Inductively Coupled Plasma Mass Spectrometry after an addition of 500 μL of nitric acid to 4.5 mL of sample. The calibration curve was validated with certified control solution 406 (SCP science, Baie-d'Urfé, Canada). Each exposure medium was sampled with two replicates per channel (i.e. eight values per condition and sampling event), totaling 16 samples per channel and 64 per condition.

Sampled slides were first rinsed with fresh Dauta medium without Th and then scraped to re-suspend the biofilm in 50 mL of Dauta medium. Three volumes of 1 mL were sampled for the subsequent analyses: chlorophyll *a* fluorescence, photosynthetic activity and microscopic identification of microorganisms. Biofilm dry weight was quantified after filtration of a 20 mL aliquot on pre-weighted and dried GF/F filters, and lyophilisation according to the NF EN 872 standard method (Afnor, 2005).

2.2. Biofilm analyses

2.2.1. Microscopy analyses

To determine the number of diatoms, live and dead cells were counted using an Axioplan Zeiss microscope. They were counted using a Nageotte cell at 200x magnification after fixation (using Lugol solution to reach a final concentration of 0.01% in each sample), following the method described in Morin et al. (2010). The taxonomic determination of diatoms was conducted using pooled replicate samples digested for 15 h with concentrated HNO_3 (68%; ACS) and then with hydrogen peroxide (30%). Subsequently, samples were mounted onto microscope slides using Naphrax (*Brunel Microscopes Ltd.*, Chippenham, UK). A minimum of 400 valves per slide were counted and identified to the species level, when possible, and results were expressed as relative abundances. The determination was conducted using a key specialized in diatoms form eastern Canada (Lavoie et al., 2008).

Micromeiofauna individuals were also enumerated in all samples, after Lugol fixation (0.01% final concentration) and using a Nageotte counting cell as described for diatoms. Determination of the protozoan composition within micromeiofauna was established with the help of the user-friendly identification key for ciliates (Foissner and Berger, 1996), and the Precis of Protistology for other protozoans, such as flagellates, heliozoans, amoebae and thecamoebians (De Puytorac et al., 1987). Rotifers were identified with the help of The Rotifer World Catalog (Jersabek and Leitner, 2013).

2.2.2. Genomic analyses

The diversity of the bacterial communities was followed over the exposure time by amplifying and sequencing the 16S ribosomal DNA in the variable V3 and V4 regions. The DNA of each sample was extracted using the FastDNA™ Spin Kit For Soil (MP Biomedicals, Thermo Fisher Scientific) recommended for biofilm (Corcoll et al., 2017). The number of gene copies was determined in each sample by qPCR in 96 well plates with PCR CFX96™ Real-Time System C1000™ Thermal Cycler (Biorad). In each well, 5 μL of DNA sample diluted at 1 ng L^{-1} with nuclease-free

water was added to 15 μL of the mix provided by the manufacturer. In order to quantify the amount of bacteria in each sample, a qPCR was realized with the following universal primers specific for members of *Eubacteria*: 341F-(5′- CCT ACG GGA GGC AGC AG -3′) and 515R (5′- ATT ACC GCG GCT GCT GGC A -3′) as described in López-Gutiérrez et al. (2004). A standard curve was established from 20 to $8{\cdot}10^7$ copies of plasmids containing a reference 16S sequence of 169 pb from the strain *Bradyrhizobium japonicum* manufactured by Invitrogen, Life Technologies (Carlsbad, CA, USA). DNA sample concentration was determined using a Thermo Scientific™ NanoDrop 2000. The sequencing of 16s rDNA of samples were realized with Illumina Miseq by the IBIS genomic platform of Laval University, Canada.

2.3. Data treatment

The number of diatoms and micromeiofauna individuals were normalized for the dry weight (dw) of the samples while the number of 16S gene copies of the bacterial community were normalized for the DNA sample weight. Shannon indices were calculated using R 3.6.1 (vegan package) (Ihaka and Gentleman, 1996). To determine significant differences between treatment groups and exposure times, ANOVA analyses were computed using Tukey's post hoc tests with R software (car and MASS packages). Significant differences between diatom growth curves were determined by an ANCOVA analysis (Fienberg et al., 2012). The taxonomic data obtained with the sequencing of 16S rDNA were analyzed following the Workflow for Microbiome Data Analysis using the Dada2 and phyloseq R packages and using the SILVA database for the taxonomy assignment (Callahan et al., 2016). A principal component analysis was performed with the FactoMineR and factoextra packages (Husson et al., 2017) on the taxonomic data (Shannon indices and log-transformed relative abundances of taxa) to provide a better overview on global effects of Th to community structures.

3. Results and discussion

3.1. Physicochemical composition

Nutrient concentrations (orthophosphates and nitrates), pH and water temperature are shown in Table 1. They remained constant during the 4-week exposure experiments and were similar between treatments (ANOVA, $p < 0.05$). Thorium concentrations in the C1 exposure conditions were lower than the targeted nominal concentrations despite the pre-equilibration of channel binding sites before the beginning of exposure. Nevertheless, the obtained concentrations were statistically higher than the background concentrations measured in the C0 exposure conditions ($p < 0.05$).

The Th speciation in the Dauta medium was calculated using the MINEQL software over the range of observed pHs (7.7–8.5). In this pH range, the tetrahydroxo-complex dominated the aqueous speciation of Th (99.5% $Th(OH)_4$) with a small presence of $Th(OH)_3$ (0.5%). The aqueous speciation of Th is mainly controlled by pH, thus, it is anticipated that for neutral and alkaline surface waters, $Th(OH)_4$ would be the dominant aqueous species present, while the less highly substituted Th $(OH)_n$ (where $n \leq 3$) species would become successively dominant at pH < 7 (Wickleder et al., 2011). Our experimental conditions, in terms of Th chemical speciation, thus reflect those expected in a natural aquatic ecosystem.

Table 1
Physicochemical conditions measured in the different treatments over the 4 weeks of exposure.

Parameter	C0	C1	C10
[Th] total (nM)	0.004 ± 0.002	0.18 ± 0.09	8.7 ± 3.4
pH	8.0 ± 0.4	8.1 ± 0.4	8.2 ± 0.4
Temperature	18.4 ± 0.1	18.5 ± 0.1	18.3 ± 0.1
Orthophosphate (mg $P\text{-}PO_4$ L^{-1})	2.9 ± 0.2	3.1 ± 0.3	3.3 ± 0.3
Nitrate (mg $N\text{-}NO_3$ L^{-1})	63 ± 8	56 ± 3	53 ± 1
Water temperature (°C)	18.4 ± 0.1	18.5 ± 0.1	18.3 ± 0.1

Values are presented as means ± standard deviations (n = 4).

3.2. Effect of Th on the growth of biofilm communities

Fig. 1 shows the variation in the number of individuals for diatoms (red), bacteria (green) and micromeiofauna (blue) over time in the different exposure conditions. A six-fold increase in the number of diatoms was observed in the first week in all conditions. Thereafter, cell concentrations appeared to be stable over the exposure time for the Th-exposed conditions with average concentrations of $8.1 \pm 2.5 \times 10^5$ (C1) and $9.7 \pm 1.9 \times 10^5$ cell mg^{-1} (C10) from weeks 1 to 4. In the control condition, no significant growth was observed between the first and the second week of exposure based on diatom density values. However, a slight decrease between weeks 1 and 2 resulted in values significantly lower in the control than in the C1 and C10 conditions at 2 weeks of exposure ($p < 0.05$). Thorium is not known to play an essential biological role in organisms; it is thus unlikely that the Th was directly responsible for the diatom growth in the Th-exposed conditions after 2 weeks. Moreover, the diatom population tended to decrease between weeks 1 and 2 in control conditions, while they remained constant in Th-exposed channels. This suggests that at week 2, Th had affected another biofilm component and decreased the competition, favoring diatom growth. The cell numbers in the control experiment increased during the two last weeks to reach $1.4 \pm 0.6 \times 10^6$ cells mg^{-1} at week 4.

The number of *Eubacteria* 16S rRNA gene copies present in the biofilm samples over the experiment (Fig. 1, data in green) showed no significant differences over exposure time and between treatments for the two Th exposure conditions with an average of $4.03 \pm 0.68 \times 10^5$ and $4.96 \pm 0.79 \times 10^5$ copies ng of DNA^{-1}. However, the control showed a significant increase between weeks 1 and 2 going from $4.79 \pm 0.61 \times 10^5$–$8.41 \pm 1.72 \times 10^5$ copies ng of DNA^{-1}, followed by a decrease over the last 2 weeks to reach $4.46 \pm 0.67 \times 10^5$ copies ng of DNA^{-1}. Thorium is already known to be internalized and to modify the cell morphology at high concentrations in bacteria genera like *Pseudomonas* (internalization characterized by transmission electron microscopy and energy dispersive X-ray microanalysis when the cells were exposed to 431 μM during 12 h), and *Bradyrhizobium* (growth inhibition of 83.6 ± 8.1% under 100 μM Th during 95 h) (Kazy et al., 2009; Santamaría et al., 2003). In this study, the results showed that Th can inhibit the growth of some bacteria in the Th-exposed conditions after 2 weeks of exposure at much lower concentrations than those typically used in the literature. However, because the 16S gene copy number present in the bacterial cells can vary from 1 to 7, depending on the species and the growth phase, we cannot make a direct link with the decrease in the total number of bacteria (Ludwig and Schleifer, 2000). Thus, those results need to be analyzed cautiously, but nevertheless they show that the presence of Th had a negative effect on bacterial community.

The total micromeiofauna individuals counted during the experiment are presented in Fig. 1 (data in blue). The number of individuals decreased over the first week in all conditions from $9.23 \pm 0.92 \times 10^3$ to $4.22 \pm 0.19 \times 10^3$ individuals mg^{-1} dw. Control values were then stable over the experiment with an average of $5.36 \pm 0.99 \times 10^3$ individuals mg^{-1} dw between weeks 1 and 4. In the Th-exposed channels, the number of individuals augmented at week 2 ($9.2 \pm 1.1 \times 10^3$ and $7.03 \pm 0.61 \times 10^3$ individuals mg^{-1} dw C1 and C10, respectively) with a significant difference for the intermediate exposure condition C1 ($p < 0.05$) compared to the control. As mentioned previously for diatoms, Th is not known to have an essential biological role, thus it is unlikely that Th directly enhanced the micromeiofauna organismal growth. Thus, increases in the number of individuals observed in the C1 condition at week 2 could be attributed to indirect effects such as the decrease of competing organisms. The number of individuals then decreased during the last 2 weeks of exposure to $1.90 \pm 0.21 \times 10^3$ and $2.46 \pm 0.39 \times 10^3$ individuals mg^{-1} dw at week 4 for C1 and C10

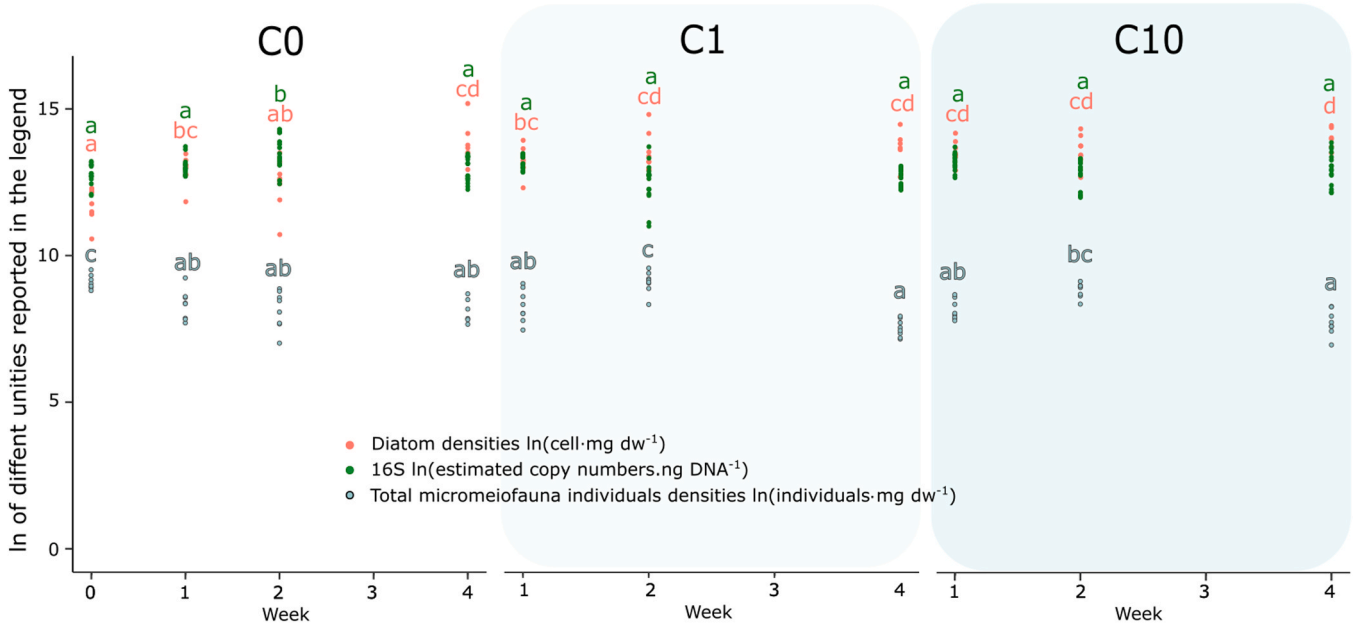

Fig. 1. Total individuals (ln) normalized by biomass (live diatoms per mg of dry weight in red, number of *Eubacteria* 16S ARN gene copies obtained by qPCR per ng of total DNA sample in green, total density of micromeiofauna individuals per mg of dry weight in blue) observed over time in control C0 (0.004 ± 0.002 nM Th) and Th exposures (C1 = 0.18 ± 0.09 nM Th and C10 = 8.7 ± 3.4 nM Th). A two-way ANOVA was performed to detect significant differences between treatments, indicated by letters ($p < 0.05$; $n = 8$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

exposure conditions, respectively.

Globally, these results showed that the two Th-exposed conditions had the same population variation pattern for the three biofilm compartments observed (diatoms, bacteria and micromeiofauna), in contrast with the pattern observed in the control condition. In the C1 and C10 exposure conditions, diatoms were clearly dominating the biofilm in the last 2 weeks of exposure, while bacteria seemed to be dominant in the control. Taxonomic diversity observed within the biofilms of this experiment is reported in Supporting Information (Table S1).

3.3. Effect on the diversity and taxonomic composition of biofilm communities

A high-throughput sequencing of the 16S rRNA genes was performed to characterize the bacterial community. The relative abundance of the 20 most prevalent bacterial 16S rRNA gene sequences are presented in Fig. 2A. Over the exposure time, eight main genera were detected and the abundance of two of them, *Pseudanabaena* and *Sphingopyxis*, varied significantly depending on time and exposure conditions. At the beginning of the experiment, the bacterial community was dominated

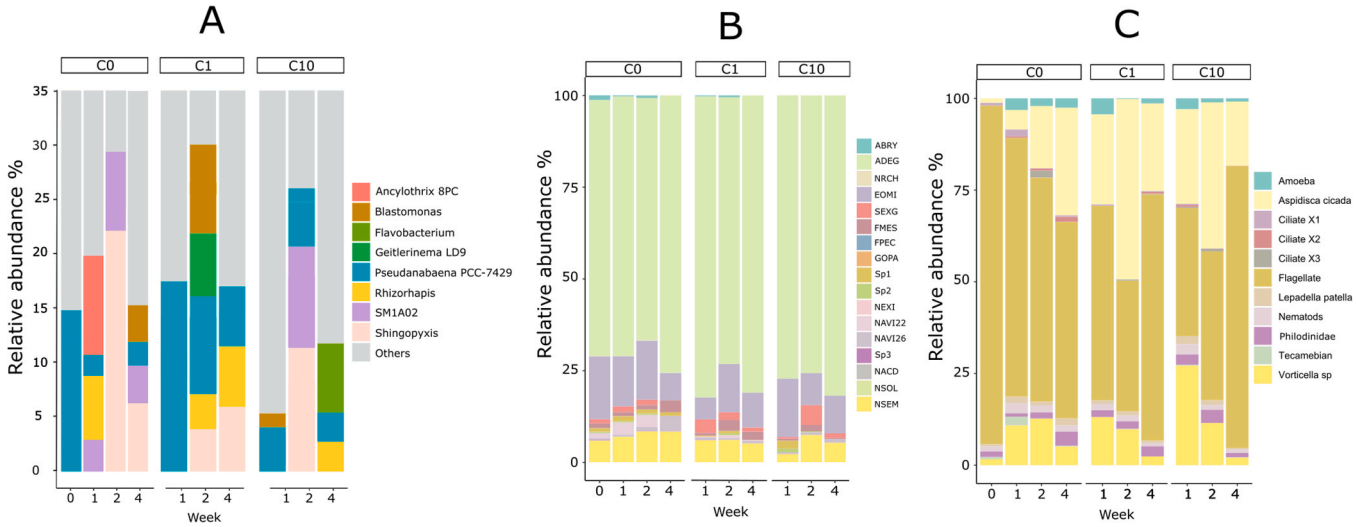

Fig. 2. Relative abundance for the percentage of bacteria (A), diatoms (B) and micromeiofauna (C) taxa counted in the biofilm sampled over time in control (C0 = 0.004 ± 0.002 nM) and Th exposures (C1 = 0.18 ± 0.09 nM and C10 = 8.7 ± 3.4 nM). Bacteria genus represented were the top 20 amplicon sequence variants (ASV) obtained by analysing the 16S rRNA gene amplicon sequencing results. Sequences were binned to Amplicon sequence variants based on their taxonomy at genus levels. The top 20 ASV represented the maximum 33% of the total genera found by the analysis, thus a scale zoom to 35% was applied to the graphic to better illustrate the results. Values shown are the averages of replicate samples for micromeiofauna ($n = 8$) and bacteria ($n = 3$); for diatoms the eight sample replicates were pooled before counting. Diatom species are *Adlafia bryophila* (ABRY), *Achnanthidium exiguum* (ADEG), *Navicula* aff. *reichardtiana* (NRCH), *Eolimna minima* (EOMI), *Stauroforma exiguiformis* (SEXG), *Fragilaria mesolepta* (FMES), *Fragilaria pectinalis* (FPEC), *Gomphonema pala* (GOPA), *Navicula exilis* (NEXI), *Navicula* sp. *22* (NAVI22), *Navicula* sp. *26* (NAVI26), *Nitzschia* cf. *acidoclinata* (NACD), *Nitzschia solgensis* (NSOL), *Navicula* cf. *seminulum* (NSEM), undetermined 1–3 (Sp1–3).

by the *Pseudanabaena* genus (15.1 ± 3.3%) composed of filamentous cyanobacteria. After 1 week of exposure, this genus decreased significantly in the control and C10 conditions with relative proportions of 2.0 ± 1.7 and 4.2 ± 1.1% of the bacterial population, respectively, while it was still the principal genus in the C1 condition with a proportion of 18.3 ± 6.9%. Then, relative abundances of *Pseudanabaena* in the C1 condition decreased significantly after the second week of exposure with a relative abundance of 3.2 ± 2.2% (ANOVA, $p < 0.001$). From the second week to the end of the exposure, values remained low and constant in all conditions. In contrary, the *Sphingopyxis* genus was not present among the eight most abundant genera from the beginning of this experiment until the second week of exposure. After two weeks, this genus abundance significantly increased to become the most abundant genus in the control at 23.1 ± 11.6% (ANOVA, $p < 0.05$) while it was also detected in the C1 and C10 conditions (4.1 ± 2.3% and 11.9 ± 8.0%, respectively). During the last 2 weeks of exposure, the *Sphingopyxis* relative abundance decreased significantly in the control to reach 6.6 ± 5.7% while values stayed constant in the two Th-exposed conditions.

Pseudanabaena is a genus of cyanobacteria in which some species are able to produce cyanotoxins such as the microcystin-LR (MCLR) (Nguyen et al., 2007). *Sphingopyxis* genus is well-known to degrade MCLR (Xiao et al., 2011). Because the number of MCLR degrading bacteria was shown to depend on this toxin concentration, Ho et al. suggested that MCLR could be a primary substrate for those bacteria in the biofilm (Ho et al., 2012, 2010). It was previously shown that copper and cadmium could modulate the transcription of genes coding for MCLR synthesis in cyanobacterial cells (Qian et al., 2012, 2010). After 2 weeks, the lower abundance of *Sphingopyxis* genus in the C1 and C10 exposure conditions could be attributed to a reduction of cyanotoxin synthesis by cyanobacteria such as *Pseudanabaena*, although subsequent analyses would need to be done to demonstrate such an effect.

Moreover, the *Sphingopyxis* genera was also reported to degrade organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) (Shokrollahzadeh et al., 2012), crude oils, diesel and kerosene (Kim et al., 2014), and polyvinyl alcohol (Yamatsu et al., 2006). A study on chromate (Cr (VI)) bioremediation by bacteria belonging to *Sphingopyxis* genera (*Sphingopyxis macrogoltabida* SUK2c) showed that their biomass reduced and complexed the Cr. The authors suggested that this metal could be bound and reduced by (–NH), (–COOH) and (P=O) groups present in the glycosphingolipids, surface proteins and on the fine peptidoglycan layer of those bacteria (Prabhakaran et al., 2019). Like Th, Cr has a "hard" character (class A metals are classified as hard spheres due to their inert gas type (d°) electron configuration), and preferably bind to N, O and F electron donor atoms (Stumm and Morgan, 1996). Thus, we can imagine that Th could follow similar complexation mechanisms on the *Sphingopyxis* bacteria surfaces observed in this work. Moreover, the sensibility of these bacteria to Th could be linked with their capacity for its bioaccumulation.

For the last week of exposure, despite the elevated variability between replicates, the composition of the bacterial community in the three exposure conditions appeared to be different from each other. In the control condition, the *Blastomonas* genus and the SM 1A02 group were present in abundance, while in the C1 and C10 conditions were not in the major genera detected by the analysis. Additionally, *Rhizorhapis* amplicon sequence variants (ASV) were part of the top 20 sequences in only the Th exposed conditions after 4 weeks, while *Flavobacterium* genus only represented 6.6 ± 8.1% of the population in the C10 exposure condition.

Relative abundance of the diatom taxa counted in the pooled samples of biofilm are presented in Fig. 2B. During the exposure time, the dominant species was *Achnanthidium exiguum* (ADEG) (Grunow Czarnecki) in all conditions. This diatom tended to be less present in the control condition during the experiment compared to the Th-exposed biofilms, with an abundance of 70.5 ± 2.8% over the 4 weeks of exposure. ADEG was more abundant in the C1 and C10 exposure conditions with relative abundances of 78.6 ± 3.6 and 78.3 ± 2.3%, respectively. This diatom appears to be tolerant to the presence of Th and is known to be found in freshwater of bad quality (Wan Maznah and Mansor, 2002). Moreover the combination of copper exposure and high temperatures (18 and 23 °C) was shown to promote ADEG's dominance over the other species of the diatom community (Morin et al., 2017). The second most abundant diatom species was *Eolimna minima* (EOMI) with 13.5 ± 3.1% in the control condition over the 4 weeks of exposure. In the C1 and C10 conditions, this diatom represented 9.5 ± 2.5 and 11.6 ± 2.7% of the diatom population over the 4 weeks of exposure. These results demonstrate a sensitivity of EOMI to Th, even though this diatom is usually described as tolerant to metallic contamination (Morin et al., 2012). After 2 weeks, *Stauroforma exiguiformis* (SEXG) (Lange-Bertalot, Flower Jones and Round) followed the inverse trend of EOMI and was more abundant in the C10 condition (5.5%) as compared to the C1 (2.1%) and control conditions (1.5%). This trend was unexpected considering that the Biological Condition Gradient (BCG) considers SEXG to be a highly sensitive species to metallic concentration disturbances (Davies and Jackson, 2006).

The relative abundances of micromeiofauna observed in biofilm samples (Fig. 2C) showed that the flagellates were the most abundant taxa in all conditions with an average of 58.3 ± 8.1% during the 4 weeks of exposure. However, they tended to be more represented at the beginning of the exposure and in the control condition, where their abundance decreased gradually over time from 70.6 ± 5.8% on week 1–53.6 ± 4.4% at the last week of exposure (week 4). The inverse was observed for the ciliate *Aspidisca cicada* for which an increase of almost 30% was observed over the exposure time.

Rotifer (*Philodinidae*) and ciliate populations varied according to the exposure conditions over the course of the experiment. All other taxa counted, such as amoeba, nematodes or flagellates, did not vary significantly between the exposure conditions.

Fig. 3. A shows the total count of ciliate individuals in the biofilm over the 4 weeks of exposure. A significant increase between the beginning of the experiment (week 0) and the first week was observed in all conditions. Afterwards, the population of the controls did not vary. The values increased between the first and the second week in the C1 and C10 exposures to reach 4987 ± 469 and 3730 ± 352 individuals mg^{-1} dw, and decreased during the last 2 weeks of exposure to 878 ± 173 and 627 ± 132 individuals mg^{-1} dw, respectively. *Aspidisca cicada* was the most abundant species observed and the number of individuals for this species had the same variation pattern throughout the exposure as the total number of ciliates for all conditions. This ciliate is commonly found as a dominant species in benthic biofilms and is characterized to be among the least sensitive to metal contamination (Gücker and Fischer, 2003; Madoni et al., 1994, 1992). *Aspidisca cicada* feeds itself mostly by grazing bacteria and algae (Curds, 1982; Gücker and Fischer, 2003; Madoni and Zangrossi, 2009). Thus, the increase in the number of ciliates after 2 weeks in the Th-exposed conditions could be linked to the higher diatom densities in those conditions for the same exposure time.

Fig. 3. B shows the rotifer individuals belonging to *Philodinidae* family counted in the biofilm over the 4 weeks of exposure. This family, found in a great diversity of ecosystems, is reported as a detritivore, microalgae and microorganism consumer (Ricci and Maria, 2000; Segers, 2007). The population in the control condition did not vary during the experiment, with an average of 104 ± 12 individuals mg^{-1} dw, as well as the C1 condition which stayed around 124 ± 12 individuals mg^{-1} dw. On the other hand, the number of individuals significantly increased after 2 weeks in the C10 exposure condition to reach 285 ± 65 individuals mg^{-1} dw. Like *A. cicada*, those high numbers of *Philodinidae* rotifer seemed to be linked with high diatom densities in the Th-exposed conditions after 2 weeks. Then values in Th exposure conditions decreased during the last 2 weeks of exposure to an average of 97 ± 25 and 53 ± 26 individuals mg^{-1} dw in the C1 and C10 exposure conditions, respectively. Other rotifers are known to be sensitive to metals

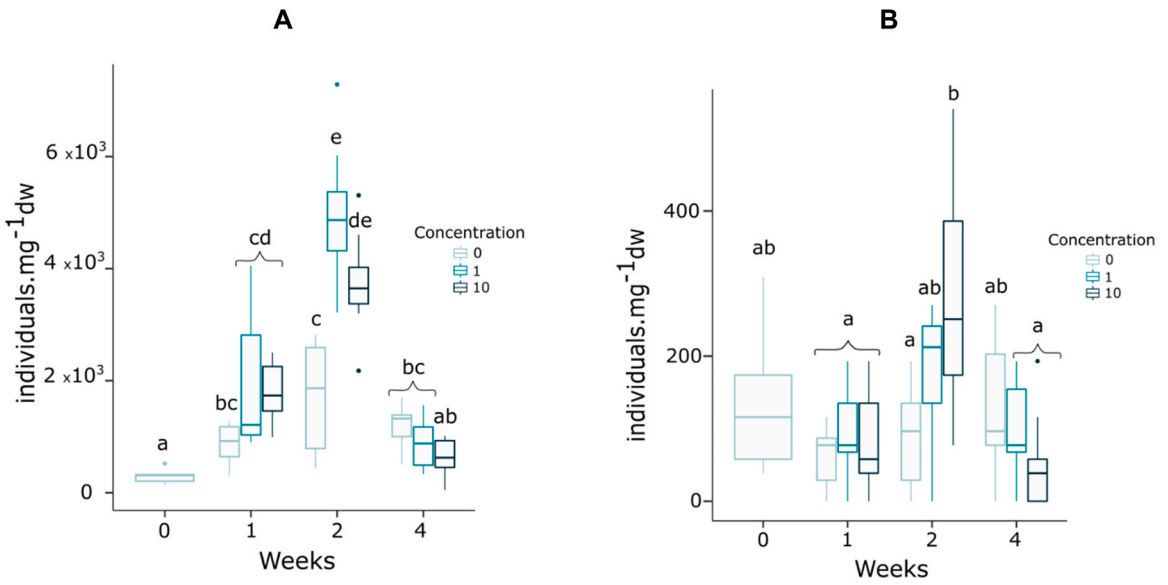

Fig. 3. Number of individuals per mg of biofilm dry weight in control (C0 = 0.004 ± 0.002 nM) and Th exposures (C1 = 0.18 ± 0.09 nM and C10 = 8.7 ± 3.4 nM) as a function of time. A. Ciliates B. Rotifers (*Philodinidae*). A two-way ANOVA was performed to detect significant differences between treatments, as indicated by letters ($p < 0.05$; n = 8).

(Alvarado-Flores and Rico-Martínez, 2017). A 96 h-LC50 of 5.8 μM Cd was observed for *Philodina* cf *roseola* and deformations appeared above a threshold concentration of 2.7 μM after 24 h of exposure (Pérez-Yañez et al., 2019). Moreover, our previous study on the impact of Zr on biofilm taxonomic structure showed that this metal can induce a dose-response effect on rotifers and the number of ciliate (*A. cicada*) individuals when they were exposed during 2 weeks to a low concentration (2.9 ± 0.3 nM Zr) (Doose et al., 2019). In contrary, Th had no negative effect on the number of individuals of both rotifers and ciliates. In fact, after 2 weeks of exposure, individuals were more abundant in Th-exposed media than in the control condition. Such effects on the micromeiofauna observed during this experiment cannot be explained by direct effects of Th on those taxa. As bacterial and diatom communities represent the main food source for ciliates and rotifers, it is

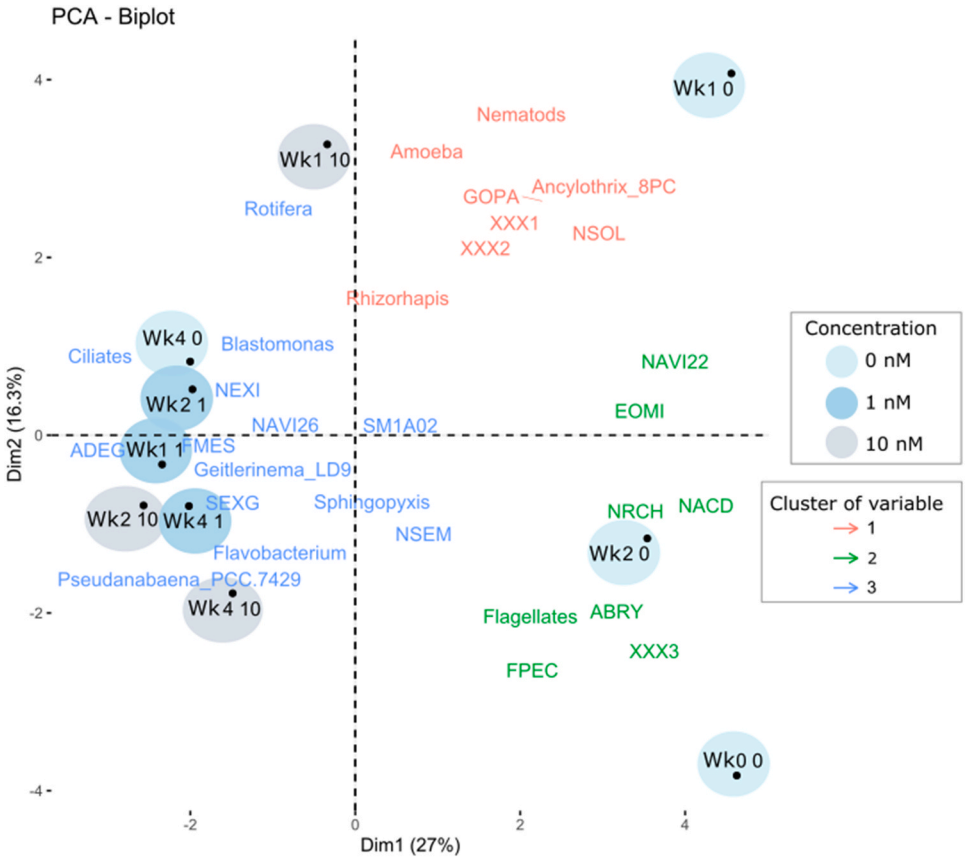

Fig. 4. Principal component analysis (PCA) performed on taxonomic data (relative abundance and Shannon index of bacterial, diatom and micromeiofaunal communities) in biofilm for control (C0 = 0.004 ± 0.002 nM) and Th exposures (C1 = 0.18 ± 0.09 nM and C10 = 8.7 ± 3.4 nM) after 0, 1, 2 and 4 weeks of exposure (Wk0, Wk1, Wk2 and Wk4). Variables were classified in three groups using the K-means algorithm. Abbreviations: bacterial 16S gene copy number (Qt), Shannon indices of bacteria, diatoms and micromeiofauna (ShanBact, ShanDiato and ShanMeio, respectively). Abbreviations of diatoms species names are described in Fig. 2.

likely that the effects of Th on the abundance and taxonomic composition of those two compartments is leading to an indirect effect on micromeiofauna by increasing the numbers of ciliates and rotifers.

3.4. Effect of Th on the global taxonomic community structure

The principal component analysis (PCA) presented in Fig. 4 was performed to better understand possible interactions between the different communities observed during this work. The two first dimensions account for 43.3% of the total dataset inertia. The PCA shows a clear separation of the control conditions from the Th-exposed conditions for the first 2 weeks along the first PCA component (27% of the explained variance). Along Dimension 2 of the PCA (16.3% of the explained variance), the dispersion of the control samples indicated that major structural changes in the biofilm community happened during the experiment. On weeks 0 and 2, the samples seemed to have higher diatom diversities with a Shannon index of 1.42 (data not shown). After 1 week, the samples were characterized by a higher micromeiofauna diversity with a Shannon index of 1.5 ± 0.2 (data not shown). Such changes highlight the natural evolution of biofilms over time and the complex interactions between biological components (e.g. competition, grazing).

Thorium-exposed conditions C1 and C10 appeared to be well discriminated from the control condition along Dimension 1, which accounts for the largest part of overall variability in the dataset. Contrasting with the temporal variability of control samples highlighted on Dimension 2, community structures in the C1 exposure condition were close to each other over the entire exposure duration. Moreover, microbial community compositions of the biofilm samples within the Th exposed biofilm samples were grouped with those of the control condition after 4 weeks of exposure on the PCA (Fig. 4), separated from the other control samples along Dimension 1. In these samples, the biofilm was characterized by low diatom diversity (Shannon index of 1.3 in the pooled samples). The less diverse taxonomic communities could be explained by the biofilm senescence after 4 weeks in the channel. The proximity of the control biofilm samples at 4 weeks of exposure with the Th-exposed conditions at all exposure times on the PCA suggests that this Th exposure rapidly and consistently affected the taxonomic structure of biofilm, accelerating senescence in the exposed biofilm. The C1 exposure conditions were characterized by ADEG and a high number of ciliate individuals which suggests that ADEG could be a preferential food source for the ciliates observed in this experiment. Higher temporal variability was found in the C10 exposure condition compared to that of C1. After 1 week of exposure, the biofilm samples were characterized by high bacterial diversity (Shannon index of 2.25 ± 0.54). Then, after 2 and 4 weeks of exposure, the community in the C10 exposure condition was characterized by the bacteria *Pseudanabaena* and *Flavobacterium*, as well as the diatom SEXG. These biofilms were characterized by low bacterial and micromeiofaunal diversity after 4 weeks with Shannon indices of 1.7 ± 0.8 and 1.0 ± 0.1, respectively. Because the relationships between diatoms and heterotrophic bacteria are known to be generally species-specific, the increase of SEXG could have favored the increase of one symbiotic bacteria species leading to a loss of bacterial diversity and selected for certain species of micromeiofauna (Eigemann et al., 2013).

The microorganism communities of biofilms are in constant interaction with each other. Benthic diatoms are reported to be the dominant primary producers in periphytic biofilms and to provide organic carbon to consumers and decomposers (Koedooder et al., 2019). Induced changes in their community by factors like seasonal variation, grazing or contamination can lead to important indirect effects on biofilm productivity, extracellular polymeric substance composition, and thus on the heterotrophic compartments of the biofilm (Passarelli et al., 2015; Wichard et al., 2007). On the other hand, reciprocal diatom-bacterial interactions can be negative (parasitic, competition) or positive with species-specific symbiosis, leading to compositional shifts in diatom species abundances (Amin et al., 2012; Zak and Kosakowska, 2015). Moreover, forcing factors such as contaminants can also influence the grazing pressure on bacterial and diatom communities provided by meiofauna and micromeiofauna by changing their taxonomic structure (Guasch et al., 2016; Neury-Ormanni et al., 2016). Thus, as previously demonstrated for other metals, Th contamination could affect this complex ecosystem equilibrium by directly affecting a biofilm compartment's sensitive species and via indirect effects on additional, or the aforementioned, biofilm compartments (Fleeger et al., 2003).

4. Conclusion

To the best of our knowledge, this is the first study to investigate the effects of Th on different periphytic biofilm communities, and on benthic micromeiofauna. Major processes of freshwater ecosystems and biogeochemical fluxes depend on biofilm health (Battin et al., 2016). In this study, significant changes were observed in bacteria and micromeiofauna taxonomic composition, with lower abundances of *Pseudanabaena* and *Sphingopyxis* genera, and higher numbers of ciliate and rotifer individuals, like *A. cicada* and *Philodinidae,* in the Th-exposed conditions compared to control. PCA results indicate a clear separation in the global taxonomic structure between the control and the Th exposure conditions over exposure time. Because the biofilms cultivated under laboratory conditions are generally less diverse taxonomically compared to field biofilms, the perturbation of natural periphytic communities in the presence of Th could be more pronounced in environmental conditions. But the species diversity is also known to favor the ecosystem's resilience. Thus, while the taxonomic structure of perturbed biofilm generally does not return to the original un-exposed state, the diversity of natural periphytic communities increases the likelyhood of being able to maintain the key functions of the biofilm during and after Th exposure compared to lab-grown communities (Lawrence et al., 2015; Romaní et al., 2016). To go further, it would be of interest to conduct field studies and to determine the impact of Th on biofilm key functions, notably by using next generation sequencing. This work shows the importance of better understanding the indirect effects of metallic contamination on periphytic micromeiofauna to improve risk assessment of metals on aquatic ecosystems based on biofilm descriptors (Barranguet et al., 2000; Singh et al., 2017).

CRediT authorship contribution statement

Soizic Morin: Supervision. **Claude Fortin:** Supervision. Caroline Doose was a Ph.D. student and lead author. She was in charge of conducting the experiments, interpreting data and writing of the first draft. Jacky Vedrenne is a research engineer specialized in micromeiofauna. He contributed to the taxonomic identification and paper review. Claude Fortin is the supervisor and Soizic Morin the co-supervisor; both contributed to the experimental design, data interpretation and paper review.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2021.112276.

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