Journal of Hazardous Materials 410 (2021) 124551

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Research paper

Periphyton as an important source of methylmercury in Everglades water and food web

Yuping Xiang [a,b], Guangliang Liu [a], Yongguang Yin [b], Yong Cai [a,b,c,*]

[a] Department of Chemistry & Biochemistry and Southeast Environmental Research Center, Florida International University, 11200 SW 8th ST, Miami, FL 33199, USA
[b] Laboratory of Environmental Nanotechnology and Health Effect, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[c] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

ARTICLE INFO

Editor: Dr. T Meiping

Keywords:
Periphyton
Methylmercury
Water
Food web
Everglades

ABSTRACT

Periphyton is ubiquitous in Florida Everglades and has a profound effect on mercury (Hg) cycling. Enhanced methylmercury (MeHg) production in periphyton has been well documented, but the re-distribution of MeHg from periphyton remains unknown. In this study, periphyton, sediments, surface water, periphyton overlying water, and periphyton porewater were collected from Everglades for analyzing the distribution of MeHg and total Hg (THg). Results showed that there were no significant differences in THg and MeHg in different types of periphyton, but they all displayed higher MeHg levels than sediments. MeHg distribution coefficients ($logk_d$) in periphyton were lower than in sediments, suggesting that periphyton MeHg could be more labile entering aquatic cycling and bioaccumulation. In water, the more the distance of water samples taken from periphyton, the lower the MeHg and dissolved organic carbon concentrations were detected. In extracellular polymeric substances of periphyton, MeHg in colloidal fractions was significantly higher than that in capsular fractions. It was estimated that approximately 10% (or 1.35 kg) of periphyton MeHg were passed on to mosquitofish entering the food web during wet season, contributing 73% of total Hg stocked in mosquitofish. These results revealed the importance of periphyton on water MeHg distribution and MeHg bioaccumulation in Everglades.

1. Introduction

Methylmercury (MeHg) is a potent neurotoxin that bioaccumulates through food chains, and highly toxic to humans and wildlife (Clayden et al., 2013). Wetlands are hotspots for mercury (Hg) methylation (Ackerman and Eagles-Smith, 2010), where sediments are generally considered the primary zones for MeHg production (Benoit et al., 1999; Drott et al., 2007; Graham et al., 2013). Recently, increasing attention has drawn to periphyton for not only MeHg production, as higher net Hg methylation potential in periphyton (up to 17% of added tracer) has been shown in comparison to sediments (up to 10% of added tracer) (Hamelin et al., 2015; Mauro et al., 2002), but also being a key route for MeHg entering food chains, especially in tropical and temperate wetlands (Cleckner et al., 1999; Planas et al., 2004).

Periphyton is a complex aggregates of algae, bacteria, archaea, fungi, protozoa, micrometazoa, and fine particulate detritus (Gaiser et al., 2011; Mauro et al., 2002), which is commonly present in three forms in aquatic ecosystems, including epiphyton, benthic mat, and floating mat, according to the matrix it is attached to (Gaiser et al., 2011). Periphyton is a distinct microcosm that contributes to MeHg production and the subsequent bioaccumulation in food webs. In one aspect, algae and bacteria within periphyton can directly take up Hg from the surrounding environment, providing ample substrates for microbial methylation. Meanwhile, the distinct redox potential gradient from the outside to inside (Lázaro et al., 2018), as well as the abundant organic matter in periphyton make Hg more bioavailable to methylators, in comparison to soil and floc (Acha et al., 2011; Correia et al., 2012; Hamelin et al., 2011; Leclerc et al., 2015; Li et al., 2012). Besides, extracellular polymeric substances (EPS) exudated by phototrophic organisms serve as adhesive agents enabling cellular attachment and maintains the structure stability of periphyton (Fang et al., 2014; Laviale et al., 2009; Xiao and Zheng, 2016), which could enhance MeHg production since attached methylators display higher Hg methylation potential than the free-living ones (Lin and Jay, 2007). Since periphyton is ubiquitous and can serve as a

* Corresponding author at: Department of Chemistry & Biochemistry and Southeast Environmental Research Center, Florida International University, 11200 SW 8th ST, Miami, FL 33199, USA.
E-mail address: cai@fiu.edu (Y. Cai).

https://doi.org/10.1016/j.jhazmat.2020.124551
Received 22 June 2020; Received in revised form 23 September 2020; Accepted 10 November 2020
Available online 12 November 2020

food web base in aquatic environments (Flemming and Wingender, 2010; Hagerthey et al., 2011; Ramanan et al., 2016; Rose and Cushing, 1970; Sanli et al., 2015), the enhanced MeHg production in periphyton should have significant implications on understanding the biogeochemical cycling and bioaccumulation of Hg.

The Florida Everglades is a typical subtropical wetland, where periphyton contributes more than half of the primary production in this ecosystem (100–10000 g m^{-2} yr^{-1}) (Gaiser, 2009; Ewe et al., 2006; Iwaniec et al., 2006). Previous studies have pointed to periphyton as an important matrix for MeHg production in the Everglades (Bae et al., 2019; Cleckner et al., 1999; Gilmour et al., 1998), and mass budget estimations showed that the annual/seasonal produced MeHg from deposited Hg in periphyton (3.5 g for dry season and 37 g for wet season) was approximately 6 folds higher than the MeHg storage in water (0.6 g for dry season and 6.6 g for wet season) in the Everglades (Li et al., 2010; Liu et al., 2008a). However, it remains unresolved as for the relation of the periphyton MeHg to the aquatic cycling and bioaccumulation of MeHg in the Everglades. Using a double isotope technique, our previous study suggested that the distribution of MeHg in Everglades water was controlled by the methylation/demethylation in periphyton, not by soil or floc (Li et al., 2012), resulting in high percentage of MeHg to THg (% MeHg) in Everglades water (~11%) (Liu et al., 2008b). In addition, previous studies highlighted the elevated Hg concentrations in food webs (Cleckner et al., 1998; Gabriel et al., 2014; Julian and Gu, 2015; Liu et al., 2008b; Loftus, 2000), suggesting that accumulation into food webs should be another primary fate of periphyton MeHg, as periphyton is an important food source for fish, crayfish and grass shrimp in the Everglades (Gaiser, 2009). On these bases, our objective was to further the understanding of the role of periphyton presence in MeHg distribution in water and accumulation in food webs on the Everglades-field scale, considering the occurrence of enhanced MeHg production in the periphyton. By analyzing THg and MeHg concentrations and ancillary geochemical parameters in different types of periphyton and relating these periphyton data to the analysis of sediments and water samples collected at different distances from the periphyton, this study reveals a new understanding of aquatic Hg cycling, in particular through integrating periphyton MeHg production, in wetland ecosystems.

2. Materials and methods

2.1. Collection of samples

Periphyton (mainly floating mat and epiphyton) were collected in 15 sites of the Florida Everglades (Fig. 1), using gloved hands and stored in 150 mL polycarbonate specimen cups. Water samples, including surface water (SW), which had a depth of about 20–100 cm depth and 1–2 m away from periphyton (not covered by periphyton), and periphyton overlying water (POW), which covered on the surface of the periphyton, were collected by filling pre-cleaned 2-L Teflon bottles with gloved hands and then kept in a cooler. For sites that were completely covered by periphyton, surface water could not be obtained. Surface soil/sediments were collected using a sediment gravity corer. Upon arrival at laboratory, a subsample of periphyton were centrifuged at 3500 g for 30 min to collect periphyton porewater (PPW) upon returning to the lab. All the liquid samples for Hg analysis were acidified with 0.5% (v/v) ultrapure HCl and analyzed within 1 week. To ensure that samples were free of exogenous contamination, two bottles of ultrapure water were put in the cooler during sampling, which were then stored under the same condition as samples until analyses. Samples from S1 were collected in April 2017 (dry season), and S2-S15 samples were collected during May to November in 2017 (wet season, Table S1).

2.2. Extraction of extracellular fractions

The periphytic EPS were manually separated into colloidal and capsular fractions (Bellinger et al., 2010), which were extracted following a modified procedure based on the previous literatures (Cyr and Morton, 2006; Leclerc et al., 2015). Briefly, the colloidal fraction was extracted by adding 20 mL of ultrapure water to 5 g (fresh mass) of periphyton and mixed at room temperature on a shaker for 1 h. The samples were then centrifuged at 20,000 g for 30 min, and the supernatant was decanted in borosilicate vials. This ultrapure water extraction was repeated, and supernatants were mixed, gaining a colloidal fraction for THg and MeHg analysis. The capsular fraction was extracted by adding 20 mL of 10 mM ethylenediaminetetraacetic acid (EDTA, Fisher Scientific) to the entire centrifugation pellets (Cyr and Morton, 2006; Leclerc et al., 2015). Samples were mixed at room temperature on a shaker for 2 h, followed by a centrifugation at 20,000 g for 30 min to get the supernatants. The EDTA extraction was repeated, and supernatants were pooled to gain a capsular fraction for THg and MeHg analysis. In this study, THg and MeHg concentrations in EPS fractions were normalized as the unit dry mass of periphyton.

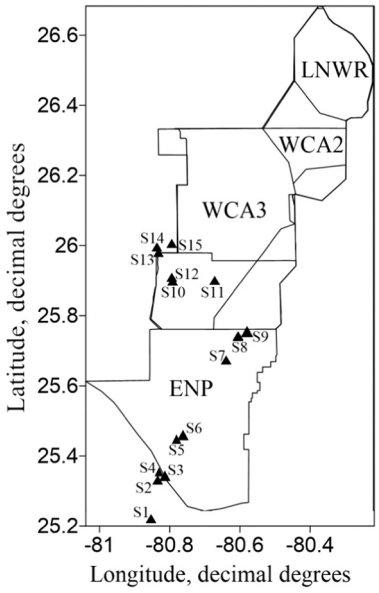

Fig. 1. Study area and the locations of sampling sites in the Everglades.

2.3. Determination of MeHg, THg, and other ancillary parameters

THg concentrations were determined by cold vapor atomic fluorescence spectrometry (CVAFS) (Merlin 10.035, PS Analytical, UK) following modified EPA method 1631E (USEPA, 2002). Briefly, THg in liquid samples were treated with UV radiation in an ultraviolet cabinet equipped with 15-watt UV lamp for 5–8 h and oxidized using 0.2 M bromine monochloride (BrCl) solution (0.5% v/v) prior to CVAFS analysis. THg in solid samples were determined following the standard operating procedures modified from EPA method 7474 (EPA 904-R-98–002). Samples were digested with concentrated HNO_3 in sealed ampoules at 105 °C for 1 h using an autoclave before cooling down to room temperature for CVAFS analysis (Li et al., 2012; Liu et al., 2008b). MeHg concentrations were determined by using a Brooks Rand MeHg system following EPA method 1630 (USEPA, 2001) and literatures (Liu et al., 2008a; Liu et al., 2008b). Briefly, MeHg in liquid samples were distilled before ethylation, gas chromatographic (GC) separation and CVAFS determination. MeHg in solid samples were extracted by $KBr/H_2SO_4/CuSO_4$ before ethylation and GC-CVAFS analysis. Quality assurance and controls (QA/QC) for THg and MeHg analyses include method blank, duplicates, and matrix spikes. Certified 1000 ppm $Hg(NO_3)_2$ standard solution (Fish Scientific, USA) and 10 ppm MeHg standard solution (Brooks Rand Ltd, USA) were used for quantification during THg and MeHg analysis, respectively. The

recoveries of matrix spikes in solid and liquid samples were 105–118% and 92–109% for THg, and 98–118% and 87–105% for MeHg, respectively.

Total organic carbon (TOC) in periphyton was determined by loss on ignition, which was reported as the percentage of ash-free dry weight (AFDW) to dry weight (DW) (%TOC = AFDW/DW×100). Briefly, 10 g (fresh weight) of periphyton was initially dried at 80 °C, weighted (dry weight, DW), and then combusted at 450 °C for 2–3 h and re-weighted (AFDW) to the nearest 0.001 g (AFDW = differences between DW and weight of ash after combustion). Chlorophyll a (Chl-a, μg dry g^{-1}) concentrations were determined after extraction with hot-ethanol (Moed and Hallegraeff, 1978). The autotrophic index (AI) was applied to reflect the ratio of heterotrophic to autotrophic communities in periphyton, which was calculated by the ratio of AFDW to Chl-a (Biggs and Close, 1989; O'Brien and Wehr, 2010). Total (TOC) and dissolved organic carbon (DOC) in water were measured following USGS SOP NU-062–1.8 on a Shimadzu TOC-5000.

2.4. Statistical analysis

All data processing and statistical analyses were conducted using SPSS 19.0. Besides the sampling map which was conducted by Surfer 10.0, all the other figures were plotted by Origin 2018. All data were tested for normality by the Lilliefors test, which is also called revised Kolmogorov-Smirnov (K-S) test. Pearson correlation (r) and Spearman's rank correlation (r_s) were conducted on normally and non-normally distributed data, respectively, by SPSS 19.0. Analysis of variance (ANOVA) and Mann-Whitney test were performed to test the differences for normally and non-normally distributed data, respectively. Statistical significances were defined at $p < 0.05$ and $p < 0.01$, indicating the "significant" and "highly significant" differences, respectively.

3. Results and discussion

3.1. Periphyton is a heterotroph-dominated algae-bacteria microhabitat in the Everglades

Chl-a concentrations (unit dry wet) of periphyton varied by one order of magnitude among 15 sampling sites (range 0.22–1.71 mg g^{-1} dw, mean 0.70 ± 0.41 mg g^{-1} dw), which did not show obvious spatial distribution in the Everglades (Fig. S1A). In periphyton community, Chl-a can best represent the total active algae, which is frequently used as an indicator of periphyton biomass. As primary producers, algae provide rich nutrients for microorganisms in periphyton microcosm, since it can convert inorganic carbon into organic carbon. In the Everglades, the total organic carbon (%TOC) of periphyton also varied widely (range 14–63%, mean 39 ± 16%) (Fig. S1B), displaying a significant positive correlation with Chl-a concentrations ($r = 0.66$, $p < 0.01$; Table S2). Besides, autotrophic index of periphyton ranged from 232 to 1399 (mean 669 ± 296, Fig. S1C), with 78% higher than 400, indicated that periphyton in the Everglades should be considered as the a community dominated by heterotroph rather than autotroph (Biggs and Close, 1989; O'Brien and Wehr, 2010).

3.2. High levels of MeHg are present in periphyton probably due to synergism of bacteria and algae

THg concentrations in periphyton ranged from 10.8 to 68.9 ng g^{-1} dw (mean 29.5 ± 18.8 ng g^{-1} dw). MeHg was in the range of 2.19–9.70 ng g^{-1} dw (mean 4.90 ± 2.36 ng g^{-1} dw), which accounted for 9.8–49% (mean 20 ± 11%) of THg (%MeHg) in periphyton (Table S1). These results are consistent with other studies in Boreal Shield Lakes, where 2–36% of the THg found in periphyton was in the form of MeHg (Desrosiers et al., 2006). Among different sampling sites, no obvious spatial distribution patterns in THg (Fig. S2A) and MeHg (Fig. S2B) were observed in periphyton, with the exception of S5-6 and S10-12 where the THg concentrations in periphyton were significantly higher. These were consistent with the characteristics of soil THg concentrations in the Everglades (Stober et al., 2001). At the same location, THg and MeHg in different types of periphyton, including floating mat, epiphyton and benthic mat, displayed no significant differences (Fig. S3A). However, the MeHg concentrations were significantly higher in periphyton than in sediment (Fig. S3A), while higher THg were observed in sediments. Similar distribution patterns were observed in porewater of different compartments (Fig. S3B).

In environmental matrices, MeHg concentration is regulated by a number of factors, particularly by the activity of Hg methylators and MeHg demethylators, the availability of inorganic Hg, as well as the uptake/release of MeHg from/into surrounding environments (Ullrich et al., 2001). In this study, THg concentration significantly positively correlated with MeHg (Fig. 2A) but negatively correlated with %MeHg (Fig. 2B) in periphyton, likely indicating that only a fraction of the THg in periphyton is available for methylators (Hsu-Kim et al., 2013; Tang et al., 2020). The higher MeHg and %MeHg in periphyton than in sediments suggests that periphyton has higher Hg methylation potentials than sediments in the Everglades, probably owing to synergism between bacteria and algae inside the periphyton for cohabitation and utilization of materials and energy (Cleckner et al., 1999; Liu et al., 2008b). Similar results were reported by former work, which found that periphyton and flocculant materials (floc) were important compartments contributing to MeHg production in the Everglades, displaying higher MeHg levels than soil in this systems (Bae et al., 2019). A positive correlation between %MeHg and AI was found in periphyton (Table S2), suggesting the significant role of heterotrophic microorganisms in Hg methylation within periphyton. Meanwhile, THg and MeHg displayed significant positive correlations with Chl-a (THg: $r_s = 0.77$, $p < 0.01$, MeHg: $r = 0.54$,

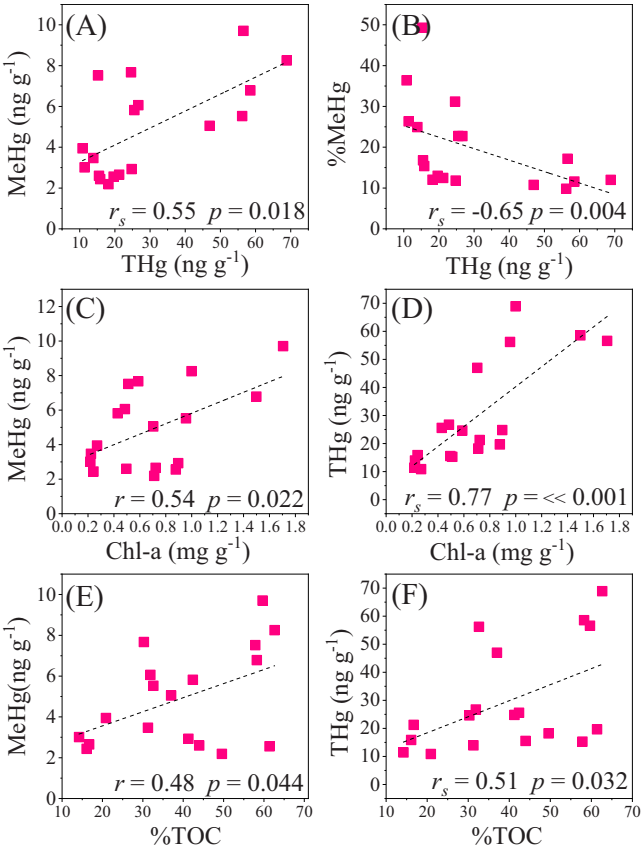

Fig. 2. The correlations of THg versus MeHg (A), THg versus %MeHg (B), Chl-a versus MeHg (C), Chl-a versus THg (D), %TOC versus MeHg (E), and %TOC versus THg (F) in periphyton.

$p < 0.05$) and total organic matter (THg: $r_s = 0.51$, $p < 0.05$, MeHg: $r = 0.48$, $p < 0.05$) in periphyton (Fig. 2C–F), suggesting the important role of phototrophs (e.g. algae, cyanobacteria and diatoms) on Hg bioavailability and/or bacterial growth in periphyton. Typically, the phototrophs are the backbones of periphyton, which serve as a substrate for cellular attachment and excrete organic substrates to support the growth of the heterotrophs (Fang et al., 2014; Ramanan et al., 2016). Moreover, the extracellular exudates released by phototrophs contain multiple functional groups (e.g. low molecular weight thiols), which might enhance the bioavailability of Hg in periphyton matrices (Leclerc et al., 2015). Overall, these results highlighted the cooperation of bacteria and algae on MeHg production in periphyton.

3.3. Periphyton MeHg release affects MeHg distribution in water

To explore the role of periphyton on Hg cycling in the Everglades, THg and MeHg concentrations in water column with varying distances (SW, POW, and PPW) from periphyton were investigated. Results found that THg concentrations (Figs. 3A and S4A) ranged from 1.30 to 3.70 ng L^{-1} (mean 2.56 ± 0.66 ng L^{-1}) in surface water, 1.07–6.73 ng L^{-1} (mean 3.73 ± 1.47 ng L^{-1}) in periphyton overlying water, and 8.67–52.6 ng L^{-1} (mean 22.7 ± 11.5 ng L^{-1}) in periphyton porewater, respectively. Overall, THg concentrations in periphyton porewater was the highest, followed by overlying and surface water, as revealed by Mann-Whitney test ($p < 0.01$ or $p < 0.05$).

Distribution of MeHg in water surrounding the periphyton was similar with THg (Figs. 3B and S4B). Periphyton porewater contained the highest MeHg, which ranged from 0.39 to 2.65 ng L^{-1} (mean 1.18 ± 0.52 ng L^{-1}). Overlying water contained the moderate level of MeHg, ranging from 0.15 to 0.91 ng L^{-1} (mean 0.49 ± 0.20 ng L^{-1}). Surface water displayed the lowest MeHg with a range of 0.11–0.27 ng L^{-1} (mean 0.21 ± 0.04 ng L^{-1}). Two possible reasons could explain this phenomenon that MeHg levels in water decreased with increasing distance from periphyton. One is that MeHg produced in periphyton will release from this matrix, which thus controls MeHg levels in water. The other is that periphyton takes up or adsorbs MeHg from water column, thus affecting the distribution of MeHg in water. The former explanation may be more reasonable in the Everglades, since our previous isotope study have already confirmed the significant influence of periphyton methylation/demethylation on MeHg levels in Everglades water (Li et al., 2012).

The distribution patterns of %MeHg were quite different from that of either MeHg or THg (Figs. 3C and S4C), which were highest in periphyton overlying water (range 3–25%, mean $14 \pm 6\%$), followed by surface water (range 4–16%, mean $9 \pm 3\%$) and porewater (range 3–14%, mean $6 \pm 3\%$). This phenomenon probably indicated that periphyton takes up inorganic Hg from the surrounding water, but only a small fraction of the inorganic Hg in the periphyton was methylated into MeHg, thus resulting in the lower %MeHg in periphyton porewater despite higher MeHg and THg concentrations there. Meanwhile, the THg in periphyton overlying and surface water was low, and the release of the MeHg from periphyton into porewater and then to the surrounding water elevated the MeHg concentrations in overlying and surface water, thus leading to higher %MeHg in periphyton overlying and surface water than in porewater. This distribution of water %MeHg around periphyton could be different from that around normal sediments (without periphyton). For instance, in stream ecosystems in Oregon, Wisconsin and Florida, %MeHg in sediment porewater could reach up to ~16% and ~9% in nonurban and urban areas, respectively (Marvin--Dipasquale et al., 2009), usually higher than in surface water. This is probably because the normal sediments are generally composed of bulk solid particles (mineral and organic) and under anaerobic conditions, where the produced MeHg could be readily retained. These results suggest that the MeHg produced in the periphyton tends to be released from the microcosms, either through microbial and algal exudates or after the decaying of periphyton, influencing the distribution of MeHg in the periphyton and surrounding water.

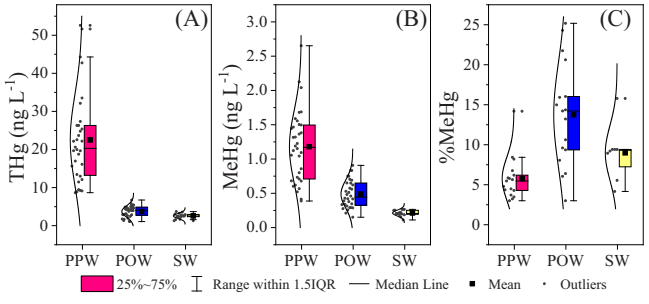

Fig. 3. THg(A), MeHg (B) and %MeHg (C) concentrations in water at different distances away from periphyton. PPW, POW and SW stand for periphyton porewater, periphyton overlying water and surface water, respectively.

3.4. EPS acts as an important route for periphyton MeHg release into water

To further explore the effect of periphyton on Hg distribution in the Everglades, sampling sites S1–S6 were taken as examples and the characteristics of THg and MeHg in the extracellular fractions of periphyton were analyzed. After normalizing the extracted EPS fractions to the per gram dry weight of periphyton, THg contents were 1.39–4.68 ng g^{-1} dw (mean 2.55 ± 1.53 ng g^{-1} dw) in colloidal EPS and 1.56–7.96 ng g^{-1} dw (mean 4.16 ± 2.75 ng g^{-1} dw) in capsular EPS, respectively (Figs. 4A and S5A). The higher THg concentrations in capsular EPS than in colloidal EPS ($p < 0.05$, Mann Whitney Test) indicated that THg (mainly as inorganic Hg(II)) should bind closely to the biofilm of periphyton (Leclerc et al., 2015). The EPS fractions only consisted 11–27% (mean $20 \pm 5.8\%$) of the total Hg in periphyton, which further suggested that live periphyton absorbs THg from the surrounding water and retain it inside the periphyton matrix.

In contrast to THg, MeHg in EPS fractions of periphyton showed a different tendency (Figs. 4B and S5B). In colloidal EPS, MeHg were in the range of 0.40–0.69 ng g^{-1} dw (mean 0.56 ± 0.11 ng g^{-1} dw), significantly higher than in capsular EPS (0.14–0.51 ng g^{-1} dw, mean 0.36 ± 0.20 ng g^{-1} dw) ($p < 0.05$, Mann Whitney Test). The EPS fractions accounted for 13–52% (mean $29 \pm 14\%$) of the total MeHg in periphyton, a relatively higher proportion ($p = 0.131$, Independent samples T-Test) than that of THg (mean $20 \pm 5.8\%$), which probably indicated that EPS played an important role in the re-distribution of periphyton MeHg. In one aspect, EPS serves as an adhesive agent enabling cellular attachment and forms a complex three dimensional biofilm matrix embedding the cells, which stimulates the growth of periphyton and enhance MeHg production (Fang et al., 2014; Laviale et al., 2009; Xiao and Zheng, 2016). Moreover, EPS is a complex high molecular weight (HMW) mixture of polymers, consisting of polysaccharides, proteins (enzymes and structural proteins), nucleic acid,

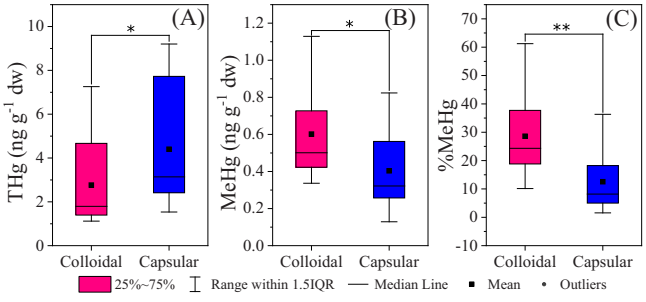

Fig. 4. THg (A), MeHg (B) and %MeHg (C) concentrations in EPS fractions of periphyton in the Everglades.

lipids, humic substances (HS), uronic acid, and inorganic components (Mishra et al., 2011; Xiao and Zheng, 2016). These components may bind MeHg when being excreted and thus affect the migration of MeHg from living organisms to the microhabitat of periphyton matrix and further to the surrounding water (Zhang et al., 2010). It was observed that the values of %MeHg (Fig. 4C) in colloidal EPS (range 10–61%, mean 29 ± 14%) were significantly higher than in capsular EPS (range 1.6–36%, mean 13 ± 10%) and periphyton (range 8–13%, mean 11 ± 2%) ($p < 0.01$, Mann Whitney Test), further suggesting the potential transport of periphyton MeHg to the surrounding water via EPS pathway.

Besides, the soluble products originated from EPS constitute of the majority of dissolved organic matter (DOM) derived from periphyton (Zhang et al., 2017). The excretion of EPS might result in elevated DOM concentrations in water, which could decrease the transmittance of UV through water and thus reduce the photo-demethylation of MeHg (Klapstein et al., 2016; Li et al., 2010). In this work, it was found that the further distances of water samples from periphyton, the lower the MeHg and DOC concentrations (Fig. S6), probably owing to the reduced photo-demethylation of MeHg in water closer periphyton matrix. The similar distribution patterns of MeHg and DOC in water also suggested the migration of periphyton MeHg into surrounding water with the release of EPS (Carlson et al., 1998; Muscatine, 1965; Lu et al., 2003).

3.5. Periphyton as a source of MeHg in Everglades water

The previously reported %MeHg in Everglades water may be, at least partially, attributed to MeHg production in periphyton and subsequent release (Liu et al., 2008b), as Everglades water itself seems not to have the ability to methylate inorganic Hg (Li et al., 2012; Mauro et al., 2002). Living organisms (bacteria and algae) in periphyton could exude MeHg (e.g., via EPS excretion) into the environment, as discussed above. Periphyton dies off and decays regularly, which will release MeHg from periphyton to the water column where MeHg could undergo further transport (e.g., sedimentation) and transformation (e.g., photo-degradation). The distribution coefficient ($logk_d$) (Eq. 1) was used to characterize the overall partitioning behavior of Hg between periphyton and water (Eckley et al., 2017; Liu et al., 2008a).

$$\log k_d = log_{10}\,[\text{Hg in solid matrix}]/[\text{Hg in (pore}-\text{)water}] \quad (1)$$

In this study, the logarithms of partitioning coefficients of THg (range 2.22–4.46, mean 3.55 ± 0.49) and MeHg (range 2.32–3.94, mean 3.19 ± 0.38) for periphyton were lower than or similar to those reported for soil (THg: range 4.51–5.05, mean 4.73 ± 0.14; MeHg: range 2.32–4.00, mean 3.27 ± 0.51) and floc (THg: 2.90–4.74, mean 4.21 ± 0.69; MeHg: range 3.42–4.60, mean 3.90 ± 0.35) in Everglades (Table S3) (Julian et al., 2016b; Liu et al., 2008a; Liu et al., 2011), suggesting lability of Hg in periphyton matrix and contribution of periphyton MeHg to water, as indicated by a line of evidence. For instance, enhanced Hg methylation in periphyton than sediment (or soil) has been found in the Everglades (Bae et al., 2019; Mauro et al., 2002). Our results showed MeHg and DOC concentrations in water decreased with the increasing distance away from periphyton (Fig. S6), probably due to periphyton MeHg migration into surrounding water with the release of DOC (Carlson et al., 1998; Lu et al., 2003). As we observed in this study, the outer-layer colloidal EPS had higher MeHg and %MeHg than the inner capsular EPS, indicating the higher re-distribution potential of MeHg from periphyton.

3.6. Periphyton as a food base contributing to food web MeHg bioaccumulation in the Everglades

Enhanced Hg methylation in periphyton has been well documented in numerous studies, but the re-distribution of MeHg from periphyton remains unknown. Through isotope analysis, it was found that $\delta^{15}N$ and $\delta^{34}S$ of mosquitofish and periphyton were significantly correlated, suggesting that periphyton may serve as an important food source in aquatic environments (Kendall and Bemis, 2005). It was reported that THg concentrations in mosquitofish (predominantly 95% as MeHg) displayed positive correlations with periphyton MeHg (Liu et al., 2008b) or THg (Julian and Gu, 2016a), suggesting that periphyton might be an important source of MeHg in the food webs (Bell and Scudder, 2005; Buckman et al., 2015; Desrosiers et al., 2006).

In this study, a model was developed to estimate the fraction of periphyton MeHg integrated into food webs via the diet uptake by using mosquitofish as an example (Eq. 2).

$$TS_{pe} = C_{pe} \times ME_{fs} \times AE_{fs} \quad (2)$$

where TS_{pe} stands for the total amount of periphyton MeHg that is transferred into fish. C_{pe} is the concentration of periphyton MeHg in the Everglades. AE_{fs} represents for the assimilation efficiency of MeHg in dietary exposure of mosquitofish. ME_{fs} means the total mass of periphyton eaten by mosquitofish seasonally, which can be calculated from the daily ingestion of fish (Eq. 3).

$$ME_{fs} = M_{fs} \times IN_{fs} \times P_{fs} \quad (3)$$

where M_{fs} is the total weight of mosquitofish in Everglades, IN_{fs} is the seasonal ingestion by mosquitofish (Newman and Doubet, 1989), and P_{fs} represents the proportions of periphyton to the total mosquitofish diet. All the related parameters were presented in Supporting Information (Table S4).

Based on this model, approximately 1.35 kg of MeHg within periphyton matrix is estimated to be passed on to mosquitofish in our sampling areas during the wet season (May-November) of the Everglades, which accounted for about 73% of the total Hg stock in mosquitofish. Apart from periphyton, some small insects, such as *Chironomid*, *Cladocera* and *P. pahitloMis*, were also important preys to mosquitofish, which might contribute to the rest Hg burdens in mosquitofish (Loftus, 2000). Besides, a small fraction (0.7–0.8%) of Hg burdens in mosquitofish could directly attribute to the aqueous exposure route (Pickhardt et al., 2006). It should be noted that the amounts of periphyton MeHg transferred into mosquitofish only account for 10% of the total MeHg in periphyton in the wet season. Besides mosquitofish, periphyton is also an important food source for other herbivorous and omnivorous fish, as well as macroinvertebrate infauna in the Everglades (Gaiser, 2009), and the fraction of periphyton MeHg integrated into the whole food web is expected to be higher. These results underscore the important contribution of periphyton on MeHg accumulation in the food web in the Everglades.

4. Conclusions

This work demonstrated the potential that periphyton MeHg can be accumulated from periphyton being a food base and MeHg released from periphyton to water column is readily available for further bioaccumulation in Everglades aquatic food webs at a 10% transfer efficiency (Lindeman, 1942). In this subtropical wetland, periphyton is considered as an algae-bacteria microhabitat dominating by heterotroph. Probably owing to the synergism between bacteria and algae, periphyton displayed significantly higher levels of MeHg and %MeHg than sediments, suggesting that periphyton is an important microhabitat producing MeHg in the Everglades. The different distribution patterns of %MeHg compared to THg and MeHg in surrounding waters of periphyton may suggested that MeHg produced in periphyton tends to be released from the microcosms, which may affect the distribution of MeHg in surrounding water of the Everglades. A line of evidences could support this observation, for instance: (1) the lability of Hg in periphyton, as evidenced by the lower or similar distribution coefficient ($logk_d$) of THg and MeHg for periphyton to those reported for soil and floc in Everglades; (2) the high re-distribution potential of MeHg from periphyton, as evidenced by the significantly higher levels of MeHg and

%MeHg in the outer-layer colloidal EPS than those in the inner capsular EPS.

Periphyton act as an important food base in the Everglades, thus a model was developed to estimate the fraction of MeHg integrated into food webs through the diet uptake by using mosquitofish as an example. The modeling results clearly indicated that periphyton is an important contributor to the fish Hg burdens in the Everglades, which accounted for 73% of the total Hg stock in mosquitofish in the wet season. There are still some drawbacks for improvement in this work. For instance, we only estimated the contribution of periphyton MeHg to mosquitofish Hg during the wet season, owing to the limitation of sampling in dry season. However, this did not affect the judgement of the overall importance of periphyton to the Hg burdens in the food webs of the Everglades. Further research is also needed to address the dynamic process of periphyton affecting the distribution of water MeHg, to gain a more comprehensive picture of the effects of periphyton presence on Hg cycling in aqueous ecosystems.

CRediT authorship contribution statement

Yuping Xiang: Conceptualization, investigation, formal analysis, visualization, writing - original draft. **Guangliang Liu:** Methodology, investigation, funding acquisition, supervision, writing - review & editing. **Yongguang Yin:** Conceptualization, writing - review & editing. **Yong Cai:** Project administration, funding acquisition, resources, supervision.

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.

Acknowledgments

This research was partially supported by Natural Science Foundation of China (21677061 and 91543103) and NSF Program of the USA (ECS1905239). The latter contribution number # is from the Southeast Environmental Research Center in the Institute of Water & Environment at Florida International University.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124551.

References

Acha, D., Hintelmann, H., Yee, J., 2011. Importance of sulfate reducing bacteria in mercury methylation and demethylation in periphyton from Bolivian Amazon region. Chemosphere 82, 911–916. https://doi.org/10.1016/j.chemosphere.2010.10.050.

Ackerman, J.T., Eagles-Smith, C.A., 2010. Agricultural wetlands as potential hotspots for mercury bioaccumulation: experimental evidence using caged fish. Environ. Sci. Technol. 44, 1451–1457. https://doi.org/10.1021/es9028364.

Bae, H.S., Dierberg, F.E., Ogram, A., 2019. Periphyton and flocculent materials are important ecological compartments supporting abundant and diverse mercury methylator assemblages in the Florida Everglades. Appl. Environ. Microb. 85, 1–17. https://doi.org/10.1128/AEM.00156-19.

Bell, A.H., Scudder, B.C., 2005. Bioaccumulation of mercury in riverine periphyton. US Geological Survey, Reston, Virginia. https://doi.org/10.3133/ofr20041446.

Bellinger, B.J., Gretz, M.R., Domozych, D.S., Kiemle, S.N., Hagerthey, S.E., 2010. Composition of extracellular polymeric substances from periphyton assemblages in the Florida Everglades. J. Phycol. 46, 484–496. https://doi.org/10.1111/j.1529-8817.2010.00829.x.

Benoit, J.M., Gilmour, C.C., Mason, R.P., Heyes, A., 1999. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. Environ. Sci. Technol. 33, 951–957. https://doi.org/10.1021/es992007q.

Biggs, B.J., Close, M.E., 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshw. Biol. 22, 209–231. https://doi.org/10.1111/j.1365-2427.1989.tb01096.x.

Buckman, K.L., Marvin-DiPasquale, M., Taylor, V.F., Chalmers, A., Broadley, H.J., Agee, J., Jackson, B.P., Chen, C.Y., 2015. Influence of a chlor-alkali superfund site on mercury bioaccumulation in periphyton and low-trophic level fauna. Environ. Toxicol. Chem. 34, 1649–1658. https://doi.org/10.1002/etc.2964.

Carlson, C.A., Ducklow, H.W., Hansell, D.A., Smith, W.O., 1998. Organic carbon partitioning during spring phytoplankton blooms in the Ross Sea polynya and the Sargasso Sea. Limnol. Oceanogr. 43, 375–386. https://doi.org/10.2307/2839087.

Clayden, M.G., Kidd, K.A., Wyn, B., Kirk, J.L., Muir, D.C.G., O'Driscoll, N.J., 2013. Mercury biomagnification through food webs is affected by physical and chemical characteristics of lakes. Environ. Sci. Technol. 47, 12047–12053. https://doi.org/10.1021/es4022975.

Cleckner, L.B., Garrison, P.J., Hurley, J.P., Olson, M.L., Krabbenhoft, D.P., 1998. Trophic transfer of methyl mercury in the northern Florida Everglades. Biogeochemistry 40, 347–361. https://doi.org/10.1023/a:1005918101773.

Cleckner, L.B., Gilmour, C.C., Hurley, J.P., Krabbenhoft, D.P., 1999. Mercury methylation in periphyton of the Florida Everglades. Limnol. Oceanogr. 44, 1815–1825. https://doi.org/10.4319/lo.1999.44.7, 1815.

Correia, R.R., Miranda, M.R., Guimaraes, J.R., 2012. Mercury methylation and the microbial consortium in periphyton of tropical macrophytes: effect of different inhibitors. Environ. Res. 112, 86–91. https://doi.org/10.1016/j.envres.2011.11.002.

Cyr, H., Morton, K.E., 2006. Distribution of biofilm exopolymeric substances in littoral sediments of Canadian Shield lakes: the effects of light and substrate. Can. J. Fish. Aquat. Sci. 63, 1763–1776. https://doi.org/10.1139/f06-079.

Desrosiers, M., Planas, D., Mucci, A., 2006. Total mercury and methylmercury accumulation in periphyton of Boreal Shield lakes: influence of watershed physiographic characteristics. Sci. Total Environ. 355, 247–258. https://doi.org/10.1016/j.scitotenv.2005.02.036.

Drott, A., Lambertsson, L., Björn, E., Skyllberg, U., 2007. Importance of dissolved neutral mercury sulfides for methyl mercury production in contaminated sediments. Environ. Sci. Technol. 41, 2270–2276. https://doi.org/10.1021/es061724z.

Eckley, C.S., Luxton, T.P., Goetz, J., McKernan, J., 2017. Water-level fluctuations influence sediment porewater chemistry and methylmercury production in a flood-control reservoir. Environ. Pollut. 222, 32–41. https://doi.org/10.1016/j.envpol.2017.01.010.

Ewe, S.M.L., Gaiser, E.E., Childers, D.L., Iwaniec, D., Rivera-Monroy, V.H., Twilley, R.R., 2006. Spatial and temporal patterns of aboveground net primary productivity (ANPP) along two freshwater-estuarine transects in the Florida Coastal Everglades. Hydrobiologia 569, 459–474. https://doi.org/10.1007/s10750-006-0149-5.

Fang, F., Lu, W., Shan, Q., Cao, J., 2014. Characteristics of extracellular polymeric substances of phototrophic biofilms at different aquatic habitats. Carbohydr. Polym. 106, 1–6. https://doi.org/10.1016/j.carbpol.2014.02.010.

Flemming, H.C., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Micro. 8, 623–633. https://doi.org/10.1038/nrmicro2415.

Gabriel, M.C., Howard, N., Osborne, T.Z., 2014. Fish mercury and surface water sulfate relationships in the Everglades protection area. Environ. Manag. 53, 583–593. https://doi.org/10.1007/s00267-013-0224-4.

Gaiser, E., 2009. Periphyton as an indicator of restoration in the Florida Everglades. Ecol. Indic. 9, 37–45. https://doi.org/10.1016/j.ecolind.2008.08.004.

Gaiser, E.E., McCormick, P.V., Hagerthey, S.E., Gottlieb, A.D., 2011. Landscape patterns of periphyton in the Florida Everglades. Crit. Rev. Environ. Sci. Tec. 41, 92–120. https://doi.org/10.1080/10643389.2010.531192.

Gilmour, C.C., Riedel, G., Ederington, M., Bell, J., Gill, G., Stordal, M., 1998. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochemistry 40, 327–345. https://doi.org/10.1023/A:1005972708616.

Graham, A.M., Aiken, G.R., Gilmour, C.C., 2013. Effect of dissolved organic matter source and character on microbial Hg methylation in Hg-S-DOM solutions. Environ. Sci. Technol. 47, 5746–5754. https://doi.org/10.1021/es400414a.

Hagerthey, S.E., Bellinger, B.J., Wheeler, K., Gantar, M., Gaiser, E., 2011. Everglades periphyton: a biogeochemical perspective. Crit. Rev. Environ. Sci. Tec. 41, 309–343. https://doi.org/10.1080/10643389.2010.531218.

Hamelin, S., Amyot, M., Barkay, T., Wang, Y., Planas, D., 2011. Methanogens: principal methylators of mercury in lake periphyton. Environ. Sci. Technol. 45, 7693–7700. https://doi.org/10.1021/es2010072.

Hamelin, S., Planas, D., Amyot, M., 2015. Mercury methylation and demethylation by periphyton biofilms and their host in a fluvial wetland of the St. Lawrence River (QC, Canada). Sci. Total Environ. 512–513, 464–471. https://doi.org/10.1016/j.scitotenv.2015.01.040.

Hsu-Kim, H., Kucharzyk, K.H., Zhang, T., Deshusses, M.A., 2013. Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: a critical review. Environ. Sci. Technol. 47, 2441–2456. https://doi.org/10.1021/es304370g.

Iwaniec, D.M., Childers, D.L., Rondeau, D., Madden, C.J., Saunders, C., 2006. Effects of hydrologic and water quality drivers on periphyton dynamics in the southern Everglades. Hydrobiologia 569 (1), 223–235. https://doi.org/10.1007/s10750-006-0134-z.

Julian, P., Gu, B., 2015. Mercury accumulation in largemouth bass (Micropterus salmoides Lacepede) within marsh ecosystems of the Florida Everglades, USA. Ecotoxicology 24, 202–214. https://doi.org/10.1007/s10646-014-1373-9.

Julian, P., Gu, B., 2016a. Appendix 3B-1: Everglades mercury hotspot study: perliminary analysis. 2016 South Florida Environmental Report.

Julian, P., Gu, B., Wright, A.L., 2016b. Mercury stoichiometric relationships in a subtropical peatland. Water Air Soil Pollut. 227, 472. https://doi.org/10.1007/s11270-016-3180-9.

Kendall, C., Bemis, B.E., 2005. Assessing the Influences of Mercury Bioaccumulation and Bioavailability in Everglades Food Webs. American Geophysical Union.
Klapstein, S.J., Ziegler, S.E., Risk, D.A., O'Driscoll, N.J., 2016. Quantifying the effects of photoreactive dissolved organic matter on methylmercury photodemethylation rates in freshwaters. Environ. Toxicol. Chem. 36, 1493–1502. https://doi.org/10.1002/etc.3690.
Laviale, M., Prygiel, J., Lemoine, Y., Courseaux, A., Créach, Stream, A., 2009. periphyton photoacclimation response in field conditions: effect of community development and seasonal changes. J. Phycol. 45, 1072–1082. https://doi.org/10.1111/j.1529-8817.2009.00747.x.
Lázaro, W.L., Díez, S., Silva, C.J.D., Ignácio, Á.R.A., Guimarães, J.R.D., 2018. Seasonal changes in peryphytic microbial metabolism determining mercury methylation in a tropical wetland. Sci. Total Environ. 627, 1345–1352. https://doi.org/10.1016/j.scitotenv.2018.01.186.
Leclerc, M., Planas, D., Amyot, M., 2015. Relationship between extracellular low-molecular-weight thiols and mercury species in natural lake periphytic biofilms. Environ. Sci. Technol. 49, 7709–7716. https://doi.org/10.1021/es505952x.
Li, Y., Mao, Y., Liu, G., Tachiev, G., Roelant, D., Feng, X., Cai, Y., 2010. Degradation of methylmercury and its effects on mercury distribution and cycling in the Florida Everglades. Environ. Sci. Technol. 44, 6661–6666. https://doi.org/10.1021/es1010434.
Li, Y., Yin, Y., Liu, G., Tachiev, G., Roelant, D., Jiang, G., Cai, Y., 2012. Estimation of the major source and sink of methylmercury in the Florida Everglades. Environ. Sci. Technol. 46, 5885–5893. https://doi.org/10.1021/es204410x.
Lin, C., Jay, J.A., 2007. Mercury methylation by planktonic and biofilm cultures of Desulfovibrio desulfuricans. Environ. Sci. Technol. 41, 6691–6697. https://doi.org/10.1021/es062304c.
Lindeman, R.L., 1942. The trophic-dynamic aspect of ecology. Ecology 23, 399–418. https://doi.org/10.2307/1930126.
Liu, G., Cai, Y., Kalla, P., Scheidt, D., Richards, J., Scinto, L.J., Gaiser, E., Appleby, C., 2008a. Mercury mass budget estimates and cycling seasonality in the Florida Everglades. Environ. Sci. Technol. 42, 1954–1960. https://doi.org/10.1021/es7022994.
Liu, G., Cai, Y., Philippi, T., Kalla, P., Scheidt, D., Richards, J., Scinto, L., Appleby, C., 2008b. Distribution of total and methylmercury in different ecosystem compartments in the Everglades: implications for mercury bioaccumulation. Environ. Pollut. 153, 257–265. https://doi.org/10.1016/j.envpol.2007.08.030.
Liu, G., Naja, G.M., Kalla, P., Scheidt, D., Gaiser, E., Cai, Y., 2011. Legacy and fate of mercury and methylmercury in the Florida Everglades. Environ. Sci. Technol. 45, 496–501. https://doi.org/10.1021/es101207f.
Loftus, W.F., 2000. Accumulation and Fate of Mercury in an Everglades Aquatic Food Web. In: Florida International University.
Lu, X., Maie, N., Hanna, J., Childers, D., Jaffé, R., 2003. Molecular characterization of dissolved organic matter in freshwater wetlands of the Florida Everglades. Water Res. 37, 2599–2606. https://doi.org/10.1016/s0043-1354(03)00081-2.
Marvin-Dipasquale, M., Lutz, M.A., Brigham, M.E., Krabbenhoft, D.P., Aiken, G.R., Orem, W.H., 2009. Mercury cycling in stream ecosystems. 2. Benthic methylmercury production and bed sediment-pore water partitioning. Environ. Sci. Technol. 43 (8), 2726–2732. https://doi.org/10.1021/es802698v.
Mauro, J., Guimaraes, J., Hintelmann, H., Watras, C., Haack, E., Coelho-Souza, S., 2002. Mercury methylation in macrophytes, periphyton, and water-comparative studies with stable and radio-mercury additions. Anal. Bioanal. Chem. 374, 983–989. https://doi.org/10.1007/s00216-002-1534-1.
Mishra, A., Kavita, K., Jha, B., 2011. Characterization of extracellular polymeric substances produced by micro-algae Dunaliella salina. Carbohydr. Polym. 83, 852–857. https://doi.org/10.1016/j.carbpol.2010.08.067.
Moed, J.R., Hallegraeff, G.M., 1978. Some problems in the estimation of Chlorophyll-a and phaeopigments from pre-and post-acidification spectrophotometrie measurements. Int. Rev. Hydrobiol. 63, 787–800. https://doi.org/10.1002/iroh.19780630610.
Muscatine, L., 1965. Symbiosis of hydra and algae. III. Extracellular products of the algae. Oceanogr. Comp. Biochem. Physiol. 16, 77–92. https://doi.org/10.1016/0010-406X(65)90165-9.
Newman, M.C., Doubet, D.K., 1989. Size-dependence of mercury (II) accumulation kinetics in the mosquitofish, Gambusia affinis (Baird and Girard). Arch. Environ. Contam. Toxicol. 18, 819–825. https://doi.org/10.1007/BF01160295.
O'Brien, P.J., Wehr, J.D., 2010. Periphyton biomass and ecological stoichiometry in streams within an urban to rural land-use gradient. Hydrobiologia 657, 89–105. https://doi.org/10.1007/s10750-009-9984-5.
Pickhardt, P.C., Stepanova, M., Fisher, N.S., 2006. Contrasting uptake routes and tissue distributions of inorganic and methylmercury in mosquitofish (Gambusia affinis) and redear sunfish (Lepomis microlophus). Environ. Toxicol. Chem. 25, 2132–2142. https://doi.org/10.1897/05-595R.1.
Planas, D., Desrosiers, M., Hamelin, S., 2004. Mercury methylation in periphyton biofilms. RMZ-M&G 51, 1309–1311.
Ramanan, R., Kim, B.H., Cho, D.H., Oh, H.M., Kim, H.S., 2016. Algae-bacteria interactions: evolution, ecology and emerging applications. Biotechnol. Adv. 34, 14–29. https://doi.org/10.1016/j.biotechadv.2015.12.003.
Rose, F.L., Cushing, C.E., 1970. Periphyton: autoradiography of Zinc-65 adsorption. Science 168, 576–577. https://doi.org/10.1126/science.168.3931.576.
Sanli, K., Nilsson, R.H., Blanck, H., Bengtsson-Palme, J., Kristiansson, E., Alm, R.M., Eriksson, K.M., 2015. Metagenomic sequencing of marine periphyton: taxonomic and functional insights into biofilm communities. Front. Microbiol. 6, 1192. https://doi.org/10.3389/fmicb.2015.01192.
Stober Q.J., Thornton K., Jones R., Richards J., Ivey C., Welch R., Madden M., Trexler J., Gaiser E., Scheidt D. and Rathbun S. 2001. South Florida ecosystem assessment: Phase I/II: Everglades stressor interactions: hydropatterns, eutrophication, habitat alteration, and mercury contamination. Monitoring for adaptive management: implications for ecosystem restoration.
Tang, W.L., Liu, Y.R., Guan, W.Y., Zhong, H., Qu, X.M., Zhang, T., 2020. Understanding mercury methylation in the changing environment: recent advances in assessing microbial methylators and mercury bioavailability. Sci. Total Environ. 714, 136827 https://doi.org/10.1016/j.scitotenv.2020.136827.
Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit. Rev. Environ. Sci. Tec. 31, 241–293. https://doi.org/10.1080/20016491089226.
USEPA, 2001. Method 1630: methyl mercury in water by distillation, aqueous ethylation, purge and trap, and CVAFS Office of Water.
USEPA, 2002. Method 1631 revision E: mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry Office of Water.
Xiao, R., Zheng, Y., 2016. Overview of microalgal extracellular polymeric substances (EPS) and their applications. Biotechnol. Adv. 34, 1225–1244. https://doi.org/10.1016/j.biotechadv.2016.08.004.
Zhang, D., Pan, X., Mostofa, K.M., Chen, X., Mu, G., Wu, F., Liu, J., Song, W., Yang, J., Liu, Y., 2010. Complexation between Hg (II) and biofilm extracellular polymeric substances: an application of fluorescence spectroscopy. J. Hazard. Mater. 175 (1), 359–365. https://doi.org/10.1016/j.jhazmat.2009.10.011.
Zhang, Z., Guo, L., Wang, Yi, Li, F., Zhao, Y., Gao, M., She, Z., 2017. Degradation and transformation of extracellular polymeric substances (EPS) and dissolved organic matters (DOM) during two-stage anaerobic digestion with waste sludge. Int. J. Hydrog. Energ. 42, 9619–9629. https://doi.org/10.1016/j.ijhydene.2017.02.201.