

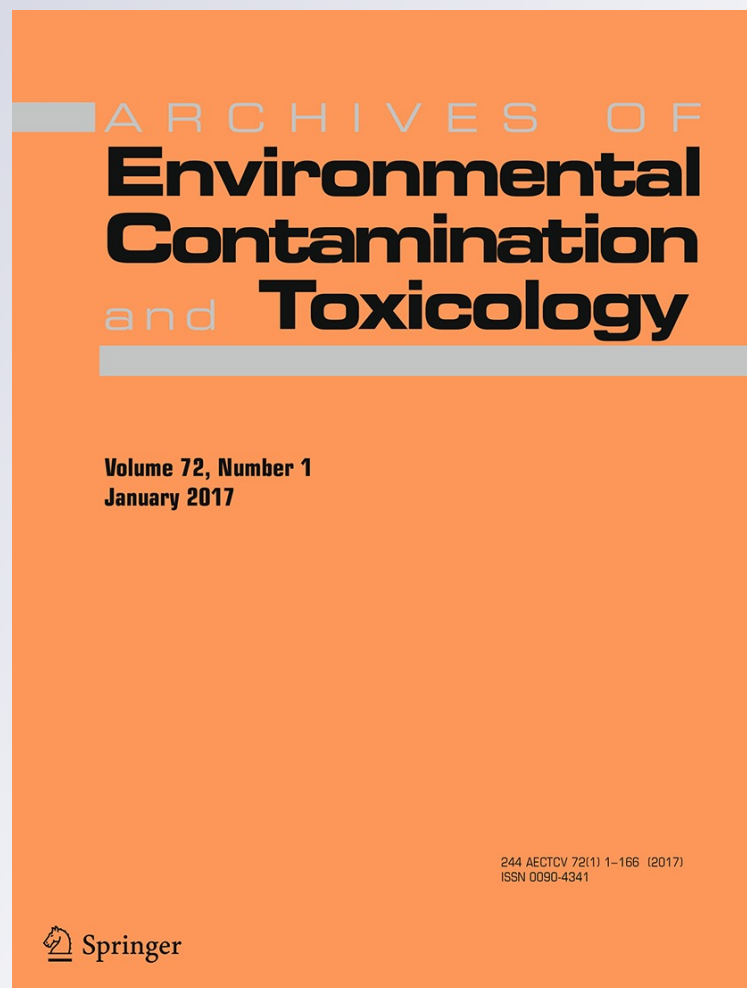
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Association of a Specific Algal Group with Methylmercury Accumulation in Periphyton of a Tropical High-Altitude Andean Lake

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Abstract Periphyton relevance for methylmercury (MeHg) production and accumulation are now well known in aquatic ecosystems. Sulfate-reducing bacteria and other microbial groups were identified as the main MeHg producers, but the effect of periphyton algae on the accumulation and transfer of MeHg to the food web remains little studied. Here we investigated the role of specific groups of algae on MeHg accumulation in the periphyton of *Schoenoplectus californicus* ssp. (Totora) and *Myriophyllum* sp. in Uru Uru, a tropical high-altitude Bolivian lake with substantial fishing and mining activities accruing around it. MeHg concentrations were most strongly related to the cell abundance of the Chlorophyte genus *Oedogonium* ($r^2 = 0.783$, $p = 0.0126$) and to no other specific genus despite the presence of other 34 genera

identified. MeHg was also related to total chlorophyll-a (total algae) ($r^2 = 0.675$, $p = 0.0459$), but relations were more significant with chlorophyte cell numbers, chlorophyll-b (chlorophytes), and chlorophyll-c (diatoms and dinoflagellates) ($r^2 = 0.72$, $p = 0.028$, $r^2 = 0.744$, $p = 0.0214$, and $r^2 = 0.766$, $p = 0.0161$ respectively). However, *Oedogonium* explains most variability of chlorophytes and chlorophyll-c ($r^2 = 0.856$, $p = < 0.001$ and $r^2 = 0.619$, $p = 0.002$, respectively), suggesting it is the most influential group for MeHg accumulation and periphyton algae composition at this particular location and given time.

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Mercury (Hg), a toxic metal, is widely distributed and persistent in the environment (Morel et al. 1998). The toxicity and bioavailability of Hg depend on its chemical form, among which methylmercury (MeHg) is the most environmentally relevant. MeHg is more relevant than other Hg species because it accumulates in the organisms and biomagnifies through the food chain (Mason et al. 1996; Watras et al. 1998) and can cause severe effects on biota and human health (Falandysz et al. 2002).

Hg methylation in aquatic ecosystems takes place in aquatic compartments such as sediments (Heyes et al. 2006), water column (Achá et al. 2012a; Eckley and Hintelmann 2006), and periphyton (Achá et al. 2011; Guimaraes et al. 2006; Hamelin et al. 2015a). Several studies show that MeHg production is higher in periphyton than in other aquatic compartments (Gentes et al. 2013; Guimarães et al. 2000; Hamelin et al. 2015a). Microbial activity mainly mediates this process. Sulfate-reducing bacteria (SRB) are frequently the most important Hg methylators (Achá et al. 2011; Compeau and Bartha 1985; Gentes et al. 2013), although iron (Fe)-reducing bacteria

(Fleming et al. 2006; Kerin et al. 2006), methanogens (Gilmour et al. 2013; Hamelin et al. 2011; Yu et al. 2013), and other bacteria (Achá et al. 2012b; Gilmour et al. 2013) may also be important Hg methylators.

Herein, periphyton is defined as a biofilm composed of algae, bacteria, fungi, microinvertebrates, and organic and inorganic detritus. All of it is held together by a mucopolysaccharide matrix (Lowe 1996). Periphyton is considered one of the main sources of food for aquatic organisms (invertebrates and fish) (Junk and Piedade 1997; Molina et al. 2010) as well as a major entry point of MeHg into the food web (Chasar et al. 2009; Molina et al. 2010). Periphyton is known to potentially accumulate high MeHg concentrations (Desrosiers et al. 2006; Hamelin et al. 2015b; Miles et al. 2001), and bioaccumulation is greater with increasing periphyton biomass (Bell and Scudder 2007; Hamelin et al. 2015b), although a dilution effect has also been documented (Desrosiers et al. 2006). Hg accumulation in periphyton can be explained in part by the Hg binding with algae and other organic compounds (naturals ligands, humic acids, thiols group, and others) (Gorski et al. 2006; Hintelmann et al. 1995; Pant et al. 1995), but little attention has been paid on how particular organisms hosting the periphyton compartment may influence MeHg accumulation.

Algal community in periphyton could affect metal accumulation (Hill and Larsen 2005). Lazaro et al. (2013) showed that conditions that favor cyanobacteria colonization in periphyton are associated with higher MeHg production, suggesting the likely relevance of algae for MeHg impacts. There is plenty of evidence regarding the algae-mediated Hg(II) reduction to Hg⁰ (Ben-Bassat and Mayer 1977; Le Faucheur et al. 2014; Mason et al. 1995), which may be regarded as a defense mechanism (Gregoire and Poulain 2014; Le Faucheur et al. 2014). Bell and Scudder (2007) reported high concentrations of MeHg in periphyton samples with high abundance of diatoms, suggesting a greater transfer of MeHg to consumers when diatoms dominate in the periphyton. Other suggest that changes in the periphyton community may influence its potential for MeHg production (Buckman et al. 2015). However, it remains unclear how other groups of algae may contribute to MeHg accumulation, in particular in high-altitude aquatic ecosystems. Here we evaluated the influence of different algal groups on the bioaccumulation of MeHg in periphyton at a given time at Lake Uru Uru (3686 m.a.s.l.), the largest source of fish for Oruro (approximately half a million people). For this purpose, we determined the algal composition in periphyton using microscopic identification, quantification, and chlorophyll analysis. We also measured MeHg, inorganic Hg (IHg), and total Hg (THg) concentrations in periphyton.

Materials and Methods

Study Area

Lake Uru Uru is part of the TDPS (Titicaca–Desaguadero–Poopó–Salar de Coipasa) hydrological system and is located in the Bolivian highlands at 3,686 m.a.s.l. It is a shallow lake (0.5–2 m) formed in the 1990s due to the human-made deviation of the Desaguadero River (United Nations Environment Programme 1996). The main water inlets are Desaguadero and Thajarita rivers. Lake Uru Uru is connected with Lake Poopó through a 30-km channel. The climate at Lake Uru Uru is cold and dry with average annual temperatures <10 °C and large temperature fluctuations between day and night (Biosca 1998). The economy of the region depends mainly on mining due to the presence of large polymetallic deposits (mainly tin, gold, silver, and copper), thus causing serious pollution in Lake Uru Uru (PPO-9505 1995; UNEP 1996), which is the main receptacle of trace metals and metalloids in the area (Garcia et al. 2005). Uru Uru water has high pH, high conductivity, and strong daily oxygen and temperature gradients (Table 1), which are larger than the seasonal gradients (Alanoca et al. 2016a). The elevated conductivity is related to a natural process of desiccation in the basin that leads to a permanent water deficit and a wide seasonal variation of the lake level.

Sampling Methods

Sampling was performed in May 2011 during the transition season (lower water) between the dry and wet seasons. Three sampling areas were selected along Lake Uru Uru: north (latitude 18°4'55.20"S and longitude 67°4'1.19"W), central (latitude 18°9'21.59"S and longitude 67°5'20.39"W), and south (latitude 18°12'21.60"S and longitude 67°4'40.08"W). Periphyton samples associated with macrophytes in each location were collected. Periphyton associated with *Schoenoplectus californicus* (Totora) was removed by scraping the surface of the macrophyte, and the scrapings collected in new, acid-cleaned 50-mL polyethylene tubes so the samples were suitable for trace-element analysis. *Myriophyllum* and its associated periphyton were manually collected in new zip-lock bags to isolate later. The hard stems were discarded, and as much *Myriophyllum* as possible was separated from the periphyton (some very small and soft portions of *Myriophyllum* were impossible to separate from the periphyton). The remaining sample was then stored in new, previously cleaned 50-mL polyethylene tubes. Subsequently, each sample was divided into three subsamples. The first was for microscopic identification and quantification and thus was

Table 1 Chlorophylls concentrations in Totora-associated periphyton and physicochemical data of the water column in Uru Uru lake

Variable	North	N	Central	N	South	N
Chlorophyll "A" (mg L ⁻¹)	20.38 ± 10.26	4	4.01 ± 2.73	3	6.81 ± 3.64	4
Chlorophyll "B" (mg L ⁻¹)	5.19 ± 2.72	4	1.96 ± 0.85	3	2.04 ± 1.28	4
Chlorophyll "C" (mg L ⁻¹)	6.39 ± 3.40	4	3.59 ± 0.85	3	1.67 ± 0.16	4
Temperature (°C)	10.37 ± 0.17	4	14.11 ± 0.72	6	11.14 ± 0.14	16
Dissolved oxygen (mg L ⁻¹)	3.55 ± 0.43	4	3.73 ± 0.44	6	4.33 ± 1.14	16
pH (Units)	8.74 ± 0.62	3	8.83 ± 0.32	4	8.74 ± 0.75	16
Conductivity (µS cm ⁻¹)	2450.5 ± 608.81	3	2285.75 ± 590.32	4	2110.58 ± 5.59	12

fixed with 4% formaldehyde. The second was preserved at -20 °C for analyzing chlorophyll-a, -b, and -c. The third fraction was preserved at -20 °C immediately after collection for THg and MeHg analysis. Such samples were later freeze-dried to determine dry weight and analysis. Physicochemical measurements were also taken in the water column (pH, temperature, dissolved oxygen, and conductivity) in each sampling area with a multiparameter probe (Hydrolab).

Microscopic Analysis

A fresh sample of approximately 1 cm³ was diluted into ultra-clean water until a clear image for identification and quantification was obtained. Algae were identified as to genus level and counted at ×250 magnification in a Sedgewick–Rafter counting chamber (1-mL volume) using an optical microscope (Carl Zeiss). The number of cells counted in 200 squares was extrapolated to 1000 squares (equivalent to 1 mL) to obtain the number of cells per milliliter of sample. Due to the size difference between eukaryotic cell (diatoms and chlorophytes) and prokaryotic cell (cyanobacteria), a correction of cell size was applied to the cyanobacteria. For filamentous cyanobacteria, approximately every 50 µm were counted as 1 cell (which was approximately equivalent to the volume of a filamentous green algae cell); and for colonies, every 50 cells were counted as a single cell (approximately the size of 1 free-living chlorophyte cell).

Chlorophyll Analysis

Chlorophyll pigments were determined according to United States Environmental Protection Agency method 446 (Arar 1997). Samples were ground in 90% acetone to obtain a homogeny extract and refrigerated at 4 °C for 24 h. Then samples were centrifuged for 10 min at 3000 rpm and supernatant filtered through a Millipore filter paper GF/F (pore size 0.7 µm). Finally, a 3-mL of sample was taken to measure absorbance at 630, 647, 664, and 750 nm corresponding to chlorophyll-c (diatoms and dinoflagellates), -b (chlorophytes), -a (all algae), respectively, and turbidity

was determined by spectrophotometer (Lambda 25, Perkin Elmer). Chlorophyll concentrations were calculated using Jeffrey and Humprey (1975) equations. The concentration of pheophytin-a was determined by acidification of the sample with 0.09 mL of 0.1 N HCl, and absorbance was measured at 665 and 750 nm after 90 s. Pheophytin-a was calculated using Lorenzen (1967) equation. The latter was only determined for *Myriophyllum* samples containing *Myriophyllum* tissue, which was impossible to separate from the algae.

Hg Analysis

Sample manipulation and digestions were performed in acid-cleaned flasks and tubes to avoid contamination. MeHg and IHg concentrations from *S. californicus* samples were determined by double-spike stable-isotope dilution using gas chromatography–inductively coupled plasma mass spectrometry (DS-ID-GC-ICPMS) (Monperrus et al. 2008; Point et al. 2007). THg was expressed as the sum of IHg and MeHg individual levels. MeHg concentrations from *Myriophyllum* samples were determined by the selective and quantitative extraction of MeHg followed by cold-vapor atomic fluorescence spectroscopy (CV-AFS) determinations according to the procedure described by Masbou et al. (2013). Because there is no periphyton-like reference material, two different biological reference materials consisting of DOLT 4 and TORT 2, obtained from the National Research Council of Canada, were tested to ensure measurement accuracy and traceability. MeHg recoveries of 98 ± 1% (*n* = 2) and 105 ± 4% (*n* = 2) relative to the certified concentrations were obtained for DOLT 4 and TORT 2 materials, respectively.

Statistical Analysis

Statistical analysis was performed using Sigma Plot 11.0 graphic package and the IBM (SPSS, Chicago, Illinois, USA) Statistics 20 statistical package. Significant differences between sampling areas and/or data groups were determined using one-way analysis of variance (ANOVA) and Kruskal–Wallis nonparametric test when residues did

not show normal distribution or failed the homogeneity of variance test. Linear regression analysis was performed to evaluate the relationship between abundance of algal groups and chlorophyll with MeHg concentration. Because the number of replicates was frequently <10 , a rigorous diagnostic was performed. In addition to testing normality (Shapiro–Wilk), constant variance and power of the test, independence of residuals (Durbin–Watson statistic), and the influence of each value on the regression was evaluated (Cook's distance, leverage and DFFITS). In addition, outliers were identified by regression diagnostic with standardized residuals and studentized residuals. In all cases, $p < 0.05$ was assumed to be significant.

Results and Discussion

Algal Abundance and Distribution

Algal abundance in the periphyton varied significantly between sampling areas (Kruskal–Wallis test, $H = 7.229$, $p = 0.017$): It was higher in the north area (Fig. 1a). Chlorophyll-*a* concentrations were also higher in the north area (Fig. S1); however, the chlorophyll concentrations may have been overestimated due to the presence of *Myriophyllum* remaining in some of the samples at the central location. The algal community was widely dominated by diatoms with chlorophytes and cyanobacteria with relatively low abundance (Fig. 1b). Diatom abundance appeared to decrease from north to south (ANOVA, $p = 0.009$), whereas chlorophytes (ANOVA, $p = 0.028$) and cyanobacteria increased their abundance; however, this change was only significant for cyanobacteria relative

abundance (ANOVA, $p = 0.025$). This variation could be linked to differences in the distribution of nutrients in the sampling areas. Indeed, the north of the lake is more directly exposed to untreated sewage waters from Oruro, which gradually becomes diluted toward the south (Ala-noca et al. 2016b).

Concentrations of chlorophyll-*a*, -*b* and -*c*, respectively, used here as indicators of total active algae, chlorophytes, and diatoms showed a slightly different picture of algae abundance and distribution in Lake Uru Uru. Chlorophyll-*a* and -*c* distribution patterns were approximately the same as those observed for cell counts (Fig. S1). However, there was more chlorophyll-*b* in the north suggesting, unlike the cell counts, higher abundance of chlorophytes toward the north of the system (Fig. S1). Such contradiction may be related to the inability of the microscopic technique to detect pico- and nano-algae (Schlüter et al. 2006) as well as distinguish between photosynthetically active and inactive cells. However, the microscopic technique has the advantage of allowing detailed taxonomical discrimination. Chlorophyll analysis enables the quantification of active populations regardless of their size, but it is limited to general groups (division) (Gocke et al. 2003).

Hg Concentrations

THg levels were on average 265.9 ± 197.4 ng g^{-1} dry weight (dw) ($n = 9$, Table S3), which is in the range of values reported for periphyton in the Bolivian (54–182 ng g^{-1} dw) and Brazilian Amazon (41.6–254 ng g^{-1} dw) (Dominique et al. 2007; Roulet et al. 2000). However, in Uru Uru, the THg concentration in periphyton was highly variable (60.6–920.7 ng g^{-1} dw) by more than one

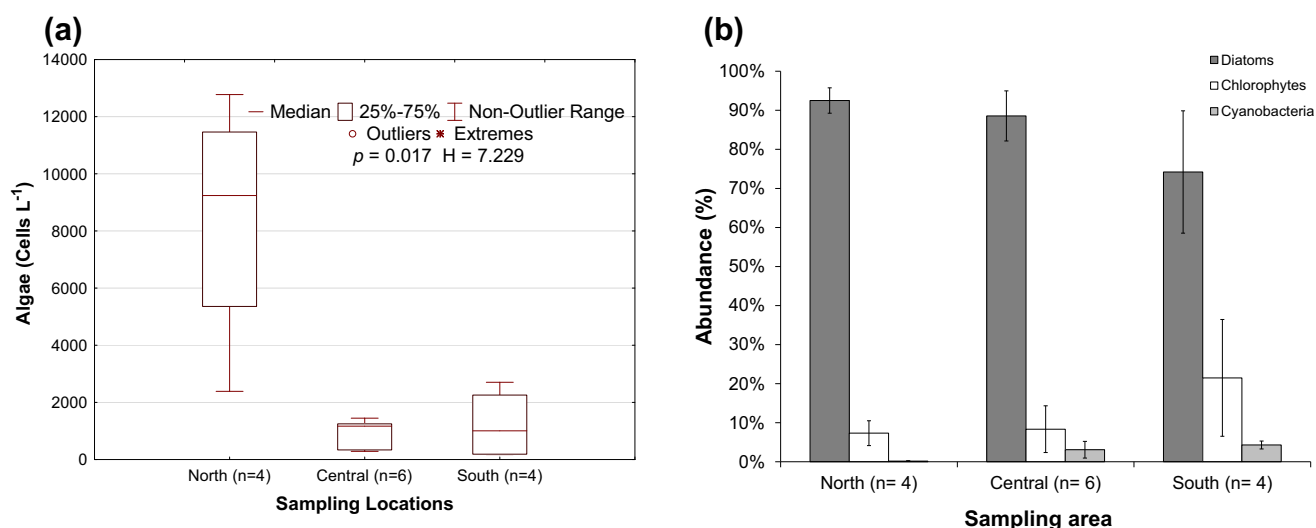


Fig. 1 **a** Algal abundance for sampling area. **b** Percentage composition of algal groups in periphyton. Central and south sites include only Totorá-associated periphyton, whereas central location also includes *Myriophyllum*-associated periphyton

order of magnitude. The highest THg values were much higher than those previously reported in the South American tropical region (Acha et al. 2005; Coelho-Souza et al. 2011; Correia et al. 2012; Molina et al. 2010).

Concentrations of MeHg ranged from 2.9 to 26.5 ng g⁻¹ dw, which is similar to those found in the periphyton of the Bolivian (7–28.2 ng g⁻¹ dw) (Molina et al. 2010) and Brazilian Amazon (2–64 ng g⁻¹ dw) (Dominique et al. 2007; Roulet et al. 2000). Concentrations were not significantly different between Totora- and *Myriophyllum*-associated periphyton. Totora accumulated 11.29 ± 5.36 ng g⁻¹ dw, whereas *Myriophyllum* accumulated 5.94 ± 0.66 ng g⁻¹ dw, but the MeHg concentration in *Myriophyllum* belongs to the macrophyte + periphyton microecosystem. The MeHg concentration in periphyton between sampling areas (Fig. 2) did not vary significantly (ANOVA, $f_{2, 11} = 4.159$; $p = 0.053$), but the power of the test is low (0.050; 0.456), meaning that it is likely that the true differences were overlooked.

MeHg concentrations for Totora periphyton were not significantly correlated to THg, unlike the findings of many other studies (Benoit et al. 2003; Drott et al. 2008), thus suggesting that other environmental factors exert primary control over MeHg accumulation in periphyton. Light as well as Hg methylation and demethylation potentials may have a major influence on MeHg accumulation (Alanoca et al. 2016a) and may help to explain our results. However, other variables, such as algal and microbial composition, may also exert control over the availability of IHg and MeHg for methylation and demethylation, respectively, and may therefore be a relevant factor controlling MeHg accumulation.

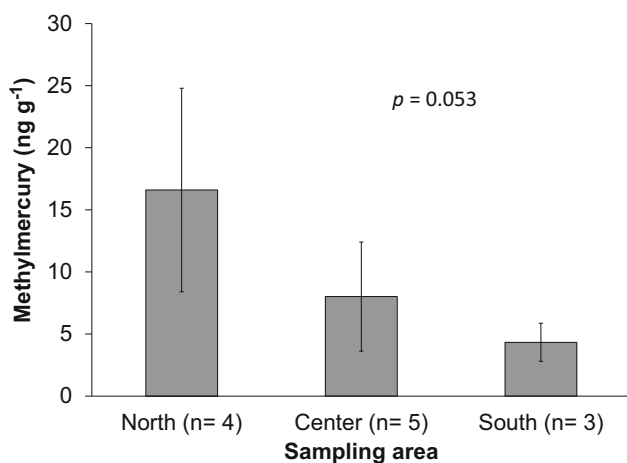


Fig. 2 MeHg concentration in periphyton (dry weight) in the three sampling areas. North and south samples only include Totora-associated periphyton, but central samples also include *Myriophyllum*-associated periphyton

Correlation Between MeHg and Algae Abundance

Although we had a small number of samples (frequently $n < 10$), all of the statistical verifications were performed to make sure that the correlations and possible relations were not artifacts. We verified normality, constant variance, the power of the performed test, regression diagnostics, and ran influence-diagnostic tests.

Overall, periphyton in Lake Uru Uru seems to play a major role in the Hg cycle (Alanoca et al. 2016a). MeHg concentrations, chlorophylls, and algal abundance had similar distribution patterns (Figs. 1, 2; Table S1). There was also a significant relation between MeHg and chlorophyll-*a* in periphyton associated with Totora ($R = 0.675$, $R^2 = 0.456$, $p = 0.0459$) (Fig. 3a). Such a relation implies that algae abundance in general may be influencing approximately 50% of the variability of MeHg concentrations in periphyton. To our knowledge, this is the first evidence of algae involvement on MeHg bioaccumulation in periphyton. The explanations for such control may be an influence in the availability of IHg and MeHg for methylation and demethylation, respectively, or simply bioaccumulation (Bravo et al. 2014). In other experiments with this periphyton, demethylation was found to be the main Hg transformation (Alanoca et al. 2016a), suggesting that periphyton may not be a source of MeHg in this ecosystem. MeHg bioaccumulation in algae may only exacerbate the role of periphyton as a sink of MeHg. Although algae abundance may also be influenced by IHg and MeHg concentrations, the toxicity in algae has only been observed at very high concentrations (Mason et al. 1996).

The relation of chlorophyll-*c* with MeHg concentrations suggests that diatoms are the most important for bioaccumulation in periphyton (Fig. 3d). However, chlorophyll-*b* (Fig. 3c) is almost as strongly related to MeHg accumulation as chlorophyll-*c*. In fact, including the data from *Myriophyllum* for chlorophyll-*c*, the relation between diatoms and MeHg becomes weaker ($R^2 = 0.461$; $p = 0.015$). We only present the data for Totora samples (Fig. 3) because there could be some overestimation of chlorophyll-*b* and -*a* in *Myriophyllum*-associated periphyton. *Myriophyllum* soft tissue was impossible to separate from the periphyton, but this should not influence chlorophyll-*c* concentrations.

Oedogonium sp. abundance was the only genus positively related to MeHg accumulation among the 35 algae genus identified and quantified (Table S4). Even compared with the relations between pigments and MeHg, the relation we observed between *Oedogonium* sp. (chlorophyte) and the percentage of MeHg (Fig. 4b) or MeHg concentration (Fig. 4a) was the strongest, suggesting that this is the most important genus for MeHg accumulation among

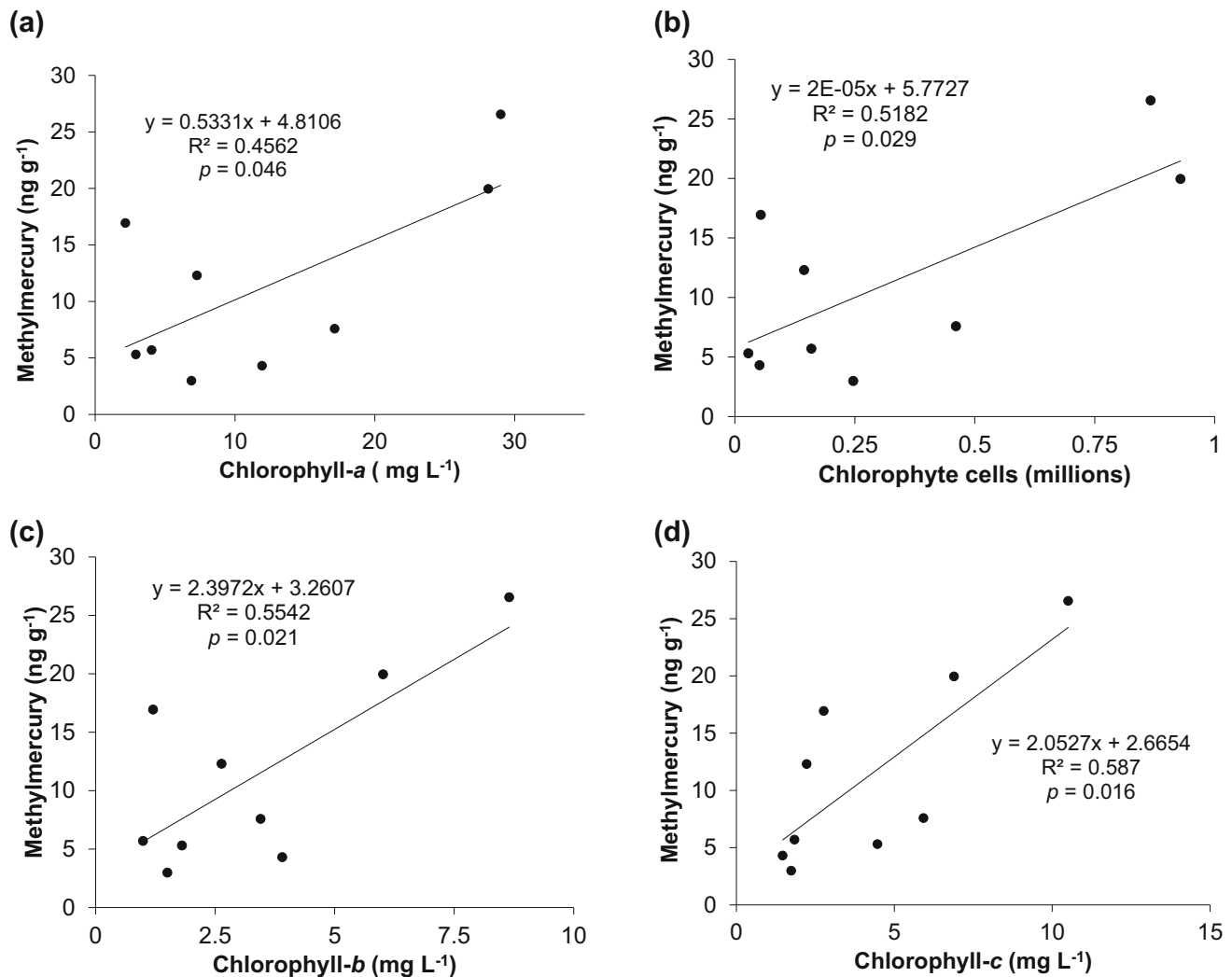


Fig. 3 Relationship between MeHg concentration with algal groups and chlorophyll pigments in periphyton associated with *Totora*. **a** MeHg versus chlorophyll-*a*. **b** MeHg versus chlorophytes. **c** MeHg versus chlorophyll-*b*. **d** MeHg versus chlorophyll-*c*

algae. This genus alone may explain $\leq 72\%$ of MeHg accumulation (Fig. 4), thus making it the strongest contributor to MeHg variability among the *Totora* periphyton samples. No other genus of chlorophytes was significantly correlated with MeHg concentrations. Culture experiments have shown that different algae species may lead to different MeHg-accumulation capacities (Moye et al. 2002).

To further verify the relation between *Oedogonium* abundance and MeHg accumulation, we evaluated whether it persisted when including data from the periphyton of *Myriophyllum* sp.. The Pearson correlation was very strong (correlation coefficient 0.808, $p = 0.0015$, $n = 12$), but the relation did not pass the constant variance test. Therefore, we transformed the data to stabilize the variance of MeHg and *Oedogonium* cells and obtained a significant relation ($R^2 = 0.463$, $p = 0.015$, $n = 12$) (Fig. 5). The relation is weaker, but it holds despite having data from periphyton

associated with a morphologically and phylogenetically different macrophytes that may even influence algae composition.

According to our data, *Oedogonium* sp. could be the most important individual group of algae for accumulating MeHg and transferring it to the food web. Such relevance of a single group of algae may be particularly relevant for the development of bioremediation and pollution-management strategies. Indeed, adding the accumulation capacities of *Oedogonium* sp. to the MeHg-demethylation potential of this periphyton described elsewhere (Alanoca et al. 2016a), makes this periphyton a sink for MeHg produced in the water column, sediments, or other biofilms (Alanoca et al. 2016a).

Little is known about the exact mechanism of accumulation of MeHg by *Oedogonium*. However, *Oedogonium* nonliving biomass appears to be a good sorbent for

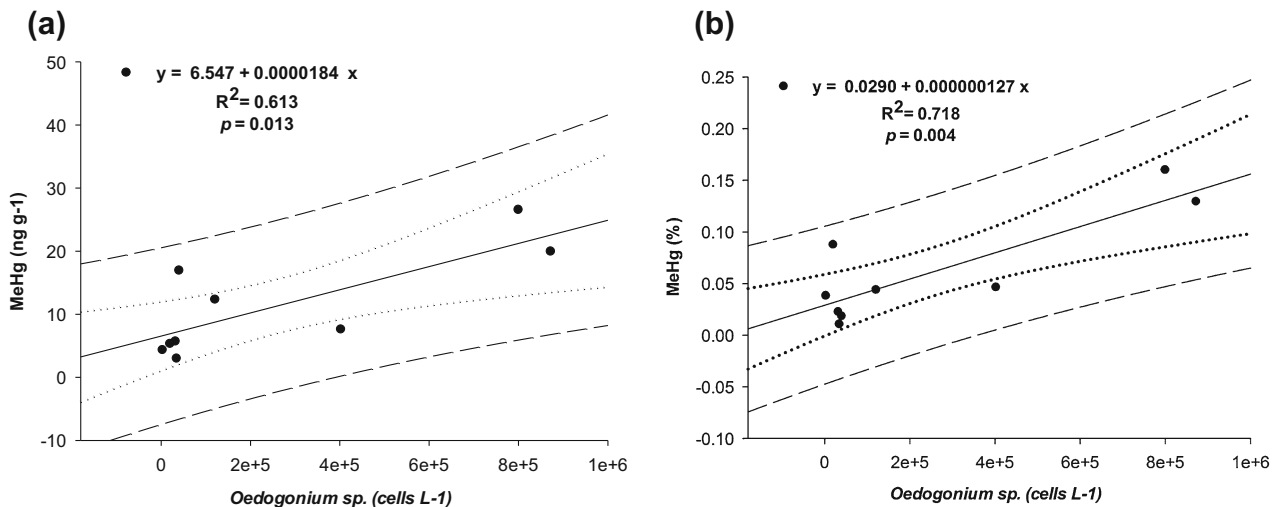


Fig. 4 Relationship between *Oedogonium* sp. cell abundance and MeHg concentration (a) (did not pass the test for independence of residuals (Durbin–Watson statistic = 3.100) or MeHg relative

concentration (% of total Hg) (b). Linear regression (solid line), prediction interval (dashed line), and confidence interval (dotted line)

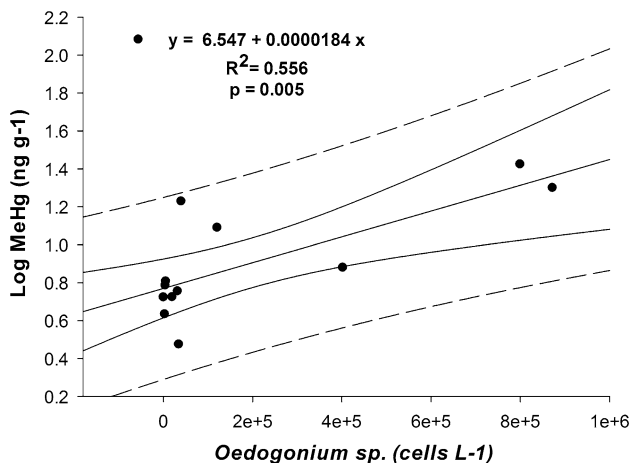


Fig. 5 Relationship between *Oedogonium* sp. cell abundance and Log MeHg concentration showing the linear regression (solid line), prediction interval (dashed line), and confidence interval (dotted line)

removal of lead(II) (Gupta and Rastogi 2008a), cadmium (II) (Gupta and Rastogi 2008b), chromium(VI) (Gupta and Rastogi 2009), nickel(II) (Gupta et al. 2010), arsenic (As), molybdate (Mo), and selenium (Se) (Kidgell et al. 2014). Living *Oedogonium* is also one of the most important algae able to concentrate Fe, and chromium (Rai et al. 2008) and has the potential for accumulating other metals such as copper (Cu), nickel (Ni), zinc (Zn), uranium, and Hg (Bakatula et al. 2014). *Oedogonium* species were found to have excellent potential for the bioremediation of wastewater polluted with Cu, manganese, Ni, cadmium, Zn, and, to a lesser extent, metalloids (arsenic, molybdenum, and selenium) (Ellison et al. 2014). The mechanism of absorption or metal binding for MeHg was not studied, but total Hg absorption in *Oedogonium* does not seem to be

influenced by pH, and there appear to be few sites specific for Hg binding (Bakatula et al. 2014). A recent study also showed higher tolerance to Hg in an algae community dominated by *Oedogonium* (Val et al. 2016).

At the level of the major algal groups, MeHg and chlorophytes abundance measured either by microscopic cell counts or by chlorophyll-*b* were statistically related ($R^2 = 0.518$, $p = 0.029$ and $R^2 = 0.554$, $p = 0.021$, respectively) (Figs. 3b, c). These results agree with previous experimental studies using cultures that have shown high MeHg-uptake rates by chlorophytes regarding other algal groups (Gorski et al. 2006; Miles et al. 2001; Moye et al. 2002). There is evidence of an active transport of MeHg in algae, particularly if they have physiological characteristics similar to chlorophytes (Moye et al. 2002). However, chlorophytes may be strongly correlated with MeHg abundance, mostly because of *Oedogonium* sp. (Fig. S2). Chlorophyte variation in the periphyton associated with *Totora* was explained in >96% by *Oedogonium* sp. cell abundance ($r^2 = 0.966$, $p = < 0.001$) despite representing <10% in some samples and, on average, <60% of the total chlorophyte cells.

Likewise, there is a strong relationship between MeHg and chlorophyll-*c* ($R = 0.766$, $R^2 = 0.587$, $p = 0.016$) (Fig. 3d), which suggests that diatoms may also actively participate in the bioaccumulation of MeHg in periphyton. Chlorophyll-*c* concentration can explain $\leq 58.7\%$ of the MeHg concentrations and 52.8% considering the degrees of freedom. However, diatom abundance determined from cell counts (cell/L) was not related with MeHg (Table S2). Such discrepancy is likely because the microscopic count cannot differentiate between active and inactive or dead cells, and MeHg in eukaryotic algae is probably mediated

by active transport (Moye et al. 2002). Moreover, in vitro cultures have found that diatoms also have high MeHg uptake rates as well as chlorophytes (Bravo et al. 2014; Mason et al. 1996; Pickhardt and Fisher 2007). However, chlorophyll-*c* is also strongly correlated with chlorophytes and *Oedogonium* sp. (Fig. S3), which makes it hard to differentiate between the influences of each group on MeHg accumulation. According to our observations, such correlation may be attributed to diatom genus, such as *Ulnaria* colonizing *Oedogonium* (Morales et al. 2013), which still makes *Oedogonium* ecologically the most important algae for MeHg accumulation. Regardless of which group does the actual accumulation, *Oedogonium* appears to control diatom abundance (Fig. S3). In fact, *Oedogonium* sp. could explain most chlorophyll-*c*- variability ($r^2 = 0.619$, $p = 0.002$), and none of the Diatom genus found was found to be significantly correlated with MeHg ($p > 0.05$).

Unlike other studies that showed some significance of cyanobacteria for MeHg production (Coelho-Souza et al. 2006; Lázaro et al. 2013), here no correlation between cyanobacteria and MeHg accumulation was observed (Table S1). MeHg accumulation in cyanobacteria could be attributed to SRB communities thriving with the electron donors produced by cyanobacteria (Baumgartner et al. 2006) or cyanobacteria accumulating MeHg (Mishra and Nanda 1997). Perhaps the low abundance of cyanobacteria in our samples explains the little if any significance they had for MeHg accumulation.

Overall, *Oedogonium* sp. found to be associated with Totora is the keystone species for MeHg contamination in the food web based on periphyton, which could be a very effective bioaccumulation pathway (Molina et al. 2010). Because little MeHg seems to be produced in the periphyton, but there is a substantial demethylation potential (Alanoca et al. 2016a), *Oedogonium* sp. could be crucial for MeHg accumulation, thus making it unavailable for demethylation and available for organisms feeding on this algae. This finding would be the first case in which MeHg in periphyton is explained partly by organisms that are not bacteria that produce it and/or decompose it. In data presented elsewhere (Alanoca et al. 2016a), periphyton methylation was negligible, but demethylation was at least two orders of magnitude higher, which did not explain MeHg accumulation in periphyton. Therefore, MeHg in periphyton may have been explained mostly by the periphyton capability to accumulate Hg given, in this case, by *Oedogonium*.

The fact that only one genus can be correlated to MeHg is also groundbreaking because the little research performed in this particular field suggested that large groups, such as diatoms or cyanobacteria, were relevant for MeHg production or accumulation (Coelho-Souza et al. 2006;

Lázaro et al. 2013). Here we show that like Hg methylation among bacteria, in situ MeHg accumulation may be a characteristic restricted to highly specific groups of algae. Such specificity suggests that changing only one or few key species could alter patterns of distribution, accumulation, and biomagnification of MeHg. Our results also indicate that many ecological interactions beyond food web structure may be overlooked and could be important to improve our understanding of the Hg cycle.

Conclusions

Algae-community composition may be a major factor for MeHg accumulation in Totora-associated periphyton. Although MeHg concentrations were correlated with different groups of algae, the filamentous green algae *Oedogonium* sp. seems to be the most influential on MeHg accumulation and algae distribution at that given place and location. Therefore, *Oedogonium* sp. has a significant potential to influence MeHg cycle and may be a keystone genus for MeHg introduction into the food web.

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