METHYLMERCURY PRODUCTION AND ASSOCIATED MICROBIAL DIVERSITY WITHIN AN OLIGOTROPHIC TEMPERATE FRESHWATER LAKE (CAZAUX-SANGUINET LAKE, SOUTH-WESTERN FRANCE)

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Introduction

Aquatic ecosystems may exhibit high methylmercury production mostly due to anoxic microbial activity (Pak and Bartha, 1998). This mercury species is one of the most toxic form, that is easily bioaccumulated and bioamplified along the food web (Cabana and Rasmussen, 1994). In Cazaux-Sanguinet Lake, mercury concentrations are low in sediments and undetectable in water (Gentès *et al*., 2013); however, methylmercury has been detected at high levels in various fish species. The recent discovery by Parks et al. (2013), of genes that encode a putative corrinoid protein, HgcA, and a 2[4Fe-4S] ferredoxin, HgcB, necessary to achieve mercury methylation, has opened new opportunities to answer this question. In this study, we explored mercury methylation capacity of all Cazaux-Sanguinet lake environmental compartments: Sediment, Water and periphytic biofilms. Moreover, we determined the 16S rRNA microbial diversity and the presence of *hgcA* genes, in order to identify biotic and abiotic environmental factors that control methylmercury production in this lake, which lead to mercury accumulation in fishes.

Methods

Seasonal microbial mercury methylation and demethylation were examined during spring and summer 2014, in four sampling sites (Figure 1). Sediment, water and aquatic plant biofilms were sampled and analysed by combining stable isotopic tracers and metabolic inhibitors, 2-bromoethane-sulfonic-acid (BESA) or molybdate, as specific inhibitors of microbial methanogenesis and sulfate-reduction, respectively (Rodriguez-Gonzalez *et al*., 2013; Compeau and Bartha, 1985). High-throughput sequencing, based on the 16S rRNA gene (V4V5 Region), was used to link the structural variations of the microbial community to the modification of environmental parameters and mercury biotransformation. In addition, the detection of mercury methylation gene, *hgcA*, was conducted on selected samples using universal primers designed by Shaefer *et al*. (2014).

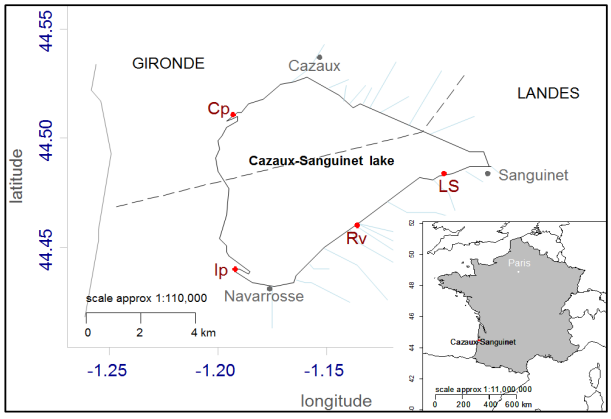

Figure 1. Map of the oligotrophic freshwater Cazaux-Sanguinet Lake, showing the investigated sites (Cp, Ip, Rv and LS). Water and sediment were sampled in spring and summer 2014 in each site, whereas periphytic biofilm were sampled only in Ip on *Lagarosiphon major* leafs and in LS on *Ludwigia grandiflora* roots.

Results

As previously reported by Gentes et al. (2013), sediments exhibited significant methylation of the inorganic mercury added (from 0.1 to 2% day^{-1}) while no methylation activity could be detected in the water column. Much interestingly, periphytic biofilms concentrated inorganic mercury at very high levels and had the highest MeHg production especially in late summer period (up to 4.51 ±0.56% day^{-1}) as presented in Table 1. The microbial alpha-diversity was higher in periphyton than in any other compartment, with a concomitant enrichment in 16S rRNA sequences from *Deltaprotebacteria* and *Clostridia* phyla. In addition, we demonstrated that methanogens and sulfate-reducers play a major role in the production of MeHg; as molybdate and BESA used as specific inhibitor, drastically decreased or enhanced mercury methylation activity, respectively (Table1). Eventually, the metabolic potential necessary for mercury methylation was confirmed by the detection of HgcA encoding genes in both sediment and periphyton samples.

Table 1. Inorganic mercury $^{199}Hg^{2+}$ methylation (M in % day^{-1}) determined in incubations of *Lagarosiphon major* and *Ludwigia grandiflora* periphyton under oxic and dark conditions. At times the samples were supplemented with metabolic inhibitors of methanogens and sulfate-reducers (e.g., 2-bromoethane-sulfonic-acid (BESA) and molybdate, respectively).

Matrix	*Lagarosiphon major* periphyton		*Ludwigia grandiflora* periphyton	
	Spring 2014	Summer 2014	Spring 2014	Summer 2014
Supplement	M % day^{-1}	M % day^{-1}	M % day^{-1}	M % day^{-1}
None	0,057 ±0,13	2,30 ±0,56	0,35 ±0,018	4,51±0,79
Molybdate	0,082 ±0,015	0,067±0,20	0,069 ±0,19	0,21 ±0,076
BESA	0,068 ±0,010	1,27 ±0,29	0,10 ±0,18	5,67 ±0,13

Conclusion

In all studied environmental compartments of the Cazaux-Sanguinet Lake, we showed that net methylmercury production significantly increased between spring and summer. The seasonal increase of temperatures, variations in microbial communities and mercury accumulation in periphyton may have a dramatic impact on MeHg production and biodisponibility to the herbivorous fishes in which it accumulates. Specific parameters such as the type and content of organic matter exudates produced by aquatic plants could increase methylmercury budget in the lake.

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