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Temporal and spatial variation in the mechanisms used by microorganisms to form methylmercury in the water column of Changshou Lake

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ABSTRACT

The microbiome in artificial lake water and its impact on mercury (Hg) methylation remain largely unknown. We selected the largest artificial lake in southeastern china, Changshou Lake (CSL), which has high background levels of Hg, for our investigation of Hg transformation microorganisms. Five different sections of the water column of CSL were sampled during four seasons. The water samples were subjected to analysis of geochemical parameters, various Hg species and microbiome information. High concentrations of total mercury (THg) were detected in CSL in comparison with those found in natural lakes. Significant differences in microbial community structure and Hg species abundance existed among seasons. High dissolved methyl mercury (DMeHg) formation and high bacterial richness and diversity occurred in the fall. The microbiome was dominated by Proteobacteria, Actinobacteria, Bacteroidetes, Deinococcus-Thermus and many unclassified bacteria. Significant correlations were found between seasonal bacterial communities and Hg levels. Hg methylation was strongly linked to the abundance of Cyanobacteria. Methylators, including *Syntrophus*, *Desulfovibrio* and *Desulfomonile* species, were detected only in samples collected in the fall. The results of enzyme functional analyses revealed that many unknown types of bacteria could also be responsible for Hg transformation. This study was the first to investigate the impact of various Hg species on the microbiome of artificial lake water. The findings of this study illuminate the role of seasonal bacteria in Hg transformation.

1. Introduction

For decades, mercury (Hg) contamination in aquatic environments has drawn special attention because of its environmental persistence, tendency for biological accumulation, and adverse effects on aquatic biota (Driscoll et al., 2013; Hsu-Kim et al., 2013). In most aquatic environmental settings, Hg exists as the elemental form dissolved gaseous mercury (DGM) (Hg°), inorganic divalent Hg(II) (including dissolved and particulate mercury), and organomercury compounds, such as monomethyl mercury (MeHg) (Driscoll et al., 2013; Lindberg et al., 2007). MeHg is the species of most concern for humans (Scheuhammer et al., 2007) because it is highly bioaccumulative (Boening, 2000; Mergler et al., 2007). In most aquatic settings, various Hg species undergo mutual transformation. Hg° can be oxidized to Hg(II) by some bacteria, such as *Bacillus* and *Streptomyces* (Smith et al., 1998), under aerobic conditions. In low-oxygen aquatic settings, dissolved Hg(II) crosses the cytoplasmic membrane and is converted to MeHg by acetyl-coenzyme A (Hsu-Kim et al., 2013; King et al., 2000). Hg(II) can also be reduced to Hg° by mercury-resistant bacteria (MRB) (Freedman et al., 2012; Siciliano et al., 2002). In addition, reductive demethylation of MeHg produces Hg°, whereas oxidative demethylation of MeHg produces inorganic Hg(II) (Oremland et al., 1991).

Natural and artificial lakes are important water systems for diverse environments around the world. Numerous studies have investigated Hg distribution patterns and the Hg cycle in natural lakes (Schaefer et al., 2004; Mason et al., 2012; Vishnivetskaya et al., 2011). These studies suggest that accumulation of MeHg in biota is greatly dependent on the MeHg concentration in lake water (Mason et al., 2005; Morel et al., 1998; Munthe et al., 2007), which is controlled by multiple transport and microbial transformation processes involved in the mercury biogeochemical cycle (Fitzgerald et al., 2007; Merritt and Amirbahman, 2009). In most natural freshwater and coastal aquatic environments, anaerobic microorganisms thriving in anoxic zones are the dominant producers of MeHg (Hsu-Kim et al., 2013). The most studied methylators of inorganic Hg(II) are sulfate-reducing bacteria (SRB) (Achá et al., 2005; Ekstrom et al., 2003; Winfrey and Rudd, 1990), and a vast majority of the microorganisms identified as Hg methylators also have the ability to degrade MeHg (Bridou et al., 2011)

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in natural lakes. In comparison with natural water, some artificial lakes are more seriously disturbed by human activities, including metal fabrication, auto garages, residential areas, farming, road construction and municipal waste disposal; as a result, artificial lakes are often polluted by complex contaminants, including heavy metals, pesticides, and organic solvents (Akoto et al., 2008). These factors lead to significant differences in contaminant concentrations and microbiome composition. Therefore, there are likely to be some differences in the mechanisms utilized by microorganisms to produce MeHg in natural and artificial lakes. In comparison with natural lakes, artificial lakes are more likely to be used as sources of water for drinking, irrigation, and aquaculture (Aziz et al., 2017). Therefore, studies of artificial lake water are important to ensure good public health, as well as for economic and environmental reasons.

Artificial lakes are considered to be a typical "mercury sensitive ecosystem" (Feng, 2011). In China, rapid development and poor regulation of the aquaculture industry have resulted in eutrophication and high background concentrations of Hg in many artificial water bodies (Xiang et al., 2014). In the 1970s, Smith found that the methyl mercury content in fish from a new reservoir was greater than the food sanitation standard ($\leq 1.0\ mg\ kg^{-1}$, wet weight) recommended by the World Health Organization (Bai et al., 2015; Smith et al., 1974). Subsequent studies indicated that mercury in artificial lake water is more easily enriched in fish in comparison with mercury in natural lake water (Da et al., 2012; Goldstein et al., 1996; Liu et al., 2012). Thus, transformation of Hg species in artificial lakes merits significant study. In most aquatic environments, microbial transformation is the main mechanism of production for most mercury species. Establishing the relationship between transformation of Hg species and microorganisms is necessary for effective remediation and control of mercury pollution in artificial lakes. In recent years, most studies focused on the impact of abiotic factors, including pH and dissolved oxygen (DO) (Bai et al., 2015; Muresan et al., 2007), on Hg transformation. Little is known concerning the relationship between microorganism-mediated methylation and Hg transformation in artificial lake water (Hsu-Kim et al., 2013).

In this study, we sought to evaluated the relationship between Hg species formation and microbial community structure, with consideration of temporal and spatial variation, in artificial lake water. We chose Changshou Lake, a large artificial lake in southeastern China, for the investigation. We tested the microbial community composition and diversity of the seasonal water column in five sections by MiSeq sequencing. Next, we assessed the interaction between Hg species and microbial community structure, as well as temporal and spatial variation in this relationship, by correlational analysis. Finally, we detected the relative abundance of most known mercury-resistant bacteria and methylators and determined their responsibility for Hg species formation.

2. Materials and methods

2.1. Study area

Changshou Lake is located in the Changshou district of Chongqing (E 106° 49′~108° 05′, N 29° 43′~30° 53′). It was created in March 1957 in the lower reach of the Longxi River, which is an important tributary of the Three Gorges Reservoir. Changshou Lake covers a surface area of $65.5\ km^2$ with an average depth of 15 m, a maximum water depth of 40 m, and an annual flow of $48.9\ m^3/s$. It is the largest artificial lake in Chongqing and plays an important role in freshwater aquaculture. The area around Changshou Lake has a subtropical moist climate with an annual average temperature of 17.7 °C. The highest annual average temperature of 40 °C occurs in August, whereas the lowest annual average temperature of − 1 °C occurs in January. In the 1990s, sewage and industrial wastewater from the Longxi River was directly discharged into this area, which led to obvious heavy metal accumulation. With the development of the fishery industry, large amounts of animal waste and fertilizers were pumped into the water until 2005. Since then, many water pollution control policies have been enacted to improve water quality, including banning the use of fertilizers in water for fish farming and the construction of sewage treatment plants. These protective measures have gradually improved the health of the aquatic environment in Changshou Lake by improving water quality.

Five sample sites were chosen in the current study (Fig. S1). Briefly, Section 1 (S1) is near the dam and the outlet of the lake. Section 2 (S2) is located in the western part of the lake near the inlet of the Longxi River, which flows through many dense villages engaged in fruit production. Section 3 (S3) is located in the northern part of the lake, which has a long hydraulic retention time. Section 4 (S4) is located in the eastern part of the lake, which has few villages and many wild orchards. Section 5 (S5), in the central part of the lake, is a tourist area with a wide variety of orchards (See Table S1).

2.2. Sample collection and assessment of physical and chemical properties

Lake water was sampled from five sections during spring, summer, fall and winter in the period from November 2014–2015. The sampling sites were located with a global positioning system (GPS). The water was collected by a Nisiki sampling apparatus. The water depth of the sampling sites varied from the surface to the sullage-water interface. The central water samples were selected for the diversity analysis. Water samples were placed into sterile bottles, stored in a cooler lined with ice bags in the field, and stored at 4 °C in the laboratory before analysis. pH and dissolved oxygen (DO) were immediately measured using a multiparameter water quality monitoring instrument (YSI 6600 V2) PT-10 (Sartorius, Germany) and a YSI 550 A instrument (Washington, USA), respectively. Water temperature (Tem) was measured using a FlashCheckPocket Probe Digital Thermometer 11000 (Delta-TRAK, USA). The physicochemical properties of the water samples are included in the Supporting information (Table S2).

The water samples for the diversity analysis were names as follows: Season (spring, summer, fall and winter)-Section (Section 1, Section 2, Section 3, Section 4 and Section 5)-Water. Spring, summer, fall and winter are abbreviated SP, SU, FA and WI, respectively. Section 1, Section 2, Section 3, Section 4 and Section 5 are abbreviated S1, S2, S3, S4 and S5, respectively, and water is abbreviated W.

2.3. Mercury analysis and quality control

The water samples were subjected to an analysis of Hg species in precipitation following approved methods (Agency, 2001; EPA, 2002). Total mercury (THg) and dissolved mercury (DHg) were measured by cold vapor atomic fluorescence spectroscopy (CVAFS, Brooks Rand model III, Brooks Rand Laboratories, Seattle, WA) following bromine monochloride (BrCl) oxidation, tin chloride ($SnCl_2$) reduction and gold-trap trapping. The THg level was subtracted from the DHg level to determine the particulate mercury (PHg) level. Reactive mercury (RHg) was measured by cold vapor atomic fluorescence spectroscopy following $SnCl_2$ reduction and gold-trap trapping. Total methylmercury (TMeHg) and dissolved methylmercury (DMeHg) were detected by the same detector after distillation, ethylation and gas chromatographic (GC) separation. The TMeHg concentration was subtracted from the DMeHg concentration to determine the particulate methylmercury (PMeHg) concentration.

The method detection limits (MDL, three times the standard deviation of seven replicate measurements of a blank solution) were approximately $0.2\ ng\ L^{-1}$ and $0.02\ ng\ L^{-1}$ for THg and MeHg, respectively. Dissolved gaseous mercury (DGM) was measured using a Tekran 2537 instrument in a temporary laboratory as described previously (Lindberg et al., 2000; Park et al., 2008). The abundance of each of the measured Hg species is shown in the Supporting information (Table S3).