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Effect of Selenium on Mercury Methylation in Anaerobic Lake Sediments

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Health hazards from mercury in the environment have caused restrictions in its use and release by industrial and other users. However, mercury from unregulated sources such as gold panning (Martinelli 1989; De Lacerda et al. 1989; Reuther 1994) can still enter the environment, and artificial dams and irrigation projects can also aggravate the level of mercury contamination (Bodaly et al. 1984; Jackson 1988). The mercury content in fish from remote lakes away from industrial mercury pollution sources can be high (Jackson 1991).

Selenium is both an essential material and an industrial pollutant. It can alleviate the toxicity of many heavy metals, including Hg. Interactions between selenium and mercury occur in many diverse biological systems, such as fish in their natural environment (Turner and Rudd 1983; Paulsson and Lundbergh 1991). These studies suggest that selenium has the potential as a chemical amelioration measure in freshwater ecosystems that have been contaminated by mercury. Our previous studies revealed that selenium (Na_2SeO_3) can reduce the methylation rate of mercury in facultative sediment. However, the effect of selenium on mercury methylation in anaerobic sediment is not known. The objective of this study was to assess the effect of selenium on mercury methylation in anaerobic sediments and examine its mechanism of action.

MATERIALS AND METHODS

Bottom sediment (approximately the top 10-15 cm) was collected in Donghu Lake of Wuhan, P. R. China. Water temperature was 10.1 °C; pH 7.45; water content of sediment was 64.03%. The sediment was a gray mud with black plant detritus and a slight sulfide odor. We pooled all sediment into a single composite prior to its use in experimental cultures, which was chemically analyzed according to Physical and Chemical Analysis of Soil (1988). Concentrations of nitrogen and phosphorous in the sediment were high (Table 1). Concentration of NH_4-N in sediment was about 4.7% of total nitrogen. Water quality indexes for Donghu Lake (Table 2) showed it to be a eutrophic lake (Liu 1996).

The compounds $HgCl_2$ and Na_2SeO_3 were added to collected sediment to yield a

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Table 1. Concentration of Kjeldahl nitrogen (KN), total phosphorous (TP), total organic carbon (TOC), and metals in sediment used in experiments (mg/kg)

KN*	TP*	TOC*	Fe*	Cu	Ni
6.35	2.52	2.85	38.7	57.8	70
Zn	Cr	Co	Mn	Pb	Hg
151	108	47.8	495	68.1	0.18

* g/kg

Table 2. Water quality index for Donghu Lake (mg/L)*

DO	COD	KN	NO_3-N	NO_2-N	NH_4^+-N	TP	PO_4^{3+}
5.62	34.2	3.86	0.74	0.099	1.96	0.712	0.261

*DO: dissolved oxygen; COD: chemical oxygen demand;
KN: Kjeldahl nitrogen; TP: total phosphorous.

Table 3. The concentration of selenium and mercury in sediment after adjusting concentrations for each experimental treatment (μ g/g wet weight)

Concentration	Experimental treatment				
	A0*	S0**	S1**	S2**	S3**
Se	0.00	0.00	0.25	2.50	5.00
Hg	50.00	50.00	50.00	50.00	50.00

* Aerating air; **Sealed with paraffin wax

concentration for mercury of 50.00 μ g/g wet weight in all samples. The concentration of selenium was adjusted to yield 0.00, 0.25, 2.50, and 5.00 μ g/g wet weight, respectively in test samples (Table 3). Sediment test samples were added to 70-mL glass bottles while aerating with nitrogen, then bottles were sealed with paraffin wax to keep them anaerobic. Additionally, sediment was placed into 500-mL glass bottles (sediment was about 5 cm high) and aerated with air during the whole incubation period (AO). All sediment test samples were incubated at 37.3 ± 1 °C. At different times (0, 4, 8, 12, 16, 21, 25, 29 days), three bottles of each experimental group were sampled by adding 2.5 mL HCl (guarantee reagent) to each test sample and mixing with a glass rod until the foam disappeared, then adding 2.5 mL of $CuSO_4$ solution (dissolved 50g $CuSO_4 \cdot 5H_2O$ in 200 mL of deionized water), stirring, centrifuging, and collecting the supernatant. The sediment sample was washed again by adding 20 mL deionized water, repeating the above process, and combining supernatants. The supernatant was adjusted to pH 3.0 with NaOH and HCl, filtered, and collected in a 125-mL separatory funnel. Methylmercury was adsorbed with thiohydroxy cotton, then desorbed with 2 mL of 1M HCl and concentrated with 1.0 mL of benzene (redistilled). The solution was shaken violently for 2 min, and the organic phase was collected for GC analysis. The recovery rate of methylmercury was 87.3%. All experimental groups consisted of triplicate cultures.

Multifactor analysis of variance with treatment group and time-in-culture as their

main factor effects was used to compare among treatment groups, $p < 0.05$ was chosen as the limit of statistical significance and, LSD (one multiple range test) was used to separate differences among means.

RESULTS AND DISCUSSION

Concentration of methylmercury increased to a peak on the 16th day before declining slowly in all treatments except S3 (Fig. 1). Multifactor analysis of variance revealed that the experimental treatments could be divided into three groups (the order of methylmercury concentration from high to low): (1) A0 (Se = 0.00 μ g/g), S1 (S =0.25 μ g/g);(2)S0 (Se = 0.00 μ g/g); (3) S2 (Se = 2.50 μ g/g), S3 (Se= 5.00 μ g/g). There were no significant differences within each group, while the differences between the groups were significant ($p < 0.05$).

Concentration of methylmercury in anaerobic sediment without selenium (S0) was lower than S1 but higher than S2 and S3 within the whole incubation process. This demonstrated that selenium at low concentration (0.25 μ g/g) could stimulate the methylation of mercury in anaerobic sediment, but reduce the methylation rate of mercury at higher selenium concentrations. This was opposite to the effect of selenium on methylation of mercury in facultative sediments. We found that selenium could reduce the methylation of mercury in facultative sediments at low concentrations (unpublished data). Most surface sediment in water was at facultative aerobic or facultative anaerobic conditions, and the surface sediment was the most important methylation site of mercury (Callister and Winfrey 1986; Berman 1989; Regnell and Tunlid 1991). Small amounts of selenium (Na_2SeO_3) could significantly reduce the formation of methylmercury in facultative aerobic and anaerobic sediments, indicating that selenium had the potential as a chemical amelioration measure in freshwater ecosystems contaminated by mercury.

Additionally, the concentration of methylmercury in experiment A0 was significantly ($p < 0.05$) higher than S0. This showed that formed methylmercury in aerated sediment was greater than in anaerobic sediment within the same incubation time. Generally, the methylation rate of mercury under anaerobic conditions is higher than aerobic conditions because of higher microbial activities. In comparison, the concentration of sulfide is higher under anaerobic cultures (Liu 1996). It is easy for Hg^{2+} to form HgS with S^{2-} and the solubility of HgS is very low (its solubility product is only 10^{-53}). Therefore, the amount of bioavailable Hg^{2+} is lower in anaerobic cultures compared to aerobic conditions. In this study, Donghu Lake was a eutrophic lake with better nutritional substances in sediment (Tables 1 and 2). Incubation of these sediments under anaerobic conditions resulted in the formation of sulfide (Liu 1996).

Methylation of mercury in sediments was a complicated biogeochemical process. Aquatic methylmercury concentrations were regulated by the concurrent processes

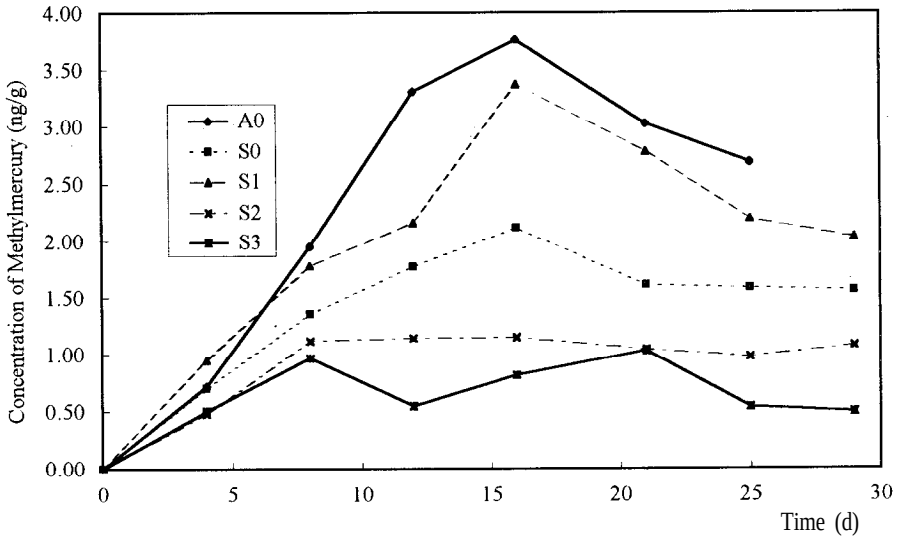

Figure 1. Changes in methylmercury concentration with additions of selenium to aerobic and anaerobic sediment cultured for 4 to 29 days

of production and degradation (Ramlal et al. 1986). To probe into the effect of selenium on this process, we presumed that the following reactive process existed:

$$\underset{A}{Hg^{2+}} \xrightarrow{k_1} \underset{B}{CH_3Hg^{+}} \xrightarrow{k_2} \underset{C}{Hg^{0}} \qquad (1)$$

where k_1 and k_2 were the reaction rate coefficients; A, B and C were the concentrations of Hg^{2+}, CH_3Hg^{2+}, and Hg^{0}, respectively.

The regression of $\log C_B$ and t (incubation time) showed that there was a significant linear correlation ($p < 0.05$) before the methylmercury level began to drift. This revealed that reaction (1) was similar to a pseudo-one-grade reaction described by the following differential equations:

$$-\frac{dC_A}{dt} = k_1 C_A$$

$$\frac{dC_C}{dt} = k_2 C_B$$

$$\frac{dC_B}{dt} = k_1 C_A - k_2 C_B$$

$$\therefore \quad C_B = \frac{k_1 C_0}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \qquad (2)$$

where C_0 was the bioavailable concentration of Hg^{2+} (mg/kg) at the start of incubation and t was the incubation time

All of the above experimental data were applied to equation (2). k_1 of S0 was 61.8% larger than A0, while C_0 was only 41.9% of A0 (Table 4). This indicated that the rate of mercury methylation (k_1) was higher under anaerobic than aerobic conditions, but the concentration of bioavailable Hg^{2+} (C_0) was lower in anaerobic sediment. The net result was the less formation of methylmercury in anaerobic sediment compared to aerobic sediment (Fig. 1).

Table 4. Parameters used to determine rates of mercury methylation at 37.3 °C

Experimental treatment*	k_1 (l/d)	k_2 (l/d)	C_0 (ng/g)	n	R^2
A0	0.00034	0.16882	1492.6	7	0.657
S0	0.00055	0.20533	625.41	8	0.729
S1	0.00036	0.18210	1238.2	8	0.655
S2	0.00021	0.25415	1292.1	8	0.759
S3	0.00028	0.44413	1119.9	8	0.509

* Same as Table 3

Among our anaerobic experiments, k_1 was highest and C_0 was lowest for S_0. This indicated that selenium significantly reduced the methylation rate of mercury and increased the concentration of bioavailable Hg^{2+}. Compared with S0, k_1 and C_0 of S1 were 65.5% and 1.98 times that of S0, respectively. This revealed that selenium at low concentration (0.25 μ g/g) increased the net formation of net methylmercury mainly by increasing the concentration of Hg^{2+} (C_0). Selenium at higher concentrations (2.50 and 5.00 μ g/g) increased the demethylation rate of methylmercury (k_2) and reduced methylation rate of mercury (k_1). This effect was more important than increasing concentration of bioavailable Hg^{2+} (C_0). So, formation of net methylmercury in S2 and S3 was lower than that in S0 (Fig. 1).

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