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5. Oxygen isotope ratios are given in per mil deviation from the ratio of a standard,

$$\delta^{18}O_c = [(^{18}O/^{16}O)_c/(^{18}O/^{16}O)_s - 1] \times 1000$$

where c is the sample of calcite and s is the standard. The standard for oxygen analyses is Pee Dee belemnite (PDB), as described by H. Craig [*Science* **133**, 1833 (1961)].
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16. We thank Dr. J. O'Neil, U.S. Geological Survey, Menlo Park, Calif., for the deuterium/hydrogen ratio measurements. We thank D. Thurber and C. Emiliani for their comments on the manuscript. This research was supported by grants from the National Research Council of Canada.

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Elemental Mercury Evolution Mediated by Humic Acid

Abstract. *Elemental mercury is formed in aqueous solution by the chemical reduction of mercuric ion in the presence of humic acid. The reduction proceeds via first order kinetics (rate constant, 0.009 hour^{-1}) and is depndent on pH. The reaction mechanism involves interaction of the ionic metal species with the free radical electrons of the humic acid.*

Most studies of mercury in the environment have been concerned either with dialkyl and diaryl organomercurials or with ionic mercury species, principally mercuric and methylmercuric ions, since these forms represent the mercury species most often introduced into the environment. However, Bongers and Khattak (*1*) have shown that anaerobic sediments treated with ionic mercury exhibit release of elemental mercury, and methylmercuric ion is converted to elemental mercury in lake sediments in the presence of microorganisms, with subsequent loss of the volatile Hg^0 (*2*).

Strohal and Huljev (*3*) have shown by radiotracer experiments that mercuric ion forms a strong but reversible complex with humic acids (they report a stability constant of 1.7×10^5 for the complex), and Szilagyi (*4*) has shown that soil humic material is capable of reducing ferric iron to ferrous iron in aqueous solution. The possibility that ionic mercury is reduced to the highly volatile elemental form by naturally occurring, ubiquitous humic acids has led us to investigate this possible pathway of mercury mobilization in the environment.

Humic acid was obtained from a farm pond sediment near the University of Georgia, Athens (*5*). Extraction and purification were accomplished by standard procedures (*6*); the purified material had the following composition, in percentages by weight: C, 48.9; H, 6.8; O, 28.0; N, 5.3; S, 1.7; and ash, 9.3. One milligram of the purified humic acid in 1 ml of 0.1*N* KOH solution was added to a 50-ml solution containing 200 μg of Hg^{2+} as $HgCl_2$ and 5 μc of carrier-free $^{203}Hg^{2+}$ and buffered to the desired *p*H with borate. Controls at each *p*H were identical solutions without the humic acid. All experiments were performed at ambient temperatures (25°C). High-purity N_2 was bubbled through the reaction solutions and volatile mercury was trapped in a potassium permanganate–sulfuric acid liquid trap. Measurements of evolved mercury were made by removing the acid-permanganate trap, clearing the trap with 5 ml of 25 percent hydroxylamine hydrochloride solution (weight to volume), and subsequently counting a portion of the solution. Sealed standards were prepared and counted along with the samples to correct for decay and daily counter fluctuations. Control experiments have shown that less than 10 percent of the activity in the traps was lost during clearing with hydroxylamine solution (*7*).

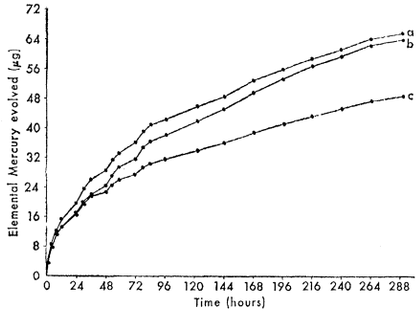

Fig. 1. Evolution of Hg^0 plotted against time at *p*H (a) 6.5, (b) 7.5, and (c) 8.2.

Elemental mercury is evolved at a slow but significant rate, which appears to be *p*H-dependent. Figure 1 shows plots of the elemental mercury released as a function of time at three different *p*H values. Almost 33 percent of the initial mercuric ion added to the solution was reduced to elemental mercury after 290 hours at *p*H 6.5, while 24 percent evolved over the same period at *p*H 8.2. All three curves show almost identical release for the first 8 hours and then diverge. This behavior would seem to suggest two reaction rates and perhaps two different reaction mechanisms and rate constants for these reactions.

Since an inordinate period of time would have been required to reach 99 percent completion of the reaction, the following method of evaluating the data was employed. The process was assumed to be describable by first-order kinetics, and a preliminary estimate of the rate constant (k^*, hour^{-1}) was obtained from the half-life equation. The half-life was taken as the time necessary to acquire half of the total quantity of Hg^0 evolved. The amount evolved at equilibrium or in infinite time (y_∞) was estimated from the preliminary rate constant (k^*) and the last data point (y', t')

$$y_\infty = y'/(1 - e^{-k^*t'})$$

The rate constant was then recalculated as the slope of the right member of Eq. 2, where y_t represents the concentration of evolved Hg^0 at any time t

$$\left(\ln \frac{y_\infty - y_t}{y_\infty} = -kt\right) \qquad (2)$$

The curve calculated from Eq. 2 for the reaction at *p*H 6.5 is a straight line with the general equation $a = -0.090 - 0.009b$, with a correlation coefficient $r = .99$. Similar plots for *p*H 7.5 and *p*H 8.2 are described by $a = -0.06 - 0.008b$ ($r = .99$) and $a = -0.155 - 0.009b$ ($r = .98$), respectively. These curves show that the reactions are first order over the entire time period examined. The close agreement of the rate constants for the three experiments indicates that *p*H influences the total amount of mercury which may be reduced, but is not involved in the rate-determining reaction.

In an attempt to rule out microbial contamination, several microscope slides were prepared at the end of the experiment and examined by light microscopy (× 1000). No organisms and no movement indicating the presence of organisms could be detected. While failure to observe microorganisms is not sufficient evidence for discounting their presence, it does set an upper limit of 10^6 per milliliter at one organism per field on the number present. In addition, any organism present had to be introduced either as an airborne contaminant or with the humic material. If introduced with the humic material, the organism or its spore had to survive *p*H changes from 1 to 12 units, centrifugation at 23,600*g*, and 12 hours exposure to HF in hydrochloric acid (5 percent by volume) during the extraction and purification. To be introducted as an airborne contaminant, the organism had to be viable under a nitrogen atmosphere and in a highly refractory organic substrate. In both cases, the organism would have to be active at a concentration of 4 mg of Hg^{2+} per liter. In these conditions, it is difficult to envision organisms as an important constituent of this reaction.

Another possible mechanism for the reduction may involve donation of electrons from the humic material. Humic acids are known to contain a free radical component, most likely of the quinone or semiquinone type (*8*). To see whether free radicals were involved in the reaction, we examined electron spin resonance (ESR) spectra (Fig. 2).

Figure 2A shows the characteristic singlet signal from humic acid (*9*). After the signal in Fig. 2A was recorded, mercuric ion as $HgCl_2$ was added to the remaining solution of humic acid. Figure 2, B and C, shows the signal 10 minutes and 2 hours after the addition.

In many cases, the peak height of an ESR signal may be used to determine quantitatively the concentration of free radicals present in the system (*10*). During this experiment no adequate standard was available. However, if the spectra in Fig. 2 are due to the same species, ratios of the peak heights provide a semiquantitative measure of the spin concentration in each solution. After the initial 10 minutes, the ratio of peak heights for Fig. 2, B and A, was 0.32. This ratio was followed with the samples sealed in their ESR cells. The peak height ratio after 2 hours was 0.12, and it remained at that value until the experiment was terminated at 505 hours, indicating no apparent further change in the spin concentration of the humic acid–mercuric ion solution. However, during this period a gray solid was observed in the ESR cell containing humic acid and mercury. The composition of this solid was not determined, but it appeared in all replicate experiments.

The explanation for the rapid initial decrease in spin signal followed by no further apparent change during the course of the reaction is not clear. One possibility is that humic acid may be acting as a catalyst or mediator in the reaction, with the electrons used in the reductions being supplied from some other source. Alternately, there may be more than one radical species in the humic material, and the Hg^{2+} may be destroying some but not all types. Also, only one radical type may be present, but steric factors may retard interaction of Hg^{2+} with some of these radicals. Finally, the peaks may represent a Hg^{2+}–humic acid complex formed before reduction of the mercury. To examine this, we determined the *g* values of the singlets. Since the *g* value is inherent to a specific radical, different *g* values indicate different radicals. The spectrum in Fig. 2A exhibited a *g* value of 2.0040 ± 0.0001, while that in Fig. 2C gave a value of 2.0034 ± 0.0002. This difference, while small, is significant, and it indicates the presence of a second radical species. Hence, we observe the rapid loss of one radical species in the presence of Hg^{2+} with little or no change of the second radical during the course of the reaction. The significance of these observations coupled with the possible catalytic nature of the humic acid must be the subject of further experimentation to elucidate the reduction mechanism.

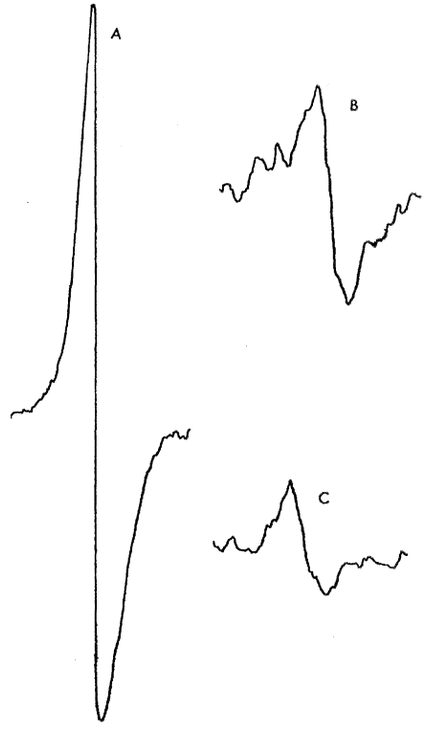

Fig. 2. Electron spin resonance signal of (A) 1 percent humic acid solution in 0.1*N* KOH, (B) the remaining solution from (A) 10 minutes after the addition of 2 mg of Hg^{2+} per milliliter as $HgCl_2$, and (C) the same solution as (B) 2 hours after the addition of $HgCl_2$. All spectra were recorded on a Varian V-4502 electron paramagnetic resonance spectrometer system: modulation amplitude, 400; signal level, 400; scan, 25 gauss.

Our results indicate that elemental mercury can be formed by interaction of mercuric ions with naturally occurring humic acids under conditions that favor chemical reactions over biologically mediated processes. The formation appears to proceed as a first-order reaction with a slow reaction rate ($k = 0.009$ hour^{-1}) and is dependent on some naturally occurring electron donor—humic acids. From these and other results (*1*, *2*) it appears that elemental mercury evolution must be considered as a possible mobilization pathway for mercury and its compounds in the environment.

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6. Sediment was extracted with 0.1*N* KOH for 12 hours and centrifuged at 2500 rev/min. The supernatant was decanted, acidified with 6*N* HCl to *p*H 1, and centrifuged at 2500 rev/min, and the precipitated humic acid was collected. The humic material was then redissolved and reacidified twice again following the above procedure. Before the final acidification, the solution was centrifuged at 14,000 rev/min to remove fines. After acidification, the humic acid was dialyzed for 24 hours against several changes of distilled water and finally shaken overnight with 5 percent HF in HCl. The sample was centrifuged, washed, and finally freeze-dried.
7. To ensure that the material being trapped was elemental mercury, two additional experiments were performed. The reaction solutions were sparged directly into an atomic absorp-

tion spectrophotometer equipped with a flameless mercury cell. Elemental mercury was observed. Also, a liquid trap of cysteine was placed in the flow system immediately before the acid-permanganate trap. This trap was counted along with the acid-permanganate trap and showed no accumulated activity during the course of the experiment. Since the cysteine trap would remove mercury and methylmercuric ions but not elemental mercury from the nitrogen flow, the mercury species trapped by the oxidizing acid-permanganate trap must have been elemental mercury. Further direct measurements of evolved Hg^0 with the atomic absorption spectrophotometer would have required excessively large concentrations of mercury, and the rate of release was so slow that this method of analysis was discontinued.

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Eutrophication and Recovery in Experimental Lakes: Implications for Lake Management

Abstract. *Combinations of phosphorus, nitrogen, and carbon were added to several small lakes in northwestern Ontario, Canada, at rates similar to those in many culturally eutrophied lakes. Phosphate and nitrate caused rapid eutrophication. A similar result was obtained with phosphate, ammonia, and sucrose, but recovery was almost immediate when phosphate additions only were discontinued. When two basins of one lake were fertilized with equal amounts of nitrate and sucrose, and phosphorus was also added to one of the basins, the phosphate-enriched basin quickly became highly eutrophic, while the basin receiving only nitrogen and carbon remained at prefertilization conditions. These results, and the high affinity of sediments for phosphorus indicate that rapid abatement of eutrophication may be expected to follow phosphorus control measures.*

Although the U.S.–Canada Water Quality Agreement (*1*) was signed on 15 April 1972, legislation prohibiting the use of phosphorus in detergents and controlling inputs of phosphorus to the St. Lawrence Great Lakes has not been passed by many states (*2*). Much of the foot-dragging on anti-eutrophication laws undoubtedly still results from the controversy and confusion surrounding the debate over the effectiveness of controlling phosphorus in influents to freshwater lakes (*3*). Among the main points debated (often on the basis of inconclusive evidence) have been:

1) Is phosphorus really responsible for eutrophication problems?

2) If sufficient phosphorus is available, can carbon limit the growth of undesirable algae?

3) Is phosphorus removal alone an effective means of overcoming eutrophication problems?

4) Are already culturally eutrophied lakes recoverable? Can this be done by controlling inputs of phosphorus alone?

5) What concentration of phosphorus can be considered safe?

Answers to these questions have been sought in a series of whole-lake experiments conducted in the Experimental Lakes Area of northwestern Ontario. Lakes in the area are set in Precambrian Shield bedrock. Chemically and biologically they are similar to more than 50 percent of the waters draining to the St. Lawrence Great Lakes (*4*).

In an early experiment, phosphate and nitrate were added to lake 227, which has an extremely low content of dissolved inorganic carbon, to see whether shortage of carbon would prevent the eutrophication of such a lake (*5*). The lake was transformed into a teeming, green soup within weeks after nutrient additions were begun. Algal standing crops up to two orders of magnitude greater than those in unfertilized lakes of the area have been observed (*6, 7*). No increase in phosphate concentration was observed, and any added phosphate disappeared in minutes because of uptake by plankton (*8*). Gas-exchange studies revealed that some of the additional carbon required for production of this algal bloom was drawn from the atmosphere, and a comparison of dissolved inorganic carbon concentrations and parameters affecting gas exchange indicated that there was no possibility that shortage of carbon could prevent the eutrophication of the St. Lawrence Great Lakes or any other water body of economic importance (*9*).

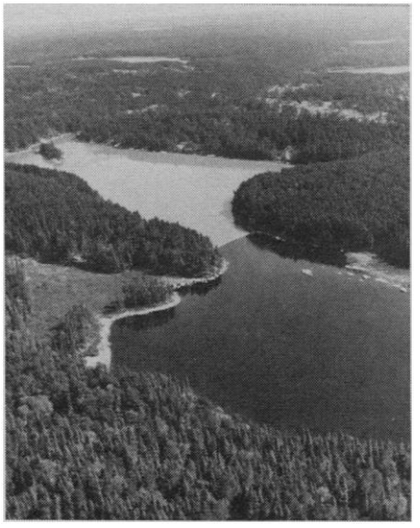

Fig. 1. Lake 226, demonstrating the vital role of phosphorus in eutrophication. The far basin, fertilized with phosphorus, nitrogen, and carbon, was covered by an algal bloom within 2 months. No increases in algae or species changes were observed in the near basin, which received similar quantities of nitrogen and carbon but no phosphorus.

Experiments conducted in smaller enclosures (2 to 3 m^3) in the same lake revealed that if phosphorus was not supplied, algal blooms did not occur (*10*). In order to test the validity of this conclusion on a whole lake, an experiment was begun in 1973 in another small lake, 226. This lake, which has two similar basins separated by a shallow neck (see Fig. 1), was divided into two equal areas by using a sea curtain (60 by 6 m) of vinyl reinforced with nylon (Kepner Plastics, Torrance, California), which was sealed into the sediments and fastened to the bedrock in the narrow section of the lake. Beginning in late May 1973, additions of nitrogen and carbon were made equally to both basins, but phosphorus was added only to the northeast basin of the lake (*11*).

The photograph in Fig. 1 was taken on 4 September 1973, when a bloom of the blue-green alga *Anabaena spiroides* covered that basin receiving phosphorus. Throughout the year, phytoplankton species and standing crops in the basin that received only nitrogen and carbon remained similar to those before fertilization was begun, consisting chiefly of *Tabellaria fenestrata*, *Synedra acus*, and other diatoms. The results indicate the efficacy to be expected from controlling phosphorus content of the influents to such waters as a means of preventing eutrophication.

A common belief is that phosphate, returned from anoxic sediments in