Aluminum Leaching Response to Acid Precipitation: Effects on High-Elevation Watersheds in the Northeast

Abstract. *Atmospheric inputs of sulfuric acid and nitric acid to noncalcareous higher-elevation watersheds in the White Mountain and Adirondack regions lead to comparatively high concentrations of dissolved aluminum in surface and ground waters. This phenomenon appears to result from modern increases in soil aluminum leaching. Transport of this aluminum to acidified lakes can lead to fish mortality. Combined results from areas of silicate bedrock in the United States and Europe suggest that aluminum represents an important biogeochemical linkage between terrestrial and aquatic environments exposed to acid precipitation.*

Numerous investigators have recently examined the effects of regional acid precipitation on terrestrial and aquatic ecosystems. Much of the emphasis in these studies has been directed toward evaluating the influence of meteoric sulfuric and nitric acids on soil leaching and fertility, forest productivity, and the survival of *p*H-sensitive freshwater communities (*1-3*). It now appears from results obtained in the northeastern United States, Canada, Norway, Sweden, and Germany (*4-6*) that one of the most dramatic effects of acid precipitation upon watershed ecosystems is the increased aluminum mobilization which may result from long-term atmospheric inputs of H_2SO_4 and HNO_3.

We present here summary data illustrating the unexpected importance of dissolved aluminum in the solution chemistry of ground and surface waters from high-elevation watersheds exposed to regional acid precipitation. We then discuss the effects of increased aluminum leaching upon pedogenetic processes, watershed biogeochemical processes, and fish populations inhabiting acidified aquatic systems.

The two investigations described in this report were conducted independently in two separate locations in the northeastern United States: (i) the subalpine balsam fir zone of Mount Moosilauke (71°50′W, 44°1′N) in the White Mountains of New Hampshire (*6*) and (ii) the Adirondack Mountain lake district of New York State (73°30′ to 75°15′W, 43°15′ to 44°30′N). Both cool temperate research areas receive similar inputs of acid precipitation (mean *p*H between 4.0 and 4.5) and are characterized by generally comparable forested landscapes (*6, 7*).

Field sampling methods and analytical techniques have been described elsewhere (*3, 6, 8, 9*). For the New Hampshire study of ion transport, samples were collected between 1975 and 1977 in two study sites located at an elevation of 1250 m in the balsam fir ecosystem. Water samples were collected in four strata: bulk precipitation, canopy throughfall, forest floor and A2-horizon percolate, and spring seeps (*6*). In the Adirondack study of high-elevation lakes, we characterized the surface water chemistry by sampling all lakes above an elevation of 610 m (219 lakes) during the period 24 to 27 June 1975 (*2, 8*). The population and community structures for fish inhabiting these lakes were determined from netting inventories conducted by the New York State Department of Environmental Conservation between 1975 and 1976. During the winter of 1976-1977, experiments were carried out in the watershed of Little Moose Lake (74°55′W, 43°44′N) to examine toxicity effects associated with snowmelt changes in the stream water chemistry. In these studies we evaluated behavioral responses, pathological changes in gill structure, and mortality among captive populations of brook trout (*Salvelinus fontinalis*) exposed to both lake and stream water. In both laboratories, considerable effort was made to obtain accurate estimates of the dissolved aluminum present in sample solutions (*3, 6, 8, 9*).

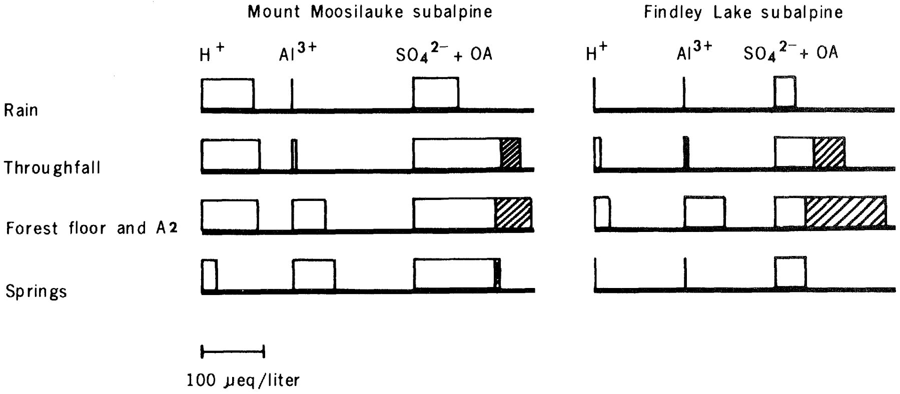

Fig. 1. Average trends of change in the ionic concentrations of free hydrogen, aluminum, sulfate, and organic anions (OA) as rainfall penetrates two contrasting ecosystems, both of which exhibit podzolized soils: the H_2SO_4-dominated soils of the Mount Moosilauke subalpine zone and the organic acid-dominated soils of the Findley Lake watershed in Washington (*17, 26*). The aluminum concentrations in each case actually represent the summed equivalents of the three ionic species: Al^{3+}, $AlOH^{2+}$, and $Al(OH)_2^+$. The concentration of OA is indicated by the shaded portion of the bars in the SO_4^{2-} + OA columns. A certain percentage of the aluminum and OA equivalents may exist together in solution as complexes.

The mean concentrations of dissolved materials in bulk precipitation, canopy throughfall, forest floor and soil percolate, and spring seepage for the New Hampshire subalpine study sites (Table 1) indicate the following: (i) the solution chemistry throughout the balsam fir ecosystem is heavily influenced by precipitation-borne H_2SO_4; (ii) in terms of charge equivalents, H^+ and SO_4^{2-} are two of the most prominent ions in every stratum of the system; and (iii) leaching of aluminum is pronounced in the soil profile, making this element the first or second most abundant cation, on a charge equivalent basis, throughout the soil solution of the entire soil profile (*10*).

The high concentrations of aluminum in solution through the fir zone soil profile represent a distinctive and presumably modern phenomenon that may be important in soil-forming processes and in a host of other watershed processes. The element aluminum is derived principally from rock weathering and exhibits a very *p*H-dependent solubility that drops drastically in the *p*H range between 5 to 7 (*11*). In the presence of organic ligands, the solubility of aluminum can be greatly enhanced by the formation of soluble organic-aluminum complexes (*12, 13*). Current understanding of pedogenesis suggests that in well-drained acid soils where there are significant inputs of organic acids (*14*) from litter decay and canopy leaching, aluminum and iron are transported from upper soil horizons into the B horizon by means of such soluble organometallic complexes. In the B2 illuvial horizon of these soils, the iron and aluminum gradually precipitate by one of two primary mechanisms (*15*). The results of Graustein (*16*) from the mountains of New Mexico and Ugolini *et al.* (*17*) from the mountains of Washington illustrate the classic pattern of aluminum and iron transport for such northern, podzolized

0036-8075/79/0420-0304$00.50/0 Copyright © 1979 AAAS

Table 1. Mean concentrations of dissolved materials in bulk precipitation, canopy throughfall, forest floor and A_2-horizon percolate, and spring seepage during the 1975 and 1976 growing seasons at the Mount Moosilauke fir zone sites; n.d., no data.

Sample	*p*H	Concentration (microequivalents per liter)													
		H^+	Ca^{2+}	Mg^{2+}	K^+	Na^+	NH_4^+	Al*	Fe^{2+}	Mn^{2+}	SO_4^{2-}	Cl^-	NO_3^-	HCO_3^-	Organic anions†
Bulk precipitation	4.08	83	9	3	3	4	13	n.d.	n.d.	n.d.	75	7	21	0	12‡
Throughfall	4.02	95	36	14	37	3	6	5	1	4	143	13	12	0	33
Percolate	4.04	91	25	15	16	7	5	54	4	2	137	16	8	0	58
Springs	4.66	22	26	19	10	13	3	67	2	1	132	7	15	0	9

*Aluminum represents the summed total of Al^{3+}, $AlOH^{2+}$, and $Al(OH)_2^+$. †Organic anions were estimated as described by Cronan *et al.* (*3*). ‡Because of the limited number of individual bulk precipitation samples collected in this study, we obtained the precipitation mean chemistry by averaging several additional studies from Mount Moosilauke and nearby Hubbard Brook Forest (*6*). Hence, the precipitation "organic anion" component is probably an artifact of averaging (*6*).

forest soils (*18*). In both investigations comparatively high concentrations of aluminum, iron, and organic anions were observed in the soil solution of the upper soil horizons, but the concentrations of these solutes were negligible in the B horizon and below (Fig. 1).

The balsam fir zone of the northeast presents an interesting contrast to the studies cited above. The profile development in the fir zone Typic Cryorthod (Podzol) soils and the cool, moist fir zone climate indicate that these soils probably developed historically under the influence of organic chelation and leaching (*19*). Now, with meteoric H_2SO_4 dominating the fir zone leaching process, the historical trend of aluminum accumulation in the B2 horizon is apparently being altered. Solution chemistry data from Mount Moosilauke show that dissolved aluminum reaches high concentrations in the forest floor and continues to increase with soil depth. Although organic ligands may participate in that cation transport, it appears that the high concentrations of dissolved aluminum through the soil profile may be accounted for entirely by the low *p*H of the H_2SO_4-dominated soil solution (*20*).

For perspective, we can review the geochemical transport pattern at Mount Moosilauke as contrasted with a comparable site at Findley Lake, Washington (Fig. 1). In most podzolized soils, the solution acidification and organometallic complexation promoted by organic acids result in the transport of aluminum and iron into lower horizons. By contrast, aluminum mobilization and leaching in the fir zone Spodosol appear to result primarily from the strong acidity of H_2SO_4 introduced by atmospheric precipitation. In the B horizon of northern podzolized soils, iron and aluminum leave solution in response to the increasing *p*H of the soil solution and the removal of organic ligands by immobilization or decomposition. Again in contrast, the fir zone soils show relatively limited increases in solution *p*H with soil depth; consequently, amorphous and exchangeable aluminum are mobilized and tend to be transported in solution through the soil profile and into streams.

Limnological studies in the Adirondack lakes region have indicated that much of this soil-derived aluminum may be transported from the surrounding watershed to acidified lakes. The synoptic survey data from the higher-elevation lakes of the Adirondacks showed that aluminum concentrations in acidified lakes are 10 to 50 times higher than concentrations in circumneutral waters from the same region (*8*). Similar patterns have been described for lakes in Norway and Sweden (*21*); these results suggest that the processes of lake acidification associated with contemporary "acid rain" may commonly result in increased aluminum transport to both streams and lakes.

One of the important ecological consequences of the increased movement of aluminum through soils and into lakes is that the balance of aquatic communities may be disturbed. Field and laboratory experiments in the Adirondacks have demonstrated that, in acidified waters, toxic conditions for fish may be produced by dissolved inorganic aluminum even at lake *p*H values that are not physiologically harmful (*9, 22*). In controlled laboratory studies, brook trout exposed to synthetic acidic solutions and natural Adirondack water with aluminum concentrations above 0.2 mg/liter showed a specific toxic response to aluminum in the *p*H range 4.4 to 5.9. Significant sublethal reductions in brook trout growth were found in the laboratory at aluminum concentrations of 0.1 to 0.3 mg/liter (*9, 22*). Mortality during exposure to acutely toxic concentrations of aluminum appeared to result from severe necrosis of the gill epithelium.

These results from the higher elevations of the White Mountains and Adirondack region indicate that soil leaching and mineral weathering by acid precipitation lead to comparatively high concentrations of dissolved aluminum in surface and ground waters. Such increased aluminum concentrations can lead to fish mortality at concentrations of approximately 0.2 mg/liter or higher. With the increased transport of aluminum to aquatic systems, there may be indirect effects upon phosphorus availability through increased inorganic precipitation of aluminum phosphates (*23*). Accelerated aluminum leaching may also have implications for soil-forming processes, the health of plant communities (*24*), and clay mineralogy (*25*). Other noncalcareous watersheds exposed to long-term acid precipitation may show similar patterns of accelerated aluminum leaching, depending upon (i) seasonal variations in the atmospheric deposition of mineral acids to the soil, (ii) the rates of organic acid release to the soil from decomposition processes, (iii) the neutralization kinetics and acid-buffering capacity of the vegetation and soil, and (iv) the size of the watershed drainage area.

CHRISTOPHER S. CRONAN
Department of Biological Sciences,
Dartmouth College,
Hanover, New Hampshire 03755
CARL L. SCHOFIELD
Department of Natural Resources,
Cornell University,
Ithaca, New York 14853

References and Notes

1. L. N. Overrein, *Ambio* **1**, 145 (1972); F. H. Braekke, Ed., *Impact of Acid Precipitation on Forest and Freshwater Ecosystems in Norway* (SNSF Project, NISK, 1432 Aas-NLH, Norway, 1976); L. S. Dochinger and T. A. Seliga, Eds., *Proceedings of the First International Symposium on Acid Precipitation and the Forest Ecosystem* (Department of Agriculture, Washington, D.C., 1976).
2. C. L. Schofield, *Ambio* **5**, 228 (1976).
3. C. S. Cronan, W. A. Reiners, R. C. Reynolds, Jr., G. E. Lang, *Science* **200**, 309 (1978).
4. L. Wiklander, *Geoderma* **14**, 93 (1975).
5. G. Abrahamsen *et al.*, in *Impact of Acid Precipitation on Forest and Freshwater Ecosystems in Norway* (SNSF Project, NISK, 1432 Aas-NLH, Norway, 1976), pp. 37-63; E. T. Gjessing, A. Henriksen, M. Johannessen, R. F. Wright, *ibid.*, pp. 64-85; T. C. Hutchinson and L. M. Whitby, in *Proceedings of the First International Symposium on Acid Precipitation and Forest Ecosystem* (Department of Agriculture, Washington, D.C., 1976), pp. 745-767; R. Mayer and B. Ulrich, in *ibid.*, pp. 737-745.
6. C. S. Cronan, thesis, Dartmouth College (1978).
7. J. G. Holway and J. T. Scott, *State Univ. N. Y. Albany Atmos. Sci. Res. Center Rep. 92* (1969); D. G. Sprugel, *J. Ecol.* **64**, 889 (1976); J. E. Witty and R. W. Arnold, *Soil Sci. Soc. Am. Proc.* **34**, 653 (1970).

8. C. L. Schofield, *Federal Aid Final Report F-28-R* (New York State Department of Environmental Conservation, Albany, 1976).
9. ———, *Research Project Technical Completion Report A-072-NY* (Office of Water Research and Technology, Department of the Interior, Washington, D. C., 1977).
10. This study did not include soil solution sampling in the B horizon; however, a more recent lysimeter study by C.S.C. has shown that aluminum concentrations in solution remain as high as 1 part per million (ppm) in transit through the B2 horizon (C. S. Cronan, W. A. Reiners, R. C. Reynolds, Jr., unpublished data).
11. K. Rankama and T. G. Sahama, *Geochemistry* (Univ. of Chicago Press, Chicago, 1950).
12. C. J. Lind and J. D. Hem, *U.S. Geol. Surv. Water Supply Pap. 1827-G* (1975).
13. W. H. Huang and W. D. Keller, *Clay Miner.* **20**, 69 (1972).
14. We broadly define organic acids to include any high- or low-molecular-weight organic compounds containing acidic functional groups.
15. Either the metals precipitate from solution as hydroxides and sesquioxides when the organic molecules are decomposed by microbes, or the organic molecules complex with increasing numbers of metal atoms during transport, eventually reach metal complexation saturation, and precipitate as organomineral colloids.
16. W. C. Graustein, *Geol. Soc. Am. Abstr. Programs* **8**, 891 (1976).
17. F. C. Ugolini, R. Minden, H. Dawson, J. Zachara, *Soil Sci.* **124**, 291 (1977).
18. The subalpine soil in New Mexico is classified as a Cryocrept, whereas the subalpine Washington soil is a Cryandept.
19. The soils were classified and were characterized for extractable aluminum by R. J. Bartlett, University of Vermont. As would be expected in a Spodosol, the vertical distribution of extractable aluminum and mineral soil organic matter peaked in the B2 horizon.
20. Lind and Hem (*12*) have estimated that waters in the *p*H range of Mount Moosilauke spring water (*p*H 4.6) can maintain approximately 0.85 ppm of aluminum in solution without the benefit of organic complexation. The average aluminum concentration of 0.75 ppm in fir zone spring water is within the limit of that calculated aluminum solubility value.
21. R. F. Wright and E. T. Gjessing, *Ambio* **5**, 219 (1976); W. Dickson, *Institute for Freshwater Research, Drottningholm, Report 54,8* (1976).
22. C. L. Schofield and J. R. Trojnar, in preparation.
23. W. Dickson, paper presented at the International Association of Limnology 20th annual meeting, Copenhagen, 1977.
24. R. J. Bartlett and D. C. Riego, *Soil Sci.* **114**, 194 (1972).
25. R. C. Reynolds, Jr., personal communication.
26. D. W. Johnson, thesis, University of Washington (1975); D. W. Johnson *et al.*, *Arct. Alp. Res.* **9**, 329 (1977).
27. We thank K. H. Cronan, W. A. Reiners, R. C. Reynolds, Jr., W. C. Graustein, D. W. Johnson, D. Deuring, J. F. Hornig, D. Kruesi, R. K. Olson, R. J. Bartlett, and J. Trojnar for their generous help and advice on various portions of this work. C.S.C. was supported in part by grants from the Cramer Fund of Dartmouth College, Sigma Xi, National Science Foundation grant DEB 76-09984, and Department of Energy grant EE77-S-02-4498. C.L.S. was supported in part by Federal Aid to Fisheries grant F28-R from the New York State Department of Environmental Conservation and Office of Water Research and Technology, Department of the Interior grant for project A-072-NY.

3 November 1978; revised 10 January 1979

Calcium Transport Across the Plasma Membrane: Stimulation by Calmodulin

Abstract. *Active transport of calcium into inside-out vesicles of red blood cell membranes was stimulated equally by (i) the purified protein activator of calcium-activated, magnesium-dependent adenosinetriphosphatase isolated from red cell hemolyzates and (ii) calmodulin, a protein activator of cyclic nucleotide phosphodiesterase isolated from bovine brain. The results provide further evidence for the identity of red blood cell activator and calmodulin and show that this cytoplasmic protein may participate in the regulation of plasma membrane calcium transport.*

The plasma membrane of the human red blood cell (RBC) contains an active Ca^{2+} transport system that uses adenosine triphosphate (ATP) as an energy source (*1*, *2*). Membranes isolated from human RBC's contain a Ca^{2+}-activated, Mg^{2+}-dependent adenosinetriphosphatase activity that is believed to be an expression of this transport process (*3*). In isolated RBC membranes this activity can be enhanced by the addition of a small acidic protein found in RBC hemolyzates (*4*, *5*). This protein, referred to here as RBC-activator, shares many of the properties of a protein recently named calmodulin (*6*). Calmodulin, also known as modulator protein (*7*) and Ca^{2+}-dependent regulator (CDR) (*6*), has a molecular weight of approximately 17,000 and has been shown to activate adenosine 3′,5′-monophosphate (cyclic AMP) phosphodiesterase (*8*) and adenylate cyclase (*9*). Jarrett and Penniston (*10*) reported similar amino acid compositions and electrophoretic mobilities of human RBC activator and beef brain calmodulin.

Calmodulin was recently shown to mimic RBC activator in stimulation of (Ca^{2+} + Mg^{2+})-adenosinetriphosphatase of isolated RBC membranes (*11*, *12*), and preliminary reports indicated that both crude hemolyzate (*13*) and purified RBC activator and calmodulin (*14*) were capable of stimulating active transport of Ca^{2+} into inside-out (IO) vesicles of RBC membrane.

Red blood cell activator and calmodulin are part of a family of Ca^{2+}-binding proteins that includes the Ca^{2+}-binding regulatory protein of skeletal muscle, troponin C (TnC). In some systems TnC and calmodulin substitute for one another (*15*, *16*). Troponin C was found to be about 1000 times less potent than calmodulin in stimulating the (Ca^{2+} + Mg^{2+})-adenosinetriphosphatase activity of RBC membranes (*11*). Considering the similarities between RBC activator and calmodulin, we compared these two proteins along with TnC in their capacities to stimulate Ca^{2+} transport.

The IO vesicles were made by the methods of Steck and Kant (*17*) with certain modifications (*18*). The vesicle preparation contained 45 to 55 percent IO vesicles, as determined by measuring acetylcholinesterase (AchE) activity in the presence and absence of Triton X-100 (*17*). Data are expressed in terms of the IO membrane protein. We did not separate IO vesicles from right-side-out and broken membranes. The dextran gradient normally used for this (*17*) caused significant loss of Ca^{2+} uptake activity.

Calcium uptake experiments were performed as described in the legend of Fig. 1 except that ouabain was not always present. Uptake was the same with or without ouabain. Some experiments were performed with calmodulin or TnC rather than RBC activator. Calcium uptake was approximately a linear function of time for only the first 6 minutes. Samples were taken every 2 minutes and transport rates were determined by linear regression of the four data points.

Adenosinetriphosphatase assay conditions were the same as transport conditions except that in all cases 0.1 m*M* ouabain was present as well as 1 μM ionophore A23187. Ouabain-insensitive activities were assayed for by measuring the concentration of inorganic phosphate (P_i) 5 minutes after the addition of ATP and again 90 minutes later; P_i was measured by an automated method of Fiske and Subbarow (*19*).

The RBC activator protein was purified from RBC hemolyzates by a method that will be described in detail elsewhere (*20*). Vanaman *et al.* (*21*) found a molecular weight for calmodulin of 16,723 based on amino acid analysis, and this value was assumed in our calculations.

Concentrations of RBC activator, calmodulin, and TnC were determined by the Bradford protein assay (*22*), using bovine serum albumin as the standard. Ionophore A23187 was from Eli Lilly & Co., $^{45}CaCl_2$ from ICN Chemical and Radioisotope Division, and ATP from Boehringer Mannheim.

Uptake of Ca^{2+} into IO vesicles can be stimulated by addition of purified Ca^{2+}-binding proteins. Figure 1 shows data from a single experiment with RBC activator. Maximum activation of uptake was achieved with an RBC activator concentration of approximately 0.39 μg/ml (23 n*M*). The results show that Ca^{2+} is taken up into IO vesicles in an ATP-de-

0036-8075/79/0420-0306$00.50/0