trends computed from the grid point data derived from the analyzed maps with the trends computed from all of the individual reporting stations suitably grouped and averaged over latitude. The results are essentially the same, although, as expected, the series based on the individual station data had larger short-period variations.

It is obvious that there is a change in slope of the trend lines (sometime in 1961) associated with the curves in Fig. 2. This change is particularly evident for the Northern Hemisphere curve. Each series was therefore divided into two subparts based on this apparent change, and separate regression lines were calculated with the following results and probable errors: for the Northern Hemisphere: August 1957 through March 1961, −4.7 ± 1.5 percent per decade; April 1961 through May 1970, +11.3 ± 2.3 percent per decade; and for the Southern Hemisphere: August 1957 through September 1961, +2.5 ± 2.3 percent per decade; October 1961 through May 1970, −1.1 ± 1.6 percent per decade.

It can be seen from these results that the upward trend in observed total ozone that occurred during the 1960's was primarily a Northern Hemisphere phenomenon and started in that hemisphere in the spring of 1961. Although these values might reflect, in part, systematic drifts in the calibration of the various instruments, there is no a priori reason why the calibration errors should be more pronounced in the Northern than in the Southern Hemisphere.

We have yet to establish whether these changes show a definitive worldwide pattern and to determine in what manner these trends are associated with changes in solar radiation, general stratospheric photochemistry, or fluctuation in the stratospheric circulation. The marked hemispheric difference in the trends, however, would seem to preclude any direct solar influence.

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References and Notes

1. W. D. Komhyr, E. W. Barrett, G. Slocum, H. K. Weickmann, *Nature (Lond.)* **232**, 390 (1971); W. D. Komhyr, R. D. Grass, G. Slocum, *Pure Appl. Geophys.* **106–108**, 981 (1973).
2. P. Goldsmith, A. F. Tuck, J. S. Foot, E. L. Simmons, R. L. Newson, *Nature (Lond.)* **244**, 545 (1973); J. K. Angell and J. Korshover, *Mon. Weather Rev.* **101** (No. 5), 426 (1973).
3. F. Urbach, Ed., *The Biologic Effects of Ultraviolet Radiation* (Pergamon, New York, 1969).
4. R. A. Craig, *The Upper Atmosphere, Meteorology and Physics* (International Geophysics Series 8, Academic Press, New York, 1965); H. U. Dütsch, in *Climate of the Free Atmosphere*, D. F. Rex, Ed. (World Survey of Climatology, vol. 4, Elsevier, Amsterdam, 1969), pp. 383–432.
5. H. Johnston, *Science* **173**, 517 (1971); H. Harrison, *ibid.* **170**, 734 (1970); *Inadvertent Climate Modification*, Report of the Study on Man's Impact on Climate (MIT Press, Cambridge, Mass., 1971).
6. The term "milliatmosphere centimeter" (matm cm) represents the equivalent depth in 10^{-3} cm if all the ozone molecules in a vertical atmospheric column of unit cross section were brought to standard temperature and pressure (STP).
7. Ozone data are centrally collected and published in monthly volumes of *Ozone Data for the World* (published by the Meteorological Branch, Canadian Department of Transport, in cooperation with the World Meteorological Organization, Downsview, Ontario).
8. G. M. B. Dobson and C. W. B. Normand, *Ann. Int. Geophys. Year* **16**, 161 (1962).
9. See, for instance, R. D. Bojkov, *Ann. Geophys.* **15**, 293 (1969); G. P. Guschin, *Studies of Atmospheric Ozone* (Hydro-Meteorological Press, Leningrad, 1963) (in Russian).
10. See, for instance, J. E. Lovill, *Colo. State Univ. Atmos. Sci. Pap. No. 180* (1972).
11. Research supported by the Atmospheric Sciences Section, National Science Foundation, and the Department of Transportation through NSF grant GA-28688X1.

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Lead Aerosol Pollution in the High Sierra Overrides Natural Mechanisms Which Exclude Lead from a Food Chain

Abstract. *Most of the lead contained in sedge and voles (mountain meadow mice) within one of the most pristine, remote valleys in the United States is not natural but came from smelter fumes and gasoline exhausts. In a food chain, natural mechanisms do not allow lead to accompany the bulk of the nutritive metals as they proceed to higher trophic levels. This exclusion can be expressed quantitatively by a comparison of lead/calcium ratios at successive trophic levels. This ratio decreased by an overall factor of 200 in proceeding from rock, to soil moisture, to sedge, to vole. This factor would have been 1200 if lead aerosols had not collected on sedge leaves and circumvented the tendency by sedge to exclude lead from the nutritive metals it absorbed from soil moisture.*

We report here on our investigations of a common but unproved assumption, that natural concentrations of lead exist within plants and animals in open country; our conclusions are based on measurements of lead inputs and outputs for a natural ecosystem, on identification of the natural and industrial leads within the ecosystem, and on measurements of the distribution of lead within a food chain within the ecosystem. Our studies were carried out in Thompson Canyon which is located in the crest of the High Sierra in California and which lies within a single batholithic rock type (Cathedral Peak quartz monzonite) that was stripped clean by Wisconsin glaciation. The soils of the canyon were formed during post-Wisconsin weathering of local quartz monzonite. The input of lead-containing aerosols less than 1 μm in diameter collected by dry deposition on foliage, measured with a deposition instrument, was about 1 kg of lead entering the 14-km^2 watershed during the four summer months. The input of lead-containing aerosols coprecipitated in snow within 14 km^2 was estimated to be 12 kg of lead for the eight winter months. Rain contributes only 5 percent of the total water input and was not analyzed. About 97 percent of the aerosol lead entering Thompson Canyon is, on the basis of $Pb/(K + Ca)$ ratios, of industrial origin. The value of this ratio is 2.0 in Los Angeles air, 0.02 in Thompson Canyon snow, 0.01 in Thompson Canyon foliar dust, and 0.0005 in Thompson Canyon rocks. Mass balance estimates of the output of industrial lead aerosols from both the Los Angeles and San Francisco areas [based on the 5 metric ton/day emission from Los Angeles estimated by Huntzicker and Friedlander (*1*) and a 2 ton/day emission from San Francisco obtained by normalizing to the Los Angeles value on the basis of population ratios] suggest that the share of this emission available for deposition within Thompson Canyon from a uniform dispersal area 2000 km in diameter should be about 10 kg of lead per year, which is within the limits of the observed input of 13 kg of lead per year. We determined the output of lead from the canyon by streams by analyzing the lead content of stream waters and using water output data to obtain the total average yearly lead output, 0.2 kg of lead per year from 14 km^2. Stream runoffs and snow accumulations were monitored throughout the year by Hinkley (*2*).

The difference between lead input and lead output shows that, at present, about 98 percent of the industrial lead entering the valley as aerosols remains there. We believe that the strongly

Table 1. Isotopic compositions of Thompson Canyon leads and urban sources.

Sample	$^{206}Pb/^{204}Pb$	$^{206}Pb/^{207}Pb$	$^{206}Pb/^{208}Pb$
Los Angeles air filter*	18.47	1.196	0.4894
San Francisco air filter*	18.40	1.185	.4870
Snow pack*	18.32	1.183	.4800
Humus (0 to 15 cm)	20.17	1.289	.5098
Groundwater (70 cm)	19.10	1.211	.4897
Humus (70 to 80 cm)	19.92	1.279	.5070
Thompson Canyon rock leach	25.87	1.631	.5929

* Lead accumulated sometime during the period from September 1972 to February 1973.

chelating humus fraction of the canyon soil probably extracts most of the industrial lead from snow meltwaters. The total reservoir of humus and lead sesquioxide film now present in 14 km^2 of the canyon is about 3 tons, as estimated from the concentration of lead in humus, the amount of humus in soil, the area covered by soil, and its average depth.

We used the historical record of industrial lead aerosols deposited in northern Greenland snow strata, normalized to the present lead concentrations in Thompson Canyon snow, to integrate the total industrial lead input to Thompson Canyon via snow over the past 80 years (*3*), and it came to 0.5 ton of lead per 14 km^2, divided about equally between smelter fume and gasoline exhausts. This is an appreciable fraction of the amount now retained in humus and indicates that natural concentrations of lead in Thompson Canyon humus were smaller three centuries ago.

It is likely that the natural lead from Thompson Canyon rocks originally contained in vegetation has now been exchanged for industrial lead. We investigated to see whether or not the isotopic composition of humus lead had been changed by industrial lead. We assume that lead leached from crushed quartz monzonite with dilute acid represents the natural lead originally occurring in humus because the latter probably came from minerals readily decomposed by weathering. This is likely since 60 percent of the canyon surface is bare rock and gruss, and existing soils are thin, immature, and only a few centuries to a few thousand years old. As shown in Table 1, the isotopic composition of lead in meadow topsoil humus is intermediate between that of the snow and rock leads but lies close to that of snow lead, suggesting that 2 tons of industrial lead may have been added to 0.5 ton of natural lead in humus within Thompson Canyon. The isotopic composition of humus lead in meadow soil at a depth of 80 cm was found to be about the same as in humus lead at 10 cm, but this may have resulted from the exchange of industrial lead in melted snow with humus at a depth of 80 cm, as shown by the isotopic composition of lead in groundwater flowing through the humus at a depth of 80 cm. The total mass of such deep humus in the canyon is negligible.

Concentrations of nutritive calcium and nonnutritive strontium, barium, and lead were determined in a food chain in Thompson Canyon which consisted of quartz monzonite, soil moisture, sedge, and meadow vole. If these metals are arranged simply as ratios of one of the nonnutritive metals to nutritive calcium in the food chain materials at successive trophic levels, the biological distribution of the metals in the ecosystem can be understood in a way that is much more satisfactory than if absolute amounts of nonnutritive lead in wet, dried, or ashed materials are compared at successive trophic levels. Data on the metal ratios in the food chain are displayed in Table 2. We see a pattern of depletion of strontium, barium, and lead with respect to calcium at successive trophic levels. On a calcium basis, voles appear to have acquired only 13 percent of the strontium, 2 percent of the barium, and 0.5 percent of the lead from rocks they would have if calcium enrichment processes were not operative in their food chain.

Although the mass of lead aerosols deposited in 14 km^2 of Thompson Canyon on foliage during the summer ($\sim$ 1 kg) is small compared to the winter deposition of lead aerosols in snow ($\sim$ 12 kg), it is apparent from the relations shown in Table 2 that small amounts of lead aerosols deposited on sedge leaves and eaten directly by voles may pollute them as seriously as large amounts of lead aerosols coprecipitated in snow and adsorbed in humus. Some 5/6 of the total lead in sedge leaves was found in acid-washings (10 percent HCl) of the leaf surfaces. The rate of lead aerosol accumulation estimated from the acid-washing of natural sedge leaves, 0.03 ng of lead per square centimeter per day (assuming none was removed by rain), compares favorably with the rate determined from plastic sedge leaves, 0.016 ng of lead per square centimeter per day in a 10 km/hour wind. These measurements indicate that at least 80 percent of the lead ingested by meadow voles in Thompson Canyon collects on leaf surfaces by aerosol deposition and originates from lead mines. The body burdens of lead in unpolluted voles living in Thompson Canyon more than 300 years ago were probably less than 0.01 part per million (ppm), compared to the 0.06 ppm now observed, because pollution lead on leaf surfaces occurs in the form of inorganic salts which may be more easily assimilated than lead in plant tissues, and because lead burdens in mammals tend to increase more or less linearly with an increase in nutritional exposure.

These findings show that a widespread assumption, that lead pollution is mainly confined to urban complexes and is essentially absent in open country, is improbable; instead they show that there is no place in the United States where one can assume that grass or mice contain amounts of lead that are even approximately natural. This report demonstrates that, until investigators make careful mass balance studies of lead pollution inputs and outputs and determine the influences of lead pollution on food chains within any remote area they wish to use as a natural baseline reference, the flora and fauna in all such areas must be suspected of serious lead pollution. This report also shows that lead cannot be assumed to accumulate at the higher ends of terrestrial food chains in the way that DDT does; instead, it shows that multiplicative natural purification processes exclude lead from the upper end of the vole food chain. Thus it is possible for those small amounts of industrial lead that circumvent natural purification processes to seriously pollute higher organisms.

Analytical methods and study area. Stable isotope dilution mass spectrometric analytical techniques were used, together with clean-laboratory procedures, to determine the concentrations of lead, calcium, strontium, and barium in all substances studied. The

Table 2. Moles of metal per 10^6 moles of calcium within a Thompson Canyon ecosystem.

Metal	Rock	Soil moisture	Unwashed sedge leaves	Vole
Ca	1,000,000	1,000,000	1,000,000	1,000,000
Sr	16,000	12,000	7,300	2,000
Ba	15,000	3,800	2,000	330
Pb	280	210	54	1.4
		*After acid-washing of the sedge**		
Pb	280	210	9	0.2

* Acid-washing theoretically reduces the body burden of lead in the vole by 83 percent.

14-km^2 study area of Thompson Canyon is located at 38°09'N, 119°30'W, at an altitude of 3170 m within the northern part of Yosemite National Park in the crest of the High Sierra, 46 km from Yosemite Village, 480 km from Los Angeles, 240 km from San Francisco, 17 km from the nearest road ending, and 3 km from the nearest park foot trail (all direct line). Toiyabe National Forest and the Hoover Wilderness Area lie between the edge of the park where the canyon lies and outlying grazing lands and small communities. More than half the exposed surface of the interior part of the canyon is barren rock. The canyon is sparsely forested, contains no lakes, and its streams contain no fish, but it does possess one broad, poorly drained meadow.

Deposition and snow lead input. We obtained dry aerosol deposition data in the canyon by exposing 1300 pieces of glycerin-coated, acid-cleaned, roughened nylon, cut to simulate sedge blades under known conditions, and then acid-washing them. Our measured accumulation of 0.016 ng of lead per square centimeter per day in 10 km/hour wind was scaled up on the basis of estimated vegetative cover to include the entire foliar surface in Thompson Canyon.

We determined the lead content of the snow pack by analyzing an entire vertical section sampled at the time of maximum mass. The lead content of the 7 April 1973 snow pack (depth, 3.3 m; density, 0.44 g/cm^3) averaged 0.59 part per billion (ppb). The surface of aged snow will show high lead concentrations because of dry aerosol deposition. The lead concentration was 3.5 ppb in a 4-cm surface layer on the 4 May 1972 snow pack and 57 ppb in a layer 2 cm thick on the 4 June 1973 snow pack.

Lead output. The average concentration of lead in stream runoff from Thompson Canyon was 0.015 ppb, which is 1/30 of the median lead concentration and 1/20 of the analytical sensitivity limit for 170 High Sierra lakes reported by Bradford *et al.* (*4*). These investigators bailed their samples out of the lakes from a helicopter which was fueled with leaded gasoline. This could account for their excessively high lead values. The lead content in free-flowing groundwater within the saturated water table zone in the meadow was 0.05 ppb.

Integrated lead accumulation. The general trends of industrial lead aerosol concentrations with time in the atmosphere of the Northern Hemisphere during the past several centuries can be obtained from data on the lead content of Greenland snow (*3*), and this record can be used to estimate the relative rates of past deposition of lead aerosols in the High Sierra. Lead concentrations in Sierra snow in 1940 were probably only one-third of present concentrations, the rise after 1940 being caused by increases in the rates of burning of lead alkyls. From about 1890 to 1940 lead concentrations in Sierra snows were regulated largely by smelter fumes. A major primary lead smelter producing tons of lead aerosols annually operated in San Francisco from 1886 to 1970. Our integral is a lower limit, since there were substantial lead inputs for a century further back in time.

Lead in humus. The top 15 cm of a typical meadow soil in the canyon contained 9.2 ppm of lead (wet basis), but only 10 percent of this lead was found to reside in humus and in sesquioxide films [15 ppm (dry) at a depth of 10 cm, 4 ppm (dry) at 80 cm], the remainder being contained in igneous minerals. This was determined by a brief $0.1N$ HNO_3 leach of the soil after it had been completely oxidized at room temperature in an excited oxygen plasma. Residual igneous minerals contained most of the lead in the soil but, because of their stabilities and insignificant surface areas, contributed little lead to the acid leach and did not interfere with the humus lead determination. Size and mineral analyses by Hinkley (*2*) showed that amorphous clays (allophane?) are present in very low abundance in the soil, and so clay minerals probably did not contribute significantly to the lead in the acid leach either.

Isotopic composition of leads. We carried out the disaggregation of minerals in a sample of fresh rock without producing much fine powder by using a hydraulically operated mortar. The resulting mass was leached with $0.1N$ HNO_3 for 30 minutes, and the lead in the acid leach solution was analyzed. This lead comprised 0.5 percent of the total lead in the rock. The leach lead is excessively radiogenic because of contributions from readily decomposed uranium-rich, lead-poor apatite (0.3 percent abundance) and lead-poor uranothorite (present). Industrial lead from Thompson Canyon snow is much less radiogenic, and, as Table 1 shows, probably originates from San Francisco and Los Angeles. The isotopic composition of snow lead is typically nonradiogenic. During the early 1960's the $^{206}Pb/^{207}Pb$ ratio was 1.144 in Sierra snow (*5*).

Metals in the food chain. Calcium, strontium, and barium values used here for quartz monzonite and soil moisture are those reported by Hinkley and Patterson (*2, 6*): calcium, 0.63 ppm; strontium, 0.017 ppm; barium, 0.008 ppm; and lead, 0.0007 ppm in soil moisture. A soil moisture sample was collected on cleaned, dried, and weighed filter disks pressed against the wet soil in the field at sedge root depth. The disk contacting soil was discarded, and the remaining disks containing some 200 mg of moisture were weighed and analyzed. The soil is classified as Entisol: Andeptic Cryofluvent (*7*).

Food eaten by voles at our study area consisted mainly of *Carex scopulorum* (a sedge). Voles do not hibernate and apparently eat sedge nodes and rhizomes under snow in winter. We have trapped voles under snow and have also found sedge rhizomes to exist in a viable, partially unfrozen condition in soil that was only partially frozen beneath the snow pack in deep midwinter. The concentrations of all four of the metals analyzed were found to be higher in sedge leaves collected in the fall, compared to midsummer sedge leaves, showing the effects of senescence. Average values in nonacid-washed sedge leaves were as follows: calcium, 1200 ppm; strontium, 19 ppm; barium, 8 ppm; and lead, 0.33 ppm (wet). About 10 percent of the alkaline earths inside the leaves were leached out by 10 percent HCl (1 minute), mainly from the cut ends. The calcium/strontium and calcium/barium ratios in the washings were the same as those found inside the leaves but different from those in soil or in dust collected on plastic, an indication that these metals came from inside, so they were added by calculation to those found on the inside. The situation was different for lead; 83 percent of that metal was found on leaf surfaces and was collected by aerosol deposition as shown above by comparison with instrument collection data. The average concentration of lead in acid-washed sedge leaves was 0.06 ppm (wet).

Vole (*Microtus montanus*) muscle and bone from a single 30-g adult were analyzed, and metal values in these tissues were used to estimate total body burdens according to known distributions of lead and alkaline earths in mammals (*8*); the proportions of these tissues in rats (*9*) were scaled according to the body measurements of our vole specimen. The body burdens were as follows: calcium, 7800 ppm; strontium, 34 ppm; barium, 9 ppm; and lead, 0.06 ppm. The concentration of lead in vole muscle (biceps femoris) was found to be 0.0015 ppm (wet), whereas that in bone (femur) was 1.1 ppm (wet). These values were determined on an animal that had been dissected and chemically processed under strict clean-laboratory conditions which provided very low contamination levels, and whose parts had been analyzed by stable isotope dilution in a high-resolution thermal emission mass spectrometer which provided uniquely accurate concentration data. These factors, together with the nature of the collecting site, account for the difference between the above data and much higher lead concentration data reported by others in similar animals.

Of all grossly abundant nutritive metals, calcium is most useful to compare with nonnutritive lead in various components of a food chain because, like lead, the bulk of it is contained not in cell fluids in plants and animals but in and near membranes where it is relatively immobile. Calcium and lead have similar migration tendencies during senescence in plants; they are absorbed through the mucosal membrane of the gut and are excreted by the kidney in similar fashion in animals. Calcium might be expected to become purified of lead in nutritive steps involving active transport across membranes, because such transport mechanisms work specifically for certain nutritive metals but only inadvertently and less efficiently for other metals.

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References and Notes

1. J. J. Huntzicker and S. K. Friedlander, in *Background Papers for a Research Conference on California Ocean Pollution*, N. Brooks and E. Goldberg, Eds. (California Institute of Technology, Pasadena, 1973), p. 43.
2. T. Hinkley, *Geol. Soc. Am. Bull.*, in press.
3. M. Murozumi, T. J. Chow, C. Patterson, *Geochim. Cosmochim. Acta* **33**, 1247 (1969).

G. R. Bradford, F. L. Bair, V. Hunsaker, *Limnol. Oceanogr.* **13**, 526 (1968).

5. T. J. Chow and M. S. Johnstone, *Science* **147**, 502 (1965).
6. T. Hinkley and C. Patterson, *Nat. Phys. Sci.* **246**, 123 (1973).
7. *Soil Taxonomy of the National Cooperative Soil Survey* (Soil Conservation Service, U.S. Department of Agriculture, Washington, D.C., in press).
8. *Health Phys.* **3**, 1 (1960); H. P. Schroeder and I. W. Tipton, *Arch. Environ. Health* **17**, 965 (1968).
9. N. H. Donald, *The Rat* (No. 6, Memoirs of the Wistar Institute of Anatomy and Biology, Philadelphia, 1924).
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Enzyme Measurements on Single Cells

Abstract. *A simple and sensitive procedure has been developed for enzymatic assays in single cells and applied to the measurement of β-glucuronidase in single parenchymal cells of liver. Cells deposited in microdroplets under oil were ruptured by freezing and thawing and allowed to react with a fluorogenic substrate. The fluorescence is developed by diffusing an organic base into the droplets and measured in a fluorescence microscope equipped with a photomultiplier.*

We have developed a micro fluorimetric assay technique for measuring β-glucuronidase in single mammalian cells. The amount of fluorescent product formed is measured by means of a fluorescence microscope equipped with a photomultiplier microphotometer. The technique is derived from the method originally introduced by Rotman for the study of single molecules of β-galactosidase in *Escherichia coli* (*1*).

Single cell measurements of enzymes may prove useful in prenatal diagnosis after amniocentesis, and they are well suited to fundamental studies in cell biology.

Rotman's conception of using micro droplets to minimize background fluorescence and thus increase the signal-to-noise ratio embodies an important principle in developing a sensitive microassay for a fluorescent product. We found optimal those droplets whose volumes were 1 to 10 nl. Smaller droplets do not have an appreciably lower background in our system and are much harder to work with; larger droplets do have a higher background and present no compensating advantages. The micro droplets were deposited under oil on a microscope slide (25 by 75 mm). The slide was 1 mm (uniform) in thickness and was modified to contain oil by glueing glass rods 2 mm in diameter to the edges with silicone rubber. After trials with other oils, we chose Amoco white oil 35 (American Oil Company). White oil has a higher viscosity than commercial mineral oils and a lower water solubility than silicone oil. The oil (1 ml) was spread evenly over the slide just before the droplets were made.

The droplet "dispenser" was a piece of polyethylene tubing (0.3 cm, inside diameter, with a 0.15-cm wall) that was heated slowly over a small flame until transparent and then drawn to an outside diameter of 0.1 to 0.2 mm. The tubing was allowed to cool and then cut so that one end was at least 0.1 mm in diameter and the other end wide enough for attachment to a pump or aspirator. Uniformly spaced and sized droplets were deposited in rows under oil on the microscope slide by drawing