

relation function S than small particle fluctuations. In other words, in Eqs. 1 and 2 there should be size-dependent multiplicative factors associated with S_f and S_b that describe the relative amount by which the bead fluorescence correlates more efficiently than the free fluorescence.

The fact that S is dominated by the bead fluorescence is the most significant feature of this assay because it allows the elimination of separation steps. This can be illustrated by data from two systems.

First, we synthesized a test system of fluorescent latex spheres (Dow; 0.46 μm in diameter) and rhodamine dye. We made five samples with the same concentration of dye but with serially lower concentrations of fluorescent spheres. Each dilution reduced the bead concentration, and hence I_b/I_f , by one-fifth. Figure 1 shows S plotted as a function of I_b/I_f . From Eqs. 1 and 2 we expect S (and I_b/I_f where I_f is constant) to decrease in direct proportion to N_b only when $S \approx S_b \gg S_f$. From Fig. 1 we see that this is true even for $I_b/I_f \approx 5 \times 10^{-3}$.

For a clinically significant test we used the Bio-Rad gentamicin fluorescent kit (Bio-Rad Laboratories, Richmond, California). This is a competitive assay with a fixed concentration of tagged gentamicin [G^*] and a variable concentration of untagged gentamicin [G] competing for a fixed number of antibody sites on beads ($G + G^*$ in excess). As [G] is increased, the fluorescent brightness of the beads will decrease; that is, n will decrease. From Eqs. 1 and 2 we see that $S^{1/2} \propto n$ as long as S is insensitive to the free fluorescence. Samples were made from the Bio-Rad kit by mixing the G^* , G , and bead reagents together and incubating for at least 30 minutes. The free fluorescence is due to the unbound G^* in addition to extra dye that Bio-Rad adds to the G^* tracer to make that reagent obviously yellow.

Figure 2 shows $S^{1/2}$ plotted as a function of [G] for two concentration ranges. The curves are the standard curves for gentamicin determination from our correlation technique with homogeneous samples. These curves coincide with the standard curve supplied by Bio-Rad, in that a given dilution of the standard results in the same change in the measured signal. Furthermore, the midpoint of curve B is translated to higher concentrations by a factor of 10. Finally, the assay can resolve gentamicin at 1 ng/ml, ($2.6 \times 10^{-9}M$) from a total specimen volume of only 10 μl .

All points in Figs. 1 and 2 are mean values from several measurements on the same sample. The standard deviation

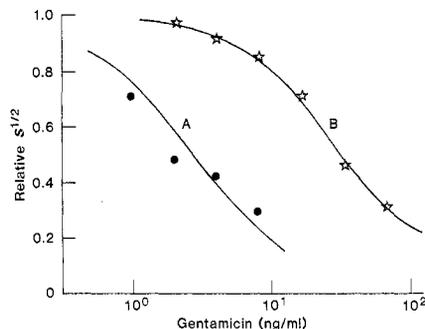


Fig. 2. Relative values of $S^{1/2}$ are plotted as a function of gentamicin concentration without separation. The $S^{1/2}$ was normalized by the measured value from a sample with a fixed concentration of gentamicin tracer and zero concentration of untagged gentamicin. The concentrations of gentamicin are those of the actual measured samples. (The concentrations of the gentamicin standards supplied with the kit before dilution range from 2 to 16 $\mu\text{g/ml}$, comparable to concentrations in serums.) Curve B gives data for samples with ten times the concentrations of G , G^* , and beads relative to curve A.

relative to the mean values of S for a given sample decreases with the inverse of the square root of the total sampling time. The scatter of points on curve A (Fig. 2) is due to shorter tests than for the samples of curve B; a typical time for

measuring a single mean value on curve B was 20 minutes. The settling of the beads during the long experiments adversely affects the deviation. We used commercially available bead reagents, but our signal-to-noise ratio would improve if we used a bead with a greater number of active sites and a density close to that of water to reduce the settling.

Our tests demonstrate the feasibility of this fluorescent immunoassay with homogeneous samples. This technique may simplify the clinical procedures for both fluorescent and radioimmunoassays (1). There is an additional advantage: without the separation steps, this technique in principle can follow the kinetics of the antibody-antigen reaction.

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3 February 1981

Fly Ash-Derived Strontium as an Index to Monitor Deposition from Coal-Fired Power Plants

Abstract. *The combustion of western U.S. coals releases significant amounts of strontium, which is relatively enriched in the fine particles of fly ash. Fly ash-derived strontium is readily absorbed by agronomic and native plant species when incorporated in soil. The strontium-87 to strontium-86 ratios of fly ash and soils were significantly different, but similar ratios were found in fly ash and plants treated with fly ash. A technique for measuring and monitoring deposition from coal-fired power plants is inferred from the enhanced plant uptake of fly ash strontium and the similarity in the isotopic ratios of fly ash and treated plants.*

In view of the nation's increasing demand for coal and the serious concerns over associated environmental impacts, knowledge of the environmental pathways of coal combustion residues is essential. Various attempts to monitor the deposition of particulate matter emitted from coal combustion onto soils and vegetation have had only limited success, mainly because of the difficulty of distinguishing between deposited and natural background materials (1). The possibility that strontium uptake in native vegetation (2) and soils (3) might be used as a monitor of power plant effluents has been suggested.

Coals from the western United States contain on the average greater concentrations of strontium than their eastern

and midwestern counterparts (4). The strontium content of western coal ranges from 93 to 500 parts per million (ppm), averaging about 260 ppm. Lignitic coals, which are abundant in the West, have the highest average strontium content (300 ppm) (5). The average strontium content of fly ash from several U.S. sources was estimated at 1334 ppm (6), and it usually increases as the particle size decreases (7, 8). Relative to typical soils (300 ppm of strontium) (9), emitted fly ash is thus highly enriched in strontium, and, depending upon the recipient soil and the amount of fly ash deposited, the strontium content of soil will increase (8).

Greenhouse experiments have demonstrated that fly ash-derived strontium is

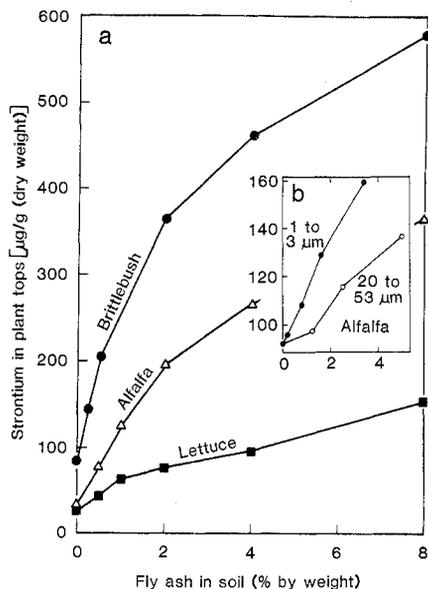


Fig. 1. (a) Effect of fly ash incorporation into soil on the strontium concentration in the leaves of brittlebush (16) and in the tops of alfalfa (17) and lettuce (18). (b) The influence of fly ash particle size on the strontium uptake by alfalfa grown on Josephine soil (19).

biologically available. Agronomic and native plant species grown on soils containing up to 8 percent (by weight) fly ash absorbed strontium in proportion to the amount of fly ash added to the soil (Fig. 1a). The uptake also appeared to be inversely related to the particle size of fly ash (Fig. 1b). Brittlebush (*Encelia farinosa* Gray), a perennial native shrub common to the western deserts (10), is particularly suitable for leaf sampling because of its broad leaf area (3). Since coal-fired power plants in the West are usually situated in remote desert areas, the choice of *E. farinosa* as a deposition indicator seems appropriate. The strontium uptake data (Fig. 1a) for this species reveal a steady increase up to 613 percent at the 8 percent fly ash level in soil. Leaves of naturally grown *E. farinosa* sampled in the vicinity of the coal-fired power plant contained up to 300 ppm strontium or more (3).

The above discussion illustrates a substantial strontium enrichment in fly ash and plants and soils treated with fly ash, but the distinction between such enrichment and the background strontium level has not yet been resolved. The isotope ^{87}Sr is the daughter product of ^{87}Rb (beta decay), whose half-life is estimated at 40×10^9 to 60×10^9 years (11). The ratio of ^{87}Sr to the stable isotope ^{86}Sr has been found useful in tracing natural processes in the lithosphere and hydrosphere (11, 12). We have measured the strontium isotopic composition ($^{87}\text{Sr}/^{86}\text{Sr}$) in fly ash, soil, and *E. farinosa*

grown on soil treated with fly ash at rates ranging up to 8 percent (by weight) (13). If it were found that the ratios for fly ash and soil were different but that the ratios for fly ash and treated plants were similar, this result would suggest a method of identifying fly ash-derived strontium and hence would serve as a technique for detecting the deposition of atmospheric emissions from coal-fired power plants.

The results indicate that the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of fly ash and soil are significantly different (0.70807 ± 0.00025 for fly ash and 0.71097 ± 0.00021 for soil). An acid soil (Redding loam, $\text{pH} \approx 5$), included in the analysis as a check, also exhibited a significantly different isotopic ratio from that of fly ash (0.71117 ± 0.00025).

The isotopic ratios of strontium in leaves of *E. farinosa* grown in control and fly ash-treated soils are shown in Fig. 2, along with the strontium isotopic ratio of fly ash ± 1 standard error of the mean (σ). The ratio declined sharply with fly ash additions up to 1 percent and then was more or less comparable to the ratio of the fly ash. For 0.25 to 8 percent fly ash in soil, the isotopic ratio in the leaf ranged between 0.70770 and 0.70833. Such a range falls within the fly ash isotopic ratio $\pm \sigma$.

The technique is apparently sensitive at low concentrations of fly ash in soil since the initial reduction in the isotopic ratio occurred within the range of ≤ 0.25 percent fly ash in the soil (Fig. 2). Various estimates indicate that, over the lifetime of a coal-fired power plant, the maximum amount of airborne fly ash deposited on neighboring soils is, in fact, well within this range (14).

The increase in the strontium concentration in the leaf (Fig. 1) and the reduction in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio with increasing concentrations of fly ash (Fig. 2) suggest that, of the strontium pool in soil, fly ash-derived strontium contributed significantly to the total uptake of strontium by the leaf. The apparent equilibrium in the isotopic ratios of fly ash and plants may, in fact, suggest that at certain concentrations of fly ash in soil (up to 1 percent), strontium uptake may essentially be entirely derived from fly ash. This is actually expected in view of the fact that the concentration of water-soluble strontium in fly ash ranged from 61 ppm at pH 12.5 (pH of fly ash as produced) to 333 ppm at pH 6.5 (3). This is 66 to 258 times as much as the mean (0.93 ppm) of water-soluble strontium found in 68 California soils (15).

The distribution of fly ash and its exchange with biological systems would depend upon local atmospheric circulation and precipitation. We expect that

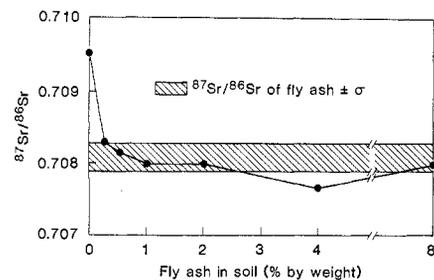


Fig. 2. The ratio of ^{87}Sr to ^{86}Sr in the leaves of brittlebush (16) grown on soil treated with various amounts of fly ash.

for fly ash concentrations lower than 0.25 percent in the natural environment a true mixing relationship would be the case rather than the saturation effects observed in this experiment. Differences between the true desert environment and the greenhouse experiments warrant further investigations in light of the importance of a hydrous environment for exchange reactions. These data could be obtained by direct sampling in the vicinity of operating coal-fired power plants or by further controlled experiments at lower fly ash concentrations under variable moisture regimes.

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19. The < 53- μ m fraction of the fly ash, which was obtained by mechanical sieving, was introduced into a microparticle classifier (Bahco 6000). Several size fractions were obtained and were subsequently identified by means of light and electron microscopy. The identified size fractions were mixed with Josephine loam soil to produce fly ash concentrations up to approximately 5 percent (by weight). The mixtures were cropped to alfalfa for approximately 1 year. The values shown in Fig. 1b are average values for samples collected from the early- and late-season clippings of the crop. Strontium in the plant materials was analyzed by neutron activation analysis.

30 September 1980; revised 23 February 1981

Magnetotactic Bacteria at the Geomagnetic Equator

Abstract. *Magnetotactic bacteria are present in fresh water and marine sediments of Fortaleza, Brazil, situated close to the geomagnetic equator. Both South-seeking and North-seeking bacteria are present in roughly equal numbers in the same samples. This observation is consistent with the hypothesis that the vertical component of the geomagnetic field selects the predominant polarity type among magnetotactic bacteria in natural environments.*

Several species of magnetotactic bacteria have been observed in aquatic sediments of the Northern and Southern Hemispheres (1-5). Each bacterium contains magnetosomes consisting of enveloped, single-domain magnetite particles. The magnetosomes are often arranged in chains with a magnetic dipole moment, parallel to the axis of motility, sufficiently large that the cell is oriented along the geomagnetic field lines as it swims (6, 7). Cells with North-seeking pole forward swim North along the magnetic field lines; cells with the South-seeking pole forward swim South. Because of the inclination of the geomagnetic field, North-seeking cells migrate downward in the Northern Hemisphere and upward in the Southern Hemisphere; South-seeking cells migrate downward in the Southern Hemisphere and upward in the Northern Hemisphere. Magnetotactic bacteria in Northern Hemisphere sediments are almost exclusively North-seeking (1-3), while bacteria in Southern Hemisphere sediments are almost exclusively South-seeking (4, 5). Thus downward directed motion is advantageous for, and upward directed motion is detrimental to, survival of these organisms; and the vertical component of the geomagnetic field selects the predominant cell polarity. If magnetotactic bacteria exist at the geomagnetic equator

where the magnetic field lines are horizontal, neither polarity should be selected. We report the observation of various morphological types of magnetotactic bacteria in aquatic sediments close to the geomagnetic equator. North-seeking as well as South-seeking bacteria are present in roughly equal numbers in the same sediment samples.

Samples of fresh water and marine sediments were collected in the vicinities of Fortaleza and Rio de Janeiro, Brazil. At these locales, the total intensity of the geomagnetic field is 0.25 to 0.28 gauss (8), approximately one-half the intensity at locales in New England and New Zealand where magnetotactic bacteria have previously been found. Fortaleza is situated close to the geomagnetic equator (inclination < 4°) while at Rio de Janeiro the inclination of geomagnetic field is 25° to 30° South. Magnetotactic responses of bacteria in sediment samples were observed in uniform magnetic fields, up to 3 gauss, provided by a pair of Helmholtz coils mounted on either side of a Nikon SMZ-10 stereomicroscope. The magnetic field axis was aligned parallel to the horizontal component of the geomagnetic field. The direction of current flow in the coils and hence polarity of the imposed magnetic field was selected with a toggle switch. Bacteria from sediment samples collect-

ed in Fortaleza migrated along the magnetic field lines, some in the field direction (North-seeking) and roughly equal numbers in the same sample opposite to the field direction (South-seeking). When the imposed field was reversed, both groups of bacteria executed U turns and swam opposite to the initial direction. Bacteria from sediment samples collected in Rio de Janeiro migrated opposite to the field direction (South-seeking) only, and also reversed direction on reversal of the field. Migration rates of bacteria from both locales were comparable to those of other magnetotactic bacteria.

The bacteria were subjected to a demagnetizing procedure by exposing them to and subsequently slowly moving them away from an alternating 60-Hz magnetic field over 1000 gauss produced by a small hand-held magnetic tape degaussing. North-seeking and South-seeking bacteria from Fortaleza were first separated by their magnetotactic response, placed in separate water drops on a microscope slide, and then exposed. Whereas before exposure all the bacteria in each drop swam exclusively in the field direction, or opposite to the field direction, after exposure each drop contained approximately equal numbers swimming in and opposite to the field direction. Similar results, previously reported for other magnetotactic bacteria (4), were also obtained with South-seeking bacteria from Rio de Janeiro. Thus each bacterial magnetic dipole is essentially a single magnetic domain and cannot be demagnetized. However, the polarity can be reversed (2) and the demagnetization procedure results in the reversal of about one-half of the dipoles of the bacterial population in each drop. Preliminary electron microscope studies show that North- and South-seeking bacteria from Fortaleza are morphologically identical and contain intracytoplasmic electron opaque particles (9). Magnetosomes consisting of intracytoplasmic, enveloped, magnetite particles are a characteristic of all magnetotactic bacteria studied to date (10-12).

In order to determine experimentally the effect of a vanishing vertical magnetic field, sediments samples from New England initially containing exclusively North-seeking bacteria were placed in a Mumetal enclosure in New England in which the magnetic field intensity was less than one hundredth the intensity outside the enclosure. The polarities of bacteria from both experimental and control samples placed outside the enclosure were monitored periodically over several weeks, that is, many bacte-