In the measurements reported here a single hair from a female head has been washed in triply distilled water, and cut into fourteen pieces approximately 3 cm in length. Each sample was then fixed to an aluminium frame. Each target was exposed to the beam of 3 MeV protons from the Rice University tandem Van de Graaff accelerator. Detector efficiency for elements lighter than Ca and the low energy bremsstrahlung background was artificially reduced by introducing a polystyrene absorber in front of the detector. The resulting X-rays were detected by a Si(Li) detector and amplified, then processed and stripped for peak intensity by an IBM–1800 computer. Figure 1 shows the X-ray spectrum obtained for one of the samples. Peaks associated with Ca, Ti, Fe, Cu, Zn, Pb, Br, and Sr K_α and K_β X-ray lines are present in all spectra.

The ratios of elements in the hair were normalized to ratios obtained from targets with known element concentrations. Figure 2 shows the ratios Ca/Zn, Cu/Zn, Sr/Zn, Fe/Zn, and Br/Zn as a function of distance along the hair from the scalp. The Ca/Zn, Cu/Zn, Sr/Zn ratios are found to increase monotonically with length along the hair except for a small dip in the 9–10 region. The Br/Zn and Fe/Zn ratios show a more complex structure, but also display a dip in 9–10 region. The Fe/Zn ratio in particular, undergoes very strong fluctuation near the base of the hair.

Two additional measurements were performed with beam-resistant and multihair targets using hair from the same female head. All three measurements were consistent. In addition, the shampoo which had been used in regular washing of the hair was found to contain no significant concentrations of metals heavier than Ca.

These results show that the concentrations of trace elements undergo large fluctuations in close proximity to the scalp. The relative concentrations of Ca, Sr, Cu, Fe, Br, and Zn change significantly with distance along the hair. The source of these variations may be associated with the environmental and biomedical history of the hair and its owner.

These facts must be considered when evaluating previous measurements of trace elements in hair. It is possible that some reported inconsistencies are due to the variation of element concentrations along single hairs and, therefore, future studies must pay attention to this problem.

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Chemical Exploration of the Microhabitat by Electron Probe Microanalysis of Decomposer Organisms

Increasing emphasis on the role of microorganisms as nutrient concentrators in ecosystems calls for the accurate, reliable chemical analysis of these cells within their microhabitats. The inability to perform such analyses has been a principal limitation of existing macrochemical techniques. Witkamp[1], Stark[2], and Todd and Cromack[3] have demonstrated that terrestrial microflora can concentrate calcium, potassium and magnesium to a point that each becomes a major nutrient pool. Microarthropods—important grazers of microflora—link the terrestrial detritus-based food chain to other chains. This paper outlines a technique which enables the chemical composition of the microfloral and faunal biomass to be analysed without its destruction or separation from the detrital matrix. With the exception of laboratory bacterial cultures, all samples were collected from the US Forest Service Coweeta Hydrologic Laboratory field site, North Carolina.

Scanning electron microscopy is currently being used to provide "visual" observations of microorganisms in terrestrial[4–6] as well as estuarine ecosystems[7]. Electron probe microanalysis utilizes a similar, focused high-energy beam of electrons to excite the constituent atoms of a sample to emit characteristic X-radiation. This radiation can be analysed and displayed as a scanned image of elemental concentration in the specimen or, in the static mode, quantitative spot analyses can be made by comparison with suitable standard samples. (With our instrument both were possible in the same run.)

The principles of electron microprobe analysis are discussed in detail by Birks[8]. He states that 1 μg samples can be analysed with one part per billion resolution for the heavier elements (atomic number greater than 12). The precision and resolution, however, depend greatly on the nature of the sample and its preparation. Impregnation with plastic or metal, polishing to a smooth, flat surface, and vacuum-coating with carbon have usually been considered necessary. In this study, however, useful quantitative as well as qualitative data were obtained using the less disruptive preparation techniques outlined by Todd *et al.*[7] for observing biological samples in the scanning electron microscope.

For examination and analysis, our specimens were affixed by double adhesive cellulose tape or silver paint to the surface of a specimen stub. The air-dried specimens were then coated (200–400 Å) with a gold-palladium alloy by vacuum evaporation. Secondary electron images were observed using a Cambridge Stereoscan Electron Microscope (Mark II A, Cambridge Instruments Co., London, England) operated at an accelerating voltage of 10 kV. X-ray and backscattered electron images as well as spot analyses were made with an electron microprobe analyser (Model 400-S, Materials Analysis Co., Palo Alto, California) operated at 20 kV accelerating voltage with 0.02 μA sample current. Both secondary electron and X-ray emission images were recorded using Type 55 P/N Polaroid sheet film (Polaroid Corp., Cambridge, Massachusetts).

Comparisons between X-ray and secondary electron images

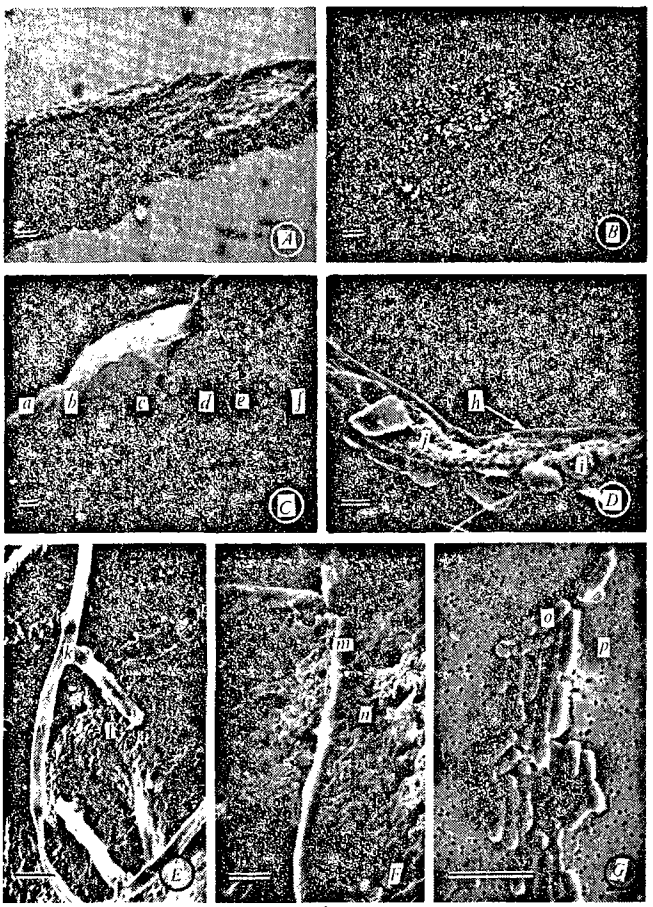

Fig. 1 *A* and *B* are electron microprobe analyser pictures of a rhizomorph sample isolated from decaying deciduous forest litter. A 100 μm marker is indicated at lower left. *A*, Image made by backscattered electrons. *B*, Calcium X-ray image. *C*, Galumnidae mite for which calcium values were determined after electron bombardment at each indicated point (*a* and *f* are background points) for 10 s periods at 20 kV. Calcium values (mg g^{-1}) for the points are: *a*, 1.5; *b*, 10.6; *c*, 18.9; *d*, 31.5; *e*, 12.7; *f*, 2.5. *D–G* are scanning electron micrographs of representative assay fields. *D*, White pine needle fragment (*g*) from litter with adhering insect wing scales (*h*) and a fungal hypha (*j*). *E*, Oak leaf (*l*) taken from decaying litter with a fungal hypha (*k*). *F*, Fungal hypha (*m*) in soil (*n*) underlying decaying oak litter. A 40 μm marker is indicated at lower left of each field. *G*, *Bacillus subtilis* cells (*o*) concentrated on the surface (*p*) of a membrane filter. A 10 μm marker is indicated at lower left.

were used to estimate the elemental content of a variety of biological specimens. These analyses have included a study of calcium, potassium, and magnesium distribution in organisms inhabiting decaying, mixed deciduous forest litter. Quantification of this technique is possible with macrochemical assay procedures such as emission spectroscopy and atomic absorption spectroscopy[8]. For example, the fungal rhizomorph illustrated in Fig. 1*A* and *B* contains 29.7 mg g^{-1} Ca as determined by these procedures. These counts can be converted to elemental values by comparison with standards of known concentrations. A calcium value of 134 $\mu g\ g^{-1}$, potassium 194 $\mu g\ g^{-1}$ and magnesium 140 $\mu g\ g^{-1}$ is approximately equal to a respective X-ray emission value of one count every 10 s.

A quantitative application of microprobe analysis to biological material is illustrated in Fig. 1*A–G* and Table 1. Fig. 1*A* is a back-scattered electron micrograph of a rhizomorph sample, and Fig. 1*A* is a calcium X-ray image of the same rhizomorph. Fig. 1*C* depicts a whole body of a Galumnidae mite, a common litter-inhabiting organism which grazes on fungi. Point analysis counts, which resulted in minimum distortion of the sample, were made at each point along a transect (*a–s*).

An application of this technique which enables nutrients to be analysed in a variety of biologically different samples located close to each other is illustrated in Fig. 1*D*. A freshly collected sample from the "L" layer of litter in a white pine stand was examined and comparison was made of the magnesium, calcium and potassium contents of a pine needle fragment, an insect wing scale and a fungal hypha, all situated within 1 mm (Table 1). All three samples contain the same relative amounts of magnesium and potassium. The calcium content of the insect wing scale, however, is eight times higher than that found in the pine litter. There is twice as much calcium in the fungal hypha as in the decaying pine needle.

Microprobe comparisons between a fungal hypha growing on a deciduous leaf and one growing in soil (Fig. 1*E* and *F*) are summarized by the numerical values for the magnesium, calcium and potassium content of these two samples in Table 1. There is no significant difference in the magnesium content of the fungal hypha colonizing the oak leaf or soil substrates. The calcium content of the fungal hypha is, however, twice that of the oak leaf, although there is no difference between the amount of calcium in the hypha and soil. Potassium seems to be concentrated within the fungal biomass of both microhabitats.

Chemical analysis of a specimen as small as an individual bacterial cell can be accomplished by the microprobe procedure, although samples of this size approach the 1 μm limit of resolution of the microprobe beam and are extremely "thin" with respect to the effective electron penetration. A stock culture of a common soil bacterium, *Bacillus subtilis*, was grown on a complex medium and the collected cells concentrated upon the surface of a 'Nuclepore' membrane filter[9]. Spot analyses were made of both the calcium and magnesium contents of the bacterial cells within the same field shown in Fig. 1*G* and are listed in Table 1. The calcium content of a *Bacillus subtilis* cell is estimated by the microprobe method as 1.1% and compares favourably with the 0.8% determined by emission spectroscopy for a mixed bacterial population grown under similar conditions[3].

Electron probe microanalysis can be used to determine concentrations of essential nutrients in biological components of the terrestrial decomposer food web, such as the detrital substrate, microbial population and microbial grazers. As decomposer organisms are of great importance in cycling of nutrients in ecosystems[10], any new technique which would give better estimates of nutrient pools contained within the decomposer food web would add substantially to the understanding of functional processes within ecosystems. Current modelling efforts being devoted to description and prediction of ecosystems by computer simulation would benefit from more accurate estimates of nutrient pools in decomposer organisms.

Several distinct advantages of this technique are evident when comparisons are made with existing macrochemical procedures. Examination of a variety of biologically different specimens located close to each other with minimum distortion of sample is possible. With the scanning electron microscope even a small specimen can be observed and uncontaminated areas can be selected for examination. Not only can estimates of microbial biomass be made from quantitative sampling techniques but it is also possible to ascertain the distribution of nutrients within the organism itself. As the specimen is not destroyed in the analytical procedure, assays of a given sample can be repeated to obtain statistical estimates of the nutrient content.

The application of this technique is not limited to investigations concerning terrestrial decomposer organisms, but will find application in any situation in which microorganisms play an integral part. The use of electron probe microanalysis will enable assessment of the role that decomposer organisms play in nutrient cycling.

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Table 1 Nutrient Analyses of Fungal and Bacterial Biomass shown in Fig. 1*D-G*

Specimen	No. of assays	Elemental content (mg/g) Magnesium	Calcium	Potassium
Fig. 1*D*				
Fungal hypha (*h*)	11	3.02±0.32* †	29.60± 2.48*	22.36±1.84
Insect wing scale (*i*)	6	2.47±0.25	112.58±14.84*	18.95±0.85
White pine needle (*j*)	7	1.72±0.20	14.56± 4.31	18.37±1.07
Fig. 1*E*				
Fungal hypha (*k*)	8	2.74±0.25	81.64± 4.67*	17.48±0.46*
Oak leaf (*l*)	5	3.30±0.36	44.62± 8.21	13.23±1.07
Fig. 1*F*				
Fungal hypha (*m*)	8	2.66±0.43	66.07± 6.95	46.21±2.81*
Soil (*n*)	4	3.66±0.98	65.15±17.66	19.90±7.46
Fig. 1*G*				
Bacteria (*o*)	12	0.08±0.08	11.32± 2.41	No data

Values reported were determined from X-ray emissions after electron bombardment of each specimen for several 10 s periods at an accelerating voltage of 20 kV.
* Indicates significant difference ($P < 0.05$) in elemental content between sample and substrate (pine needle, oak leaf, soil).
† Standard error of mean is given for all samples.

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Role of Protozoa in Waste Purification Systems

Pirt and Bazin[1] discussed possible adverse effects of bacterial predation by protozoa on the removal of substrate by activated sludge. Their analysis considered not activated sludge but a single-stage chemostat without recycle as used experimentally by Curds[2], and mathematically by Curds[3] and Canale[4], for the study of predator/prey interactions. Pirt and Bazin[1] concluded that efficient waste removal could best be achieved with two chemostats in series, eliminating protozoa from the first stage by operating it at a dilution rate in excess of their maximum specific growth rate.

Table 1 compares the concentrations of bacteria, protozoa, and substrate obtained from this system with those from a single-stage chemostat having the same overall residence time, demonstrating the better performance of the two-stage system. Table 1 also shows the effluent concentrations for a two-stage system of the same overall residence time which allows growth of protozoa in both stages. Contrary to the suggestion of Pirt and Bazin[1] the presence of protozoa in both stages improved treatment, with a further reduction in total solids concentration.

These results were obtained by assuming, as did Pirt and Bazin[1], that all the bacteria were dispersed and therefore

Table 1 Calculated Concentrations of Bacteria, Substrate and Protozoa in Effluent from Single-stage and Two-stage Continuous Culture

Single-stage operation

Dilution rate (h^{-1})	Specific growth rate (h^{-1}) Bacteria	Protozoa	Substrate	Effluent concentration (mg l^{-1}) Bacteria	Protozoa
0.225	0.9989	0.225	8,858	128	221

Two-stage operation

Stage 1				Stage 2					
Dilution rate (h^{-1})	Concentration (mg l^{-1}) Substrate	Bacteria	Protozoa	Dilution rate (h^{-1})	Specific growth rate (h^{-1}) Bacteria	Protozoa	Concentration (mg l^{-1}) Substrate	Bacteria	Protozoa
0.9	90	4,955	0	0.3	0.045	0.3	0.47	300	2,350
0.385	29.6	2,566	1,209	0.541	0.039	0.27	0.40	204	2,393

(μ_{max} bacteria = 1.0 h^{-1}, K_s bacteria = 10 mg l^{-1}, μ_{max} protozoa = 0.4 h^{-1}, K_s protozoa = 100 mg l^{-1}, Y bacteria = Y protozoa = 0.5, S_0 = 10,000 mg l^{-1}, residence time 4.4 h).