Appendix 2. Methods of Analysis for Samples of Splendid Feather Moss (*Hylocomium splendens*) for Trace Metals, Sulfur, and Nitrogen

Drying and Grinding Samples

At the laboratory, air-dried samples were first passed through a stainless steel grinder with a 20-mesh sieve and mixed thoroughly. Ground samples were dried at 65 degrees Celsius (°C) for 2 hours and cooled in a desiccator before weighing for analysis.

Total Sulfur

Total sulfur (Tot–S) was determined by combusting 150 milligrams (mg) of sample in a ceramic boat after mixing with Com-Cat Accelerator (LECO trade name for tungsten oxide compound), in an oxygen atmosphere at 1,290 °C in a LECO Sulfur Determinator, Model No. S144–DR. The sulfur dioxide (SO_2) evolved from the sample was determined by a nondispersive infrared detector empirically calibrated with LECO plant reference materials (LECO Corporation, 3000 Lakeview Dr., St. Joseph, Michigan, 49085). The determination limit was 0.01 percent dry weight.

Total Nitrogen

Total nitrogen (Tot–N) was determined by combustion. A standard reference material for nitrogen (N) included NIST 1575 Pine Needles or other similar material having a certified concentration of Tot–N. Total Kjeldahl N was determined by converting the various forms of N to ammonium (NH_4^+), measuring NH_4^+ concentration, and subtracting the weight of hydrogen. To accomplish this, 0.150 grams (g) of dry, ground plant material was digested in 3.5 milliliters (mL) concentrated sulfuric acid (H_2SO_4) with 1.5 mg potassium sulfate (K_2SO_4) and 7.5 mg selenium. This mixture was placed in an electrically heated aluminum block at 400ºC and digested for 1 hour. The NH_4^+ formed was reacted with salicylate in the presence of hypochlorite and nitro-prusside to form an emerald-green complex. Color intensity was measured spectrophotometrically on a Technicon AutoAnalyzer at 660 nanometers (nm). The method converts only partial amounts of nitrate (NO_3^-); thus, samples containing high concentrations of nitrates were pretreated with salicylic acid to ensure complete conversion.

The combustion method for total nitrogen used a LECO FP–528 Nitrogen Analyzer. A 150–500 mg sample was weighed into a gel capsule and dropped into an 850 °C furnace purged with oxygen gas (O_2). The combustion products (carbon dioxide [CO_2], water [H_2O] and nitrates), were filtered, cooled by a thermoelectric cooler to condense most of the water, and collected into large ballast. A 3 cubic centimeter (cm^3) aliquot of the ballast combustion product was integrated into a helium carrier stream. The stream first passes through a hot copper column to remove O_2 and convert nitrates to nitrogen gas (N_2). A reagent tube then scrubs the remaining CO_2 and H_2O from the stream. The N_2 content was measured by a thermal conductivity cell against a helium background and the result was displayed as a weight percentage of N. Determination limits for Tot–N and total Kjedahl N were 0.01 percent dry weight.

Nitrate as Nitrogen

Nitrates were extracted by shaking 100–200 mg of dried plant material with 30 mL of 2 percent acetic acid solution for 30 minutes. About 0.85 cm^3 of prewashed charcoal was added to each sample and shaking was done for 5 additional minutes. Samples were centrifuged or filtered through a Whatman No. 42 filter paper. Nitrate concentrations in the filtrate were determined colorimetrically by the cadmium reduction method. With this method, nitrates were reduced to nitrites in a copperized cadmium column. The nitrite ions reacted with sulfanilamide under acidic conditions to form a diazo compound, which couples with N–1–Napthylethylenediamine dihydrochloride to form a reddish purple azo dye. The resultant dye was quantified by measurement at 520 nm on an Alpkem Rapid Flow Analyzer.

Aluminum, Boron, Cadmium, Calcium, Chromium, Copper, Iron, Lead, Magnesium, Manganese, Nickel, Phosphorus, Potassium, Sodium, and Zinc

These elements were determined using simultaneous inductively coupled plasma-atomic emission spectrophotometry. Reporting limits are listed in table 2–1. For this analysis, a 0.25–0.50 g sample of dried plant material was digested with 2 mL hydrogen peroxide (H_2O_2) and 0.5 mL trace metal grade nitric acid (HNO_3) in a microwave digestion vessel for 4 minutes at 296 watts and 8 minutes at 565 watts. Analysis of the digestate for phosphorus, potassium, calcium, magnesium, sodium, aluminum, iron, manganese, zinc, copper, boron, lead, nickel, chromium, and cadmium was completed with inductively coupled plasma-atomic emission spectrophotometry. The wet digest procedure is a partial digestion of the sample that is designed to solubilize the less refractory components of plant material. Silicate matrices that may be present as contaminants of the sample are minimally solubilized by this method.

Table 2–1. Target determination limits for analysis of moss or lichen tissues by inductively coupled plasma atomic emission spectrophotometry (ICP-AES).

[µg/L, microgram per liter; µg/g, microgram per gram; nm, nanometer; ICP–AES, inductively coupled plasma-atomic emission spectrophotometry]

Element	Reporting limit (µg/L)	Determination limit (µg/g dry weight)	Analytical method	Wavelength (nm)
Aluminum	180	3.6	ICP–AES	308.215
Arsenic	40	0.78	ICP–AES	193.696
Boron	24	0.46	ICP–AES	249.773
Barium	7	0.12	ICP–AES	455.403
Beryllium	3	0.04	ICP–AES	313.042
Calcium	42	4.36	ICP–AES	317.933
Cadmium	7	0.12	ICP–AES	226.502
Cobalt	13	0.24	ICP–AES	228.616
Chromium	15	0.28	ICP–AES	205.552
Copper	27	0.52	ICP–AES	324.754
Iron	18	0.96	ICP–AES	259.94
Potassium	708	14	ICP–AES	766.491
Lithium	21	0.4	ICP–AES	670.781
Magnesium	191	3.8	ICP–AES	279.079
Manganese	4	0.06	ICP–AES	257.61
Molybdenum	12	0.22	ICP–AES	202.03
Sodium	181	3.6	ICP–AES	588.995
Nickel	23	0.44	ICP–AES	231.604
Phosphorus	36	0.7	ICP–AES	214.914
Lead	85	1.7	ICP–AES	220.353
Rubidium	2,650	53	ICP–AES	780.02
Silicon	85	1	ICP–AES	251.611
Strontium	4	0.06	ICP–AES	421.552
Titanium	16	0.3	ICP–AES	334.941
Vanadium	19	0.36	ICP–AES	292.402
Zinc	8	0.4	ICP–AES	213.856

Reporting limits are based on the concept of the Lowest Quantitatively Determinable Concentration (LQDC) and are five times the instrument detection limit. Precision at the LQDC is about plus or minus 10 percent and analytical results are quantitative. The instrument detection limit is 2 times the standard deviation of eleven replicates of a reagent water sample.

Wet Digest Inductively Coupled Plasma—Coaxial Analysis of Cadmium, Chromium, Copper, and Lead

Coaxial analysis (Perkin Elmer Optima 3000 DV inductively coupled plasma-atomic emission spectrophotometry set in axial mode) was used to analyze the wet digests to obtain lower detection limits for cadmium, chromium, copper, and lead.

Ash Yield

The percentage ash yield was determined by combusting 0.5 to 1.0 g of the oven-dried sample at 485 °C for 12 hours in a circulating air muffle furnace. The determination limit was about 0.5 percent by dry weight.

Quality Assurance and Quality Control of Reference Materials and Blanks

Quality-assurance samples included the following analyses: calibration checks, blanks, duplicates, spikes, and at least one standard reference plant tissue sample. At least one quality-assurance sample was run after every 10 unknowns.