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Differences in Chemical Composition of Plant Sample Fractions Resulting from Grinding and Screening[1]

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ABSTRACT

Alfalfa, barley straw, corn leaves, peas, red clover, and wheat straw samples were ground in a Wiley mill and screened to separate particle sizes. Particle size samples were analyzed for N, P, K, Ca, Mg, Zn, Se, and SiO_2 The percentage composition of N, P, Ca, Mg, and SiO_2 generally increased with decreasing particle sizes of three wheat straw varieties, one baled and one hand-cut sample each of barley straw and alfalfa, corn leaves, red clover, and peas. The K concentrations were unpredictable. Selenium tended to be more concentrated in the smaller than in larger particles. The variability in chemical composition because of particle sizes points out the necessity for adequate sample grinding and mixing for obtaining representative subsamples for chemical analyses and biological experiments.

Additional index words: **nitrogen, phosphorus, potassium, calcium, magnesium, silica, selenium, zinc, particle size.**

THE accuracy of plant material chemical analyses depends in part upon how well a subsample represents the entire plant sample. A degree of inaccuracy is probably introduced into many plant material analyses because of unrepresentative subsamples. This inaccuracy could account for much of the variability encountered in analyses such as total nitrogen by a Kjeldahl method. Plant material composition problems may also be found in biological experiments in which plant materials are added to soil, such as in decomposition or nitrification rate studies. The senior author became aware of these problems through previous research (9) involving alfalfa decomposition and nitrification in soil. A high degree of reproducibility was obtained in biological experiments using the alfalfa after characterizing the alfalfa by particle size and consequently by N concentration.

Many reports have dealt incidently with sample preparation and the precautions that are necessary. Piper (8) recommends that where 1- to 2-g samples are taken for analysis, sieves with round holes 0.5 mm diameter should be used. He also gives a short discussion on plant sample handling and prevention of contamination. Johnson (4) reported that dust must be removed by washing plant samples before accurate microelement analyses can be obtained. No reports were found that dealt with chemical composition of plant samples as influenced by grinding and particle size separations.

Differences in N content of alfalfa samples by particle size have been observed to range from 100 to several hundred percent. These observations led to investigations to determine the elemental composition of several plant species and the influence of plant material particle sizes on elemental composition.

METHODS AND MATERIALS

Alfalfa *(Mericago sativa)*, barley straw *(Hordeum vulgare)*, 'Lemhi' wheat straw *(Triticum aestivum)*, University of Idaho Experimental wheat straw 59-10320 *(Triticum aestivum)*, and peas *(Pisum sativum)*, were all sampled by hand-cutting from fields near Kimberly, Idaho. The alfalfa was cut very young and the peas were cut at early bloom. Another barley straw and an alfalfa sample were obtained from baled straw and hay stacks in the same area. 'Gaines' wheat straw *(Triticum aestivum)* was obtained from plots at Aberdeen, Idaho. Sweet corn leaf samples *(Zea mays)* and red clover *(Trifolium pratense)* samples were cut by hand near Kimberly, Idaho.

The alfalfa, barley straw, and Gaines wheat straw samples analyzed and reported in Table 1 were ground in a Wiley mill through a 2-mm stainless steel screen and sieved through stainless steel screens to give particle sizes of greater than 841 μ (20-mesh), 841 to 500 μ (20 to 32-mesh), 500 to 250 μ (32 to 60-mesh), 250 to 149 μ (60 to 100-mesh), and smaller than 149 μ (100-mesh). The fraction weights of these three materials were determined. All other samples were ground in a Wiley mill through an 841 μ (20-mesh) brass screen and separated into the same particle sizes as indiciated for the first-mentioned samples. The samples were not washed before drying and grinding.

Nitrogen analyses were made by a modified Kjeldahl procedure (2). Phosphorus was determined by the molybdate vanadate method reported by Chapman and Pratt (3). Potassium was determined on a flame photometer. Calcium, magnesium, and zinc were determined on an atomic absorption spectrometer (7)[3]. Selenium was determined by fluorimetry using a method described by Allaway and Cary (1).

Silica was determined colorimetrically by a method described by Jones and Handreck (5) and modified to use oxalic acid for eliminating interference from phosphorus (10).

The limits of variability for the various methods employed are about as follows: nitrogen, phosphorus, and potassium $\pm 2\%$; calcium, magnesium, and zinc $\pm 5\%$; selenium ± 10 ppb in the range 20 to 300 ppb; and silica $\pm 10\%$.

RESULTS

The fractional percentages and the N, P, K, Ca, Mg, Zn, Se, and SiO_2 contents for each particle size of barley straw, Gaines wheat straw, and alfalfa are given in Table 1. The greater than 841μ particles for the

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Table 1. Particle size distribution and elemental composition of barley straw, Gaines wheat straw, and alfalfa.

Particle size, microns	Fraction, %	N, %	P, %	K, %	Ca, %	Mg, %	Zn, ppm	Se, ppb	SiO_2, %
				Barley straw					
> 841	7.47	.19	.058	2.24	.092	.097	102	29	0.73
841-500	46.01	.27	.083	2.24	.155	.115	61	39	1.22
500-250	29.80	.41	.112	2.02	.155	.160	62	38	2.29
250-149	10.08	.52	.128	1.67	.200	.177	21	58	3.37
< 149	6.64	.67	.160	1.95	.512	.260	16	73	4.50
Weighted mean		.36	.099	2.10	.179	.143	57	42	1.94
				Gaines wheat straw					
> 841	4.28	.21	.042	1.14	.075	.053	15	27	2.00
841-500	51.43	.20	.035	.84	.070	.070	15	32	2.74
500-250	27.50	.23	.042	.94	.075	.076	10	27	3.02
250-149	10.38	.25	.035	.80	.092	.095	10	28	3.89
< 149	6.41	.34	.064	1.09	.118	.120	12	40	4.66
Weighted mean		.23	.039	.89	.077	.077	13	30	3.03
				Alfalfa					
> 841	1.73	1.86	.187	1.54	1.150	.245	26	35	0.13
841-500	33.14	1.66	.184	1.42	1.380	.272	28	36	0.29
500-250	40.13	2.09	.186	1.54	1.660	.285	21	43	0.36
250-149	14.01	2.68	.213	1.42	2.000	.375	21	58	1.00
< 149	10.99	2.18	.198	1.20	1.740	.400	27	58	4.50
Weighted mean		2.04	.190	1.47	1.623	.305	24	44	0.88

two straw samples represented a fairly small fraction of the entire sample, with the major part of the straw samples being found in the 841 to 500μ range. As particle sizes decreased below 500μ, the percentage of the total sample in each fraction also decreased, but the actual amount of material found in the very small particle sizes is rather surprising, with 6 to 11% in the smaller than 149μ size. The same particle size distribution was found for alfalfa except the 500 to 250μ fraction contained the largest percentage (40%) of the total.

The N, P, Ca, Mg, and SiO_2 percentages of the three plant materials increased with decreasing particle sizes. Some of these increases were comparatively small. For instance, the P content of alfalfa increased about 1.4 fold, and was almost constant for the three larger particle sizes. Other increases were rather large. For instance, the Ca content of barley straw increased from 0.092 to 0.512% or about 5.6 fold, and the SiO_2 in the alfalfa increased from 0.3 to 4.50% for a 35-fold increase. Most of this latter increase came between the 250 to 149μ and the $< 149\mu$ particle sizes. Other increases in component composition percentages with decreasing particle size were intermediate between those indicated.

The K concentration was variable and unpredictable in all the plant materials. This may be understandable because K does not combine chemically in the plant. In the barley straw there was a dramatic decline in Zn percentage composition with decrease in particle size. The other two plant materials showed a slight decrease, or decrease and then an increase in the smaller particles, but differences were relatively small. Barley straw contained more Zn in the larger sizes than did the other two plant materials. In the barley and wheat straw samples the smallest particle sizes contained a greater Se concentration than the larger particle sizes. The concentrations of Se were so low that the precision of about $\pm$ 10 ppb left some question as to actual differences between fraction sizes in some cases.

Weighted means were determined for the elemental percentages of the three plant materials reported in Table 1. These values are thought to be the most representative chemical composition values that are available for the entire plant sample. Comparison of the particle size fraction composition with that of the

Table 2. Chemical composition of several plant materials by particle size.

Particle size, microns	N, %	P, %	K, %	Ca, %	Mg, %	Se, ppb	SiO_2, %
			Wheat straw 59-10320				
841-500	.61	.100	1.87	.100	.058	24	1.12
500-250	.71	.120	1.82	.122	.080	28	1.44
250-149	.81	.120	1.69	.140	.096	25	2.08
< 149	1.00	.160	1.82	.205	.115	25	2.58
			Lemhi wheat straw				
500-250	.24	.060	.31	.072	.030	24	1.47
250-149	.24	.060	.27	.072	.025	28	3.11
< 149	.32	.080	.41	.110	.055	35	3.64
			Barley straw				
841-500	1.00	.080	1.05	.158	.055	32	.95
500-250	1.27	.180	.94	.325	.255	28	2.06
250-125	1.78	.240	.94	.580	.326	45	3.06
< 125	2.10	.280	.90	.690	.392	86	4.21
			Corn leaves Codo 20				
500-250	1.99	.040	.66	.715	.620	70	2.82
250-149	2.01	.039	.47	.750	.605	22	3.06
< 149	2.62	.446	.49	.720	.942	12	2.96
			Red clover				
500-250	2.20	.290	1.40	.905	.274	45	.15
250-149	2.81	.356	1.54	1.068	.290	56	.08
< 149	2.82	.360	1.50	1.045	.322	87	.71
			Peas				
500-250	2.88	.524	.33	.632	.240	41	.10
250-149	3.24	.444	.55	.910	.295	41	.10
< 149	3.36	.550	.61	.920	.320	42	.20
			Alfalfa				
500-250	3.16	.530	1.74	1.100	.190	33	.08
250-149	3.85	.584	1.87	1.410	.195	33	.06
< 149	4.03	.470	1.79	1.400	.220	51	.22

weighted mean should give some indication of the possible deviation from true values that can be obtained when separation of the sample takes place during handling.

Table 2 shows the percentage composition of N, P, K, Ca, Mg, Se, and SiO_2 in two wheat straw varieties, samples of hand-cut barley straw, sweet corn leaves, red clover, peas, and very young alfalfa, by particle sizes. As before, there was an increase in percentage composition of N, P, Ca, Mg, and SiO_2 with decreasing particle size for all plant materials. There was no predictable trend for K concentration. The Se concentrations in the experimental wheat straw and the peas remained almost constant with particle size of the plant material. Selenium concentration increased with decreasing particle sizes of alfalfa, red clover, barley straw, and Lemhi wheat straw, but the reverse was true with corn leaves.

DISCUSSION

The percentage composition of N, P, Ca, Mg, and SiO_2 generally increased with decreasing particle sizes of three wheat straw varieties, one baled and one hand-cut barley straw and alfalfa samples, corn leaves, red clover, and peas. Differences in elemental composition as particle size decreased ranged from about a 1.4-fold increase in P percentage to about a 35-fold increase for SiO_2 percentage of one alfalfa sample. Both of these extremes were in the baled alfalfa sample. The elements that increase in percentage composition in the smaller particle sizes are concentrated in the more active plant parts. For example, N and P are constituents of amino acids, proteins, enzymes, coenzymes, and nucleic materials which are concentrated in the leaves and other succulent plant tissues. Surprising as it may seem, Si is also concentrated in much higher amounts in leaves around cell perimeters than in nodes or internodes of barley plants. Jones and Handreck (6) reported that the leaf of barley con-

tained 3 times as much Si as the internode and roots, 14 times as much as the node, and 40 times as much as the seed.

When the succulent leaf parts of plants are ground in a mill, they pulverize into small particles regardless of the screen size. The structural components of the plants, such as stems and ribs, are tough and woody in comparison, and are only reduced in size as the blades cut them small enough to pass through the screen. These tougher parts do no contain the high concentrations of such elements as N, P, Ca, Mg, and SiO_2 found in more active plant parts.

Potassium was unpredictable in the differences in concentration that were observed. There were some increases and some decreases in K concentration with changing particle sizes but no patterns were evident. The observed differences were generally small. The lack of trends in K concentration changes with particle sizes was probably because K is not chemically bound in cell constituents and, therefore, does not form a permanent part of the plant constituents as is the case with other elements.

The high SiO_2 concentration in the cereals contrasts with the low concentration in leguminous plants, indicating that cereals absorb more SiO_2 than legumes. The very high SiO_2 content of the fine fraction of one alfalfa sample is an interesting exception.

It is not surprising that Se tended to be more concentrated in the smaller plant material particles because most Se in plants is combined in amino acids. As is the case with N, P, Ca, Mg, and SiO_2, Se concentration is greatest in the more active or succulent plant parts. Se and Si are not known to be required by these plants for normal growth. Yet these two elements are most concentrated in the active plant parts along with elements that we know are required for plant nutrition. The reason Se concentration decreased with particle size, in the case of corn leaves, is not known.

The wide variability in chemical composition of the different plant materials and their different particle size fractions points out the necessity for careful sample preparation such as fine grinding or very thorough mixing before representative subsamples can be obtained for chemical analyses or biological experiments.

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