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USER PERSPECTIVE ON LABORATORY ANALYSIS AND IT'S RELIABILITY

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Abstract. **Agricultural producers and the fertilizer industry in the Southeast invest millions annually on soil and leaf testing. The results are used for diagnostics to determine nutrient inputs or to justify nutritional needs in response to government regulations. Full utilization of the results has been finite due to differences in analytical procedures used and limited understanding of the significance of the numbers. In addition the result reliability of leaf and soil testing laboratories have been often questioned. A survey of five laboratories in 1995 and six in 1996 showed them to differ from each other a minimum of 7.7% (Mg) and maximum of 50.4% (Fe) when the deviation was calculated as average of all samples. Reported concentrations for nitrogen in leaf tissue varied from 0.79% to 2.1% for the lowest and 3.27% to 3.54% for the highest sample. Average cumulative error for all samples and all elements combined varied from 4.6% to 20.0% in 1995 and from 5.6% to 18.1% in 1996. Given the variation found and the potential of the results being used to comply with governmental regulation use of unified procedures and regulation of QA/QC may be necessary to assure sufficient accuracy.**

An analytical report from a soil and leaf testing laboratory is one of the primary tools growers use to evaluate success or failure of a citrus fertilization program. The composition and amount of fertilizer to be applied can be guided by leaf and soil analysis, with an attempt on the part of the grower to respond to plant needs as well as to achieve projected crop goals. The reports are used for diagnostic purposes such as determination of deficiency or toxicity, or to determine nutrient application responses. Governmental agencies seek to limit nutrient applications using leaf and soil analysis reports as basis to allocate nutritional inputs.

Soil testing is often used as a primary tool to determine fertilizer application requirements, so testing methods attempt to estimate what and how much is available for plant uptake over time. Since there are vast differences in soils and plants, several nutrient extraction procedures have been developed that attempt to estimate the availability of nutrients over a period of time. For example, such criteria as pH, soil structure and physiochemical characteristics of nutrient-soil interaction have been used to develop extraction procedures. Some of the commonly used methods include Ammonium acetate extraction at pH 7.0 or 4.8, Mehlich 1 (also called double-acid), Bray P and Mehlich III extractions. Subjecting one sample to all mentioned processes will lead to a set of results all differing from each other. Furthermore, it has been shown that conversion of results from one method to another is often not only difficult, but is also unreliable in part due to differences in chemical behavior of the soils and extraction chemicals.

Leaf analysis is useful only when sufficient data exist to correlate plant part, time of sampling and yield to concentration values in the tissue. The analytical methods for leaf tissue testing after drying and grinding of the sample generally follow two pathways: A) ashing + acid dissolution for elemental analysis, and B) acid digestion and analysis for nitrogen. Consequently, leaf analysis results are less likely to vary due to applied sample preparation procedure compared to soil analysis results. For this reason, we selected leaf tissue testing as the tool to evaluate the performance of several laboratories.

Materials and Methods

Eight (June 1995) and six (June 1996) leaf samples were sent to five (1995) and six (1996) Southeastern agricultural testing laboratories for leaf analysis. In both years the leaf samples were collected from areas where significant concentration differences were anticipated due to differential nutritional treatments. Each sample was dried, ground, mixed and split equally between all laboratories.

In 1995 one of the eight samples sent for analysis was a standard check sample from the University of Florida Institute of Food and Agricultural Sciences South West Research and Education Center soils laboratory which was analyzed for nitrogen 40 times with concentration of 1.43+-0.14%. The rest of the samples were freshly-sampled citrus leaves from areas under differing nutritional treatments.

Each of the six samples in 1996 were split into eight equal aliquots. This allowed a) sending of six separate samples to each of the six laboratories and b) utilization of one sample per laboratory as replicated sample. Initially five samples were sent to all selected laboratories. Sample #6 and the second replicate sample were sent a week later followed by one last replicate sample shipment seven days after that. Thus, each laboratory received three shipments with five, two and one samples correspondingly.

The overall performances of the laboratories were compared using four different approaches.

1. Comparison of percent deviation from best value including cumulative error.
2. Plotting of the results against each other using generally accepted citrus leaf concentration ranges.
3. Comparison of individual nitrogen values, relating magnitude of deviation and variation.
4. Assessment of the over all performance by evaluating the magnitude of the 95% confidence interval.

To evaluate the laboratories based on percent deviation from best value average percent deviation (AD) per element, cumulative average deviation (CAD) per sample per element and cumulative error (CE) were calculated based on element specific best values (BV) per sample.

Determination of a true value or a value that most probably is closest to it was difficult. This was well illustrated with the nitrogen readings where BV>Avg except in sample 8 in 1995 and BV< Avg in 1996. This is because the average N concentrations were strongly influenced by lab B in 1995 and lab E in 1996 (Table 3.). Therefore, for the purposes of comparison with an attempt to min-

imize such factors as skewness of data and contribution of outliers best value BV was determined as:

$$BV_i = \frac{Sum[Element_i]_n - [Element_i]_{nMax} - [Element_i]_{nMin}}{(n-2)}$$

Where

$[Element_i]$ = Conc. of element i in sample n
$[Element_i]_{nMax}$ = Highest conc. of element i in sample n
$[Element_i]_{nMin}$ = Lowest conc. of element i in sample n

and

AD = (Sum(($lab_j Element_i/BV_i$) x 100 - 100)/x
where x = number of laboratories

and

CAD = Sum(AD_i)
CE = Sum $|AD_i|$

Comparison of the results based on generally accepted leaf tissue ranges was accomplished by assigning numerical values for each of the concentration ranges shown in Table 1. The high optimum range was assigned value +0.5 and the low -0.5 to give 1.0 unit differential. Each successive range thereafter was assigned a value increasing/decreasing by 1.0 units. The comparison of all samples were made by accumulating assigned element specific range values in each sample for 1995 and 1996 and graphing. Only Nitrogen results are reported separately sample by sample.

Sending a set of samples to a laboratory gives only a snap shot evaluation of the laboratory performance, which may not fully reflect the reliability of the laboratory due to short-term equipment failure, erroneous calibration etc. To investigate the repeatability of results over time a triplicate sample (1996) was sent to each laboratory. Each of the six samples were replicated with a specific laboratory and shipping was arraigned so that minimum of a one week interval was maintained in between shipments.

The goodness of data can be also assessed by comparing the magnitude of the 95% confidence interval to the magnitude of the result average. Assuming standard distribution and using standard deviation (STD) and its multiples it has been shown that 95.45% of data falls within AVG +-2xSTD. Hence the magnitude of the range 2×STD compared to the average gives an insight of general result reliability and the performance of each laboratory.

Results

Using 10% or less deviation from BV_i as criteria for acceptable performance and counting the number of incidence per laboratory when AD exceeded the limits allows a comparison of average performance to be made. For laboratory E, the average deviation (AD) exceeded or was equal to BV_i +-10% for eight out of 10 elements in 1995 and six out of 10 in 1996. Laboratories A, B, C and D exceeded the limit 0, 2, 3 and 0 times out of 10 in 1995 and 2, 6, 5 and 3 out of 10 in 1996 correspondingly. The sixth laboratory included only in 1996 had AD of 5 of the 10 elements to exceed or equal to BV_i +-10%.

Cumulative analysis showed that the major analytical discrepancies were found with the micronutrients Fe, Mn, Zn, Cu and B (Fig. 1.). In 1995 the agreement between laboratories excluding Lab. E regarding macro nutrients was quite good. In 1996 the variability in test results with regards to N, P, K, Ca and Mg increased partially due to inclusion of laboratory F which with laboratory E contributed to end result with strong variability from that of BV for most elements. Average and cumulative performances for 1995 and 1996 are summarized in Fig. 1.

To determine whether any of the laboratories had a tendency to error below or above the BV_i, cumulative average deviations (CAD) were calculated. To estimate the expected average variation per element for each laboratory, Cumulative Index (CI) was calculated as CI = CE/(#Elements analyzed * #samples). Both CAD and CI are shown in Table 2.

In 1995 and again in 1996 the results showed consistent trend in variation from the BV_i. However, the magnitude of deviation was much smaller in 1996 than in 1995. The expected error calculated as CI shows that laboratories A, B and C formed rather homogenous group where CI:s were about 5% and standard deviations varied on both sides of 3.5. Laboratories D, E and F had corresponding CI values up higher at 13% to 18% with standard deviations near 10.

Table 1. Generally recognized leaf concentration ranges for Citrus. Concentration windows and corresponding indicator values used to compare laboratory results.

		Leaf Concentration Ranges									
		N%	P%	K%	Ca%	Mg%	FE (ppm)	Mn (ppm)	Zn (ppm)	B (ppm)	Cu (ppm)
Excessive		>3.2	>0.3	>2.4	>7.0	>0.8	>250	>1000	>300	>200	>25
Optimum		2.4-2.9	.12-1.8	1.2-1.7	3.0-5.5	0.3-0.6	60-120	25-200	25-200	40-150	5-16
Deficient		<2.2	<0.08	<0.7	<1.5	<0.2	<35	<17	<17	<20	<4
	Assigned value	Concentration windows used for laboratory evaluation.*									
Excessive	+3.5	3.2	0.3	2.4	7	0.8	250	1000	300	200	25
High	+2.5	3.05	0.21	2.05	6.25	0.7	185	600	250	175	20.5
M High	+1.5	2.9	0.16	1.7	5.5	0.6	120	200	200	150	16
H Op	+0.5	2.65	0.14	1.45	4.25	0.45	90	112.5	112.5	95	10.5
L Op	-0.5	2.4	0.12	1.2	3	0.3	60	25	25	40	5
M Low	-1.5	2.3	0.1	0.95	2.25	0.1	45.5	21	21	30	4.5
Low	-2.5	2.2	0.08	0.7	1.5	0.2	35	17	17	20	4
Deficient	-3.5	<2.2	<0.08	<0.7	<1.5	<0.2	<35	<17	<17	<20	<4

*The lowest value for each range is shown in the table.

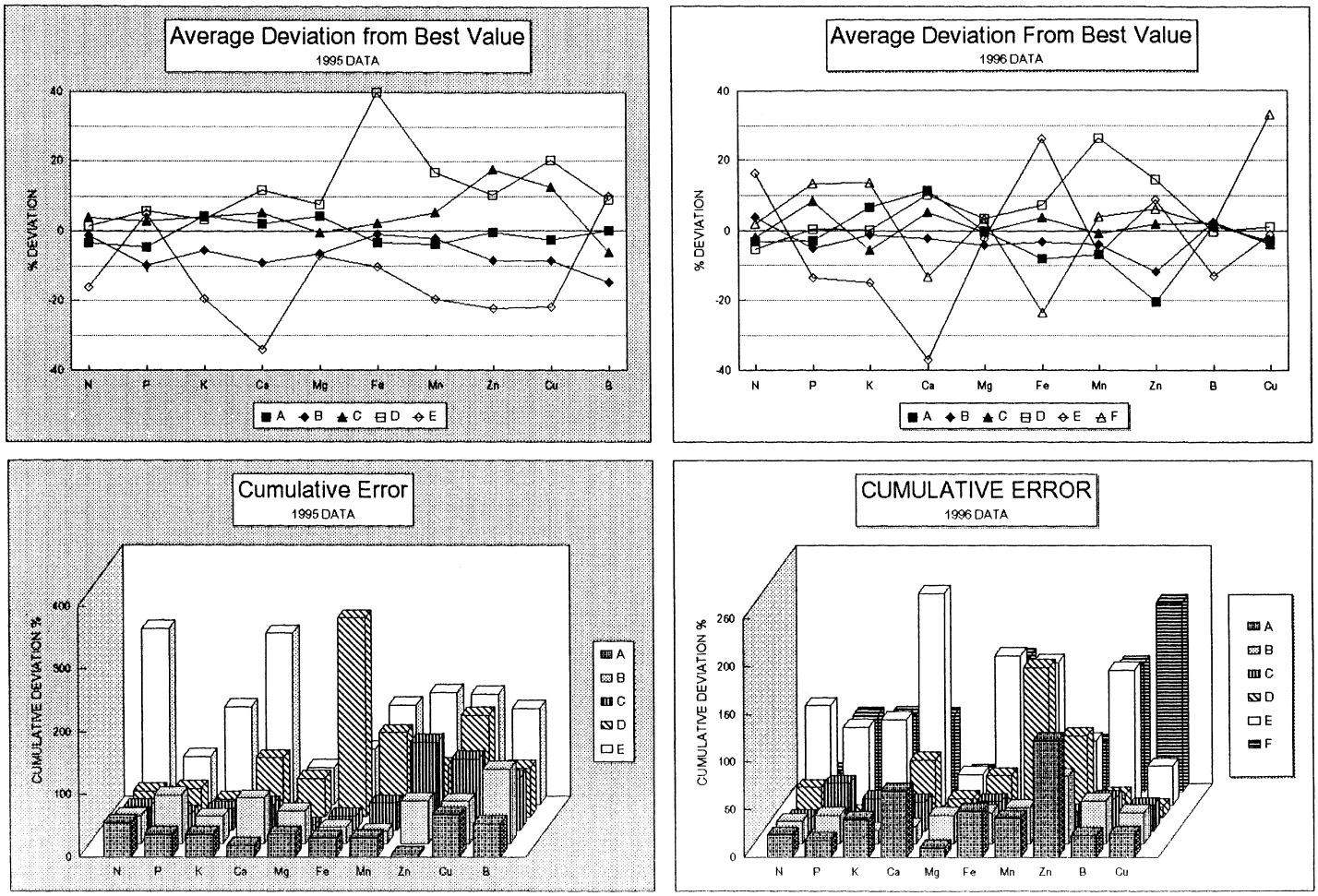

Figure 1. Average Deviation and Cumulative Error per Element. Average Deviation from BV is a result of eight samples in 1995 and six in 1996. Cumulative Deviation was calculated as sum of absolute deviations from BV.

Categorizing the results into concentration windows as shown in Table 1 resulted in most cases to single range variation between laboratories (data not shown). Beside nitrogen, the largest difference was found in 1996 with zinc and calcium in two samples where variation ranged from low optimum to deficient and high optimum to deficient respectively. In 1995 the largest difference between laboratories was found with iron and the other with calcium with variation from low optimum to low. Cumulatively laboratory E systematically showed extreme readings in 1995 and was the highest in 1996 except for sample 6.

The nitrogen values varied significantly from laboratory to laboratory. Table 3 shows individual results by sample and Fig. 2 illustrates the variability evaluated by range and percent deviation. The largest deviation difference occurred in 1995 where the lowest value was less than 50% of the highest. The smallest difference of 7.89% between the highest and lowest reported value occurred when N concentration was 3.41%, a high value. With normal N values the variability increased to 10% to 15% when laboratory B in 1995 and E in 1996 were omitted. In comparison the difference between IFAS recommended optimum and deficient leaf sample for nitrogen is only 8.33%.

The results from sending replicate samples to each of the laboratories is summarized in Fig. 3 and Table 4 showing replicate to replicate, element to element average variation to ranging from 1.76% to 11.62%. The corresponding standard deviations for the variability were 1.47 and 19.6.

The coefficient of variation (CV), which is calculated by dividing standard deviation by the mean, allows data sets of different magnitude to be compared providing yet another tool to assess the reliability of the data. In 1995 the highest CV for reported nitrogen values was 34.7% and the lowest was 7.04%. In 1996 the corresponding results were 17.1% and 3.7%. In Table 3 the concentration values for upper and lower confidence limits at 95% probability are shown. The widest difference spanned a total of 2.26 concentration units reaching from concentration of 1.40% to 3.69% for S3 in 1995. The best was 0.49 concentration units ranging from 3.15% to 3.64% for T5 in 1996 For elements other than N (data not shown) the magnitude of 95% confidence interval exceeded 10% in 68 and 20% in 43 cases out of 72 in 1995 and 51 and 38 cases out of 53 in 1996 correspondingly.

Discussion

This study demonstrates the leaf testing to have 59.72% (1995) and 74.51% (1996) of the data exceeding +-20% variation with 95% confidence intervals. The observed large variation could be due to 1) internal sample variability and 2) laboratory QC/QA.This study cannot account the full contribution of sample variability. However, the sample contribution is necessarily <<5% based on the replicated analysis of the same sample which resulted in average variability of less than 5% for three of the tested laboratories, with the lowest average variation of 1.78%. Consequently, the sample homogeneity can be assumed to be better than 95% and po-

Table 2. Direction and Tendency of Deviation and Cumulative Index per Element.

	CAD		Cumulative Index			
	1995	1996	1995	STD	1996	STD
A	-6.31	-25.18	4.58	2.18	6.98	5.27
B	-66.92	-29.66	7.39	3.48	5.63	2.53
C	+47.77	+8.14	6.97	5.09	4.65	1.71
D	+127.27	+57.93	13.19	10.17	7.61	7.42
E	-136.00	-37.20	19.97	8.87	18.13	9.32
F		+39.52			14.53	8.91

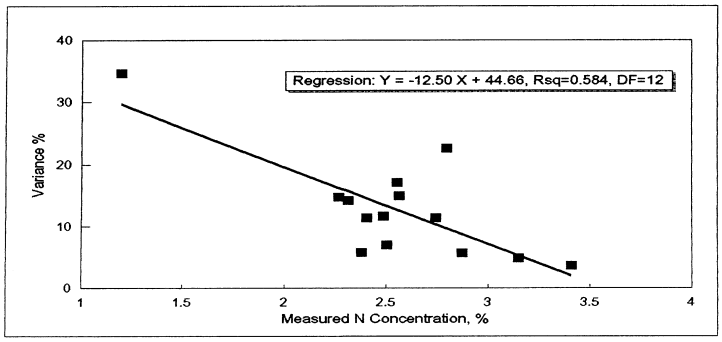

Figure 4. Correlation Between N Concentration and Variance.

ample, laboratory and year.

1995 Data						
S2	S3	S4	S5	S6	S7	S8
2.31	2.77	2.11	2.49	2.29	2.35	1.4
1.60	1.40	1.60	1.70	1.80	2.20	2.1
2.42	2.78	2.45	2.66	2.44	2.60	1.02
2.46	2.92	2.49	2.57	2.49	2.56	1.16
2.21	2.84	2.25	2.63	2.50	2.67	0.79
2.31	2.80	2.27	2.56	2.41	2.50	1.19
2.20	2.54	2.18	2.41	2.30	2.48	1.29
0.31	0.57	0.32	0.36	0.26	0.17	0.45
14.20	22.56	14.72	14.93	11.41	7.04	34.70
2.82	3.69	2.82	3.13	2.83	2.82	2.19
1.58	1.40	1.54	1.69	1.78	2.13	0.40

1996 Data				
T2	T3	T4	T5	T6
2.83	2.71	2.38	3.48	2.83
2.85	2.85	2.76	3.54	3.27
2.81	2.76	2.48	3.31	3.11
2.73	2.65	2.38	3.27	2.95
3.20	3.50	3.70	3.30	3.40
3.00	2.59	2.59	3.55	3.26
2.87	2.74	2.55	3.41	3.15
2.92	2.87	2.78	3.39	3.20
0.17	0.33	0.48	0.12	0.15
5.69	11.41	17.11	3.65	4.83
3.25	3.52	3.73	3.64	3.51
2.59	2.22	1.83	3.15	2.89

)5% confidence.

tentially reaching over 98%.

The cumulative analysis from laboratory to laboratory showed substantial variation with the minor elements, as high as >50% for Fe while of the major nutrients variation climbed to >40% for Ca and =>20% for N.

For nitrogen, the result deviation between laboratories exceeded 10% for all samples in both years and range analysis showed 4 to 5 category differentials except in one occasion in 1996. It was interesting to note that there was an inverse relationship between N concentrations and variance (Fig. 4) where increasing concentration produced decreasing variance. The resulting high bias e.g. deviation from target value is indicative of poor low range sensitivity of the method.

Precision defined as repeatability of the analysis over a 4 to 6 week period seemed to be relatively good for all laboratories, being less than 6.16% for N and <10.20% for rest of the major nutrients. For the minor elements the variability was =<18.14% except for laboratory F, which reached 70% for zinc.

Wide scatter of elemental results from one laboratory to another as well as differences in the cumulative error peaks from element to element within a laboratory indicates significant problems with bias. The observed change in year to year patterns of the cumulative deviation and relatively good short term repeatability together indicate that even though the short term precision may be good, over long term the laboratories drift in regards to the precision and experience scatter in relation to bias. Hence, the accuracy, defined as low bias combined with high precision, turns out to be poor.

These leaf analysis results can only provide relative information due to observed reliability. Even though the relative user value of the results increases with the performance of the laboratory it is questionable whether data with given reliability can be used as basis for governmental regulation or related research. This is even more emphasized as the comparison of results from one laboratory to another could not be done reliably even if the data were expressed as concentration ranges rather than values.

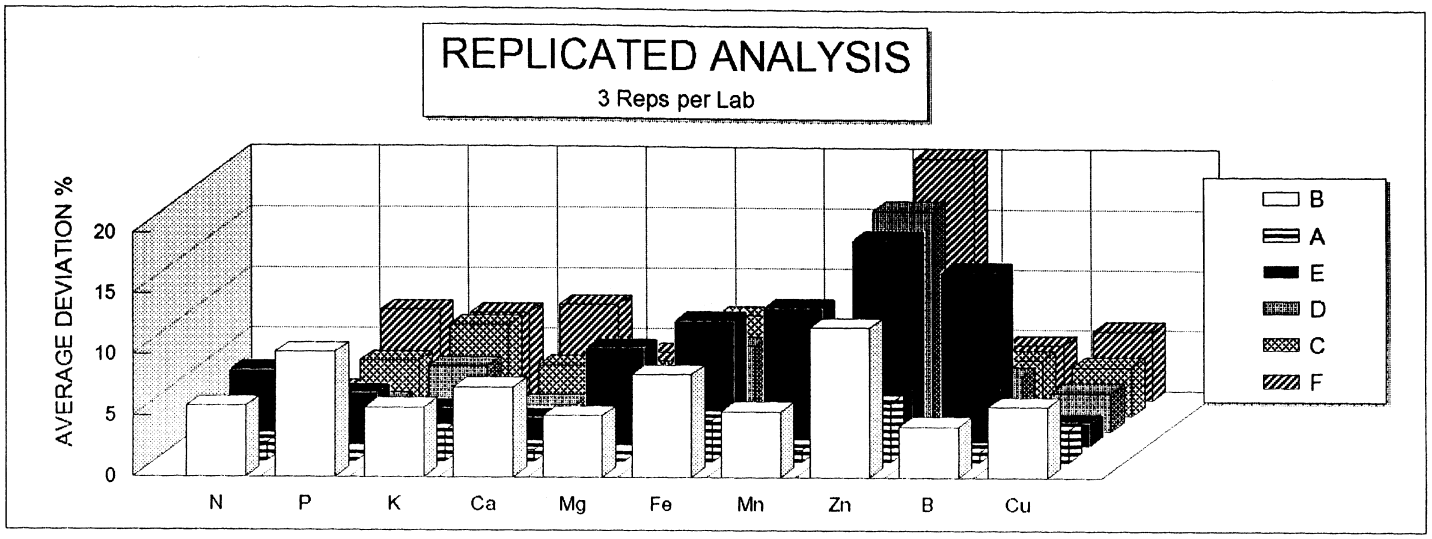

Figure 3. Average Deviation of replicated analysis.

Regardless, leaf testing is here to stay and as far as individual user is concerned decreasing deviation and increasing repeatability cater the highest value if historical data with the same laboratory exists. This in mind it is good to remember that the best of any analytical results is only as good and representative as the sample that was submitted to the laboratory.

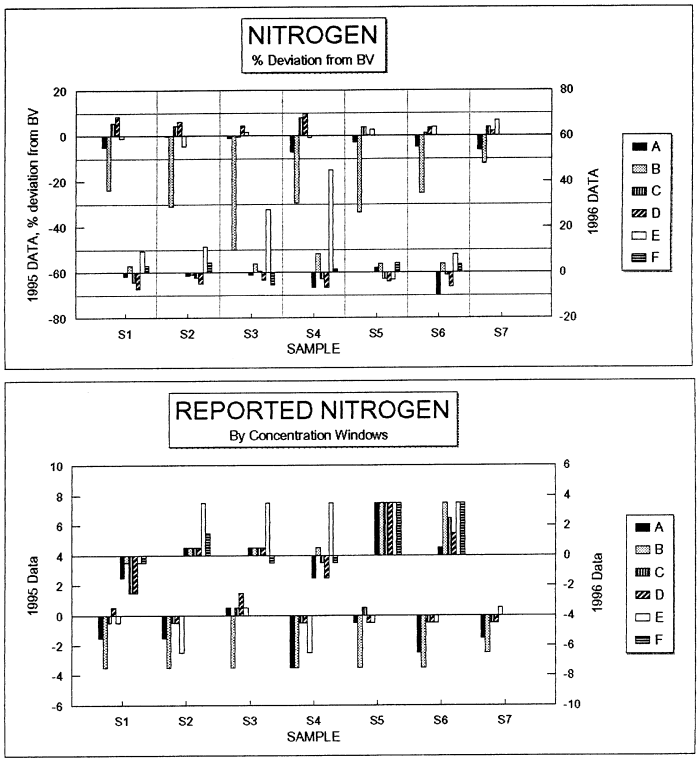

Figure 2. Variation of Reported Nitrogen Values. The variation of reported N values for 1995 and 96 is plotted parallel sample by sample. The zero line on left denotes the BV for 1995 samples and corresponding zero line on right the BV for 1996. Categorizing results into concentration windows and resulting scatter is plotted on the lower graph. The order of 1995 and 1996 data groups is reversed in the graphs.

Table 4. Replicate Sample Variation.

Lab	Cumulative*	Avg	Std	Min**	Max**
A	17.58	1.76	1.47	0.00	4.79
B	70.11	7.01	2.42	4.17	12.25
C	45.49	4.55	1.87	1.67	8.12
D	48.84	4.88	4.69	1.54	18.14
E	78.87	7.89	4.99	1.94	17.01
F	116.17	11.62	19.60	0.72	70.12

*Percent variation calculated as STD% of average.
**Element with minimum/maximum percentage of STD from the average.

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