A PRELIMINARY REPORT OF PETIOLE ANALYSIS AS AN INDICATION OF THE FERTILIZER REQUIREMENTS OF SUGAR BEETS

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At the time we were asked to prepare this paper we were pleased to be afforded this opportunity, and had outlined a number of experiments that would have made this paper more worthwhile. Due to a transfer of one of the authors to another locality during the summer, some of the experimental plots were lost and the final objectives lost or incompleted. We, therefore, wish to offer this paper only as a preliminary report in the hope that it may stimulate further experimentation by more sugar chemists.

One of us once worked with a very estimable chemist who formerly taught Chemistry in one of the better Colorado Universities, and one of his statements regarding chemists was as follows: "There are research chemists, chemists, sugar chemists and morons". We will classify ourselves somewhere in the substrata below the sugar chemist level. We have written this paper with the hope that it may benefit the average factory chemist equipped with average laboratory equipment.

During the early stages of evolution of soil sciences chemical analyses of soils furnished the chief means of soil diagnosis and the more three general systems of soil analysis practiced for a long time were total soil analysis, analysis of soil portions soluble in strong acids and analyses of dilute acid or water extracts of soils. These results were used in varying degrees to interpret the quality of soils with respect to their content of nutrients either harmful or beneficial to various types of crops.

The first two methods have given valuable information concerning chemical compositions of soils but also determine constituents not immediately soluble in soil solutions for ordinary cropping conditions. These results are therefore of little value for purposes of correlating the fertilizer requirements of soils with the analyses.

The third method named has been the most popular in latter years chiefly because of the part played by the soil solution and the easily soluble soil contents in maintaining plant growth particularly during its critical periods.

In the everlasting search for more suitable methods the Winogradsky biological method was next developed and has been commonly called the spontaneous culture method. After numerous tests and applications under practical conditions the value of this method seems to be quite limited and has been relegated to the background.

Following this, methods were developed wherein buffered solutions were used for extracting the soil. The results obtained by such methods indicated more accurately the elements available for plant growth. Various types of buffered solutions have been used and analytical methods have been developed by such eminent men as Morgan, Carolus, Thornton and Brown whose phosphate method is now used exclusively by The Great Western Sugar Company.

The aforementioned researchers then realized the possibilities and value of petiole analyses as indications of soil fertility requirements. Messrs. Gardner and Robertson were among the first to work on petioles grown on western soils.

Encouraged by the splendid results of these experiments we decided to follow up the study as it applied to our own locality. We make no claim to originality as most of our methods used are adapted with slight modifications from the methods set forth by these earlier men. Much of the past work has been carried out on plants other than sugar beets and we feel that this type of work may well be conducted by the factory chemist for the sugar beet industry.

The factory chemist is on the ground throughout the growing season and has opportunity to observe all types of cultural practices on good and poor types of farms. He also has the opportunity of sampling petioles under varying conditions of drought, moisture, hail and insect injuries as well as bacterial and virus diseases.

We know by actual observation that metabolism is affected by these conditions and that petiole analysis under such conditions is not a true measure of fertilizer requirements. Under true research conditions these adverse factors could be carefully controlled but in the practical state these factors must be considered and readily recognized in order to correlate petiole analysis with soil needs.

These studies can best be made in the field and for this reason the problem is well adapted as research for the factory chemist.

Our work on petioles was first conducted in the Lyman, Nebraska area. In this work we have assumed that healthy plants making vigorous growth on favorable soil will select the plant nutrients in concentrations most favorable to their needs. Most of the analyses reported in this paper are based on such comparisons and in our early work no attempt was made to evaluate the nutrients in terms of parts per million of plant tissue. We evaluated them in a manner similar to that used by earlier workers, i. e., comparative terms of low, medium, and high.

We tried a series of pot tests on a soil known to be deficient in phosphate and on which blackheart had definitely developed during the previous year. Seven groups of pots were set up as follows:

Group No.	Number of Pots	Treatment
1	5	100# Superphosphate per acre
2	5	200# " " "
3	5	400# " " "
4	5	200# " " "
		200# Sodium nitrate " "
5	5	200# Superphosphate " "
		6 Tons Calcium Carbonate " "
6	5	200# Sodium nitrate " "
7	20	No treatment

Seed planted on March 6th began to sprout on March 14th but due to poor conditions of temperature and light growth was slow. It was not until May 28th that the plants were large enough to sample. This first group of pot samples are referred to the color charts in the bulletin by M. F. Morgan- Number 372- "The Universal Soil Testing System"- Connecticut Agricultural Experiment Station. The terms High, Medium and Low should not be confused with the terms High, Normal and Low used in describing the field samples taken later. Even at their best the potted plants never contained the hign concentrations of the field plants. At the beginning of the experiments phosphorus was the only element determined.

On May 28th all groups were tested and all were about alike giving what we would call a medium high by the color chart. One week later all pots were again tested. Groups 1, 2, 3, 4 and 5 still gave a medium high phosphorus test. Groups 6 and 7 gave medium tests. On June 12th groups 1, 2, 3, 4, and 5 still gave medium high tests. Groups 6 and 7 had dropped to low. On June 20th groups 1, 2, 3, 4, and 5 remained medium high. Group 3 containing 400# superphosphate per acre contained no more than group 1 containing 100# of superphosphate per acre. Groups 6 and 7 had dropped to very low and contained scarcely enough phosphorus to give the test. Ten individual plants from group 7 showed little variation.

By this time the potted plants were sickly in appearance and growth had almost stopped. This was true of all groups, the untreated plants appearing as good as the treated ones. The plants in the field had far outgrown them. It was decided to abandon the experiment on pots and work on plants from the fields.

As early as June 5th we obtained plants from the fields. We selected virogous and healthy plants growing on soils that have produced good tonnage and high sugar. When we compared our tests of these with the color charts we had quite a different picture. We give here an average of several comparisons.

Nitrate Nitrogen	Very High
Phosphorus	High
Potassium	Extra High
Calcium	High
Magnesium	Medium
Aluminum	Low
Iron	Medium

These high concentrations exist in the young healthy beet, but as they increase in size and the season advances the concentration diminishes. From this point on analysis will be reported as Normal, High and Low. Normal meaning the concentrations in normal beets growing on good soils. High and Low meaning very definite differences. No attempt is made to draw fine distinctions. Many individual samples from the same soil were tested growing side by side. Then, as now, we are not concerned as to whether the petiole contains 3 or 5 parts of phosphorus per million, but whether it contains 5 or 40 ppm.

The remainder of the work consisted of testing field samples. In selecting these samples we looked for evidence of abnormal growth and in particular, fields showing evidence of blackheart. Some examples of the general results are given here.

June 29th.

Beets growing on the Meyer farm showed a slackening of growth and were not as large as average beets. They needed irrigation. The analysis of the petioles was as follows:

Nitrate Nitrogen	Very High - 4 to 5 times normal
Phosphorus	Twice as high as normal
Magnesium	Twice normal
Potassium	Thrice normal
Iron	Low

The analysis of the soil on which the beets were growing, in terms of Morgan's standards, was as follows:

Nitrate Nitrogen	70# per acre
Phosphorus	300# " "
Potassium	600# " "
Magnesium	50# " "
Iron	15# " "

At harvest time this field yielded 12.85 tons per acre with 15.8% sugar content.

July 1st

Samples taken from Foster field. These beets needed water and growth had been slight for the past ten days. Analysis of petioles is as follows:

Nitrate Nitrogen	3 to 4 times normal
Phosphorus	2 times normal
Potassium	4 times normal
Magnesium	Normal
Iron	Low

Soil from the Foster farm:

Nitrate Nitrogen	40# per acre
Phosphorus	100# " "
Potassium	800# " "
Magnesium	25# " "
Iron	15# " "

The final yield on this field was 8.53 tons per acre with 17.4 sugar content.

August 10th

A field on the Stricker farm developed what we believed to be black-heart. A large percent of the beets were affected. Apparently healthy beets were growing within two or three feet of the affected beets. The field had had a light irrigation the previous week. Samples were taken from the healthy beets and the affected ones. Analysis is as follows:

	Healthy Beets	Affected Beets
Nitrate Nitrogen	Normal	High
Phosphorus	High	Low
Magnesium	Normal	Normal
Potassium	High	High
Iron	Highest found in all tests	Low

August 12th

A field on the Walter Butcher farm had shown good growth up till August 1st. A condition resembling blackheart had developed through the field with the exception of an area on which a straw pile had burned or rotted. The beets in this area were very fine and growing well. Samples taken from both areas compared as follows:

	Beets on strawstack area	Rest of field
Nitrate Nitrogen	Normal	High
Phosphorus	High	Low
Magnesium	Normal	Normal
Potassium	High	High
Iron	High	Low

August 15th

A sample from the Shultz farm. These beets were slowing down in growth, and had been watered the week before. A large percent of the beets around the edges of the field had developed a yellowing of the leaves. This was not the characteristic yellow due to the loss of nitrogen but a more intense color, it being about half the shade of a lemon peel. In some cases there were yellow and green leaves on the same plant. Tests from the green and yellow petioles from the same plant gave the same analysis. Healthy beets were selected growing near the affected beets. The analysis was as follows:

	Healthy Beet	Yellow Leaved Beet
Nitrate Nitrogen	Normal	Normal
Phosphorus	Normal	Low
Magnesium	Normal	Normal
Iron	Trace	None
Potassium	High	Highest in any test

August 24th

A field of beets on the J. K. Butcher farm developed spots of blackheart. Samples were taken from the affected beets and about five feet away samples were taken from healthy beets. These tested as follows:

	Unaffected Beets	Affected Beets
Nitrate Nitrogen	High	Slightly below Normal
Phosphorus	Low	Very Low
Potassium	Normal	Normal
Magnesium	Normal	Low
Iron	Low	Trace

Further study and more information from other authors previously mentioned in this paper influenced us to change our original technique. Later experience has taught us that while the dyphenylamine tests for nitrates gave good results in Mr. Morgan's hands we were unable to obtain consistent results with it.

The changed technique also led to a discovery which we believe to be of importance. We were working on petioles which gave low phosphate tests. The procedure for this analysis at that time was to grind two grams of petioles with approximately .25 grams of Darco and 10 ml of a 2% acetic acid solution. This was filtered and 5 ml were mixed with 5 ml of Brown's reagent, Truog indicator added for color development and comparisons made after ten minutes. The solutions given by these petioles showed a greenish cast which interfered with final color comparisons. In the effort to overcome this we tried charring two grams of petioles in a platinum dish over a flame at a dull red heat. On taking up this charred residue with the two percent acetic acid solution and filtering, we obtained a clear solution and much to our surprise obtained a higher phosphorus test. We repeated the experiment with like results.

After this discovery we made it a point to ash all petiole samples found low in phosphorus and obtained a much higher reading. This led us to believe that some of the phosphorus was in an organic form. We tried ashing petioles with magnesium nitrate and taking up both with acetic and hydrochloric acid. We did not obtain higher phosphorus tests on these samples than we did on the charred samples. From then on we made it a practice to char all petiole samples. This gave us much more consistent results.

We found that charring petioles giving a high phosphorus by the old method did not increase the test materially, but in low phosphorus tests charring materially increased it.

We are not prepared to say definitely what function the organic phosphorus plays in plant metabolism. We do know however that beets beginning to be affected with blackheart show a rapid decrease in both organic and inorganic phosphorus. We observed one case wherein beets growing on soil which had previously given a doubtful phosphorus soil test began to develop typical blackheart. Beets across the road on good soil gave a total phosphorus content of approximately 60 ppm. The beets beginning to show blackheart contained approximately 12 ppm. total phosphorus. Test rows of these beets were treated with superphosphate in the equivalent of 600# per acre. In spite of the fact this was late in July these test rows had remarkable recovery and within two weeks contained more total phosphorus than the healthy beets to which they were compared. At harvest time these test rows yielded about 8 or 9 tons per acre, while the remainder of the field was practically a total loss. Most of our district soils have been so well phosphated in past years that few fields can be found showing blackheart.

This affords little opportunity for determining the extreme phosphorus deficiency at which blackheart will probably occur.

As regards other necessary elements for plant growth, such as potassium and calcium, we found these present in large quantities in the petioles in all beets grown in our territory. This would indicate that our soils have a large sufficiency of these elements, and even though the beet has an enormous appetite for these salts, we felt it was not necessary to make analyses for these salts a routine procedure, as it will be a long time before addition of

such salts to the soil may be necessary.

Other lesser elements of plant growth, such as magnesium, manganese, and iron, were always found to be present in slight traces, but distinctions as to the amount could not be made between good and poor beets. It was therefore decided to abandon these as routine tests.

The sugar beet seems to be able to select the optimum amount of phosphorus necessary for vigorous growth, petioles of beets growing on soil containing 200# of phosphorus per acre showing almost the same amount of phosphorus as petioles of beets growing on soil containing 1000# of phosphorus per acre.

This, however is not true of nitrates, for beets will absorb nitrates in proportion to the concentration of nitrates in the soil. High nitrogen in the petiole is therefore direct evidence of high nitrogen in the soil with the exception of cases of beets suffering injury or drouth, wherein the nitrates may increase to large proportions, even on soils of low nitrate content. As evidence of this a field sampled early in June was injured by hail. Three days later the nitrogen content had increased about four times.

As this is a preliminary report it might be well to state our tentative plans for future work wherein periodic analyses will be made on selected fields of both good and poor beets. The petioles will be analyzed for phosphorus and nitrates with occasional analyses for some of the less important elements.

After trying various methods we have adopted the two following methods for the determination of phosphorus and nitrates viz. Thornton's method for phosphorus with slight modifications, and the phenol-disulphonic acid method for the nitrate test:

Sampling. Petioles are selected from several plants at random over the field. These are cut in pieces about 2 mm. thick and mixed.

Method. Two grams of the sample are weighed out and ground with, .25 grams of Darco (phosphate free) and 10 ml of Thornton's phosphorus reagent. This is then filtered through a 12.5 cm. Whatman paper No. 5. The filtrate is placed in a test tube. A small amount of stannous oxalate or La Motte Turog reducer is added. (The amount that can be picked up on the end of a toothpick is enough.) The color is allowed to develop for five minutes and the solution is compared with standards. (Actual standards can be matched with a solution of night blue and alcohol.)

In addition to this 2 grams of petioles are carbonized at a dull red heat, after cooling 10 ml of Thornton's phosphate reagent are added. The carbonized petioles are broken up with a glass stirring rod and the mixture filtered. The procedure is then the same as before.

Thornton's Phosphate Reagent: The test solution is prepared by dissolving 4 grams of ammonium molybdate in 500 cc. of distilled water and adding to it slowly and with constant stirring a mixture of 437 cc. of distilled water and 63 cc. of concentrated hydrochloric acid. This gives an acid concentration of approximately 0.75N. Where large quantities of the reagent are to be used it may be more conveniently made up in more concentrated stock solutions and diluted with distilled water as needed. The more concentrated solu-

tions will keep indefinitely if properly stored while the dilute solution, as prepared for the test, often develops an appreciable blue color on standing for several weeks. Storage of Pyrex bottles in a cool place will prevent such deterioration, to a large extent.

Nitrate Nitrogen.

Sampling. Take 2 gms. of finely cut petiole tissue and place in a small 50 c.c. mortar containing 10 ml of 2% acetic acid and .25 gms. of phosphorus free Darco. Grind the mixture intermittently for 10 minutes and then filter through a 12.5 cm. Whatman No. 5 filter paper. The filtrate is now ready to use in determining nitrogen. One gram of plant tissue is represented by 5 c.c. of the filtrate.

Method. With phenol-di-sulphonic acid.

Evaporate to dryness 1 - 2 c.c. of the filtrate, to which has been added .5 - 1 c.c. of 10% NaOH, in a pyrex beaker over a water bath or an alcohol lamp. Dissolve the salts with 2 c.c. of phenoldisulphonic acid and allow to stand 5 minutes. (Phenoldisulphonic acid is prepared by dissolving 25 gm. of pure phenol in 150 c.c. of concentrated H_2SO_4. Add 75 c.c. of fuming sulfuric acid and heat in a boiling water bath for two hours.) Add water and 30% NaOH until the yellow color has developed to its maximum intensity. Make to 50 or 100 c.c. depending on density of color and compare with a standard in a colorimeter.

A standard solution containing 100 ppm. of NO_3-N results when .722 gms. of KNO_3 are dissolved in a liter of water. One-half, 1, 1½ and 2 c.c. of this solution equals a comparative concentration of 250, 500, 750, 1000 p.p.m. in the plant when 1 c.c. of the filtrate is used and both solutions are made to the same volume.

Summary and Conclusions

1. Preliminary tests indicate that the petiole analysis is a better index to soil requirements than soil analyses especially in beets grown on Western soil.

2. The methods are simple and can be worked in any factory laboratory.

3. The factory chemist is on the ground and has ample material and conditions of growth on which to experiment.

4. Analyses of petioles under adverse growing conditions or injury may not be indicative of the soil fertility.

5. Phosphate determination should be made on green petioles and the carbonized petioles. They should be studied in conjunction with each other.

We close with an apology for the meagre data we are able to supply, but if we have encouraged anyone to take up this work we feel that it will eventually result in benefit to the industry.