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CLI. THE DETERMINATION OF CELLULOSE IN SOIL¹.

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INTRODUCTORY.

DURING the course of an investigation into the decomposition of green manures in soil it was necessary to determine the amount of cellulose that underwent changes at the different stages of fermentation. Charpentier [1920] has shown that when cellulose, in the form of filter-paper, is mixed with soil it can be recovered quantitatively by extraction with Schweitzer's reagent, an observation later confirmed by Barthel and Bengtsson [1924]. This method, however, cannot be applied directly to soils mixed with plant materials, as the plant tissues contain, amongst other constituents, hemicelluloses and lignin which interfere with the extraction of cellulose with Schweitzer's reagent. The hemicelluloses are to a certain extent soluble in Schweitzer's reagent and are precipitated with alcohol. Lignin on the other hand acts as a barrier to the complete dissolution of cellulose in Schweitzer's reagent.

Waksman and Tenney [1927] suggested treating the material with a 5 % solution of NaOH for 30 minutes at 15 lbs. pressure in order to remove the hemicelluloses and lignin before extracting the cellulose with Schweitzer's reagent. Solutions of sodium hydroxide of strength greater than 1-2 % are known to attack cellulose, so that any treatment in which the plant material is exposed to the action of alkali of more than 1-2 % strength at high temperatures is bound to give a low yield of cellulose. Bengtsson [1924] proposed treating the soil mixed with plant materials with a solution of sodium bisulphite in hydrochloric acid at a temperature of 100° for 72 hours and then extracting cellulose with Schweitzer's reagent. Apart from the fact that the sulphite process of separating cellulose gives lower yields than the Cross and Bevan method, the period of digestion required for the preliminary treatment of the material makes the process too long.

With a view to the elimination of these disadvantages an attempt was made to find a suitable method for the estimation of cellulose in plant materials mixed with soil.

¹ This paper is an abridged form of part of a thesis approved for the degree of Ph.D. in the University of London.

EXPERIMENTAL.

Some preliminary experiments were carried out on a sample of oat straw whose cellulose content was known. It contained 39.3 % cellulose by Jenkins's method [1930] which gives as good results as the Cross and Bevan method. In all subsequent experiments this figure was taken as the standard with which the results of the various methods tried were compared.

An attempt was first made to see if Schweitzer's reagent could extract all the cellulose from the straw after the hemicelluloses were removed as suggested by Waksman [1927]. One g. portions of straw were treated with 100 cc. of 1 % NaOH and 1 % H₂SO₄ at boiling temperature for half an hour with each reagent. The residue, after thorough washing and drying, was shaken with 100 cc. of Schweitzer's reagent for one hour¹ and the cellulose estimated according to Charpentier's process. The residue was re-extracted twice with Schweitzer's reagent and cellulose precipitated each time as before. The results (Table I) show that even by extracting the material three times, a part of the

Table I.

	1st Extraction	2nd Extraction	3rd Extraction	Total
Cellulose on dry matter, %	29.5 27.6	2.94 3.05	2.29 1.66	34.37 32.31

cellulose only could be recovered. There is a progressive dissolution of cellulose, its complete dissolution being hindered by lignin with which it is supposed to be encrusted.

The use of chlorine gas for removing lignin, apart from its other drawbacks, is ruled out where the material under treatment is mixed with soil. The sulphite process of Bengtsson, already referred to, was tried on one g. portions of the oat straw (Table II).

Table II.

	1st Extraction	2nd Extraction	Total
Cellulose % on dry matter	36.7 37.5	0.86 0.50	37.56 38.00

Though the removal of lignin has increased the amount of cellulose that could be extracted with Schweitzer's reagent, yet the yield is not up to the standard. This suggests that a part of the cellulose has been attacked during the preliminary digestion with the bisulphite. It is clear that it is necessary to remove lignin from the plant tissues before the cellulose can be completely dissolved by the Schweitzer's reagent, and yet the reagent employed must be such as to have a minimum effect on the cellulose. Jenkins's method of chlorinating lignin by means of sodium hypochlorite in an alkaline solution suggested itself and was next tried. One g. portions of the straw were subjected to the

¹ In some preliminary experiments it was found that shaking for one hour extracted as much cellulose as shaking for 6 or 24 hours.

dilute alkali and acid treatments as before and the residues therefrom were treated twice with sodium hypochlorite as described by Jenkins. The excess of hypochlorite was removed and the residues, after drying, were extracted with 100 cc. of Schweitzer's reagent and cellulose was estimated as before (Table III).

Table III.

No. of determination	Cellulose % on dry matter
1	40.15
2	39.96
3	40.05
4	39.07
5	40.82
Mean value	40.01 ± 0.28

The Schweitzer's reagent is now able to extract the whole of the cellulose at one extraction and the results agree well with the standard.

The straw was now mixed with a light sandy soil, and the mixture subjected to the same treatments as with straw alone. 10 g. of the soil mixed with 1 g. of the straw were used for analysis (Table IV).

Table IV.

No. of determination	Cellulose in soil %	Cellulose in mixture of soil + straw	
		Amount obtained	Amount calculated
1	0.0627	0.3955	
2	0.0546	0.3987	
3	0.0627	0.3915	
4	—	0.3858	
5	—	0.3851	
Mean value	0.0600 ± 0.0027	0.3913 ± 0.0027	0.4060

The results show that almost the whole of the cellulose added to the soil in the form of straw can be recovered by the method of analysis employed. A part of the cellulose, 0.0147 g. could not be recovered from the mixture. This loss of cellulose which was also observed by Charpentier and others is attributed by them to adsorption by the soil. Whatever the explanation be, it seems certain that a part of the added cellulose, in whatever form it is added, is always retained by the soil and is not recovered by the method of analysis used.

The method was then applied to different plant materials and to mixtures of these plant materials with soil. In all cases 10 g. of the soil together with 1 g. of the plant material were taken for analysis. Table V shows the results of analysis of plant materials alone. In Table VI the figures are given for the same plant materials mixed with the soil.

The results show that the cellulose added to the soil in the form of plant materials of widely different nature and age can be recovered almost quantitatively.

Table V.

No. of determinations	Cellulose on 100 g. dry matter					
	Young tares		Young mustard	Sugar beet tops		Mature mustard
	by Jenkins's method			by Jenkins's method		
1	9.83	9.62	9.42	7.18	7.87	22.90
2	10.48	10.11	8.60	8.12	7.79	23.75
3	9.21	—	—	8.09	—	—
4	9.52	—	—	7.61	—	—
5	9.83	—	—	6.53	—	—
6	9.28	—	—	6.79	—	—
Mean value	9.69	9.87	9.01	7.39	7.83	23.32
Standard error	0.18	—	0.51	0.26	—	0.42

Table VI.

	Cellulose in 10 g. soil + 1 g. plant material, expressed on dry matter			
	Cellulose g.	Average value	Amount calculated	Difference
Young tares + soil	0.0926 0.1015	0.0970	0.1028	0.0058
Young mustard + soil	0.0968 0.0833	0.0901	0.0960	0.0059
Sugar beet tops + soil	0.0731 0.0731 0.0745 0.0731	0.0735	0.0798	0.0063
Mature mustard + soil	0.2375 0.2350	0.2362	0.2391	0.0029

Description of method.

Ten g. of soil are heated in a beaker with 100 cc. of 1 % NaOH and allowed to boil for 20–30 minutes, maintaining a constant volume during heating. The soil is allowed to settle; this may be hastened by making the suspension slightly acid. The upper liquid is filtered off and the residue washed twice with hot water. The material on the filter is transferred to the beaker and the soil heated with 100 cc. of 1 % hydrochloric acid for about 20 minutes, maintaining a constant volume of liquid. After settling, the upper liquid is filtered off and the residue washed free of acid with hot water. The material on the filter-paper is returned to the beaker, 5 cc. of sodium hypochlorite, having 15 % available chlorine, are added and the volume is made up to 100 cc. The liquid must remain alkaline. It is allowed to stand for half an hour in the cold with three or four shakings during the interval. The upper liquid is filtered and the material on the filter returned to the beaker, another 5 cc. of the sodium hypochlorite solution added and the solution made up to 100 cc. and allowed to stand for half an hour with shakings as before. As much as possible of the upper liquid is filtered and to the residue in the beaker a dilute solution of H_2O_2 (10 cc. of 20 vol. H_2O_2 made up to 100 cc.) is gently added till effervescence

ceases, excess being avoided. The suspension is allowed to settle, being hastened with HCl if necessary, and the clear liquid is filtered through the same filter-paper. The residue in the beaker is transferred to an evaporating dish and washed thoroughly free of acid with hot water by decantation. The material on the filter-paper is transferred to the dish and the residue evaporated to dryness on a water-bath.

When the residue is completely dry, it is transferred to a suitable bottle for extraction with Schweitzer's reagent. 100 cc. of Schweitzer's reagent are added and the material shaken for one hour. It is then allowed to settle, preferably overnight. More than 50 cc. are filtered, a suction pump being used if necessary. To 50 cc. of the filtrate in a 400 cc. beaker 200 cc. of 80 % alcohol are added, and the precipitate is allowed to settle overnight. It is then filtered by decantation through a suitable filter aided by suction, Jena glass filter-crucible No. 1G4 being very convenient. After the upper liquid has all been filtered, 50 cc. of a mixture of alcohol and HCl (40 cc. of 80 % alcohol *plus* 10 cc. HCl) are added to the precipitate; the mixture is shaken till all the copper hydroxide is dissolved and is then allowed to stand for about an hour. The upper liquid is filtered through the same filter, the precipitate washed free of copper with hot water, and transferred to the crucible, washed further with hot water, then with alcohol and ether in turn as directed by Charpentier. The crucible is dried in an oven for about one hour; the cellulose is transferred to a weighed crucible, weighed, ignited and again weighed. The loss of weight on ignition multiplied by 2 represents the amount of cellulose in 10 g. of the soil.

SUMMARY.

1. A method is described for the determination of cellulose in soil mixed with plant materials. It is essentially a combination of the Jenkins and Charpentier methods. The soil is treated with hot dilute alkali and acid and then with a solution of sodium hypochlorite in the cold. Cellulose is then extracted from the residue with Schweitzer's reagent, precipitated with alcohol and determined by loss of weight on ignition.

2. The results obtained by this method on plant materials alone agree very closely with those given by the hypochlorite method of Jenkins.

3. It is shown that it is necessary to remove the lignin from the plant tissues, otherwise it is not possible to obtain a quantitative yield of cellulose by extraction with Schweitzer's reagent.

4. The method has proved its value in recovering almost the whole of the cellulose added, when different plant materials are mixed with soil. A part of the cellulose is, however, retained by the soil and is not extracted by the Schweitzer's reagent.

5. No attempt has been made to apply the method to different types of soil.

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