STUDIES ON SOIL ORGANIC MATTER
PART II. THE EXTRACTION OF ORGANIC MATTER FROM SOIL BY NEUTRAL REAGENTS

By J. M. BREMNER AND H. LEES, *Rothamsted Experimental Station, Harpenden, Herts*

(With Two Text-figures)

Despite the enormous amount of work that has been carried out on the problem very little progress has been made in the elucidation of the chemical nature of soil organic matter. The problem is essentially an empirical one, and real progress cannot be expected until the major practical difficulties of the subject are overcome. The first, and probably the most serious, difficulty encountered in the study of soil organic matter is that of separating the organic complexes from the inorganic material of the soil. Considering its importance, surprisingly little attention has been given to this problem. Caustic alkali has been used almost exclusively for the extraction of soil organic matter since Achard (1786) first employed it to isolate 'humic acid'. Generally a dilute (0·5 M) solution of sodium, potassium, or ammonium hydroxide is used, but more concentrated solutions have often been employed. Although caustic alkali is the most efficient of the extractants so far tested, it is a drastic reagent, and its use as extractant is likely to cause grave alteration in the physico-chemical properties of the organic complexes. Milder extractants, such as neutral solutions of salts of the alkali metals (Simon, 1929, 1930; Pozdena, 1937 a, b; Scheffer, 1936) and aqueous solutions of hexamethylene-tetramine (Souci & Schmitt, 1937; Zeile, 1935) and urea (Zeile, 1935), have occasionally been employed but have not found favour owing to their inefficiency.

The object of the present investigation was to survey the extractive powers of various neutral reagents and to define the conditions under which maximal extraction of organic matter from soil by such reagents can be achieved.

Preliminary investigations showed that a significant fraction of the organic matter of soil was extracted by solutions of the sodium or potassium salts of acids forming either insoluble precipitates or soluble co-ordination complexes with calcium ions. The organic matter extracted from soil by such salt solutions was precipitated by calcium salts. These facts are commonly described by saying that sodium humate is soluble and calcium humate insoluble. This is in harmony with the accumulation of humus in chernozems and rendzinas (soils rich in calcium) and the mobility of humus in 'black alkali' soils (containing much exchangeable sodium). Humus also forms insoluble complexes with a number of other cations, notably those of the heavy metals. With many of these metals there are several possible modes of combination—the cation may form insoluble salts or complexes or there may be mutual coagulation between negative humus sols and positive sols of basic salts of the metals. Whatever the mechanism of the combination, it appeared likely that soil organic matter might be extracted fairly efficiently by neutral reagents capable of forming either insoluble precipitates or soluble co-ordination complexes with the heavy metals. Preliminary work exploring this possibility has already been published (Bremner, Heintze, Mann & Lees, 1946). Further developments are described below.

EXPERIMENTAL

The nitrogen-extracting power of a reagent has been used throughout as an index of its ability to extract organic matter. The validity of this index is discussed by one of us (J.M.B.) in Part III of this series.

Extractions were performed in stoppered conical flasks which were shaken at intervals to assist solution, and the soil was finally separated from the extract by centrifuging and filtering. Except where there is a contrary statement, experimental results refer to a single overnight extraction of the soil (5 ml. extractant/g. of soil).

Six of the seven soils used have already been described (Bremner, 1949). The other soil (Burwell) was a peat containing shell fragments from the Cambridgeshire fen district. It contained 29·6 % C, 2·04 % N and 30·3 % $CaCO_3$ (calculated on dry-matter basis). Nitrogen was determined by the micro-Kjeldahl method previously described (Bremner, 1949).

RESULTS

(1) *Survey of extractants*

Table 1 gives the results obtained in a survey of the nitrogen-extracting powers of various neutral sodium salt solutions; the results obtained with 0·5 M-NaOH, the classical extractant, and 0·5 M-Na_2CO_3 are given for comparison.

It can be seen that fluoride and pyrophosphate are the most efficient of the neutral inorganic extractants tested. Of the two, pyrophosphate has proved to be the more generally useful. The fact that pyrophosphate invariably exhibits an extractive power superior to that of orthophosphate indicates that its efficiency is derived from an ability, which is not shared by orthophosphate, to form soluble co-ordination complexes with metals. Although sodium hexametaphosphate is a mild detergent and can form non-ionized calcium complexes (Hatch & Rice, 1939), it is of little value as an extractant of soil organic matter. Fluoride is by far the most efficient of the halides in extraction. Its superiority would appear to be due to its special ability to dissociate

There would appear to be some correlation between the efficiency of a reagent and the solubility of its calcium salt. The only outstanding anomaly is citrate, but it is known that the ionization of calcium citrate involves the formation of a complex anion.

Although citrate and oxalate are generally as efficient as pyrophosphate, the latter is obviously preferable as an extractant of soil organic matter since it is an inorganic reagent and its presence in soil extracts does not introduce the practical difficulties caused by the presence of citrate or oxalate.

The abilities of several reagents not listed above to extract organic matter from soil were also tested, but none proved satisfactory; among these reagents

Table 1. *A comparison of the nitrogen-extracting powers of various neutral sodium salt solutions*

A, Allotment soil; B, Broadbalk soil; C, Hoosfield soil; D, Burwell soil.

	Nitrogen extracted (% total soil N)				Solubility of corresponding Ca salt (g./100 ml. H_2O)
	A	B	C	D*	
Sodium salts of inorganic acids					
0·1 M-pyrophosphate	9·3	11·0	11·2	22·0	—
0·5 M-fluoride	7·3	7·6	10·0	21·9	0·0016
1 % (w/v) hexametaphosphate	3·7	8·6	4·8	7·2	—
0·1 M-orthophosphate	3·9	5·6	4·1	3·8	0·02†
0·1 M-borate	3·2	5·6	—	3·0	
0·5 M-chloride	1·2	2·5	1·3	1·2	60
0·5 M-bromide	1·2	2·4	—	1·2	125
0·5 M-iodide	1·1	2·5	—	1·1	66
Sodium salts of organic acids					
0·2 M-oxalate	11·1	9·8	10·4	24·9	0·00067
0·2 M-citrate	9·0	8·6	—	19·5	0·25
0·2 M-tartrate	5·4	7·7	—	15·2	0·0266
0·2 M-malate	3·8	5·5	—	5·0	0·812
0·2 M-tricarballylate	3·8	4·1	—	3·4	—
0·2 M-salicylate	3·0	4·6	—	3·1	2·70
0·2 M-benzoate	2·0	2·1	—	1·7	2·67
0·2 M-succinate	1·7	2·2	—	0·8	1·52
0·2 M-p-hydroxybenzoate	1·6	2·1	—	0·8	—
0·2 M-acetate	1·3	2·2	1·2	0·8	37·4
Compare					
0·5 M-sodium hydroxide	26·0	26·0	20·0	24·9	0·185
0·5 M-sodium carbonate	9·0	11·2	11·8	25·5	0·0015

* 10 ml. extractant/g. of soil.   † $CaHPO_4 2H_2O$.

the metallo-organic complexes of soil by precipitating calcium or removing other metals as soluble co-ordination complexes. The other halides dissolve practically no organic matter from soil. The pH of a fluoride extract rises when left in contact with the soil. For example, overnight treatment of 5 g. of Allotment soil with 25 ml. of 0·5 M-sodium fluoride (pH 7·0) gave an extract of pH 9·5. This rise in pH may be due to replacement of hydroxyl ions in the ion exchange complex of the soil by fluoride ions (cf. Dickman & Bray, 1941).

Citrate and oxalate, the most efficient of the organic acid extractants tested, both have a 'calcium-masking' action; oxalate removes calcium in the form of an insoluble precipitate, and citrate forms non-ionized complexes with the cation.

were hexamethylene-tetramine, dodecyl sulphate and strong solutions of urea, formic acid and phenol. Water and aqueous solutions of salts other than those of the alkali metals dissolved practically no organic matter from soil.

(2) *The extraction of soil organic matter by pyrophosphate*

The results obtained in a study of the factors affecting the extraction of organic matter from soil by pyrophosphate are given below; for comparison, the results obtained with caustic alkali are occasionally included.

Time of extraction. Fig. 1 shows the effect of time on the extraction of nitrogen from Allotment soil by caustic soda and neutral sodium pyrophosphate;

similar results were obtained with other soils. It can be seen that whereas the quantity of nitrogen extracted by neutral pyrophosphate is almost maximal in a few hours, caustic soda continues to extract more nitrogen the longer it is left in contact with the soil.
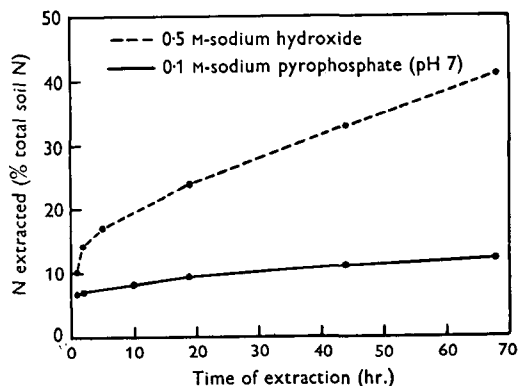

Fig. 1. The effect of time on the extraction of nitrogen from Allotment soil by pyrophosphate and alkali. 5 ml. extractant/g. of soil.

Temperature of extraction. As shown in Fig. 2, practically no advantage is gained by raising the temperature of pyrophosphate extraction, although there is an apparent advantage with caustic soda. Arnold & Page (1930) and Puri & Sarup (1938–9) used hot caustic soda for the extraction of soil organic matter.
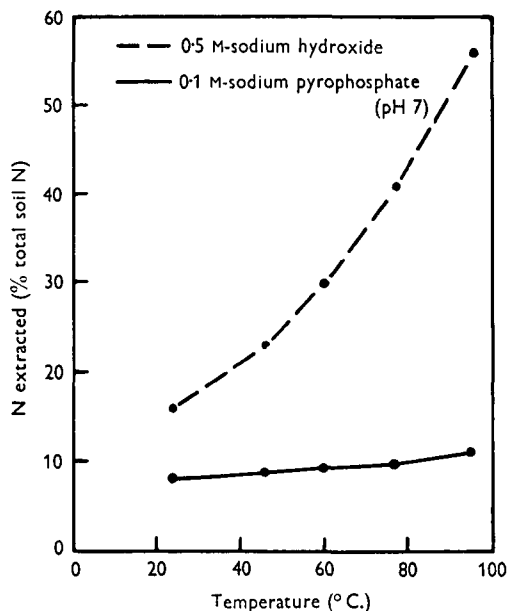

Fig. 2. The effect of temperature on the extraction of nitrogen from Allotment soil by pyrophosphate and alkali. 5 ml. extractant/g. of soil; time, 5 hr.

Volume of extractant. The results given in Table 2 show that the volume of extractant used per g. of soil is not critical.

Table 2. *Extraction of nitrogen from soil by 0·1 M-sodium pyrophosphate* (pH 7). *The effect of varying the volume of the extractant*

A, Allotment soil; B, Broadbalk soil.

	N extracted (% total soil N)	
ml. extractant/g. of soil	A	B
3	9·2	10·3
5	9·3	11·0
7	10·1	10·9
10	10·6	10·8
20	10·8	10·9

Concentration of extractant. The concentration of the pyrophosphate solution used for extraction is also uncritical so long as it exceeds a certain minimum value (Table 3). For the soils tested this value was about 0·1 M. In contrast, the amount of organic matter extracted when a soil is treated with alkali is greatly affected by the concentration of the alkali. For example, extracts obtained by treating 10 g. samples of Allotment soil overnight with 50 ml. of 0·1, 0·5, 1·0 and 2·0 M-sodium hydroxide contained 22·5, 26·0, 29·9 and 35·5 % of the total soil nitrogen, respectively.

Table 3. *Extraction of nitrogen from soil by sodium pyrophosphate solution* (pH 7). *The effect of varying the concentration of the extractant*

A, Allotment soil; B, Broadbalk soil.

	N extracted (% total soil N)	
Concentration of pyrophosphate	A	B
0·050 M	5·3	10·2
0·075 M	7·1	10·6
0·100 M	9·3	11·0
0·150 M	9·5	11·1
0·200 M	9·8	11·2

pH *of extractant.* Although the efficiency of pyrophosphate extraction increases as the pH of the extractant is raised (Table 4), the danger of damage to the extracted material is also increased. So little is known about the chemical nature of soil organic matter that no upper limit to the pH of extracting fluids can, as yet, be set. There is some evidence, however, that soil organic matter contains protein material, and many proteins are known to be altered above pH 8·0. This pH may, therefore, be suggested as an arbitrary upper limit for certain purposes.

Shaking. The effect of shaking on extraction of nitrogen from soil by pyrophosphate and alkali is shown in Table 5. It can be seen that no advantage is gained by shaking continuously during extraction.

Moreover, it is very difficult to filter extracts obtained by this method.

Table 4. *Extraction of nitrogen from soil by* 0·1 M-*sodium pyrophosphate. The effect of varying the* pH *of the extractant*

A, Allotment soil; B, Broadbalk soil.

pH of extractant	N extracted (% total soil N)	
	A	B
4	7·1	6·8
5	—	8·4
6	8·2	9·7
7	9·2	10·9
8	10·3	11·2
9	11·4	11·8

Table 5. *The effect of shaking on the extraction of nitrogen from soil by pyrophosphate and alkali*

A, Hoosfield soil; B, Broadbalk soil.
1, occasional shaking; 2, continuous shaking.

	N extracted (% total soil N)			
	A		B	
Extractant	1	2	1	2
0·1 M-sodium pyrophosphate (pH 7·0)	11·2	11·4	11·0	11·3
0·5 M-sodium hydroxide	20·0	20·4	26·0	26·5

Pretreatment of soil before extraction

(a) *Ball-milling.* The effect of ball-milling Broadbalk soil before extraction with pyrophosphate and alkali is shown in Table 6; the soil was milled for 4 days with agate balls. Ball-milling does not always lead to greater extraction. For example, it has no effect on the extraction of nitrogen from Allotment

Table 6. *The effect of ball-milling on the extraction of nitrogen from Broadbalk soil by pyrophosphate and alkali*

	N extracted (% total soil N)	
Extractant	Unmilled soil	Milled soil
0·1 M-potassium pyrophosphate (pH 7·0)	11·1	13·0
0·5 M-potassium hydroxide	30·3	33·3

and Hoosfield soils by pyrophosphate. Practical difficulties are introduced by ball-milling, since the milled soil tends to give a colloidal suspension which is difficult to centrifuge or filter. It may be noted here that Wolf & Schlatter (1930) found that stable humus sols were obtained by shaking sandy soils with water for several days but that no organic matter was dissolved if the sand was first removed. This suggests that in their experiments the dispersion of the organic matter was caused by coarse sand acting as a colloid mill. No dispersion

of organic matter was observed when the soils used in this work were shaken with water.

(b) *Acid-leaching.* It is well known that a preliminary leaching of the soil with cold dilute acid leads to a considerable increase in the amount of organic matter soluble in alkali. As shown in Table 7, this pretreatment of the soil also has some effect on the extraction of organic matter by pyrophosphate. Acid-leaching was effected by shaking the soils with 0·1 N-hydrochloric acid for about two hours and then washing the acid-extracted soils thoroughly with distilled water; practically no organic matter was removed from the soils by this process. Since inorganic nitrogen is removed when a soil is leached with acid, the organic, not the total, nitrogen contents of pyrophosphate extracts of untreated and acid-leached soils are compared in Table 7. When soils are leached with acid prior to extraction with neutral pyrophosphate care must be taken to ensure that the pH of the extractant is not lowered by residual acid in the soil; it is sometimes necessary to add a few drops of alkali to the soil/pyrophosphate suspension after extraction has been proceeding for about an hour in order to adjust the pH to 7·0.

Table 7. *Extraction of organic nitrogen from soil by pyrophosphate and alkali. The effect of acid-leaching the soil before extraction*

A, 0·5 M-sodium hydroxide; B, 0·1 M-sodium pyrophosphate (pH 7·0).

	Organic N extracted (% total soil N)	
Soil	A	B
Hoosfield	19·0	10·2
Acid-leached Hoosfield	35·2	10·6
Broadbalk	24·2	9·2
Acid-leached Broadbalk	40·0	11·1
Allotment	25·2	8·5
Acid-leached Allotment	33·8	9·3

Frequency of extraction. Table 8 gives the amounts of nitrogen extracted from various soils by one treatment with 0·1 M-sodium pyrophosphate (pH 7·0) and by repeated treatments with the same reagent. It can be seen that a single extraction of a soil with pyrophosphate removes practically all of the organic matter soluble in the reagent.

Table 8. *The amounts of nitrogen extracted from soil by* (a) *one treatment with* 0·1 M-*sodium pyrophosphate* (pH 7·0) *and* (b) *repeated treatments with the same reagent*

	N extracted (% total soil N)	
Soil	(a)	(b)
Hoosfield	11·2	12·0
Broadbalk	11·0	11·2
Allotment	9·3	9·5

The effect of soil type. As shown in Table 9, pyrophosphate extracts a much greater proportion of the

organic matter from highly organic than from mineral soils.

Table 9. *The amounts of nitrogen extracted from different soils by* 0·1 M-*sodium pyrophosphate* (pH 7·0)

Soil	Total N content of soil (%)	N extracted (% total soil N)
Hoosfield	0·10	11·2
Barnfield	0·27	7·8
Broadbalk	0·25	11·0
Allotment	0·49	9·3
Burwell	2·04	22·0
Swaffham	2·38	31·4

(3) *The organic matter extracted by pyrophosphate*

The organic matter in a neutral sodium pyrophosphate extract of soil can be separated into the customary acid-insoluble ('humic') and acid-soluble ('fulvic') fractions by acidifying the extract with dilute mineral acid. No attempt has been made to compare the properties of the humic fractions isolated from pyrophosphate extracts of different soils since no criteria of homogeneity have been developed by which the purity of the preparations could be assessed. The fulvic complexes of pyrophosphate extracts are readily adsorbed from acid solution and can be separated by the use of suitable adsorbents and eluents. For example, a useful resolution of the fulvic fraction can be achieved using charcoal as adsorbent and acetone, water and neutral pyrophosphate as eluents (cf. Forsyth, 1947).

Most of the inorganic material in a pyrophosphate extract of soil can be removed by simple dialysis of the extract through cellophane against distilled water; practically no organic matter is lost in this process. The organic matter in dialysed pyrophosphate extracts is largely precipitated on addition of metallic salt solutions (excepting those of alkali metals), and it is worthy of note that in general the metallo-organic precipitates exhibit the same type of solubility as that of soil organic matter. Thus the metallo-organic complexes obtained by addition of soluble calcium, manganese, copper or iron salts to dialysed extracts are far more soluble, for example, in pyrophosphate than in orthophosphate, and are practically insoluble in water. This metallic precipitation technique can be used to concentrate dilute solutions of soil organic matter. Copper salts are particularly effective for this purpose. It is merely necessary to add a soluble copper salt to the solution, remove the precipitate by centrifugation, redissolve it in a small volume of pyrophosphate and dialyse. Although this method of concentration involves some slight loss of organic material it is likely to prove useful in the study of soil organic matter.

DISCUSSION

The results obtained in this investigation support the view that soil organic matter is intimately associated with metals and that its solubility in neutral reagents is largely determined by the nature and extent of this association. The efficiency of a neutral salt extractant is apparently dependent upon the ability of its anion to remove interfering metals either as insoluble precipitates or as soluble co-ordination complexes. For efficient extraction the cation of the neutral salt must be sodium, potassium or ammonium since other cations cause precipitation of the organic matter. The efficiency of 'calcium-masking' reagents (oxalate, citrate, etc.) indicates that calcium is one of the chief interferents in the extraction of organic matter from soil.

Neutral pyrophosphate, the most useful of the neutral reagents tested, is not quantitatively as effective an extractant as caustic alkali. This is to be expected since soil organic matter almost certainly contains organic complexes which, by their nature, are insoluble in neutral reagents. For certain investigations on soil organic matter neutral pyrophosphate is obviously preferable to caustic alkali as extractant; for others, the more complete extraction by caustic alkali may be preferred, even though it is achieved at the cost of some damage to the organic complexes.

SUMMARY

A study of extracting reagents has been made to find mild but reasonably efficient methods of extracting organic matter from soil. Sodium pyrophosphate proved to be the most satisfactory of the neutral reagents tested, and the optimum conditions for extraction of organic matter from soil by this reagent have been defined.

REFERENCES

Achard, F. K. (1786). *Crell's Chem. Ann.* 2, 391.

Arnold, C. W. B. & Page, H. J. (1930). *J. Agric. Sci.* 20, 460.

Bremner, J. M. (1949). *J. Agric. Sci.* 39, 183.

Bremner, J. M., Heintze, S. G., Mann, P. G. J. & Lees, H. (1946). *Nature, Lond.*, 158, 790.

Dickman, S. R. & Bray, R. H. (1941). *Soil Sci.* 52, 263.

Forsyth, W. G. C. (1947). *Biochem. J.* 41, 176.

Hatch, G. B. & Rice, O. (1939). *Industr. Engng Chem.* 31, 51.

Pozdena, L. (1937a). *Bodenk. PflErnähr.* 2, 55.

Pozdena, L. (1937b). *Bodenk. PflErnähr.* 3, 315.

Puri, A. N. & Sarup, A. (1938–9). *Soil Res.* 6, 122.

Scheffer, F. (1936). *ForschDienst.* 1, 422.

Simon, K. (1929). *Z. PflErnähr. Düng.* A 14, 252.

Simon, K. (1930). *Z. PflErnähr. Düng.* A 18, 323.

Souci, S. W. & Schmitt, F. (1937). *BrennstChemie,* 18, 21.

Wolf, L. & Schlatter, H. (1930). *Cellulose-Chem.* 11, 254.

Zeile, K. (1935). *Kolloidzschr.* 72, 211.

(*Received* 1 *March* 1949)