

A New Sequential Extraction Method for Soil Organic Matter Fractionation

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ABSTRACT. *A new sequential extraction method for soil organic matter fractionation is proposed. This method was evaluated with another three methods vi. i) direct application of strong acid method (Bremner, 1965), ii) the conventional method (Waksman, 1939) and iii) Persson's method (Persson, 1968). The main criteria used to compare the new method were the separation of humus materials and non-humus materials and the time required for the acid hydrolysis.*

Two soils, widely different in carbon and nitrogen contents, were used in this investigation. The organic matter of the soils were fractionated using these 4 methods. The extracted organic matter fractions were analyzed for carbon and nitrogen.

The results showed that the newly proposed method was promising and proved to be much better than the other methods studied. It separated the soil organic matter into different fractions and makes these fractions available for further study. This would lead to better understanding of the organic matter decomposition in soil. The separation of humus and non-humus organic materials was achieved by the new method as equally well as the direct, and the Persson's methods. The time required for acid hydrolysis was less compared to other methods, and as such the proposed method was less laborious.

INTRODUCTION

Single extractants used in organic matter extraction separate only a part of the organic matter, leaving a large amount within the soil (Mortenson, 1965). They cannot be used satisfactorily to separate the stabilized old humus materials from fresh actively decomposable soil

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organic materials (Persson, 1968; Jansson, 1972). The most popular single extractant used in humified organic matter extraction is sodium hydroxide since it was first introduced by Achard (1786).

However, there is a common criticism that the extracted humus materials have always been contaminated by non-humus materials (Jansson and Persson, 1968; Persson, 1968). Therefore, different sequential extraction methods have been tried in various soil organic matter studies to separate the non-humified decomposable organic matter from humified fraction. In these methods, acid hydrolyses were introduced and carried out before the alkaline extraction, to hydrolyze and dissolve the non-humified decomposable fresh organic materials such as carbohydrates (Choudhri and Stevenson, 1957) and proteinous materials prior to the extraction of the humus materials (Mortenson, 1965; Jansson and Persson, 1968; Felbeck, 1971; Jansson, 1972). The different soluble organic fractions, obtained by acid hydrolysis, make them available for further study in relation to their biological importance. However, among the numerous methods available so far, no standardized or universally applicable fractionation method is available in organic matter fractionation studies.

The aim of the study was to evaluate a newly proposed method (Nandasena, 1990) with another three methods, namely the direct application of strong acid method (Bremner, 1965), the conventional method (Waksman, 1939) and Persson's method (Persson, 1968). The main criteria used to compare the new methods are :

1. The separation of humus materials and non-humus materials and
2. The time required for the acid hydrolysis.

MATERIALS AND METHODS

Two soils widely different in carbon and nitrogen content, collected from the farm fields of Gasthuisberg and Gent were used in this investigation. Soil samples taken from the 0-30 cm depth were thoroughly mixed and air dried. Soil samples were then passed through a 2 mm sieve and stored at 18 C prior to analysis. Some of the soil characteristics are given in Table 1.

Table 1. The general characteristics of soils.

Soil	pH (water:soil) (2:1)	C %	N %	C/N ratio	Clay % 0-2 μ m	Silt % 2-50 μ m	Sand % >50 μ m
Gasthuisberg	7.20	0.86	0.08	10.7	12.0	77.3	10.7
Gent	7.75	1.79	0.20	8.9	14.2	32.9	52.9

The organic matter contents of the two soils were fractionated using four organic matter extracting procedures namely the newly proposed method (Nandasena, 1990), Persson's method (Persson, 1968), the conventional humus analysis method (Waksman, 1939) and the direct application of strong mineral acid method (Bremner, 1965) in this investigation. The extracted organic matter fractions were analyzed immediately for carbon and nitrogen. For the determination of carbon and nitrogen of the extracted fractions, the standard Kjeldahl method (Bremner, 1965) and the dichromate oxidation method (Bremner and Jenkinson, 1960) were used respectively. All fractionation procedures and analyses were replicated 4 times.

RESULTS AND DISCUSSION

The amounts of extracted carbon and nitrogen of different fractions of each of the different methods for the two soils are given in Tables 2 to 5. For comparison, the carbon and nitrogen figures were presented as relative percentage values. In this study, the soil organic matter extracted by all the treatments except the alkaline extraction of each method, were considered as the non-humified organic materials of the soil. In each method, the organic matter extracted by the alkaline extraction (it employs to extract humic acids from soil) and the remaining soil residue (humus) together were considered as the humified organic materials of the soil.

In sequential soil organic matter extraction procedures, acid hydrolyses are performed prior to the alkaline extraction to hydrolyze and remove carbohydrates, proteinaceous components and other easily decomposable materials prior to the extraction of humus

Table 2. The relative percentage of extracted carbon in soil 1 (Gasthuisberg) by different methods.

Fraction	Relative percentage of carbon			
	Newly proposed	Persson's	Conventional	Direct
Water	0.41			
0.5N K ₂ SO ₄		1.48	1.32	
H. Water	4.94			
0.5N H ₂ SO ₄	22.09	4.16	5.04	
0.55N H ₂ SO ₄		28.54		
3.5N H ₂ SO ₄	18.25			
6N H ₂ SO ₄	12.79	18.73		44.92
0.5N NaOH	17.18	21.57	57.89	16.81
Res.	24.34	25.52	35.74	38.27
Non-humus-C*	58.48	52.91	6.37	44.92
Humus - C**	41.52	47.09	93.63	55.08

* Non-humus-C = total carbon - sum of 0.5N NaOH and Res. carbon.

** Humus-C = sum of 0.5N NaOH and Res. carbon.

Table 3. The relative percentage of extracted carbon in soil 2 (Gent) by different methods.

Fraction	Relative percentage of carbon			
	Newly proposed	Persson's	Conventional	Direct
Water	1.67			
0.5N K ₂ SO ₄		2.24	2.12	
H. Water	4.16			
0.5N H ₂ SO ₄	25.09	4.41	4.12	
0.55N H ₂ SO ₄			32.93	
3.5N H ₂ SO ₄	22.92			
6N H ₂ SO ₄	9.25	21.55		59.48
0.5N NaOH	28.77	30.59	39.74	30.91
Res.	8.14	8.27	54.02	9.61
Non-humus-C*	63.09	61.14	6.24	59.48
Humus-C**	36.91	38.86	93.76	40.52

* Non-humus-C = total carbon - sum of 0.5N NaOH and Res. carbon

** Humus-C = sum of 0.5N NaOH and Res. carbon

Table 4. The relative percentage of extracted nitrogen in soil 1 (Gasthuisberg) by different methods.

Fraction	Relative percentage of carbon			
	Newly proposed	Persson's	Conventional	Direct
Water	0.90			
0.5N K ₂ SO ₄		1.66	1.72	
H. Water	4.85			
0.5N H ₂ SO ₄	17.15	2.88	2.97	
0.55N H ₂ SO ₄			35.13	
3.5N H ₂ SO ₄	35.11			
6N H ₂ SO ₄	20.92	39.60		81.52
0.5N NaOH	12.91	12.49	72.78	9.78
Res.	8.17	8.24	22.53	8.70
Non-humus-N*	78.92	79.27	4.69	81.52
Humus-N**	21.08	20.73	95.31	18.48

* Non-humus-N = total nitrogen - sum of 0.5N NaOH and Res. nitrogen.

** Humus-N = sum of 0.5N NaOH and Res. nitrogen.

Table 5. The relative percentage of extracted nitrogen in soil 2 (Gent) by different methods.

Fraction	Relative percentage of nitrogen			
	Newly proposed	Persson's	Conventional	Direct
Water	1.29			
0.5N K ₂ SO ₄		2.06	2.64	
H. Water	4.54			
0.5N H ₂ SO ₄	17.39	2.93	2.51	
0.55N H ₂ SO ₄		32.75		
3.5N H ₂ SO ₄	40.24			
6N H ₂ SO ₄	17.99	42.05		85.79
0.5N NaOH	14.59	16.00	58.51	11.11
Res.	3.97	4.20	36.34	3.09
Non-humus-N*	81.44	79.80	5.15	85.80
Humus - N**	18.56	20.20	94.85	14.20

* Non-humus-N = total nitrogen - sum of 0.5N NaOH and Res. nitrogen.

** Humus-N = sum of 0.5N NaOH and Res. nitrogen.

(Jansson and Persson, 1968; Schnitzer and Schuppli, 1989). Therefore, the idea of using acid hydrolysis before the alkaline extraction is to separate the non-humified organic materials from humified materials. According to Kononova (1966) these hydrolyzable, easily decomposable materials belong to the compounds of non specific nature or non-humified organic materials in soil. Further, soil biomass, dead microbial tissues, secondary microbial degradation and synthesis products may also be included into the non-humified fraction.

As a rule, humus materials are extracted with alkaline solutions such as sodium hydroxide (Stevenson, 1965). The humified organic matter may contain lignin and their derivatives such as phenolic compounds, for example, vanillic acid, vanillin, syringaldehyde and syringic acid which are mainly found in woody plant materials (Hurst and Burges, 1967). Lignin is very difficult to separate because of its resistance to acid hydrolysis. It exists in plant in intimate association with structural polysaccharides and can only be separated from these by rather strong extractions. For example, the 72% H_2SO_4 acid extracts these polysaccharides without removing lignin from soil organic matter (Hurst and Burges, 1967). Schnitzer (1978) estimated that 65 to 75% of the organic matter predominantly in inorganic soils consists of humus materials, namely humic acids, fulvic acids and humin. However, this figure seems to be somewhat high because the extraction was made without extracting the fresh organic debris with the use of acids. According to Flaig *et. al.*, (1975) the remainder is mainly composed of polysaccharides and protein like substances which undoubtedly belong to the non-humus components in soil organic matter.

Many workers used 6N acid extraction as a final step of the acid hydrolysis in sequential acid extraction procedures to hydrolyze and separate non-humified organic matter fraction from humified fraction (Jawson and Elliott, 1986; Stevenson, 1982; Persson, 1968). In this study also, all the extractable organic matter upto 6N acid extraction were considered as non-humified organic matter fraction of soil. The alkaline (sodium hydroxide) extract and remaining residue were considered the humified fraction of soil. Broadbent (1968) showed that the 6N acid extracts all the newly immobilized nitrogen from soil. Probably this nitrogen is not transformed into the resistant humus nitrogen. Bremner (1965) used the 6N acid hydrolysis to separate non-humus materials from stabilized humus materials. Jawson and Elliott (1986) used it to separate the biologically active fraction from the

stable humus materials in soil. In all of these studies, the 6N acid hydrolysis was used to separate the decomposable fresh non-humified organic matter from soil. However, the hydrolysis time was not exactly same in these studies. Therefore, sometimes it is difficult to compare the results.

The present study showed that the amount of organic matter extracted by the 6N acid in the direct method was comparable with the total amount of extractable organic matter by all the treatments, except alkaline treatment and residue in the new and Persson's methods (Tables 2 to 5). About 79 to 85% of the nitrogen was extracted as non-humified nitrogen from both soils by all the methods except the conventional method. Rest of the nitrogen (about 15 to 21%) were taken as humus nitrogen (total of the alkaline extractable and the residual humin nitrogen) in both soils. (Tables 4 and 5).

The carbon contents of the non-humified fraction of the Gasthuisberg soil, extracted by the new, the Persson's and the direct method fluctuated between 45 and 58%. In Gent soil it fluctuated between 59 and 63%. Almost similar amounts were extracted by these methods. On the other hand, the conventional method recorded very low amounts for the non-humus carbon (about 6%) (Tables 2 and 3).

The humus materials left after the 6N acid hydrolysis were almost the same in these three methods (Tables 2 to 5). However, it should be mentioned, that particularly the humus carbon content by the direct method showed a slightly higher figure compared with that by the other two methods. Humus nitrogen remained more or less in similar quantities. This is possibly due to the formation of insoluble materials during prolonged acid hydrolysis in the direct method. Jansson and Persson (1968), in their fractionation studies, indicated that the alkaline extractables and the residual fraction together were the humus materials of soil organic matter. Approximately, similar amounts of humus materials were separated from the non-humus materials by the newly proposed method as revealed by the carbon and the nitrogen figures (Tables 2 to 5).

The newly proposed method also divides the non-humus fraction again into several fractions according to the hydrolyzability and solubility of the various compounds by different acid concentrations as well as water. This is an advantage over the direct method. This decomposable

non-humus fraction is not considered as one unit or one pool by several scientists in their studies (Jansson and Persson, 1968; Paul and Juma, 1981 and Van Veen *et. al.*, 1984). It comprised several different pools. Therefore, this method makes it easy to study the organic matter decomposition and transformations in detail after splitting the fraction into several decomposable sub-fractions.

In the conventional method the amount of nitrogen extracted by the acid was only about 5% in both soils. About 95% of nitrogen remained with humus materials. This figure seems to be very high. Such an amount of humus nitrogen cannot be present in normal mineral soils and some of the decomposable organic materials are still present in the humus materials. Some workers reported that the acid insoluble nitrogen fraction was around 20 to 35% (Bremner, 1965 and Stevenson, 1965).

The main criterion used to compare the methods was the content of humus material (humic acid and humin) which was left after the acid extractions. The new method separates the non-humus and humus materials as effectively as the Persson's method. It gives an idea about the soil organic matter status and the amounts of decomposable non-humified fraction as well as the resistant humus fraction of soil. This information would be helpful in soil fertility studies to maintain soil organic matter level. Therefore, the division may be considered a biologically meaningful separation.

The second criterion was the total acid hydrolysis time used by the different methods. Total time of acid hydrolysis was 6, 9 and 16 hours for the new, the Persson's and the direct method respectively (Table 6). Generally, these types of extractions are time consuming processes and are laborious (Gonzalez *et. al.*, 1984; Gonzalez-Prieto and Carballas, 1988). It is important to reduce the time used in fractionation methods when selecting a new method which at the same time separates the organic matter into meaningful fractions. The new method show an improvement in this aspect. A higher hydrolysis time makes soluble organic materials into insoluble forms, perhaps forms artifacts and leads to wrong interpretation of the results (Bremner, 1950; Asami and Hara, 1970; Ogner, 1970; Janel *et. al.*, 1979).

Table 6. The acid hydrolysis time (hours) used by the different fractionation methods.

Fraction	Newly proposed	Persson's	Direct
0.5N H ₂ SO ₄	1	1	
0.55N H ₂ SO ₄		5	
3.5N H ₂ SO ₄	2		
6N H ₂ SO ₄	3	3	16
Total	6	9	16

CONCLUSION

The newly proposed method is promising and proved to be much better than the other methods studied. It separates the soil organic matter into different fractions and makes them available for further study to gain better understanding of the organic matter decomposition. The separation of the humus and the non-humus organic materials is achieved by the new method as equally well as the direct and the Persson's methods. The time required for the acid hydrolysis is less compared to the other methods and as such the method is less laborious.

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