Studies on Soil Humus with Special Reference to Its Molecular Weight Systems

By AKIO INOKO*
MAKOTO TAMAI**

* Department of Soils and Fertilizers, National Institute of Agricultural Sciences
** Faculty of Agriculture, Miyazaki University

Numerous information concerning soil organic matter and/or soil humus has been accumulated through century-long studies. Nature and properties of soil humus, however, are still obscure at present, owing mainly to its extremely complicated composition.

According to reviews and reports on soil humus problems (Dubach et al.[2] and others), soil humus in a broader sense can be conveniently divided into two groups of substances: non-humic and humic substances. Non-humic substances include all classes of compounds occurring in living organisms, e.g., carbohydrates, proteins, lignins, and others. They consist mainly of substances of high molecular weights, while the substances having lower molecular weights, such as sugars, amino acids, and phenolic compounds are found scarce in soils. Humic substances, as Heteropolycondensates (Kononova[5]), consist of acidic substances, with relatively high wide-ranged molecular weight, and yellow to dark brown color, that are produced in soils and probably not belonging to any of organic compounds classified in organic chemistry. Therefore, it should be noted that soil humus including non-humic and humic substances shows complicated characteristics with respect to their molecular weights and compositions.

For the research on such complicated substances as those polymers, it is generally understood that charatceristics variations of their nature with respect to their molecular weights as well as their molecular weight distributions should be clarified at the first step of the research (Inoko[3]). From this point of view the authors[4] carried out a study on characteristics variations of extensively fractionated humic acid samples with respect to their molecular weights by using the gel permeation chromatographic techniques.

Estimation of molecular weight distributions of soil humic matter

Referring to the dispersion for molecular weights of humic acids repotred by Mehta et al.[6], the authors examined the molecular weight distributions by fractionating soil humic matter with respect to molecular weight by using the gel permeation chromatographic techniques. As a gel matrix, Sephadex -dextran gel was seletced for this purpose (Morris et al.[7]).

In this research, seven soil humic fractions with different nominal molecualr weights were prepared by the gel permeation technique using seven kinds of Sephadex gels with varying exclusion limits. It was observed that these fractions were eluted from the Sephadex G-200 column in the order of their molecular weights. As shown in Fig. 1, it was also found that there is a linear relationship between partition coefficients, Kd values, of each humic fraction from the column and the logarithm of molecular weights of the fractions.

Using this relationship, curves of molecular weight distributions can be obtained from the elution curves of soil humic matter and/or humic acids by converting elution volumes to

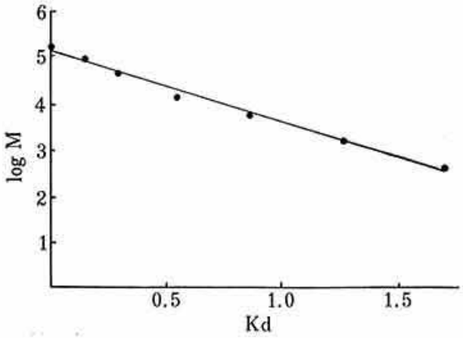


Fig. 1. Relationship between Kd values from the Sephadex G-200 column and molecular weights of humic fractions
Kd : partition coefficient
M : molecular weight

molecular weight values. The gel permeation procedure using Sephadex G-200 monocolumn was developed by the authors for the estimation of molecular weight distribution and/or fractionation with respect to molecular weight.

Soil humic matter and/or humic acids examined in this research were prepared from two kinds of soils, Kuriyagawa humic allophane soil (C 11.03%, N 0.69%) and Fujishima mineral paddy soil (C 1.23%, N 0.11%), through sodium pyrophosphate extraction. As shown in Fig. 2, it was found that the molecular weight of soil humic matter ranged from 0.1×10^4 to more than 20×10^4 with the average molecular weight from 0.5×10^4 to 1.0×10^4.

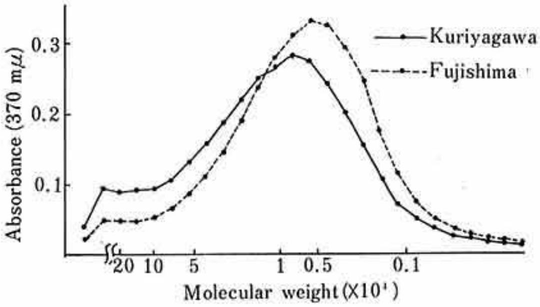
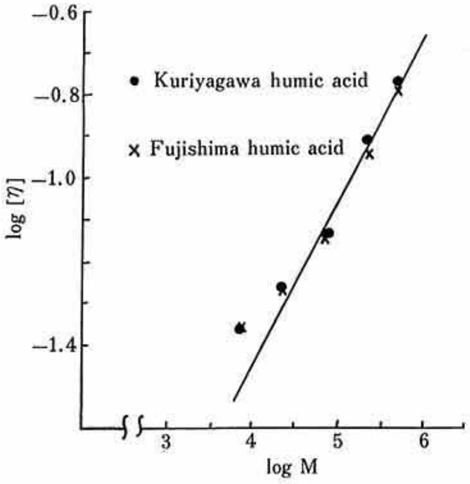


Fig. 2. Molecular weight distribution curves of Kuriyagawa and Fujishima humic matter



M : molecular weight
[η] : intrinsic viscosity

Fig. 3. Relationship between intrinsic viscosities and molecular weights of humic acid fractions

It was observed that intrinsic viscosity of each humic acid fraction increases with the increase of molecular weight of the fractions, showing a linear relationship between logarithm of molecular weight of the fractions and those of their intrinsic viscosities as revealed by Staudinger's equation (Fig. 3). The average molecular weights of the unfractionated humic acids, 3.7×10^4 for Kuriyagawa soil and 3.6×10^4 for Fujishima soil calculated by the Staudinger's equation from the data of intrinsic viscosities show a satisfactory coincidence with those figures calculated from the weight distributions of each humic acid fratcion by the author's procedure, 5.8×10^4 and 4.7×10^4 respectively. From these results it was recognized that this procedure is reasonably applicable to the fractionation of soil humic matter and/or humic acid with respect to their molecular weights.

Physico-chemical properties of humic acid fractions

Some physico-chemical properties of humic acids fractionated by the authors' procedure were correlated with their molecular weights. As shown in Table 1, absorbance at 370 mμ and C/N decrease generally with increasing molecular weights of the fractions, whereas no definite trend was found between $\Delta \log k$ and molecular weights of the fractions.

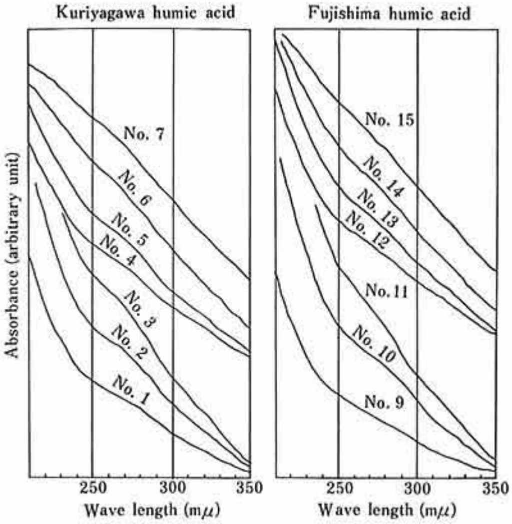
Spectral absorption characteristics of humic acid also vary with the molecular weights of the fractions. In the visible wave regions, logarithm of absorbances varys with wave lengths, following the linear regression equations with standard errors of estimates, listed in Table 2. From these equations, with a few exceptions, it is recognized that the absorbance at any wave length decrease with increasing molecular weights of the fractions. The particular pattern of absorption at 280 mμ, which originated from phenolic compounds derived from lignins, becomes more

Table 1. Some chemical properties of humic acid fractions

molecular weight ($\times 10^4$)	$\Delta \log k$*	absorbance**	C/N
	Kuriyagawa humic acid		
>20	0. 65	11. 5	10. 5
20—10	0. 62	16. 7	9. 6
10— 5	0. 61	18. 1	10. 2
5— 1	0. 61	21. 8	11. 5
1—0. 5	0. 63	27. 0	11. 0
0. 5—0. 1	0. 67	37. 7	14. 2
<0. 1	0. 66	18. 6	17. 8
	Fujishima humic acid		
>20	0. 73	7. 3	9. 9
20—10	0. 76	9. 8	11. 6
10— 5	0. 74	14. 1	9. 8
5— 1	0. 74	17. 3	11. 1
1—0. 5	0. 78	20. 4	12. 5
0. 5—0. 1	0. 85	28. 1	13. 2
<0. 1	0. 85	26. 7	19. 6

* logarithmic figures of the difference between absorbance at 400 mμ at 600 mμ

** $-\log T$ (370 mμ/C mg/ml)



No. 1, 9, molecular weight $>20\times10^4$; No. 2, 10, $(20-10)\times10^4$; No. 3, 11, $(10-5)\times10^4$; No. 4, 12, $(5-1)\times10^4$; No. 5, 13, $(1-0.5)\times10^4$; No. 6, 14, $(0.5-0.1)\times10^4$; No. 7, 15, $<0.1\times10^4$

Fig. 4. Ultraviolet spectra of humic acid fractions

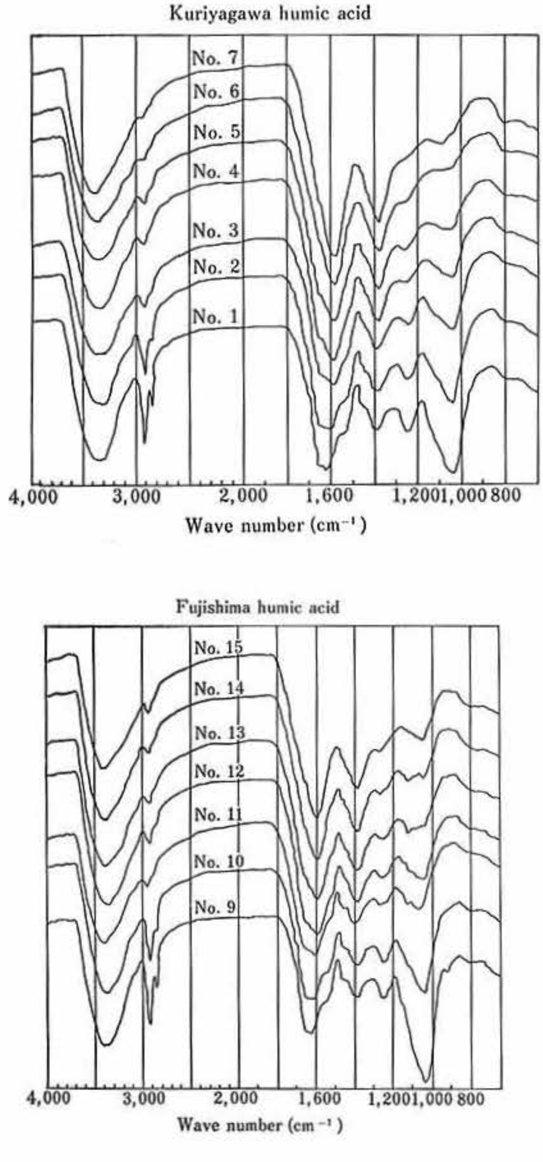
Table 2. Light absorption of humic acid fractions, as expressed by regression equations between logarithmic figures of absorbances and wavelengths in visible regions

molecular weight ($\times 10^4$)	Kuriyagawa humic acid		Fujishima humic acid	
	regression coefficient	standard error of estimate	regression coefficient	standard error of estimate
>20	$y=0.99-3.23x$	0. 0198	$y=1.37-5.14x$	0. 0637
20—10	$y=1.02-3.05x$	0. 0133	$y=1.04-3.78x$	0. 0303
10— 5	$y=1.33-3.50x$	0. 0497	$y=1.39-4.32x$	0. 0531
5— 1	$y=1.28-3.09x$	0. 0175	$y=1.51-4.36x$	0. 0404
1—0. 5	$y=1.12-2.81x$	0. 0559	$y=1.70-4.69x$	0. 0662
0. 5—0. 1	$y=1.27-3.13x$	0. 0763	$y=1.96-5.06x$	0. 0727
<0. 1	$y=1.75-3.90x$	0. 0658	$y=1.96-4.79x$	0. 0741

$y=\log$ (absorbance), $x=$ wave length(μ)
$[\text{Absorbance}]^{0.01\%}_{1\text{cm cell}}$

distinct with increasing molecular weights of the fractions (Fig. 4).

Some of the absorption bands in the infrared wave region of each humic acid fraction appear to have a correlation with their molecular weights (Fig. 5). The absorptions at 2,910–2,850 cm^{-1} for C-H stretching of aliphatic compounds, at 1,700–1,600 cm^{-1} for aldehydes and Ketones, at 1,550–1,500 cm^{-1} for C=C and nitrogen containing heterocyclic compounds, at 1,450 cm^{-1} for methyl and methylene groups, and at 1,040 cm^{-1} for O-H stretching of primary alcohol increase with increasing molecular weight of the fractions. It was also found that the absorptions of shifted 1,600 and 1,400 cm^{-1}, originated from the functional group of dissociated carboxylate ions decrease with increasing molecular weights of the fractions.



No. 1, 9, molecular weight $>20\times10^4$; No. 2, 10, $(20-10)\times10^4$; No. 3, 11, $(10-5)\times10^4$; No. 4, 12, $(5-1)\times10^4$; No. 5, 13, $(1-0.5)\times10^4$; No. 6, 14, $(0.5-0.1)\times10^4$; No. 7, 15, $<0.1\times10^4$

Fig. 5. Infrared spectra of humic acid fractions

Biochemical properties of humic acid fractions

As shown in Fig 6, the distribution of nitrogeneous compounds after acid hydrolysis

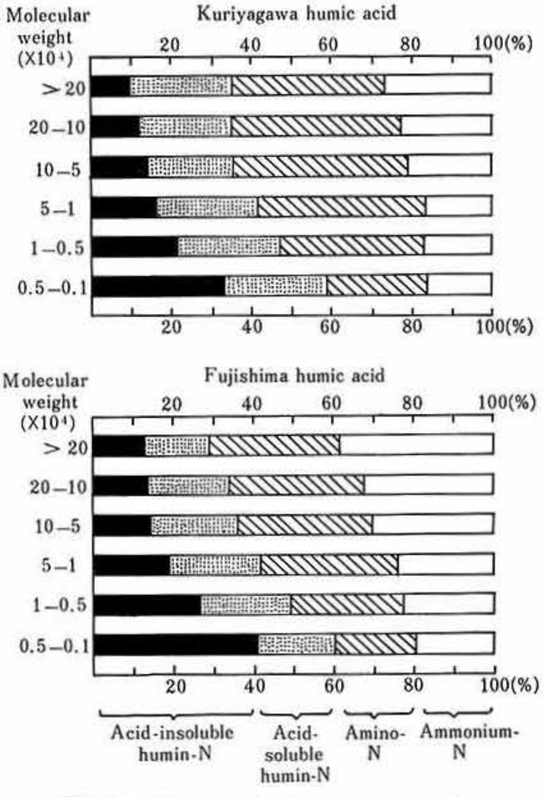


Fig. 6. Nitrogen distributions of humic acid fractions

Table 3. Nitrogen contents of humic acid fractions which are hydrolysable by proteolytic enzyme, pronase

molecular weight ($\times 10^4$)	Kuriyagawa humic acid		Fujishima humic acid	
	percent for total-N	percent for amino-N	percent for total-N	percent for amino-N
>20	5.7	15.2	5.1	15.7
20—10	5.2	12.6	4.1	12.3
10—5	4.8	11.3	3.2	9.7
5—1	2.1	5.7	2.3	6.5
1—0.5	1.2	3.6	1.3	4.6
0.5—0.1	0.2	0.8	—	—

Table 4. Sugar contents of humic acid fractions (equivalent glucose percent)

molecular weight ($\times 10^4$)	Kuriyagawa humic acid	Fujishima humic acid
>20	7.13	7.23
20—10	4.30	5.84
10—5	3.63	5.15
5—1	2.15	2.54
1—0.5	0.85	1.09
0.5—0.1	0.05	0.34

is found to have a close relationship with their molecular weights. For instance, the nitrogen distribution of acid-insoluble humin which seems to be the most resistant to microbial attack, markedly decreases with increasing molecular weights of the fractions. The distributions of acid-hydrolysable ammonium and amino nitrogen also increase with increasing molecular weights of the fractions.

The contents of nitrogen in the fractions of humic acids which are hydrolysable by a proteolytic enzyme, pronase, increase with increasing molecular weights of the fractions. This means that a protein-like substance having peptide bonds should exist in the fractions of high molecular weight regions (Table 3).

Sugar content of each humic acid fraction, determined by the anthrone method, increases with increasing molecular weights of the fractions (Table 4). The sugar content of the highest molecular weight fraction, namely more than 20×10^4, reaches up to 7%, whereas it is extremely low in lower molecular weight fractions. Occurrence of polysaccharide in higher molecular weight fractions is also assumed.

Discussion and conclusion

A gel permeation procedure developed by the authors seemed to be reasonably applicable to the estimation of molecular weight distribution and/or fractionation of soil humic matter with respect to their molecular weight. Using this procedure, it was found that the molecular weights of soil humic matter examined ranged from 0.1×10^4 to more than 20×10^4.

From the results of correlations of some properties of humic acid fractions with their molecular weights, it can be summarized as follows;

1. Characteristic properties of humic acids vary successively with their molecular weights, as reported by Butler et al.[1] and Swift et al.[9]
2. The fraction with high molecular weights holds much stronger aliphatic properties, which decreases the light absorbance and the carboxyl contents, and is rich in non-humic substances such as proteins, polysaccharides, and phenolic compounds derived from lignins.
3. In the fractions with lower molecular weights, these non-humic substances mentioned above decrease rapidly together with the aliphatic propetries, whereas their light absorbances and carboxyl contents of the fractions increase.
4. The characteristic humic substances,

which should show high light absorbances and strong aromatic properties, are assumed to be contained in the fractions of less molecular weight than 1×10^4. This means that apart from the really high molecular group such as proteins, lignins, polysaccharides, etc., the humic substances is formed in the range of molecular weight at about 1×10^4 separately.

In relation to the authors' assumption for the formation of humic substances in soil as shown in para. 4 mentioned above, it is noted here that the fractions with lower molecular weights obtained by the authors consist of stable substances. Similar assumption for soil humus formation is reported by Swift et al.[9] In the beginning, there are many organic residues in soil left from the animals, plants, and microbes; most of them are supposed to be lignins, proteins, polysaccharides, and others, known as high molecular weight substances. They might be decomposed by microbial activities since the residues are left in soil. At the first step, it seems that a light colored humic substance is formed through the decomposition of organic residues (Swaby et al.[8]). At this step, there still remain easily decomposable residues, but the rate of the decomposition might become slower gradually with the progress of that change. It is apparently true that the molecular weights of the whole organic matters at this step are just lowering by the decomposition; namely during such process the aliphatic properties may decrease, carboxyl functional group may increase and other tremendous chemical reactions proceed. Finally the aromatic properties may increase more with the increases of double bonding, resulting in the formation of stable humic substances which must show high light absorbances and dark colors. Incidentally, the humus is formed along the direction of decreasing molecular weights of the original residues. Therefore, the substances with molecular weights in the range of about 1×10^4, that was obtained by the authors, seemed to be composed of real humic materials. They are not the materials for humus formation.

This concept is clearly opposite to the current theory on humus formation, i.e. humic substances must be the endproducts formed through many step of polymerisation and condensation of organic compounds. Due to the fact that natural humus covers a very wide range in the molecular weight distribution, it may be true that the process of humification is in the direction of lowering molecular weights instead of increasing them. In any case, it is very difficult to separate non-humic and humic substances by any chemical reaction except the molecular-sieve methods developed by the authors and others.

References

1) Butler, J. H. A. & Ladd, J. N.: The effect of extractant and molecular size on the optical and chemical properties of soil humic acids. *Aust. J. Soil Sci.*, **7**, 229–239 (1969).
2) Dubach, P. & Mehta, N. C.: The chemistry of soil humic substances. *Soils and Fertilizers*, **26**, 293–300 (1963).
3) Inoko, A.: Approach to the humus research from polymer science. Soil science and plant nutrition in modern agriculture, 3, Tokyo, Yokendo, 36–43 (1972) [In Japanese].
4) Inoko, A. & Tamai, M.: Studies on soil humus with special reference to its molecular weight systems. *Bull. Nat. Inst. Agr. Sci.*, B. 28, (1976) [In Japanese].
5) Kononova, M. M.: Soil organic matter, 2nd ed., Oxford, Pergamon Press, 94–110 (1972).
6) Mehta, N. C., Dubach, P., & Deuel, H.: Untersuchungen über die molekulargewichtsverteilung von Huminstoffen durch Gelfiltration an Sephadex. *Z. Pflanzenernähr. Düng Bodenk.*, **102**, 128–137 (1963).
7) Morris, C. J. O. R. & Morris, P.: Separation method in Biochemistry, London, Pitman, 365–448 (1964).
8) Swaby, R. J. & Ladd, J. N.: Chemical nature, microbial resistance, and origin of soil humus. Trans. Joint Meeting Comm. IV and V, Intern. Congr. Soil Sci., Wellington, Wright and Carman, 197–202 (1962).
9) Swift, R. S., Thornton, B. K., & Posner, A. M.: Spectral characteristics of humic acid fractionated with respect to molecular weight using an agar gel. *Soil Sci.*, **110**, 91–99 (1970).