

MOLÉCULAR SIZE DISTRIBUTION OF HUMIC ACIDS EXTRACTED FROM WATER STABLE AGGREGATES OF DIFFERENT SIZES

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ABSTRACT

Humic Acids (HA) extracted by 0.1M NaOH-pyrophosphate mixture from water stable aggregates of different sizes (0.25, 0.5, 1.0, 2.0 mm) were fractionated using Sephadex gels into molecules of different size ranges. It was observed that smaller sized aggregates contained greater proportion of high molecular weight of humic acid molecules (> 10,000) in contradistinction to larger aggregates which contained more of small molecules (< 700). The size range of HA molecules as a factor in determining aggregate size in soil is discussed.

Keywords: Soil aggregation, Humic acid, Water stable aggregates, Molecular size.

1. INTRODUCTION

A good soil structure, essential for healthy plant growth, is a function of water stable aggregates therein. Good tilth also requires that the aggregates be present in the soil must be of such size range that they permit optimum plant growth. Attempts have been made to develop indices to denote the aggregates status of soils for comparative purposes [1, 2].

The ability of soil aggregates to withstand collapse on wetting is imparted by both inorganic and organic cementing materials. While there is controversy over the relative importance of these two groups of substances in bringing about aggregate stability, carbohydrates and humic acids (HA) are the organic substances most commonly implicated in the phenomenon [3-6]. The binding of the soil particles is due to the free functional groups in the organic substances. The type, number and availability of these groups are related to the nature of the organic substance under study.

Allison [7] who wrote one of the finest critiques on soil aggregation had this to say about the role of humic acids in the phenomenon: "Very large

humic acid or humate molecules are ineffective as binding agents because they are too big, are spherical instead of linear, have an undesirable configuration, and have only a limited number of functional groups that are free to attach themselves to clay particles. By making conditions more suitable for root ramifications this stable type of humus does indirectly increase aggregation and as already stated helps to keep the aggregate small". Since it is known that HA is polydisperse and occur over a wide size range I wondered whether there would be any relation between the size of soil aggregates and the molecular weight of HA present in them. While these studies were in progress Dell'agnola and Ferrari [8] reported that the proportion of larger molecular weight HA was more in aggregates of greater water stability, whereas the less stable aggregates contained more of smaller sized HA. They also found that the stability of aggregates was related to functional groups present, particularly to the OH:COOH ratio.

2. EXPERIMENTAL

(a) *Source of soil and aggregates*

Red sandy loam soil (Sand 58.9%, Silt 4.8%, Clay 36.3%, Carbon 1.02%, Nitrogen 0.08-0.1%, pH 7.0-7.4, CEC 14-16 meq/100 g, WHC 33.3 ml/100 g) from a virgin area in the Institute campus (top 10 cm) was collected and without any further treatment was wet sieved through a nest of sieves of 2.00 mm, 1.0 mm, 0.5 mm, 0.25 mm mesh size. Water stable aggregates retained on each sieve was collected, air dried, and stored. Water stable aggregates of different sizes were also obtained by the following way. For this the above soil was gently powdered and air dried. After air drying the soil was passed through 0.125 mm sieve and the fraction that passed through the sieve was collected. About 2 kg of this sample was amended with starch and ammonium sulfate (to 1.0% and 0.1% levels respectively) and transferred to one litre beakers. Moisture was brought to 60% and was incubated at room temperature for over a year. The soil was then wet sieved and water stable aggregates of different sizes were collected.

(b) *Extraction of Humic Acids*

Humic acids from the whole soil or the different sized aggregates were extracted with NaOH. $\text{Na}_2\text{P}_2\text{O}_7$ mixture (0.1 M with respect to each of the compounds). HA was precipitated with conc. H_2SO_4 at pH 2.0. The precipitate was washed with distilled H_2O dissolved in 0.1 N NaOH and reprecipitated with H_2SO_4 . The reprecipitation was repeated several times till the supernatant on addition of acid was colourless. The precipitate

was then dissolved in 0.1 N NaOH and was dialysed against distilled water till there was no change in the pH of the water. This gave stable solutions of HA.

(c) *Estimation of organic carbon*

Organic carbon in the soil and HA was determined by dichromate digestion [9]. HA was also determined by measuring the optical density (OD) of the solutions at 450 nm in a Bausch and Lomb colorimeter. A separate standard curve was found to be essential for each molecular size range.

(d) *Determination of molecular size range*

The proportion of different sized molecules present in HA was determined by fractionating them through Sephadex Gel Columns of different grades, following essentially the methods of Rashid and King [10]. Two ml of HA solution containing about 2 to 5 mg C was added on top of the column. After all the sample has entered the Gel bed the column was eluted with glycine-NaOH buffer at pH 10. The HA fraction coming out with the void column was collected in 3 to 5 ml, and the 'organic matter' was estimated by checking the OD at 450 nm as described above and also by dichromate digestion. The amount retained by the Gel was obtained by calculating the difference between the amount added on top and the amount excluded by the Gel column. The proportion of any molecular weight range was obtained by calculating the difference the portion excluded by one grade of the gel and the portion retained by the next higher grade. Sephadex G10, G15, G25, G50 and G75 were used in the studies.

3. RESULTS AND DISCUSSION

Humic acids from soil and sediments are known to occur over a wide size range. Though the distribution of different sizes has not been determined in every instance their sizes have been reported from a few hundreds to millions. Mehta *et al.* [11] and Dubach *et al.* [12] have reported a range from 2,000 to 40,000 molecular wt. and Posner [13] found that over 55% of HA from soil had molecular weights under 10,000. In these studies the molecular weight HA from Bangalore soil did not exceed 50,000. In all cases there was no exclusion from G75 Sephadex column. The use of gel filtration methods for determining the molecular weights of materials is now a common practice. However Cameron *et al.* [14] have cautioned the advisability of calibrating the columns for each class of materials separately instead of relying upon the manufacturer's data. For instance, Sephadex

columns calibrated for globular proteins may not be suitable for HA type molecules. However in the present studies the approach was to determine the fraction of HA excluded by each grade of Sephadex and the results obtained gave reliable data.

The distribution of different sized molecules of HA obtained from whole soil as well as the water stable aggregates obtained from wet sieving 0.125 mm soil incubated in the laboratory is presented in Table I. The

TABLE I

*Organic carbon content and molecular size distribution of humic acids in water stable aggregates of different sizes**

Aggregate size	Organic Carbon mg/100 g		Distribution of Humic Acids according to molecular wt ranges (%)				
	Total	In Humic Acid**	700 (G 10)	700- 1,500 (G 15)	1,500- 5,000 (G 25)	5,000- 10,000 (G 50)	10,000- 50,000 (G 75)
Whole soil	630	286	16.7	15.3	8.0	40.0	20.0
0.25 mm	707	318	2.0	39.0	8.0	9.0	42.0
0.50 mm	614	302	12.0	38.0	12.0	3.0	37.0
1.00 mm	631	252	18.0	24.0	12.0	29.3	17.7
2.00 mm	568	274	19.0	43.0	13.0	12.5	12.5

* Aggregates obtained from wet sieving soil (< 0.125 mm) incubated in the laboratory for 12 months.

** Acid precipitable fraction of 0.1 M NaOH-pyrophosphate extract. Over 50,000 (G 75)—No exclusion in all cases.

pattern shows interesting relationships with the size of the soil aggregates. In general the bigger the aggregate size the greater was the proportion of the small molecular weight HA present in the extract. Conversely high molecular weight HA (10,000 to 50,000) accounted for bulk of the HA in small sized aggregates (0.25 mm). The intermediate sized HA molecules showed a somewhat mixed trend. It is likely that as the HA molecule becomes bigger fewer reactive groups are available for binding soil particles, which may tend

to keep the aggregates small; whereas small molecular weight HA may still be linear with more functional groups available for keeping soil particles together. The results reported here appear to be at variance from those of Dell'agnola and Ferrari [8] mentioned earlier; but these authors studied only larger sized aggregates and restricted their investigations to only higher molecular size range. Further they were trying to correlate the water stability of aggregates and size of HA molecules. It is quite likely that high molecular weight HA bestows greater hydrophobicity and thus stability to aggregates.

The size of HA in soil may grow in size by the activities of organisms building up humus, while another group of organisms may degrade it and keep the molecular size small. The nature of equilibrium between these two opposing activities may play a part in deciding the aggregate size. The absorption of HA by clay particles is mainly brought about by cation bridges and such binding plays an important role in bringing about soil aggregation. Rashid [15] reported that the cation complexing ability of HA was inversely related to the molecular size. He stressed the importance of low molecular weight fractions in metal complexing phenomenon. Fractions of less than 700 molecular weight complexed 3 to 4 times more cations than high molecular weight fractions. These observations impart considerable significance to our results on low molecular weight HA in aggregate formation and stabilisation.

Small sized molecules may still be linear with their reactive groups exposed; but as the molecule elongates and grows in size, they may bend and form globular structures. Larger sized globular molecules can have more reactive groups but all of them may not be available for binding soil, but they may bestow more hydrophobicity to the aggregates.

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