CHAPTER - III

MATERIALS AND METHODS

Preparation of Samples

A. Isolation, Fractionation and Purification of Soil and Peat Humus

(1) Soil Humus

Humic matter, subsequently fractionated into fulvic, humic and hymatomelanic acids, was isolated from the forest soil (0-15 cm depth) of Raja Rammohanpur in the district of Darjeeling (India). The organic matter content of this soil was found to be 1.41%, determined by Walkely and Black method[1]. Below is furnished the procedure followed in the extraction, fractionation and purification of this humus with 0.3 M (NaOH) solution used as an extracting agent.

About 5 kg of the soil moistened overnight with water was shaken with 0.05 M HCl and left over a day for the decomposition of the carbonates. Finally washed free of HCl, the soil was mixed with about 3 litres of 0.3 M (NaOH) at the room temperature followed by intermittent shaking of the mixture and lastly it was kept overnight to stand. The dark coloured liquied thus obtained in the process was centrifuged in the refrigerated centrifuge, model K-24, MLW (G.D.R.) at 20,000 r.p.m. to remove the clay substances and the humin fraction. The crude humic acid was separated from the centrifugate by acidifying it with the dil. HCl to pH 1-2 and freed from the supernatant by centrifugation. The centrifugate containing fulvic acid was preserved for subsequent treatments.

The crude humic acid was redissolved in dilute NaOH, reprecipitated and separated as before. This process was repeated a few times. The precipitate was then dialysed against distilled water through the dialyser tubing (Arthur H. Thomas Co., U.S.A.) after appropriate treatment[2]. The dialysed material was subsequently dried by an infrared lamp and then subjected to the soxhlet extraction by 95% ethanol. The alcohol insoluble fraction containing humic acid and the alcohol soluble fraction, called hymatomelanic acid, were dissolved separately in alkali and precipitated at pH 1-2. The precipitated humic and hymatomelanic acids were washed with distilled water and subjected to extensive dialysis as before.

As fulvic acids in solution slowly polymerise and they remain more stable in the form of Ba-salts, this acid contained in the centrifugate mentioned above, was precipitated as Ba-fulvate by adding $BaCl_2$ and raising the pH of the solution to 7.0 with dilute NaOH[3]. The precipitate was separated by centrifugation and washed with distilled water. Finally it was extensively dialysed with distilled water. Fulvic acid was regenerated from the Ba-fulvate by passing the aqueous suspension of the latter through a column of Amberlite IR - 120 resin in the H-form .

(2) Peat Humus

The peat humus, supplied by Fluka AG, was dissolved in 0.3 M (NaOH) solution and centrifuged to remove the alkali insoluble

humin fraction. The subsequent operation leading to the fractionation of this peat humus into fulvic and humic acids, and their purification were exactly similar to those adopted in case of soil humus .

Preparation of Model (Synthetic) humic acid

The model humic acid was synthesised from the mixture of benzoquinone and ammonium chloride. The methods of ladd and Butler[4] involving oxidative coupling of quinone were adopted in the preparation of these model compounds. The method is described below.

54.0 gms solid P-benzoquinone was added to the solution of 40.0 gms of ammonium chloride in 0.1 M potassium phosphate at pH 8.0, the total volume of the reaction mixture being 400.0 ml. The system was incubated at 45°c for 24 hours. The solution darkened rapidly on the addition of P-benzoquinone and the pH of the mixture dropped to the acidic region within an hour of the commencement of the incubation process. The pH was brought back to the original value (8.0) with alkali. The incubation period over, the mixture was centrifuged and the dark brown supernatent was retained. The residue was suspended in 0.3 M NaOH, stirred for five minutes, centrifuged and the centrifugate was added to the earlier one. Now the centrifugate obtained was subjected to the similar treatments as adopted with soil and peat humus to effect its fractionation into purified synthetic humic acid, the acid soluble fraction was however discarded .

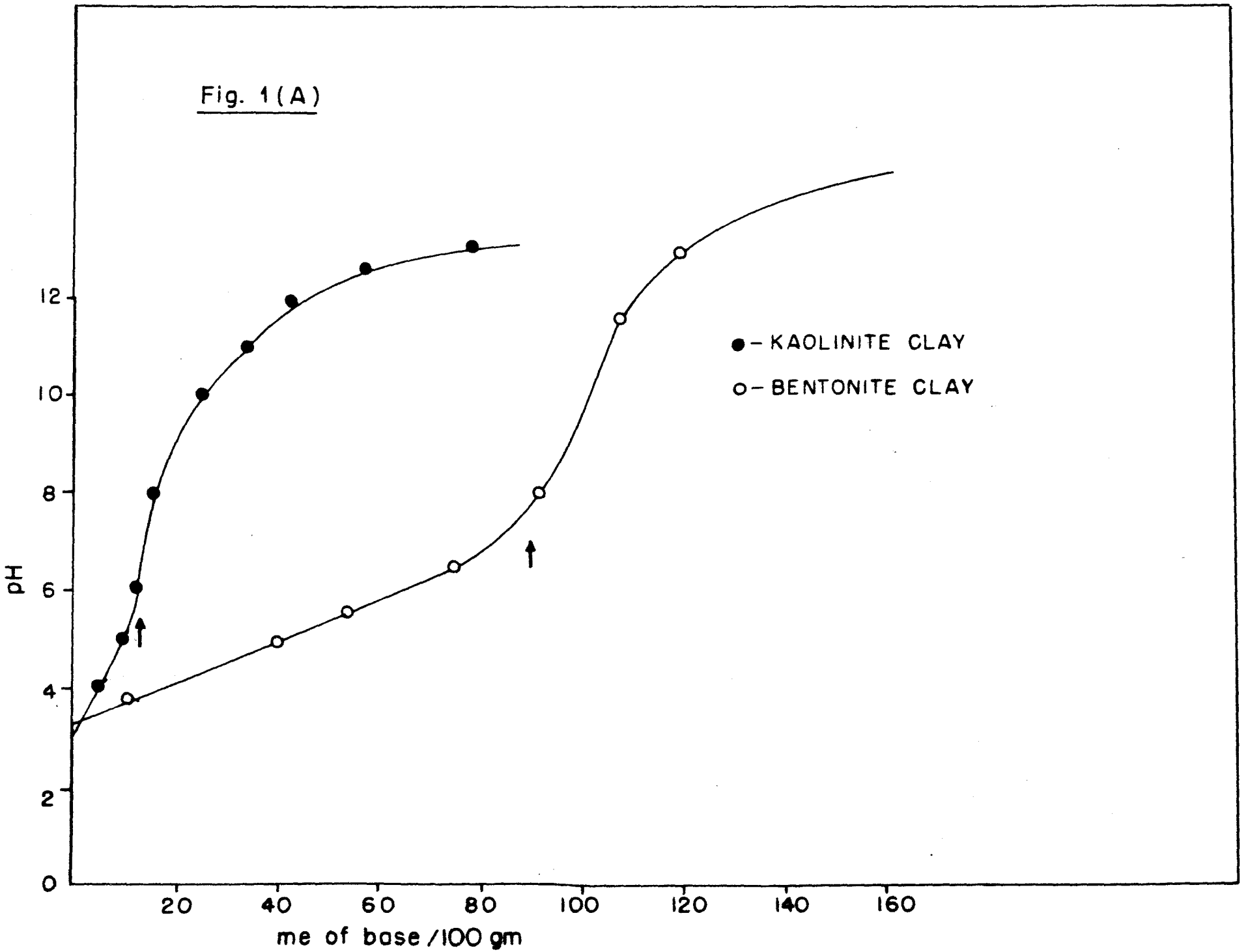
Symbol Used

(a)	Soil Humic Acid	-	SHA
(b)	Soil Fulvic Acid	-	SFA
(c)	Soil Hymatomelanic Acid	-	SHYA
(d)	Peat Humic Acid	-	PHA
(e)	Peat Fulvic Acid	-	PFA
(f)	Synthetic Humic Acid	-	SYNHA

C. Preparation of Clay Samples

In the present work two different samples of clay have been used, of which one belong to the Bentonite clay mineral (Source - Evans Medical Ltd. England, Batch No. C-57084) and the other one is the kaolinite clay mineral (Source - B.D.H., Bombay, Batch No. C-82703). The original samples of each kind were dispersed in water according to the international method[5] using NH_4OH as the dispersing agent. The clay fraction ($< 1\ \mu$) of each was collected at a depth of 10 cm at the end of 24 hours settling. The clay suspension was slightly coagulated with dilute hydrochloric acid and thoroughly treated with hydrogen peroxide to destroy organic matter. It was then subjected to electrodialysis. The Sol chamber is made of thick drawing paper (Kent paper) wrapped round a wooden frame and carefully paraffined at the junctions to prevent leakage. The anode chamber is a porous porcelain tube. The cathode is a galvanised iron gauge made into the form of a cylinder round the sol chamber and placed in the cathode vessel. The anode is a platinum foil dipped in water placed in the anode chamber. A continuous automatic flow of water was maintained from the anode to the cathode compartment through the sol chamber. The completion of electrodialysis was marked by absence of alkalinity in the dialysate. A period of 6-10 days was usually required for the complete

conversion of 40-50 gms of the material into H-clay. Finally these dialysed H-clay materials were shaken with cation exchange resin (Amberlite IR-120 in the H-form) for 12-14 hours and then slowly passed repeatedly through the same resin columns to get H-clay in the purest form. After this, the clay was dispersed in conductivity water in jena bottles in a mechanical shaker. The pH values varied between 3.2 and 3.8. The cation exchange capacity of the pure H-clay was determined by half-saturated KCl-KOH method[6] and also from pH titration with standard NaOH solution (Fig. 1A).



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REFERENCES (CHAPTER - III)

1. Walkley, A. and Black, I.A. - Soil Sci., 37, 29 (1934).

2. Staufter, R.E. - Technique of organic chemistry, Vol. III (Ed. A. Weiseberger) Inter Science, N.Y., 313 (1950) .

3. Datta, C. and Mukherjee, S.K. - J. Indian Chem. Soc. 45, 555 (1968).

4. Ladd, J.N. and Butler, J.H.A. - Aust. J. Soil Res., 4, 41 (1966).

5. Robinson, G.W. and Richardson, M. - Imp. Bur. Soil Sci. Tech. Bull., 26, 1 (1933) .

6. Ganguly, A.K. - J. Phys. and Colloid, Chem. 55 1417 (1951) .