

STUDIES ON THE INTERACTION OF FOLIC ACID, 2, 4-D, AND MALATHION WITH NATURAL, SYNTHETIC AND MICROBIAL HUMIC ACIDS

M. ADHIKARI, B. MANDAL *and* P. SEN

College of Agriculture, Calcutta University, Calcutta

(Communicated by Dr. S. P. Raychaudhuri, F.N.A.)

(Received 20 December 1978)

A study has been made on the interactions of folic acid, 2, 4-D and malathion with natural, synthetic and microbial humic acids by means of electrometric and IR spectroscopy. The bonding sites on humic acid are considered to be $-\text{COOH}$, $-\text{OH}$ (phenolic), $-\text{C}=\text{O}$, $>\text{NH}$, $-\text{NH}_2$. The relative interacting power of the different humic acids are synthetic $>$ microbial $>$ natural and in most cases H-bonding is mostly responsible for such interactions although other weak forces are taking part in it.

INTRODUCTION

HUMIC substances are known to interact with metal ions, hydrous oxides and clays. Recently (Hayes *et al.*, 1968), much interest is being shown on the ability of humic substance to interact with a variety of organo-chemicals because they can act as key factor in the behaviour of such chemicals in soil as well as in aquatic environment. Bioactivity, persistence, biodegradability and leachability bear a direct relationship with the humus content (Bailey & White 1964). Information as to how these toxic pollutants react with humus will provide a more rational basis for their effective use, thereby reducing the undesirable side effects due to carryover and contamination. Because of the unique properties of humus materials to adsorb a diverse group of substances present in soil, it is also expected that they are able to interact with some of the vitamins and antibiotics present in the soil. Though such studies are very rare, they have much importance in connection with the soil microbial ecology. B-vitamins are present in fertile soils and the presence of these vitamins play indirect role in establishing the microbial equilibrium since a large number of soil organisms require or produce these compounds and among the different vitamins required for the growth of micro organisms, vitamin B₁₂ (folic acid) is one of the most commonly required factors. On the other hand, work of Kozar *et al.* (1971) on the influence of vitamin B₂, folic acid and humic substances (sodium humates and humophos) on yield and oil formation in sunflower seeds shows an increase in oil percentage.

Adsorption of folic acid by humic substances will help to minimize the leaching loss of this valuable vitamin from the soil and in maintaining the microbial equilibrium.

In the present paper, the interaction products of folic acid, 2, 4-D and malathion with natural, synthetic and microbial humic acids separately have been studied by means of electrometric titrations and IR spectroscopy. The object of this study is to find the structural modifications of humic acid through these interactions, the bind-

ing sites involved and at the same time to compare the interacting power of natural humic acid with that of synthetic and microbial humic acids.

MATERIALS AND METHODS

Natural humus was isolated from pond sediments (P. S.) (0-15 cm depth, Bighra, Burdwan) by 0.5 (N) Na_2CO_3 solution and humic acid was precipitated by raising the pH to 2-3 by dil HCl (Adhikari *et al.*, 1970). The methods of preparation of synthetic humic acid and production of microbial humic acid were followed as described in the previous communication (Adhikari *et al.*, 1978). The usual procedures of fractionation and purification were followed in all the cases.

METHODS OF INTERACTION OF FOLIC ACID, 2, 4-D (SODIUM SALT) AND MALATHION WITH HUMIC ACID

0.1 per cent solution of folic acid (BDH, AR) in distilled water was made with the addition of dilute NaOH just to obtain a clear solution. The pH of the resultant solution was 7.6. The different compositions of the complexes, that is, the humic acid is to folic acid ratio were determined from the breaks/inflexions in graphical plot of the resultant pH vs. sodium folate added. The mixtures were allowed to equilibrate for seven days at room temperature with occasional shaking. The plots for each of the natural, synthetic and microbial humic acid are shown in Fig. 1.

A 200 ppm solution of 2, 4-D (Na-salt) was prepared by dissolving it in double distilled water. A saturated solution of malathion (Technical grade supplied by Cynamide India Ltd.) containing 150 ppm was made in double distilled water.

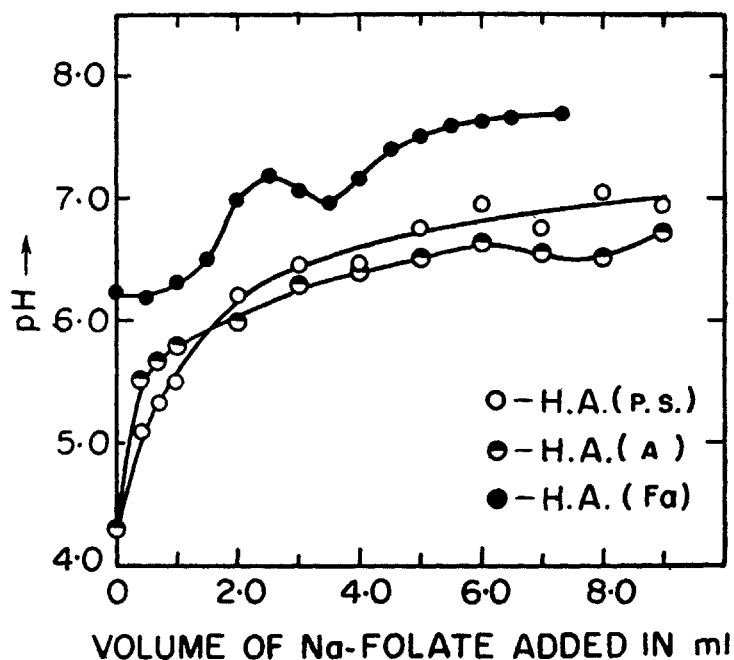


FIG. 1. Titration curve of H.A. vs. Na-Folate.

For the interaction of 2, 4-D (Na-salt) and malathion with different humic acids, known amounts of the different humic acids were taken in dialysis tubings and immersed in different sets of 2, 4-D and malathion solutions and was allowed to equilibrate for three days at room temperature following the technique of Hayes *et al.* 1968.

Electrometric studies

Both continuous and discontinuous potentiometric titrations of the different humic acids and of their respective complexes with folic acid, 2, 4-D and malathion were carried out with the aid of glass electrode pH meter using standard solutions of NaOH (BDH, AR) and Ba (OH)₂, (BDH, AR). Total acidities were determined from titration with Ba(OH)₂ solution up to pH 9.0 and —COOH groups by shaking with calcium acetate solution, allowing to equilibrate overnight and then titrating the resultant solution with standard NaOH solution up to pH 9.0 (Schnitzer & Khan, 1972). The amount of phenolic —OH was determined in each case by subtracting carboxylic acidity from the total acidity.

IR Spectroscopy

Thoroughly dried samples (1.5 mg each) were dispersed in KBr (200 mg) and pressed disc were prepared. The spectra were recorded at room temperature with the aid of a Beckmann model 20 infrared spectrophotometer fitted with a NaCl prism.

RESULTS AND DISCUSSION

The data of the electrometric titrations and IR spectroscopy for the different humic acids were shown in the previous paper (Adhikari *et al.*, 1978). Among the different synthetic humic acids, the results of the interaction products of one viz., humic acid from hydroquinone and glycine (symbolized as *HA (A)*) and that of one microbial humic acid from strachybotrys atra (symbolized as *HA (F_a)*) are presented here as their representatives. The different electrochemical parameters viz. initial pH, *pK_{av}* (pH at 50 per cent neutralisation) total acidity, decrease/increase in acidity, decrease/increase in COOH groups and phenolic —OH groups after interaction are shown in Table I. Comparing these values with that for the respective parent humic acid, it is seen that the initial pH values of the complexes with folic acid and 2, 4-D are slightly higher than their respective parent counterpart. This is partly due to the neutralization of the acidity of humic acid as these interacting substances were added as their Na-salt and partly due to the blocking of —COOH and phenolic —OH groups of the humic acid through complexation. Thus, there is a decrease in total acidity of the complexes than the original humic acid. This decrease for folate complexes are much more for synthetic humic acid followed by microbial and natural humic acid. Decrease in individual —COOH groups and phenolic —OH groups also follows this order, synthetic > microbial > natural. Folic acid having N-containing groups like =NH, —NH₂—N =etc., is capable of forming H-bonds with C=O groups on the humic acid molecule such as

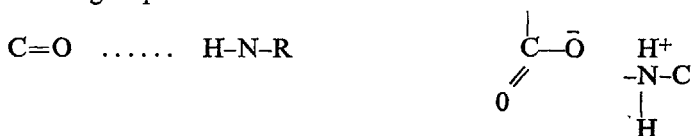


TABLE I
 Electrometric data

Sample	Initial pH	pK_{av}	C.E.C. (pH metric) meq/100 g	C.E.C. (H.A)— C.E.C. complex meq/100 g	—COOH groups blocked in meq/100 g	Phenolic—OH groups blocked in meq/100 g
H.A. (P. S.)	1. 6.2	6.8	370	55	38	17
+	2. 6.45	7.1	363	62	44	18
Folic Acid	3. 6.75	7.3	352	73	46	27
H. A. (A)	1. 5.8	6.4	280	73	46	27
+	2. 6.3	7.05	272	81	53	28
Folic Acid	3. 7.5	7.2	267	86	55	31
H. A. (F _a)	1. 7.2	7.6	326	60	38	22
+						
Folic acid	2. 7.4	7.9	312	74	48	26
H. A. (P. S.)						
+	4.8	6.55	388	37	25	12
2, 4-D						
H. A. (A)						
+	4.2	6.4	297	56	38	18
2, 4-D						
H. A. (F _a)						
+	4.5	6.6	345	41	26	15
2, 4-D						
H. A. (P. S.)						
+	4.2	7.15	390	35	9	26
Malathion						
H. A. (D)						
+	3.9	6.6	290	46	14	32
Malathion						
H. A. (F _a)						
+	4.0	6.7	346	40	12	28
Malathion						

Synthetic and microbial humic acids having more simpler structure than the natural and having the functional groups more exposed, can form such type of linkages more easily than the natural humic acid. Moreover formation of H-bonding with the C=O of the interacting substance with —NH, —NH₂, or —OH groups in the humic acid can not be ruled out. And such types of H-bonding are more expected in the case of 2, 4-D and malathion complexes.

For malathion complexes, the initial pH values are more or less same as it was for the parent humic acid and the total acidities are slightly increased. This may be partly due to the hydrolysis of the ester group (—COOC₂H₅) of malathion and H-bonding through —COOH groups of humic acid with 'P' or 'S' of malathion may occur. Hence the amount of —COOH groups are more or less same as it was before whereas the amount of phenolic —OH are of somewhat lower value which also indicates a type of H-bonding with the C=O of the ester group and with 'P' or 'S' of the malathion molecule. In all cases, the Van der Waals type of forces are also involved.

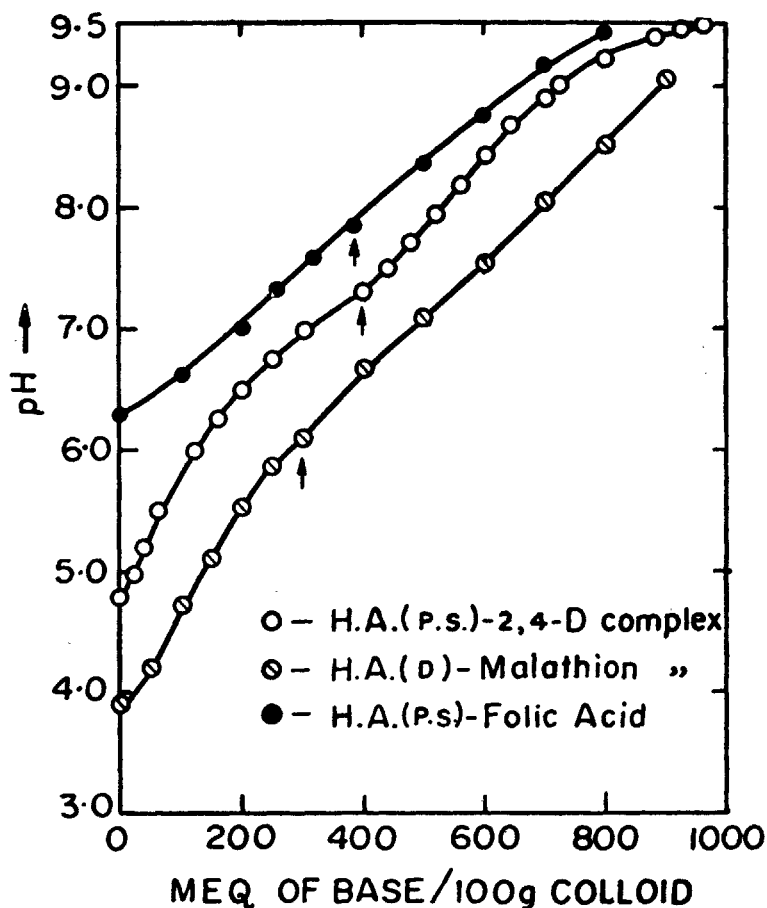


FIG. 2. Titration curve of the complexes.

A representative of the potentiometric titration curves of the complexes (natural humic acid-folic acid complex, natural humic acid—2, 4-D complex and another syn. HA-malathion complex) is shown in Fig. 2. The nature of the titration curves are quite different from that of the parent humic acid. In case of the 2, 4-D and malathion complexes, the curves steadily rise with sharp inflexion points. This indicates that only one type of acidic group (*viz.*, $-\text{COOH}$) are involved in the neutralization process in the former case whereas more complexities arise in the case of the humic acid folate complexes where different functional groups are taking part in the neutralization process.

IR spectroscopy data of the complexes as obtained from the frequency *vs.* absorbance plots (Fig. not shown) and the tentative assignments as done (Adhikari *et al.*, 1978; Schnitzer & Khan, 1972; Sullivan & Felbeck Jr., 1968) are recorded in Table II. From the table, it is seen that all the products have some common absorption bands as was found for the respective parent counter parts.

The main bands are at 3400 cm^{-1} (H bonded $-\text{OH}$), 2380 cm^{-1} (Zwitter ions of NH^+ and NH_2^+ etc.), 1700 cm^{-1} ($\text{C}=\text{O}$ of CO_2H and ketones), 1650 cm^{-1}

TABLE II
IR Spectra (Frequency in cm^{-1})

1	H. A. (P. S.) + Folic acid		H. A. (A) + Folic acid		H. A. (F ₈) + Folic acid		H. A. (P. S.) + 2,4-D		H. A. (P. S.) + 2,4-D		H. A. (D) + Malathion		H. A. (F ₈) + Malathion		Asymments	
	2	3	1	2	3	1	2	1	2	1	2	1	2	1		2
3400	(b, w)	3400	(b, w)	3400	(b, w)	3400	(b, w)	3400	(b, w)	3400	(b, w)	3400	(b, w)	3400	(b, w)	H bonded OH and NH stretch
2380	(sp, st)	2380	(sp, st)	2400	(sp, m)	2380	(sp, m)	2320	(w)	2380	(w)	2380	(w)	2380	(w)	Zwitter ions of NH ⁺ and NH ₃ ⁺ etc. or p-H stretch
1700	(m, b)	1700	(m, b)	1700	(b, w)	1700	(b, w)	1720	(b, w)	1700	(st, b)	1700	(st, b)	1700	(st, b)	C=O stretching of CO ₂ H, esters and ketones
1610	(m, b)	1610	(m, b)	1650	(st, b)	1620	(st, b)	1630	(st, b)	1650	(st, b)	1650	(st, b)	1650	(st, b)	Aromatic C=C, —NH bending vibration
1540	(m, b)	1540	(m, b)	1550	(m, b)	1540	(m, b)	1550	(m, w)	1550	(m, w)	1550	(m, w)	1550	(m, w)	Aromatic C=C stretching
1300	(b, m)	1400	(sp, st)	1400	(w)	1400	(w)	1400	(w)	1400	(w)	1450	(w)	1460	(w)	vibration secondary amines
1180	(sp, w)	1180	(sp, w)	1200	(w)	1180	(w)	1190	(w)	1200	(w)	1200	(w)	1200	(w)	Aliphatic CH deformation
1130	(sp, w)	1130	(sp, w)	1100	(w)	1100	(w)	1100	(w)	1100	(w)	1030	(w)	1040	(w)	(1460) salts of COOH (1300–1400)
860	(w)	860	(w)	840	(w)	840	(w)	840	(w)	800	(w)	800	(w)	800	(w)	C=O stretch and OH
																deformation of CO ₂ H
																C=O of cyclic and aliphatic esters and alcohols
																Aromatic CH ₂ deformation
																in COOH groups or C—Cl stretching vibration

sp — sharp, m — medium, b — broad, st — strong, w — weak

(Aromatic C=C), 1515 cm^{-1} (C=C stretching vibration, secondary amines), 1470–1420 cm^{-1} (aliphatic —CH deformation), 1390–1332 cm^{-1} (salts of COOH), 1280–1137 cm^{-1} (C=O stretch and OH deformation of CO_2H). The spectra for the complexes when compared to the spectra of their respective parent humic acid show some changes in functional group absorptions. For the humic acid—folate complexes, the intensity of absorptions of CO_2H at 1700 cm^{-1} are reduced for all the cases and a strong absorption at 1610 cm^{-1} and 1300–1400 cm^{-1} due to the carboxylate anion are found. Moreover the intensity of absorption at 3400 cm^{-1} is also reduced in case of the complexes and compared to their parent counterpart. The absorption band at 2380 cm^{-1} (due to —NH⁺, —NH₂⁺ etc.) was very weak for the untreated humic acids, but the intensity of the absorption are increased after complexation with folic acid. These indicate that the reactive sites are mainly —COOH and phenolic —OH in humic acid and they are expected to form H-bonds with amino/imino groups present in the folate ion. This type of possible linkage for S-triazine-humic acid complex has been shown by Sullivan & Felbeek (1968) from IR spectroscopy. It is thus found that some of the COOH and phenolic —OH groups present in humic acid are blocked through complexations.

In the case humic acid 2, 4-D complexes when compared with the parent humic acids it is noticed that the intensity of absorption at 3400 cm^{-1} due to H bonded OH and —NH stretch as well as that at 1700 cm^{-1} due to COOH groups are reduced. A strong broad absorption is found at 1650 cm^{-1} . This band is due to the aromatic C=C vibration and presence of the carboxylation. A new peak at 800 cm^{-1} which is due to the C—Cl stretching vibration is found for all the 2, 3-D humic acid complexes. The absorption peak at 1390 cm^{-1} — 1332 cm^{-1} due to the salts of —COOH group are not obtained in these complexes. Thus here the binding sites on humic acid are H bonded —OH, —NH and —COOH groups. As the C=O absorptions (1700 cm^{-1}) are reduced with the absence of the peak at 1390 cm^{-1} (for a salt of a carboxylic acid) in the humic acid — 2, 4-D complexes there is the possibility of intermolecular H-bonding. Similar possibility of intermolecular H-bonding has been suggested by Sullivan & Felbeek (1968) for S-triazine humic acid complex.

For the humic acid-malathion complexes increased intensity of absorption at 1700 cm^{-1} and 1030 cm^{-1} mainly due to the ester group of the malathion molecules is observed. Moreover, the peak at 1450 cm^{-1} due to aliphatic —CH deformation are also obtained. Another interesting features in the absorptions bands for these complexes are that the intensity of absorption at 3400 cm^{-1} (due to —CH, NH etc.) are reduced, but the intensity of absorptions at 2380 cm^{-1} increased and the P=S stretching vibration at 600–750 cm^{-1} are absent.

Here the increased absorption at 2380 cm^{-1} may be assigned to P—H vibration. Thus in this case the phenolic —OH and —COOH groups of humic acid may be involved whereas the binding sites on malathion are P and S atoms.

The probable binding of humic acid involved in the interaction process are, therefore phenolic —OH, —COOH and (> NH⁺), NH₂ etc. To have a more clear picture of these interaction phenomena, other physico-chemical studies on these interaction products have been undertaken.

ACKNOWLEDGEMENTS

One of the authors, B. Mandal, is grateful to C.S.I.R. for the assistance of a Junior Fellowship. Thanks of the authors are due to Cynamid India Ltd., Bombay for supplying the sample Malathion.

REFERENCES

- Adhikari, M., Manda¹. B. and Mukherjee, T. K. (1978). Studies on physico-chemical properties of natural synthetic and microbial humic acids. *Proc. Indian natn. Sci. Acad.*, **44**, A, 202-210.
- Adhikari, M., and Hazra, G. C. (1970). Study of chelation of humic acid with bivalent metal ions. *Indian J. appl. Chem.*, **33**, 322-328.
- Bailey, G. W., and White, J. L. (1964). Review of adsorption and desorption of organic pesticides by soil colloids, with implications concerning pesticide Bioactivity. *J. agric. Food Chem.*, **12**, 314-332.
- Hayes, N. H. B., Stacey, M., and Thomson, J. M. (1968). In : *Isotopes and Radiation in Soil Organic Matter Studies*. Int. Atomic Energy Agency, Vienna, 75-90.
- Kozar, D. G., and Sovan, A. S. (1971). In : *Trans. int. Symp. 'Humus et Planta'*, (Pragne) p. 591.
- Schritzer, M., and Khan, S. U. (1972). *Humic Substances in the Environment*. Marcel Dekker Inc., N. Y.
- Sullivan, Jr., and Felbeek Jr. (1968). A study of the interaction of S-triazine herbicides with humic acids from these different soils. *Soil. Sci.*, **106**, 42-52.