9

Dacca University Studies, B, XXVII (1) : 39—46, 1979 (January)

OCCURRENCE OF HYDROLYSIS PRODUCTS OF PHOSPHOLIPIDS IN SOME SUB-HUMID TROPICAL SOILS OF BANGLADESH

R. MANDAL and A. ISLAM

Department of Soil Science, University of Dacca, Bangladesh.

Asbtract

Hance and Anderson's method for determining lipid phosphate was found best among the four methods used due to double over-night pretreatments. Air-dried samples yielded lower lipid values than the fresh ones due to changes in the nature of the organic matter itself on drying, or adsorption and binding of lipids to clay fractions. Concentrations of phosphatidyl choline, phosphatidyl ethanolamine and glycerophosphate ranged from 0.65 to 9.50, 0.30 to 3.60 and 0.15 to 2.25 ppm, respectively and each was significantly correlated with organic phosphate, total phosphate, organic carbon and total nitrogen content of the samples. All the fractions, however, showed better correlation to the cumulative effect of the same variables.

Introduction

Fractionation studies on soil organic phosphate have shown that a small proportion of soil phosphate is present in the class of biologically important phosphate ester, phospholipids (Hance and Anderson 1963a ; Kowalenko and McKercher 1971) and is nutritionally valuable in soil fertility (Pierre and Bower 1945) following its mineralization, the rate of which depends on the chemical nature and quantity of the compound. The quantitative variation of the compound in soil is associated with the type of soil, mode of extraction and presence of other soil constituents. Moreover, scientists are of the opinion that hydrolysis of soil phospholipids releases phosphatidyl choline, phosphatidyl ethanolamine, glycerophosphate and serine (Hance and Anderson 1963b ; Simoneaux and Caldwell 1965) but very little work has been done to determine their amounts in soils.

Thus, laboratory experiments have been performed to assess the occurrence of phospholipids as measured by four different methods. An attempt has also, therefore, been made to estimate the relative amounts of their hydrolysis products in some sub-humid tropical soils of Bangladesh and evaluation of their relationships with soil properties.

Materials and Methods

Soils

Samples of surface soils (0 to 15 cm) were collected from different sites in Bangladesh containing high organic matter which represented ten soil

Table 1. Certain physical and chemical properties of the soil samples studied*

Soil Series	USDA soil texonomy	General type	Texture	Clay <0.002 mm (%)	pH	Org.C (%)	Total N (%)	Org. P (ppm)	Total P (ppm)
Karail	Fluvaquentic Haplaquept	ABC	Clay	60	4.3	2.38	0.15	205	445
Jatrabari	Fluvaquentic Haplaquept	GFS	Clay	50	4.5	2.39	0.15	180	405
Siddirganj	Aeric Fluvaquentic Haplaquept	NDGFS	Silty Clay	50	4.6	2.78	0.17	250	520
Kajla	Fluvaquentic Haplaquept	NDGFS	Silty Clay	45	4.6	2.68	0.18	220	425
Magra	Fluvaquentic Haplaquept	NDGFS	Silty Clay	41	5.5	3.94	0.30	320	500
Harta	Histosols, nndifferentiated	Peat	Clay	50	5.8	12.94	0.88	560	850
Naldanga	Typic Hydraquent	NDGFS	Silt loam	27	5.9	12.04	0.86	530	780
Paysa	Typic Haplaquoll	NDGFS	Silt loam	28	6.6	7.09	0.58	450	680
Sarala	Aeric Fluvaquentic Haplaquept	NDGFS	Clay	55	7.2	2.55	0.16	240	505
Lashkara	Aeric Fluvaquentic Haplaquept	NDGFS	Clay	48	7.3	3.84	0.27	300	490

*Soil samples were collected through the courtesy of Soil Survey Department, Dacca.

ABC=Acid basin clay; GFS=grey floodplain soil; NDGFS=non-calcareous dark grey floodplain soil.

series and five major soil groups. The soils were air dried, mixed and sieved through a 100 mesh screen. Fresh soil samples were also preserved properly. Data on certain physical and chemical properties of the soil samples studied are presented in Table 1.

Analytical methods

Analyses were made for pH by the Pye glass electrode method (soil : solution ratio 1 : 2.5), organic carbon by wet oxidation method as described by Walkley and Black (1934), total nitrogen by the Kjeldahl method, organic and total phosphate by Mehta *et al.* (1954) and the hydrolysis products of phosholipids by Hance and Anderson (1963b). Extraction and estimation of phospholipids were undertaken by the methods of Sokolov (1948), Rask and Phelps (1925) and Hance and Anderson (1963a).

Results and discussion

Comparison of methods for determining phospholipids

The amounts of lipid phosphate obtained from ten soils samples by four different methods are summarized in Table 2. Concentrations of phospholipids ranged from 0.30 to 5.55, 0.12 to 0.96, 0.20 to 1.90 and 1.20 to 15.50 ppm for Sokolov, modified Sokolov, Rask and Phelps, and Hance and Anderson's method respectively. The values obtained are well comparable with those previously reported by Hance and Anderson (1963a, b), Kowalenko and McKercher (1971) and Islam and Ahmed (1973). The modified method of Sokolov yielded the lowest lipid values among the four methods. However, Hance and Anderson's method proved to be the best one for the extraction of phospholipid due to double overnight pretreatments with HCI/HF, followed by washing with water. The efficiency of the methods can be arranged as follows :

Modified Sokolov$<$Rask and Phelps$<$Sokolov$<$Hance and Anderson

Tables 2 and 3 showed that extracted lipid phosphate content in fresh samples (Table 3) was higher than those of air-dried ones (Table 2) in all methods except the Hance and Anderson's method. However, Hance and Anderson's method, extracted lipid phosphate in equal amounts from both the fresh and air-dried samples except one (Harta) where a slightly higher value was observed in the fresh sample. The reduction in solubility might have been due to changes in the nature of the organic matter itself on drying. or adsoiption and binding of the lipids to the clay fractions of the samples (Hance and Anderson 1963a).

Table 2. Phospholipid content of soil smaples estimated by four methods

Soil series	SOKOLOV			Modified SOKOLOV			RASK and PHELPS			HANCE and ANDERSON		
	ppm	%*	%**	ppm	%*	%**	ppm	%*	%**	ppm	%*	%**
Karail	0.30	0.15	0.07	0.12	0.06	0.03	0.20	0.10	0.04	1.65	0.80	0.37
Jatrabari	0.33	0.18	0.08	0.14	0.08	0.30	0.21	0.12	0.05	0.50	0·83	0.38
Siddirganj	0.50	0.20	0.10	0.23	0.90	0.04	0.38	0.14	0.07	1.20	0.48	0.24
Kajla	0.88	0.40	0.21	0.48	0.22	0.11	0.46	0.21	0.19	2.75	1.25	0.65
Magra	1.05	0.33	0.21	0.18	0.08	0.04	0.39	0.12	0.08	3.50	1.90	0.70
Harta	5.55	0.99	0.65	0.77	0.14	0.08	1.55	0.28	0.18	15.50	2.77	1.82
Naldanga	3.25	0.61	0.42	0.96	0.18	0.12	1.90	0.36	0.24	11.40	2.15	1.46
Paysa	2.02	0.45	0.30	0.48	0.11	0.07	0.90	0.20	0.13	7.20	1.60	1.06
Sarala	1.10	0.46	0.22	0.44	0.18	0.09	0.43	0.18	0.09	3.80	1.58	0.75
Lashkara	1.05	0.35	0.21	0.34	0.11	0.07	0.65	0.22	0.13	4.50	1.50	0.92
Minimum	0.30	0.15	0.07	0.12	0.06	0.03	0.20	0.10	0.04	1.20	0.48	0.24
Maximum	5.55	0.99	0.65	0.96	0.22	0.12	1.90	0.36	0.24	15.50	2.77	1.82
Mean	1.60	0.41	0.25	0.41	0.12	0.07	0.71	0.19	0.12	5.26	1.41	0.84
L. S. D. (P=0.01)	0.11			0.06			0.18			0.85		

* Percentages were calculated on the basis of organic phosphate.

** Percentages were calculated on the basis of total phosphate.

Table 3 Concentration of phospholipids in fresh soil samples determined by four methods

Soil series	SOKOLOV	Modified SOKOLOV	RASK and PHELPS	HANCE and ANDERSON
	Phospholipids in ppm			
Karail	0.50	0.25	0.40	1.65
Jatrabari	0.45	0.30	0.38	1.50
Siddirganj	0.56	0.39	0.45	1.20
Kajla	1.05	0.68	0.68	2.75
Magra	1.35	0.33	0.85	3.50
Harta	6.80	1.40	3.08	15.65
Naldanga	4.95	1.65	3.80	11.50
Paysa	2.90	0.90	2.40	7.20
Sarala	1.60	0.88	1.27	3.80
Lashkara	1.85	0.70	0.98	4.50

Distribution of phospholipids and their hydrolysis products

The values for lipid phosphates extracted by Hance and Anderson's procedure varied signifcantly from soil to soil (Table 2) and ranged between 1.20 and 15.50 ppm (mean 5.30 ppm) accounting from 0.48 to 2.77% of the organic phosphate (mean 1.41%) and 0.24 to 1.82% of the total phosphate (mean 0.84%). Concentrations of phosphatidyl choline, phosphatidyl

Table 4. Hydrolysis products of phospholipids of soil samples

Soil Series	Phosphatidyl choline			Phosphatidyl ethanolamine			Glycerophosphate		
	ppm	%*	%**	ppm	%*	%**	ppm	%*	%**
Karail	0.80	48.5	0.38	0.50	30.3	0.22	0.25	15.1	0.12
Jatrabari	0.65	43.3	0.36	0.56	33.3	0.28	0.20	13.3	0.11
Siddirganj	0.71	59.2	0.28	0.30	25.0	0.12	0.15	12.5	0.06
Kajla	1.75	63.6	0.80	0.65	23.6	0.30	0.25	9.3	0.11
Magra	2.10	60.0	0.66	0.80	29.9	0.25	0.45	12.9	0.14
Harta	9.50	61.3	1.70	3.25	21.0	0.58	2.25	14.5	0.40
Naldanga	6.40	56.1	1.21	3.60	31.6	0.68	1.30	11.4	0.25
Paysa	4.00	55.6	0.89	2.50	34.7	0.56	0.60	8.3	0.13
Sarala	1.80	47.4	0.75	1.40	36.8	0.58	0.40	10.5	0.17
Lashkara	2.50	55.6	0.83	1.00	22.2	0.33	0.75	16.7	0.25
Minimum	0.65	43.3	0.28	0.30	21.0	0.12	0.15	8.3	0.06
Maximum	9.50	63.6	1.70	3.60	36.8	0.68	2.25	16.7	0.40
Mean	3.20	55.1	0.78	1.45	28.1	0.39	0.66	12.4	0.17
LSD (P=0.01)	0.31			0.22			0.18		

* Percentages were calculated on the basis of phospholipids.

** Percentages were calculated on the basis of organic phosphate.

ethanolamine and glycerophosphate ranged from 0.65 to 9.50 ppm (mean 3.20 ppm), 0.30 to 3.60 ppm (mean 1.45 ppm) and 0.15 to 2.25 ppm (mean 0.66 ppm) respectively (Table 4). These constituents constitute about 43.3 to 63.6%, 21.0 to 36.8% and 8.3 to 16.7% of the lipid phosphate respectively and 0.28 to 1.70%, 0.12 to 0.68% and 0.06 to 0.40% of the organic phosphate respectively. Table 4 also shows that 89.9 to 99.1% of the lipid phosphate was identified as phosphatidyl choline, phosphatidyl ethanolamine and glycerophosphate and the rest unidentified fraction might have been attributed to serine and related compounds. The unidentified fraction of phospholipids was presented in Table 5.

Table 5. Unidentified proportion of soil phospholipid fractions

Soil series	Total identified Phospholipids components			Unidentified phospholipids components		
	ppm	%*	%**	ppm	%*	%**
Karail	1.55	93.9	0.74	0.10	6.10	0.05
Jatrabari	1.45	89.9	0.75	0.05	10.10	0.08
Siddirganj	1.16	96.7	0.46	0.04	3.30	0.02
Kajla	2.65	96.5	1.21	0.10	3.50	0.04
Magra	3.55	95.8	1.05	0.15	4.20	0.04
Harta	15.00	96.8	2.68	0.50	3.20	0.09
Naldanga	11.30	99.1	2.14	0.10	0.90	0.01
Paysa	7.10	98.6	1.58	0.10	1.40	0.02
Sarala	3.60	94.7	1.50	0.20	5.30	0.08
Lashkara	4.25	94.5	1.41	0.25	5.50	0.09
Minimum	1.16	89.9	0.74	0.04	0.90	0.01
Maximum	15.00	99.1	2.68	0.50	10.10	0.09
Mean	5.14	95.6	1.40	0.16	4.40	0.05

* Percentages were calculated on the basis of phospholipids.

** Percentages were calculated on the basis of organic phosphate.

The values obtained in the present study compared well with findings of other investigators. Phospholipids varied between 0.6 and 0.9% of the organic phosphate in five British soils (Hance and Anderson 1963a), 0.89 and 3.50% in a number of Saskatchewan soils (Kowalenko and Mckercher 1971.), and 0.5 and 7.0% in ten Bangladesh soils (Islam and Ahmed 1973). Phosphatidyl choline and phosphatidyl ethanolamine accounted for 0.5 to 1.9% and 0.39 to 1.60% of the organic phoshate respectively and about 40 and 30% of the lipid phosphate respectively in some Saskatchewan soils (Kowalenko and Mckercher 1971). Glycerophosphate constituted 52 to 86% of the lipid phosphate in some British soils (Hance and Anderson 1963b).

Association of hydrolysis products of phospholipids with soil properties

Correlation coefficients calculated between the phospholipids content as derermined by Hance and Anderson (1963b) method and various soil physical and chemical parameters are presented in Table 6. Multiple correlation suggested that the amounts of lipid phosphates and their hydrolysis products were significantly correlated with organic phosphate, total phosphate, organic carbon, total nitrogen, clay and pH of the soil samples. Individually, phospholipid was found to be significantly correlated with organic phosphate, total phosphate, organic carbon and total nitrogen at

Table 6. Simple and multiple correlation coefficients of phospholipids and their hydrolysis products with various soil properties

Correlation between	Correlation coefficient (r)
Phospholipids and organic P	+0.953*
Phospholipids and total P	+0.948*
Phospholipids and organic C	+0.974*
Phospholipids and total N	+0.962*
Phospholipids and clay	+0.216
Phospholipids and pH	+0.391
Phosphatidyl choline and organic P	+0.944*
Phosphatidyl choline and total P	+0.941*
Phosphatidyl choline and organic C	+0.968*
Phosphatidyl choline and total N	+0.953*
Phosphatidyl choline and clay	+0.207
Phosphatidyl choline and pH	+0.349
Phosphatidyl ethanolamine and organic P	+0.945*
Phosphatidyl ethanolamine and total P.	+0.944*
Phosphatidyl ethanolamine and organic C	+0.949*
Phosphatidyl ethanolamine and total N	+0.960*
Phosphatidyl ethanolamine and clay	+0.415
Phosphatidyl ethanolamine and pH	+0.476
Glycerophosphate and organic P	+0.889*
Glycerophosphate and total P	+0.875*
Glycerophosphate and organic C	+0.921*
Glycerophosphate and total N	+0.890*
Glycerophosphate and clay	+0.174
Glycerophosphate and pH	+0.342
Phospholipids and all factors	+0.986*
Phosphatidyl choline and all factors	+0.980*
Phosphatidyl ethanolamine and all factors	+0.994*
Clycerophosphate and all factors	+0.973*

Significant at 0.1% level.

the 0.1% level. The best correlation of the compound was with organic carbon. Simple correlation also revealed that phosphatdiyl choline, phosphatidyl ethanolamine and glycerophosphate significantly correlated with the same factors as phospholipids. Siganificant correlations between phospholipids and organic phosphate, total phosphate and organic matter have been reported earlier (Islam and Ahmed 1973). Variation of phospholipids as well as their hydrolysis products with organic carbon and organic phosphate was due to the fact that these components are integral part of organic matter. Soils with high pH values also favour the resistance of the components in the soil. Glycerophosphate is known to be adsorbed by clay minerals (Anderson and Arlidge 1962) and its stability is thought to be associated with this property. Evidence from the present study supports this idea as the amounts of phospholipids as well as their hydrolysis products are positively correlated with clay contents of the samples.

References

Anderson, G. and E. Z. Arlidge, 1962. The adsorption of inositol phosphate and glycerophosphate by soil clays, clay minerals and hydrated sesquoxides in acid media. J. Soil Sci. **13** : 216-224.

Hance, R. J. and G. Anderson 1963a. Extraction and estimation of soil phospholipids. Soil Sci. 96 (2) : 94-98.

Hance, R. J. and G. Anderson, 1963b. Identification of hydrolysis products of soil phospholipids. Soil Sci. 96 (3) : 157-161.

Islam, A. and B. Ahmed, 1973. Distribution of inositol phosphates, phosphlipids, and nucleic acids and mineralization of inositol phosphates in some Bangladesh soils. J. Soil Sci. 24 : 193-198.

Kowalenko, C. B. and R. B. McKercher, 1971. Phospholipid components extracted from Saskatchewan soil. Can. J. Soil Sci. **51** : 19-22.

Mehta, N. C., J. O. Legg, C. A. I. Goring and C. A. Black, 1954. Determination of organic phosphorus in soils. I. Extraction method. Soil Sci. Soc, Am. Proc. **18** : 443-449.

Pierre, W. H. and C. A. Bower, 1945. A review of recent works on soil organic matter. J. Soil Sci. **2** : 67-82.

Rask, O. S. and I. K. Phelps, 1925. Extraction and estimation of lipoids in cereal products. Ind. Eng. Chem **17** : 187-189.

Simoneaux, B. J. and A. G. Caldwell, 1965. Phospholipids selected soils. Agron. Abstr. Annu. Meet. Am. Soc. Agron. pp. 77.

Sokolov D. F. 1948. The presence of several organic phosphorus compounds in the soil. Pochvovedenie. pp. 502-505.

Walkley, And I. A. Black, 1934, An examination of the deltijareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37 : 29-39.