

ORGANIC MATTER-PHOSPHORUS ASSOCIATIONS AS A SINK IN P-FIXATION PROCESSES IN ALLOPHANIC SOILS OF CHILE

FERNANDO BORIE and HUGO ZUNINO

Universidad de Chile, Departamento de Análisis Químico, Casilla 233, Santiago 1, Chile

Summary—In fertilized and unfertilized plots of ten Chilean allophanic soils the fraction of total-P associated with organic matter and the organic carbon content were determined. Cultivation with P fertilization for 40 yr or more increased the organic C and organic P in most of the soils. It is hypothesized that these effects are related to the high biochemical activity of these soil environments which are dominated by actinomycetes and fungal species as well as to the chemical and physical properties of the soils. It appears that, following its fast adsorption onto active allophanic surfaces, added-P is subjected to reactions resulting in the formation of organic matter-P associations or complexes possibly through Al bridges. The humus-P complexes compose the major portion of total-P in these soils and may be viewed as a P sink in the overall P-cycling process. There is a need for further research on the specific mechanisms by which plant roots utilize P-sinks as sources of P. Based on microbial activity a model of the P-cycle operating in these soils is hypothesized.

INTRODUCTION

Fixation of phosphate in allophanic soils is one of the major factors limiting their agronomic use. The mechanisms involved in such fixation processes have been widely investigated using short-term equilibria procedures. The conclusion has been reached that phosphate retention in allophanic soils is mainly due to the ability of allophane (amorphous aluminum-silicate of variable Al to Si ratios) to strongly adsorb the phosphate specifically by ligand-exchange (Parfitt, 1980). The rapid disappearance of phosphate from an equilibrating solution is thus kinetically related to adsorption isotherms and mechanistically to adsorption reactions onto active allophanic colloids.

It is also generally accepted that repeated application of inorganic P results in an organic P buildup and that this form of phosphorus participates somehow in the P-cycle and may contribute to the pool of available P (Anderson, 1975). However, few attempts have been made with allophanic soils to investigate the biological transformations of adsorbed phosphate and its relation to plant availability.

Volcanic soils of Chile with high allophane content are basically characterized by their unusually high organic matter content and by a microbial ecology dominated by actinomycetes and fungi (Zunino *et al.*, 1982a). These characteristics and interactions with allophane create a soil environment highly reactive from the biochemical standpoint. This is reflected, among others, in the following properties of such soils: (a) strong stabilization of the indigenous organic matter and of extraneous organic materials and compounds (Zunino *et al.*, 1982b), (b) high rates of synthesis of microbial humic-type macromolecules (Zunino *et al.*, 1982c), and (c) high enzymatic activity (Borie, unpublished data; and Borie and Fuentealba, 1982). Therefore the phosphate added to these soils after being rapidly adsorbed by allophanic clay

materials, should be affected by all the biotic factors active in such an environment. To what extent the biotic factors are related to the chemical combinations of phosphate, and consequently to its availability, is not yet fully understood. The fact that mycorrhizal infection is widespread in roots of both indigenous and cultivated plants growing in these soils (Borie, unpublished data), suggests that this type of symbiosis might be the key factor in P-nutrition. It follows that a full knowledge of the dynamic changes to fertilizer-P in such soils is of great practical importance.

We have been attempting to elucidate the effect of biotic factors on phosphorus cycling and changes in allophanic soils in relation to phosphorus availability. The occurrence and significance of humus-P associations in several representative soil samples taken from cultivated and uncultivated field plots have been investigated.

MATERIALS AND METHODS

Surface samples of 10 volcanic ash-derived soils (typic Dystrandeps) were collected. Samples of each soil were taken from cultivated and uncultivated areas. The sampling sites chosen were located near old country roads. The cultivated samples were taken in the field at least 15 m away from the road fence while the uncultivated samples were taken from undisturbed sites near the road cuts and at least 2 m from the fence. The allophane contents of these soils are of the order of 30% (Zunino *et al.*, 1982b).

According to the records of the local Experiment Station the fields under cultivation (mainly wheat, sugar-beet, barley) have been receiving phosphate fertilization for at least 40 yr. A typical application is 120 kg P_2O_5 ha⁻¹ as superphosphate. The sites classified as uncultivated have not been fertilized for the last 25–30 yr.

The samples were sieved (<2-mm) and analysed as follows:

Organic carbon

This was determined by a dry Dumas Method using a Coleman nitrometer adapted for C determination. This method appears to be the most reliable and accurate for soils high in organic matter.

Total P

This was determined by the method of Dick and Tabatabai (1977). This involves the complete destruction of organic matter by a freshly prepared NaBrO solution at 260–280°C followed by an acid extraction with a mixture of HCOOH and 1 N H₂SO₄. Phosphorus was determined in the resulting extract by the sulfomolybdic acid method using ascorbic acid as reductant.

P associated with organic matter

This was determined by the method of Steward and Oades (1972) modified by Borie for application to volcanic soils high in organic carbon (1983). After a mild extraction with 1 N HCl successive extractions with 0.5 N NaOH under ultrasonic vibration are made. Inorganic, or "free" phosphate, is determined directly in the combined extracts by diluting the sample and measuring the phosphate as above. Total P is determined in the same combined extracts after destroying the organic matter with NaBrO. The fraction of P associated with organic matter (called herein after "humus-P") is calculated by difference. Undoubtedly not all the phosphorus grouped in this category is strictly organic in nature, but it largely represents the sum of actual organic-P (inositol phosphates, phospholipids, nucleic acids, etc.), and phosphorus bound to organic macromolecules directly or through Al or Fe atoms. This is why we prefer the term organic matter-phosphorus association (or humus-P association). Since part of the P present in the soil is only weakly associated with humus, the figures for humus-P given in the tables may be lower than the actual values due to the hydrolytic splitting of the associations during the extraction stages.

Humic and fulvic phosphorus

The alkaline extract is carefully adjusted to pH 2.0 with dil. H₂SO₄. Then inorganic (or "free") phos-

phate and total-P are determined in the solution after filtering and washing the humic precipitate. Fulvic-P is designated as total-P of the acid solution minus inorganic-P of the same solution. Humic-P is calculated as the humus-P of the combined extracts minus fulvic-P determined in the acidic solution.

Available phosphate

This was determined by using the Bray and Kurtz No. 2 method (Jackson, 1958).

RESULTS AND DISCUSSION

The data in Table 1 show the effects of long-term phosphate fertilization and continuous cultivation on the P-fractions and organic C levels in ten allophanic soils. The organic C tended to increase in all the fertilized soils, with the exception of Arrayán and Victoria 3 soils. This effect can be related to the microbial activity of these soils. It has been shown that they possess a unique microbial ecology dominated by actinomycetes and fungal species some of which synthesize humic-like polymers (Zunino *et al.*, 1982a,c). Soils under continuous fertilization and cultivation management are moderately high in available P, have relatively large annual organic inputs, and have greater plant root activity than the corresponding uncultivated ones. These factors would tend to stimulate microbial activity and, consequently, organic polymer synthesis through specific biochemical pathways (Zunino *et al.*, 1982b; Martin *et al.*, 1982). The newly-incorporated organic residues are metabolized and stabilized by incorporation into humic-allophane complexes. Therefore it seems logical to expect an organic C build-up under these circumstances.

The levels of total-P in the fertilized samples are much higher than in the corresponding unfertilized ones, corroborating that in allophanic soils the applied phosphate does not move downward significantly.

The P associated with organic matter accounts for approximately 60% (average figure) of the total-P in both fertilized and unfertilized samples. Furthermore as noted in Table 2, the increment in the humus-P (Δ humus-P) compared to the increment of total-P (Δ total-P), is of the order of 76% (average figure) with the exception of Arrayán and Loncoche soils. These

Table 1. Distribution of P and C content of some allophanic soils of Chile

Soil	Total-P		Humus-P		Humus-P		Available-P		Organic-C	
	($\mu\text{g g}^{-1}$)		($\mu\text{g g}^{-1}$)		(% of total)		($\mu\text{g g}^{-1}$)		(%)	
	F*	U*	F	U	F	U	F	U	F	U
Corte Alto	2387	1513	1257	835	53	55	19	3	7.6	5.7
Arrayán	1982	1562	1057	1150	53	74	47	4	4.6	4.6
Loncoche	3102	1503	1290	723	42	48	37	5	6.0	5.5
Malihue	1724	1542	1280	973	74	63	12	17	6.3	5.9
Osorno	3560	3000	2384	2159	67	72	73	20	6.5	5.8
San Pablo	3074	2812	1879	1729	61	61	29	26	6.0	5.0
Victoria	2380	1950	1409	1063	59	55	12	5	7.7	6.4
Puerto Varas	2175	1371	1560	1040	72	76	49	6	9.8	9.6
Metrenco	4011	2135	3197	1145	80	54	165	9	5.8	4.9
Victoria 3	1422	1150	870	650	61	57	12	9	5.8	6.0

*F = fertilized plot; U = unfertilized plot.

Table 2. Increments of P fractions in soils under fertilization

Soil	Δ total-P* ($\mu\text{g g}^{-1}$)	Δ humus-P† ($\mu\text{g g}^{-1}$)	$\frac{\Delta \text{ humus-P}}{\Delta \text{ total-P}}$
Corte Alto	874	422	0.51
Arrayán	380	-93	0
Loncoche	1600	567	0.35
Malihue	182	307	1.68
Osorno	560	225	0.40
San Pablo	262	150	0.57
Victoria	430	346	0.80
Puerto Varas	804	520	0.65
Metrengo	1876	2052	1.09
Victoria 3	272	220	0.81

* (total-P fertilized plot) - (total-P unfertilized plot).

† (humus-P fertilized plot) - (humus-P unfertilized plot).

results indicate that the fertilized-P incorporated in the system, and quickly adsorbed onto the surface of allophanic colloids, subsequently reacts with the organic components of the soils to form stable associations. The data in Table 3 support this view since they show that the incidence of humic-P, within the humus-P fraction, increases noticeably with fertilization and cultivation in all soils with the exception, again, of Arrayán soil. Separate experiments have indicated that, the Arrayán soil has low enzyme activity, has the lowest organic C content and does not show clear actinomycete and fungal dominance. These differences in biological properties may have influenced the P-chemistry of this soil in spite of its allophanic nature. Levels of humus-P associations in non-allophanic soils have been repeatedly reported and in general the values are below 50% of total-P (Finkl and Simonson, 1979), but differences in the analytical methodology avoid more reliable comparisons.

The increases upon fertilization of humic-P, within the humus-P fractions is related to the simultaneous increase in the organic-C (see Table 1). Undoubtedly the high biosynthesis rate of humic-like polymers, taking place in the fertilized soils, should influence the association of phosphate with these new polymers formed *in situ* which resist further microbial degradation. Active aluminum may behave as a bridge facilitating coupling reactions by mechanisms similar to that described by Appelt *et al.* (1975) in the same allophanic soils and in some Japanese andosols (Wada and Gunjigake, 1979). According to the hypothesis of Zunino and Martin (1977), the newly formed microbial polymers can be classified as type-2 complexants, e.g. organic macromolecules whose active functional groups are not coordinated yet with cationic species. Therefore, considering the fact that the activity of aluminum in these soils is elevated, one could expect a high probability that the so-called Al bridges do form between phosphate and the organic polymers.

Knowledge of phosphate reactions in high P-adsorption capacity soils, is fundamental to evaluate the availability of this essential element to plants. A better understanding of these transformations could help to find better management choices to improve P-absorption by crops. The results reported in this paper and their interpretation allow one to suggest a

general hypothesis to illustrate how the added phosphate, after being physico-chemically adsorbed, can be biologically transformed and sink into an organic polymer-P-allophane complex. The hypothesis is presented as a P-cycle in Fig. 1. For simplicity only Al-surfaces are ascribed to Center I; even though less frequent, active Fe-surfaces should behave similarly.

Fertilizer-P upon application is readily adsorbed by positively charged adsorbate-free sites onto the allophanic surface (Center 1). Since the naturally-occurring allophanic-humus interactions primarily involve external surfaces (due to the spongy structure of both colloids) adsorbate-free allophane surfaces are available to link phosphate directly (Appelt *et al.*, 1975). Eventually part of the adsorbed phosphate would become incorporated into the microbial metabolism cycles. The well-developed branched structure of fungi and actinomycetes species should facilitate physical contact and consequently the transportation of P into the biomass. Upon death of microbes and after tissue decay the P is re-incorporated into the soil media as organic P. Recently phosphatidyl-ethanol-amine and phosphatidyl-choline have been definitely identified by gas and thin layer chromatography in these soils (Borie, unpublished data).

Humic-type macromolecules synthesized by Center II can interact with Center I surfaces forming a mixed group of varying nature as is partially illustrated in Fig. 1. Upon aging, part of these mixed groups may split from Center I constituting two types of discrete phases: P-organic matter associations (preferably through Al bridges) and variscite-like compounds. Center I would thus be partly freed of the adsorbed phosphate by loosing its most external layer, and would recover its anion-adsorption capacity (H. Appelt; personal communication). This process would resemble a kind of peeling of the allophane surface through which buried aluminosilicate layers would arise as new active adsorption sites within the soil media. With this interpretation adsorption of phosphate in allophanic soils would be an endless process unless the active allophanic Center I became completely disrupted. This would explain the unusually high P-fixing capacity maintained in these soils even after years of heavy fertilization with phosphate.

In general, all forms of P in soil are potential sources of this element to plants (Bielisky, 1973). The degree of actual availability will depend on the tenacity with which the P is attached to the source. In the case of allophanic soils the main permanent sources should be Center I, Center II, the sink

Table 3. Organic phosphorus associated with the humic acid fraction

Soil	Humic-P (% of humus-P)	
	F	U
Corte Alto	85	81
Arrayán	65	66
Loncoche	83	66
Malihue	64	43
San Pablo	59	56
Victoria	89	80
Puerto Varas	70	65
Metrengo	95	76

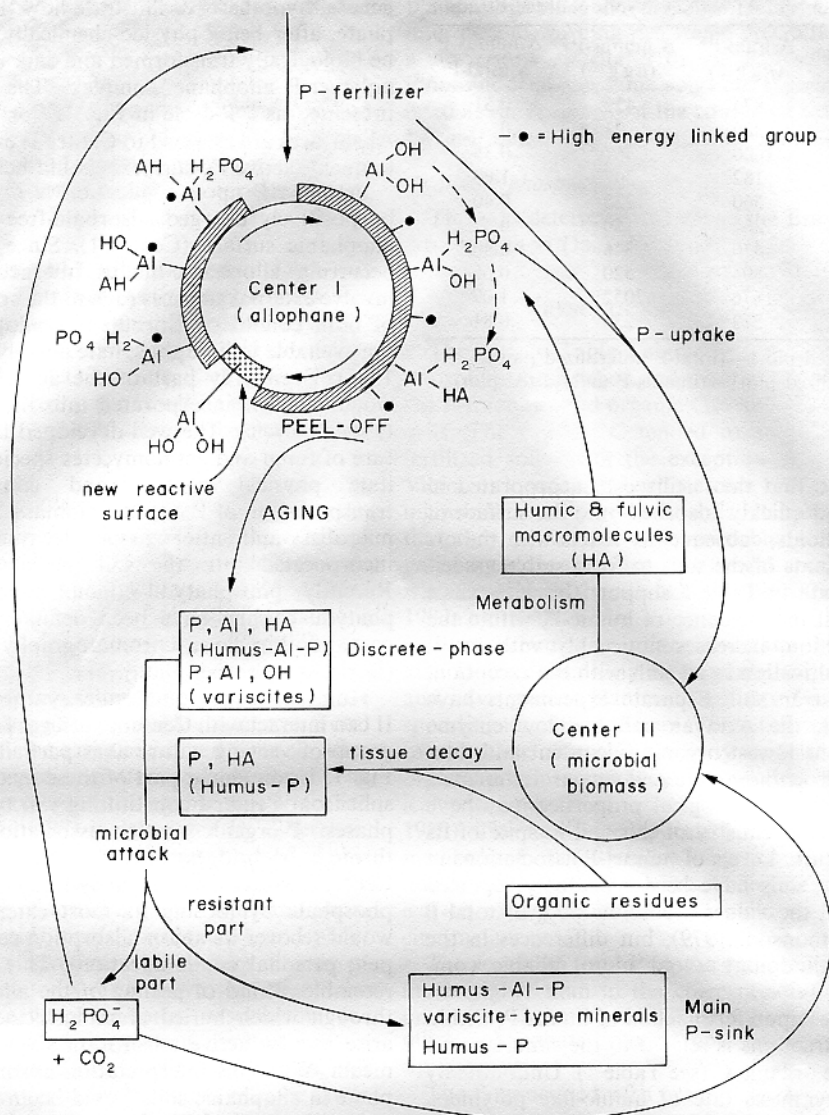


Fig. 1. Hypothetical scheme depicting the influence of microbial activity on the fate of fertilizer-P in allophanic soils.

humus-P, humus-Al-P associations and variscite-like minerals. The tenacity with which each component binds phosphate (both indigenous and added P) brings about the scarcity of available P for plant roots.

There exist several possible ways to increase P-availability in soil. The traditional ones include the use of slow-release fertilizers, foliar applications, special methods of fertilizer application in the field, etc. Even though some of them alleviate P-fixation in allophanic soils none adequately solve the problem. In these soils improvements in P-nutrition have to be sought by shifting the P-equilibrium toward root absorption, by taking into account the special environment and the composition of P-sinks. To achieve this it is essential to understand the mechanisms by which native vegetation absorbs enough P to satisfy its P requirements. It has been demonstrated that phosphatase activity is very high in these soils

wherein the enzyme is stabilized by interaction with allophanic clays and humus. Also the number and variety of phosphate-solubilizing fungi are important. Both of these mechanisms are considered to be good alternatives deserving close attention. However, it seems that the most attractive and practical way to help P-nutrition would be based upon mycorrhizal infection of plant roots. Mycorrhizas merit strong attention in allophanic soils, given the fact that the symbiotic fungi present the great advantage of their branched hyphal structure constituting a very effective exploring system reinforced by the high biochemical activity of the fungi. Studies in progress have shown that nursery-infected plants growing in allophanic soils of Chile show increased P-absorption capacity.

Besides the efforts to characterize and understand the phosphate adsorption mechanisms occurring at the allophane surface level, additional research on the

potential availability of humus-P and humus-metal-P associations should be encouraged. More research, by applying newly-improved techniques (Brookes *et al.*, 1982; Hedley and Stewart, 1982), is also needed to evaluate the amounts of P contained in the microbial biomass of these soils; this type of P is considered an important stage in P-turnover in soil (Jenkinson and Ladd, 1981). Assuming a P-content of 0.3% in the microbial population and that the biomass represent 3% of the total C, about 8–9% of the organic P could be in the microbial cells. These figures would confirm that microbial-P plays an important and specific role in P-cycling operating in allophanic soils.

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REFERENCES

- Anderson G. (1975) Other organic phosphorus compounds. In *Soil Components, Organic Components* Vol. 1, (J. K. Gieseking, Ed.), pp. 305–333. Springer-Verlag, New York.
- Appelt H., Coleman N. T. and Pratt P. F. (1975) Interactions between organic compounds, minerals and ions in volcanic-ash derived soils: II. Effects of organic compounds on the adsorption of phosphate. *Soil Science Society of America Proceedings* **39**, 628–630.
- Bielisky R. L. (1973) Phosphate pools, phosphate transport and phosphate availability. *Annual Review of Plant Physiology* **24**, 205–252.
- Borie F. (1983) Fósforo orgánico en suelos volcánicos de Chile. *Agricultura Técnica (Chile)* **43**(3). In press.
- Borie F. and Fuentealba R. (1982) Bioquímica de suelos derivados de cenizas volcánicas. 2. Actividad ureásica. *Agricultura Técnica (Chile)* **42**, 135–142.
- Brookes P. C., Powlson D. S. and Jenkinson D. S. (1982) Measurement of microbial biomass phosphorus in soil. *Soil Biology & Biochemistry* **14**, 319–329.
- Dick W. A. and Tabatabai M. A. (1977) An alkaline oxidation method for determination of total phosphorus in soils. *Soil Science Society of America Journal* **41**, 511–514.
- Finkl C. W. and Simonson R. W. (1979) Phosphorus cycle. In *The Encyclopedia of Soil Science* Part 1, (R. W. Fairbridge and C. W. Finkl, Eds), pp. 370–376. Dowden, Hutchinson and Ross, New York.
- Hedley M. J. and Stewart J. W. B. (1982) Method to measure microbial phosphate in soils. *Soil Biology & Biochemistry* **14**, 377–385.
- Jackson M. L. (1958) *Soil Chemical Analysis*. Constable, London.
- Jenkinson D. S. and Ladd J. N. (1981) Microbial biomass in soil-measurement and turnover. In *Soil Biochemistry* (E. A. Paul and J. N. Ladd, Eds), Vol. 5, pp. 415–471. Marcel Dekker, New York.
- Martin J. P., Zunino H., Peirano P., Caiozzi M. and Haider K. (1982) Decomposition of ¹⁴C-labeled lignins, model humic acid polymers, and fungal melanins in allophanic soils. *Soil Biology & Biochemistry* **14**, 289–293.
- Parfitt R. L. (1980) Chemical properties of variable charge soils. In *Soils with Variable Charge* (B. K. G. Theng, Ed.), pp. 167–194. New Zealand Soil Bureau, D.S.I.R. Lower Hutt, New Zealand.
- Steward J. H. and Oades J. M. (1972) The determination of organic P in soils. *Journal of Soil Science* **23**, 38–49.
- Wada K. and Gunjigake N. (1979) Active aluminum and iron an phosphate adsorption in andosols. *Soil Science* **128**, 331–336.
- Zunino H. and Martin J. P. (1977) Metal-binding organic macromolecules in soil. I. Hypothesis interpreting the role of soil organic matter on the translocation of metal ions from rocks to biological systems. *Soil Science* **123**, 65–76.
- Zunino H., Borie F., Aguilera M., Peirano P., Caiozzi M. and Martin J. P. (1982a) Bioquímica de suelos derivados de cenizas volcánicas. I. Ecología microbiana y su relación con algunas propiedades físico-químicas de ellos. *Agricultura Técnica (Chile)* **42**, 55–60.
- Zunino H., Borie F., Aguilera M., Martin J. P. and Haider K. (1982b) Decomposition of ¹⁴C-labeled glucose, plant and microbial products and phenols in volcanic-ash derived soils of Chile. *Soil Biology & Biochemistry* **14**, 37–43.
- Zunino H., Aguilera M., Peirano P., Caiozzi M. and Rex A. (1982c) Bioquímica de suelos derivados de cenizas volcánicas. 3. Síntesis microbiana de polímeros húmicos y su capacidad de adsorción de Zn(II) y Mg(II). *Agricultura Técnica (Chile)* **42**, 287–292.