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ORGANIC PHOSPHORUS IN SOILS WITH SPECIAL INTEREST IN SOILS OF THE TROPICS

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It is the purpose of this study to review and summarize the existing knowledge and literature on organic phosphorus in highly weathered soils of the tropics. This review includes many references which are concerned primarily with organic phosphorus as present in soils of temperate regions. This is included to serve as a starting point for investigations in tropical areas.

The three major topics covered are: identification and determination of specific forms of organic phosphorus; mineralization of organic phosphorus; and determination of total organic phosphorus. Less attention is given to microbiological aspects of the problem.

Specifically, the author discusses and reviews the following topics: inositol phosphates; phospholipids; nucleic acids; correlation with organic nitrogen and carbon; effects of cultivation and presence of plants; effects of soil organic phosphorus-clay mineral interactions; extraction methods; ignition methods and comparative studies of extraction versus ignition methods.

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K. J. Roberts and R. M. Weaver

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Organic Phosphorus in Soils with Special Interest in Soils of the Tropics[1]

K. J. Roberts and R. M. Weaver[2]

Significant deficiencies of plant available phosphorus occur in many of the highly weathered Ultisols and Oxisols of the tropics. For adequate crop production, these deficiencies must be overcome by addition of phosphate fertilizers or else implementation of soil management practices that bring about an increase in the rate at which the largely unavailable, total soil phosphorus becomes available for plant uptake. In most cases, fertilizer application has been and is, the preferred method used to correct the phosphorus deficiencies. However, the many problems of soil retention of added phosphates in the tropics (Ahenkorah, 1968) may require fertilizer expenditures that are beyond the economic resources of the subsistent farmer. In this case, soil management practices that enhance the availability of the total soil phosphorus would be in order. In this regard, it is very likely that for highly weathered soils of the humid tropics, the organic forms of soil phosphorus may be of more interest than the inorganic forms. There are two main reasons for this. First, the organic forms of phosphorus in highly

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weathered soils of the humid tropics constitute in general, a higher proportion of the total soil phosphorus than in less-weathered soils of temperate regions (Bornemisza and Igue, 1967; Blasco et al., 1968; Omotoso, 1970). Secondly, humid tropical regions have a rapid rate of production and decomposition of organic matter, and organic phosphorus should be mineralized rather quickly. Indeed, the potential of organic phosphorus in soils of the humid tropics to act as a reserve pool for available phosphorus by undergoing mineralization has not been overlooked (Friend and Birch, 1960; Cunningham, 1963; Smith and Acquaye, 1963; Omotoso, 1971). There is, however, very little information on the exact forms and relationships of the various organic phosphorus compounds as they exist in tropical soils. Nor is there detailed knowledge of the real value of organic phosphorus to plant nutrition in the tropics, nor of the exact chemical, physical or biological factors which influence its mineralization and subsequent reactions.

It is the purpose of this review to summarize the existing knowledge and literature on organic phosphorus in highly weathered soils of the tropics. This review will include many references which are concerned primarily with organic phosphorus as present in soils of temperate regions. This is not to imply that the results obtained in temperate zone research are applicable to soils of the tropics, but is meant to serve as a starting point for investigations in tropical areas. The three major topics covered; identification and determination of specific forms of organic phosphorus, mineralization of organic phosphorus, and determination of total organic phosphorus; cover most of the range of soil organic phosphorus problems. Less attention is given to microbiological aspects of the problem (See Alexander, 1961; 1971; Barrow, 1961).

FORMS OF SOIL ORGANIC PHOSPHORUS

Anderson (1960) suggests that "there may be a distinct qualitative difference in the phosphate esters present in various soil types or soils of different regions. Alternatively, the degree of success with which a procedure enables a given soil phosphate ester to be estimated may depend on the nature of the soil." A great deal of work has been directed at positive identification and quantitative estimation of specific organic phosphorus constituents. Excellent reviews of these forms are given by Anderson (1967) and Cosgrove (1967).

Anderson (1967) notes that there are three major identifiable groups of organic phosphorus compounds present in the soil: the phospholipids, the nucleic acids, and the inositol phosphates. There is also evidence that phosphoproteins and sugar phosphates (glucose-1-phosphate) exist in the soil. A large portion of soil organic phosphates, ranging from 33% to over 50% of the total, remains unidentifiable at the present.

About 1% of the total organic phosphorus is in the form of phospholipids (Hance and Anderson, 1963a,b); from 5-10% is nucleic acid phosphorus (Anderson, 1958, 1961); and a wide range of values for inositol phosphates with estimates as great as 60% have been reported (Anderson, 1956, 1967; Williams and Anderson, 1968; Omotoso and Wild, 1970; Martin and Wicken, 1966).

Inositol Phosphates

Inositol phosphates represent the most significant portion of organic phosphorus in soils. Cosgrove (1966) has noted that soils contain a number of stereoisomeric forms of the inositol phosphates found nowhere else in nature. Inositol itself (hexahydroxycyclohexane) is present in virtually

all tissue.

Forms of inositol phosphates occurring in soil include polyphosphates of five of nine possible stereoisomers, several stereoisomeric forms of inositol penta- and hexaphosphates (Cosgrove, 1963; Martin and Wicken, 1966), and myoinositol hexaphosphate, which comprises most of the soil inositol phosphate. Very small amounts of other myoinositol polyphosphates and lower esters of the other isomers of inositol have been identified (Smith and Clark, 1951; Anderson, 1956; Wild and Oke, 1966).

Reliable methods of inositol phosphate isolation and identification date back to Smith and Clark (1951), who utilized anion exchange chromatographic analysis. Procedures developed since then have improved reliability; but are still in a state of flux. Perhaps the most widely used is that of Anderson (1956, 1964). To extract inositol phosphates, the soil is leached with cold 0.2 N HCl to remove calcium and then extracted with 1 N NaOH. The esters are separated by chromatography on anion resin after preliminary chemical fractionation of the extract. Both neutral salt solutions and acids have been used to elute the esters from the resins. Cosgrove (1963) describes a separation of myoinositol phosphates and hexaphosphates by gradient elution with acid. Further pretreatment to break up certain inositol complexes with proteins is described by Anderson and Hance (1963). Gel chromatography, in place of paper chromatography, is described by Steward and Tate (1971). Martin (1970) suggests measurement of inositol phosphates by isotope dilution analysis, and obtained excellent results with the method. A colorimetric procedure for inositol phosphate determination is outlined by Lornitzo (1968).

Halstead and Anderson (1970) examined inositol phosphate content in three types of soil extracts; alkali, acid, and aqueous acetylacetone. They found that NaOH removed the most inositol phosphate. The lesser amount removed by HCl was attributed not to hydrolysis but to the presence of insoluble complexes of inositol phosphates. The use of a mild extractant (0.2 M acetylacetone, pH 8) also indicated that the inositol phosphate in soil exists in complex forms and that the degree of complex formation varies considerably with the soil. Omotoso and Wild (1970b) explain such results by hypothesizing that organic phosphates in soil are separated from the bulk of soil organic matter when humic acid is precipitated. Under mild extractions such as acetylacetone, some inositol phosphates remain in organic complex.

This issue of organic phosphorus complexes in the soil has received increased attention recently. Previously, few studies had attempted to reveal how compounds such as inositol phosphates or nucleic acids actually exist in the soil and how they relate to other organic matter compounds. Moyer and Thomas (1970) isolated inositol phosphates from soils with a molecular weight range of 1,000 to 50,000 by using a gel filtration technique which separates compounds on a physical basis according to molecular size. In addition, they found a fraction which contained 36% of the total organic phosphorus with molecular weight greater than 50,000. Thomas and Bowman (1966) had initially examined high molecular weight organic phosphates in soil and described a mild extraction procedure utilizing a chelating resin. Veinot and Thomas (1972) suggest that it is unlikely that compounds with such high molecular weights as reported by Moyer and Thomas (1970) are true molecular species. They indicate that they are most likely complexes formed from simpler organic

compounds. In an attempted examination of the constituents of these high molecular weight complexes, they found that iron and aluminum are partially responsible for the complex formation. But, after noting a high molecular weight complex relatively low in aluminum or iron, they concluded that there are other agents responsible for complex formation. In an examination of the stability of such high molecular weight complexes, Veinot and Thomas (1972) found that even relatively mild extraction treatments for removing organic phosphorus will alter the molecular weight distribution. Zunino et al. (1972) report a theoretical derivation of stability constants of metal-soil organic matter complexes based on the use of the resin exchange method of Clark and Turner (1969). Previous work on characterization of soil organic matter-metal-phosphate complexes was done by Levesque (1969), who found that significant amounts of complexed inorganic phosphorus were intermixed with organic phosphorus compounds in the soil extracts.

Phospholipids

As result of the relatively small fraction of total organic phosphorus in soil comprised of phospholipids, much less work has been directed at their determination. The extractability of phospholipids is complicated by several factors. The nature of the predominant lipids, their linkage with protein or carbohydrates, the clay content of the soil, and the drying of the soil all affect phospholipid extractability. Hance and Anderson (1963a) review these factors and previous methods of phospholipid extraction and estimation. In the sequel paper, Hance and Anderson (1963b) outline procedures for the identification of specific phospholipids: glycerophosphate was identified by paper chromatography after alkaline hydrolysis of isolated phospholipids

with boiling 0.5 N alcoholic NaOH for four hours and was estimated by periodate oxidation to represent 52-86% of total lipid phosphorus. Choline and ethanolamine were identified in an acidic (boiling 6 N alcoholic HCl for 4 hours) hydrolyzate. More recently, Kowalenko and McKercher (1970) compared several methods of phospholipid extraction, and concluded that Hance and Anderson (1963a,b) was the most satisfactory.

Nucleic Acids

Again it is Anderson that has made the greatest contribution toward the identification and estimation of nucleic acids in the soil. Anderson (1958) reviewed previous research on nucleic acids in soil, and noted that the purine bases guanine and adenine, and the pyrimidines, cytosine, thymine and uracil, have been identified in soil extracts. These bases are characteristic of DNA; and Anderson (1958) estimated that the DNA phosphorus in certain Scottish soils accounts for less than 1% of total organic phosphorus. In a later paper, Anderson (1961) improved his method and found values for DNA phosphorus as high as 2.4% of total organic phosphorus. But it was not until rather recently that Anderson (1970) was able to confirm the existence in soil of characteristic structural units of DNA. An excellent review of nucleic acids in soil may be found in Anderson (1967).

FORMS OF ORGANIC PHOSPHORUS IN SOILS OF THE TROPICS

As is the case with many chemical and physical characteristics of tropical soils, there is a notable lack of published data concerning organic phosphorus in soils of the tropics in the literature. On the other hand, a comparatively large amount of work has been done on characterizing inorganic forms of phosphorus in tropical soils (see Kamprath, 1972 for a review of work done

in Latin America). In the process of inorganic characterization, mostly by the methods of Chang and Jackson (1957) or some modification thereof, many of these investigations indirectly determined an organic phosphorus content by assuming it to be the difference between total phosphorus and the "Chang and Jackson" inorganic fractions. Such an assumption is likely to be untrue as it is possible that some organic phosphorus is calculated as part of the inorganic phosphorus fraction.

Bornemisza and his co-workers (1967, 1968) have contributed to investigation of total organic phosphorus in tropical soils, and reported values for organic phosphorus ranging from 35% to 77% of total soil phosphorus in Costa Rican soils. In two different highly weathered soils, reported values were 35% and 63%. Enwezor and Moore (1966) report a mean organic phosphorus content of 42% of total phosphorus in Nigerian soils. Apparently organic phosphorus can comprise a significant proportion of total soil phosphorus, particularly in the more highly weathered Ultisols and Oxisols. Some results, however, have been reported (Dahnke et al., 1963; Westin and De Brito, 1969; Moura Filho et al., 1972) that would suggest that more highly weathered soils have a decreased proportion of phosphorus in the organic form. While this may be true for some cases, it has been shown by Bornemisza and Igue (1967) that these results may very likely be a consequence of using methods that are less efficient for the extraction of organic phosphorus from highly weathered soils in comparison to less weathered soils. There has been very little work done on characterization of the specific forms of organic phosphorus in tropical soils and the relationship of this breakdown to a potential "reserve pool" of phosphorus in the soil. Omotoso and Wild (1970a) reported on inositol phosphates of several Nigerian soils, none of them highly weathered.

The Smithsonian Information Exchange reports that Oliver and Bang (1971) were studying the problems presented by the content and evolution of organic phosphates in the soils of Madagascar. It appears from the project summary that the study was primarily concerned with how the organic phosphates entered the inorganic phosphate "pool" and not with the specific forms of organic phosphorus. No publication is known from this work.

MINERALIZATION OF ORGANIC PHOSPHORUS

The mineralization and immobilization of organic phosphorus brought about by microbiological activity is certainly an important part of the general phosphorus cycle. The concept of organic phosphorus as an important reserve for replenishing plant available phosphorus in some soils is relatively widespread, and may be particularly important in certain soils of the tropics (Enwezor, 1967). The significance of organic phosphorus in such a case is primarily dependent on mineralization rates and subsequent reactions in the soil, i.e., fixation.

Cosgrove (1967) notes that there are two types of investigations of mineralization processes. One is based on the measurement of the phosphatase activity of organisms isolated from the soil; and the second is based on the measurement of the phosphatase activity of the soil as a whole. The latter approach would include the activity of any free enzymes in the soil.

Cosgrove (1967) reviews previous work done on determining activity of certain microorganisms with respect to organic phosphorus mineralization; but he finds that most evidence obtained was conflicting. The variation in reported values is attributed by Cosgrove to difficulties in substrate use, particularly pH, in experimental procedure; and to the use of commercial samples of phytate. A method of investigation of the phosphatase activity of

the whole soil is described by Halstead (1964) as adapted from Kramer and Yerdei (1959).

Cosgrove (1967), in attempting to explain the large accumulation of inositol phosphates while a wide variety of microorganisms exhibit phytase activity, notes that iron and aluminum phytates are very insoluble. These forms are likely to predominate in acid soils, thus raising the pH of an acid soil would likely cause increased mineralization of phytate phosphorus. This could very well be why that liming of highly weathered soils often results in enhanced phosphorus availability (Olson and Engelstad, 1972).

Organic phosphorus mineralization has been found to increase with increasing temperature by Bower (1949), Van Diest and Black (1959b) and Acquaye (1963). This effect is particularly significant above 30°C. Repeated wetting and drying cycles were found by Birch and Friend (1961) to significantly increase mineralization of organic phosphorus.

The effect of added inorganic phosphorus fertilizers on the mineralization of organic phosphorus has been considered by several workers. Acquaye (1963) attempted a study of organic phosphorus mineralization rates upon addition of N-P fertilizers in soils of Ghana and found that increased mineralization occurred. Similar results were reported by Smith and Acquaye (1963). Weir and Black (1968a) reviewed previous research which had concluded that the addition of inorganic phosphorus, while increasing organic phosphorus levels, significantly increased organic phosphorus mineralization. They suggested and tested the following hypothesis: by competition with organic phosphorus for the cations responsible for its slightly soluble form, the inorganic phosphorus increases the solubility of the organic phosphorus and its susceptibility to mineralization. In spite of previous work on the topic,

Weir and Black were not able to find an important influence of added inorganic phosphate on the rate of mineralization of organic phosphorus.

Correlation with Organic Nitrogen and Carbon

Thompson and Black (1949) and Thompson et al. (1954) indicated that organic phosphorus correlated positively with organic carbon and total nitrogen and that its mineralization correlated positively with concurrent carbon and nitrogen mineralization. In addition, the amounts of mineralized organic phosphorus, nitrogen and carbon roughly corresponded to the proportions in which these constitutents occurred in soil organic matter. This observation, however, has not been settled; and it should be noted that organic phosphorus behavior is not necessarily correlated with that of carbon and nitrogen.

In a review of nearly twenty references, Black and Goring (1953) suggested a generalized C:N:org P ration of 110:9:1 for soils. This generalization is unacceptable, for it is now known that such a ratio varies considerably with changing soil conditions. John et al. (1965) demonstrated this by measuring C:org P ratios ranging from 46 to 648, and N:org P ratios ranging from 5 to 57. John et al. suggest a primary dependence of this ratio on pH and note the possibility of influence by iron and aluminum.

Nye and Bertheux (1957), in a study of phosphorus distribution of soils of Ghana, were among the first to note that Black and Goring's suggested C:org P ratio of 110 is not applicable to soils of the tropics. Enwezor (1967), in a study of a wide range of Nigerian soils, concluded that organic phosphorus was not correlated with the C:org P ratio. No significant correlation between the C:org P ratio and mineralized phosphorus was found.

Bornemisza (1968) reported widely divergent values for C:org P and N:org P between soils of Costa Rica and more weathered soils of the Amazon Basin. Respective values were 64 and 5.3 for Costa Rica and 300-400 and 20-40 for the Brazilian samples.

Studies concerning the effect of pH and liming on mineralization of organic phosphorus (Thompson et al., 1954; Halstead et al., 1963; Awan, 1964) were interpreted by Cosgrove (1967) as implying the generalization that alkaline soils would usually have a higher C:org P and N:org P ratios than acid soils.

Such a generalization, however, may be dangerous in light of the complex relationships of organic phosphorus and the lack of detailed information concerning the full range of forms of organic phosphorus as they exist in the soil. The values reported by Bornemisza (1968) demonstrate the fallability of such a generalization.

Effects of Cultivation and the Presence of Plants

Evidence obtained by Sekhon and Black (1969) indicated that the presence of plants decreases the organic phosphorus content of the soil external to the roots. Successively lower contents of extractable organic phosphorus were found in samples that had been (a) air dried initially and stored in that condition, (b) incubated in a moist condition but without a crop, and (c) planted to four successive crops, the roots being removed prior to soil analysis. Differences were significant. Sekhon and Black (1969) investigated the possibility that the decrease in organic phosphorus was concentrated in a thin layer around the roots. Measurements of organic phosphorus in aggregates adhering to the roots and in the bulk of the soil were compared

and no significant differences were found. Thompson and Black (1970a) questioned Sekhon and Black's (1969) sampling techniques and assumptions and attempted a study of plant effects on organic phosphorus mineralization in a simulated rhizosphere in order to avoid sampling error. Thompson and Black (1970b) suggested two possible mechanisms for plant induced decrease in soil organic phosphorus: (a) enhanced phosphatase enzyme activity in the soil around the roots causes an increase in the rate of mineralization of organic phosphorus (the "Phosphatase hypothesis"), and (b) direct absorption of soil organic phosphorus by roots (the "direct absorption hypothesis"). The latter explanation, generally dismissed, was given serious consideration by Thompson and Black, but they were unable to reach any final conclusions regarding either hypothesis. Van Diest and Black (1959a,b) investigated the mineralization of organic phosphorus during a crop cycle as related to the phosphorus nutrition of that crop, and concluded that a significant fraction of the phosphorus taken up by plants was initially present in the organic form and was mineralized while the plants were growing. Sekhon and Black (1968) confirmed this. In a discussion of this "current contribution" of phosphorus during plant growth, Weir and Black (1968b) note that the most direct evidence obtained for existence of a current effect is the finding that biological indices of phosphorus availability increase with the amount of mineralization of soil organic phosphorus or with the amount of organic phosphorus, independently of the labile inorganic phosphorus present initially.

Effects of Soil Organic Phosphorus - Clay Mineral Interactions

The adsorption of organic phosphorus compounds on clay mineral surfaces would increase their resistance to mineralization. The extent and nature of

this adsorption would depend on the specific surface and the net electric charges of the clay mineral, the equilibrium pH of the adsorbing medium, and the nature of the adsorbed organic phosphorus molecules. The possibility that the enzyme is adsorbed rather than the organic phosphorus also exists, and such an adsorption would also tend to decrease mineralization rates. Most research has concerned temperate zone soils in which the clay minerals were predominantly of the montmorillonite group which would generally adsorb significantly more organic phosphorus than kaolinitic clays. The significance of such a mechanism in soils of the tropics may be questionable, but there has been little discussion of this possibility in the literature. Anderson and Arlidge (1962) report on the adsorption of inositol phosphates and glycerophosphate in acid media. An investigation of the adsorption of organic phosphates in tropical soils and identification of the specific forms, if any, which are adsorbed would be of interest.

DETERMINATION OF TOTAL ORGANIC PHOSPHORUS IN SOILS

The problem of total organic phosphorus determination is one which still has not been completely solved. Nearly all work on the problem has concerned indirect methods of measurement. These methods can easily be divided into two classes, extraction methods and ignition methods:

<u>Extraction Methods</u>: The organic phosphorus and much of the inorganic phosphorus are extracted from the soil. The organic phosphorus in the extract is converted to orthophosphate and the content of organic phosphorus is determined from the increase in inorganic phosphorus compared with the original extract.

Ignition Methods: The organic phosphorus is converted to inorganic phosphorus by ignition of the soil at some specified temperature. The organic phosphorus is determined from the difference between the inorganic phosphorus content of extracts of ignited and non-ignited samples.

For much time, direct evaluation seemed impracticable because of the intimate association of both organic and inorganic forms of phosphorus with other soil constituents. After the most extensive investigation of methods of organic phosphorus determination had passed (in the decade 1954-1964), Anderson and Black (1965) developed a procedure for direct evaluation. They used mechanical and chromatographic filtration for separating organic phosphorus from inorganic phosphorus. The technique involves adding an acidified extract to a column of carbon, washing the column with dilute HCl to remove the inorganic phosphorus and analyzing the carbon for residual phosphorus. They report that more accurate results are obtained by filtering an extract through a carbon column rather than by adding carbon to the extract and removing it by filtration, as Goring (1955) had done in different investigations. Anderson and Black conclude that inorganic orthophosphate retained from the extracts is negligible in comparison with organic phosphorus. On the other hand, if large excesses of inorganic orthophosphate are added, their retention by the carbon is great enough to require correction by an appropriate control. Nevertheless, they maintain that accurate results for organic phosphorus can still be obtained, whereas large error would be introduced in this case using indirect methods. The method has rarely appeared in the literature since.

Extraction Methods

These methods have been the most used. The various methods proposed are rather time consuming and sometimes difficult, yet they generally give more accurate results than ignition type methods if proper caution is taken. Possible sources of serious error in extraction methods are:

(a) incomplete extraction of organic phosphorus,

(b) hydrolysis or interconversion of organic forms by strong extracting agents.

In addition, extraction methods tend to give variable results under varying experimental conditions.

Early discussion of extraction procedures is given by Kaila and Virtanen (1955) and Saunders and Williams (1955). Most presently used methods are a modification of Mehta et al. (1954). In an extraction method the soil is generally first leached to remove most of the calcium, magnesium, iron and aluminum which are considered to bind phosphorus compounds in the soil. Initial leaching may remove some organic phosphate, but this is determined and included in the final results. Initial extraction methods have varied. Their chief requirement is the removal of polyvalent cations under conditions where more labile soil organic phosphorus compounds are not hydrolyzed. Mehta et al. (1954) used concentrated HCl for one hour at elevated temperature. This has the disadvantage that acid-labile phosphorus esters may be hydrolyzed. Anderson (1960) showed this to be true and proposed a modification of the Mehta procedure by performing a dilute alkali extraction (0.3 N NaOH) before extracting with HCl. Other pretreatment steps have involved the use of 4 N H_2SO_4 for 18 hours (Kaila et al., 1955), acetone (Dormaar and Webster, 1963), 8-hydroxy quinoline (Boswell and DeLong, 1959), and disodium-

EDTA (Harrap, 1963). The Mehta-Anderson method is outlined below:

(1) One gram of soil is ground to pass a 100 mesh sieve and is treated with 70 ml 0.3 N NaOH, shaken overnight in a 100 ml centrifuge tube.

(2) The tube is centrifuged, the supernatant is transferred to a 100 ml volumetric flask and filled to volume.

(3) The soil residue is treated with hot concentrated HCl for one hour (60 ml) then 0.5 N NaOH for one hour (60 ml), then hot 0.5 N NaOH for eight hours (50 ml). The supernatants are collected in a 200 ml volumetric flask. (Anderson suggests modification if 250 ml volumetric is used.)

(4) Anderson recommends phosphorus determinations by the method of Dickman and Bray (1940) or Martin and Doty (1949).

Anderson concludes that phosphate esters are least stable to hydrolysis at pH 4 and the order of their stability is: nucleic acids > inositol phosphates > glycerophosphate.

Bornemisza et al. (1967) found in an investigation of soils of the Amazon Basin, that enough iron can be extracted from certain soils high in iron by the outlined procedure above that serious interference in subsequent spectrophotometric determination may occur. They recommend that extracts from soils likely to be significantly high in iron be passed through a strong acid cation exchange resin prior to making spectrophotometric measurements.

More recently, extraction methods involving ultrasonic vibration techniques have been advanced. Halstead and Scott (1966) described a method of organic matter extraction by means of ultrasonic dispersion in aqueous acetylacetone, earlier examined by Greenland and Ford (1964). Steward and Oades (1972) outline a specific procedure for determination of organic phosphorus

using ultrasonic vibration. The advantage of such a procedure is that 3 minutes of ultrasonic vibration in 0.5 N NaOH was found as effective as a 16 hour shaking extraction with hot 0.5 N NaOH. They conclude that in normal shaking procedure a very long period of time is required for the NaOH to penetrate "hidden" recesses of clay particles to extract organic materials, whereas immediate access is provided by ultrasonic dispersion. Experimentally, though, it may be difficult to avoid loss of sample.

Other extraction methods proposed include sodium bicarbonate extraction (MacLean, 1965).

Ignition Methods

Various ignition methods have also been proposed (Legg and Black, 1955; Saunders and Williams, 1955; Walker and Adams, 1958; Olsen and Dean, 1965). Despite the fact that the method is relatively rapid and simple, the accuracy of the method is extremely endangered by the following (Williams et al., 1970; Dormaar and Webster, 1964):

(1) incomplete oxidation of organic phosphorus up to 650°C;

(2) volatilization of phosphorus at temperatures greater than 400°C;

(3) possibility of incomplete extraction of mineralized organic phosphorus;

(4) formation of insoluble complexes as in the case of phytin, and likely RNA;

(5) changes in the extractability of inorganic phosphorus resulting from ignition;

(6) hydrolysis of organic phosphorus during the acid extraction of the unignited soil.

Mehlich (1963) made findings which also raise doubts about the validity of organic phosphorus determinations done by ignition methods, especially on tropical soils. Although temperature was found to have little effect on phosphorus mobilization in soils containing predominantly montmorillonite; mobilization in limonite and in soils containing mainly allophane and halloysite greatly increased by heating to a maximum of 250-350°C; heating to 350-550°C was required for increasing phosphorus solubility in kaolin and kaolinite containing soil; and heating to 750°C progressively increased acid soluble phosphorus in goethite and in soils containing two- and three-layer minerals in varying mixtures (kaolinite/halloysite, kaolinite/illite, etc.). Mehlich found that the effects of temperature on acid soluble phosphorus in clay minerals are apparently associated with dehydration and crystallization conditions.

The techniques of ignition methods vary from that of Legg and Black (1955) where a low (240°C) ignition temperature is used, followed by a strong extraction with hot concentrated HCl; to that of Saunders and Williams (1955) where a high (550°C) ignition temperature is used, followed by a milder extraction with 0.2 N sulfuric acid. Dormaar and Webster (1964) found that the Legg-Black method gave incomplete combustion and low recoveries and was most likely biased toward the Canadian soils used in their experiment. They found that 500°C was a good compromise for ignition temperature if a quick approximate measure is desired. But they cautioned against relying on the procedure for quantitative results. Even then, one should recognize the implications of Mehlich (1963).

Comparative Studies of Extraction versus Ignition Methods

Several studies have been made comparing ignition and extraction methods for tropical soils exclusively, several have appeared investigating soils of New Zealand (Martin, 1964; Williams et al., 1970; Williams and Walker, 1967) and still others investigating temperate zone soils (Saunders and Williams, 1955; Hance and Anderson, 1962).

Enwezor and Moore (1965), in a comparison of Mehta et al. (1954) and the ignition procedure of Saunders and Williams (1955) on Nigerian soils of savanna region, found significantly higher results with ignition than with extraction. It is likely that increased solubility of inorganic phosphorus occurred upon ignition, and either incomplete extraction or, more likely, hydrolysis of organic phosphorus occurred in the extraction method. Both values are therefore questionable and Enwezor and Moore suggest that they be used as relative maxima and minima for organic phosphorus levels. It appears that their investigations would have been more worthy if they had used a modified Mehta procedure, i.e., Anderson (1960).

Bornemisza and Igue (1967) compared Anderson (1960) with both high and low temperature ignition techniques in a study of soils of Costa Rica, and found the extraction method to give the highest and most accurate results. They explained the unusual lower values of high temperature ignition methods by hypothesizing the formation of aluminum phosphates during ignition of soils high in sesquioxides.

Williams et al. (1970) found higher results in high temperature ignition than extraction, with low temperature ignition giving the lowest values. Their conclusion that extraction procedures are superior to ignition proce-

dures in accuracy has been the conclusion of most comparisons in temperate regions, save Hance and Anderson (1962) who recommend ignition by reason of its rapidity. The implication given by Anderson (1960) that varying soil conditions will give different results as to which method is best must be considered. Omotoso (1970), in a study of cacao growing soils of Southern Nigeria, concluded that the high temperature ignition method of Saunders and Williams (1955) was superior to the method of Anderson (1960) for reasons of simplicity. The ignition method gave higher values originally, but upon repeated extraction the Anderson method was able to recover nearly the same amount of organic phosphorus. It appears that technical ability and aversion of the many potential sources of error may be the most important factors determining the accuracy of either method. Additional comparative studies on soils of the tropics would be useful.

The time of sampling may also be a factor. Although the seasonal variation in organic phosphorus investigations of Dormaar (1972), Saunders and Metson (1971) and Williams (1962) cannot be directly applied to tropical studies; an investigation of possible variation of organic phosphorus levels due to time of sampling might be of value.

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