RETENTION, HYDROLYSIS AND PLANT AVAILABILITY OF PYROPHOSPHATE APPLIED TO ORGANIC SOIL MATERIALS

by

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ABSTRACT

Pyrophosphate retention and half-life values, as well as pyrophosphatase activity, were determined in 24 organic soil materials containing < 20% ash. Pyrophosphate retention was correlated with ash content (r = 0.876^{**}) but still was weak. Pyrophosphatase activity [11,6 to 148,1 mmol.kg⁻¹.(2h)⁻¹] was higher in virgin than in cultivated materials and was promoted apparently by nonspecific acid phosphatases. The interaction between water-soluble pyrophosphate and pyrophosphatase activity explained 77% of the variation in log-transformed half-life values ranging from 0,1 to 3,7 days. Copper significantly decreased pyrophosphatase activity. However, pyrophosphate hydrolysis rate was not affected significantly by Cu contents up to 1177 mg.kg⁻¹ in humic materials. Because of rapid rates of pyrophosphate hydrolysis in humic and mesic materials compared with rate of P uptake by onions, no significant difference in bulb yield and P uptake were obtained at harvest between pyrophosphate and orthophosphate fertilizers.

RESUME

La rátention et la demi-vie du pyrophosphate de même que l'activité pyrophosphatasique furent déterminées dans 24 matériaux organiques contenant < 207 de cendres. Une corrélation significative (r = 0,876**) fut établie entre le contenu en cendres et la retention, toutefois lâche, du pyrophosphate. L'activité pyrophosphatasique, attribuable à des phosphatases acides non spécifiques, était plus élevée en matériaux vierges que cultivés. L'interaction entre le pyrophosphate hydrosoluble et l'activité pyrophosphatasique expliqua 777 de la variation dans les valeurs de demi-vie (0,1 - 3,7 jours) transformées en logarithmes. Le Cu inhiba l'activité pyrophosphatasique. Toutefois, des teneurs en Cu atteignant 1177 mg par kg de matériaux humiques n'ont pas ralenti significativement l'hydrolyse du pyrophosphate. A cause des taux rapides d'hydrolyse du pyrophosphate en sol organique par rapport au taux de prélèvement du P par l'oignon, aucune différence significative n'existait entre fumures pyrophosphatées et orthophosphatées quant aux rendements des bulbes et aux prélèvements de P.

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I dedicate this work to my wife, Catherine, for her patience, selfcontrol and love, and to our four children, for whom Dad was often elsewhere, even at home.

CONTRIBUTION TO KNOWLEDGE

Using pyrophosphate reactions reported for mineral soils, a general model was developed to explain the fate of pyrophosphate in organic soils by five major reactions: retention of soluble pyrophosphate, hydrolysis of soluble pyrophosphate into soluble orthophosphate, retention of soluble orthophosphate, plant absorption of soluble pyrophosphate and plant absorption of soluble orthophosphate. New findings have been made on the capacity of organic soils to retain pyrophosphate and on the rate of pyrophosphate hydrolysis in these soils.

The present research provides original findings as follows:
1) Pyrophosphate retention in organic soil materials was highly correlated with ash content in the range of 1 to 19% ash; low-ash materials retained little or no pyrophosphate, while high ash materials retained pyrophosphate
weakly, as noted by small half-life values (< 3,7 days) for pyrophosphate incubated in these materials. Thus the effect of retention was short-lived.
2) Pyrophosphatase and phosphatase activities were assayed under comparable conditions by developing a new assay method for organic soils. A 0,2 M Tris-citric acid buffer at pH 6,4 was used. Pyrophosphatase and phosphatase activities were highly correlated and apparently promoted by nonspecific acid phosphatases. The activation energy of pyrophosphate hydrolysis in fibric and humic materials was 22 and 33 kJ.mol⁻¹, respectively.
3) The theoretical interaction between pyrophosphate retention and pyrophos-

5) The theoretical interaction between pyrophosphate retention and pyrophosphatase activity was described by a multiple regression equation which explained 77% of the variation in log (half-life) values of pyrophosphate hydrolysis in 24 organic soil materials.

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- 4) The enzyme inhibitor Cu, widely used as a fertilizer, was found to reduce pyrophosphatase activity in 24 organic soil materials, but was not likely to reduce pyrophosphate effectiveness as a fertilizer in humic materials containing up to 1177 mg of Cu per kg.
- 5) Differences between pyro- and orthophosphate as P sources for onions grown on humic and limed mesic materials were found to be nonsignificant, although heavy applications of Ca(OH)₂ to the mesic material increased considerably pyrophosphate retention and reduced pyrophosphatase activity as compared with the original material. Pyrophosphate hydrolysis rate was rapid in organic soils as compared with the rate of P absorption by onions. Onions could accumulate excessive amounts of P from both pyrophosphate phate and orthophosphate fertilizers.

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GENERAL INTRODUCTION

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INTRODUCTION

Nearly 15 000 ha of organic soils are under vegetable production in Canada. These soils have unique chemical, physical and biological properties that make them ideally suited to intensive crop production, but at the same time these soils require specialized management techniques. Fertilizers are used extensively, but fertilizer reactions are dependent on complex chemical and biological reactions. Organic soils require generally less fertilizer N and P, but more K, Mg and micronutrients that mineral soils. こうい、日本語を見ていたので、「「「「「「「「」」」」

The P fertilizers generally used on organic soils are granulated orthophosphate. However, liquid ammonium polyphosphates, mainly composed of ortho- and pyrophosphates, may be used advantageously on these soils, since polyphosphates can complex many micronutrients and can be applied uniformly either as solutions or as suspensions. Indeed, little research has been carried out on the behavior of pyrophosphates in organic soils, as opposed to the many studies conducted on mineral soils.

Byrophosphates hydrolyze to orthophosphate, and this is influenced by enzymes. Enzyme activity in organic soils could be complicated by absorption reactions and microbial activity. Yet plants prefer orthophosphate to pyrophosphate as a P source. Thus reactions of pyrophosphates in organic soils must be studied to clarify the effects of hydrolysis rates on P uptake by plants. It is the objective of this work to study these reactions.

The relevant literature will be reviewed and the hypotheses will be set up in Chapter I. In Chapter II, pyrophosphate hydrolysis, pyrophosphatase

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activity and pyrophosphate retention will be investigated in 24 organic soil materials from Eastern Canada. In Chapter III, the influence of a pyrophosphatase inhibitor (Cu), widely used as a fertilizer, will be studied with regard to mitigation of pyrophosphate hydrolysis in organic soils. Finally, in Chapter IV, the rate of pyrophosphate hydrolysis will be compared to the rate of P uptake by onions grown on humic and limed mesic materials. The effect of Ca(OH)₂ on pyrophosphates activity and pyrophosphate retention will be investigated.

MANNUS CONTRACTOR AND A MANUAL

These observations should allow us to define rate factors involved in pyrophosphate hydrolysis and, thus, to predict the efficiency of pyrophosphate fertilizers as a source of P for crops grown on organic soils.

CHAPTER I

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PYROPHOSPHATE REACTIONS

IN ORGANIC SOILS

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The use of liquid fertilizers has been increasing in North America since the early sixties. The breakthrough for this rapid expansion was the production of high-grade superphosphoric acid by the Tennessee Valley Authority in 1960. Superphosphoric acid is composed of orthophosphate and polyphosphates. The formation of polyphosphates from orthophosphoric acid is a polycondensation reaction (Jung and Jurgens-Gschwind 1975):

2 H_3PO_4 (- H20) $H_4P_2O_7$ Pyrophosphoric acid 3 H_3PO_4 (- 2 H20) $H_5P_3O_{10}$ Triphosphoric acid Thus, nH_3PO_4 (-(n-1) H20) H(n+2)P(n)O(3n+1) Polyphosphoric acids

The most widely used polyphosphate fertilizer is ammonium polyphosphate. Normally, it has an analysis of 10-34-0 and contains 55% of its phosphate in the form of orthophosphate, 39% in the pyrophosphate form, and the rest in more condensed P forms. Any difference in efficiency between ammonium polyphosphate and orthophosphate fertilizers has been attributed generally to the non-orthophosphate fraction of ammonium polyphosphate (Sutton and Larsen 1964).

Availability of pyrophosphates to plants

Pyrophosphate has been used as a model compound to study the availability of more complex polyphosphates to plants.

A classic paper was published by Sutton and Larsen in 1964. They showed that the availability of pyrophosphate to plants was related to the

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rate of hydrolysis of pyrophosphate to orthophosphate. Phosphorus uptake by ryegrass¹⁾ from either pyrophosphate or orthophosphate was found equivalent in a soil possessing a high biological activity; in contrast, a soil having a low biological activity was not as effective in supplying P to ryegrass fertilized with pyrophosphate as compared to orthophosphate. In a water culture experiment, Sutton and Larsen (1964) tested barley²⁾ seedlings receiving similar amounts of P as pyrophosphate or orthophosphate. The rate of P uptake was 2,4 times less from pyrophosphate over a 3,5 hour period. The possibility of pyrophosphate hydrolysis was excluded. Sutton and Larsen (1964) attributed the difference to the valencies of the component ions, since P uptake from pyrophosphate at pH 5,0 was similar to P uptake from orthophosphate at pH 9,0. In both cases the component ions were largely divalent.

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Gilliam (1970) confirmed that pyrophosphate ions could be absorbed by plant roots, and showed that pyrophosphate and orthophosphate were equally effective in supplying P to P-deficient wheat³⁾, corn⁴⁾ and barley seedlings. Gilliam (1970) explained his results by an increase of phosphatase activity in seedlings that were grown previously in a P-deficient medium.

From field experiments held at 95 sites in Great Britain, Sutton *et al.* (1966) concluded that the effect of pyrophosphate on yield of a particular crop could be related to a series of interactions between the rate of hydrolysis in soil, the time of maximum P requirement by crops and the P status in soil. Low temperature was the main cause of widespread reduction in hydrolysis rates, since reduced P uptake from pyrophosphate was associated 1) Lolium sp. 2) Hordeum vulgare L. 3) Triticum aestivum L. 4) Zea mays L.

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with low soil temperatures (7-12 degrees) regardless of biological activity. Correspondingly, Engelstadt and Allen (1971) found that pyrophosphate was less effective than orthophosphate as a source of P for corn grown in a cool = soil (16 °C), whereas both sources were equally effective in a warm soil (24 °C). The relationship between the P status of a soil and the availability of polyphosphates was studied by Rhue *et al.* (1981). They showed that under conditions of low P availability in soil, the yield of tubers was lower in the case of potatoes fertilized with ammonium polyphosphate as compared to those fertilized with diammonium phosphate. At higher levels of available P, differences in tuber yield were negligeble , although differences in tissue P content were still measurable (Rhue *et al.* 1981). Apparently, no attention was given to the time-dependency of the P requirement of the crop compared with the rate of pyrophosphate hydrolysis in soil.

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The results of many field and greenhouse experiments have indicated that polyphosphate fertilizers containing a high proportion of orthophosphate were comparable to orthophosphate fertilizers soluble in water and citrate (Dobson *et al.* 1970; Jung and Jurgens-G² chwind 1975).

Sequestration

Solubility in water of ammonium polyphosphate even in presence of Al-Fe impurities was attributed to the ability of polyphosphates to sequester Al-Fe in solution (Slack *et al.* 1965). Bremner *et al.* (1946) were the first to show evidence that pyrophosphate, the most efficient of the polyphosphate series, could form coordination complexes with polyvalent ions. 1) Solanum tuberosum L.

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The influence of polyphosphate additions to a soil system on the solubilization of micronutrients was studied by Giordano et al. (1971). They found that the zinc level in the soil solution of a Harstells fsl was influenced more by pH and liming than by the source and the rates (0;200;2000 mg.kg⁻¹) of applied P. The level of zinc was increased by triammonium pyrophosphate during the first day of application only, since rapid hydrolysis precluded any further sequestration. Mortvedt and Osborne (1977) confirmed that solubilization of soil micronutrients by polyphosphates did not appear to play an important role in plant nutrition. They attributed some reported superiority in yield of crops fertilized with polyphosphates over those fertilized with orthophosphate to micronutrient contaminants in polyphosphates. Asher and Bar-Josef (1982) found that the ability of pyrophosphate to sequester zinc in a montmorillonite solution was low at a pH of less than 6,0 and that even at a higher pH, a P concentration of 2000 mg.kg⁻¹ was too low to be effective. Hence, they concluded that the short-term increase of zinc concentration in soils treated with pyrophosphate was caused by organic matter solubilization.

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Hydrolysis of pyrophosphate

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Seven factors were reported by Van Wazer (1958) to influence pyrophosphate hydrolysis: temperature, pH, enzymes, colloidal gels, complexing cations, concentration and ionic environment. The effect of temperature predominates in soils (Sutton *et al.* 1966).

According to Van Wazer (1958), it would take 200 hours and one month,

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respectively, to hydrolyse 5% of a 1% solution of pyrophosphate adjusted to pH 4,0 and 7,0. At neutral pH and room temperature, the half-life of pyrophosphate is a matter of years. Van Wazer (1958) also indicated that colloidal gels such as hydrated oxides of iron and aluminium could accelerate the hydrolysis of polyphosphates. However, Hashimoto *et al.* (1969) rejected the hypothesis that colloidal gels such as clays, gibbsite and goethite could contribute effectively toward pyrophosphate hydrolysis in soil.

When pyrophosphate is reacted with soils, retention-release mechanisms could affect considerably pyrophosphate concentrations. Results obtained so far indicate that mineral soils retain more pyrophosphate than orthophosphate (Sutton and Larsen 1964; Gunary 1966; Hashimoto *et al.* 1969). Philen and Lehr (1967) showed that calcium and magnesium carbonates reacted readily with pyrophosphate at room temperature, and that clay minerals and hydrous oxides of Fe-Al reacted slowly in a matter of weeks and months. Many reaction products of pyrophosphate with soil constituents have been identified (Philen and Lehr 1967; Subbarao and Ellis 1975; Yadav and Goshal 1981; El Zahaby and Chien 1982). The retention-release mechanisms were thought to be responsible for the 10-35% of added pyrophosphate remaining resistent to hydrolysis after a long period of incubation in soils (Gilliam . and Sample 1968; Hossner and Melton 1970; Racz and Savant 1972; Juo and Maduakor 1973).

Huffman and Fleming (1960) observed that the addition of a Hartsells soil to a one liter solution containing one gram of polyphosphates reduced the half-life of hydrolysis from 1059 to 83 hours. The half-life of pyrophosphate in soils varies from 0,6 to 100 days (Sutton and Larsen 1964;
Blanchar and Hossner 1969; Hossner and Phillips 1971). Sutton and Larsen (1964) could explain 78% of the variation in half-life values with CO2 evolution and soil pH.

Gilliam and Sample (1968) studied the course of pyrophosphate hydrolysis in three soils containing 0,10 to 0,35% of organic carbon and having a common pH of 4,8. By comparing the amount of pyrophosphate hydrolyzed in natural and autoclaved soils after 129 days of incubation, they calculated that chemical factors contributed 25 and/50% toward pyrophosphate hydrolysis in Norfolk and Piedmont soils, respectively. Since the reaction was first-order and not zero-order, the contribution of chemical and biological factors were not appraised concurrently. In a situation where the substrate.concentration is no longer rate-limiting, it can be shown from Gilliam and Sample's data that, for the first six days of incubation, the contribution of chemical factors fell to 2% and 17% in Norfolk and Piedmont soils, respectively. Even then, soil sterillization may have been incomplete (Dick and Tabatabai 1978). In any event, biological hydrolysis was still largely dominant in soils with a low organic matter content. ここの思想はなどのないのないというないのないのである

Under field conditions, provided that microclimate and cultural methods were similar, Sutton *et al.* (1966) found a positive correlation between pyrophosphate efficiency and soil phosphatase activity

and pH. Phosphatase activity was assumed by Sutton et al. (1966) to be linked in some manner to pyrophosphatase activity. The activation energy

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of pyrophosphate hydrolysis was found to vary from 32 to 43 kJ.mol⁻¹ (Dick and Tabatabai 1978) as compared with 85-170 kJ.mol⁻¹ for pyrophosphate hydrolysis in solution (Van Wazer 1958), thus supporting the hypothesis that the reaction was enzyme-catalyzed (Racz and Savant 1972).

Eiyazi and Tabatabai (1977) reported that the term 'phosphatase' had been used currently in the literature to name a broad group of abiontic enzymes that catalyse hydrolysis of esters and anhydrides of phosphoric acid. In mineral soils, buffer pH optima were found at 5,8 in a citrate buffer (Douglas *et al.* 1977) and at pH 8,0 in a modified universal buffer (Dick and Tabatabai 1978). However, if the soil is described as a system of humus and clay immobilized enzymes (McLaren 1975), the optimum pH for enzyme activity at the colloid surface may be 1 to 2 units less than the suspension pH (McLaren and Packer 1970). Thus, acid to neutral pyrophosphatase systems are probably active in mineral soils.

Aye Paw and Hughes (1974) found a peak in pyrophosphatase activity of soil microorganisms at pH 8,5. However, the optimal pH could vary from 5,9 to 8,6 depending on the activating cation (Searle and Hughes 1977). Mg^{++} , Zn^{++}, Co^{++}, Mn^{++} and Fe^{++} activated the constitutive pyrophosphatase of soil microorganisms, whereas Ca⁺⁺, Cu⁺⁺, Al⁺⁺⁺ and Fe⁺⁺⁺ depressed it (Searle and Hughes 1977). Microorganisms could also perform pyrophosphate hydrolysis with an inductive alkaline phosphatase which was 25-fold less efficient than the highly specific alkaline pyrophosphatase (Josse 1966). An acid pyrophosphatase was reported in plant cells to have an optimal pH of 5,0-5,3 and to be inhibited by Mg^{++} (Rauser 1971; Kar and Mishra 1974).

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Pyrophosphatase activity has been shown to be influenced by liming (Hossner and Melton 1972), orthophosphate additions (Savant and Racz 1972), [Mg]/[Ca`+ Mg] ratio (Tabatabai and Dick 1979), formaldehyde, fluoride, oxalate, carbonate and sterilization (Dick and Tabatabai 1978). Toluene, K^+ , NH_4^+ , Cl-, NO_3 , NO_2^- , SO_4^- and EDTA had no effect on pyrophosphatase activity (Dick and Tabatabai 1978).

Thus, soil management, particularly liming and micronutrient applications, could affect pyrophosphatase activity.

Modelling

Pyrophosphate hydrolysis in mineral soils could be modelled as a function of pyrophosphatase activity and pyrophosphate retention and release (Racz and Savant 1972). Since the pyrophosphate uptake by plants would be slower than that of orthophosphate (Sutton and Larsen 1964), pyrophosphate hydrolysis in soil could be considered as an availability index of pyrophosphate. The following model gives a simplified pathway of pyrophosphate availability to plants:

soluble pyrophosphate $\frac{1}{4}$ organisms $\frac{5}{5}$ soluble orthophosphate

Reaction 3 is the most important reaction of the operating system, since reaction 4 has been shown to be less efficient than reaction 5 (Sutton and Larsen 1964). Since organic soils fix orthophosphate to a very small extent (Fox and Kamprath 1971), reaction 2 would be of little significance in the model. Reaction 1, as well as reaction 3, are unknown for organic soils. Since pyrophosphate reacts primarily with mineral matter (Philen and Lehr 1967), pyrophosphate retention would be related to ash content of organic soil materials. Since phosphatase activity is high in organic soils (Kavanagh and Herlihy 1975), pyrophosphate hydrolysis is probably rapid in organic soils.

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Retention sites in soils can compete with active sites on enzymes for available pyrophosphate. Reactions 1 and 3 could be analyzed as independent variables and half-life values of pyrophosphate as the dependent variable in a regression model. Reaction 1 would provide a measure of the retentionrelease mechanism under the condition that pyrophosphate concentration in solution remains relatively constant. Pyrophosphatase activity could be assayed kinetically under conditions yet to be defined for organic soils.

From the review of literature, it appears that pyrophosphate absorption by plants can follow two routes: reactions 1-4 or reactions 1-3-5. If the most rapid pathway is 1-3-5, any additive that can decrease the rates of reactions 1 and 3 should decrease the rate of pyrophosphate absorption by plants. The 1-3-5 pathway can be analyzed in two steps: reactions 1 and 3, as described previously, and reactions 1-3-5. A comparison between pyrophosphate and orthophosphate in a fertilizer trial would integrate reactions 1, 3 and 5.

Hypotheses

Main hypothesis

Pyrophosphate is hydrolyzed rapidly to orthophosphate in organic soils as a result of high pyrophosphatase activity and low 'pyrophosphate retention, and thus pyrophosphate should be as effective as orthophosphate in supplying P to plants.

Subhypotheses

1- Pyrophosphatase activity in organic soils is related primarily to a system of nonspecific acid phosphatases and to soil pH and past management.

2- Pyrophosphate retention in organic soils is related to ash content.

3- Half-life values of pyrophosphate in organic soils are related to pyrophosphatase activity and pyrophosphate retention.

4- Since Cu can inhibit pyrophosphatase activity in organic soils, the rate of pyrophosphate hydrolysis is slower with higher Cu contents.

5- Since liming materials can decrease pyrophosphatase activity and increase pyrophosphate retention, heavy liming of acid organic soils can decrease the rate of pyrophosphate hydrolysis.

6- Pyrophosphate hydrolysis is rapid in organic soils, as compared with the rate of P uptake during onion growth.

7- Pyrophosphate and orthophosphate are equivalent sources of P for onions.

These hypotheses have been tested and are discussed in subsequent chapters.

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CONNECTING PARAGRAPH

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In Chapter f, a general model of pyrophosphate availability to plants was introduced. This model includes three major reactions: enzymatic hydrolysis of pyrophosphate, physico-chemical retention of phosphates and plant absorption of P. In Chapter II, hypotheses about pyrophosphatase activity and pyrophosphate retention in organic soils are tested. It was hypothesized that most of the variation in $_{0}$ half-life values of pyrophosphate in 24 organic soil materials could be accounted for by enzymatic activity and physico-chemical retention. Furthermore, enzymatic activity is analyzed as to substrate specificity, and retention is related to ash content in fibric, mesic and humic materials. The influence of crops, either as a sink for P or as a factor promoting pyrophosphate hydrolysis, has been excluded. C

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CHAPTER II

PYROPHOSPHATE HYDROLYSIS

IN ORGANIC SOILS

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SUMMARY

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Pyrophosphatase and phosphatase activities were assayed with 1 g of organic soil material and 750 µmol of either sodium pyrophosphate or phenyl disodium phosphate dissolved in 20 mL of 0,2 M tris-citric acid buffer at pH 6,4 and incubated at 37°C for 2 h. Activation energy of pyrophosphate hydrolysis was 22 kJ.mol⁻¹ for virgin fibric materials and 33 kJ.mol⁻¹ for cultivated humic materials. Enzymes promoting pyrophosphatase activity were easier to extract from fibric than from humic materials. Twenty-four fibric, mesic and humic materials were selected for studying enzyme activities and pyrophosphate retention and hydrolysis. Phosphatase and pyrophosphatase activities were highly correlated $(r = 0.947^{**})$ and were apparently promoted by nonspecific acid phosphatases. Enzymatic activities varied from 11.6 to 148.1 mmol.kg⁻¹ (2h)¹, and were higher in virgin than in cultivated materials. Half-life values and retention levels of 8,1 mmol.kg⁻¹ of pyrophosphate in organic soil materials were determined at room temperature. Soil retention increased with an increase in ash content (r=0,876**), and was apparently weak, as noted by complete pyrophosphate hydrolysis after 4 to 8 days of incubation. Half-life values varied from 0,1 to 3,7 days, and were highly correlated with enzymatic activities and levels of water-soluble pyrophosphate. Half-life values less than 0.5 days corresponded to enzymatic activities exceeding 45-50 mmol.kg⁻¹ (2h)¹ and to more than 7,2 μ mol of watersoluble pyrophosphate. A regression model, involving an interaction between pyrophosphatase activity and level of water-soluble pyrophosphate, accounted for 77% of the variation in log (half-life) values. The interaction could be interpreted as a decrease in hydrolysis rate with reduced levels of water-soluble substrate.

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Les activités phosphatasiques et pyrophosphatasiques ont été mesurées en sol organique en utilisant 1 g de sol et 750 µmol de substrat sous forme de pyrophosphate de sodium ou de phényl disodium phosphate dans 20 mL d'une solution-tampon 0,2 M composée de Tris et d'acide citrique à pH 6,4. L'incubation dura 2 h à 37°C. L'énergie d'activation de l'hydrolyse du pyrophosphate s'éleva à 22 kJ.mol⁻¹ chez des matériaux fibriques vierges et à 33 kJ. mol⁻¹ chez des matériaux humiques cultivés. Des enzymes catalysant l'hydrolyse du pyrophosphate furent extraits de matériaux fibriques avec plus de facilité que de matériaux humiques. Vingt-quatre matériaux fibriques," mésiques et humiques furent étudiés pour leurs activités enzymatiques, la rétention et la rémanence du pyrophosphate. Une corrélation hautement significative fut établie entre les activités phosphatasiques et pyrophosphatasiques, lesquelles furent associées à des phosphatases acides non spécifiques. Les activités enzymatiques se situaient entre 11,6 et 148,1 mmol.kg⁻¹.(2h)¹, les plus hautes valeurs correspondant à des matériaux vierges. La rétention et la rémanence du pyrophosphate dans les matériaux organiques furent déterminées avec 8,1 mmol.kg⁻¹ à la température de la pièce. La rétention était liée au contenu en cendres (r = 0,876**), mais demeurait apparemment faible puisqu'il y eu hydrolyse complète du pyrophosphate en dedans de 4 à 8 jours. La demi-vie du pyrophosphate variait de 0,1 à 3,7 jours et montrait des relations hautement significatives avec les activités enzymatiques et les niveaux de pyrophosphate hydrosoluble. Les demi-vies inférieures à 0,5 jour correspondaient à des activités supérieures à 45-50 mmol.kg⁻¹.(2h)¹ et à plus de 7,2 mmol.kg⁻¹ de pyrophosphate hydrosoluble. Un modèle de régression, impliquant une interaction entre

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l'activité pyrophosphatasique et le niveau de pyrophosphate hydrosoluble, expliqua 77% de la variation dans les valeurs de demi-vies transformées en logarithmes. Cette interaction est attribuable à une baisse de substrat hydrosoluble produisant un ralentissement du taux d'hydrolyse.

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INTRODUCTION

Availability of pyrophosphate fertilizers to plants is increased by hydrolysis of pyrophosphate into orthophosphate (Sutton and Larsen 1964). The rate of pyrophosphate hydrolysis in solution is influenced by temperature, pH, enzymes, colloidal gels, complexing cations, concentration and ionic environment (Van Wazer 1958). The effects of temperature, enzymes and pyrophosphate concentration predominate in soils (Sutton *et al.* 1966; Hashimoto *et al.* 1969; Chang and Racz 1977).

Activation energy values of 32,5 to 43,2 kJ.mol⁻¹ $(10^{\circ}C \le T \le 50^{\circ}C)$ for pyrophosphate hydrolysis in mineral soils agreed with the range of values reported for potato and yeast pyrophosphatases (Dick and Tabatabai 1978). However, the enzymatically catalyzed pyrophosphate hydrolysis may not be enzyme-specific. The general name "phosphatases" has been given to a group of widely distributed enzymes that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Eivazi and Tabatabai 1977). In fact, a significant correlation between phosphatase activities and half-life values of pyrophosphate in soils has been observed (Sutton *et al.* 1966), suggesting that phosphatase and pyrophosphatase activities in soil are correlated.

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A number of procedures with different buffer compositions have been proposed to assay phosphatase and pyrophosphatase activities in soil (Kramer and Yerdei 1959; Halstead 1964; Hoffmann 1968; Tabatabai and Bremner : 1969; Douglas *et al.* 1977; Dick and Tabatabai 1978). Optimum pH values ranged from 5 to 8. Tris (tris-hydroxymethyn-aminomethane) and citrate are generally accepted as buffer components and allow an exploration of a wide range of pH values between 3 and 9. Tris, a phosphate acceptor, accelerates the rate of monoester phosphate enzymatic hydrolysis as the concentration is raised from 0,02 M to 0,5 M (Neumann 1969). Citrate effecti-" vely chelates calcium (Norvell 1972), which has been shown to inhibit pyrophosphatase activity (Aye Paw and Hughes 1974). Thus, a Tris-citric acid buffer would accelerate the hydrolysis rate of pyrophosphate and phenyl. phosphate in soils and increase the precision on measuring low activity levels after a short period of time.

Soil enzymes are thought to be mainly fixed on humus and clay particles (McLaren 1975). According to McLaren and Packer (1970), as the ionic strength of an assay solution is increased, the zeta potential of colloidal particles is reduced and the difference in optimum pH between free and adsorbed enzymes is reduced. Some enzyme desorption may even occur at buffer concentrations exceeding 0,1 M (Martin-Smith 1963). Thus both buffer composition and concentration are likely to influence the optimum pH value for soil enzyme activities.

Assays of pyrophosphatase activity in soils should provide an index of hydrolysis rate of pyrophosphate fertil/izers. However, a hydrolysis index

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alone was found nsufficient to explain pyrophosphate behavior in soils, since complex retention-release mechanisms could control pyrophosphate concentration in solution. Racz and Savant (1972) showed that pyrophosphate hydrolysis was more rapid in solution than in adsorbed state. Therefore, a description of pyrophosphate hydrolysis in soils should include an enzyme activity index and a pyrophosphate retention index.

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Soils

Pyrophosphate hydrolysis is probably rapid in organic soils, since pyrophosphatase activity is correlated with organic C content in soil (Tabatabai and Dick 1979) and since pyrophosphate reacts mainly with soil minerals (Philen and Lehr 1967; Subbarao and Ellis 1975). Within the organic soil group, however, organic materials vary widely in origin, degree of decomposition, pH, colloidal properties and ash content. Those characteristics may affect pyrophosphate hydrolysis.

The aims of the present study were: (1) to develop a methodology using Tris and citrate buffers for assaying pyrophosphatase and phosphatase activities in organic soil materials under similar conditions, and (2) to describe pyrophosphate behavior in organic soils by pyrophosphatase activity and amount of non-retained pyrophosphate in 24 materials from Eastern Canada; selected according to degree of decomposition, pH and ash content.

MATERIALS AND METHODS

Twenty-four organic soil materials (Table 1) were selected from 36 mate-

rials (0-20 cm) collected in Eastern Canada. These materials contained less than 20% ash. Soil materials were analyzed for C/N ratios, pyrophosphate indices (PPI) and cation exchange capacities (CEC) by standard procedures (Day et al. 1979) in order to characterize their decomposition state (Levesque and Dinel 1982). Sample distributions of the three variates (C/N, PPI, CEC) were normalized using log-transformations. A cluster analysis was performed with the three variates according to the method of Jeffers (1978), in order to classify soils as to their state of decomposi-From this analysis, a final soil selection comprised eight fibric, tion. eight mesic and eight humic organic soil materials (Table 1). The fibric materials were mainly derived from moss. The mesic and humic materials were dominantly herbaceous-woody. Prior to hydrolysis studies, all air-dried and"2 mm-sieved materials were remoistened to a -10 kPa matric potential and incubated in plastic bags for at least two weeks.

Half-life of pyrophosphate

Half-life values, as measures of the hydrolysis rate of pyrophosphate in soils, were estimated by incubating one g of soil adjusted to a -10 kPa matric potential with one mL of 8,1 mole L^{-1} P as Na₄P₂O₇.10H₂O adjusted to soil pH.

The incubation period depended on the hydrolysis rate and ranged from 6 h to 8 days. The course of pyrophosphate hydrolysis was followed in 24 organic soil materials by extracting orthophosphate with 20 mL of 0,658 N H_2SO_4 , which resulted in a 0,5 N H_2SO_4 solution during extraction (Racz and Savant 1972). The extraction proceeded for 15 minutes on a reciprocal shaker

at 200 rpm. After filtration, orthophosphate in solution was determined colorimetrically (Technicon 1977) with the thermostatic bath of the phosphate module disconnected to avoid pyrophosphate hydrolysis. A control sample was extracted for native P. Means of duplicates were fit the mathematical models by simple linear regression. The models selected were hyperbolic of the form Y = Ax/(b + X) and logarithmic of the form $Y = f (\log X)$. Hyperbolic models were linearized either to 1/Y = (1/A) + (b/A) (1/X) or to X/Y = (b/A) + (X/A), where A was the maximum value of Y at $X + \infty$, and b was the value of X at Y = A/2. The linearization showing the highest correlation coefficient was selected:

Enzyme assays

The assay mixture was buffered with a 0,2 M Tris solution adjusted to the desired pH with a 0,2 M citric acid solution. We found, in preliminary experiments at buffer pH of 8 (Dick and Tabatabai 1978), that 750 mmol.kg⁻¹ of substrate produced zero-order, concentration-independent, reactions in assay mixtures containing fibric or humic materials. Toluene was not added as a bacteriostatic agent since it has produced no effect on phosphatase and pyrophosphatase activities in short-term experiments. (Speir and Ross 1978; Dick and Tabatabai 1978).

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The duplicated assay mixtures comprised 1 g of soil, 5 mL of distilled water, 5 mL of 150 mmol.L⁻¹ substrate solution and 10 mL of 0,4 M Tris-citric acid buffer solution. The substrates used were sodium pyrophosphate (SPP) and phenyl disodium phosphate (PDP) from Fisher Scientific. After a 2 h incubation at 37° C in 75 mL test-tubes, the pH of suspensions was measured

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with a GK 24036 combined electrode. Twenty mL of 1N H₂SO₄ was added to extract orthophosphate. A 15 minute extraction at 200 rpm on a reciprocal shaker was found to be 98-100% efficient. The suspension was filtered through Whatman #42 paper. Orthophosphate in filtrates was analysed colorimetrically (Technicon 1977). A control was performed by using this technique but adding the substrate solution at the end of the incubation period.

Pyrophosphatase activity within 85% of maximum value was obtained at a Tris content in the buffer of 66-78% with fibric materials, 73-78% with mesic materials and 73% with humic materials. The corresponding values for phosphatase activity were 66%, 66-78% and 78-86%. Thus, a medium value of 73% Tris content was selected to assay pyrophosphatase and phosphatase activities. Within a 95% confidence interval, the average solution pH of 24 assay mixtures was $6,5 \pm 0,6$ in presence of SPP and $6,0 \pm 0,9$ in presence of PDP.

The reaction rate of SPP and PDP at optimum pH was determined in duplicates, as described above after 1, 2, 3 and 4 h of incubation. The results were statistically analyzed by an orthogonal polynomial procedure.

Enzyme solutions were prepared in quadruplicate by incubating 1 g of humic or fibric materials with 40 mL of buffer at optimum pH for 1 or 2 h at 37° C. The suspension was filtered through Whatman #42 paper. Ten mL aliquots of filtrates were assayed with 5 mL of 150 mmol.L⁻¹ SPP solution and 5 mL of 0,4 M buffer solution at optimum pH. Control samples were

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performed as above. Sterile water was added as a treatment to account for chemical hydrolysis of SPP during the incubation period. Soil samples were also assayed as described earlier, to estimate the contribution of extracted enzymes to SPP hydrolysis by soil materials.

The activation energy of the hydrolysis reaction in the assay mixtures at optimum pH was estimated by assaying pyrophosphatase activity at 12, 24, 36 and 48° C. The rate factors at each temperature were then fit to the linearized Arrhenius equation (Williams *et al.* 1978) by means of a simple linear regression procedure:

 $\ln k = (\text{constant}) - (\text{Eak}^{-1}\text{T}^{-1})$

when T was the absolute temperature, R, the gas constant [8,314 J.(mol-K)⁻¹] and Ea, the activation energy.

Pyrophosphate retention

In order to measure pyrophosphate retention independently from pyrophosphate hydrolysis, one gram of organic soil material adjusted to 500% H_2^0 in 75 mL test tubes was autoclaved in duplicates for one hour at 121°C. Then one mL of a 8,1 mmole.L⁻¹ sterile P solution as sodium pyrophosphate adjusted to soil pH was added to the autoclaved materials. A control was performed with one mL of distilled water to account for native P. A 4 h incubation period was found to be sufficient to attain a relatively constant retention value with organic soil materials. After completion of the incubation period, 34 mL of autoclaved distilled water was added to the mixture, which was then shaken for 30 minutes at 200 rpm on a reciprocal shaker. A five mL aliquot was pipetted in both treatment and control, and

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hydrolyzed for two hours at 100° C on a hot plate with five mL of 1N H₂SO₄ (Racz and Savant 1972). Pyrophosphate remaining in solution was computed by difference in orthophosphate concentration between treatment and control (Technicon 1977). On the average, 2% of pyrophosphate added was hydrolyzed during the incubation period.

Statistical models

A completely randomized design was used for all experiments. Simple linear correlation and regression techniques were applied to analyses involving two variables. The multiple regression equations were computed by a stepwise procedure. Cate-Nelson diagrams (Nelson and Anderson 1977) were drawn to partition organic soil materials in two groups by minimizing or maximizing the number of points in diagonally opposite quadrants.

RESULTS

An amount of 750 µmol in assay mixtures produced zero-order reactions, provided that less than 16% of added SPP (Table 2) and less than 24% of added PDP (Table 3) were hydrolyzed. Only one fibric material exceeded the 16% • limit for the linearity of SPP hydrolysis over a 2 h incubation period.

The buffer solution extracted pyrophosphatase from both humic and fibric materials (Table 4). Most of the extraction occurred during the first hour of incubation. Pyrophosphate hydrolysis in sterile water was low and not statistically different from zero. Pyrophosphatase activity in extracts from humic materials was different from zero at the 10% probability

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level, and accounted for 12% of pyrophosphatase activity in the comparable soil material. Pyrophosphatase activity in extracts from fibric materials contributed to 32% of total activity, and was different from zero at the 0,01 level of significance. Thus, enzymes having pyrophosphatase activity were more easily extracted from fibric than from humic materials.

The effect of temperature on pyrophosphatase activity (Figure 1) indicated that the hydrolysis rate declined or levelled off cubically above $36^{\circ}C$ (Table 5). From 12 to $36^{\circ}C$, Q_{10} values varied from 1,3 to 1,8.

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Between $12^{\circ}C$ and $36^{\circ}C$, the activation energy of the reaction was computed as 22,4 kJ.mol⁻¹ for fibric materials and 32,8 kJ.mol⁻¹ for humic materials. There was a correlation of -0,99 between lnk and 1/T, indicating correspondence with the Arrhenius equation.

Pyrophosphatase activity varied from 11,6 to 148,1 mmol.kg⁻¹. $2h^{-1}$ in fibric materials, from 12,2 to 109,3 mmol.kg⁻¹ in mesic materials and from .17,6 to 35,6 in humic materials (Table 6). Pyrophosphatase activity was negatively correlated with soil pH (r = -0,577^{**}) and exceeded 40 mmol.kg⁻¹. $2h^{-1}$ in virgin materials associated with bog and swamp vegetation.

Similarly, phosphatase activity varied from 15,8 to 145,4 mmol.kg⁻¹. $2h^{-1}$ in 24 organic soil materials (Table 6) and was highly correlated with pyrophosphatase activity (r = 0,947^{**}). Phosphatase activity was also highest (> 50 mmol.kg⁻¹.2h⁻¹) in virgin materials, as compared to materials derived from cultivated and newly broken soils. On the average, phosphatase and

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pyrophosphatase activities, were not different ($t_{23} = 0,891$). However, in virgin materials, phosphatase activity exceeded significantly pyrophosphatase activity by a mean difference of 12,0 mmol.kg⁻¹.2h⁻¹ ($t_7 = 2,712^*$). Part of that difference may be due to the fact that SPP hydrolysis rate was underestimated under nonzero-order conditions in material #2. In mesic and humic materials, phosphatase and pyrophosphatase activities were not significantly different, although pyrophosphatase activities tended to be generally higher than phosphatase activities.

The amount of water-soluble pyrophosphate was expressed in mmol of pyrophosphate per kg of soil. The values ranged from complete solubility in water (8,1 mmol.kg⁻¹) to low amounts of water-soluble pyrophosphate (Table 6). Water-soluble pyrophosphate levels and ash content were negatively correlated (r =-0,876^{**}) and, thus, pyrophosphate retention was directly correlated with ash content of organic soil materials. The hydrolysis of 8,1 µmol of pyrophosphate by one g of soil material was completed after 4 to 8 days of incubation at room temperature. The course of hydrolysis was fit either to a hyperbolic (22 soils) or to a logarithmic curve (2 soils: #21 and #24). With 3 to 5 df, the r^2 - values were 0,90 to 0,99 for the linearized hyperbolic models, and 0,83-0,96 for the logarithmic models. Correspondingly, all simple correlation coefficients were significant at the probability level of 5%. Half-life values, obtained by interpolation, were generally low, from 0,06 to 3,74 days (Table 6). Log-transformed half-life values were highly correlated with pyrophosphatase (r = -0,659^{**}) and phosphatase (r = -0,765^{**}) activities,

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with amounts of water-soluble pyrophosphate $(r = -0,691^{**})$ and with soil pH $(r = 0,624^{**})$.

Organic soils materials were partitioned in two groups with respect to half-life of pyrophosphate (Figure 2). Soil materials having half-life values under the 0,5 day limit had enzymatic activities in excess of 47-51 mmol.kg⁻¹. $2h^{-1}$ and amounts of water-soluble pyrophosphate in excess of 7,2 mmol.kg⁻¹. Materials promoting rapid hydrolysis originated from virgin acidic soils of low ash content.

Assuming that pyrophosphatase activities (E) and amounts of watersoluble pyrophosphate (S) interacted in their apparently curvilinear relationship with log-transformed half-life values of pyrophosphate in organic soil materials (H), a multiple regression model was computed as the following:

 $H = 0,259 - (0,403)(10^{-3})ES^2 + (0,014)(10^{-3})E^2S, (R^2 = 0,77).$

Thus, amounts of water-soluble pyrophosphate did influence the rate of pyrophosphate hydrolysis, as shown by the regression equation. The interaction between pyrophosphatase activites and amounts of water-soluble pyrophosphate was described by factors ES^2 and E^2S . The two factors, of opposite sign, would cancel one another at an E/S ratio of 29. Half-life values increased with an increase in E/S ratio, indicating that both enzyme activity and substrate availability were rate-limiting. Predicted half-life values deviated more from observed values in humic materials, compared with mesic and fibric materials. Humic materials clustered about a half-life value of

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1,8 days. In mesic and fibric materials, predicted values varied from 1,1 to 1,7 days, and from 0,1 to 1,4 days, respectively.

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DISCUSSION

Activation energy values of 22 to 33 kJ.mol⁻¹ for SPP hydrolysis in assay mixtures were characteristic of an enzyme-catalyzed reaction. The value of 33 kJ.mol⁻¹ found for humic materials was in the range of values found for mineral soils (Dick and Tabatabai 1978). Activation energy values of 39,8 and 41,6 kJ.mol⁻¹ have been obtained for yeast pyrophosphatase (Kunitz 1952) and bone phosphatase (Sizer 1943). Comparatively, chemical hydrolysis of SPP would require 85 to 170 kJ.mol⁻¹ (Van Wazer 1958).

Enzymes are largely immobilized in soils by inorganic and organic macromolecular components (McLaren 1975) and, as such, resist considerably heat denaturation (Tulskaya 1982). Since pyrophosphatase activity was more easily extracted from fibric than humic materials, and was less efficiently protected against heat inhibition by fibric vs humic materials, the soil colloid-enzyme interactions would be weaker in fibric than in humic materials. The fact that humic materials showed activation energy values similar to those of mineral soils would indicate the occurrence of enzymes systems tightly bound to humus and/or clay colloids. In contrast, fibric materials, which derive their colloidal properties mainly from polyuronic acids (Moore and Bellamy 1974), would immobilize soil enzymes on a nonextractable form to a lesser extent than humic materials. This "locus" effect (McLaren and Packer 1970) was consistent with lower activation energy required in fibric compared with humic materials.

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Enzyme systems capable of SPP and PDP hydrolysis in assay mixtures were more active at acid to neutral suspension pH. Since acidic colloidal particles could have surfaces up to 100 times more acid than a suspending medium (McLaren and Packer 1970), even a neutral pH in assay mixtures would correspond to an acid pH at colloid surface. Thus, acid phosphatase systems promoted SPP and PDP hydrolysis in organic soil materials. There is evidence that such enzyme systems are, as a whole, nonspecific.

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Firstly, acid phosphatases can be active not only toward most monophosphate esters, but also toward pyrophosphate (Newark and Wenger 1960; Ikawa et al. 1964). Correspondingly, a good correlation should be established between SPP and PDP hydrolysis promoted by nonspecific acid phosphatase systems. Indeed such a correlation was found to be highly significant. Secondly, if two phosphatase systems having affinities for SPP or PDP paralleled each other with a high degree of correlation, the hydrolysis of both SPP and PDP would follow a zero-order reaction model up to a high proportion of hydrolyzed substrate. On the contra^{TY}, zero-order reactions proceeded until less than 16 to 24% of SPP or PDP hydrolysis was completed. Thus, organic soil phosphatase systems would be mostly nonspecific. Phosphatase and pyrophosphatase activities were higher in virgin than in cultivated materials. In virgin materials derived from *Sphagnum* moss, phosphatase activity has been found to be high as a result of the symbiosis between Ericoid plants and mycorrhizal fungi (Burgeff 1961). Land clearing during reclamation could destroy part of the enzyme-rich topsoil. In

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addition, activity of extracellular enzymes, among which are phosphatases (McLaren 1975), is reduced in cultivated peats on account of more proteolytic activity than in virgin peats (Kavanagh and Herlihy 1975) and of continual applications of enzyme inhibitors such as lime (Halstead 1964; Hossner and Melton 1970), orthophosphate (Savant and Racz 1972; Speir and Ross 1978) and trace elements (Tyler 1974). Thus organic soil genesis and management were determinant as to enzyme activities obtained.

The high correlation found between water-soluble pyrophosphate and ash content was consistent with the reaction of many inorganic soil components with pyrophosphate (Philen and Lehr 1967). Indeed, organic materials did not retain pyrophosphate because all pyrophosphate added to low-ash fibric materials remained water-soluble. Thus pyrophosphate behaved like orthophosphate, which has been found to have no affinity for organic colloids (Fox and Kamprath 1971). Whatever the retention capacity of organic soil materials for pyrophosphate, retained pyrophosphate was weakly held, as noted by complete pyrophosphate hydrolysis after 4 to 8 days of incubation.

Although pyrophosphatase activity alone accounted for 43% of total variation in half-life values of pyrophosphate in 24 organic soil materials, the addition of water-soluble pyrophosphate to the regression model increased the R²-value to 77%. The interaction between pyrophosphatase activity and pyrophosphate retention supported the theoretical model of Racz and Savant (1972).

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CONCLUSIONS

1- Activation energy required for pyrophosphate hydrolysis under assay conditions was less with fibric materials than with humic materials, probably due to differential "locus" effects at the colloid-enzyme interface.

2- Phosphatase and pyrophosphatase activities in 24 organic soil materials were highly correlated. Nonspecific acid phosphatase systems were apparently involved in both pyrophosphate and phenyl phosphate hydrolysis.

3- Phosphatase and pyrophosphatase activities as well as amounts of pyrophosphate retention, were related to soil pH, ash content, soil genesis and soil management. Low-ash, acid virgin, organic soil materials showed highest enzyme activity and lowest pyrophosphate retention.

4- The theoretical model of Racz and Savant (1972), relating pyrophosphate hydrolysis in soil to pyrophosphatase activity rates and pyrophosphate retention states, was confirmed by a multiple stepwise regression procedure.

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Class	-Material	C/N	CEC (mmol [½Ba ⁺²] .kg ⁻¹)	Pyrophosphate index	pH (0,01M CaCl ₂)	Ash (%)
	1 V1)	82	980	. 9	3.0	1.8
-	2 V2)	43	1050	10	3.2	2,6
,	3 V3)	49	1270	12	2,6	1,6
e	4 V3)	37	1020	16	2,7	3,6
ribric	5 V4)	44	1300	18	3,4	4,2
1	6 C2)	36	1110	14	5,7 ·	12,4
	7 C2)	30	1070 -	14	5,8	13,2
	8 C1)	/ 39	1320	26.	5,2	9,1
یس جیر خدة عال جی خذہ طل		r — — ez ₄₅ 47 —	و جدد بدن این که بله دو دورند این که آور دید پیپیند بله دو ا	ه سه بینه کام استار بانی نباه کام بیده این این این در این	ین خبر دو دو دو بازی این دارد در این این این	ببجهين ويق يمله الله البينا فقع بهزو
	9 B3)	29	1200	42	2.7	2.0
	10 C4)	31	1360	47	5,7	9,7
,	11 C4)	28	1550	34 '	5,9	13,6
no of o	12 C3)	27	1270	52	4 (8	9,1
mearc	13 V3)	21	1140 r	42	3,2	9,6 **
	14 C3)	19	1570	21	3,7	7,5
	15 C3)	22	1470	42	3,8	6,5
	16 C3)	26	1690	54	4,2	7,8
ک (در میزین خد س	بي مصحم جي زمة بيونية تصفية مصار علامي		الله الله الي حد الله الله الله الله الله الله الله الل	و بيه بيه المراشد بين يوريه بي عن المراشر بين ال	خدب ها، _ا خذ علی نده هگ کا ا خرور بر	\sim
	17 C3)	21	1740	41	4,5	10,1
	18 C3)	18	1950	30	5,0	14,2
	19 C3)	15	1900	34	5,1	17,4
numic	20 C3)	16	1910 .	41	5,1	18,2
	21 C3)	18	1560 -	107	5,3	14,9
	22 C3)	16	1690	122	· 5 , 4	18,1
	23 B3)	20	2380	85	6,3	18,6
	24 C3)	16	2020	بر129	6,0	18,5

Table 1. Characterization of 24 organic soil materials of Eastern Canada

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V, C, B: virgin, cultivated and newly broken, respectively.

1) New-Brunswick 2) Newfoundland 3) Quebec 4) Nova Scotia

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						· /
Organic	Inc	ubatio	n peri	period Polynomial F _{1,48} value		F _{1 48} value
soil material	1h	2h	3h	4h	degree	· ·
	% hydro	lysis	of SPI	added	ه خل های در این خود همونون خرو بری هم بین باند این وی هو در	ور باین و بین می این این این این این این این این این ای
				5 . 1	•	**
Fibric	13	21	28	· 33	Q.	. 22
Fibric	7	13	17	20	Q	29
Fibric	` 6	11	14	16	Q	42
Fibric	3	5	7	8	L	350,
Mesic	1	2	•3	4	L	88
Humic	. 1	2	- 3	* 3	L	56

Table 2.Rate of pyrophosphate (SPP) hydrolysis by six organic soilmaterials assayed in a 0,2 M Tris-citric acid buffer at pH 6,4

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Table 3. Rate of phenyl phosphate (PDP) hydrolysis by six organic soil materials assayed in a 0,2 M Tris-citric acid buffer at pH 6,4

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Organic	Inc	ubatic	on peri	.od	Polynomial	F _{1.48} value	
soil material	<u>1h</u>	<u>2h</u>	<u>3h</u>	4h	degree	_, , , , , , , , , , , , , , , , , , ,	
*	% of hy	drolys	is of	PDP added		و هذه میله منه روی هی هم خان وی چوردی می وی در ا	
Fibric	12	21	28	34	0	12.**	
Fibric '	10	17	22	24	ġ	26	
Fibric	6	11	16	20	Ľ	575	
Fibric	6	11	15	19	• L	479	
Mesic	1	2	4	5	L	32**	
Humic	1 -	2	4	5	L	່ 39 ົົ	

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	0 0	· 0	0 0	0 0	1 1	-1 1	0 -2	< 1 701**
Orthogonal contrasts	0	1 1	-1 1	-2	0 0	0 0	0 0	< 1 214**
1)		`	-				-	
و منه چهری اور ورو های ورو های ورو	0,4	3,5	3,4	29,8	21,9	23,7	70,5	
			- rate of	pyrophosphate	hydrolysis	$(mmo1. 2h^{-1})$		
	25	1b extract	2h extra	ect material	lh extra	ct 2h extra	t material	1,28 value
	^H 2 ⁰		humic	بین سے میڈیو: بھا کہ علل جو سے جو سے م	fibric			F _{1.2}

Table 4. Pyrophosphatase activity in sterile water, in humic and fibric materials, and in filtrates of soil materials extracted with a 0,2 M Tris-citric buffer (pH 6,4) for 1h and 2h at 37°C

CV < 1%

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+, **: significant at the 10% and 1% levels, respectively

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1) Steel and Torrie (1980)

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Table 5. Highest polynomial degree of the course of pyrophosphate hydrolysis at 12, 24, 36 and 48° C, and activation energy (Ea) of the hydrolysis reaction in the $12-36^{\circ}$ C range computed from Arrhenius equation (r-value given for n = 3)

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Soil material	Polynomial d	egree	Activation e	energy L)
	degree F ₁	,30 value	Ea	r-value
Fibric	cubic	8,28**	22,4	-0,996**
Humic	cubic	4,44*	32,8	-0,997**

CV = 5%

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Table 6. Pyrophosphatase (SPP) and phosphatase (PDP) activities in a 0,2 M Tris-citric acid buffer (pH 6,4), half-life values and amounts of water-soluble pyrophosphate (PP) in 24 organic soil materials

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Materia	1	SPP mmol.kg ^{_1}	.2h ⁻¹	PP in sol'n mmol.kg ⁻¹	Half-life days
	1	74,1	100,6	7,6	0,08
÷	2	148,1	145,4	- 8,1	0,06
Tri Landa	3	67,0	102,1	8,0	0,09
	4	39,7	51,0	5,5	1,03
FIDITC	5	47,2	50,6	7,2	0,41
	· # 6	35,8	44,1	4,6	1,47
	7	34,1	40,0	4,1	3,13
و جمل الس حصر حامل مندر المان م	8	11,6	19,9	5,2	1,03
	٥	14.2	17 0	7.0	1 00
Mesic	10	14,5	19.0	7,0	1,09 2 92
	11	12,2	10,0	5,1	Z ,02 T ,02
	10	12,5	10,0	4,0	1,93 0,70
	12	100.2	4/,0	3,0	1 20
	1/	25 7	103,3	4,0	1,20
	14	, cc	20,14	. ⊃,⊥ 	L ,04
	15	44,0 20 5	30,7	4,⊥ 2,2	2,08
ه ۵۰ سم برده هم هما ۵۰ مر			27,5,*	د و د 	1,10
	17	34,4	26,2	° 0,6	2,09
	18	28,3	26,9	1,9	0,76
	19	35,6	30,6	1,2	0,70 .
T2	20	30,5	25,5	1,2	0,68
Humic	21	33,3	33,1	0,8	1,92
	22	35,6	33,4	0,5	3,55
	23	13,9	17,7	2,1	1,16
	24	17,6	15,8	0,1 -	3,74
F23,23	values	7379**	345**	45**	
CV (%)		4	6	14	-

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Figure 2. Cate-Nelson diagrams of log (half-life) of 8,1 mmol.kg⁻¹ P as sodium pyrophosphate vs pyrophosphatase activity (PPASE), phosphatase activity (PASE) and water-soluble pyrophosphate (PP in sol'n).

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CONNECTING PARAGRAPH

In Chapter II, the course of pyrophosphate hydrolysis was described by a complex interaction between enzymic activity and physico-chemical retention. If retention values are kept constant, half-life values of pyrophosphate hydrolysis (H) would be a quadratic function of pyrophosphatase activity (E), such that $H = aE^2 + bE + c$, where a, b and c are constants. Thus, variation in E could affect H values. Are these variations of any significance?

Enzyme activity can be reduced by adding Cu as an inhibitor. Cu is selected since heavy applications (20 to 300 kg.ha⁻¹) are essential to achieve high standards of productivity in organic soils and to decrease the oxidation rate of these soils. Thus, the question of whether Cu additions to organic soil materials can affect pyrophosphatase activity and, consequently, decrease half-life values of pyrophosphate will be discussed in Chapter III.

CHAPTER III

INFLUENCE OF CU ON PYROPHOSPHATE

HYDROLYSIS IN ORGANIC SOILS

SUMMARY

The objectives of this study were to investigate the effect of Cu levels on pyrophosphatase activity of 24 organic soil materials differing in organic N content, cation exchange capacity (CEC) and degree of decomposition and to measure pyrophosphatase activity and half-life values of pyrophosphate in four humic materials similar in all respects except Cu content. Cu added at a rate of 5 g.kg⁻¹ decreased significantly pyrophosphatase activity in fibric, mesic and humic materials (t = 8**). The magnitude of the decrease in pyrophosphatase activity was highly correlated with pyrophosphatase activity in the 24 materials (r = 0,725**), but was not significantly correlated with organic N and CEC. Pyrophosphatase activicy in four organic soil materials was 19% lower in materials containing 360 and 1177 mg of total Cu per kg than in materials containing 22 and 126 mg of total Cu per kg. Thus, a "critical" range was established between 126 and 360 mg of Cu per kg. This range was comparable to the critical range of 150 to 220 mg Cu per kg of organic soil materials, estimated from other sources, as to inhibition of CO2 evolution and 8, enzymic activities. However, a common course of pyrophosphate hydrolysis was obtained under laboratory and field conditions in materials containing 22 and 1177 mg Cu.kg⁻¹. A half-life value of 1 day was computed for pyrophosphate incubated with these two materials at room temperature. Since most Quebec organic soils contain less than 120 mg $Cu.kg^{-1}$ as total Cu, it was apparent that pyrophosphate hydrolysis, and thus pyrophosphate efficiency, as a P-fertilizer, would not be affected importantly by Cu inhibition of pyrophosphatase activity.«

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Le but de cette recherche était d'évaluer l'effet du Cu sur l'activité pyrophosphatasique de 24 matériaux organiques montrant des différences quant au N organique, à la capacité d'échange cationique (CEC) et au degré des décomposition et de mesurer l'activité pyrophosphatasique dans quatre matériaux humiques semblables en tout point sauf le contenu en Cu. L'addition de Cu à un taux de 5 g.kg⁻¹ a réduit significativement l'activité pyrophosphatasique dans les matériaux fibriques, mésiques et humiques (t = 8**). L'amplitude de cette réduction était très significativement liée à l'activité pyrophosphatasique des matériaux (r = 0,725**) mais ne montrait aucune corrélation significative avec le contenu en N organique et la CEC. L'activité pyrophosphatasique dans quatre matériaux organiques était de 19% inférieure dans les matériaux contenant 360 et 1177 mg de Cu total par kg par rapport à ceux qui contenaient 22 et 126 mg de Cu total par kg. Ainsi, une plage "critique" a été établie entre les valeurs de 126 et 360 mg de Cu par kg. Cette plage critique est comparable aux seuils critiques déjà établis de 150 à 220 mg de Cu par kg de matériel organique pour l'inhibition de la production de CO₂ et de l'activité de huit systèmes enzymatiques. Toutefois, une courbe commune d'hydrolyse du pyrophosphate fut établie au champ et au laboratoire chez des matériaux contenant 22 et 1177 mg Cu.kg⁻¹. La valeur de la demi-vie du pyrophosphate incubé avec ces deux matériaux à température de la pièce fut de 1 jr. Comme 1a plupart des sols organiques du Québec contiennent moins de 120 mg.kg⁻¹ de Cu total, l'hydrolyse du pyrophosphate et, par conséquent, la valeur fertilisante de cette source de P, ne devraient pas être affectées de façon importante par ces niveaux de Cu.

RESUME

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INTRODUCTION

Searle and Hughes (1977) have shown that Cu can inhibit pyrophosphatase activity derived from soil microbial cells. Since the hydrolysis of pyrophosphate, the main non-orthophosphate component of polyphosphate fertilizers, has been found to be mainly enzyme-catalyzed in soils (Hashimoto *et al.* 1969), the addition of Cu to Cu-deficient soils could decrease the rate of pyrophosphate hydrolysis into orthophosphate. A decrease in pyrophosphate hydrolysis could depress the rate of P uptake by plants since pyrophosphate has been shown by Sutton and Larsen (1964) to be taken up less rapidly by plants than orthophosphate.

Several annual Cu applications totaling 300 kg.ha⁻¹ have been recommended for organic soils (Gilbert 1952). Such an amount-would correspond to 600 mg.kg⁻¹ of Cu in the humic surface layer (0-20 cm) of organic soils having a bulk density of 0,25 kg.L⁻¹. A total amount of 20 to 30 mg Cu.kg⁻¹ is required for responsive crops grown on organic soils (Lucas and Knazek 1977). A survey of 87 Quebec organic soils in 1979 indicated that 32 surface materials contained less than 20 mg.kg⁻¹ of total Cu, 51 materials contained 30 to 120 mg Cu.kg⁻¹ and 4 materials contained 128 to 310 mg Cu.kg⁻¹ (unpublished data in Appendix III). Thus most Quebec organic soils contained less than 120 mg.kg⁻¹ of total Cu and 37% of these soils were probably Cu-deficient.

By applying the Cate-Nelson partitioning procedure (Nelson and Anderson 1977) to the results of Mathur *et al.* (1979) and Mathur and Sanderson (1980), r Cu contents of 220 mg.kg⁻¹ in organic soil materials were found to reduce CO, evolution and Cu contents of 150 mg.kg⁻¹ reduced 8 enzymic activities.

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Thus, a decrease in enzymic activities was expected upon Cu fertilization up to 600 mg.kg^{-1} .

Since organic soil materials can adsorb considerable amounts of Cu (Parent and Perron 1983), and since soil enzymes have been shown to be active primarily as abiontic enzymes (Kiss *et al.* 1975) fixed on soil colloids (McLaren 1975), organic soil materials could compete for Cu with soil enzymes and thus protect soil enzymes to a certain extent against Cu inhibition. Apparently, Cu could be fixed by nitrogenous compounds (Goodman and Cheshire 1973) and through coordination links with functional groups containing oxygen (Bloom and McBride 1979). Thus, organic N contents and cation exchange capacities (CEC) could influence the relative decrease in pyrophosphatase activity upon Cu additions to organic soil materials.

The aims of the present study were (1) to investigate the effect of Cu additions on pyrophosphatase activity in 24 organic soil materials differing in organic N content, CEC and degree of decomposition and (2) to determine the influence of Cu contents of up to 1177 mg.kg⁻¹ on pyrophosphatase activity and rate of pyrophosphate hydrolysis in four similar organic soil materials except for Cu content.

MATERIAL AND METHODS

Assays with 24 materials

Twenty-four organic soil materials from Eastern Canada, eight from each of the major groups (fibric, mesic and humic), were analyzed in duplicate for total N, N-NH₄⁺ (McKeague 1976) and for CEC (Thorpe 1973) (unpublished data in Appendix IV). Organic N was obtained by difference between total N and the N-NH₄⁺ fraction.

The materials were assayed in duplicate for pyrophosphatase activity (see Chapter 2) with and without the addition of 5 g.kg⁻¹ of Cu as sulfate (Mathur and Rayment 1977). Pyrophosphatase activity was determined by incubating, at 37° C for 2h, one g of material with 750 µmol of sodium pyrophosphate \pm 5 mg of Cu, dissolved in a 0,2 M Tris-citric acid buffer solution of pH 6,4. To account for native P, a control sample was analyzed by adding pyrophosphate at the end of the incubation period. Orthophosphate in assay mixtures was extracted with 20 mL of 1 N H₂SO₄ on a reciprocal shaker for 15 min. The suspension was filtered through Whatman #42 paper. Orthophosphate in filtrates was determined colorimetrically (Technicon 1977). The pH of assay mixtures having received Cu was 0,1 unit lower than that of assay mixtures not treated with Cu.

The significance of the differences between the two treatments was tested with the t-test for paired samples (Steel and Torrie 1980). The absolute decrease and the arcsin ∇ -transformed percentage of decrease (Steel and Torrie 1980) were correlated with organic N and CEC. The relative decrease in pyrophosphatase activity values was computed by dividing the difference between the two treatments by the difference between the sample not treated with Cu and the control sample.

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Laboratory studies on four humic materials

Humic materials were obtained from field IV microplots previously treated with four rates of Cu on the Agriculture Canada experimental farm at Sainte-Clotilde, Quebec. The total amounts of Cu in these humic materials were 22, 126, 360 and 1177 mg.kg⁻¹, respectively (Mathur *et al.* 1983). These materials, similar in all respects except Cu content (Table 1), were analyzed in duplicate for pyrophosphatase activity as described earlier, and for retained pyrophosphate and orthophosphate by the following procedure. One g of autoclaved (121°C, 1h) material containing 5 mL of distilled water was incubated with 1 mL of 500 mmol. L^{-1} solutions of either sodium pyrophosphate or dibasic sodium phosphate adjusted to soil pH. The incubation was run at room temperature for 4h. The incubated mixture was extracted with 34 mL of distilled water on a reciprocal shaker for 30 min. The suspension was filtered through Whatman #42 paper. Orthophosphate was determined in filtrates of orthophosphate treatments (Technicon 1977). For pyrophosphate treatments, a 10 mL aliquot of the filtrate was hydrolyzed in 0,5 N H₂SO₄ for 2h at 100°C on a hot plate (Racz and Savant 1972). Control soil samples were analyzed for native P.

The half-life values of pyrophosphate in the humic materials containing/ extreme amounts of Cu (22 vs 1177 mg.kg⁻¹) were assessed at room temperature by incubating in duplicate 1 g of material, 5 mL of distilled water and 1 mL of a 500 mmol.L⁻¹ sodium pyrophosphate solution adjusted to the pH of the material. At five selected times (6-12-24-28-96 h) duplicate samples were taken at random for orthophosphate determination (Technicon 1977). Orthophosphate was extracted with 0,5 N H₂SO₄, as described earliers. The

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thermostatic bath of the auto-analyzer module was disconnected to prevent the hydrolysis of pyrophosphate during, color development. Control soil. samples for native P were analyzed in duplicate at the beginning and at the end of the incubation period.

Field study

C

The course of pyrophosphate hydrolysis was monitored in the same microplots as mentioned above. To these microplots, solutions of $K_4P_2O_7$ prepared by the method of Gilliam (1970) was applied in triplicate at a rate of 112 kg P.ha⁻¹. The bulk density of the humic materials under study was 0,14 kg.L⁻¹ (Mathur *et al.* 1983). Fertilizer P was raked into the surface 10 cm. The experimental units were completely randomized with four Cu levels. Each microplot was sampled 10 times in the surface layer from May the 28th to August the 2nd, 1982. Duplicated control plots were sampled 5 times during the season to estimate native P. The samples were extracted with 0,5 N H₂SO₄ and analyzed for P (Technicon 1976). Rainfall and soil temperatures near the experimental area were recorded daily.

The hydrolysis data were fit to a quadratic hyperbola of the form ' Y = A X / (b + X), where Y was the amount of orthophosphate in the material (mg.kg⁻¹), corrected for native P, X was the time in days, A was the estimated Y maximum and b was the estimated X value at Y = A/2. The hyperbola was linearized as X/Y = b/A + X/A before analysis by a simple linear regression procedure.

RESULTS AND DISCUSSION

Assays with 24 materials

The decrease of pyrophosphatase activity upon Cu addition to 24 organic soil materials ranged from 1,1 to 20,7 mmol.kg⁻¹.2h⁻¹, with corresponding relative decreases of 3 to 43% (Table 2). The t-value of the difference between Cu-treated and non-treated materials was highly significant (t = 8**). On the average, pyrophosphatase activity was 14% lower in treated than in untreated samples. However, no significant correlation was shown between absolute and relative values of the decrease (r = 0,157) since pyrophosphatase differed widely from one material to another. The greater the pyrophosphatase activity, the greater was the absolute decrease (r = 0,725**) and the lower was the relative decrease (r = -0,491*).

No significant correlations were established between absolute decrease in pyrophosphatase activity and either organic N (r = 0,040) or CEC (r = -0,229), as well as between relative decrease and organic N (r = 0,369) and CEC (r = 0,325). Thus, with a Cu concentration of 5 g.kg⁻¹, organic N and CEC did not reduce enzyme inhibition by Cu.

The decrease in pyrophosphatase activity by adding Cu to organic soil materials was consistent with the effect of Cu on other enzyme activities in mineral and organic soils (Juma and Tabatabai 1977; Mathur *et al.* 1979). It was apparent however that Cu inhibition of pyrophosphatase activity in organic soil materials was related primarily to the amount of active enzyme already present in the materials rather than to organic N or CEC.

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Studies on four humic materials.

Pyrophosphatase activity was found to be significantly higher in low-Cu than in high-Cu humic materials (Table 3). Ordination of Cu contents by orthogonal contrasts was as following:

 $(Cu_{22} \neq Cu_{126}) > (Cu_{360} > Cu_{1177}).$

All comparisons were significant at the 0,01 probability level. A higher activity value in Cu_{22} than in Cu_{126} could be attributed to the response of previously grown crops to Cu addition. Thus, a "critical" interval of Cu contents that would decrease significantly pyrophosphatase activity in the humic materials was found between 126 and 360 mg.kg⁻¹. Such a range was consistent with "critical" values of 150 to 220 mg.kg⁻¹ (Mathur *et al.* 1979; Mathur and Sanderson 1980).

Despite a 19% difference in pyrophosphatase activity between $(Cu_{22} + Cu_{126})$ and $(Cu_{360} + Cu_{1177})$ materials; no significant difference in the course of pyrophosphate hydrolysis was found between Cu_{22} and Qu_{1177} materials under laboratory conditions. Neither the effect of material $(F_{1,12} = 3,81)$ nor the material X time interaction $(F_{5,12} = 1,75)$ was significant at the 0,05 probability level. However, the time factor was highly significant $(F_{5,12} = 278**)$ and resulted in a single hydrolysis curve for the two treatments (Figure 1). The coefficient of variation was 5%. The interpolated half-life value of pyrophosphate hydrolysis in Cu_{22} and Cu_{1177} materials was approximately 20 h.

The course of pyrophosphate hydrolysis under field conditions had a similar trend as under laboratory conditions (Table 4). However, the coefficient of variation of 20% was higher than in laboratory test. Only the time factor was significant and resulted in a common hydrolysis curve for the 4 treatments (Figure 2). Pyrophosphate hydrolysis was more rapid during the first two weeks than later during the season. Soil temperature increased between the 40th and the 60th day with a corresponding apparent increase in orthophosphate production. However, a lower orthophosphate recovery at the 68th day as compared with the 54th day after pyrophosphate application could be attributed in part to some leaching of orthophosphate by the 80 mm of rainfall recorded between the two sampling dates.

The course of pyrophosphate hydrolysis was described by the following equation:

- <u>5</u>`,

$$Y = \frac{380X}{6+X}$$
; $R^2 = 0,965$; $n = 9$;

with variables and constants as defined earlier. A half-life value of 6 days was subject to the formal assumption that the maximum value of P recovery was 380 mg.kg⁻¹. However, an amount of 800 mg P.kg⁻¹ was present in the sampled layer at the beginning of the experimental period. The difference between these two values could be attributed in part to phosphate leaching outside the sampled layer or to incomplete pyrophosphate hydrolysis. In fact, the retention experiment under laboratory conditions indicated that, on the average, 85% of the pyrophosphate added was retained by the 4 humic materials, whereas no orthophosphate was retained. Thus, orthophosphate was

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practically not retained by the 4 humic materials and could have been leached away in part as pyrophosphate was hydrolyzed into orthophosphate during the incubation period in the field. A very low capacity for orthophosphate adsorption has been observed also by Fox and Kamprath (1971) in acid organic soils for North Carolina.

Thus, it was apparent that, in the range of 22 to 1177 mg Cu per kg of humic materials, a common course of pyrophosphate hydrolysis could be obtained under laboratory and field conditions despite a significant difference of 19% in pyrophosphatase activity between high-Cu and low-Cu materials. That range of total Cu corresponded to a range of 10 to 480 mg.kg⁻¹ of 1N HCl-extractable Cu.

CONCLUSION

Linear correlation studies indicated that neither organic N nor cation exchange capacity, two parameters measuring a number of functional groups in organic soil materials, was significant for mitigating pyrophosphatase inhibition by Cu in fibric, mesic and humic materials.

However, Cu values exceeding 360 mg.kg⁻¹ as total Cu or 200 mg.kg⁻¹ as 1N HCl-extractable Cu produced a significant decrease in pyrophosphatase activity that did not reduce importantly half-life values of pyrophosphate incubated under both laboratory and field conditions. Since most Quebec organic soils surveyed contained less than 120 mg.kg⁻¹ of total Cu, it was apparent that pyrophosphate hydrolysis would not be depressed by the Cu values found in these soils.

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Humic	pH in	C/N	DA(1) ^{extract}	table 1N	HC1 ex	tracta	able	
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Table 1. Chemical characteristics of 4 humic materials of different Cu \odot contents (means of duplicates)

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Table 2. Decrease of pyrophosphatase (PPase) activity upon Cu addition of 5 g.kg⁻¹ as related to organic N and cation exchange capacity (CEC) and degree of decomposition of 24 organic soil materials (means of duplicates)

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... **Table 3.** Orthogonal contrasts for comparing the rate of pyrophosphatase activity in 4 humic materials similar except for Cu content (means of duplicates)

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CV = 2%

**: significant at the 0,01 level

1) Steel and Torrie (1980)

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Table 4. Analysis of variance of the rate of K₄P₂O₇ hydrolysis in four organic soil materials similar except for Cu content (field conditions)

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CV = 20%

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**: significant at the 0,01 level



are 1. Average course of pyrophosphate hydrolysis applied in duplicate at a rate of 500 µg of P to one g of two humic materials containing 22 and 1177 mg of Cu per kg, respectively.

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CONNECTING PARAGRAPH

In Chapter III, the study of the influence of pyrophosphatase activity on the course of pyrophosphate hydrolysis was a simplification of the regression model developed in Chapter II. However, the general model introduced in Chapter I involves not only soil-related factors, but also factors related to plant growth and development. Thus, in Chapter IV, the rate of P uptake by onions is compared with the rate of pyrophosphate hydrolysis in organic soil materials. Two major types of organic soil surface materials are found in Quebec: acid mesic materials not yet reclaimed and old-cultivated humic materials. Since acid mesic materials must be heavily limed before cropping, the influence of liming materials on pyrophosphatase activity and pyrophosphate retention should be investigated. In Chapters II and III, it was observed that half-life values of pyrophosphate in organic soil materials did vary between 0,1 and 3,7 days under laboratory conditions and that small changes in pyrophosphatase activity did not produce a significant decrease in the course of pyrophosphate hydrolysis in humic materials. Thus, pyrophosphate and orthophosphate should be equivalent sources of P for crops grown on organic soils. It is the scope of Chapter IV to apply the general model to onions grown in vitro on humic and limed mesic materials.

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CHAPTER IV

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A COMPARISON OF PYROPHOSPHATE AND ORTHOPHOSPHATE FERTILIZATION

OF ONIONS GROWN ON ORGANIC SOIL MATERIALS

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SUMMARY

The objectives of this study were to monitor the rate of onion growth and development in comparison with the rate of pyrophosphate hydrolysis in humic and limed mesic materials and to compare pyrophosphate and orthophosphate fertilizers as to onion yield and uptake of P and Cu. Pyrophosphate hydrolysis in organic soil materials incubated at a -10 kPa matric potential was 100% complete after 8 days in the humic material and 95% complete after 13 weeks in the limed mesic material. The relatively slow rate of pyrophosphate hydrolysis in the limed mesic material was attributed to the action of the liming material. The addition of 4,5% Ca(OH), to the mesic material decreased pyrophosphatase activity by 29% and water-extractable pyrophosphate by 100%, resulting in an apparent increase in half-life values from 1 to 28 days as compared with the original material. Concurrently, the course of onion growth indicated a period of high P demand between the 5th and the 12th weeks after seeding, extending from the leek stage to bulb maturity. However, no significant differences in bulb yield and P uptake by onions were found at harvest between pyrophosphate and orthophosphate as sources of P. Only Cu uptake was significantly higher in orthophosphate-, vs pyrophosphate-fertilized materials particularly at the lowest P rate applied to the limed mesic material. Bulb yields increased linearly with P rates in the humic material, but decreased linearly in the limed mesic material. P uptake increased also '_ linearly with P rates in both materials. A 8% reduction in bulb yield in the limed mesic material was associated with excessive levels of 4,0% P in bulbs and 3,1% P in leaves.

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RESUME

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Le but de ce travail était double: suivre la courbe de croissance de l'oignon par rapport à la courbe d'hydrolyse du pyrophosphate dans des matériaux mésiques chaulés et humiques, et comparer la valeur fertilisante du pyrophosphate à celle de l'orthophosphate en terme de rendement de bulbes et de prélèvement en P et Cu de l'oignon. L'hydrolyse du pyrophosphate dans du matériel organique incubé à un potentiel matriciel de -10 kPa a été complète après 8 jours dans du matériel humique, mais fut complétée à 95% au bout de 13 semaines dans du matériel mésique chaulé. Le taux relativement faible d'hydrolyse en matériel mésique chaulé a été attribué à l'action de la chaux. L'addition de 4,5% de Ca(OH), à du matériel mésique a réduit l'activité pyrophosphatasique de 29% et la quantité de pyrophosphate hydrosoluble de 100%, faisant passer la valeur de demi-vie à 28 jours par rapport à une valeur calculée de 1 jour pour le matériel originel. Concurremment, la courbe de croissance de l'oignon indique qu'une période de demande élevée en P s'est étendue de la 5e à la 12e-semáine après le semis, entre le stade poireau et la maturité du bulbe. Cependant, aucune différence significative entre le pyrophosphate et l'orthophosphaten a été observée dans les rendements des bulbes et le prélèvement de P. Une différence dans le prélèvement de Cu, favorisant l'orthophosphate, a été observée, particulièrement au taux d'application le plus faible sur matériel mésique chaulé. Le rendement des bulbes a augmenté proportionnellement au taux de P en matériel humique, mais a diminué proportionnellement au taux de P en matériel mésique chaulé. Le prélèvement de P a augmenté proportionnellement au taux de P appliqué aux deux matériaux. Une réduction de rendement d'oignons de 8% a été observée sur du matériel mésique

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chaulé et a été associée à des niveaux excessifs de 4,0% P dans les bulbes et de 3,1% P dans les feuilles.

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INTRODUCTION

In southern Quebec, nearly 7 000 ha of organic soils are devoted to vegetable production. The traditional source of P for plants grown on these soils is in the form of granulated orthophosphate fertilizers. During the last decade, increasing amounts of liquid sources of P as polyphosphate fertilizers have become available on the market. However, comparisons as to yield and nutrient uptake between orthophosphate and pyrophosphate, the main non-orthophosphate component of polyphosphate fertilizers, have been carried out primarily on mineral soils. The comparative efficiency of pyro- and orthophosphate has not been studied on organic soils.

Although pyrophosphate could be absorbed by plants (Gilliam 1970), Sutton and Larsen (1964) found that, in short-term water culture experiments, orthophosphate was taken up 2,4 times more rapidly than pyrophosphate by barley seedlings. However, on account of pyrophosphate hydrolysis to orthophosphate, any difference between each source of P would be small for P-deficient plants producing high amounts of phosphatases (Gilliam 1970) and for plants growing in mineral soils of high biological activity (Sutton and Larsen 1964) at mean soil temperatures exceeding $12-15^{\circ}C$ (Sutton *et al.* 1966). Sutton *et al.* (1966) concluded that the effect of pyrophosphate on final yields of a particular crop would depend on the rate of pyrophosphate hydrolysis in the soil, the P status of the soil and the time of maximum P requirement by the crop.

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Although pyrophosphate can complex Cu, Mn and Fe in soils (Bremner et al. 1946), it has been advocated that, due to rapid hydrolysis, polyphosphate concentrations of up to 2000 mg.kg⁻¹ in soil would soon fall below levels likely to enhance uptake of native heavy metals (Hashimoto and Wakefield 1974; Mortvedt and Osborne 1977). Asher and Bar-Josef (1982) found that, in acid soils, pyrophosphate concentration would be too low to sequester Zn effectively. Thus, the rate of pyrophosphate hydrolysis in soil would influence P uptake to a greater extent than uptake of heavy metals.

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The rate of pyrophosphate hydrolysis in soils has been described to be controlled by pyrophosphatase activity and pyrophosphate retention (Racz and Savant 1972). Since liming materials can increase pyrophosphate retention (Philen and Lehr 1967; Sharma 1977) and decrease activity of phosphatases (Halstead 1964), applications of up to 22 t.ha⁻¹ of liming materials to newly broken organic soils (Jasmin and Heeney 1962) would probably influence retention and hydrolysis of pyrophosphate. The activity of phosphatase would be reduced also[°] upon clearing virgin organic soils as a result of the common practice of scraping the topsoil during reclamation. Thus, pyrophosphate hydrolysis could be reduced in organic soils recently cleared as compared with organic soils cultivated for a long period of time.

The aims of the present study were (1) to follow the course of onion growth and development as compared with the course of pyrophosphate hydrolysis in two organic soils differing in liming management and fime under cultivation and (2) to compare the efficiency of pyrophosphate and ortho-

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phosphate fertilizers as to crop yield and uptake of P and Cu by onions grown on these two organic soils.

MATERIALS AND METHODS

Mesic and humic materials were obtained from the surface (0-30 cm) of a newly broken mesisol near Farnham, Quebec, and from a cultivated mesisol near Sainte-Clotilde, Quebec, respectively. Onions grown previously on these organic soils were known to respond to P fertilization. The mesic materials were less dense, more acidic and lower in ash than the humic materials (Table 1). The mesic materials were limed to a pH $(0,01 \text{ M CaCl}_2)$ of 5,5 by adding 45 g.kg⁻¹ of reagent-grade hydrated lime. Lime requirement was determined by incubating the mesic materials, held at a -10 kPa matric potential, with increasing amounts of Ca(OH)₂ for a period of two weeks.

Four experiments were conducted under laboratory and greenhouse conditions.

Pyrophosphate hydrolysis

The course of pyrophosphate hydrolysis was followed in quadraplicate by incubating either 232 mg or 394 mg of P as $K_4P_2O_7$ with 1 kg of humic and mesic materials, respectively. The P concentrations corresponded to a P rate of 0,664 g per pot used in the onion growth experiment. The soil materials were held at a -10 kPa matric potential in plastic bags during an incubation period of 13 weeks at 25 \pm 5°C. The $K_4P_2O_7$ was prepared by heating reagent-grade K_2 ^{HPO}₄ at 300°C for 4 h and at 525°C overnight (Gilliam 1970). According to Van Wazer (1958), K_2 HPO₄ starts to dehydrate

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at 250°C and becomes pure $K_4P_2O_7$ when held at 400°C and above. The product was found to be 99,8% non-orthophosphate (Technicon 1977). The thermostatic bath of the auto-analyzer module was disconnected to prevent P_2O_7 hydrolysis during color development.

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At selected times, orthophosphate was extracted from 5 g of incubated material with 0,5 N H_2SO_4 (Racz and Savant 1972) for 15 min on a reciprocal shaker at 200 rpm. The suspension was filtered through Whatman #42 paper and analyzed colorimetrically for P as described above. Control soil samples containing no pyrophosphate were analyzed also in quadruplicate to account for native P.

Effect of Ca(OH₂) on pyrophosphate hydrolysis and retention

Pyrophosphatase activity was assayed in five replicates with 1 g of soil material and 750 μ mol of sodium pyrophosphate dissolved in 20 mL of a 0,2 M Tris-citric acid buffer of pH 6,4 (see Chapter II). Incubations were conducted in 75 mL test-tubes at 37°C for 2 h. A control sample was analyzed by adding pyrophosphate at the end of the incubation period. The assay mixture was extracted with 20 mL of 1 N H₂SO₄ for 15 min on a reciprocal shaker. After filtration through Whatman #42 paper, the extract was analyzed colorimetrically for P (Technicon 1977). The pH of the assay mixture was taken at the end of the incubation period with a combined electrode.

A measure of pyrophosphate retention was obtained by incubating, in duplicate, 1 g of autoclaved humic, mesic or limed mesic materials $(121^{\circ}C,$ 1 h) containing 5 mL of distilled water with 1 mL of a 500 mmol.L⁻¹ sodium

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pyrophosphate solution adjusted to the pH of the material (see Chapter II). After a 4 h - incubation period in 75 mL test-tubes at 25° C, the materials were extracted with 34 mL of distilled water for 30 min on a reciprocal shaker. After filtration of the suspension through Whatman #42 paper, a 10 mL aliquot of the filtrate was hydrolyzed in 0,5N H₂SO₄ for 2 h at 100^oC on a hot plate (Racz and Savant 1972). Orthophosphate in solution was determined colorimetrically (Technicon 1977). Control samples of mesic and humic materials containing no pyrophosphate were analyzed to account for native P.

P and Cu uptake by onions

The course of P and Cu uptake by onions (Allium cepa L. cv. Early Harvest) grown on the limed mesic material was monitored in separate pots during a period of 13 weeks in the greenhouse at a temperature of $25 \pm 5^{\circ}$ C. The daylength was increased weekly from 14 h at seeding to 16 h at bulb formation, and then gradually decreased to 14 h at harvest.

Rectangular pots, 12 L in volume and 28 cm in height, were filled with 1,68 kg of the mesic material. The water content was adjusted to 50% water-filled porosity and maintained at that level (375% H₂O by weight) during the growth period by weighing regularly three selected pots. Each pot received 75 g of Ca(OH)₂, 1,33 g of N as NH_4NO_3 or KNO_3 , 3,35 g of K as KNO_3 or K_2HPO_4 , 1,64 g of Mg as MgSO₄, 664 mg of P as K_2HPO_4 , 220 mg each of Cu-Zn-Mn as sulfates, 133 mg of B as borax and 1,3 mg Mo as $Na_2MoO_4.2H_2O$. All products were applied in solution, except for Ca(OH)₂.

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Six holes, equally spaced, were made at the soil surface to receive 5 or 6 seeds. The crop was thinned to six seedlings per pot 18 days after sowing. Onion growth was recorded every 7 days by harvesting the plants in individual pots from the 34th day to the 13th week. Also, the date at which a given phenological stage was completed at 50% was recorded. Ten phenological stages were considered (Rey *et al.* 1974): grain, loop stage, flag stage, stall of cotyledon, leek stage, fall of first leaf, bulb formation, bulb growth; leaf collapse and bulb maturity. The experimental design was a randomized complete block with 7 replicates and 10 treatments or harvesting periods. After harvesting, bulbs and tops were separated, diced and dried at 70°C to constant weight, ground in a Wiley mill and dry-ashed at 550°C for 4 h. Ash was dissolved in 3 mL of concentrated HCl on a hot plate at 40°C. Five mL of 2N HNO₃ was added to the mixture, which was then completed to 50 mL with distilled water. The final solution was analyzed for P colorimetrically (Technicon 1977) and for Cu by atomic absorption spectrophotometry.

Added P, bulb yield and nutrient uptake

The response of onions as to bulb yield and P and Cu uptake was analyzed at three rates of $K_4P_2O_7$ and K_2HPO_4 applications to the humic and the limed mesic materials. A factorial experiment was replicated six times and arranged in a randomized complete block design. Soil preparation, basic fertilization, seeding and thinning were as in the onion growth experiment, except for P fertilization. The three rates of P were 0,332, 0,664 and 1,328 g of P per pot. After 13 weeks of growth, onion bulbs were weighed. Bulbs and tops were prepared and analyzed for P and Cu as described previously.

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Pyrophosphate hydrolysis

The half-life of pyrophosphate in the humic material was approximately 2 days, as compared to 4 weeks in the limed mesic material (Figure 1). Correspondingly, pyrophosphate hydrolysis was 100% completed in the humic material after 8 days of incubation, but only 95% completed in the limed mesic material after 13 weeks of incubation.

ESULTS

The addition of hydrated lime to the mesic material decreased significantly pyrophosphatase activity by 29%, and water-extractable pyrophosphate by 100% (Table 2). The pH of the assay mixtures was 7,0, 6,1 and 6,9 with the humic, mesic and limed mesic materials, respectively. The humic material had greater pyrophosphatase activity than the unlimed and the limed mesic material (Table 2). However, the limed mesic material retained higher amounts of pyrophosphate against water extraction than did the humic material. Thus, the shorter half-life of pyrophosphate in the humic as compared with the limed mesic material was consistent with higher pyrophosphatase activity and lower 'pyrophosphate retention in the humic than in the limed mesic material.

Onion growth and development

During the first 5 weeks of development, onion seedlings passed through 5 phenological stages (Figure 1). However, at leek stage, only 2% of maximum growth was attained. Dry matter increased to 90% of the maximum value from the 5th to the 12th week, as a result of leaf growth between leek stage and fall of the first leaf, and of bulb growth from bulb formation to bulb maturity.

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In the period from leek stage to bulb maturity, relative values of P and Cu accumulated in plant material were higher than those of dry-matter production. Between the 5th and the 12th week, the trend of P and Cu uptake was approximately parallel to that of dry-matter production.

From the 6th week, the crop accumulated increasing amounts of P and Cu from the soil materials. By this time, all the pyrophosphate applied to the humic material was completely converted into orthophosphate (Figure 1). In the limed mesic material , however, one third of the pyrophosphate applied was found to be unhydrolyzed in incubated soil material. Thus, a difference in onion response between $K_4P_2O_7$ and K_2HPO_4 should occur primarily in the limed mesic materials.

Effect of K4P207 vs K2HPO4 on bulb yield and P and Cu uptake

Bulb yield and P uptake were not significantly affected by the source of P, but were significantly influenced by the soil material and the P level (Table 3). In contrast, Cu uptake was significantly influenced by the source of P and the soil material, but not by the P level.

The level of P influenced bulb yield apparently by increasing P uptake by plant material (Table 4), since P uptake increased linearly with increasing rates of P applied to humic and limed mesic materials. However, bulb yield increased linearly in the humic material but decreased linearly in the limed mesic material with increasing P rates. Thus, P would have reached excessive levels in onions grown on the limed mesic material.

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The concentration of P in bulbs increased linearly with P rates (Table 4). The P concentration in tops increased quadratically with increasing P rates in onions grown on the limed mesic material, but increased linearly with P rates in onions grown on the humic material. At P concentrations of up to 3,6% in bulbs and 2,5% in tops bulb yields of onions grown on the humic materials were not depressed. However, P concentrations of 4,0% in bulbs and 3,1% in tops were related to a reduction of 8% in bulb yield of onions grown on the mesic material, as compared with maximum yield. A 10% reduction in bulb yields in the limed mesic material was related to P concentrations of 4,4% in bulbs and 5,4% in tops.

The influence of P source on Cu uptake could be explained by the second-order interaction (Table 3), which was significant at the 10% level $(F_{2,55} = 2,56)$. Onlons grown on the humic material had similar Cu uptake values whatever the P source and the P level (Table 5). In contrast, Cu uptake by onions grown on the limed mesic material were generally similar between the P sources except at the lowest P rate. Thus, at a low rate of P application to limed mesic material , $K_4P_2O_7$ -fertilized onions were apparently less effective at taking up Cu than K_2HPO_4 -fertilized onions, although bulb yields were comparable at harvest.

DISCUSSION AND CONCLUSIONS

These results support the hypothesis that under warm soil conditions, the efficiency of pyrophosphate as a source of P to plants is equivalent to that of orthophosphate (Sutton *et al.* 1966). The rate rather than the source of P influenced yield until excessive levels of P were attained in the plant material.

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According to Loneragan *et al.* (1982), plants likely to suffer from P toxicity have shown P concentrations from 1,2 to 4,5% in their leaves. Thus, it was apparent that P concentrations exceeding 4,0% in bulbs and 3,1% in tops have reached excessive levels reducing bulb yield significantly.

Since P uptake by onions was similar between the P sources in limed mesic materials despite partial pyrophosphate hydrolysis in incubated materials during bulb growth, factors related to plant growth could have influenced pyrophosphate availability. Plants could absorb pyrophosphate per se at a rate that depends on P demand, whereas P demand by the plant could induce the production of phosphatases by the root cells (Gilliam 1970).

The effect of calcium hydroxide on pyrophosphate hydrolysis was important. The half-life values of pyrophosphate, computed from an empirical equation involving pyrophosphatase activity and amount of water-soluble pyrophosphate (see Ghapter II), were 2 and 1 days for the humic and the unlimed mesic materials, respectively, as compared with 2 days and 28 days found for the humic and the limed mesic materials, respectively. The empirical equation was not applicable to the limed mesic material since the parameter values exceeded the boundaries of the equation, which were between 0,1 and 8,1 mmol.kg⁻¹ for water-extractable pyrophosphate and between 11,6 and 148,1 mmol.kg⁻¹.2h⁻¹ for pyrophosphatase activity. Thus, the decrease in hydrolysis rate of pyrophosphate upon liming the mesic material was consistent with a decrease in pyrophosphatase activity and an increase in pyrophosphate retention.

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The decrease in phosphatase activity upon liming was related to a change in soil pH (Halstead 1964), presumably beyond the optimum pH of an acid phosphatase system. A pH increase of assay mixtures was also observed upon liming mesic materials. Hossner and Melton (1970) attributed the reduction of pyrophosphate hydrolysis upon liming acid mineral soils with 20 g $CaCO_3$.kg⁻¹ to biological, phosphatase-related, factors rather than chemical changes in soils, since $CaCO_3$ had been incubated previously with soils for 3 weeks prior to pyrophosphate additions. However, Sharma (1977) found that pyrophosphate retention increased with increasing $CaCO_3$ applications of up 30 g.kg⁻¹ of slightly acid to neutral mineral soils. Thus, the effect of hydrated lime applied to the mesic material in this experiment was consistent with the effect of other liming materials on reducing pyrophosphatase activity and increasing pyrophosphate retention, as found for mineral soils.

Since the decrease of water-soluble pyrophosphate was considerable upon liming the mesic material , $K_4P_2O_7$ could have reacted promptly with hydrated lime to produce Ca-K pyrophosphates (Brown *et al.* 1964). However, the newly formed Ca-K pyrophosphate would not alter to markedly less available phosphates (Lehr *et al.* 1964), since no significant difference in bulb yield and P uptake by onions was shown between the two sources of P.

Consequently, pyro- and orthophosphate were equivalent sources of P for onions grown in organic soil materials under greenhouse conditions, although the hydrolysis rate of pyrophosphate was relatively low in the limed mesic material as compared with the humic material. Increasing P rates increased P uptake to levels that could reduce bulb yield significantly.

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*Excessive levels of P were attained in plant materials whether pyrophosphate
or orthophosphate were the source of P.

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Table 1.	Physical a	and chemical	characteristics of t	he humic	and mesic
,	materials	before limin	ng and fertilization	•	•

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Soil characteristic	Humic	Mesic	1
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Rubbed fibers (< 0,150 mm) (%)	6	25	pad
Bulk density (kg.L ⁻¹)	0,24	0,15 _/	· · ·
Ash (%)	18,5 ⁱ	2,0 -	. ,
pH (0,01 M CaCl ₂)	. 6,0	2,7	•
DA ⁽¹⁾ -extractable P (mg.kg ⁻¹)	28	24 .	-

(1): Double acid (0,025 N $H_2SO_4 + O_8O5$ N HC1)

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Table 2. Pyrophosphatase activity (means of 5 rep, CV = 8,8%) and amount of water-soluble pyrophosphate (means of 2 rep, CV = 4,7%) in mesic, limed mesic and humic materials

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Materials		5 1. 1.10	Pyrophosphatase activity (mmo1.kg ⁻¹ .2h ⁻¹)	Water-soluble pyrophosphate (umol.kg ⁻¹)	
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Мевіс	۰ ۱. ۵	•	/ 12,6	7,0	. 11
Limed mesic	v	• •	9,0	0,0	.
Humic	•	[~] °•	23,4	_. 1,3	بر ۲ ۱
Lsd ($\alpha = 0,05$)	بر جند المحمد المحم المحمد المحمد	ی هم می خلاطی چو چو هم می می در ر	· 3,5	0,1	

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Table 3. Analysis of variance of the influence of three rates of P application as $K_4P_2O_7$ or K_2HPO_4 on bulb yield, P and Cu uptake of onions grown on humic or limed mesic materials

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Source of variation	df	Bulb yield	P uptake	Cu uptake	
	• •	ه هم همه هم همگری بر بین بین می می در باری می این می بین می این می ای این هم این	F-values	برین هیی است. دینی می این این این این این این این این این ای	,
Material	1	139,58**	327,73**	45,61**	u
P source	ຳ 1	0,16	° 0,51	4,54*	≯
P`level °	2	0,84	43,59**	0,08	
Material x P source	1	3,15	3,22	1,76	
Material x P level	2	5,33*	0,83	1,16	•
P source x P level	2	1,24	0,29	1,67	
Material x P source x P level	2	1,19	0,24	[*] 2,56	•
EMS	55	775,2	284,0	746,6	•

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*, **: significant at the 0,05 and the 0,01 levels, respectively

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Table 4. Bulb yield, P uptake and P concentration in onions fertilized at three rates of P in humic and limed mesic materials (means of 6 replicates)

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				•	•
Material	P level	Bulb yield	P uptake	<u> </u>	P in
	(g per pot)	(g per pot)	'(mg per pot)	Bulbs	Tops
	• 0 332		 56	2 0	1 8
Humito	0,664	137	72	2,3	2,2
, , ,	1,328	162	95 ·	3,6	2,5
	/ 0.332	· 235	123	3.3 '	2.2
Mesic	0,664	217	142	4.0	3.1
	1,328	212	174	4,4	5,4 '
<u></u>	Model		- F _{1,55} value	8	
Humic	Linear	6,67*	85,99**	74,56**	7,61**
	Quadratic	, 1,61	1,70	0,06	< 0,01
Mesic '	° Linear	4,10*	(1)	(1)	154,10**
·	Quadratic	0,75	ین بین بین بیزیانی وید می می بین بین بین ا		16,75**
CV (Z)		15,2	15,3	9,9	21,5
 	د است همه هم خدر شد می مده می هم هم هم هم بر است معهد می خدر می می می می می می	بندي بسيد به به خذ بمبن د مه به به	، خلیار سن جنب کا جاند ایک مقابلی جند بین ولد که س	ک سے میں عبرت ذیرت پری سے خینہ بڑی خاند اس پندے *	، جنب جنی کی کو کی ڈیل جنیل جی کی کی جنی ہیں ہے ہے

(1) matevial x P level nonsignificant at the 0,05 level

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Table 5.	Cu uptake fertilize	by onions grown on d at three rates of	K ₄ P ₂ O ₇ and K ₂ H	i mesic materiais an ?04	a
Material		P level	<u>Cu uptake (µg per pot)</u>		
	-	(g per pot)	[₭] 4 [₽] 2 ⁰ 7	к ₂ нро ₄ /	` ,
Humic °	, 	0,332 0,664 1,328	80 90 80	80 80 100).
Mesic ·	¢ /	0,332 « 0,664 1,328	110 120 120	160 130 120	

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CV = 25,5%

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GENERAL CONCL

GENERAL CONCLUSIONS

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The mechanism of pyrophosphate availability to plants grown on organic soils involved three major reactions: pyrophosphate retention, pyrophosphate hydrolysis and orthophosphate absorption.

Organic soil materials have shown little affinity for pyrophosphate retention. Although organic soil materials retained increasing amounts of pyrophosphate with increasing ash contents, this retention capacity was short-lived, since pyrophosphate was hydrolyzed rapidly to orthophosphate in a matter of days at room temperature. Nevertheless, it was apparent from stepwise regression analyses that increased pyrophosphate retention delayed enzymic hydrolysis and the production of orthophosphate. Indeed, pyrophosphate hydrolysis was primarily enzymatic in organic soil materials, as noted by activation energy values. Since the enzymic system was acid and apparently nonspecific, acid phosphatases were probably involved in pyrophosphate hydrolysis.

Pyrophosphatase activity was inhibited by Cu addition. Since pyrophosphatase inhibition by Cu to 80% of the reference value did not produce significant decreases in pyrophosphate half-life values, the application of Cu at recommended rates should not reduce significantly the rate of pyrophosphate hydrolysis in organic soils.

However, the heavy application of hydrated lime to acid mesic materials not only decreased pyrophosphatase activity to 55% of the original value but also increased considerably pyrophosphate retention. Despite the consequent delay in orthophosphate production due to the liming material applied, the

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rate of P uptake by onions was equivalent from pyrophosphate - and orthophosphate - fertilized mesic materials. Since pyrophosphate and orthophosphate were also equivalent sources of P for onions grown on humic material, it seems likely that the pyrophosphate hydrolysis process was an efficient pathway to increase P availability to plants in both freshly limed mesic and old cultivated humic materials.

These studies indicate that pyrophosphate would make an acceptable P fertilizer equivalent to orthophosphate in organic soils of Eastern Canada.

Consequently, because of rapid rates of pyrophosphate hydrolysis in organic soil materials compared with rate of P uptake by crops, pyrophosphate retention and hydrolysis rates did not appear to limit pyrophosphate availability to plants grown on organic soils.

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Appendix I

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Influence of suspension pH on pyrophosphate (SPP) hydrolysis by three organic soil materials assayed in a 0,2 M Tris-citric acid buffer

Suspensio	on pH.after	incubation	% of max	imum SPP h	ydrolysis	6 0	- <u>e</u>
Fibric	Mesic	Humic	Fibric	Mesic	Humic	و منه و د و د و د و د و د و د و د و د و د و	
3.7	3.8	3.9	29	28	15		
4.1	4,4	4.2	36	31	19°	ß	
4.7	4.9	4.7	63	45	27		
5.5	5.5	5.2	87	62	33		
5.9	6.1	5,9	100	88	5 5 [°]	N 1	
6.6	6.8	6.8	. 95	100	100	`	
7.4	7.6	7.6	57	53	83	59	
7.9	8.1	7.9	24	43	54		
8.2	8.3	° 8.3	7	84	30		
8,5	8,5	8,6	1 O	12	25		
8,8	8,9	9,0 _	0	4	17		_
F.			234**	23**	130**	,	
10 Castelais	للو مەر 10 مىر 10 م	bion (9)	, o,	20	0	a	
Coerricie	nt of varia	cion (%)	, 0	20	0	,	
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Appendix II

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Influence of suspension pH on phenyl phosphate (PDP) hydrolysis by three organic soil materials assayed in a 0,2 M Tris-citric acid buffer

Fibric	Mesic	Humic	Fibric	Mesic	" Humic		
		و میں ایش خود سور ایس جرار میں میں کر پر اور میں میں اور				0	
3,3	3,3	3,4	• 20 ·	38	49 🔥		
3,8	3,8	3,8	37	46	60		
4,3	4,3	4,2	· 68	67	- 62		•
4,7	4,9	4,7	100	91	75		•
5,5	5,5	5,4	68	100	. 76	-	•.
5,8	6.1	6,2	46	93	100		
6,8	7,1	7,2	26	75	[·] 100		,
7,3	· 7,6	7,8	16	。54	100		
8,0	8,0	8,2 '	6	30	87 .		
8,2	8,4	8,4	3	1 5 ·	75		
8,6	8,7	8,7	1	12	61		
F ₁₀	'values	ک جنہے بیش شک نہیں کی ایک میں کی دی کر ایک	147**	296**	12**		
rv, Coefficie	nt of vari	ation (%)	11	5	10		

Appendix III

Class	Number '	Class	Number	Class	Number
09	, 6	110-119	2 [°]	210-219	O
10-19	26	120-129	1	220-229	Ó
20-29	10	130-139	·	230-239	0
30-39	13	140-149	0	240-249	0
40-49	11	150-159	0	250-259	0
50-59	6	160-169	• 0	260-269	0 ^
60-69	3	170-179	0	270-279	1
70-79	• 3	180-189	0	280-289	0
80-89	0	190-199	0	290-299	°~ 0
90-99	2	200-209	0	300-309	1
100-109	1			••	

Classes of total Cu contents (mg.kg⁻¹) of 87 cultivated surface (0-20 cm) organic soil materials collected in Quebec in 1979

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Appendix XV

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Total N, N-NH₄⁺, organic N and N-NO₃⁻ contents of 24 organic soil materials from Eastern Canada (%)

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Material	Total N	N-NH4+	Organic N	N-N03	
· 1	0,6	0,1	0,5	0,0	
2	1,1	0,1	1,0	0,0	4
3 🖌	1,0	0,1	0,9	0,0	
4	1,2	0,2	1,0	0,0	
5.	1,0	0,1	0,9	0,0	
6	1,1	0,0	,1,1	0,1	
7	1,3	0,0 .	4,3	0,1	
8 .	1,1	0,0	1,1	0,1	Ð
9	-1,8	0,1	1,7	• • • • • • • • • • • • • • • • • • • •	-
10 ,	1,4	0,0 .	1,4	0,1	
11	1,5,	0,0	. 1,5	0,1	
12	1,8	0,0	1,8 '	0,1 .	• -
13	2,3	0,2	2,1	0,0	
14 *	2,4	0,1	2,3	0,1	
15	2,2	0,1	2,1	0,1	
16	1,9	0,0	1,9	0,1	
17	* 2,2	0,0	2,2	0,1	
18	2,4	0,0	2,4	0,1 ,	
, 1 9 ° :	2,8	0,0	2.8	0,1	
20	2,6	0,0	2,6	0,1	
21	2,4	0,0	2,4	0,2	
22	2,6	, 0,0 ~	2,6	• 0,2	
23	2,1	0,0	2,1	0,1	
24	2,5	0,0	2,5	0,1	

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•	
✓ ♣	VITA
₀ 1950	Born in Greenfield Park, Quebec
^{0,} 1973	B.S.A. from Laval University
1976	Two month visit to micromorphology centers in Paris, Wageningen
	and Stuttgart
. 1977	M.Sc. in soil micromorphology (Histosols) under Dr. F.J. Pauze of
•	Laval University
/ 1977– 1979	Agronomist for the Quebec "Relance" development programme in Gaspésie
i	and Iles-de-la-Madeleine
. 1979	Organic soil physicist, St-Jean Research Station. Two missions for
• •	the Newfoundland organic soil development project.
1980	Tour of Quebec major organic soil deposits
1983	Two week mission on peatland use in USSR under the Canada-USSR
	agricultural cooperation agreement
1984	Attendance to the international peat congress held at Dublin
198 4	Ph.D. in soil fertility
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