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SOIL AND COLLOIDAL PHOSPHORUS DYNAMICS IN THREE KY SOILS: BIOAVAILABILITY, TRANSPORT AND WATER QUALITY IMPLICATIONS

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ABSTRACT OF THESIS

SOIL AND COLLOIDAL PHOSPHORUS DYNAMICS IN THREE KY SOILS: BIOAVAILABILITY, TRANSPORT AND WATER QUALITY IMPLICATIONS

Particulate P constitutes a significant portion of the total P found in surface runoff water. Water dispersed P-containing particles can travel long distances via surface runoff and reach water bodies causing decrease in water quality. The main objective of the study was to evaluate the potential facilitation of P transport by the water dispersed soil colloids (WDC) using three KY soils with a long-term record of poultry manure, and fertilizer P applications. Sequential fractionation for both whole soils and colloidal samples revealed that the WDC had a greater total and labile P content than the soil as a whole. Also, application of manure and fertilizer P seemed to decrease colloidal organic P fractions and increase the inorganic P fractions over the period of a growing season (May to September). Laboratory settling kinetics experiments were set up for the clay-colloidal fractions of the soils. It was shown that particulate P fractions paralleled WDC settling kinetics whereas dissolved P fractions remained in solution even after 36 hours. Field taken intact soil cores were leached with colloidal suspensions to test the effect of WDC on the vertical P movement. Results illustrated the preferential flow of particulate P though the macropores. When water was applied to the manure amended soil, dissolved P levels increased significantly over the control. WDC additions lowered dissolved P levels to the manure-amended columns, by sorbing to the WDC particles, but still greater than the dissolved P levels of the columns that had not been applied with manure.

Keywords: Soil Phosphorus, Water Dispersed Colloids, Fractionation, Water Quality.

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2002

SOIL AND COLLOIDAL PHOSPHORUS DYNAMICS IN THREE KENTUCKY SOILS: BIOAVAILABILITY, TRANSPORT AND WATER QUALITY IMPLICATIONS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science
in the College of Agriculture at the University of Kentucky

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CHAPTER ONE

LITERATURE REVIEW

PHOSPHORUS CHEMISTRY AND FORMS

Phosphorus (P) is one of the most important nutrients in the cycle of life. It is found in humans, animals, plants, and microorganisms varying in amount, chemical structure, and forms. Its complexity appears in soil and water systems, partitioning itself into several P fractions in minerals and organic compounds with differing bioavailability and bonding strength.

Phosphorus' strong affinity for the soil's solid phase causes it to exhibit low concentrations in the soil solution, as it is in dynamic equilibrium with soil's solid phases. Its low concentration in soil solution has led farmers to use fertilizers and manures in order to maintain a level sufficient for plant growth (0.02 ppm P for corn).

During the last several decades, excess amounts of animal waste have been surface-applied to some fields, affecting the soil's P sorption capacity. In cases where manure application exceeds the soil P sorption capacity, unusually elevated P levels are found at the soil surface, causing P to become more susceptible to movement with surface runoff water. These elevated P levels in surface runoff contribute significantly to eutrophication of water bodies, leading to algal blooms.

Thus, it is important to define and isolate the different P fractions in surface soils in order to assess their bioavailability. Free ortho-phosphates and metal-P ion pairs constitute the soluble, most bioavailable, P forms. It has been found that hydrolysis and degradation of more chemically stable P compounds is caused either by biotic (enzymes) or abiotic (metal-promoted hydrolysis) processes. These processes result in the release of P compounds that are relatively available to microorganisms.

There is no typical distribution of P between the organic and inorganic P forms in the environment. The P distribution varies with soil type, texture, and the timing and history of manure application (type, and method of application). Animal waste applications and soil management practices largely affect organic P distribution in soils. According to Stevenson (1994), organic P compounds can be classified into four clusters, based on their abundance in soils: inositol phosphates (2-50%); phospholipids (1-5%); nucleic acids (0.2-2.5%); phosphoproteins and metabolic phosphates (traces).

Inositol phosphates are esters of hexahydrocyclohexane. The most commonly found inositol phosphate is phytic acid, or its hexaphosphate ester, which occurs also in cereal grains. Caldwell and Black (1958) were among the first to detect large quantities of phytic acid in manures. Its strong resistance to physical and biochemical degradation makes it a large reservoir of organic P in the soil.

Phospholipids are insoluble in water and they are abundant in localized colonies of microorganisms. Nucleic acids (RNA, and DNA), constitute a major portion of living cells, and they are synthesized during decomposition of plant and animal remains by soil microorganisms.

Humic-P compounds in soils are more significant as compared to their concentrations in water bodies. Minear (1972) found that the organic P content of lake water constituted a very small fraction of the dissolved organic matter (DOM). DOM contains both humic and nonhumic dissolved organic substances. Humic P compounds have low concentrations that hinder their characterization and isolation in both soil and water systems.

On the other hand, inorganic P is also a significant portion of total soil P and its forms vary with pH. Over a wide range of pH, inorganic P can be “free” metal-P ion-pairs or orthophosphate ions in solution. At neutral to basic pH levels, inorganic P can be in the form of Ca and Mg phosphates with different solubilities, depending on the degree of crystallization of the Ca-P minerals. Organic matter is an important factor that hinders the nucleation of calcium phosphates (Krom and Berner, 1980). At neutral to low pH values, inorganic P can be loosely-bound to clay minerals or highly-occluded within the mineral oxides. Allophane minerals adsorb the most P on their surfaces, followed by ferrihydrite > goethite > gibbsite > hematite > kaolinite > montmorillonite. Allophanes can adsorb large amounts of phosphates due to their high specific surface area.

HUMIC SUBSTANCES AND PHOSPHORUS

Humic substances comprise 50-70 % of the total soil organic matter, (Stevenson, 1994). Fulvic, humic acids and humin are the main components of soil humic substances. Fulvic acids are soluble at any pH, contrary to humic acids that become insoluble as the pH drops below seven. The humin fraction contains neutral insoluble organic moieties. Fulvic acids are low molecular weight compounds, greater in total acidity, carboxylic, phenolic, and alcoholic OH contents than the higher molecular weight humic acids. Also, most of the oxygen content is encountered in the surface functional groups of humic acids, whereas for fulvic acids they are evenly distributed throughout the whole chemical formula.

Humic P substances play a significant role in many geochemical processes, despite their low concentrations in soil and water systems. Plechanov et al (1983) reported that phosphorus comprised less than 1% of the humic substances. Humic substances form complexes with anions, and polyvalent metals (Levesque and Schnitzer, 1966). Schnitzer (1995) reported that these complex-bridges are more important than single complexes in neutral or weakly acid solutions.

The effect of humic substances on mineral formation and dissolution phenomena is well established. Baker (1973) recognized the role of humic substances in dissolving minerals by releasing metal cations in solution. Nelson et al. (1990) found that as the soil clay content increases, the mean dissolved organic carbon in stream waters decreased, probably due to adsorption of organic carbon onto clay minerals. Surface and interlayer adsorption of humic acids has been observed (Schnitzer and Kodama, 1966). They observed that fulvic acid could be adsorbed in the interlayer of montmorillonite with subsequent expansion. This is especially true at low pH levels, where the fulvic acid moiety “shrinks” and more easily penetrates the mineral’s structure. This is where fulvic acid can form either inner or outer sphere complexes with the structural cations, especially Fe^{3+} (Kodama and Schnitzer, 1973), depending on the affinity of the cations for water molecules (expressed with the metal’s hydrolysis pK_h). This mechanism involves the generation of acid protons that can gradually degrade the mineral’s structure.

Complexes of humic compounds with metal oxides and phosphates can alter the soil colloids’ net surface charge and the zero point of charge (PZC) (Seaman et al., 1995).

As the quantity of these metal-humus complexes increases, greater phosphate adsorption occurred in Thai and Korean Ultisols, and Alfisols (Tokashiki et al., 1983). Humus complexed with Fe and Al bring about decreases in the humic compound’s net negative charge, by blocking ionized carboxyl and phenolic OH functional groups.

MYTHS AND PITFALLS ABOUT P

Several classic perceptions about soil P behavior are gradually being rejected as new scientific literature emerges. One of those classic perceptions was that an effective common field practice to increase crop yield was to apply manure to meet crop N requirements. Manure’s low N: P ratio causes this practice to result in much more added manure-P than the crop actually needs or removes at agronomically optimum yields. Sims and Wolf (1994) observed that P leaching occurred when the input of fertilizer and manure P exceeded crop requirements.

A lake water concentration of P greater than 0.02 ppm accelerates eutrophication, whereas soil solution P values critical for plant growth are 0.2 to 0.3 ppm (Sharpley et al., 1999). These differences in soil and water P concentrations illustrate the contribution of P losses through surface runoff and subsurface leaching to the elevated P levels observed in water bodies.

Further, the classical perception that P does not move through the soil profile needs to be reconsidered. When P inputs exceed the soil P sorption capacity, especially in coarse-textured soils, then P transport phenomena are more likely to occur (He et al., 1999). Much of the “eluviating” P is sorbed to soil colloids, calling into question the classical perception that P moves through the soil only as dissolved inorganic P (Rolston et al., 1975; Sharpley, 1985; Sinaj et al., 1998).

MANURE-P CHARACTERIZATION

Manure is composed of organic and inorganic P compounds, heavy metals, and recently, the sex hormones estradiol and testosterone (Finlay-Moore et al., 2000). Poultry manure is normally an alkaline material with pH values ranging from 7.5-8.5 (Sims and Wolf, 1994). The total P concentration in manure varies with type of manure. Dairy manure averages 9 g P kg^{-1}, while other values include 25 g P kg^{-1}, for poultry manure, 20 g P kg^{-1}, for poultry litter and 30 g P kg^{-1}, for swine slurry (Barnett, 1994a). Furthermore, animal diet and manure collection, treatment, and storage are all important factors that explain much of the variation found in total P values for manure.

There is an increasing need for detailed characterization of manure-P. So far, a modification of the traditional soil P fractionation has been used for manure P analysis. Sharpley and Moyer (2000) found that much of the P, in several types of manures and composts, was inorganic (63 to 92%). In an earlier effort, Barnett (1994b) tried to characterize the different P forms in manures by employing a chemical fractionation scheme. Lipid-P, organic P, inorganic P and residual P were the main fractions, with inorganic P constituting about half of all the manure P. Dinel et al. (1998) isolated the colloidal fraction of hog manure and spectroscopically characterized the chemical P compounds. By means of mass spectroscopy, they were able to find large amounts of sterol and lipid-P in manure, which are relatively easy to hydrolyze in natural soil environments.

EFFECT OF MANURE APPLICATION ON CHEMICAL PROPERTIES OF SOILS

Animal waste application to agricultural land is a common crop management practice employed by farmers to enrich the soil with necessary plant growth nutrients. Besides other nutrients, animal waste contains P, mainly as dicalcium phosphate, and also heavy metals, some growth hormones and plenty of enteric microbes. In the past few decades, manure has been applied to the surface of limited agricultural areas more as a means of disposal, rather as a source of nutrients. Manure's inorganic and organic components interact with the soil particles, even months after application, affecting the soil's physicochemical properties.

Manure's effect on soil pH is contradictory. According to lyamuremye et al (1996), addition of manure to soils with a high P fixation capacity leads to an increase in soil pH. In contrast, Sims (1986) found that broiler litter raised the pH of an Evesboro loamy sand soil from 6.5 to 7.5 immediately after application, but caused the final soil pH to fall to about 5.5 after 20 weeks.

Similar results were found for soil CEC (Gao and Chang, 1996). They concluded that long-term manure application increases CEC by increasing total organic carbon (TOC). However, the soil CEC increase per unit weight of soil organic matter attributable to manuring was less than that published for indigenous soil organic matter. Manure application also increases the soil electrical conductivity values over that of unamended soils. Manure's high content of salts and sodium causes increases in salinity and SAR levels of manure-amended soils.

Manure application often has a positive effect on soil test P values. No-till soil management, and pasture management practices, typical for southern eastern regions of the country's agricultural lands, often imply the use of surface applied manure. This practice will lead to the generation of a considerable amount of dissolved or particulate P (PP) at the surface of the soil, which can be transported from the field via surface runoff.

WATER-DISPERSED SOIL COLLOIDS

The colloidal phase refers to particles and discontinuities of matter with size dimensions in the range of 1nm to 1µm (Hayes and Bolt, 1991). In the case of soil colloids, we refer to a sizeable portion of the clay fraction of the soil (<2µm), which plays an important role in environmental and plant nutritional issues.

Soil colloids can be classified as inorganic and organic. Inorganic colloids are clay minerals such as montmorillonite, kaolinite, smectites, and metal (Fe, Al, and Mn) oxohydroxides. On the other hand, organic soil colloids can be polysaccharides, lipids, polypeptides, and amorphous macromolecular humic substances (Hayes and Bolt, 1991). Baldock et al (1992) reported that finer soil particles exhibited a higher proportion of aliphatic materials and a lower proportion of aromatics and carbohydrates, compared to coarser soil particles.

It is easy to understand that the complexity of soil colloids makes them difficult to characterize. That complexity retards the better understanding of their specific role in aquatic systems. An operationally defined separation of water dispersed soil colloids (WDC), based on extraction with water and centrifugation, has been widely used (Seta and Karathanasis, 1997b).

The clay minerals found in soils usually occur in mixtures that play a major role in many geochemical processes. The most commonly found minerals are those of the kaolinite subgroup (1:1 layer silicates), and the mica subgroup (2:1 layer silicates). Kaolin based minerals are not expandable, and they have a lower number of active surface charge sites as compared to 2:1 minerals. Thus, they are less chemically involved in sorption/desorption processes than the mica subgroup.

Metal oxides are fine clay particles that play a major role in colloidal systems. They are extremely active due to their high surface charge density, high specific surface area, and the fact that each metal at the edge of those minerals is singly coordinated to oxygen or hydroxyls that are protonated or not, depending on the pH of the soil. As such, they have a variable surface charge. When the pH is the same as the pKa of the oxide, 50 % of the surface charge is protonated to give $OH_2{}^{1/2+}$ groups, and the other half is carrying a $OH^{1/2-}$ charge creating a net zero surface charge. This property can be exploited to destabilize and flocculate colloidal suspensions.

Another property of the metal oxides is their tendency to form strong bonds with phosphates. Cations like Ca, Mg, Al, Fe, and Mn act as a bridge between phosphates and negatively charged colloids. Calcium and Mg form outer sphere complexes that are not irreversibly bound to P. On the other hand, Al, Fe, and Mn can form inner-sphere complexes, which bind P more strongly, making P less available. The tunnel and layer type structure of oxides enables solid or liquid state diffusion of P ions into their network. At pH values above the PZC, oxides carry a net negative charge, whereas at pH values below the PZC, they carry a net positive charge. At natural pH levels (5-7), Fe and Al oxides carry a net positive charge, making them strong sorbents for phosphorus. As the metal oxides age they come to carry a net negative charge, due to a charge reversal phenomenon (Liang and Morgan, 1990). It has been speculated that as the concentration of phosphate increases, it occupies more surface sites on the oxide, lowering its net positive charge. This process results in a gradual increase in the oxide's net negative charge in phosphate abundant environments. Furthermore, Mn, Fe and Al oxides act as Lewis acids at the pH values common to soils, thus cleaving rings and oxidizing organic compounds containing phosphates (Lehmann and Cheng, 1988; Shindo and Huang, 1984).

EFFECT OF WDC ON PHOSPHORUS MOBILITY

Numerous studies confirm the enhancement of contaminant mobility by soil colloids (Kaplan et al., 1997). Their small size, coupled with their large specific surface and a large number of reactive surface functional groups per unit mass, makes them efficient sorbents for certain contaminants (O'Melia, 1989). Small colloids are transported mainly by diffusion while larger ones move with gravity. Particles with diameter on the order of a micron have the greatest ability to migrate through the soil since colloid-sized substances may be transported much farther than substances associated with larger sized particles (McCarthy and Zachara, 1989). Accelerated transport by soil colloids has been shown for organic pesticides (Seta and Karathanasis, 1997a).

Water dispersed colloids can be generated from soil aggregates interacting with dissolved solutes and the soil matrix. Surface colloid chemistry often uses the term "colloid stability" to describe the case where colloids remain in suspension without observing aggregation phenomena due to specific chemical conditions (e.g. low ionic strength or considerable changes in total net variable charge).

Changes in solution chemical factors such as pH, ionic strength, sorption of polyvalent cations, and/or total organic carbon can induce colloidal suspension stability or flocculation phenomena (Gschwend et al., 1990). Lowering ionic strength can increase suspension stability, thus making particles more prone to transport. Increases in ionic strength cause compression of the diffuse double layer between the soil charged particles and flocculation could occur, clogging small pores and reducing transport. Fluctuations in pH can affect the net surface charge distribution, and destabilize colloidal suspensions, but if and only if compression of the double layer has been achieved due to a decrease in the net surface charge.

It has been hypothesized that manure application will alter physicochemical soil conditions during the growing season and possibly favor the dispersion of colloids. Manure's acidic functional groups usually carry a net negative surface charge, thus interacting less with the soil colloid surfaces. This potentially contributes to increased manure colloids' stability. Under these conditions, the inorganic and organic P compounds associated with manure colloids might be expected to show enhanced mobility within the soil profile. Earlier research has shown that P movement in the soil profile depends on the rate of P application, P reactions with the soil solid phase, and the consistency and type of the applied waste (Reddy et al., 1980).

Soil P sorption capacity plays a major role in P mobility. Several studies have observed a decrease in P sorption capacity in heavily manured soils. This may contribute to long-term increases in P leaching or surface P runoff (Simard et al., 1993). Organic residues having P concentrations less than 0.3% increased P sorption, whereas manures having a P concentration greater than 0.3% decreased P sorption (Singh and Jones, 1976). Organic acids have been shown to compete for the same adsorption sites as $H_2PO_4^{2-}$ and reduce the capacity of minerals to retain $H_2PO_4^{2-}$ (Fox and Kamprath, 1971). Thus, one might expect accelerated downward P transport in soils high in organic matter (Fox and Kamprath, 1971). It has been shown that organic forms of P may move to greater depths than soluble inorganic P due to their less reactive nature (Rolston et al. 1975). Eghball et al. (1996) demonstrated that manure P moved deeper than P coming from fertilizer P, even at similar P loading rates. Cogger and Duxbury (1984) attributed P leaching losses from cultivated organic soils to mineralization of large quantities of organic P, combined with a low content of Al and Fe oxides.

Another important factor regulating P release is E_h. Anaerobic decomposition of organic matter causes the reduction of Fe^{3+} in P-containing iron hydroxides, resulting in the release of P into drainage water (Mitsch and Gosselink, 1986; Ponnamperuma, 1972).

In general, soil P losses from manure application via surface runoff are considered greater than P losses due to subsurface transport phenomena, especially in cases where manure application and low infiltration result in a surface soil “seal” (Smith et al., 1998). The amount of P lost in surface runoff from land receiving animal waste depends on the waste application rate, the timing of application, and the time interval between application and the runoff event (Sharpley et al., 1994). Sauer et al. (2000) reported that poultry litter application increased molybdate reactive P (MRP) and particulate P (PP) levels in runoff relative to the controls. Also, inorganic P (IP) was much higher than organic P (OP). Usually, P content in runoff is dependent on the number of rainfalls since application, rather than the quantity of rainfall or runoff (McLeod and Hegg, 1984).

Hooda et al (2000) found that water desorbable P was better correlated with the degree of soil saturation with P (%) = (P * 100) / (Fe + Al) (mmol/kg)) rather to the total soil P. This indicates the significance of soil colloids (metal Fe, and Al oxides) to runoff-P, especially in noncalcareous soils. Hooda et al (2000) tried to correlate water desorbable P from 29 soils with five different soil test P procedures (Olsen, Mehlich III, NH_4-oxalate, Fe_20_3 coated paper, and distilled water). Distilled water extractable P explained 96% of the variation in water desorbable P from the soils, NH_4-oxalate extractable P explained 19% of the variation, and Mehlich III explained 81% of the variability. The excellent correlation between distilled water extractable P and dissolved P urged the European Union to establish water extractable P as the test to use for fertilizer P recommendations (Breeuwsma and Silva, 1992). Khiari et al. (2000) found a high degree of correlation (~90%) between Mehlich III extractable P and water extractable P. Actually, the lower the colloidal Al content, the higher the water extractable P.

Sharpley and Smith (1995) found that two soils with the same Mehlich III extractable P values had 0.28 and 1.36 mg MRP L^{-1}. Cox et al. (2000) found that soils with higher clay content (32%) tend to have lower MRP and higher total P levels than soils with low clay content (6%). Also, the amount of sediment was significantly higher from soils with greater amounts of clay.

On the other hand, fertilizer P losses in the form of particulate P (PP) and MRP, via subsurface leaching, are relatively small (1% of applied P) when compared to losses through surface runoff (9% of applied P) (Sharpley and Withers, 1994). Dolfing et al

(1999) observed flocs of humic materials, Al, Fe and P in aqueous soil extracts. They found that these colloidal flocs could be transported through the soil profile via electrostatic repulsion between the negatively charged humic-P compounds and the negatively charged soil matrix.

PARTICULATE P FORMS AND P IN SURFACE RUNOFF

Except for the classical perception that P moves only as orthophosphates in solution, evidence has led scientists to begin taking into account particulate P (PP), which is the colloidal portion of P where $0.45\mu m < PP < 2\mu m$ (Haygarth and Sharpley, 2000). Studies by Hilger et al. (1999) have shown that under high discharge conditions, the fraction of total P as PP is triple that as MRP.

The association of colloidal particles and P has been reported to be significant for the clay fraction of soils and manures and much less important for the silt and sand fractions (Leinweber et al., 1997). Phosphorus inputs from animal waste will be preferentially retained by the smaller sized particles, and the added P will not be redistributed uniformly across the whole soil (House et al., 1998). The selective erosion of these fine soil particles, which can lead to increased PP in surface runoff, is usually underestimated in cases where analysis involves the whole soil (Sharpley, 1985). This PP fraction is drastically increased when soil disruptive events such as intense rainfall take place (Kronvang et al., 1997), and the resulting drainage waters or surface runoff contain large amounts of suspended solids (House et al., 1998). Pionke et al. (1997) have demonstrated that 90 percent of the annual algal-available P exported from watersheds comes from only 10 percent of the land area during a few large storms.

Best management practices (BMP) for preventing P transport are riparian zones, conservation tillage, and buffer strips. It has been shown that BMPs could reduce PP but they are not as efficient to lower MRP (Young et al., 1982; Gray et al., 1986). MRP encapsulates not only the dissolved phosphates but also phosphates hydrolyzed from organic and inorganic colloids due to the acidic background in the colorimetric methods, which use molybdate to complex orthophosphates, or due to eukaryotic enzymatic activity (Baldwin, 1998; Tarapchak, 1983; Sinaj et al., 1998; Baldwin et al., 1996).

FACTS ABOUT MOLYBDATE-REACTIVE P (MRP)

It was the classic paper by Murphy and Riley (1968) that established a global P determination protocol based on the assumption that molybdate reacts only with inorganic P to form a reductant sensitive complex, 12-molybdophosphoric acid. Since then, P has been transformed from a "well-behaved" nutrient to an element that causes algal blooms and declines in the quality of many water bodies. Elevated levels of molybdate-reactive P (MRP), otherwise termed dissolved inorganic phosphate, have been well correlated with algal blooms.

The bioavailability of MRP remains somewhat in question. The classical perception is that in estimating MRP, you obtain the actual amount of P that is available to microorganisms. The validity of this depends on the chemical and physical properties of soils and water systems. Sauer et al. (2000) found a good correlation between MRP and Mehlich III extractable soil P that occurred only in the untreated plots. Poultry litter plots generated constant MRP levels in runoff, despite a wide range in soil Mehlich III values (45-160 ppm P). That means that MRP in runoff was mostly coming from the litter P, extracted with the rainwater.

The fact that MRP is found in runoff does not imply that all of it will be bioavailable. The different forms of MRP vary in bioavailability. Molybdate-reactive P could be free phosphate ion or metal-ion pairs that are bioavailable to microorganisms. MRP could also be a part of dissolved organic compounds, loosely bound to their surface functional groups, which can be released relatively easily or not, into solution by hydrolytic means. Except for the fact that MRP can be attached to organic colloids, it can also be loosely bound to inorganic clay minerals and metal oxides.

Except for the “free” portion of the total MRP in the sample, organic and inorganic colloidal MRP forms could release free orthophosphates in solution by degradative means, either prior to, or during the P colorimetric analysis. Hydrolysis of organic compounds due to enzyme activity has been documented. Phosphatases (Cembella et al., 1984) and nucleotidases (Azam and Hodson, 1977) are representative examples of enzymes responsible for biotic hydrolysis of organic P compounds. The catalytic action of metal oxides and divalent heavy metals, such as Cu^{2+}, on organic P moieties, has also been observed by researchers (Baldwin, 1995). Furthermore, chemical extractants such as HCl and NaOH are also known to hydrolyze organic compounds (Ivanoff et al., 1998).

Finally, the acidic medium used in all colorimetric P methods could be responsible for the degradation of inorganic and organic colloids. This process releases free PO_4 into solution that otherwise would not have been detected as MRP. Thus, it is important to keep in mind the possible contributions to MRP as it is now measured.

MRP contains different size-defined classes of compounds that differ in reactivity. Buffle et al. (1978) fractionated organic and inorganic moieties in aquatic systems. The finer class was comprised of dissolved ions and ligands, both organic and inorganic. The second class ranged from 200-10000 Da, and consisted mainly of fulvic acids. The third class ranged from 10000-1000000 Da, and contained humic acids, proteins, phospholipids and metal oxides associated with phosphates. Finally, the last class was comprised of suspended colloidal particles having a diameter > 0.05 µm. Haygarth et al. (1997) used ultrafiltration and composite cellulose nitrate/acetate membranes under vacuum to fractionate soil solutions and river waters into different MRP fractions. They found that in sediment samples taken from runoff, no difference in MRP concentrations existed in samples with particles in the size ranges of 0.45-0.22, 0.22-0.1 and 0.1-0.025 µm. There was a difference only in the ultrafiltered samples (<10000Da and <1000 Da). Unfiltered samples were much higher in MRP content than the <0.45 µm filtered sample. Shand et al. (2000) also used a series of micron size filters in an effort to separate different size MRP and OP classes. They found that unfiltered MRP levels were much higher than in <0.45 µm filtrates. Also, no difference in MRP levels between 0.45 and 0.22 µm filtrates existed for the soil solution taken from a peat soil. Finally, most of the organic P in the <0.45 µm filtrate was of a size > 10 kD.

The main objective of the study was to evaluate the potential facilitation of P transport by the water dispersed soil colloids (WDC), by using three KY soils with a long-term record of poultry manure, and fertilizer P applications.

CHAPTER 2

Physicochemical Properties of the Soils

The soils used in this study were selected based on their use in three long-term field experiments involving poultry manure and fertilizer P applications. They are the following: a Maury silt loam (fine-silty, mixed, mesic, Typic Paleudalf), at the University of Kentucky's Spindletop experimental station farm, near Lexington, KY; a Pope silt loam (coarse-loamy, mixed, active, mesic, Fluventic Dystrochrept), at the University of Kentucky's Robinson Forest substation farm near Quicksand; and a Tilsit silt loam (fine-silty, mixed, semiactive, mesic, Typic Fragiudult), at the University of Kentucky's West Kentucky Research and Education Center, near Princeton, KY. Field treatments included an unamended control, poultry manure, and fertilizer P. Poultry manure was applied at a rate of 33 kg P ha^{-1} every two years since 1993 to the Pope and Tilsit soils, and every year since 1994 at a rate of 101 kg P ha^{-1} to the Maury soil. Fertilizer P as triple superphosphate (TSP) was applied only to the Pope and Tilsit soils at a rate of 33 kg P ha^{-1} every other year since 1986. These application rates are near agronomically optimum levels for both the Pope and Tilsit, but not for the Maury soil. No fertilizer P was applied to the Maury soil due to its high content of phosphatic limestone.

P Terminology (Based on Haygarth and Sharpley, 2000)

MRP = Molybdate Reactive P. This is the dissolved inorganic P (DRP) or the inorganic P after filtering the sample (<0.45µm).

DOP = Dissolved Organic P. This is the dissolved organic P portion of the total dissolved P. This is usually operationally defined as the difference between total dissolved P (TDP) and MRP.

IPP = Inorganic Particulate P. This inorganic P is associated with suspended mineral colloids. This is part of the particulate (>0.45µm) fraction retained on the filter.

OPP = Organic Particulate P. This organic P is associated with suspended organic colloids. This is part of the particulate (>0.45µm) fraction retained on the filter.

Soil Chemical Analyses

Soil samples (0-7.5 cm depth) were collected during the 1999 growing season. The soils were air-dried and passed through a 2 mm sieve. Soil samples were analyzed for the following physicochemical properties: CEC at pH 7 by the 1M ammonium acetate method, soil texture (Gee and Bauder, 1986), exchangeable cations (Ca, Mg, Na, K), and available P according to the Mehlich III P procedure (Mehlich, 1984), soil pH, and EC performed on a 1:1 soil: water suspension. Total C was determined using a Carlo-Erba (Milan, Italy) dry combustion analyzer. Mineralogical composition of the clay fraction of the three soils was quantitatively assessed by x-ray diffraction (Karathanasis, 1991).

P Analyses

MRP determination was accomplished by using the malachite green method (Van Veldhoven and Mannaerts, 1987). The method relies upon mixing the sample with acidified ammonium molybdate and the subsequent formation of a heteropoly phospho-molybdate complex. The sample also reacts with a reagent containing a basic dye and polyvinyl alcohol (PVA). The reagents used are described below. A 1.75% (w/v) ammonium heptamolybdate tetrahydrate solution in 6.3 N sulfuric acid was prepared by dissolving 8.75 g of ammonium molybdate in 400 mL of deionized water and then adding 87.5 mL of concentrated sulfuric acid, after which the solution was cooled at room temperature and diluted to 500 mL with deionized water. A 0.035% (w/v) solution of malachite green carbinol hydrochloride (Aldrich) in 0.35% (w/v) aqueous polyvinyl alcohol (MW = 50,000, Aldrich) was prepared by dissolving 1.75g of PVA in 450 mL of deionized water preheated to 80 C, and adding 0.175 g of malachite green to the PVA solution after cooling, and then diluting to 500 mL with deionized water. The two reagents were stable at room temperature for several months. The acidity of the solution must be maintained around 0.9 N H^+ in order for the absorbance of the solution to be proportional to the inorganic P concentration, and to avoid incomplete reaction. This can be accomplished by both adjusting the acidity of the sample, and using the 6.3 *N* ammonium molybdate reagent, or by using the 1.26 *N* ammonium molybdate in acidified samples. Fourty µL of the acid added with the ammonium molybdate solution plus 200 µL of the sample were loaded into a disposable plastic microplate. The microplate was shaken for 10 minutes. Following, another 40 µL of the malachite green plus the PVA solution was added in the sample. The microplate was shaken for another 20 minutes to ensure complete mixing of the dye with the sample. Then, the microplate was transferred to a microplate autoreader (Biotek model EL 311) to measure the optical density of P-containing samples at a 630 nm wavelength.

In cases where the interference of organic compounds during MRP determination needs to be avoided, a correction factor was employed. Besides the original set of samples, another set of the *same* samples was used except that no ammonium molybdate solution was added (the acid portion was still added to maintain optimum acidity). The absorbance generated from this set of samples was mostly due to background organic components and is subtracted from the absorbance of the ammonium molybdate-treated set of samples to get the final MRP concentration.

Total P was determined colorimetrically after wet digestion of the soil or WDC with HNO_3 / $HClO_4$ (9:1 v/v). Two mL of P-containing solution was placed in a 50-mL Erlenmeyer flask and evaporated at 80 C. Afterwards, 5 mL of the acid digestion mixture was added, with watch glasses covering the flasks, to permit refluxing, and better digestion of the organic P compounds. This required a total of three hours at 80 C. Finally, samples were heated to 400 C for three hours, hydrolyzed with 10 mL of 1.26 N H_2SO_4, ultrasonicated, and then placed on a hot plate (80 C) for 20 minutes to hydrolyze any metaphosphates and polyphosphates formed during the digestion process. A 200 µL sample was subjected to the malachite green method for MRP (TP) determination. Organic P was calculated as the difference between TP and MRP.

In order to extract the WDC from the soils the method by Seta and Karathanasis (1997a) was employed. Duplicate samples of ten grams of air-dried soil were each added to two hundred mL of deionized water, end-over-end shaken at 96 rpm for 16 hours, and then centrifuged for 3.5 min at 750 rpm. The colloidal suspension/supernatant was retained. The WDC content of the supernatant was determined gravimetrically by drying out duplicate 25 mL aliquots at 110 degrees Celsius. The absorbance of the colloidal suspensions was measured at a wavelength of 540 nm. Prior to absorbance measurements, calibration curves for the WDC of each soil were constructed by sequential dilution of each colloidal suspension to extinction. Treatment differences in each soil over the growing season (April to September) were explored with the Design-Expert statistical software package employing the least significant difference method, which makes use of the Student's t-test (a =0.05).

Results and Discussion

The three soils were analyzed for bulk chemical properties, and the mineralogical composition of their clay fraction (<2 µm) (Tables 1, and 2, respectively). All three soils had a similar particle size distribution with an average of 81% silt, 13% clay, and 6% sand. Maury had a higher soil pH, Mehlich extractable cations (Ca, Mg, Na, K), and Mehlich extractable P over the Pope and Tilsit soils probably due to Maury's indigenous high levels of phosphatic limestone. However, the Pope soil had greater organic matter (OM) content (~5.5%) than the Tilsit (3.5%), and the Maury (4.5%) soils.

Analysis of soil samples collected during and right after the end of the growing season for the Pope soil showed that CEC values were significantly greater ($p<0.05$) in fertilized with P and manured plots (4.8 cmol kg^{-1}) over the unamended control (4.4 cmol kg^{-1}) (Table 1). Similarly, exchangeable Ca levels were significantly greater in manure (1240 mg kg^{-1}), and fertilizer (1210 mg kg^{-1}) amended plots over the unamended control (1060 mg kg^{-1}). Mehlich III extractable P levels in P-amended with manure (10.5 mg kg^{-1}), and TSP (12.5 mg kg^{-1}) plots were significantly greater than the unamended control (6.5 mg kg^{-1}). Organic matter content of the Pope soil was significantly greater in plots treated with fertilizer (6.1%), and manure (5.6%) long-term applications, over the unamended control plots (4.9%). The soil pH did not change significantly with manure (5.5) or fertilizer (5.3) additions over the unamended control (5.3).

Analysis of soil samples collected during and right after the end of the growing season for the Pope soil showed that for the Tilsit soil, CEC values were significantly greater in manure (5.3 cmol kg^{-1}), but not in fertilized plots (4.9 cmol kg^{-1}) over the unamended control (4.7 cmol kg^{-1}) (Table 1). Exchangeable Ca levels were significantly greater in manured (1490 mg kg^{-1}), but not fertilized with P plots (1810 mg kg^{-1}) over the unamended control (1290 mg kg^{-1}). Mehlich III extractable P levels in P-amended with manure (8.5 mg kg^{-1}), and TSP (9 mg kg^{-1}) plots were significantly greater than the unamended control (6.5 mg kg^{-1}). Organic matter content of the Tilsit soil was not significantly increased with fertilizer (3.7%), and manure (3.4%) applications, over the control (3.3%). The soil pH did not change significantly with manure (5.8) or fertilizer (5.6) additions over the unamended control (5.5).

On an average of the growing season for the Maury soil, manure application significantly increased soil CEC levels (7.5 cmol kg^{-1}) over the unamended control (6.7 cmol kg^{-1}) (Table 1). Exchangeable Ca levels were significantly greater in manure-amended plots (1810 mg kg^{-1}) over the unamended control (1550 mg kg^{-1}) due to high limestone levels found in the Maury soil. Mehlich III extractable P levels in manure-amended (233 mg kg^{-1}) plots were significantly greater than the unamended control (146 mg kg^{-1}). Organic matter content of the Maury soil was not increased significantly with manure (4.5%) long-term applications, over the control (4.4%). Similarly, the soil pH did not change significantly with manure additions (from 6.1 in control to 6.4 to manure-amended plots).

The clay mineralogical analysis revealed the abundance of phyllosilicate minerals in all three soils (Table 2). As expected, no consistent trend was observed in the mineralogical composition of the soils due to manure or fertilizer applications. The Pope soil's clay fraction was dominated by equivalent amounts of mica and kaolinite (~30% of the clay fraction), followed by quartz (19%), and smaller amounts of 2:1 minerals, feldspars, and hydroxylated interstratified vermiculite (HIV). The striking feature of the mineralogical analysis of the Pope soil was the identification of crystalline goethite (4.6%) in the plots that only manure had been applied. Perhaps significant amounts of Fe were present in the poultry manure because of pre-land application effort to reduce manure-P by adding Fe-based salts.

The Tilsit soil was dominated by quartz (30%) and 2:1 minerals (22%). HIV, and mica were present in smaller (~17%) quantities, followed by small amounts of kaolinite and feldspars (<10%). The clay fraction of the Maury soil was dominated by quartz (45%). HIV, 2:1, and mica were present in nearly equal amounts (15%). Kaolinite and feldspars were detected in smaller amounts (11, and 4%, respectively).

The knowledge of the type of clay minerals present in the soils will contribute to the better understanding of the rate of P sorption reactions. For example, kaolinite and oxides exhibit a greater affinity and they adsorb ions faster than 2:1, and mica minerals, due to the readily available sites of kaolinite and oxides versus the multitype sites of 2:1, and mica minerals (Jardine and Sparks, 1984). The information obtained so far will be useful to help us interpret diferences with treatment and soil effects in P fate and transport.

DETERMINATION OF WATER DISPERSIBLE COLLOIDS (WDC) FROM SOIL

INTRODUCTION

Transport and deposition of suspended solids generated during a rain event occur during several soil processes. These suspended solids or soil colloids are characterized by their small size particles (< 2µm), coupled with a large specific surface area, and a large number of reactive surface functional groups per unit mass that makes them efficient sorbents for contaminants (O' Melia, 1989). The water dispersed soil colloids (WDC) may travel through the soil profile via soil macropores carrying with them sorbed masses of contaminants (McCarthy and Zachara, 1989). For a detailed review of colloidal transport in soils please check the review of Kretzchmar et al (1999).

Several field (El-Farhan et al., 2000), and laboratory (Saiers et al., 1994) experiments have been conducted to test the effect of soil colloids to contaminant transport through leaching during saturated flow. The association between colloidal particles and phosphorus has been reported for the clay fraction (<2µm) of soils and manures but that association is much less for the silt and sand fractions (Leinweber et al, 1997). Phosphorus inputs from fertilizer or animal waste will be preferentially retained by the smaller sized particles, as the added P will not be distributed uniformly across the whole suite of soil particles (House et al., 1998).

Our objective was to quantify this chemically active fraction of the soil, and examine whether fertilization, manuring or aging through the growing season would affect WDC concentrations in soil suspensions.

RESULTS AND DISCUSSION

On average, across all soils and treatments, the soil's WDC content was about 4% over the growing season. The average WDC content, expressed as a proportion of the clay fraction of the soils, was approximately 23%.

In the Pope soil, WDC concentrations from the manured treatment remained constant throughout the growing season, while that of the unamended control decreased over time (Figure 2-1). This can be partially explained by the manure's ability to prevent WDC from falling out of suspension due to electrostatic repulsive forces between charged soil particles. Where fertilizer had been added to the Pope soil, WDC concentrations followed the same trend that manure exhibited. Fertilizer amendments did not differ in WDC generation ability as compared with organic amendments.

In the Maury soil, WDC levels were approximately the same across the growing season, with the unamended control's WDC levels being slightly higher than that for manured soil (Figure 2-2). The fact that aging of manure or fertilizer treated soil was not able to increase or decrease WDC levels in the Maury soil could be attributed to the high level of Fe oxides in this soil. That is probably the reason why even in the control treatment we observe high amounts of generated WDC, which remain constant throughout the growing season.

Finally, the Tilsit soil exhibited different behavior (Figure 2-3); The WDC concentrations decreased over the growing season for all treatments.

To conclude, there was little consistency in trends for the generation of WDC in these soils. No clear treatment or soil effect was detected in the generation of the WDC. The WDC remained more or less constant during the growing season, except for the Tilsit soil, which exhibited a decline towards the end of the growing season.

Table 1. Selected properties of the soils ¶

Soil	Treatments	CEC cmol kg^{-1}	Exchangeable Cations †								EC dS m^{-1}	% Org Matter (LOI)	pH ‡
			Clay	Silt	Sand	Ca	Mg	K	Na	P			
			---------------%	-------------		----------------------------mg kg^{-1}	---------------------------						
Pope	unamended control	4.4 ±0.4	14.1	77.4	8.5	1060 ±91	226±18	81±8	11±0.6	6.5±0.6	0.6	4.9	5.3 ±0.4
	TSP	4.8 ±0.4	12.4	79.4	8.2	1210 ±89	247±23	93±18	11±0.6	12.5±1	0.5	6.1	5.3 ±0.4
	poultry manure	4.8 ±0.2	11.9	80.0	8.1	1240 ±98	237±43	117±21	23±0.2	10.5±1	0.5	5.6	5.5 ±0.5
Tilsit	unamended control	4.7 ±0.7	11.4	84.2	4.4	1290 ±98	92±12	80±12	13±0.5	6.5±0.6	0.9	3.3	5.5 ±0.6
	TSP	4.9 ±0.3	9.8	84.6	5.6	1460 ±110	96±11	61±6	14±0.5	9±0.1	0.7	3.7	5.6 ±0.9
	poultry manure	5.3 ±0.3	9.4	85.2	5.4	1490 ±102	118±13	70±8	20±0.6	8.5±0.2	0.7	3.4	5.8 ±0.2
Maury	unamended control	6.7 ±0.4	13.6	81.3	5.1	1550 ±102	301±43	253±32	12±0.3	146±11	1.0	4.4	6.1 ±0.5
	poultry manure	7.5 ±0.8	15.6	79.1	5.3	1810 ±110	358±31	295±34	19±0.3	233±23	0.6	4.5	6.4 ±0.3

‡ At soil:solution ratio of 1:1

† Use of Mehlich III soil test

¶ All numbers represent mean± standard deviation, mean is the average of six months (April-September)

Table 2 Mineralogical analysis of the clay fraction of the three soils

Soil	Treatments	2:1	Inter stratified	Mica	Kaolinite	Quartz	Goethite	Feldspars
		% of the clay fraction of the soil						
Pope	unamended control	9.6	10.2	28.7	28.2	19.0	nd	4.3
	TSP	13.1	8.7	32.0	28.8	13.4	nd	3.8
	poultry manure	6.3	6.1	29.4	35.2	14.3	4.6	4.0
Tilsit	unamended control	23.6	17.8	13.8	10.6	28.0	nd	6.2
	TSP	21.6	24.1	12.5	nd[‡]	36.0	nd	5.9
	poultry manure	24.3	16.0	13.0	8.8	33.3	nd	4.5
Maury	unamended control	17.3	15.4	13.4	9.2	40.8	nd	3.7
	poultry manure	12.7	15.6	9.1	8.9	48.4	nd	5.0

[‡] not detected

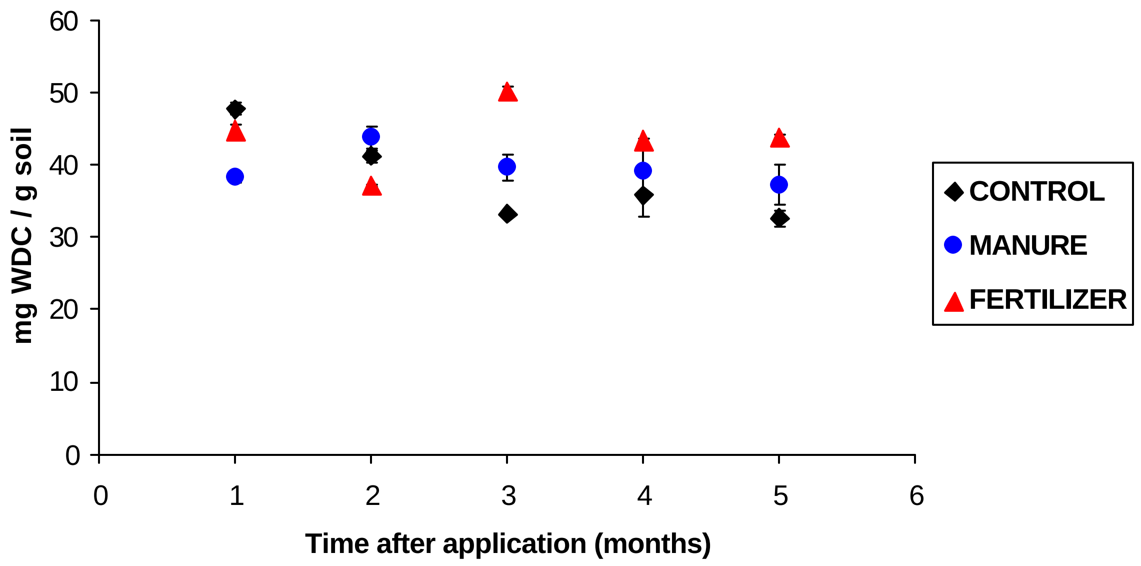


Fig 2- 1. Changes in WDC from the Pope soil during a growing season.

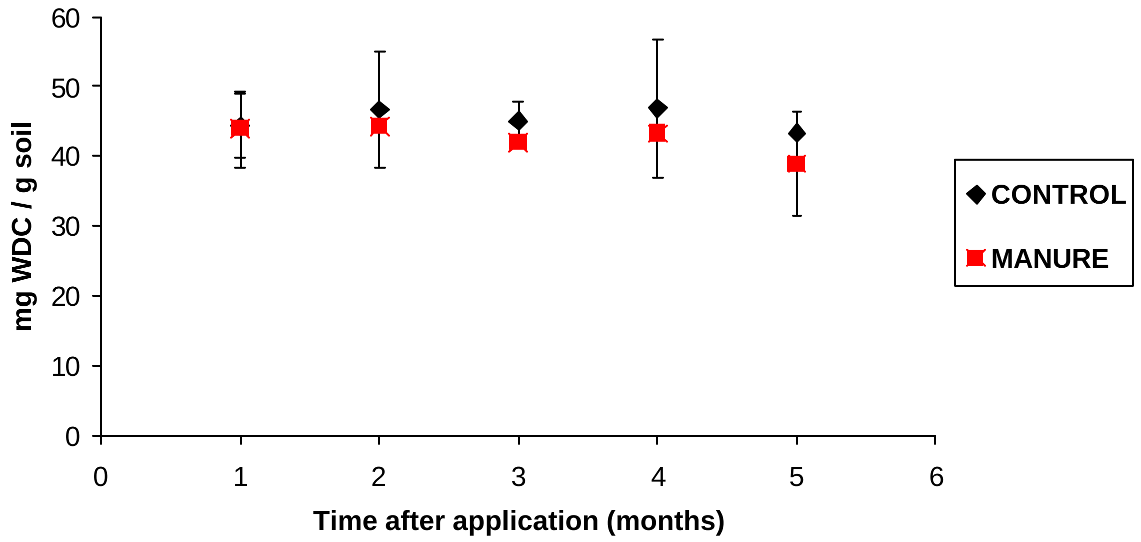


Fig 2- 2. Changes in WDC from the Maury soil during a growing season.

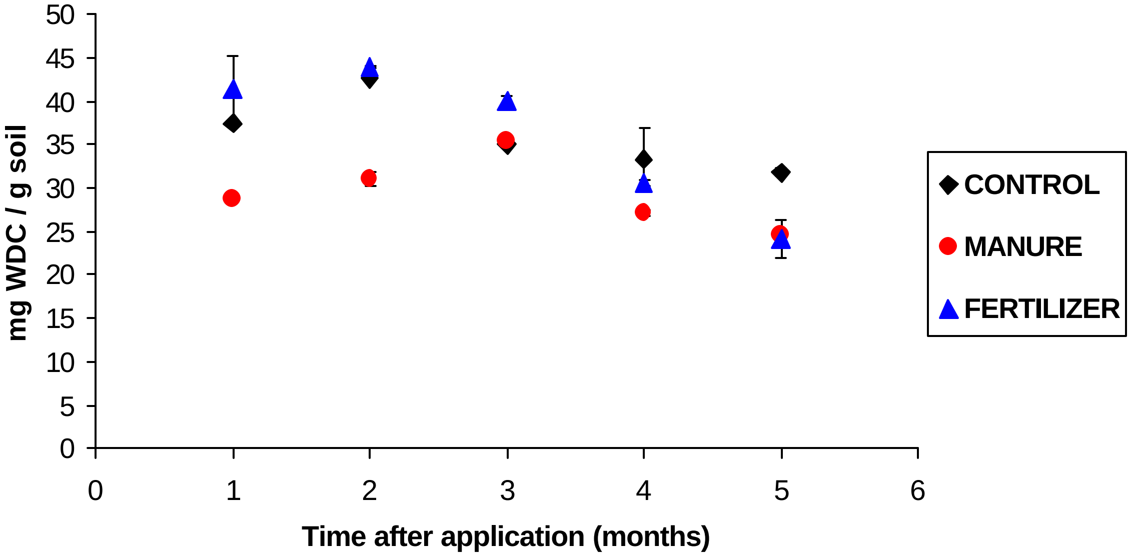


Fig 2- 3. Changes in WDC content from the Tilsit soil during a growing season.

CHAPTER 3

Hydrolysis of Organic Phosphorus (OP) Compounds in Soil / Water Environments by the Malachite Green Method.

INTRODUCTION

Excess amounts of inorganic and organic phosphorus can be found in soils, streams and groundwater. Excessive P often comes from heavy animal waste and fertilizer P applications to agricultural areas. There is a growing need for accurate and precise measurements of orthophosphates in soil-aquatic systems in order to follow the P loading of soils and for a better understanding of phosphorus transformations.

One of the methodological dilemmas is related to the potential interference of organic P (OP) compounds during the determination of orthophosphates. More specifically, the susceptibility of OP compounds to phosphate hydrolysis differs from one compound to another, depending on their chemical properties and the background solution chemistry.

The hydrolysis of OP compounds has well been documented in the chemistry literature (Schmidt, 1982). One of the types of hydrolysis that occurs in the natural environment (soils and water bodies) is the metal-catalyzed hydrolysis of organic compounds (Stone and Torrents, 1995). Their results indicated an accelerated hydrolysis of phosphoro(thion)ate pesticides in the presence of copper and zinc. The affinity of these metals for organic ligands and subsequently for the P atoms is high compared to that for Ca and Mg, especially in the near-neutral pH environment encountered in many soils.

Higher charge metals may also serve as catalysts. Under specific chemical conditions iron and aluminum oxide surfaces act as catalysts for reactions between iron and aluminum hydroxy-complexes and organic ligands. The mechanism behind this hydrolysis (Smolen and Stone, 1997) is the attack, at the organic phosphorus atom, by hydroxyls or water molecules, which coordinate themselves around the organic P atom, and gradually convert the parent polyester to triester, diester and monoester products. Another pathway of hydrolysis is the attack of water molecules at the carbon atoms within the alcoholate ester linkages. Both thionate (P=S) and oxonate esters (P=O) are thought to be hydrolyzed via these mechanisms.

Treatment of soil and water solutions with different chemical extractants, like HCl and NaOH, can result in significant hydrolysis of OP compounds like p-nitrophenyl-phosphate and glycose-6-phosphate, respectively, but phytic acid has exhibited strong resistance to hydrolysis by chemical extractants (Ivanoff et al., 1998). They also found that shaking time and strength of the extractant played major roles in the hydrolysis of OP compounds. Increasing the contact time and the concentration of the acid or alkaline extractant increased the hydrolysis of the organophosphorus compound. The soil to extractant ratio was critical for some extractants (NaOH), but not for others (HCl) (Ivanoff et al., 1998).

Hydrolysis may also occur during the addition of molybdate, due to the acidic conditions used in colorimetric P detection methods (Tarapchak, 1983; Stainton, 1980). A soil or water sample collected from the field usually contains both organic and inorganic P compounds. During the determination of inorganic P, molybdate will complex not only free phosphates but also phosphates that are a part of the surface of clay minerals or organic compounds. Thus, when employing the acidic background of all the P colorimetric methods, a full awareness of the potential hydrolysis of the OP compounds in the sample must be considered (Anderson, 1960).

One of the methods for determining phosphates that is gaining acceptance is the malachite green method (Van Veldhofen, 1987). This method has several advantages over traditional methods (Murphy and Riley, 1962) including reagent stability, non time-consuming, avoiding transition metal interference (Ciavvatta et al., 1990) and high sensitivity at low soil and water P concentrations (Ohno and Zibilski, 1991).

The method is based on the formation of a heteropoly phospho-molybdate complex with the use of a basic dye (malachite-green). This basic dye has high color stability and is able to cover the full range of phosphomolybdate complexes, changing its color according to the amount of P within a sample, the more P, the bluer and less yellow the color. The acidity of the assay has to be around 0.9 $N\ H^+$ in order to avoid precipitation and agglutination phenomena or incomplete reaction of molybdate with phosphate.

Hydrolysis of OP compounds during colorimetric analyses would cause an overestimation of soluble inorganic P (IP). Thus, it would be beneficial to isolate these OP compounds. Tiessen et al. (1984) showed that acidification of the colored soil extract solution to a pH of about 2, followed by cooling and centrifugation, provided a clear solution for colorimetric P determination. The major pitfall in this strategy is that using such low pH values may cause dissolution of clay particles containing phosphates. These phosphates would likely not be bioavailable under normal soil conditions. Gerke and Jungk (1991) used an organic flocculent (polyethylenamine) and an ultrafiltration method to separate inorganic P from humic-P complexes in alkaline soil extracts. This method proved to be a slightly more efficient than the previous one but was time and money consuming.

Little is known about naturally occurring OP compounds and much less about their degree of hydrolysis in the acidic media used in the malachite green method. For this reason, model OP compounds representing the most abundant species found in aquatic and soil systems were used to assess hydrolysis under the acidic conditions of the malachite green method.

Materials and Methods

A series of OP compounds were spiked with inorganic P to determine their degree of hydrolysis. The model OP compounds used in this study were phytic acid, the dodecasodium salt hydrate; 2- aminoethylphosphonic acid; beta-D-glucose-6-phosphate, the monosodium salt; adenosine 5'-monophosphate monohydrate; 4-nitrophenyl phosphate, the disodium salt hexahydrate; glycerol 2-phosphate, the disodium salt hydrate; and sodium tripolyphosphate. The OP stock standards were prepared in water to provide a concentration of 100 mg P/L. Afterwards, they were further diluted with H_2O to give a range in OP concentration from 0 to 8 ppm P. Standard inorganic P (IP) solutions were prepared by dissolving a standard P solution (Fisher) in H_2O to give a range in IP concentration from 0 to 0.4 ppm P. Then, one mL of each of the OP standard solutions was mixed with one mL of each of the IP standard solutions and shaken for 20 minutes. In the following text, the corrected, after dilution, concentrations will be reported.

After shaking, a 1.75% ammonium molybdate solution and a 6.3 M H_2SO_4 solution were prepared for the malachite green method. The mixed solutions (200µL) were shaken with 40 µL of acidified ammonium molybdate for 10 min and were finally shaken for 20 min with the malachite green dye-PVA (polyvinyl alcohol) complex. Afterwards, the absorbance was determined on a microplate autoreader (Biotek EL 311) at a wavelength of 630 nm. The degree of hydrolysis was calculated with the following equation:

{IP measured in solution (ppm P) – IP added to solution (ppm P)} / OP added to solution (ppm P).

Recently, a graphitized carbon black, Carbopack B, has been employed to fractionate and separate acidic and basic compounds based on its complex, both positively and negatively charged, surface framework (Di Corcia and Marchetti, 1991). This complexity permits strong adsorption of organic compounds, but not so irreversibly as occurs with the traditional activated carbon (Darco). This property of graphite was employed in order to separate organic P compounds from the inorganic moieties in water samples, leaving behind only the inorganic P.

Subsamples of the solutions that were subjected to the malachite green method were shaken with 0.12 g of graphitized carbon black for 2 hours and then passed through 0.45 µm filters. The filtrate P concentrations were then determined colorimetrically as previously described.

RESULTS AND DISCUSSION

With the exception of the beta-D-glucose-6-phosphate and the phytic acid (Fig. 3-1), the tested OP compounds confirmed our hypothesis that organic P hydrolysis does occur under acidic conditions, during molybdate addition, in the malachite green method (Figs 3-2, 3-3, 3-4 and 3-5). Little overestimation of inorganic P took place when phytic acid was in the sample. This is in agreement with other findings, which confirmed the resistance of phytic acid to degradation via biotic and abiotic factors (Anderson, 1960, Ivanoff et al., 1998). The degree of hydrolysis varied among the P compounds, with adenosine 5'-monophosphate (AMP) having the highest degree of hydrolysis and phytic acid the lowest (Table 3-1). In general, the degree of hydrolysis was independent of the concentration of OP compounds, over the range tested here.

Compounds like adenosine 5’ monophosphate (AMP), aminoethylphosphonic acid, and the tripolyphosphates can significantly alter orthophosphate levels via hydrolysis. The AMP exhibited the highest degree of hydrolysis (Table 3-1). This latter OP compound is a vital part of living cells and is abundant in soil systems (Stevenson, 1994). Although this degree of hydrolysis would be considered insignificant in total P determinations, it is critical in inorganic P measurements, which are often at the ppb range. Also, the variation in hydrolysis among the model OP compounds illustrates their differences in P bonding strength.

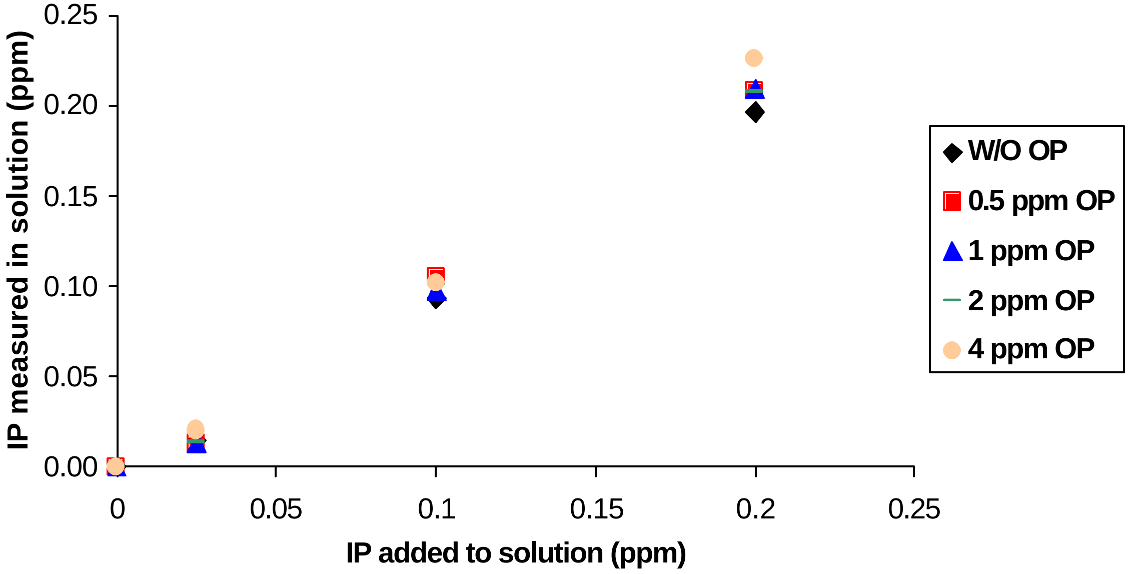


Fig 3-1: Phytic acid hydrolysis under the acidic conditions of the malachite green method.

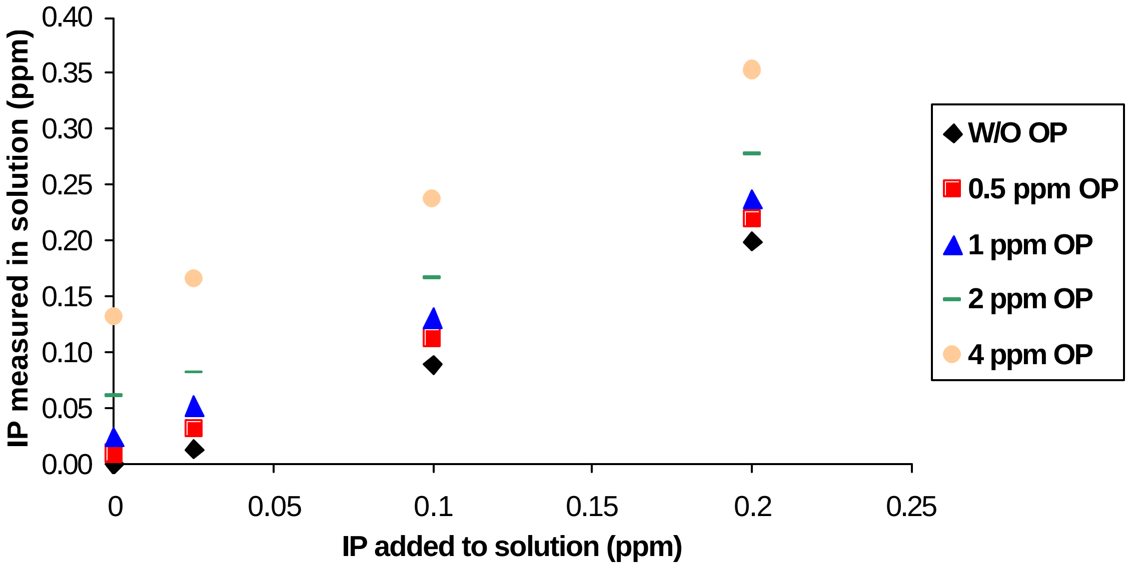


Fig 3-2: 4-nitrophenyl phosphate hydrolysis under the acidic conditions of the malachite green method.

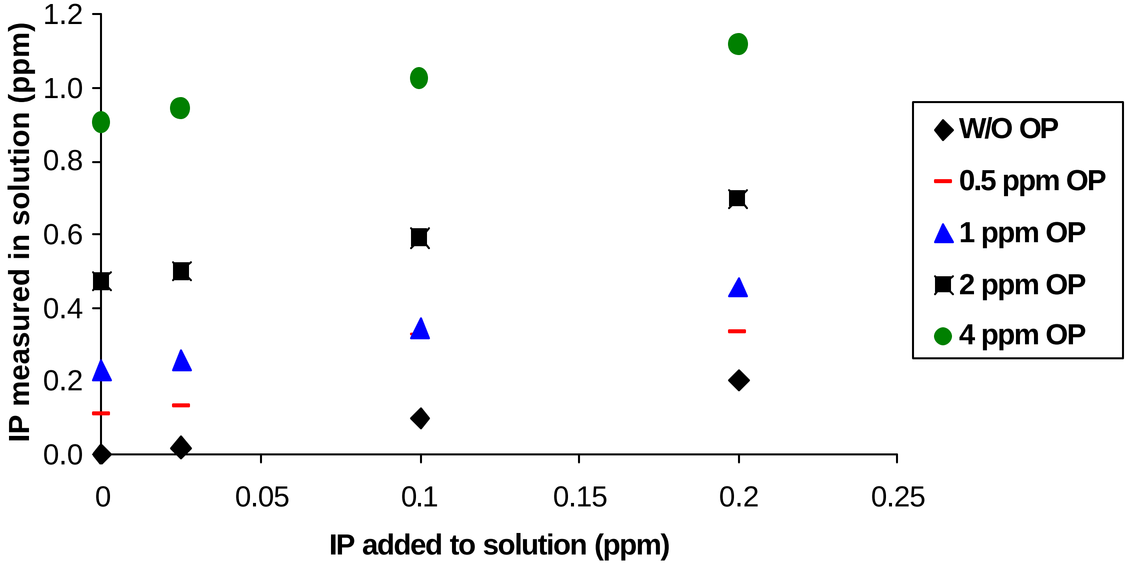


Fig 3-3: Adenosine 5'-monophosphate hydrolysis under the acidic conditions of the malachite green method.

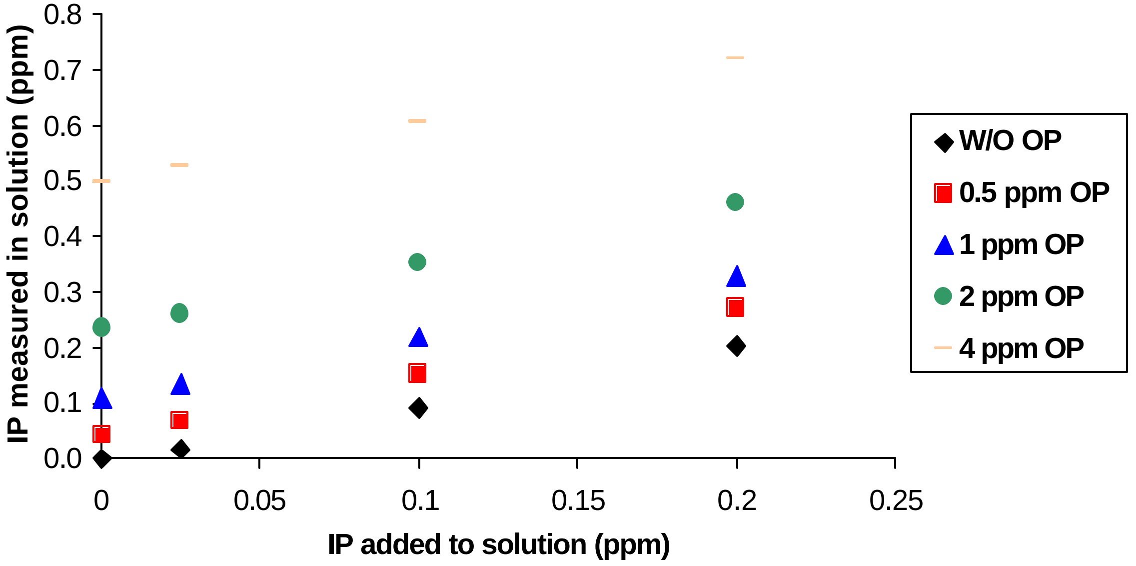


Fig 3-4: 2-aminoethylphosphonic acid hydrolysis under the acidic conditions of the malachite green method.

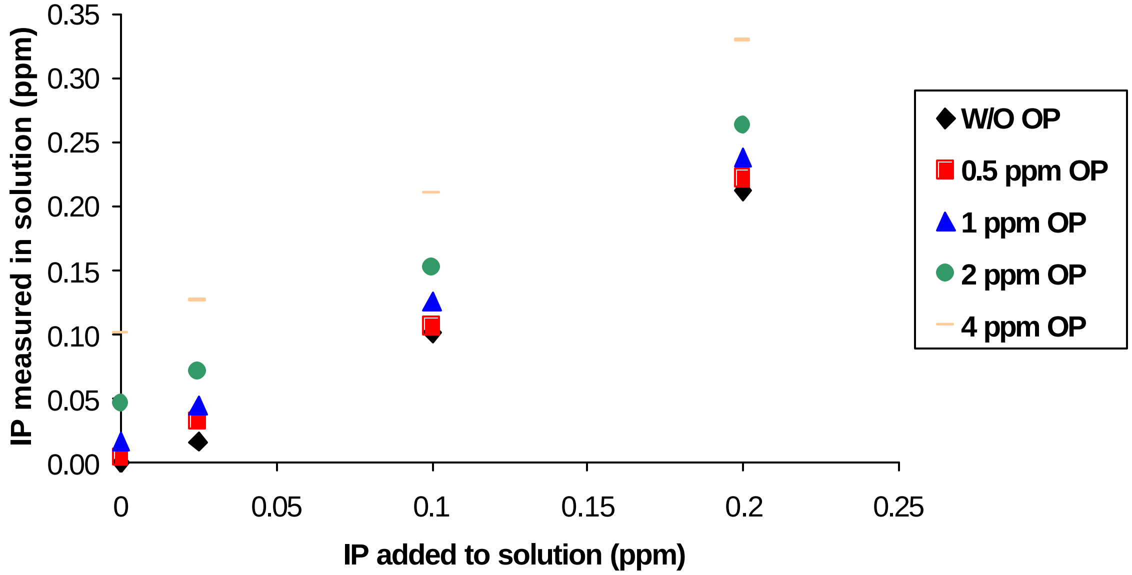


Fig 3-5: Glycerol 2-phosphate hydrolysis under the acidic conditions of the malachite green method.

Table 3-1: Ranking of the model OP compounds according to their degree of hydrolysis (% of total OP) during the malachite green method.

Adenosine 5'-monophosphate, monohydrate	23.0
2- Aminoethylphosphonic acid	14.0
Sodium tripolyphosphate	6.0
Glycerol 2-phosphate disodium salt hydrate	3.0
4-nitrophenyl phosphate salt hexahydrate	3.0
Beta-D-glucose-6-phosphate, monosodium salt	1.0
Phytic acid, dodecasodium salt hydrate	0.0

The graphitized carbon black (Carbopack B) was used in an attempt to separate organic P from inorganic P compounds. The Carbopack B was found to neither sorb nor release inorganic P (Fig 3-6). However, it seems that none of the tested OP compounds was retained on the surface groups of the Carbopack B. Hydrolysis of 2-aminoethylphosphonic acid was the same, in the presence or absence of Carbopack B (Fig 3-7). The same result occurred with the other tested OP compounds (Figs 3-8, 3-9 and 3-10). Digestion of the filtrate solution revealed the presence of each of the organic P compounds, confirming that the compound was not adsorbed on the Carbopack B.

Although preliminary results showed that Carbopack B was able to adsorb the colored high molecular weight humic compounds, it was unable, under our experimental conditions, to retain fulvic-OP compounds. These organic compounds were more hydrophilic than the humic substances.

The fact that nonenzymatic hydrolysis of organic compounds in the presence of mineral phases has been observed previously (Baldwin, 1998; Baldwin et al., 1995) is consistent with this type of hydrolysis and further increases the risk of overestimating the inorganic P concentration in soil solution and water samples.

Our study confirmed that the hydrolysis of organic P compounds occurs under these analytical conditions, causing overestimation of dissolved inorganic P when organic P compounds are present in the sample.

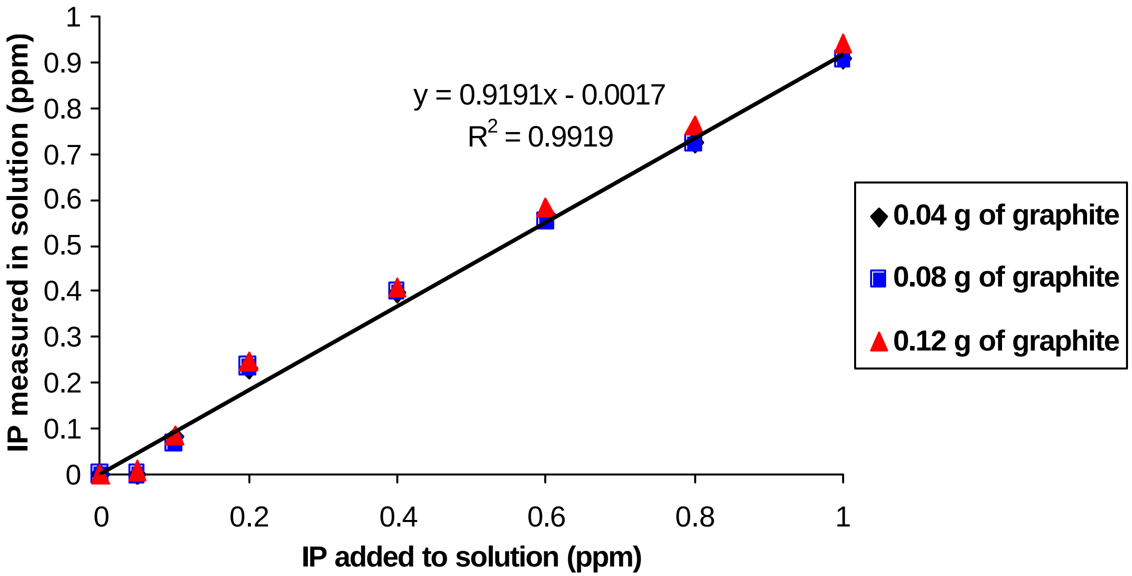


Fig 3-6: Inorganic P concentration in the presence of increasing levels of the graphitized Carbopack B.

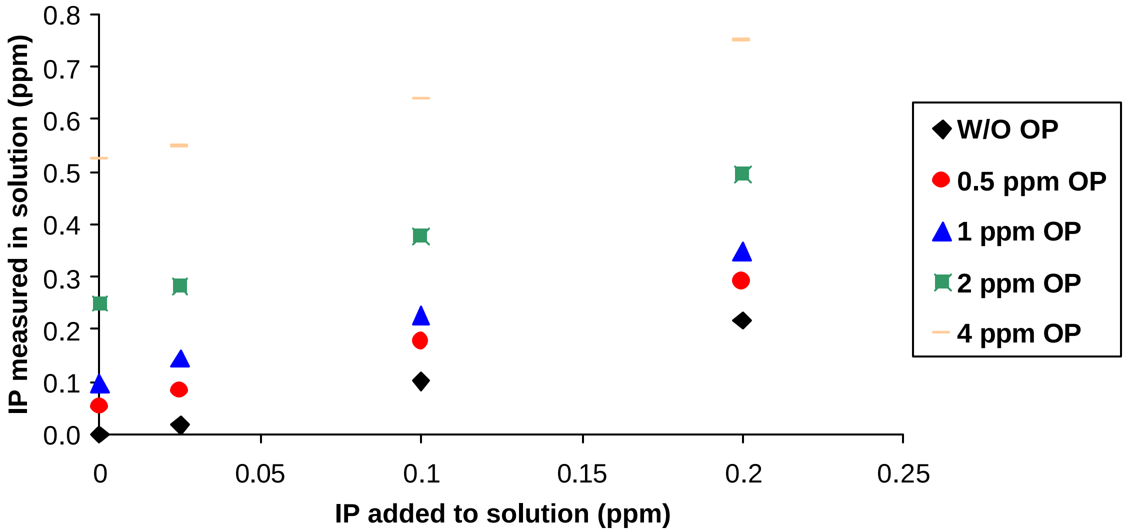


Fig 3-7: 2-aminoethylphosphonic acid hydrolysis in the presence of the graphitized Carbopack B.

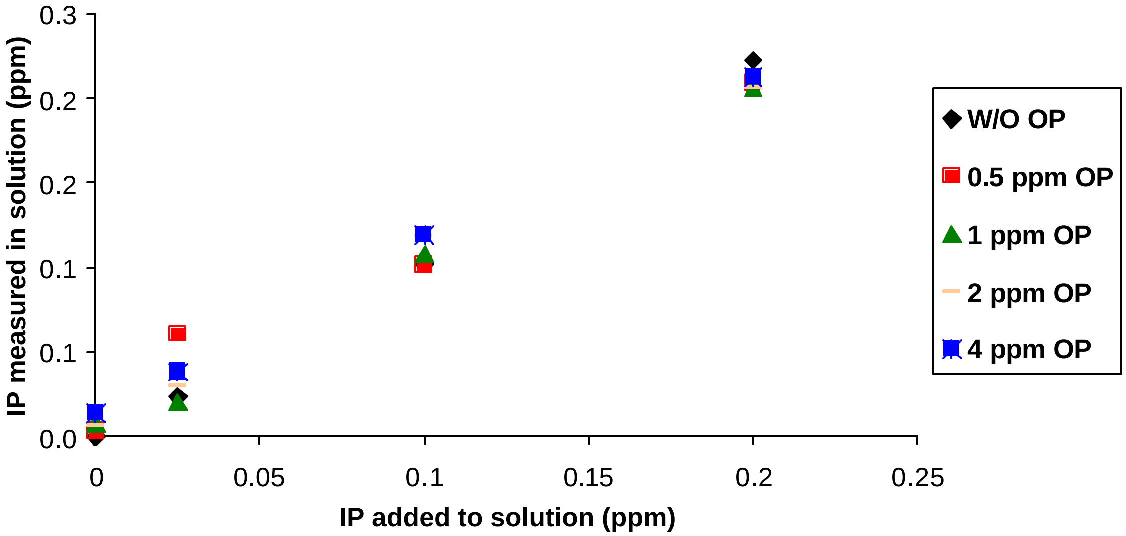


Fig 3-8: Phytic acid hydrolysis in the presence of the graphitized Carbopack B.

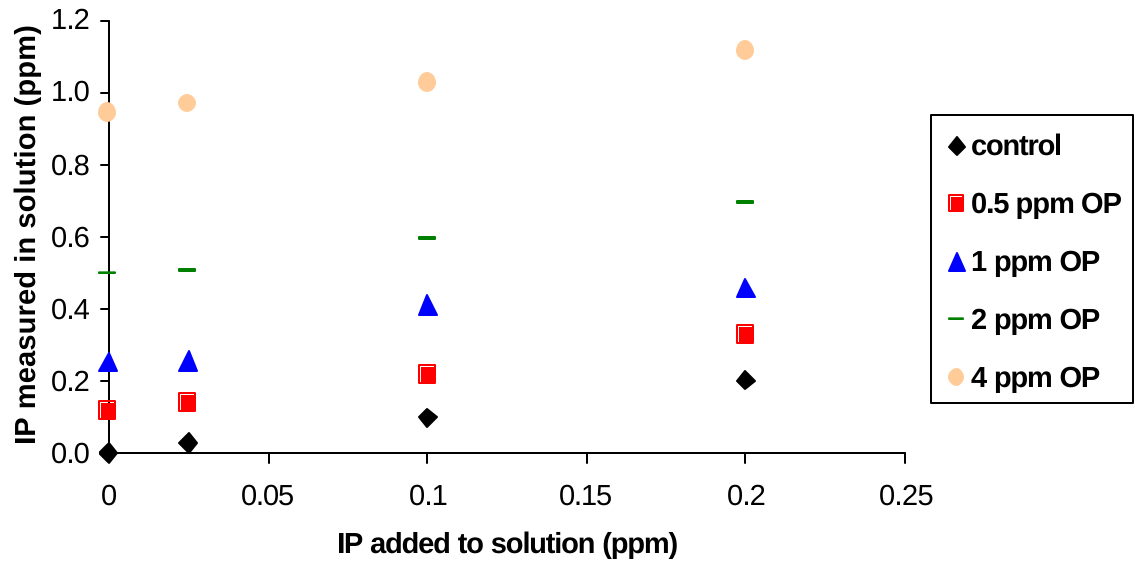


Fig 3-9: p-nitrophenyl phosphate hydrolysis in the presence of the graphitized Carbopack B.

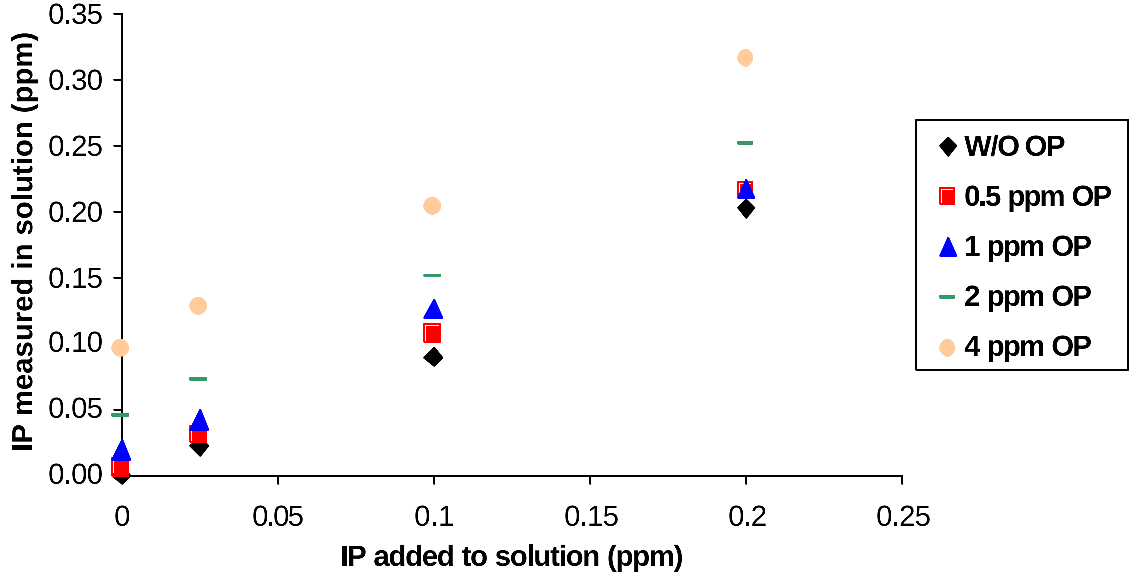


Fig 3-10: Glycerol 2-phosphate hydrolysis in the presence of the graphitized Carbopack B.

CHAPTER FOUR

Colloidal versus Soil Phosphorus Dynamics during a Growing Season in Soils Amended with Different P Sources

Introduction

The sequential fractionation scheme approach has been widely used to characterize P cycling and reactions in soils. The measured P fractions represent, more or less, the forms / bonding of P to soil organic and inorganic components. In the past, fractionation schemes were exclusively developed to assist agronomic interpretation of P availability for plant growth.

Phosphorus fractionation schemes seem to be better than soil P tests because they can generate detailed information on the different chemical P forms in soil. Subsequently, their results can be correlated to soil properties and used in management programs (McCallister et al. 1987). Phosphorus fractionation can be used to characterize the biological availability of P to algae (eutrophication). This can be achieved by monitoring the fluctuation, over time, of specific P fractions. For example, $NaHCO_3$ and resin P fractions are both thought readily available to microorganisms, algae, and plants.

One of the first who actually practiced organomineral, rather than mineral P fractionation schemes was John Hedley. Hedley et al. (1982b) reported on the changes in P fractions due to different cultivation practices conducted over a period of 65 years. His principal idea was that by using chemical extractants of different strength and selective preference, he would be able to isolate and extract different P forms; some of them immediately plant available, others are available in the long-term, and occluded forms of P that are insoluble for even longer periods of time. He assumed that each fraction could be clearly separated from the others. Hedley also assumed that all P fractions are in equilibrium with each other, tending to replenish changes in any fraction.

P fractionation schemes have been applied to soils with strong P fixation capacity. In highly weathered acid soils, such as Ultisols, P levels are generally low and plant available P comes not only from the resin and $NaHCO_3$ P fractions, but also from the NaOH-extractable P fraction. The latter extracts mainly metal phosphates. This fraction is usually considered to supply P over the long-term (Zhang and MacKenzie, 1997). Equilibria between these three P fractions help to maintain levels of available P sufficient for plant growth.

In cases where available inorganic P is low, then organic P forms often come into play, supplying mineralized P concentrations sufficient to maintain "reasonable" labile P levels. That was the case for an unfertilized crop system on an Ultisol in Brazil (Beck and Sanchez, 1994). They found that the NaOH fraction was a major sink for P but that rapid desorption followed. The high content of metal oxides in these Ultisols explains the high P affinity of the NaOH-extractable P fraction.

There is an increasing need for a better understanding of transformations of the different soil P fractions when manure and fertilizer P have been applied to soils. Sharpley and Smith (1995) found that manure increased all inorganic P fractions, as compared to the unamended control, and that there was a decrease in the NaOH and residual OP fractions, but not in the bicarbonate OP fraction. The HCl-P fraction was most affected since manure is high in Ca-P. These soils were subjected to remarkably high manure application rates (35-270 kg P ha^{-1} yr^{-1}). Reddy et al. (1999) reported, in a short-term field experiment, that addition of increasing fertilizer P rates, alone or combined with a single rate of manure P (88 kg P ha^{-1}), increased NaHCO3 and NaOH-extractable organic and inorganic P fractions, but not HCl-extractable and residual P fractions. Robinson and Sharpley (1997) found that simultaneous addition of manure and fertilizer P resulted in greater P availability from fertilizer than manure P. Iyamuremye et al. (1996) studied the effect of different organic amendments on P fractions after a month of incubation. They found that organic residues or manures could increase the amount of labile P (resin and bicarbonate extractable), and the amount of chemisorbed P (NaOH extractable). Increasing the pH, and reducing the exchangeable Al and Fe by adding CaCO3, caused no changes in the P fractions. However, incubation studies need to be very carefully monitored for changes in pH and ionic strength during the course of the incubation, which can account for fluctuations in labile P levels (Agbenin and Tiessen, 1995).

Hedley's scheme has recently been applied to "pure" manure and compost samples in order to isolate different P forms (Sharpley and Moyer, 2000). Inorganic P fractions were found to constitute about 85% of the total P in poultry litter. Inorganic P fractions varied with manure and compost type. Leinweber et al. (1997) used a modified Hedley procedure to determine the different P fractions in animal manures, whole soils and texture–sized separates. Resin-IP extracted from whole soil samples averaged 7% of the total P extracted, but was, on average, 3 times higher in the chicken manure. Clay sized particles contained much more P in all fractions, compared to coarser particles.

A modified Hedley P fractionation scheme was employed on "pure" manure samples in order to determine susceptibility of manure P to surface runoff as well as P bioavailability (Dou et al., 2000). They concluded that water was able to extract 50% of the P in manures, implying great potential for contribution to eutrophication. The same scheme was also applied to biosolids (i.e. anaerobically treated sewage sludge) (Sui et al., 1999). They found that water extractable P from those materials was only 1% of total P. This contrasts quite markedly with manures. When applied to soil, biosolids increased all P fractions except NaOH-OP and residual-P, compared to the unamended control.

The purpose of this experiment, was to characterize and isolate the different P fractions found in the WDC of three soils amended with different P sources. This would identify the lability of P sorbed by the WDC, and its potential for surface / subsurface movement.

MATERIALS AND METHODS

Three soils, part of field research sites where manure and fertilizer P treatments have been applied for years, were used in this study. Location and soil properties are presented in earlier chapters of this thesis (Chapter 2). Three treatments from each field site were selected: the unamended control, only poultry manure amended, and only fertilizer P amended. Their application rates are presented in an earlier section of the thesis. Surface soil samples (0 to 7.5 cm) were collected during the 1999 growing season, approximately one month after P source application. The actual dates of the P source application were 29 March for the Maury, 1 April for the Pope, and 2 April, 1999 for the Tilsit. The water dispersible clay (WDC) fraction of the soils was isolated using the procedures of Seta and Karathanasis (1997a). Portions of resulting colloidal suspensions were dried at 35C. Duplicate whole soil (1g) and WDC (0.5g) samples were subjected to a modified Hedley P fractionation scheme (Beck et. al., 1994).

This fractionation scheme assumes that the different P fractions are distinctively separated from each other. It is also assumed that no hydrolysis of P compounds in the organic P fractions takes place. Another assumption is that there is no organic P in the resin-IP fraction, although we observed some cases where this assumption was not valid. Also, aerobic conditions were maintained throughout the long (16h) shaking time used to determine all P fractions.

For the whole soil P fractionation, samples were used only from the first sampling date for all soils (early May), whereas all sampling dates were used for the WDC samples. The focus of this study was to study possible changes in the WDC-P fractions over the growing season in P amended soils. The outline of the fractionation scheme is presented in Table 4-1. The details of the scheme are as follows: thirty mL of deionized water and 0.4g of anion exchange resin (DOWEX 1X8-50, chloride form) enclosed in a porous cotton bag, shaken with either whole soil (1g) or the WDC sample (0.5g) for 16h in a 50-mL centrifuge bottle. Use of the bag minimizes losses from soil or WDC material becoming attached to the resin beads. After shaking, the bag with the resin was washed thoroughly with deionized water and transferred to another centrifuge bottle. The bag was then shaken with 30mL of 0.5N HCl for 16h to release P adsorbed onto the resin. The centrifuge bottles with the soil or WDC were centrifuged at 7500 rpm for 10 min. The clear supernatant was decanted and the bottle, soil and entrained solution weighed prior to the next extraction in the sequence. Solution entrained in the remaining soil or WDC particles was taken into account in determining all P fractions after the resin-P fraction.

Table 4- 1: Soil and WDC P fractionation scheme (modified from Hedley et al., 1982b).

Kind and sequence of extractant	Designated P fractions	P forms
deionized water + anion exchange resin	Resin- IP	Mainly inorganic available P
0.5M $NaHCO_3$ pH = 8.5	$NaHCO_3$- IP and OP fractions	IP and OP labile P
0.1N NaOH	NaOH- IP and OP fractions	IP and OP metal oxide-P
1N HCl	HCl- IP	Mainly calcium phosphates
9/1 v/v HNO_3 / $HClO_4$	Residual P	Occluded IP and OP

After the resin-IP fraction was determined, 30mL of 0.5M $NaHCO_3$ at pH = 8.5 were added to the remaining soil or WDC mass and shaken for 16h. Samples were then centrifuged for 10 min at 7500 rpm and the supernatant was retained. The same procedure was followed for the P fractions extracted with 0.1N NaOH and 1N HCl. The last P fraction (residual-P) was generated by digesting the residual soil or WDC with a mixture of 9:1 concentrated (v/v) HNO_3 / $HClO_4$. Independent digestion of original soil or WDC samples was done in order to calculate the recovery of total P by the fractionation scheme.

All of the supernatants were passed through a 0.45µm filter and neutralized to a pH of 6 before determining both dissolved inorganic P (MRP) and total P (after nitric / perchloric acid wet digestion) concentrations with the malachite green method (Van Veldhofen and Mannaerts, 1987). Organic P was calculated as the difference between total P and MRP. Statistical analysis was done by using SAS (GLM-SAS procedures, SAS 1985).

RESULTS AND DISCUSSION

In average, whole soil P fractionation recovered about 90% of the total P values, and they were consistently lower than the total P values obtained from an independent P acid digestion method (Table 4.2). This is in good agreement with values reported by Hedley et al. (1982b). The total P values obtained from the sum of all the P fractions in the three soils followed the following order: Maury > Pope > Tilsit. Differences in total P among the three soils could be explained by differences in the amount and frequency of P source application, as well as differences in parent material P concentration. The Maury soil's indigenous total P content was five times higher than that of the other two soils. This is largely due to its high P parent material (Ordovician phosphatic limestone). Total P values for the three treatments sampled from the Pope and Tilsit soils followed a similar pattern: fertilizer P > manure > unamended control (Table 4-2). Poultry manure application to the Maury soil raised total P levels from 3082 mg P kg^{-1} (control) to 4219 mg P kg^{-1} (manure-amended). The fact that fertilizer P increased total P values to a greater extent than manure in the Pope and Tilsit soils is due to the longer duration of fertilizer P application.

The distribution of P among the fractions of the whole soil samples varied considerably among the three soils (Tables 4-2 and 4-3). Inorganic P distribution among the fractions was similar for the Pope and Tilsit soils but these were different from the Maury (Table 4-3). The Maury soil's total inorganic P content was approximately 80 percent of total P. The other two soils had an average of 15 percent of their total P as inorganic P. For the inorganic P fractions of the whole soil samples, the NaOH-extractable P fraction extracted the greatest amount of P in the Pope and Tilsit soils (~7% of total P), and the HCl-extractable P fraction extracted the greatest amount of P from the Maury soil (~50% of total P). This fraction consists mainly of calcium phosphates. The Maury soil contains the Ca-P mineral apatite. The poultry litter also contained a good deal of Ca-P, only reinforcing the dominance of this fraction in the Maury soil. Resin-extractable P was approximately 3% of the total P values for all three soils. $NaHCO_3$-extractable P levels were less than 1% for all three soils.

Organic P also varied between these soils. Organic P averaged 40% of total P in the Pope and Tilsit, but was only 10% of total P in the Maury. Also, the NaOH-OP fraction was consistently higher than the $NaHCO_3$-OP fraction in all soils (Tables 4-2 and 4-3). Residual P accounted for 50% of the total P found in the Pope and Tilsit soils but only 22% for the Maury soil.

Table 4-2: Whole soil (0-7.5 cm depth sampled in early May 1999) P fractionation: P concentrations.

INORGANIC P FRACTIONS

Soil	Treatment	Resin	$NaHCO_3$	NaOH	HCl
		--------µg P g^{-1} soil--------			
Pope	CONTROL	15±0.5	nd	23±2.5	25±3.0
	FERTILIZER	21±1.1	5±1.1	51±1.5	31±1.1
	MANURE	17±1.6	3±0.0	33±4.3	24±3.8
Tilsit	CONTROL	6±1.1	nd*	20±0.7	1±0.1
	FERTILIZER	12±0.7	1±0.2	26±3.1	10±0.2
	MANURE	9±0.1	2±0.4	22±1.4	5±1.4
Maury	CONTROL	50±9.3	45±3.8	511±17.3	1472±117
	MANURE	123±3.4	86±1.8	637±51.0	2157±410

ORGANIC, RESIDUAL P FRACTIONS, TOTAL P

Soil	Treatment	$NaHCO_3$	NaOH	Residual	Total[@]	Total P[$]
		--------µg P g^{-1} soil--------				
Pope	CONTROL	39±1.5	138±7.2	228±38.7	528±2.2	468
	FERTILIZER	49±0.1	169±4.2	220±4.5	657±8.3	546
	MANURE	44±6.2	159±16.1	256±40.8	608±31.5	535
Tilsit	CONTROL	32±4.2	82±4.8	145±27.1	329±9.5	285
	FERTILIZER	40±12.0	81±4.3	145±32.6	408±27.4	316
	MANURE	29±2.1	71±1.8	165±4.1	355±0.8	302
Maury	CONTROL	37±5.1	294±1.3	673±16.5	3117±72.2	3082
	MANURE	32±0.7	263±56.0	920±51.0	5329±71.7	4219

[@] Total P = original soil samples subjected to an independent P digestion.

[$] Total P = the sum of all P fractions.

*nd = not detected.

Table 4- 3: Whole soil (0-7.5 cm depth sampled in early May 1999) P fractionation: Fraction of total P.

Soil	Treatment	INORGANIC P FRACTIONS ------------------% of total P--------------			
		Resin	$NaHCO_3$	NaOH	HCl
Pope	CONTROL	3	0	5	5
	FERTILIZER	4	1	9	6
	MANURE	3	0	6	4
Tilsit	CONTROL	2	0	7	1
	FERTILIZER	4	0	8	3
	MANURE	3	0	7	1
Maury	CONTROL	2	1	17	48
	MANURE	3	2	15	51

Soil	Treatment	ORGANIC, RESIDUAL P FRACTIONS, TOTAL P ------------------------% of total P--------------------			
		$NaHCO_3$	NaOH	Residual	Total
Pope	CONTROL	8	30	49	100
	FERTILIZER	9	31	40	100
	MANURE	8	30	48	100
Tilsit	CONTROL	11	29	51	100
	FERTILIZER	13	26	46	100
	MANURE	10	24	55	100
Maury	CONTROL	1	10	22	100
	MANURE	1	6	22	100

Among the different inorganic P fractions found in the Pope and Tilsit soils, NaOH contained the highest proportion of inorganic P, followed by HCl > Resin > $NaHCO_3$ (Table 4-3). For the Maury soil, the HCl-P fraction was the largest, followed by NaOH > Resin > $NaHCO_3$. It is interesting to note that this sequence is not necessarily an indication of bioavailability. The two labile P fractions (resin and bicarbonate P) constitute the lowest proportion of inorganic P in all three soils. This fact should not lead one to underestimate their potential as bioavailable P sources to microorganisms, since ppb-level concentrations of inorganic P are sufficient to cause P related water quality problems. No significant differences in P amendment treatments were found in the inorganic P fractions of the three soils (Table 4-3).

Table 4-4 gives the amount of resin-P extracted from water dispersible colloids (WDC) and the corresponding whole soil samples. It is interesting to note that in all soils and treatments, WDC contained much more resin-P than the whole soil samples. This fact suggests that once dispersed from soil aggregates by raindrop splash, there is considerable potential for P transport with the moving water dispersible soil colloids.

Table 4-4: Resin-P data averaged over the growing season from whole soil and water dispersible colloids (WDC).

	RESIN-IP FRACTION (µg P g^{-1} of WDC or soil)					
	POPE		TILSIT		MAURY	
	WDC	SOIL	WDC	SOIL	WDC	SOIL
CONTROL	46±6.7[@]	15±1.5	24±2.3	6±0.5	595±21	50±3.6
FERTILIZER	76±1.0	21±0.5	19±2.5	12±0.5		
MANURE	59±6.5	17±0.5	32±0.7	9±0.2	1310±45	123±9.0

[@] mean (n=2) ± standard deviation.

There is a considerable scientific literature on soil P fractionation but reports on colloidal P fractionation are negligible. Table 4-5 shows the proportion of total P found in the different fractions for both whole soil and WDC samples. In all soils, the resin-P and $NaHCO_3$-P fractions contained significantly ($p<0.05$) higher proportion of total P in the WDC than in the whole soil. Both resin-P and $NaHCO_3$-P fractions are considered to be the more labile P fractions that are bioavailable to microorganisms, algae and plants. Another important observation from Table 4-5 is that both organic and inorganic NaOH-P fractions appear to represent the same proportion of total P in both WDC and whole soil samples.

Table 4-6 shows the effect of P amendment on the proportion of total P found in the inorganic P fractions of the WDC over the sampling period. For the most bioavailable P fractions (resin-P and $NaHCO_3$-IP), both fertilizer and manure P significantly increased the proportion of total P found in these fractions, relative to the unamended control. Fertilizer amendment resulted in the highest proportion of total P in these P fractions, followed by poultry litter addition (Table 4-6). That was true for both Pope and Tilsit soils and can be partially explained by the shorter history of manure application, relative to P fertilization, for these two experimental sites. The Maury soil had the highest proportion of total P in the two more bioavailable P fractions (resin-P and $NaHCO_3$-IP), probably due to the very high levels of P in parent material. Inorganic P removed by HCl tended to be greater when manure was applied (Table 4-6). As HCl mainly extracts calcium phosphates, the results support other

Table 4-5: The P fractions as a proportion of the total P: Statistical comparison of whole soil and WDC samples.

WDC-P fractions that were a significantly (*p<0.05*) higher proportion of total P, as compared to the whole soil.

Maury	Pope	Tilsit
OP-$NaHCO_3$	OP-$NaHCO_3$	IP-HCl
IP- $NaHCO_3$	IP- $NaHCO_3$	IP- $NaHCO_3$
IP-Resin	IP-Resin	
Residual P		

WDC-P Fractions that were **not** a significantly (*p>0.05*) higher proportion of total P, as compared to the whole soil.

Maury	Pope	Tilsit
OP-NaOH	OP-NaOH	OP-NaOH
IP- NaOH	IP- NaOH	IP- NaOH
IP-HCl	IP-HCl	IP-Resin
	Residual P	Residual P
		OP-$NaHCO_3$

Table 4-6: Effect of P amendment on the proportion of total P found in the inorganic P fractions of the WDC from the three soils.

	Resin-P			NaHCO$_3$-IP			NaOH-IP			HCl-IP		
	Pope	Tilsit	Maury	Pope	Tilsit	Maury	Pope	Tilsit	Maury	Pope	Tilsit	Maury
Control	3.2c	2.1b	10.3b	2.1c	1.5c	2.7b	3.2c	5.8c	12.9a	4.2b	0.0b	25.7b
Fertilizer	5.2a	2.8a		4.1a	3.4a		6.6b	12.2a		6.8a	0.0b	
Manure	4.4b	2.7a	17.1a	3.4b	3.1b	3.3a	8.3a	9.0b	12.2a	7.4a	9.5a	35.7a

Within a column, means with different superscripts are significantly different at the 95% level of confidence.

work which found that manure contained large amounts of calcium phosphates (Sharpley and Smith, 1995).

Table 4-7 illustrates the effect of P amendment on the proportion of total P in the organic and residual P fractions from the WDC of the three soils. Manure application decreased the proportion of total P as NaOH-OP and residual-P in the WDC of all three soils. These fractions act as P sources in the redistribution of P to more available, either organic or inorganic, P fractions. Guo et al. (2000) stated that the contribution of the organic P fractions, relative to the inorganic P fractions, to plant P absorption from P amended soils remains to be determined. They did not find any changes in either $NaHCO_3$-P or NaOH-P fractions with plant removal of P from soil. Beck and Sanchez (1994) found that organic P did not significantly contribute to plant available inorganic P in an Ultisol that received repeated P applications. Finally, Huffman et al. (1996) tried to test the effect of soil texture on labile organic P fractions. They found that organic P fractions were not significantly changed, but stayed rather constant during a three-month period. It appears from these reports that most of the time it is difficult to pinpoint changes in the organic P fractions by working with whole soil samples. Most of the P reactions appear in the fine colloidal sub-fraction of the clay fraction of the soil, as was done in our study. Thus, the WDC need to be acknowledged for their major contribution to P cycling and transformations in agricultural soils amended with different P sources.

Table 4-7: Effect of P amendment on the proportion of total P found in the organic and residual P fractions of the WDC from the three soils.

	Residual-P@			$NaHCO_3$ -OP			NaOH-OP		
	Pope	Tilsit	Maury	Pope	Tilsit	Maury	Pope	Tilsit	Maury
Control	62.40a	53.5a	37.3a	6.3b	12.1a	2.3a	18.3a	24.7b	8.5a
Fertilizer	52.89b	43.8b		6.4b	10.7b		17.8a	26.8a	
Manure	52.81b	51.2a	25.1b	10.3a	12.5a	0.8b	13.0b	11.7c	5.4b

@ Residual-P is a mixture of highly occluded organic and inorganic moieties.

Within a column, means with different superscripts are significantly different at the 95% level of confidence.

Temporal changes in the concentrations of P found in the P fractions found in the WDC are shown in Tables 4-8 and 4-9. Though the tables provide detail, graphical presentation of selected data provide easier visualization of seasonal changes in P fractions in these soils.

Initially, there was a decline in resin–IP in through late June-early July (third sampling date) for soil WDC, coinciding with the period of greatest crop growth and P uptake (Figures 4-1, 4-2 and 4-3). Towards the end of the growing season, when crop P uptake declines, both resin-IP and $NaHCO_3$-IP (Figures 4-4, 4-5 and 4-6) increased considerably, compared to earlier dates. As the redistribution from organic P to inorganic P takes place during a growing season, the resin-IP and $NaHCO_3$-IP rose, especially towards the end of the growing season, and especially in manured and fertilized soils (Figures 4-1, 4-2, 4-3, 4-4, 4-5 and 4-6).

Other researchers have also identified that increases in loosely bound IP occur in a greater extent in the autumn rather than in the spring (Yli-Halla et al., 1995). Disoolved reactive P concentrations in August runoff were double that found in May in the study of Pote et al. (1996). The fact that the colloidal P fraction of these soils exhibited the behavior observed for whole soils in previous studies illustrates the likely contribution of WDC to previous observations. It is likely that desorption of loosely bound P from WDC contributes to elevated levels of dissolved IP at the end of the growing season.

Table 4-8: Effect of P amendment on the concentrations (μg P g^{-1} WDC) found in the inorganic P fractions of the WDC (1 to 5 represent sampling dates from May to September).

Soil	Resin-IP			$NaHCO_3$-IP			NaOH-IP			HCl-IP		
	Pope	Tilsit	Maury	Pope	Tilsit	Maury	Pope	Tilsit	Maury	Pope	Tilsit	Maury
Control 1	45.46 ±0.6	23.81 ±7.2	595.1 ±57.3	17.27 ±3.3	13.7 ±4.6	184.6 ±10.0	74.3 ±0.0	72.3 ±25.1	813.3 ±63.0	30.3 ±2.9	nd[@]	1512 ±158.3
Control 2	51.70 ±8.1	16.56 ±3.1	721.9 ±42.8	25.50 ±0.8	14.3 ±1.1	174.9 ±21.0	37.7 ±2.0	73.7 ±0.5	894.2 ±158.9	5.9 ±3.3	nd	1572 ±311.1
Control 3	31.98 ±5.4	15.45 ±0.8	462.7 ±4.0	34.99 ±3.0	13.1 ±0.1	165.8 ±6.9	61.6 ±6.9	71.3 ±16.8	792.3 ±38.5	59.3 ±1.5	nd	1756 ±275.0
Control 4	32.74 ±2.0	24.85 ±1.3	669.9 ±47.3	35.78 ±2.9	19.8 ±0.9	179.8 ±5.5	18.7 ±1.7	44.1 ±31.1	710.5 ±80.1	85.9 ±7.5	nd	1401 ±42.3
Control 5	55.63 ±6.4	31.42 ±0.7	725.1 ±11.2	32.88 ±0.0	21.8 ±2.2	131.2 ±24.8	23.4 ±10.2	46.1 ±21.7	772.8 ±73.3	56.1 ±7.6	nd	1694 ±117.6
Manure 1	58.76 ±2.7	32.45 ±3.2	1306.2 ±110.8	36.55 ±3.1	22.0 ±1.6	233.4 ±2.7	145.6 ±10.3	87.0 ±11.2	1157 ±26.5	126.8 ±6.6	84.2 ±14.9	3418 ±274
Manure 2	68.20 ±1.1	15.15 ±3.9	1321.2 ±163.2	51.27 ±0.6	19.5 ±2.2	278.4 ±43.6	102.8 ±3.6	99.3 ±13.8	1196 ±124	90.4 ±1.5	97.7 ±0.0	3751 ±124
Manure 3	42.01 ±2.2	15.42 ±2.3	1193 ±104	43.91 ±1.9	25.5 ±1.4	323.5 ±20.4	122.9 ±3.8	101.7 ±7.2	1193 ±99	105.7 ±4.0	103.4 ±6.4	3413 ±96
Manure 4	64.82 ±6.8	42.75 ±3.3	1760 ±438	52.89 ±0.8	49.3 ±1.1	344.7 ±57.4	103.1 ±10.4	101.3 ±22.8	951.7 ±120.6	97.3 ±5.5	106.8 ±2.2	2567 ±879
Manure 5	72.66 ±6.8	30.14 ±0.2	2118 ±388	53.12 ±0.4	38.8 ±3.0	329.4 ±54.2	105.7 ±0.1	64.4 ±22.3	1036 ±171	94.4 ±2.0	78.3 ±0.6	3067 ±236

[@] nd = below detection limit

Table 4-8 (cont.nd): Effect of P amendment on the concentrations (µg P g^{-1} WDC) found in the inorganic P fractions of the WDC (1 to 5 represent sampling dates from May to September).

Soil	Resin-IP			$NaHCO_3$-IP			NaOH-IP			HCl-IP		
	Pope	Tilsit	Maury	Pope	Tilsit	Maury	Pope	Tilsit	Maury	Pope	Tilsit	Maury
Fertilizer 1	76.09 ±7.8	19.16 ±5.5		44.71 ±1.2	28.8 ±0.2		98.0 ±22.7	128.2 ±21.3		89.3 ±22	nd	
Fertilizer 2	74.09 ±5.2	33.37 ±2.4		72.35 ±3.6	47.6 ±2.2		88.4 ±6.8	164.0 ±1.8		92.7 ±18	nd	
Fertilizer 3	63.30 ±0.8	35.55 ±2.5		68.83 ±1.4	38.7 ±0.9		145.4 ±4.4	141.4 ±3.0		107.1 ±11.1	nd	
Fertilizer 4	108.7 ±8.2	33.71 ±7.9		70.61 ±4.1	36.4 ±3.5		121.1 ±7.7	139.3 ±21.4		117.7 ±1.4	nd	
Fertilizer 5	83.09 ±2.0	57.43 ±8.2		61.02 ±8.7	69.5 ±0.3		73.3 ±38.0	197.5 ±14.4		124.1 ±39.9	nd	

Table 4-9: Effect of P amendment on the concentrations (μg P g^{1} WDC) found in the organic and residual P fractions of the WDC (1 to 5 represent monthly sampling dates from May to September).

	Residual-P$^{@}$			**NaHCO$_3$-OP**			**NaOH-OP**		
	Pope	Tilsit	Maury	Pope	Tilsit	Maury	Pope	Tilsit	Maury
Control 1	713.5 ±58.4	494.2 ±7.4	2362.7 ±31.2	101.9 ±4.4	114.5 ±10.6	117.0 ±16.2	377.0 ±36.1	279.0 ±41.4	477.4 ±11.4
Control 2	898.0 ±12.2	499.5 ±4.4	2392.4 ±52.2	117.2 ±2.3	124.7 ±4.3	134.3 ±5.4	208.7 ±10.2	333.1 ±21.0	500.3 ±50.7
Control 3	833.5 ±1.9	448.0 ±25.4	2186.2 ±108.5	54.0 ±0.5	127.3 ±0.7	153.3 ±8.8	231.5 ±8.7	304.4 ±12.3	612.0 ±77.1
Control 4	828.3 ±25.3	760.3 ±11.0	2299.0 ±25.1	87.7 ±29.2	140.7 ±40.6	133.7 ±9.5	213.3 ±27.0	186.7 ±23.9	519.1 ±44.2
Control 5	922.1 ±48.0	667.3 ±12.5	2220.7 ±97.1	67.2 ±3.7	139.4 ±32.0	168.7 ±6.6	202.5 ±37.8	194.5 ±28.9	502.1 ±108.8
Manure 1	675.6 ±0.5	605.7 ±38.2	2266.2 ±16.2	214.7 ±8.2	104.0 ±8.9	77.3 ±18.6	254.0 ±9.8	96.0 ±3.6	467.0 ±77.3
Manure 2	957.4 ±17.0	507.0 ±92	2269.9 ±12.4	131.2 ±6.7	120.9 ±13.6	99.8 ±33.8	127.8 ±13.4	92.8 ±6.9	493.3 ±36.6
Manure 3	647.3 ±20.0	500.8 ±11.8	2239.0 ±11.5	142.7 ±4.4	142.6 ±15.3	98.3 ±18.8	198.8 ±16.2	157.2 ±32.8	568.8 ±32.9
Manure 4	599.4 ±0.8	613.2 ±27.6	2259.3 ±101.4	133.5 ±19.7	104.2 ±8.8	53.3 ±8.1	171.1 ±11.2	127.4 ±4.0	436.4 ±102.9
Manure 5	821.8 ±6.2	344.1 ±67.0	2286.5 ±20.6	95.4 ±8.3	135.4 ±11.9	68.2 ±8.6	151.0 ±6.4	107.9 ±9.9	479.1 ±64.2
Fertilizer 1	771.2 ±101.1	612.3 ±6.9		165.7 ±6.4	154.3 ±41.8		464.4 ±78.3	362.8 ±46.8	
Fertilizer 2	769.7 ±15.8	552.8 ±28.2		69.7 ±6.7	148.2 ±18.0		234.2 ±9.1	353.9 ±8.0	
Fertilizer 3	925.8 ±0.8	479.1 ±19.0		144.8 ±16.8	139.7 ±20.3		269.8 ±3.6	315.2 ±4.9	
Fertilizer 4	833.7 ±9.3	525.3 ±8.2		75.5 ±18.6	122.3 ±5.1		223.7 ±1.6	327.1 ±24.9	
Fertilizer 5	823.9 ±42.7	590.6 ±12.2		64.6 ±4.8	111.1 ±1.5		221.6 ±4.6	329.1 ±6.2	

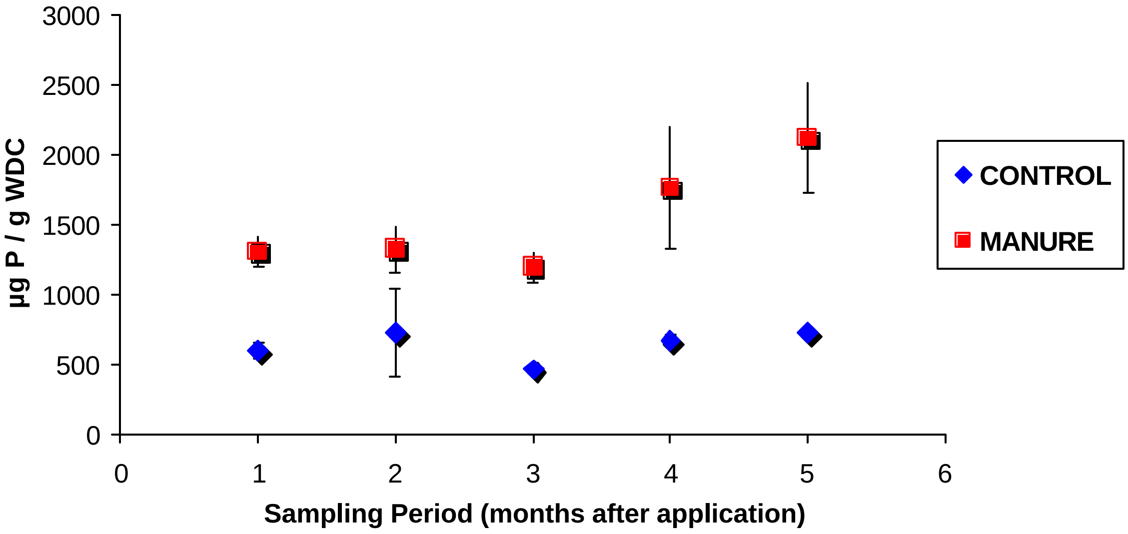


Figure 4-1: Temporal changes in resin-IP in the Maury WDC.

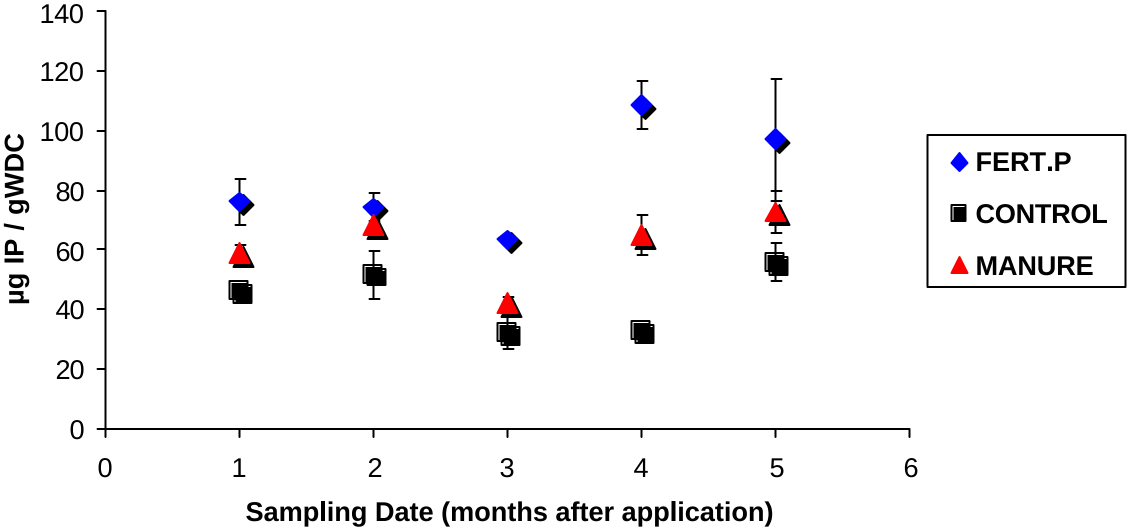


Figure 4-2: Temporal changes in resin-IP in the Pope WDC.

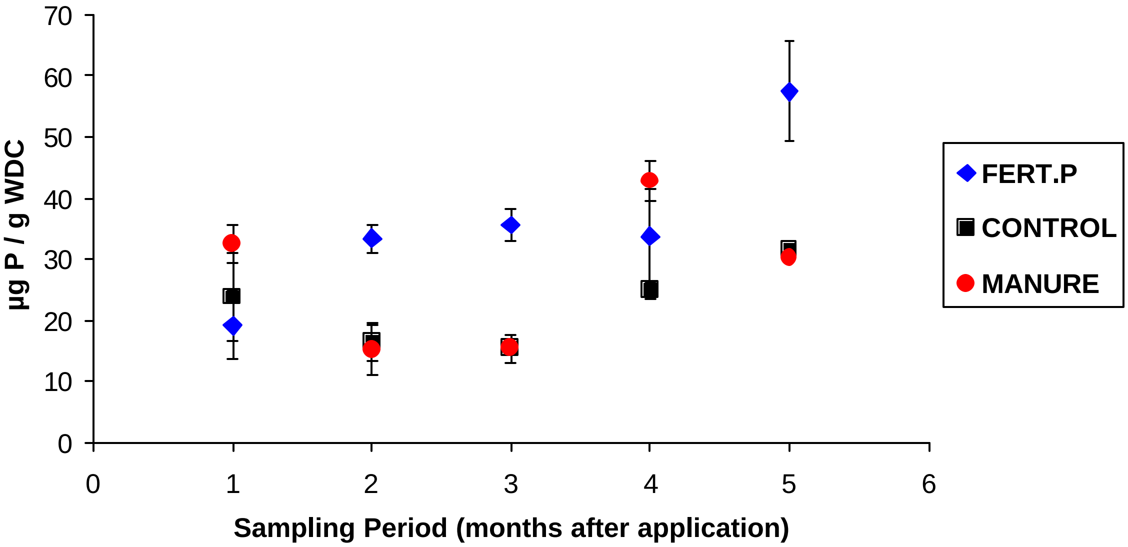


Figure 4-3: Temporal changes in resin-IP in the Tilsit WDC.

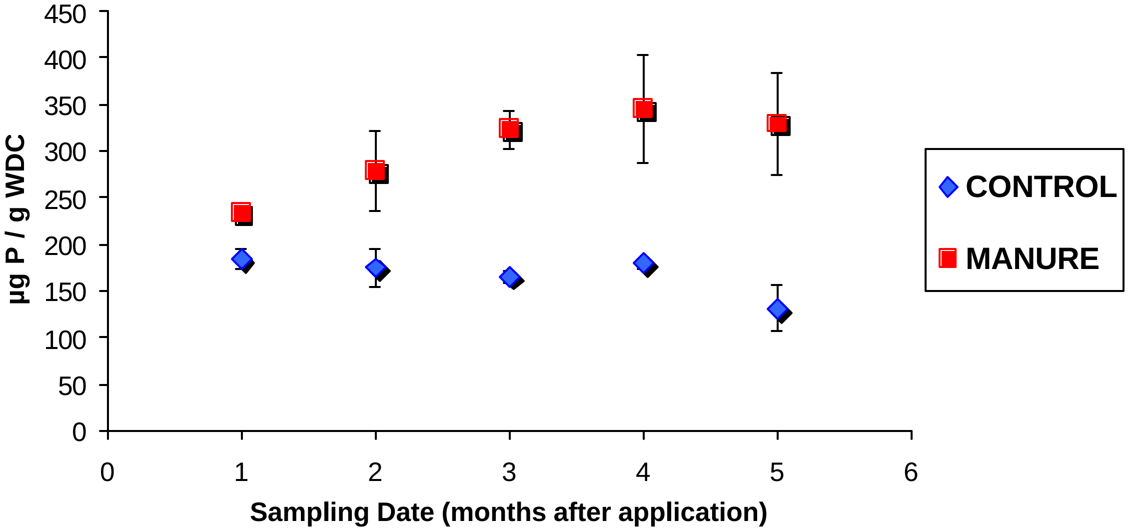


Figure 4-4: Temporal changes in $NaHCO_3$-IP in the Maury WDC.

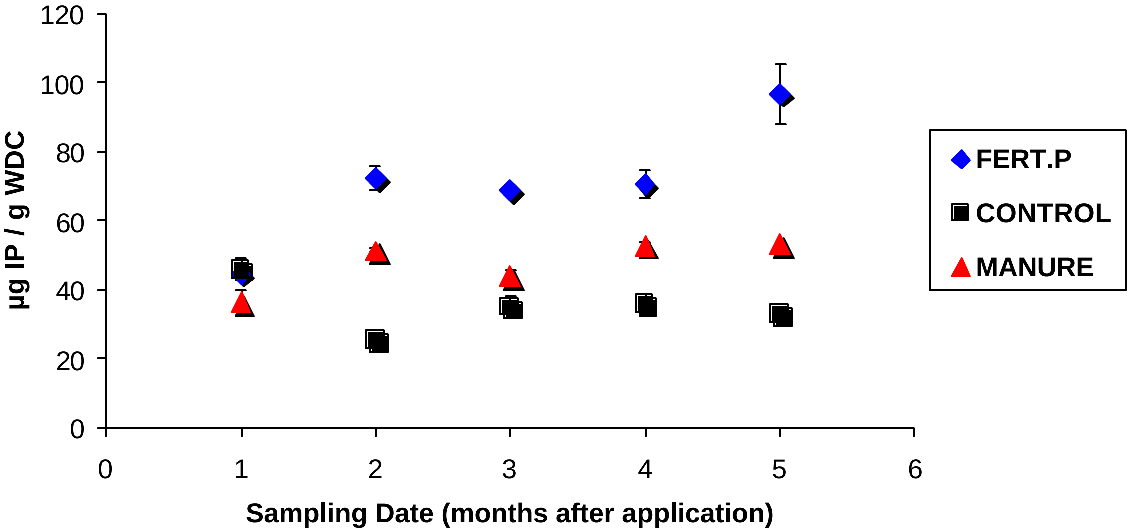


Figure 4-5: Temporal changes in $NaHCO_3$-IP in the Pope WDC.

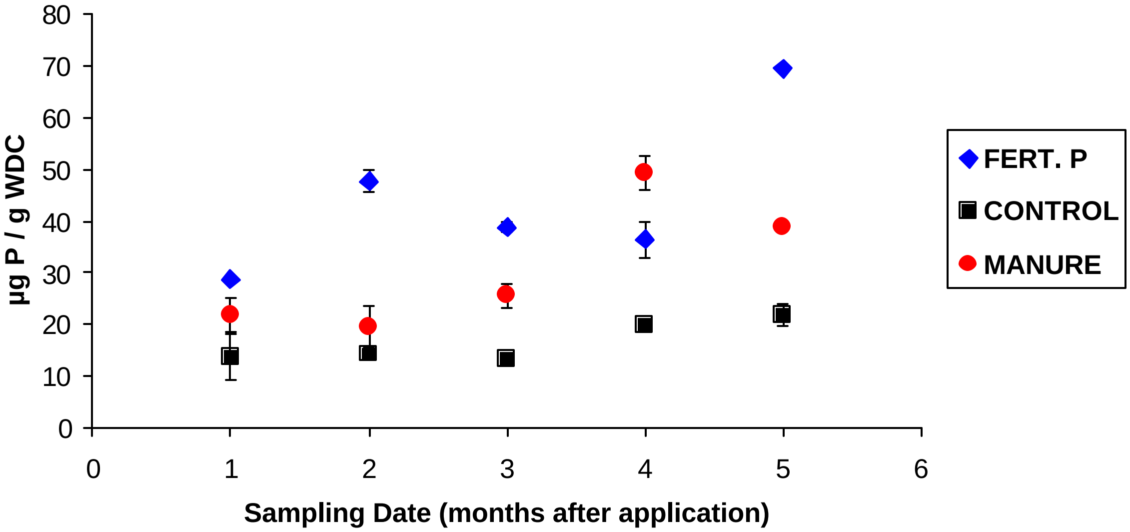


Figure 4-6: Temporal changes in $NaHCO_3$-IP in the Tilsit WDC.

On the other hand, organic P in the WDC fraction of the manured soils was either constant (Figures 4-7, 4-9, 4-10 and 4-11) or tended to decrease (Figures 4-8 and 4-12), especially later in the growing season. That latter behavior somewhat balances the seasonal increase in WDC inorganic P reported earlier. The fact that there was little difference between P sources in these temporal trends suggests that the organic P being mineralized in these fractions is derived more from past crop residue and microbial biomass P, rather than the current season's manure amendment.

Finally, one of the P fractionation hypotheses was that resins also adsorb colloidal P in addition to the "free" orthophosphates. Baldwin et al. (1996), Cooperband et al (1999) demonstrated the hydrolysis of an OP compound by iron oxide impregnated filter paper, an adsorbent similar to the resin in P extractability. If the resin adsorbs organic particulate P (OPP), and during the recovery of P from the resin the OPP hydrolyzes and releases MRP detectable with the malachite green method, then overestimation of resin-IP occurs. Figure 4-13 shows that as solution OPP increases, the more MRP is detected, probably due to hydrolysis of these organic P compounds.

To conclude, this study revealed that WDC-P fractionation extracts more P, also more bioavailable P, than from whole soil samples. The WDC from manured and fertilized soils contain a much higher P concentration as compared to WDC from unmanured soils. Manure and fertilizer P addition increased the proportion of P in bioavailable fractions. In manured soil WDC, P was redistributed to more bioavailable forms towards the end of the growing season.

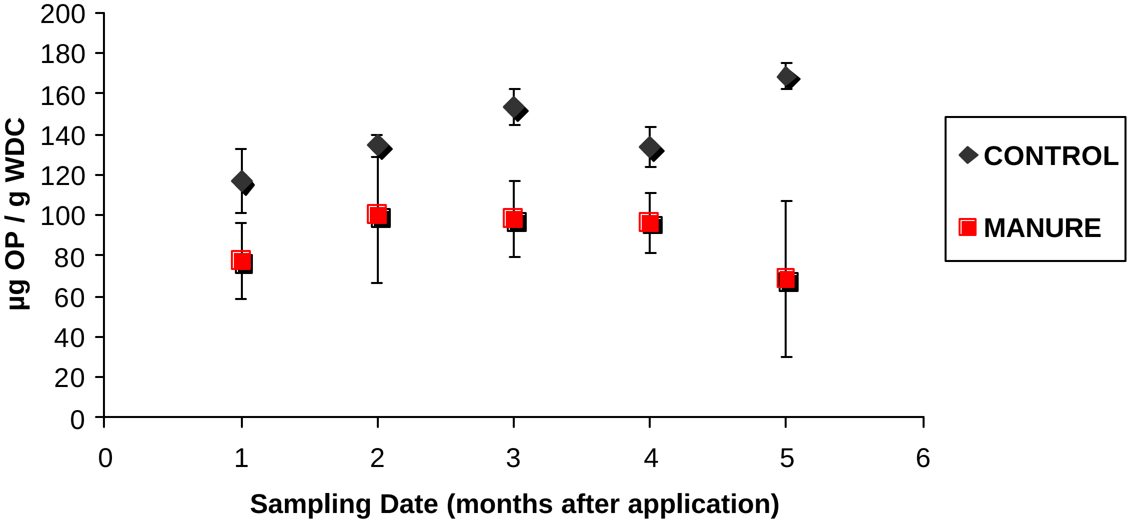


Figure 4-7: Temporal changes in $NaHCO_3$-OP in the Maury WDC.

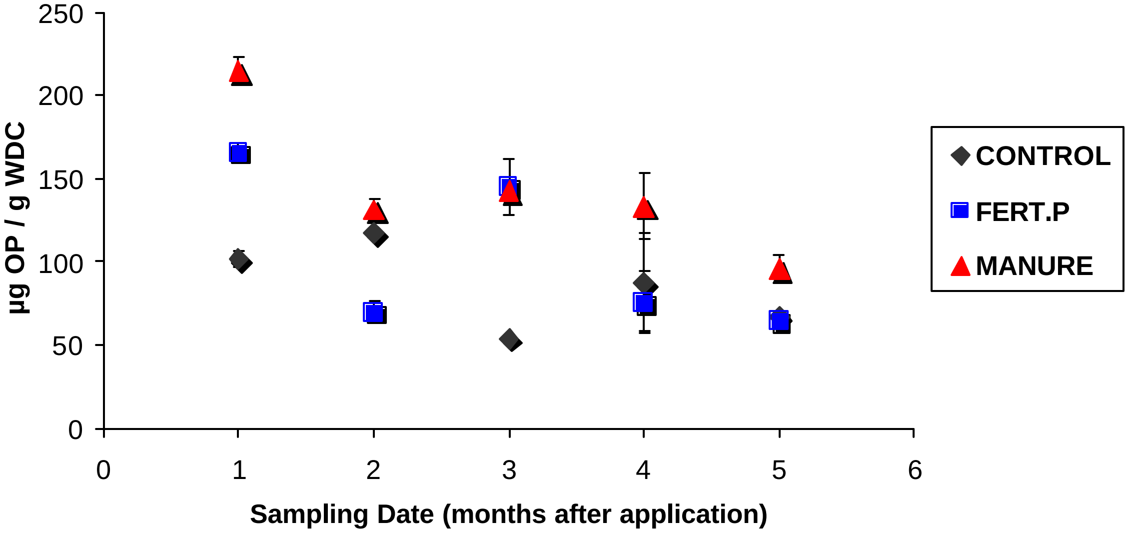


Figure 4-8: Temporal changes in $NaHCO_3$-OP in the Pope WDC.

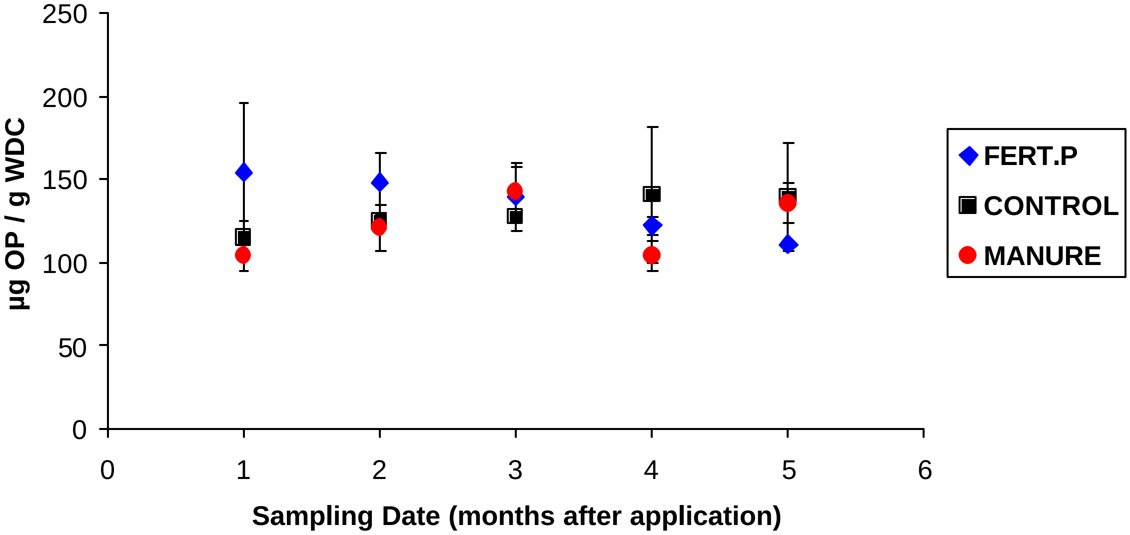


Figure 4-9: Temporal changes in $NaHCO_3$-OP in the Tilsit WDC.

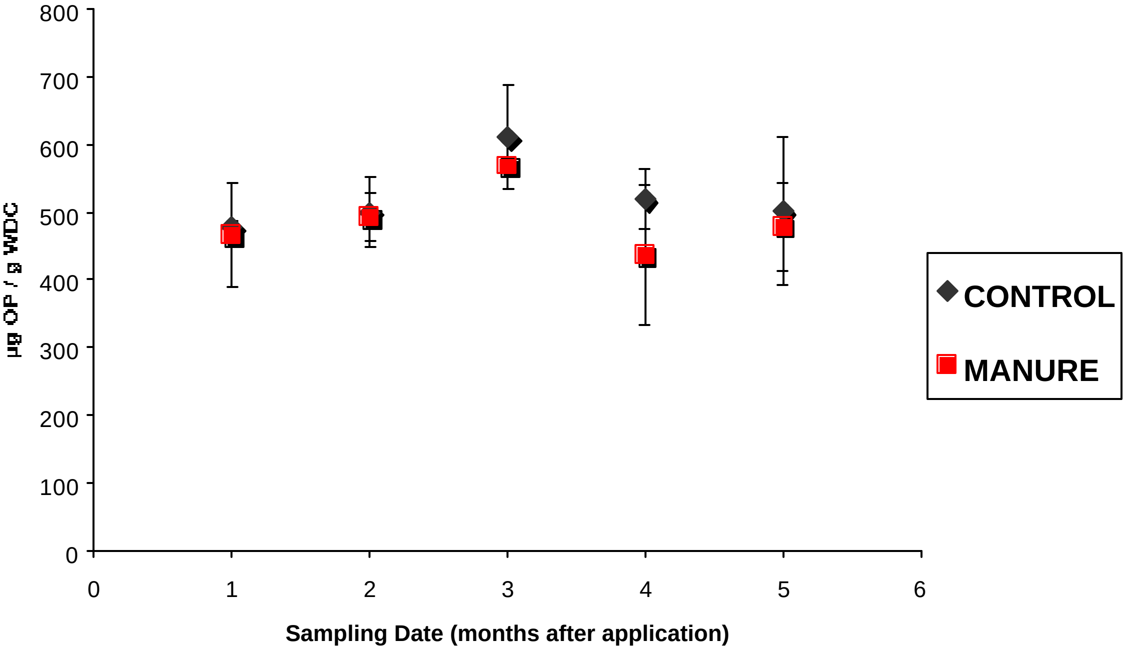


Figure 4-10: Temporal changes in NaOH-OP in the Maury WDC.

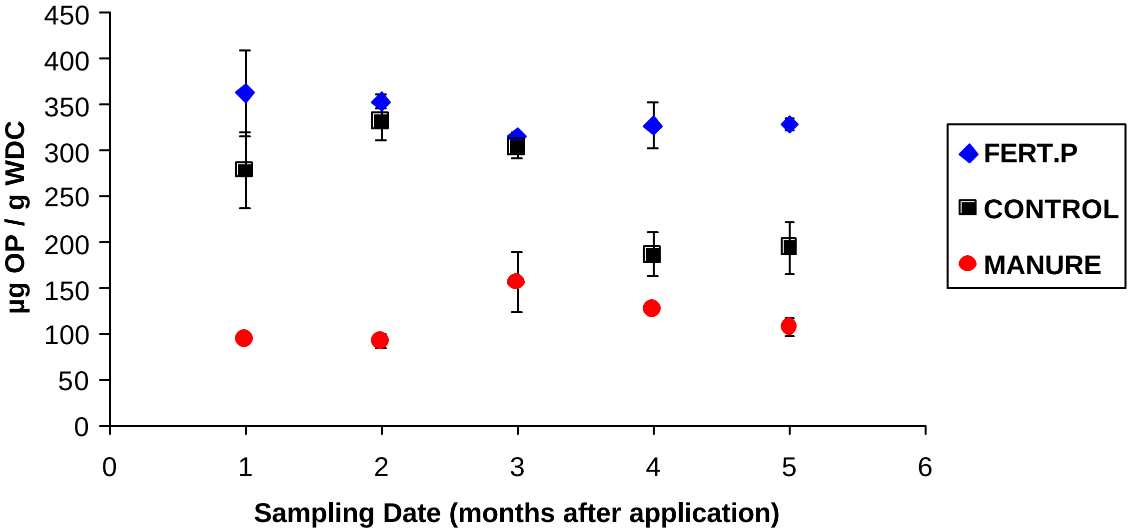


Figure 4-11: Temporal changes in NaOH-OP in the Tilsit WDC.

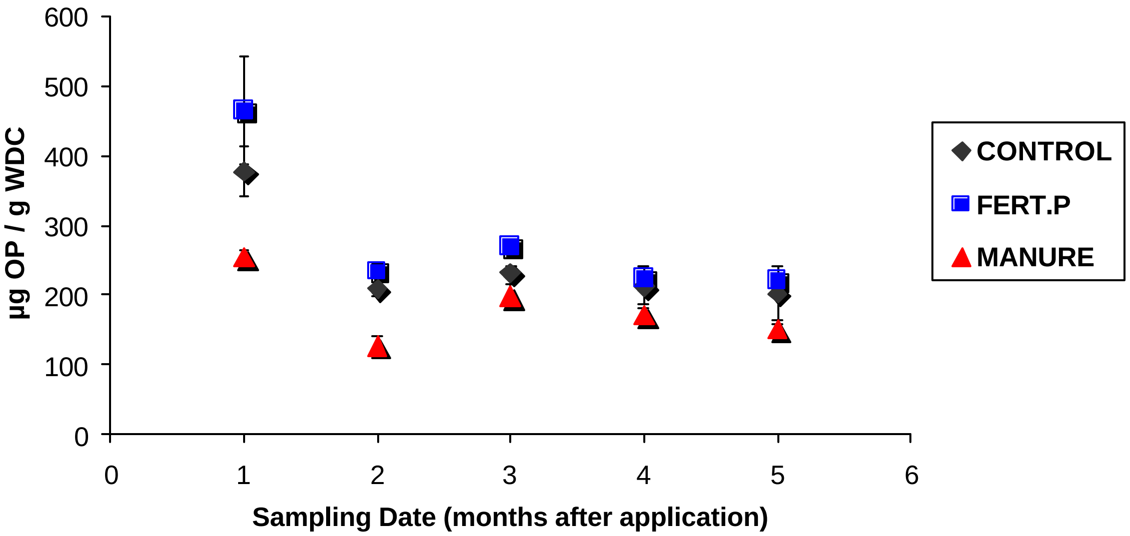


Figure 4-12: Temporal changes in NaOH-OP in the Pope WDC.

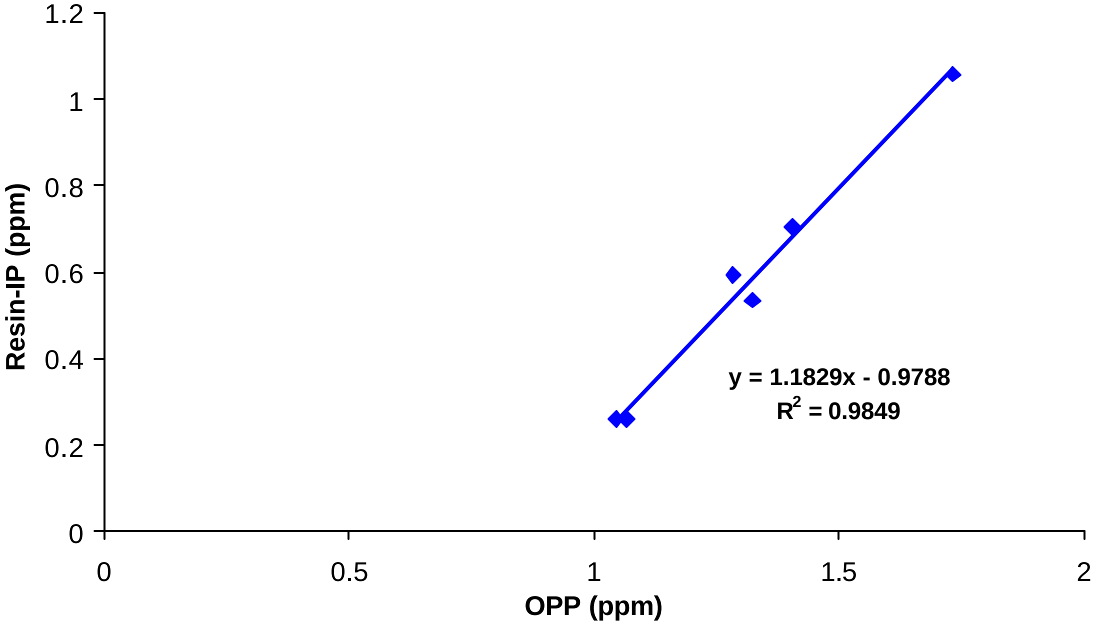


Figure 4-13: Relationship between organic particulate P (OPP) and resin-IP in the three soils.

CHAPTER FIVE

Settling Kinetics Behavior of Colloidal P And Water Dispersible Soil Colloids (WDC) from Soils Amended with Different P Sources

INTRODUCTION

Soil disruption during rainfall events can generate water dispersible soil colloids (WDC) that are susceptible to movement. Losses of these particles, caused by lateral surface water movement, are a major factor in particulate P (PP) losses. Some studies have found a close relationship between soil test P, a measure of bioavailable P, and P in runoff (Pote et al., 1996; Sharpley, 1985). Cox (2000) demonstrated the effect of soil clay content on the dissolved P levels in runoff after addition of organic and inorganic P amendments to soils with varying clay content. Soils with high clay content required much higher soil test P levels in order for their runoff P concentration to reach thresholds of environmental consequence. Hooda et al. (2000) found that soil texture was a crucial factor affecting P found in runoff. Krogstad (1986) reported that the suspended solids contained about 1000 ppm colloidal P, which constituted about 95% of the total P in runoff water. This proportion of total P declined to 25% when the WDC content also decreased to 10 ppm. Krogstad (1986) also found that dissolved MRP concentrations were unaffected by changes in WDC concentrations.

In cases where fields generate runoff with low WDC content, such as meadow fields under no-till soil management, then high runoff P concentrations can only be explained by mineralization of surface residues or by recent surface application of soluble P sources (Lundekvam, 1998). In cases where fields generate runoff with a high concentration of WDC after a rainfall event, then an understanding of the chemical behavior and settling kinetics of these particles is important. The longer the WDC stay in suspension, the higher the risk of P loss via WDC losses in surface runoff.

The objectives of this study were: a) to determine the settling kinetics of the soils' WDC and size-separated P fractions; b) evaluate the effect of manure application on the stability of WDC and size-separated P fractions; c) establish relationships between the colloidal-P fractions and the soil test P values used to assess risk of surface P loss from agricultural soils.

MATERIALS AND METHODS

Soils (Maury, Tilsit and Pope silt loams) located at three field experimental sites amended with fertilizer-P and / or poultry litter were used in this study. The Maury was amended only with poultry litter due to its high native P status. The experimental design was identical to the one mentioned in preceding chapters. Surface (0-7.5 cm) soil samples, taken approximately three months after application of the two P sources, were used to generate the WDC. The procedures of Seta and Karathanasis (1997a) were employed: ten grams of each soil were shaken for 16 hours with 200 ml deionized water and centrifuged for 3.5 min at 750 rpm. The supernatant was retained and mixed with a solution of 0.01 M $CaCl_2$ (1:1 volume ratio) to maintain a constant ionic strength. Following this, 50 mL of the mixed solution was placed in 50 cm long glass test tubes and allowed to settle. Within each test tube, aliquots were taken from a constant depth after 0, 12, 24 and 36 hours of settling.

Half lives of the WDC and colloidal P particles were estimated following a first-order kinetics approach:

$$-d(A) / dt = kA$$

where A = the WDC concentration, k= the settling rate constant, and t = settling time. The above equation was integrated, then transformed to its linear form in order to calculate the time at which the A parameter was reduced to the half of the initial WDC content

($A = A_o/2$).

Measurements of pH, total organic carbon (TOC), WDC, molybdate reactive P (MRP) and total P were made at each interval on the unfiltered samples. The PP was found by difference between unfiltered and filtered (0.45µm) portions of the aliquot. Organic P fractions were estimated by difference between total and inorganic P values.

The TOC was measured by dry combustion on a LECO autoanalyzer, WDC were calculated from calibration curves after measuring the absorbance at a wavelength of 540 nm on a microplate reader (Seta and Karathanasis, 1997a). MRP was measured with the malachite green method (Van Veldhoven and Mannaerts, 1987). Total P was also determined by the malachite green method following a wet digestion of the sample with a mixture of concentrated HNO_3/$HClO_4$ (9:1 v/v). A detailed description of the different operational P forms and size-fractions is given in Tables 5-1 and 5-2, and Figure 5-1.

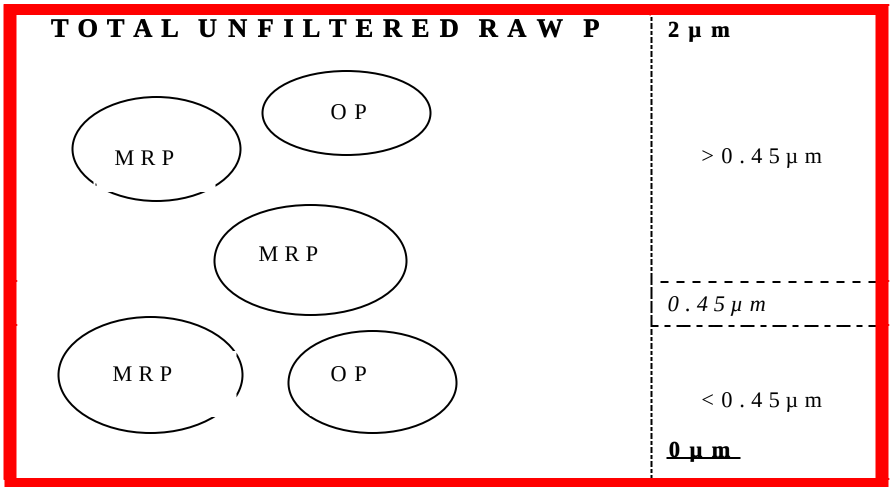
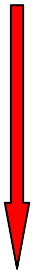


Table 5-1: P forms defined in the study – prior to filtering.



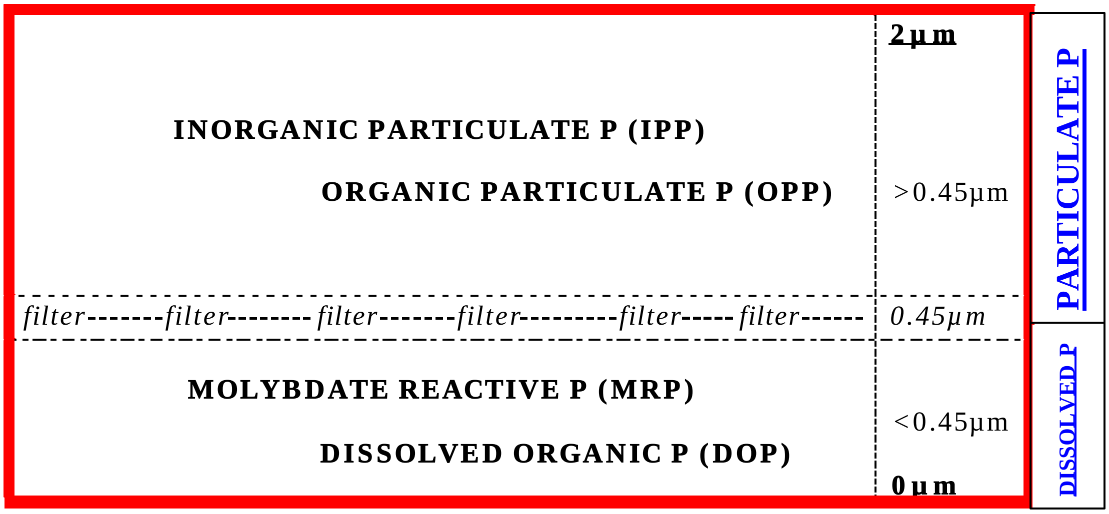
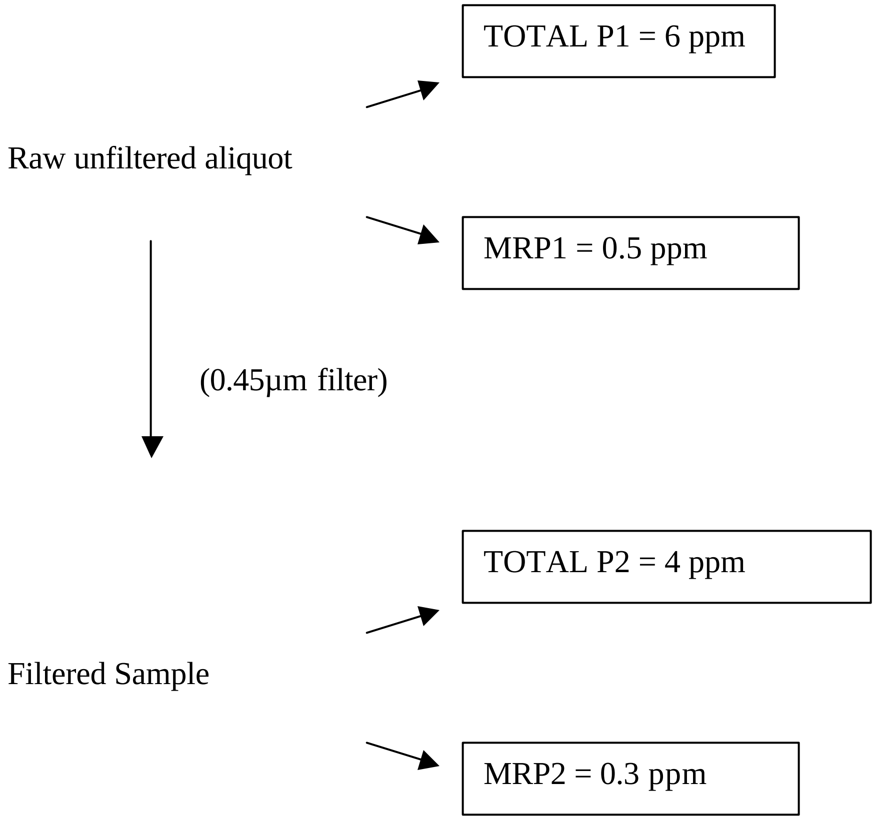


Table 5-2: P forms defined in the study – after filtering.

Figure 5-1: An example flow chart for the analyses and calculations of the P forms in the dissolved and colloidal portions of an example sample.



TPP=TOTAL P1 - TOTAL P2 = 6.0 – 4.0 = 2.0 ppm

IPP=MRP1 - MRP2 = 0.5 - 0.3 = 0.2 ppm

DOP=TOTAL P2 - MRP2 = 4.0 - 0.3 = 3.7 ppm

OPP=TPP – IPP = 2.0 - 0.2 = 1.8 ppm

RESULTS AND DISCUSSION

The pH of the colloidal suspensions remained nearly constant around 6, for the three soils. In all three soils, there was little difference in the WDC settling rate between amended and unamended soils (Figures 5-2, 5-3 and 5-4). However, there were differences between the soils in the initial WDC generated. The Tilsit soil generated the greatest initial amount of WDC. Though TOC settled with time (Figure 5-5), there was a strong negative correlation between TOC and the initial amount of WDC generated may partially explain that observation (Figure 5-6). Initially, the Tilsit WDC had the least amount of organic carbon, a likely cementing agent. This lack of TOC likely assisted the initial dispersion of WDC.

Particulate P (PP) concentrations fell during settling, paralleling declines in WDC. Inorganic particulate P concentrations decreased during settling, especially when manure or fertilizer P amendments had been made to the soil (Figures 5-7, 5-8 and 5-9). Organic particulate P (OPP) settling followed WDC settling behavior, being no different in trendline due to P amendment (Figures 5-10, 5-11, and 5-12).

The settling half lives for the WDC particles (Table 5-3), averaged 23 hours for the Pope WDC, 16 hours for the Tilsit WDC, and 25 hours for the Maury WDC. The Tilsit colloids were the least stable in suspension despite the fact that they had the highest initial concentration. In most soils, the OPP fraction settled out faster than the IPP fraction, which remained in suspension longer than the WDC.

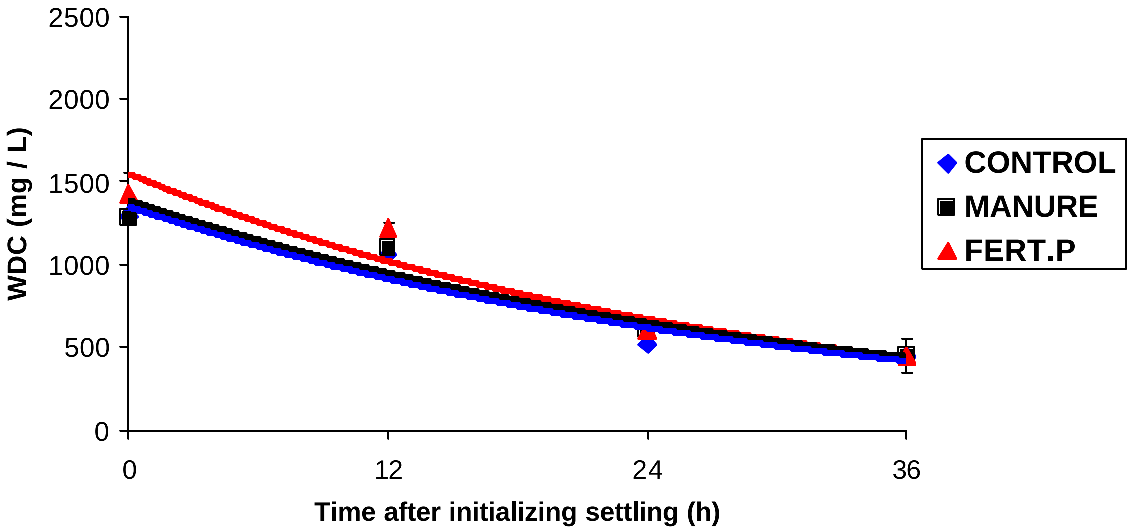


Figure 5-2: Settling of WDC from the Pope soil.

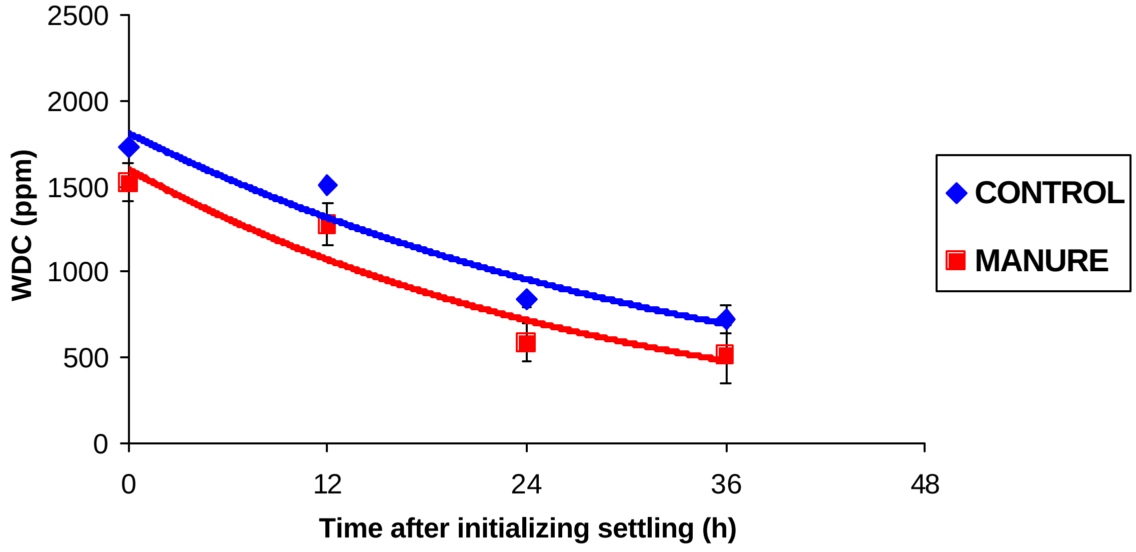


Figure 5-3: Settling of WDC from the Maury soil.

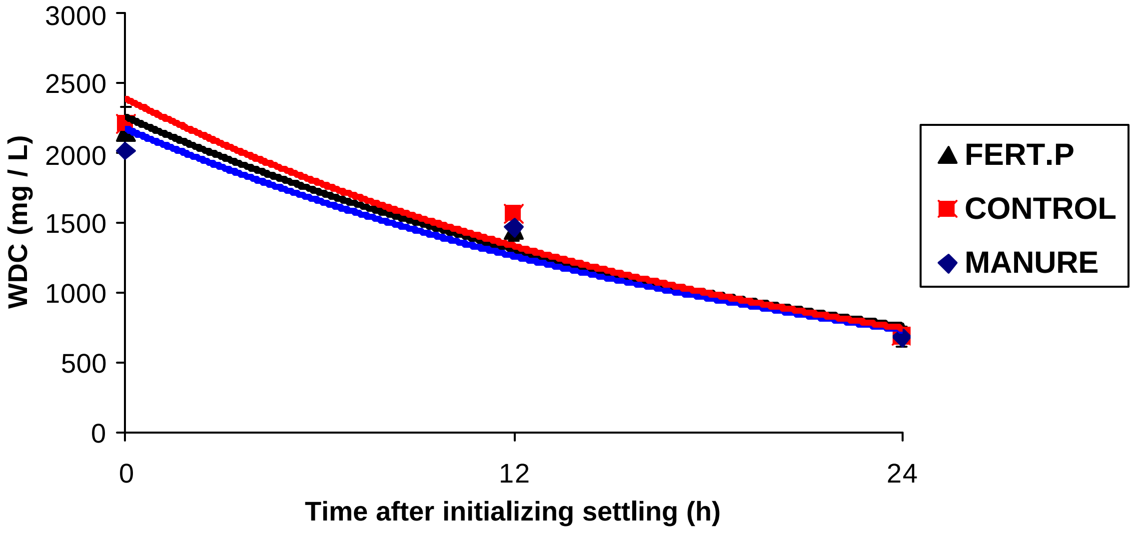


Figure 5-4: Settling of WDC from the Tilsit soil.

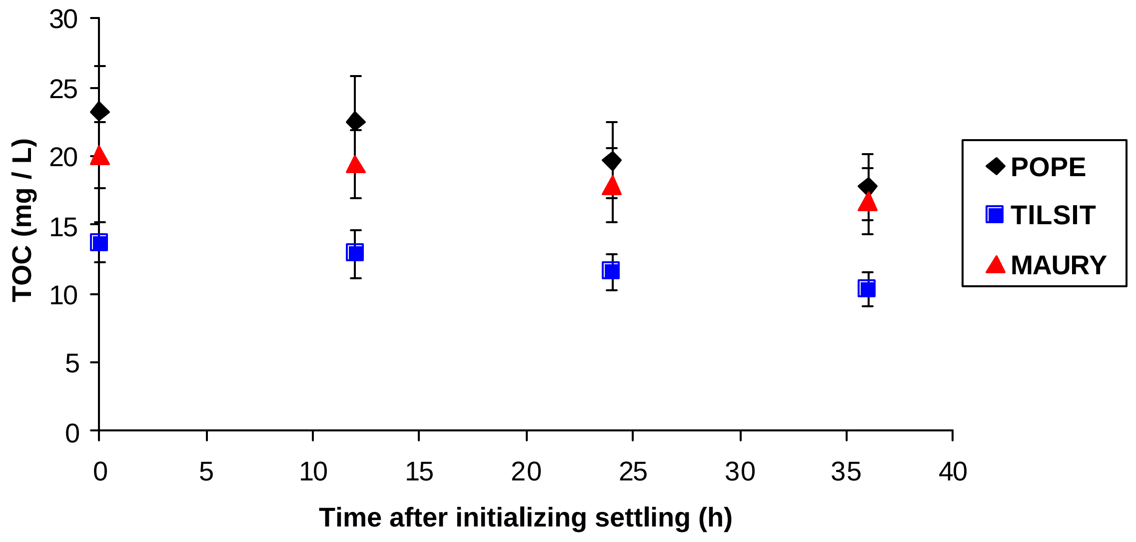


Figure 5-5: Soil TOC concentrations, averaged over the amendment treatments, during the experimental settling period.

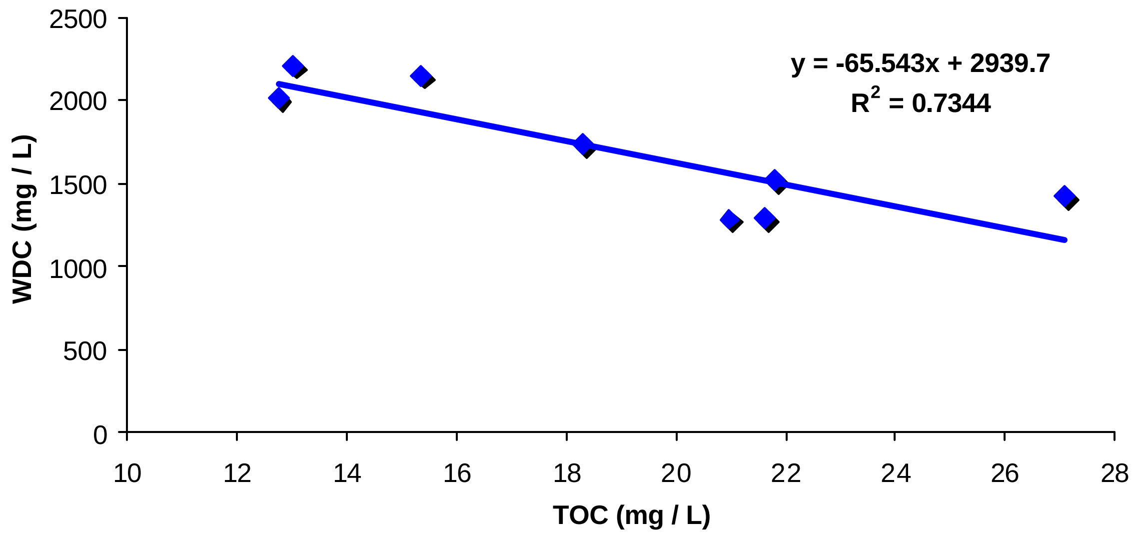


Figure 5-6: Initial WDC vs. initial TOC for three soils amended with different P sources.

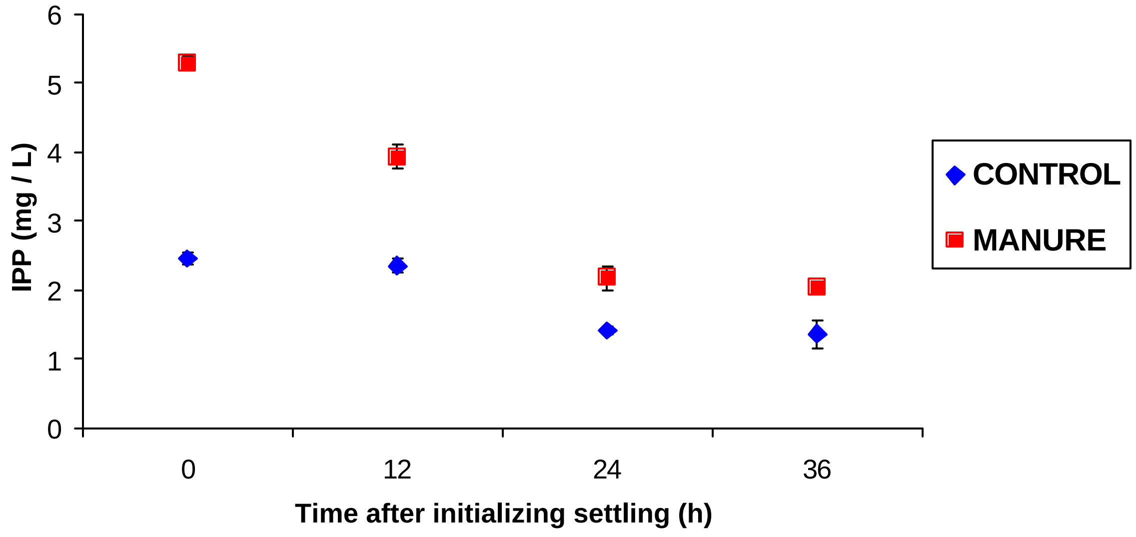


Figure 5-7: Settling of IPP from the Maury soil.

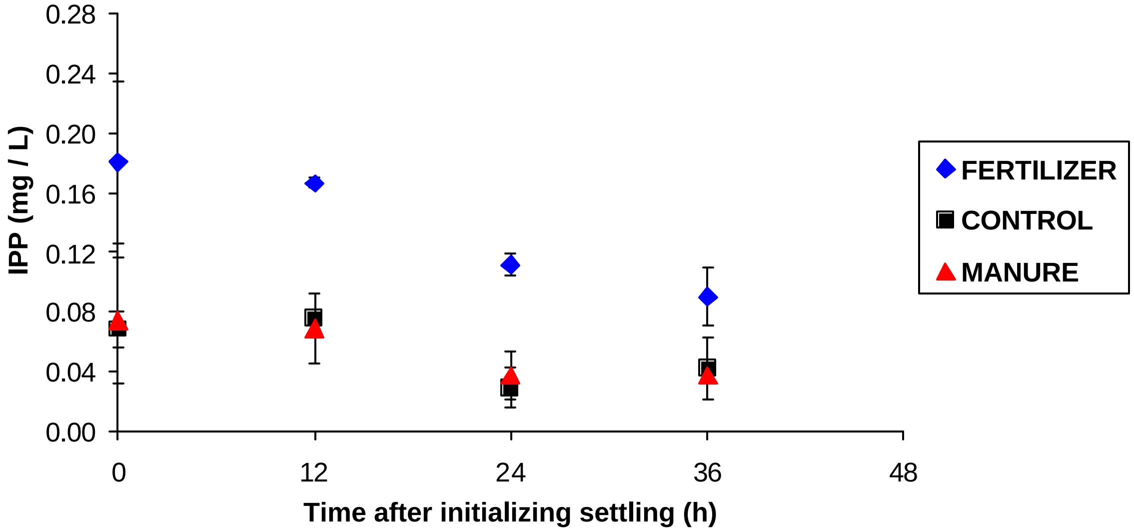


Figure 5-8: Settling of IPP from the Pope soil.

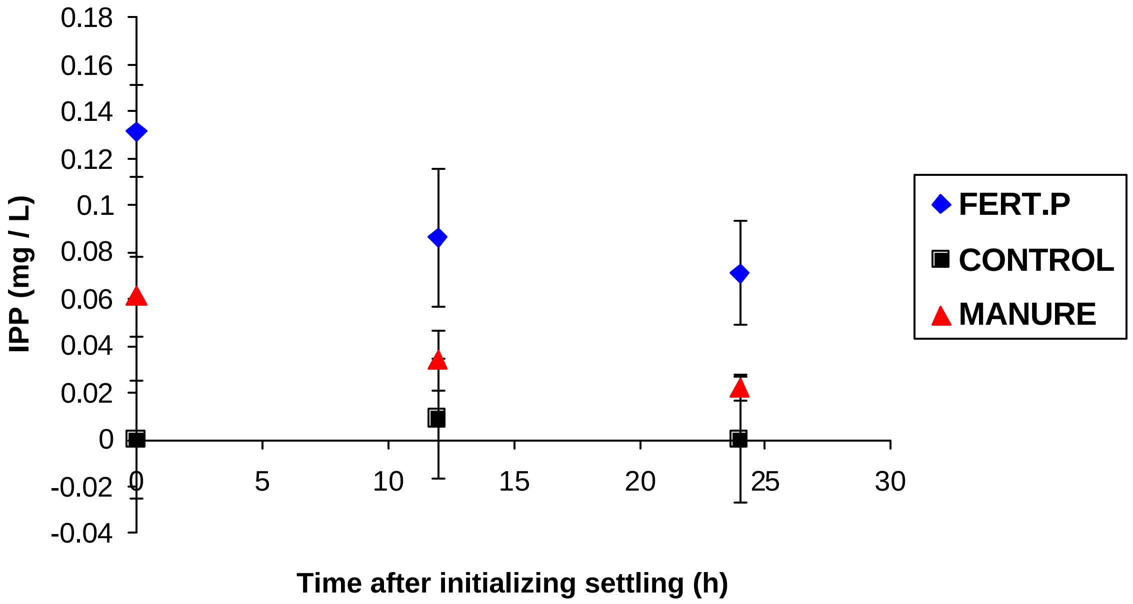


Figure 5-9: Settling of IPP from the Tilsit soil.

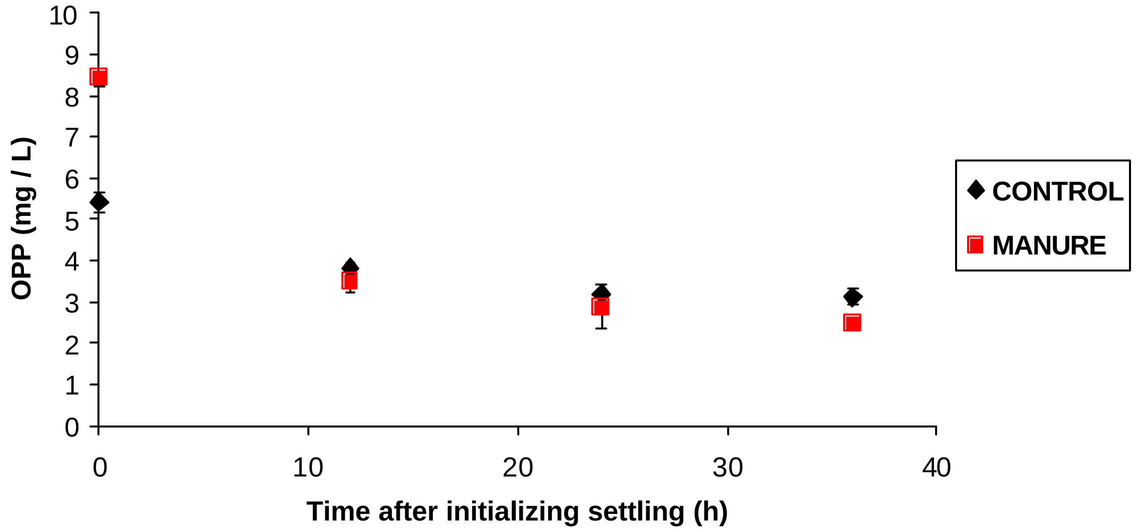


Figure 5-10: Settling of OPP from the Maury soil.

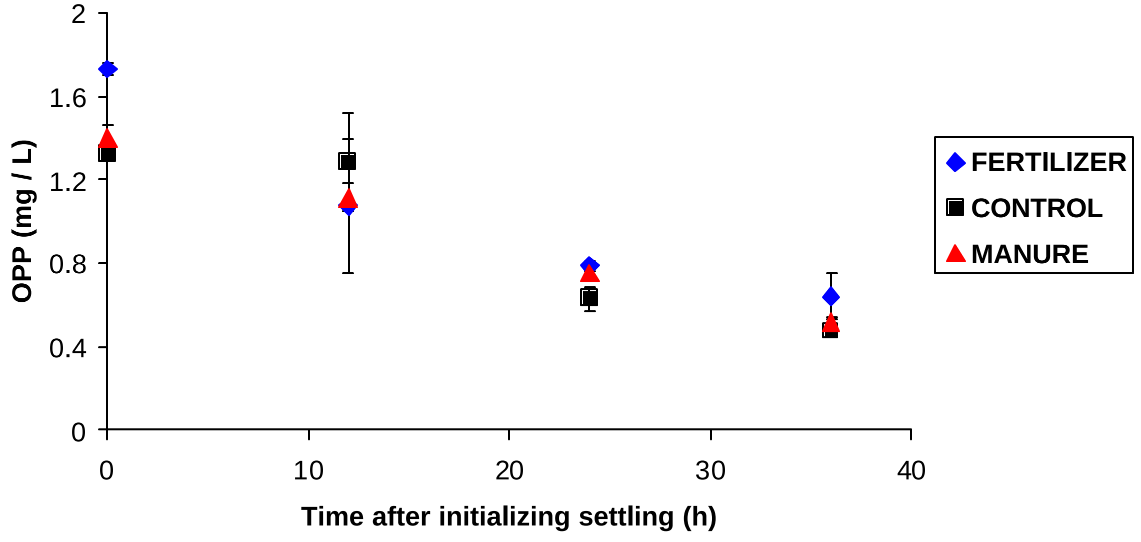


Figure 5-11: Settling of OPP from the Pope soil.

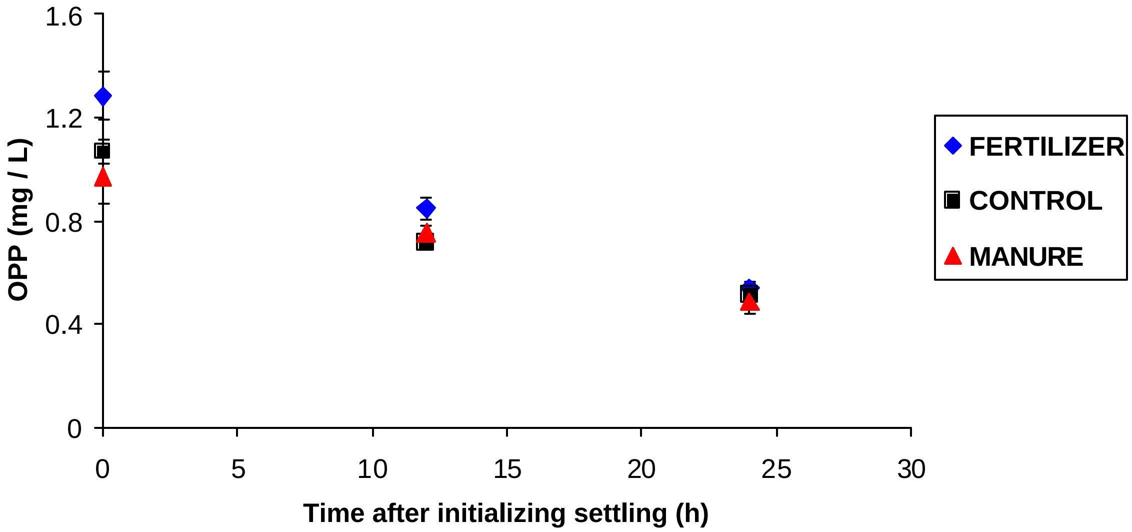


Figure 5-12: Settling of OPP from the Tilsit soil.

Table 5-3. Settling half lives for colloidal particles and P fractions.

SOIL TREATMENT		**TPP**	**IPP**	**OPP**	**WDC**
		-------------(hours)------------------			
POPE	CONTROL	26.6	36.8	26.1	22.5
	FERTILIZER	23.7	36.3	22.6	22.3
	MANURE	27.0	32.6	26.8	24.3
TILSIT	CONTROL	22.3	bdl¶	22.2	15.9
	FERTILIZER	19.7	25.4	19.3	16.4
	MANURE	22.0	15.8	22.4	16.9
MAURY	CONTROL	39.4	38.7	39.8	27.7
	MANURE	16.0	23.5	11.8	22.3

¶ bdl = below detection limit.

TPP = total particulate P

IPP = inorganic particulate P

OPP = organic particulate P

WDC = water dispersible colloids

This phenomenon paralleled the observation that less WDC were generated with more TOC in suspension.

Across all soils and treatments, the PP (0.45 µm < PP < 2µm) and dissolved P (< 0.45µm) fractions constituted approximately 90 and 10%, respectively, of the unfiltered total P (Table 5-4). Within these size fractions, organic P fractions constituted 60-80%, and the inorganic fractions 20-40%, of the total P. In all soils, positive relationships were found between WDC and PP concentrations (Figure 5-13).

The suspension MRP concentration was generally constant during settling for all soils during settling (Figures 5-14, 5-15 and 5-16). The suspension MRP concentrations were higher than the unamended control where fertilizer P had been added to Pope and Tilsit soils, but not where manure had been applied. This was not the case for the Maury soil, where the manured soil exhibited MRP levels three times higher than those of the control. Added P may be much more loosely bound in the Maury soil due to its high native P levels. The dissolved phosphates were generally unaffected while P attached to the colloidal particles started to fall out of suspension.

Another possible explanation for the general lack of change in MRP could be that cations bound to functional surface groups on the organic colloids were in equilibrium with solution phosphates, thus exhibiting a net negative surface charge. So, additional solution phosphates were not attached to colloidal surface sites during settling because they were already in equilibrium. The WDC, through electrostatic attraction between inorganic metal oxides and variable surface charge clay minerals like kaolinites, experienced double layer

Table 5-4. Average initial suspension concentrations of the several P fractions.

SOIL	TREATMENT	TPP	IPP	OPP	TDP	MRP	DOP
		----------------------(mg P L^{-1})----------------------					
POPE	CONTROL	1.45	0.07	1.37	0.15	0.03	0.12
	MANURE	1.49	0.08	1.40	0.15	0.03	0.12
	FERTILIZER	1.90	0.18	1.75	0.25	0.08	0.17
TILSIT	CONTROL	1.10	bdl¶	1.05	0.17	0.03	0.14
	MANURE	1.15	0.06	1.03	0.16	0.03	0.12
	FERTILIZER	1.41	0.14	1.28	0.18	0.06	0.12
MAURY	CONTROL	8.00	2.50	5.70	0.85	0.61	0.25
	MANURE	15.00	5.20	10.0	2.49	2.16	0.25

¶ bdl = below detection limit; TPP = total particulate P; IPP = inorganic particulate P

OPP = organic particulate P; TDP = total dissolved P; DOP = dissolved organic P

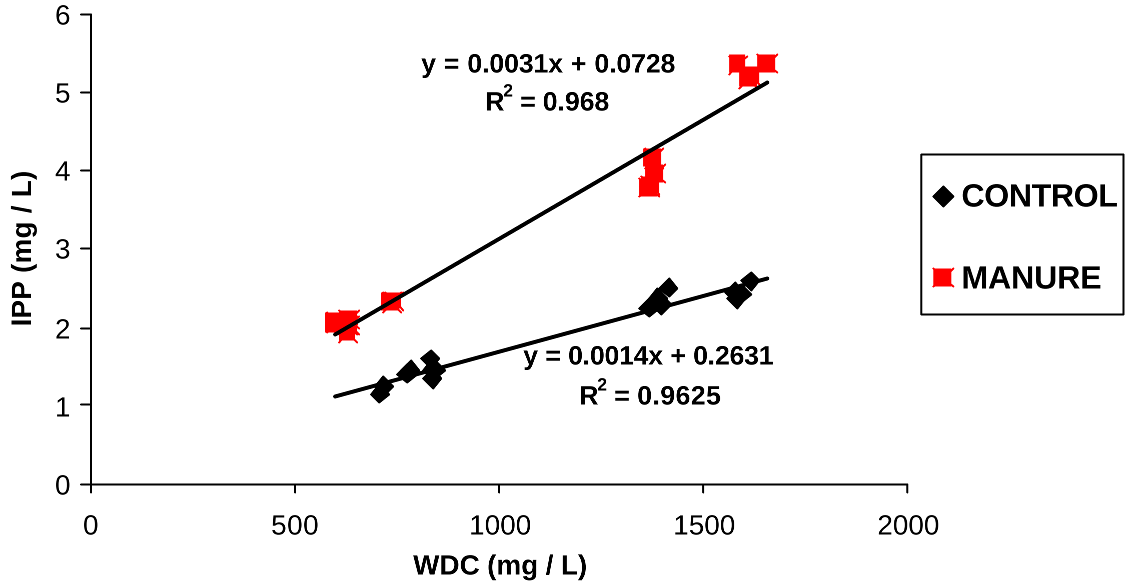


Figure 5-13: Relationship between WDC and inorganic particulate P (IPP) in the Maury soil.

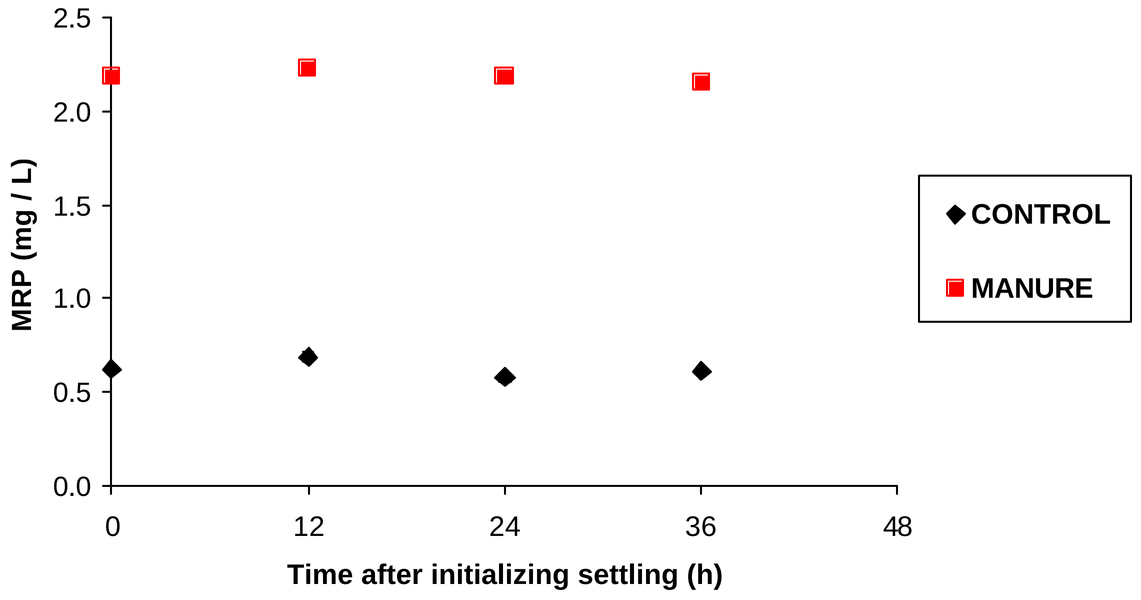


Figure 5-14: Changes in MRP during suspension settling - Maury soil.

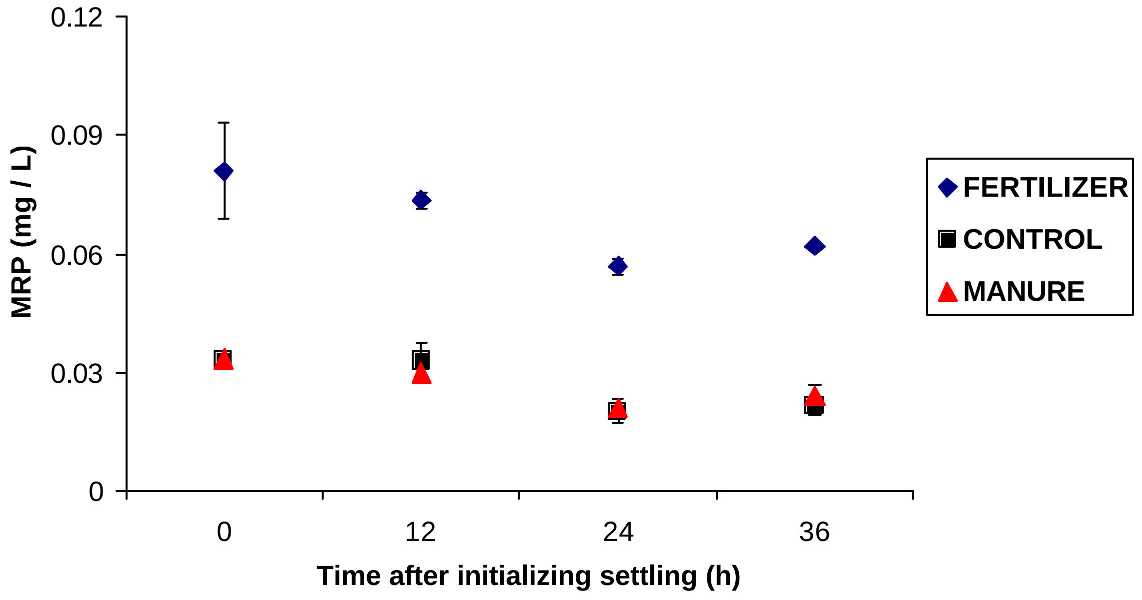


Figure 5-15: Changes in MRP during suspension settling - Pope soil.

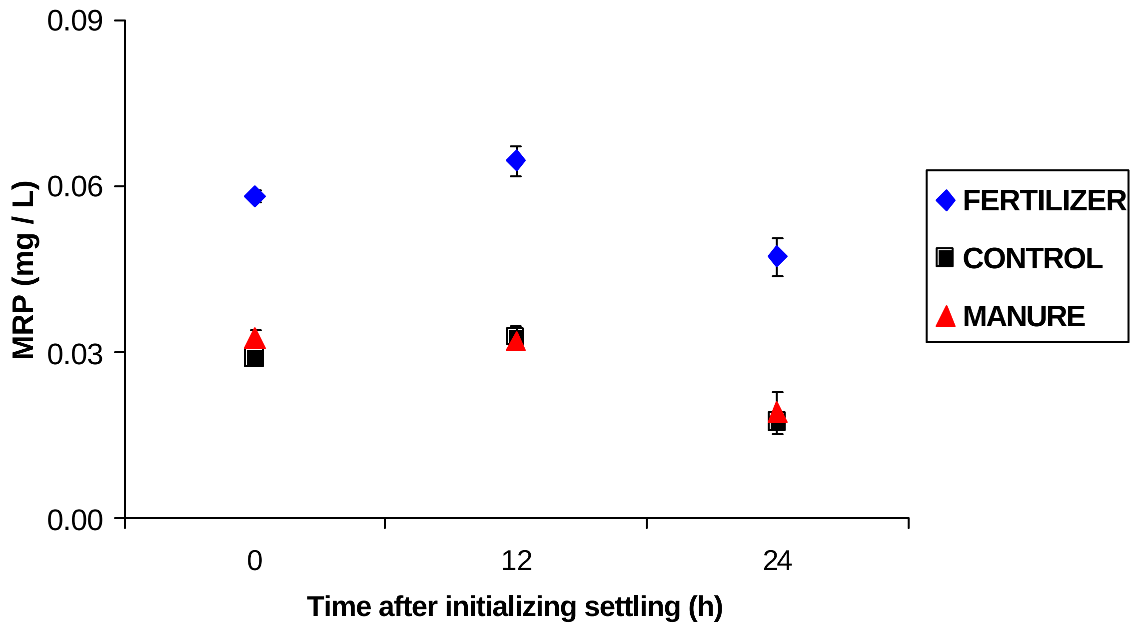


Figure 5-16: Changes in MRP during suspension settling - Tilsit soil.

compression and closer approach. This closer approach increased the frequency and intensity of collisions among colloidal particles, leading to flocculation. So, though manure or fertilizer application reduced the amount of WDC initially generated, these amendments did not reduce suspension MRP concentrations.

Dissolved organic P (DOP) concentrations also remained generally unchanged during the settling period, regardless of whether manure or fertilizer P were added to these soils (Figures 5-17, 5-18 and 5-19). This P fraction is believed to be relatively bioavailable to microorganisms and, like MRP, poses a threat to water quality. Application of manure in excess amounts (Maury soil) significantly increased both MRP and DOP concentrations over those of the unamended control.

Soil test (Mehlich III) P values were positively correlated with concentrations of several P fractions in the unfiltered colloidal suspensions of the three soils (Figures 5-20, 5-21 and 5-22).

Figure 5-21 is the same graph as 5-22, but also includes the Maury soil. Most of the graphs do not include the Maury soil due to the great difference in P concentration between it and the other two soils. This gap in the data makes it difficult to draw a true trendline and could cause distortions in the interpretation of the data. When correlating Mehlich III P values with total dissolved P and dissolved MRP, a second-degree polynomial proved to give the best model fit to the data (Figs. 5-23 and 5-24). The model predicts that when Mehlich III P exceeds 35 ppm (the inflection point), a large increase in MRP will be observed. It is likely that as P amendment

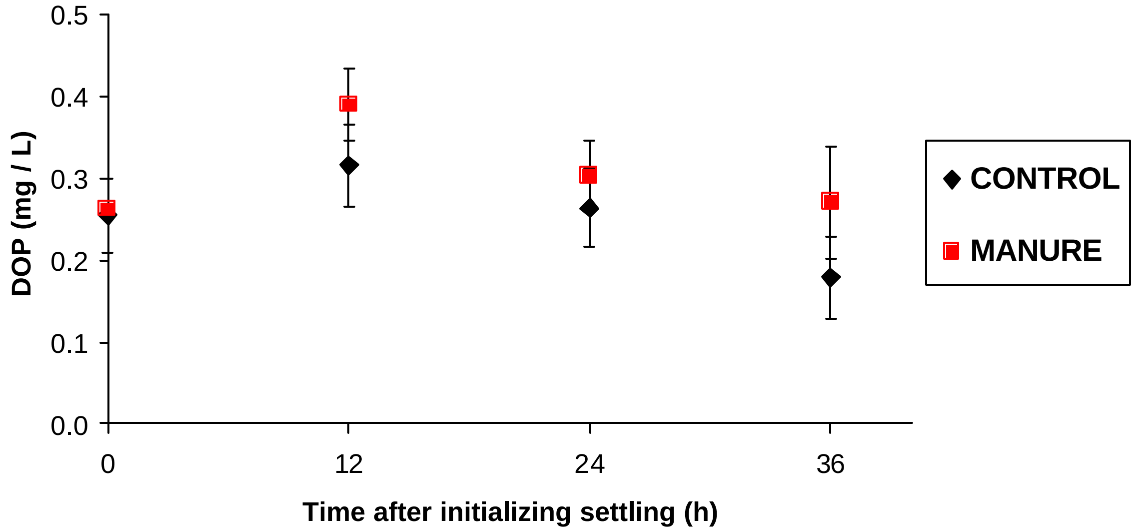


Figure 5-17: Changes in DOP during suspension settling - Maury soil.

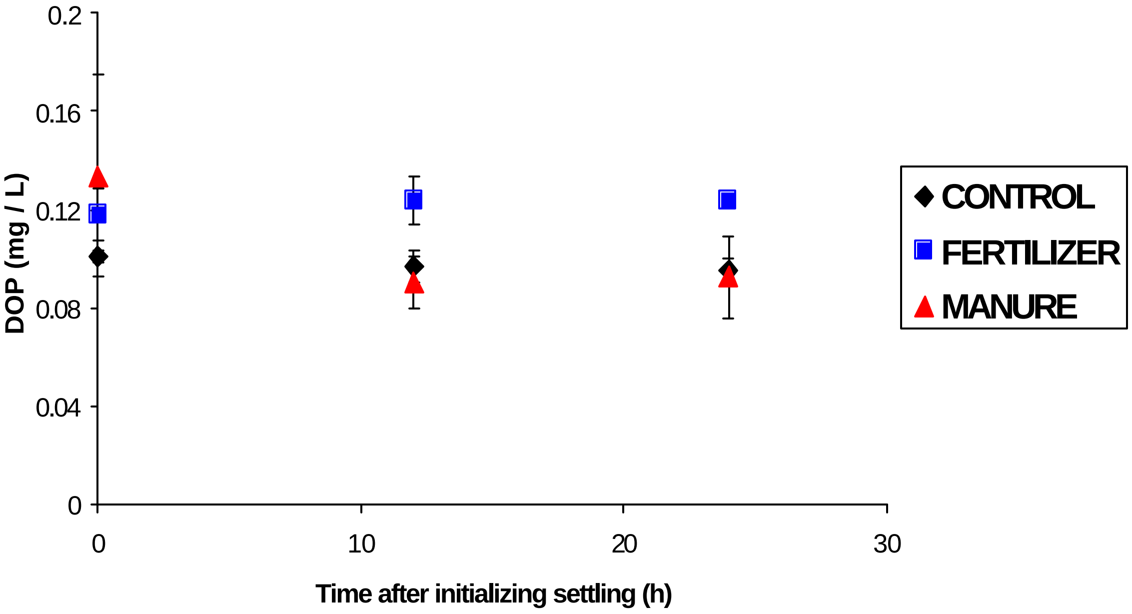


Figure 5-18: Changes in DOP during suspension settling - Tilsit soil.

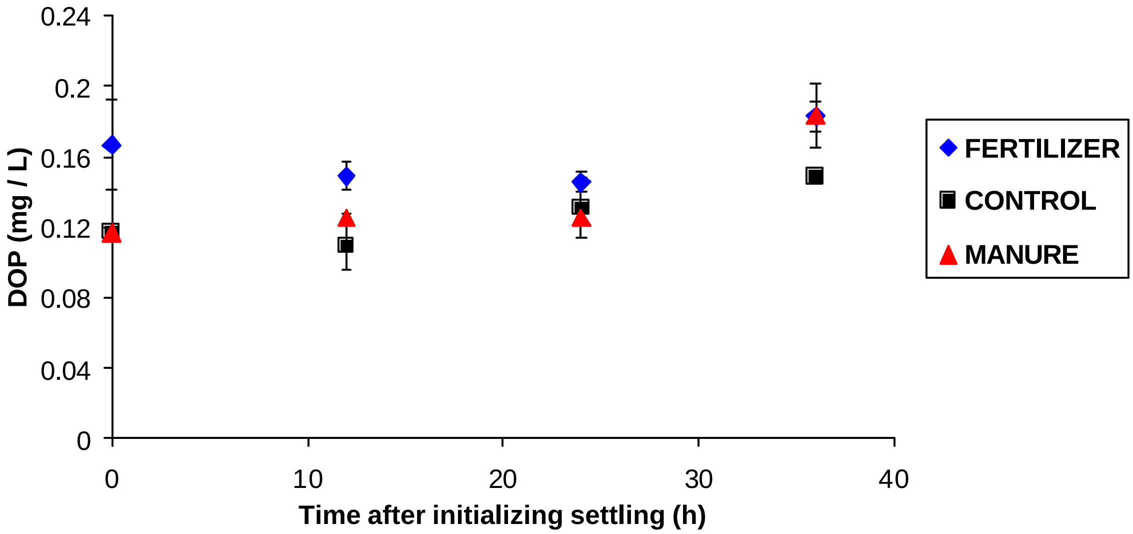


Figure 5-19: Changes in DOP during suspension settling - Pope soil.

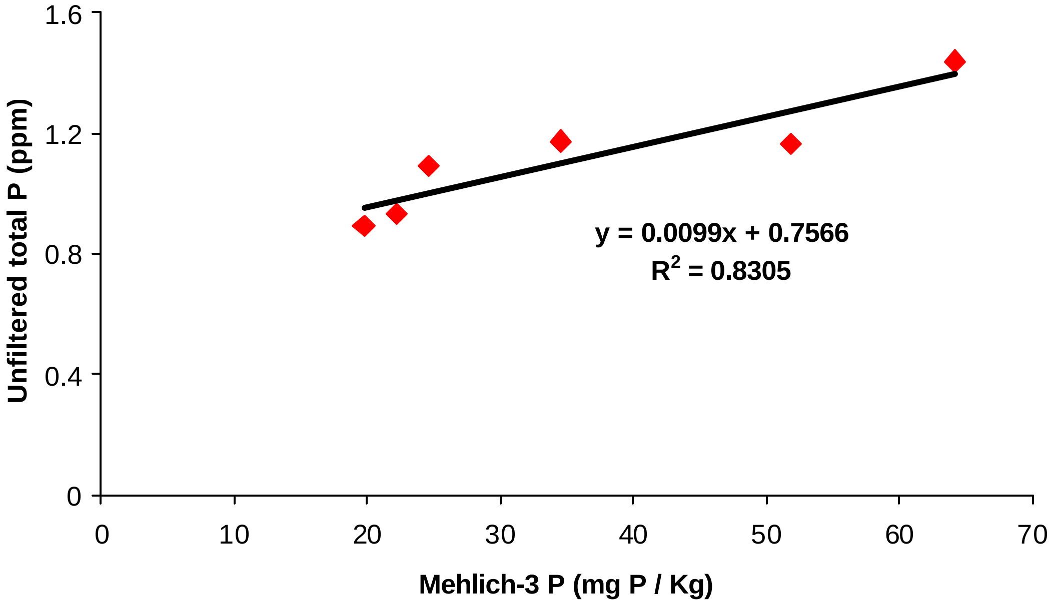


Figure 5-20: Unfiltered total P vs. Mehlich-III P in the Pope and Tilsit soils.

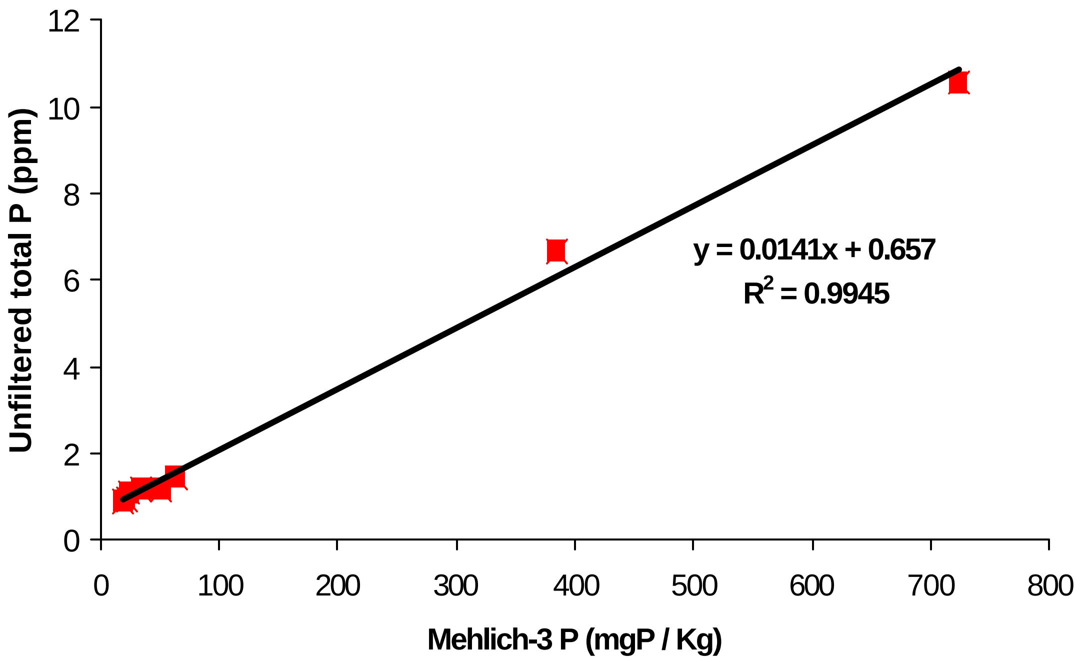


Figure 5-21: Unfiltered total P vs. Mehlich III P in the Pope, Tilsit and Maury soils.

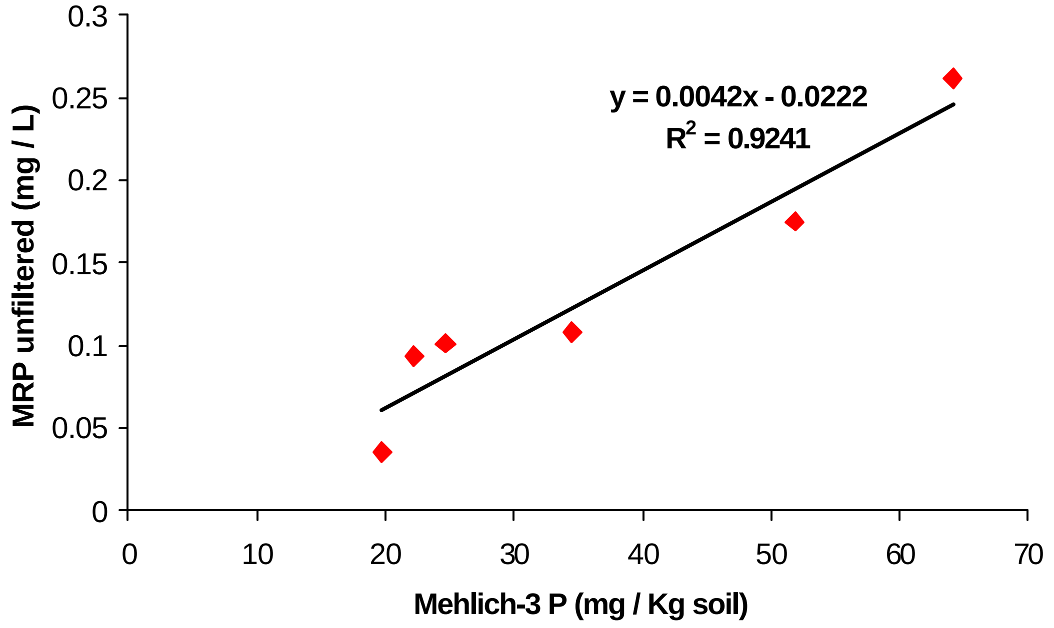


Figure 5-22: Unfiltered MRP vs. Mehlich III P in the Pope and Tilsit soils.

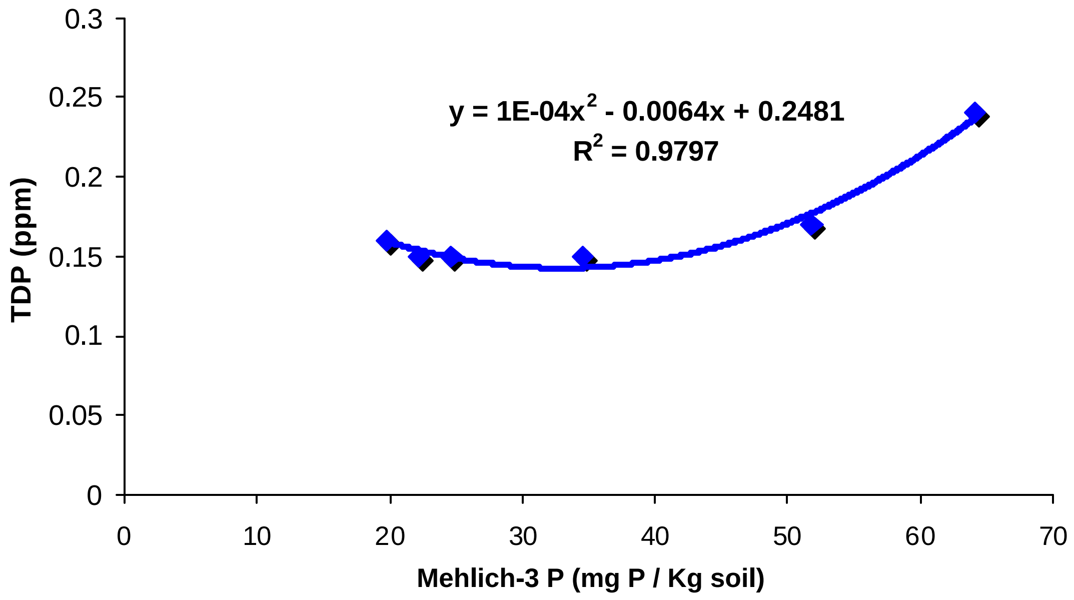


Figure 5-23: Total dissolved P vs. Mehlich III P in the Pope and Tilsit soils.

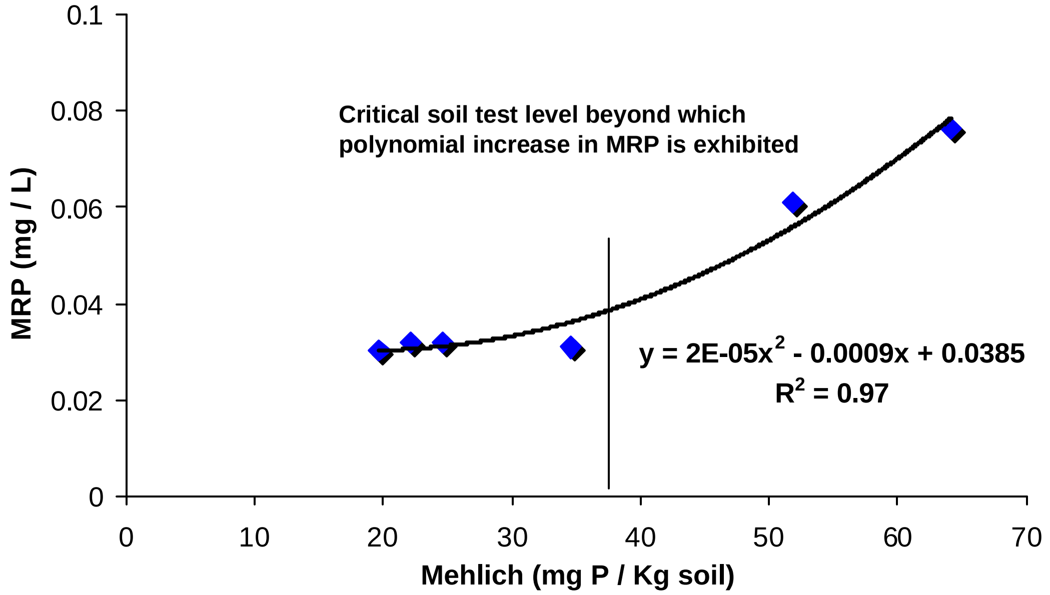


Figure 5-24: MRP vs. Mehlich III P levels in the Pope and Tilsit soils.

exceeds the soil P adsorption capacity, elevated MRP levels reflect the 35 ppm P inflection point. Such "inflection" points have been observed by other researchers (Heckrath et al., 1995; Siddique et. al., 2000) and have been explained using Quantity:Intensity (Q/I) theory. Mehlich III P concentrations in the soil represent the quantity factor. As Q goes up, the I factor (MRP) goes relatively higher, depending on the buffer capacity of the soil. When the buffer capacity (dQ/dI) falls below a critical point, the I factor increases to a much greater extent, reflected in the inflection point.

Resin P, presumably all inorganic P, represents the most bioavailable colloid P fraction. The MRP was strongly related to resin P (Figure 5-25, $r^2 = 0.86$). This is a good indicator of the role that WDC play in P bioavailability and interactions among the various labile P fractions.

Soil test P values not only represent near-term bioavailable P but also P that will be bioavailable over a longer period of time. The Mehlich III P procedure extracts much more P than resin and $NaHCO_3$ combined. These two labile P fractions are considered to be quickly bioavailable and represent what algae and other microorganisms use. Extractable P above that found in these two labile P fractions could be considered less accessible to microorganisms in eutrophic water bodies. However, in oligotrophic bodies, this situation can be reversed and algae use P that under

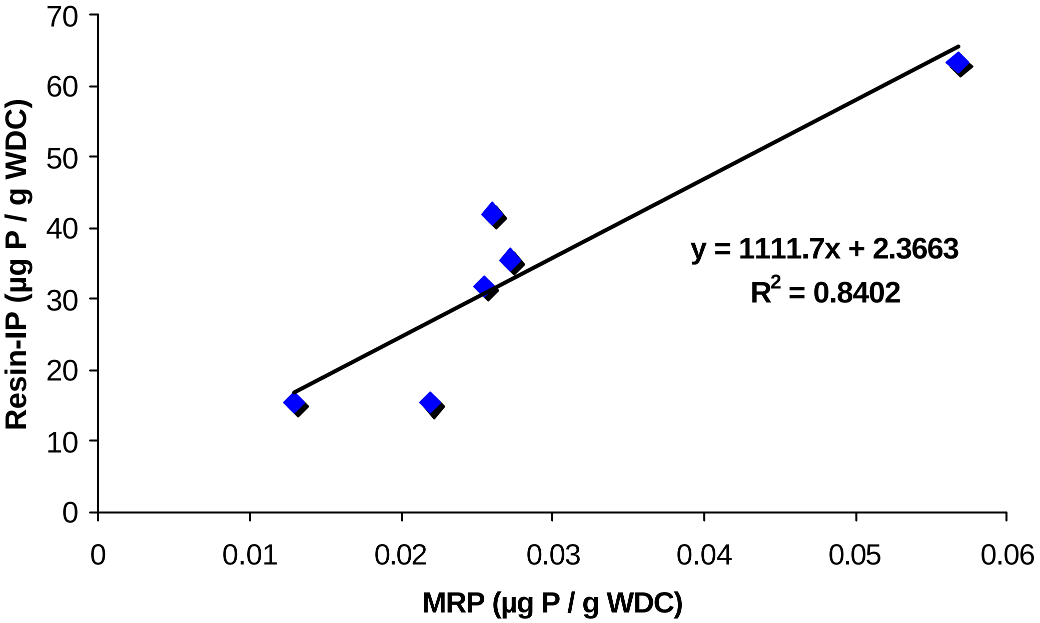


Figure 5-25: Relationship between MRP and resin P in the Pope and Tilsit soils.

other circumstances would not have been bioavailable. Thus, it would have been better if Mehlich III P values had been used only for agronomic purposes. A soil test more environmentally sensitive to changes in P bioavailability should be established that would also take into account the P associated with the WDC fraction of the soil.

To conclude, particulate P fractions parallel WDC settling behavior, although inorganic particulate P tended to stay in suspension longer than the P associated with particulate organic fractions. Dissolved P fractions tend to be unaffected by changes in colloid concentrations remaining in suspension. These WDC P fraction dynamics need to be taken into account in runoff assessments.

CHAPTER SIX

Vertical P Transport Facilitated by water-dispersible colloids in Intact Soil Cores Taken from a Manure-Amended Soil

INTRODUCTION

The stability of colloidal particles in solution influences their mobility and consequently the potential for colloidal P transport. Dispersed colloids tend to remain in suspension when the repulsive potential between two planar colloid surfaces is maximal (Sposito, 1984). Changes in pH and ionic strength of the suspension can lead to a decrease in the repulsive forces between the colloid surfaces and an increase in the electrostatic attractive forces. This causes a gradual destabilization of the system and the particles begin to fall out of suspension.

Although suspended particles can be generated in the soil profile, their mobility is reduced, even halted, by filtration processes. Particle filtration is a physicochemical process that occurs through interception, diffusion and sedimentation (Vinten and Nye, 1985). However, particle filtration is reduced in cases where preferential pathways exist through the soil profile. Preferential flow is the process whereby much of the water and chemical movement through a porous medium follows favored flow paths, bypassing other parts of the medium (Gupta et al., 1999). Preferential flow is conducted through large pores or macropores. These macropores exist in soils high in “cracking” clay content, or having considerable decayed root channels, and macroorganisms such as earthworms, which can build a considerable macropore network in soils. Hydrologically, preferential flow occurs when the intensity of a rainfall exceeds the infiltration capacity of the soil, thus causing surface ponding to take place (Shirmohammandi et al., 1991). This process initiates a significant hydrostatic pressure, causing the larger pores to conduct water and solutes.

Preferential flow has been shown to positively influence subsurface P transport in several research reports (Gachter et al., 1998; Jensen et al., 1998). It has been suggested that losses of particulate and total P are water flux dominated in structured soils (Ulen et al., 1998). Gupta et al. (1999) reported that significant preferential flow and P transport occurred in soil monoliths with radioactive P^{32} phosphate solutions.

Phosphorus movement within the soil has received much less attention as compared to lateral P transport across the soil surface. Still, there are reports of vertical P transport in soils receiving long-term applications of animal waste (Sims et al., 1998; Lucero et al., 1995). Subsurface P movement has also been observed in soils heavily amended with sewage sludge or fertilizer (Siddique et al., 2000). Campbell and Racz (1975) observed vertical P movement beneath an animal feedlot on a coarse textured soil. Novak et al. (2000) detected elevated dissolved inorganic P levels in wells located in shallow ground water, beneath a spray field receiving animal waste.

Different P forms have different mobility. Organic forms of P move to greater depths than soluble inorganic P, apparently because of their lesser reaction with soil colloids (Hannapel et al., 1964; Chardon et al., 1996). Thus, soils receiving large amounts of organic wastes may be more likely to contaminate shallow groundwater via subsurface P movement.

There are conflicting observations as regards whether dissolved or particulate P constitutes the majority of P in percolate. Some studies report that dissolved P constitutes the majority of leached P (Heckrath et al., 1995). Others report that particulate P is the dominant P form found in drainage (Beauchemin et al., 1998).

Water flow dynamics seem to affect the forms of P found in drainage waters. Simard et al. (2000) found that with rapid flow particulate P was the major P form found in drainage, whereas with slow flow, dissolved organic P was the major P form. Another factor that influences P leaching is a change in ionic strength. Hergert et al. (1981) studied the P lost in tile effluent from manured soils. He found that P concentrations increased with increasing water flow (low ionic strength) and decreased with decreasing water flow (higher ionic strength of solution).

Most of the above-cited research referred to P losses from tile drains. Little information exists for P losses in naturally drained soils. Turner and Haygarth (2000) observed subsurface P transport in four grassland soils that differed in P status. Leachate from these naturally drained soils contained 0.1-0.2 ppm total P in their monolith lysimeter studies.

Phosphorus losses through leaching can occur as a function of different processes (Magid et al., 1999): (i) chemical desorption of P from the soil matrix to solution; (ii) P release via reductive dissolution of iron in precipitated iron phosphates; (iii) P dissolution from manure or fertilizer P enriched particles and subsequent entry into preferential pathways; (iv) in situ generation and colloidal associated P transport via macropores.

The last process was the main focus of this study. The objectives of this study were: a) to determine the effect of WDC on the forms and concentrations of P found in the leachate of manured and unmanured intact soil columns; and b) to evaluate whether the potential for P transport was greater in manure amended columns.

MATERIALS AND METHODS

The soil used was a Pope silt loam, located at the Robinson Forest Experiment Station near Quicksand, Kentucky. Sampled field treatments included the unamended control and manure amended, both with four replications. Soil samples were collected to a depth of 22 cm. PVC cylinders with beveled edges (33 cm height, 7.5 cm internal diameter) were used to collect intact soil columns. The cylinders were pushed carefully into the ground and excavated. Extra care was taken to minimize compression of the soil inside the PVC columns. The soil extending below each column was carefully trimmed. Following excavation, they were sealed in polyethylene bags and stored at room temperature. All columns were saturated with deionized water in order to remove air pockets and obtain identical conditions for all columns. The bottom of the columns was covered with cotton cloth to prevent soil loss.

A peristaltic pump was used to supply the two treatments, deionized water or deionized water plus the colloidal suspension, at a rate of 2 mL min^{-1}. Tap water was not used because it has been shown to disperse the soil (Jacobsen et al., 1997). A constant water head (3 cm) approach was used and steady-state flow conditions were maintained. Extra care was taken to maintain a constant flow rate during the experiment. Also, during addition of solutes or suspensions, care was taken to minimize generation of *in situ* WDC at the surface of the soil columns.

All experiments were performed at 20°C. Deionized water or 0.01M $CaCl_2$, the latter also containing 300 mg WDC L^{-1}, were applied to the surface of vertically oriented soil columns taken from the two soil treatments, unamended and manured, both replicated four times. In the columns treated with the colloidal suspension, deionized water was used before applying the WDC. The WDC were isolated, according to procedures mentioned previously, from the corresponding field treatments (unamended or manured) of the Pope soil. In order to evaluate the breakthrough curve for a non-reactive solute, Cl^- was employed as a tracer.

A number of assumptions had to be made. A constant macropore water velocity was assumed and no WDC generation occurred at the column surface. Finally, no hydrodynamic dispersion of particles occurred within the columns during the experiment.

ANALYSES

The WDC concentration was measured with a microplate reader at a wavelength of 540 nm. Calibration curves were constructed after a sequential dilution of WDC to extinction. For calibration, concentrations of WDC were determined gravimetrically by drying duplicate aliquots at 110°C. Drainage water MRP and total P concentrations, in both unfiltered and filtered (0.45µm) portions, was determined using the malachite green method.

RESULTS AND DISCUSSION

The initial concentrations of the WDC and the different colloidal P forms added to the columns are shown in Table 6-1. During the experiment, the pH of the leachate was rather stable, with values ranging over 0.1 to 0.3 pH units from the mean. The leachate pH, averaged over all columns, was 6.0. The saturated hydraulic conductivity was nearly constant throughout the experiment for all columns, except for one manured soil column receiving only water. This column had a more substantial macropore system that conducted the water through the column at an unexpectedly high rate. The behavior of that column did not influence average saturated hydraulic conductivity of that treatment combination (Fig. 6-1).

Table 6-1: Concentrations of different P forms in the initial WDC suspensions taken from unamended (n=3) and manured (n=4) soils.

	UNAMENDED	MANURED
	-----------------(mg L^{-1})	---------------------
WDC	320 ± 19	243 ± 14
MRP	0.09 ± 0.003	bdl[¶]
DOP	0.07 ± 0.004	0.15 ± 0.004
IPP	0.04 ± 0.002	0.03 ± 0.002
OPP	0.42 ± 0.005	0.43 ± 0.006

[¶] bdl = below detection limit.

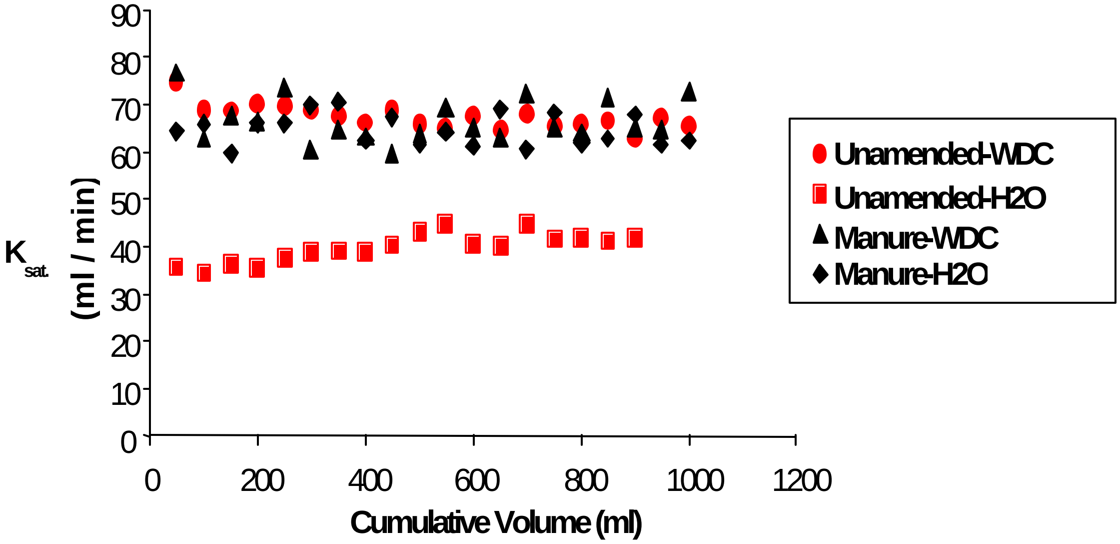


Figure 6-1: Average saturated hydraulic conductivity for the columns in the experiment (n=4 for unamended soil columns and n=3 for manured soil columns).

When water was applied to both manured and unamended soils, no WDC detachment from the soil matrix was observed (Figs. 6-2 and 6-3). When the colloidal suspension was added, a breakthrough curve for the added WDC was exhibited for both manured and unmanured soil columns (Figs. 6-2 and 6-3). Only thirty percent of the initial WDC concentration was detected in the leachate of both manured and unamended columns at the close of the experiment. This may not be the maximal concentration of the WDC that would be transported through the columns. The addition of a maximum of 4 pore volumes of solution/suspension may be insufficient to fully describe the behavior of colloidal particles in this system. However, the transport of colloidal suspensions through the columns and the processes taking place within the surface layer of this soil (adsorption, sedimentation etc.) were not the main issues of this study. Our focus was on P transport and how quickly P might be transported through the columns in the presence or absence of WDC.

Figures 6-4 and 6-5 show the breakthrough curves for inorganic P (IPP) associated either with mere water of the WDC suspensions. When mere water was applied, no IPP came through the unamended soil columns. However, a small amount of IPP was found in the effluent of the manured soil columns. No WDC were found in these columns. This discrepancy may be due to a weakness in the WDC determination method or that the P-bearing colloids were too small to be detected. When WDC were applied, colloidal IP reached 0.6 and 0.8 C/C_o for the unamended and manured soils, respectively. However, IPP breakthrough in the manured soils was much later than

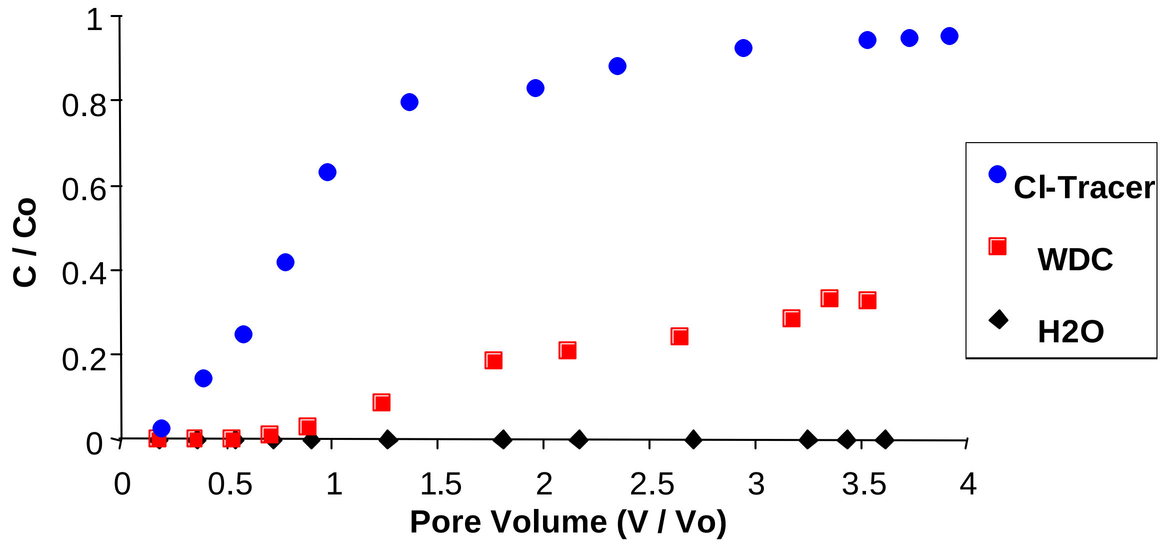


Figure 6-2: Average breakthrough curve for WDC and non-reactive tracer (Cl) in the unamended Pope soil.

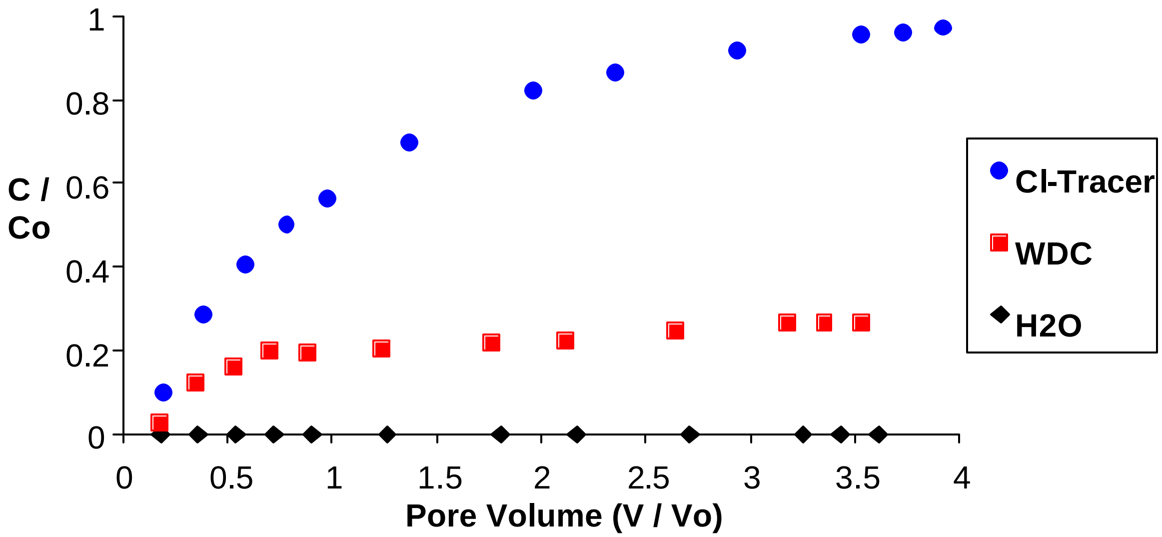


Figure 6-3: Average breakthrough curve for WDC and non-reactive tracer (Cl) in the manured Pope soil.

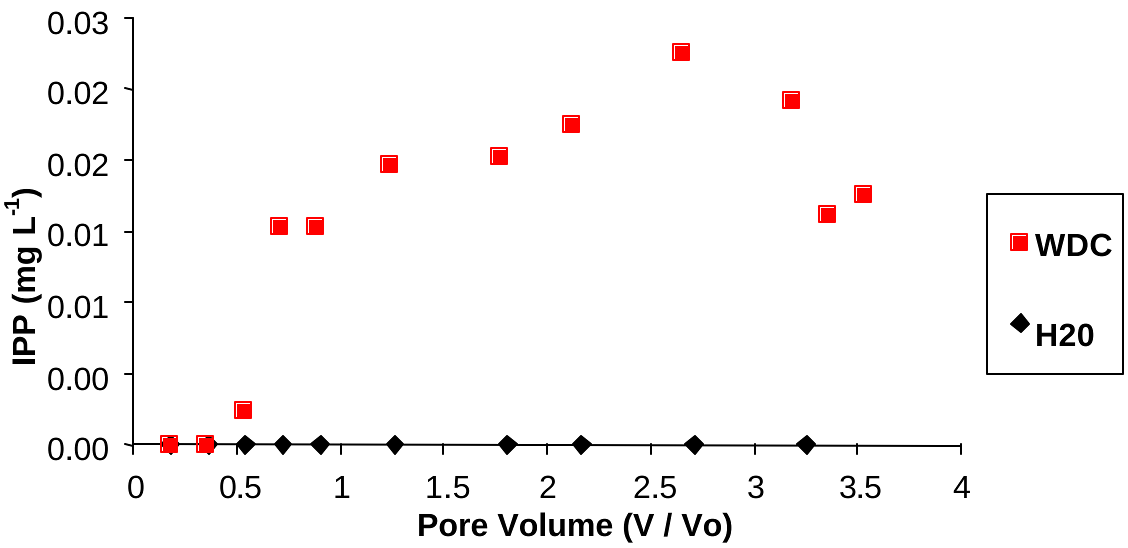


Figure 6-4: Average breakthrough for IPP in the unamended Pope soil.

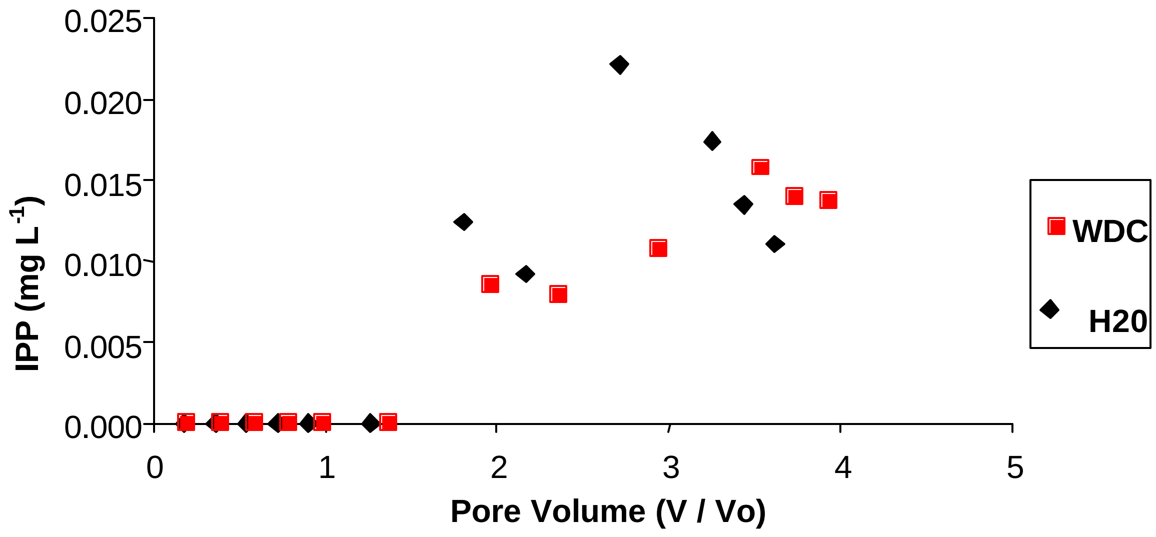


Figure 6-5: Average breakthrough for IPP in the manured Pope soil.

in the unamended soil columns.

Among all the colloid P fractions, the IPP fraction found in this experiment proved to be well correlated only with the resin-IP fraction (Figure 6-6). The correlation of IPP with the resin-IP fraction, the most bioavailable P fraction, suggests that the colloid-facilitated P migrating through the soil profile is also bioavailable.

Organic P associated with the WDC transport (OPP), exhibited breakthrough behavior similar to IPP in the unamended soils (Figures 6-7 and 6-8). In the manured soil columns, OPP breakthrough concentrations were higher than those observed in the unamended soil columns, but all tended to become constant after 4 pore volumes of leaching.

Dissolved P fractions breakthrough behavior was affected by the presence of WDC. Dissolved inorganic P (MRP) exhibited an interesting behavior: in both manured and unamended columns treated with deionized water the MRP concentration drifted slowly upward throughout the experiment, starting at 30% of the initial MRP concentrations in the colloidal suspensions, and finishing at about 50% of those concentrations. A considerable amount of MRP was desorbed from the soil matrix when only water was applied to the unamended soil (Figure 6-9). In the manured soil, even greater concentrations of MRP were hydrolyzed when mere water was applied (Figure 6-10).

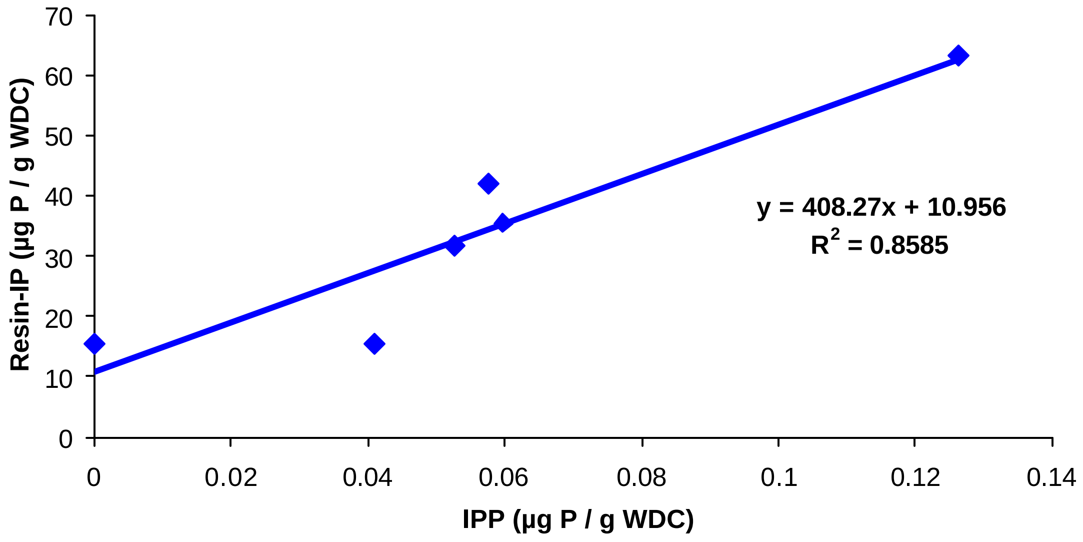


Figure 6-6: Relationship of the average IPP concentration versus resin-IP in the Pope soil columns (*total of 7 columns/points-one is overlapped*).

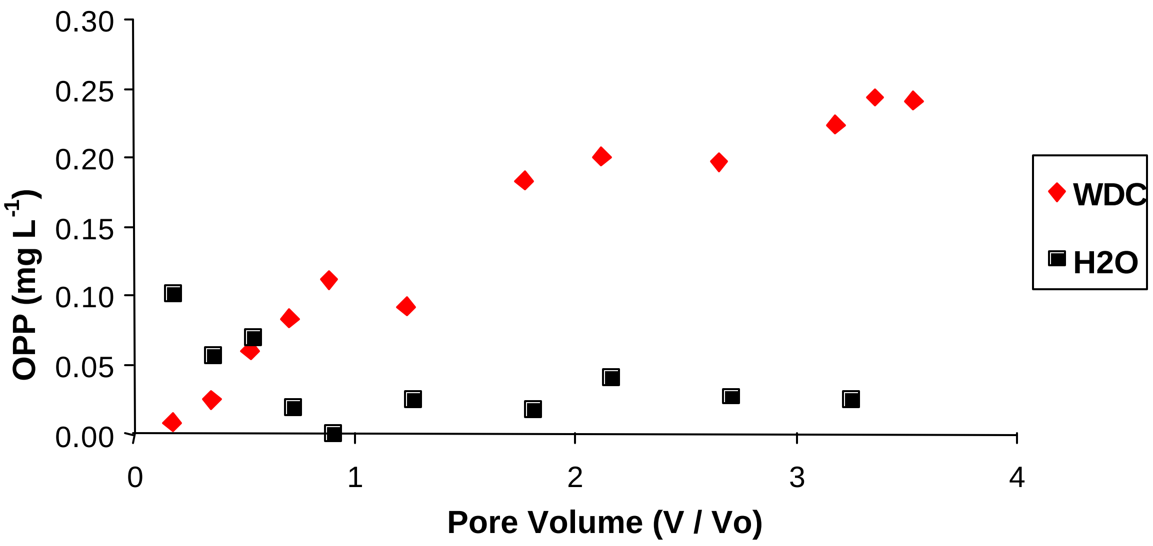


Figure 6-7: Average breakthrough curve for OPP in the unamended Pope soil.

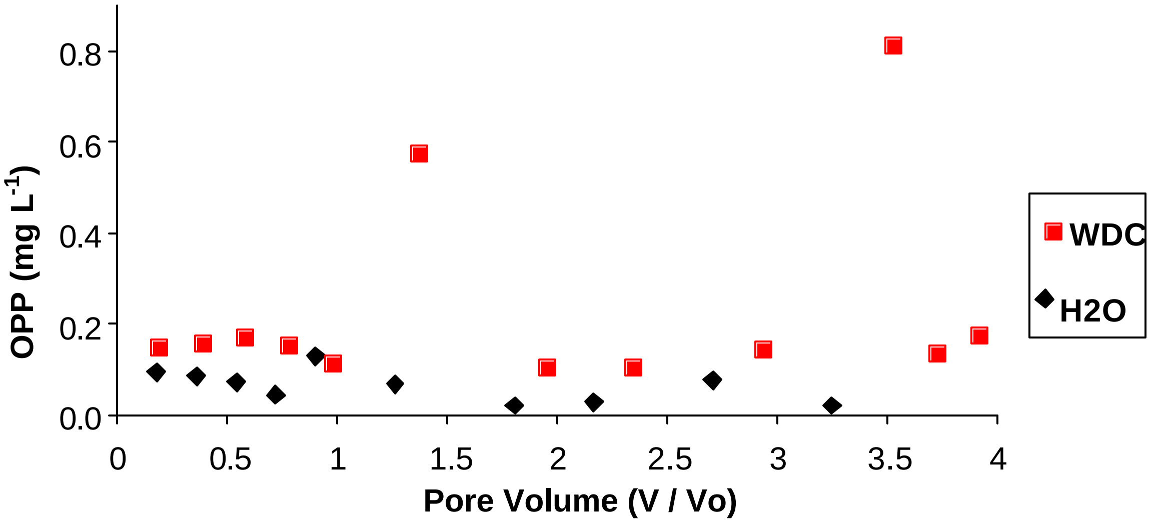


Figure 6-8: Average breakthrough curve for OPP in the manured Pope soil.

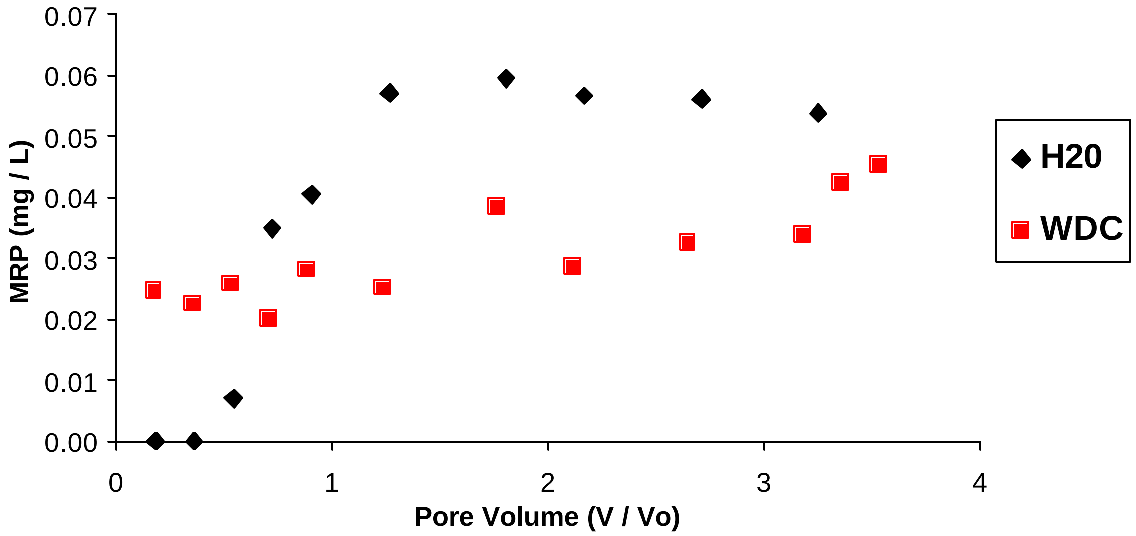


Figure 6-9: Average breakthrough curve for dissolved MRP in the unamended Pope soil.

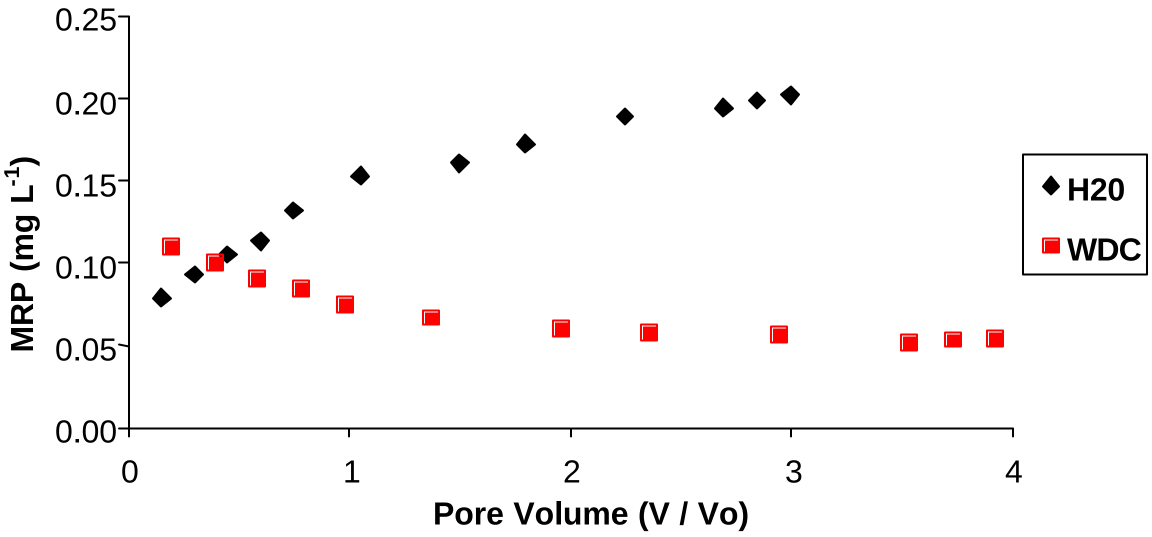


Figure 6-10: Average breakthrough curve for dissolved MRP in the manured Pope soil.

The opposite trend in MRP leachate concentrations was exhibited when WDC were introduced into columns of the manured soil (Figure 6-10). Dissolved MRP levels were initially high, but gradually decreased as the WDC concentration in the effluent increased. It is likely that the WDC were able to adsorb a portion of the MRP from solution and likely carried that sorbed P within the soil column until filtered by the soil. Despite this adsorption/transport process, a considerable amount of MRP was still in the effluent at the close of the experiment. The concentrations of MRP in the manured soil were higher than that in the leachate of the unamended columns.

Burwell et al. (1974) reported that part of the P released in runoff could be readsorbed by soil material during transport. Sharpley et al. (1981) observed the phenomenon of soil colloids adsorbing MRP in a surface runoff study. They found that as the suspended sediment concentration increased the concentration of soluble P decreased. Also, they observed that runoff samples from fertilized areas exhibited greater soluble P sorption by soil material, as compared to unfertilized areas.

In this study, MRP from the manured columns treated with WDC had C/C_0 values approaching six, despite the fact that a portion of the MRP was adsorbed to the WDC. This observation is very important with regard to long-term manure amendment of agricultural fields, since it confirms the potential of manure to contribute to subsurface MRP and colloidal P movement.

The other dissolved P fraction, DOP, was unaffected by the breakthrough behavior of WDC since it remained constant throughout the experiment (Figures 6-11 and6-12).DOP

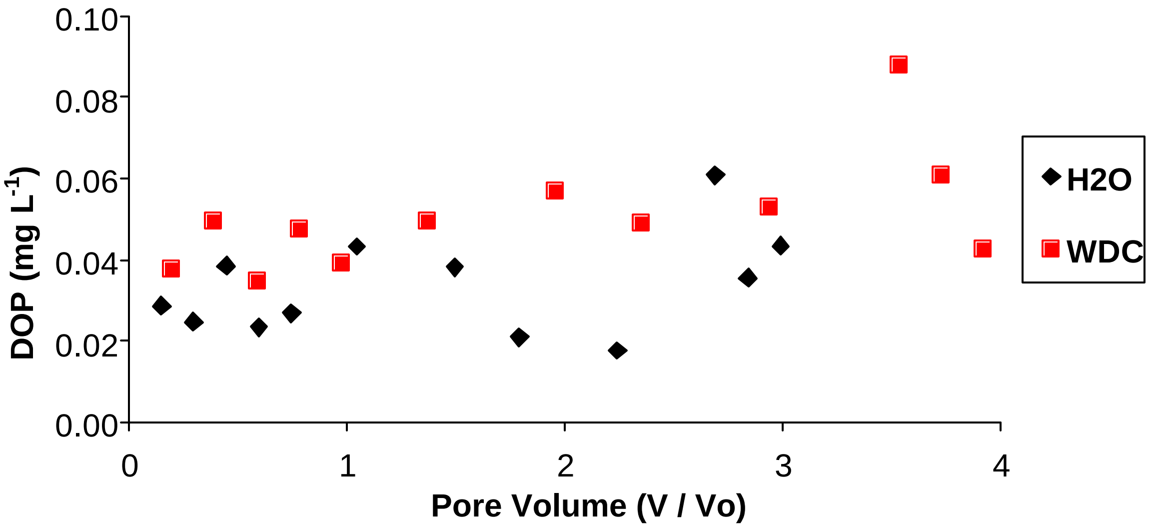


Figure 6-11: Average breakthrough curve for DOP in the manured Pope soil.

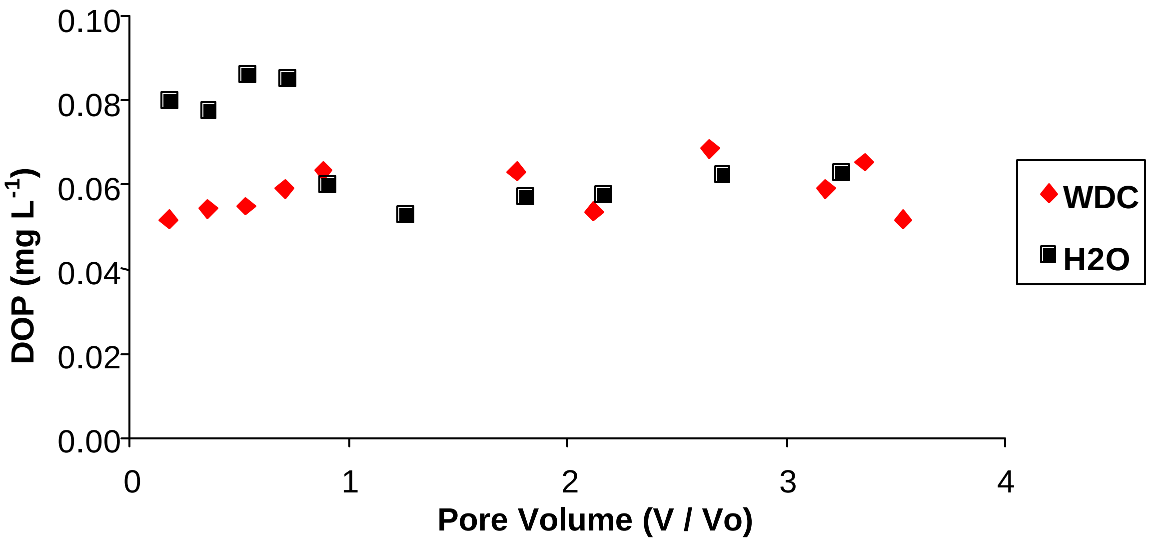


Figure 6- 12: Average breakthrough curve for DOP in the unamended Pope soil.

breakthrough concentrations were around 80% of initial concentrations when WDC were present. In the absence of WDC, leachate DOP concentrations were nearly constant and equal to those observed in the presence of WDC in both manured and unamended columns.

To conclude, particulate P transport via colloid movement through the soil columns was observed. The dissolved P fractions remained unaffected by colloidal P breakthrough behavior in unmanured columns. Dissolved inorganic P concentrations were increased with water movement through both unamended and, to a greater extent, manured, soil columns. It appears that some of the manure P compounds were hydrolyzed as water moved through the soil columns. Potential generation of WDC may reduce the MRP concentration in the moving suspension because of the partial adsorption of the dissolved inorganic P by the WDC. However, this does not reduce the overall negative effects of manure application since these WDC were then able to migrate through the soil columns and carry the newly sorbed MRP along.

These processes could be very important in agricultural fields amended with manure. It seems that intense rainfall events can generate a considerable amount of dissolved and particulate P that is susceptible not only to surface runoff but also to subsurface transport.

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Vita

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