TRANSFORMATION OF PHOSPHORUS AND ITS AVAILABILITY TO PLANTS IN COAL MINE SOILS

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SUMMARY

The work of this thesis is concerned with the study of some aspects of phosphorus cycling in coal mine soils. The introductory chapter presents a short review of some aspects of phosphorus behaviour in soil. The main emphasis has been given to phosphorus forms and their practical significance, reaction and interaction in soils, mobility and crop requirements. A short account of phosphorus behaviour and its problems in coal mine soils has also been discussed.

Chapter 2 deals with the various methods and procedures adopted during the present study. Both routine published methods and methods developed to meet specific problems encountered with coal mine soils have been described.

Although phosphorus is a thoroughly studied plant nutrient in agriculture, much remains to be learned about its status and behaviour in coal mine soils. Chapter 3 deals with the phosphorus status and chemical and biological turnover in coal mine soils. Thirty samples of widely varying properties were taken and various properties measured. All types of extractable phosphorus (acetic acid, anion exchange resin and sodium bicarbonate) measured in the waste material were very low. Carbon dioxide evolution and phosphorus turnover rates showed that a high level of carbon, but very little phosphorus, was turned over. Carbon mineralization correlated with

soil pH. Due to either loss of phosphorus or very low mineralization rate, the effect of soil pH on phosphorus turnover was not pronounced. The present results support the contention that chemical immobilization of phosphorus can be substantial in these coal mine soils. The biological immobilization can also not be ignored. The generally low values of acid phosphatase activities measured in these soils also suggest that phosphorus mineralization may be slower on mine soils. The high values of adsorption maxima, calculated from Langmuir plots, in some soils highlight the great ability the material has for removing phosphate from solution.

Phosphorus is the second most restrictive element to plant growth after nitrogen in coal mine soils. Chapter 4 discusses the problem of different levels of available phosphorus, crop response and the capacity of soil to fix phosphorus after cropping. A growth experiment under controlled conditions, in which varying levels of phosphorus fertilizers were added to five different coal mine soils, was set up. Since it was expected that either biological or chemical immobilization of phosphorus, or both, might play an important role in the lack of response of phosphatic fertilizers to crops in the coal mine soils, both acid phosphatase activities and adsorption maxima were also measured. Three rates of phosphorus, representing the addition of 0, 50 and 100 kg ha^{-1} as sodium dihydrogen phosphate were compared for both crop yield and phosphorus uptake by perennial ryegrass.

The results showed that phosphorus deficiency is a major factor limiting plant growth in most of the coal mine soils. Lolium perenne can benefit from the addition of phosphorus. However, there is a danger that such an increase in phosphorus addition can cause phosphorus losses. Soils which did not show any phosphorus losses exhibited low adsorption maxima indicating low phosphorus fixation. pH and acid phosphatase activities did not show any relationship with crop response to phosphate fertilizer. The high amount of phosphorus mineralized in some soils might partly be due to high acid phosphatase activities. The poor correlations of acetic acid extractable phosphorus with phosphorus uptake and crop yield indicate that either acetic acid is extracting an unavailable pool due to low pH of coal mine soil or has limitations in the analysis of soils derived from different parent materials. Some coal mine soils contain partially weathered or unweathered materials which might play an important role in the poor acetic acid P-crop uptake relationship by exposure of new soil surfaces from which phosphate ions are not taken up by ryegrass.

There is a great deal of controversy surrounding the effect of liming on both phosphorus supplying power and phosphorus extraction of highly weathered acid soils. A laboratory incubation experiment was carried out (Chapter 5) to study the effect of lime on both native and added phosphorus in two selected samples of coal mine soils with different properties. The effect of two extractants, namely acetic acid and ammonium fluoride, was also studied and compared. Three rates of phosphatic fertilizer, 0, 50 and 100 mg P kg^{-1} soil, were applied with and without lime. One level of lime representing the amount required to raise the pH of soil to 6.5 was applied as $CaCO_3$. This study supported the contention that, in the majority of cases, lime induces no effect on phosphorus availability. However, in one soil, lime significantly increased the acetic acid extractable phosphorus.

A brief, concluding chapter discusses some management practices in the light of the experimental results described previously.

CHAPTER 1

GENERAL INTRODUCTION TO SOIL PHOSPHORUS

1.1 Introduction

Phosphorus makes up about 0.12 percent of the earth's crust (van Wazer, 1958), while the phosphorus content of most mineral soils falls between 0.02 and 0.05 percent. Nearly 200 phosphorus minerals have been reported to occur in nature. The most important of all these minerals is the apatite group which constitutes the bulk of the commercial sources of phosphates. Fluorapatites are most easily weatherable and are therefore not commonly found in sediments and soils as primary minerals. The secondary phosphates present in sediments belong to the apatite group.

The significant role of phosphorus in building up and sustaining land fertility has long been recognized by agricultural workers and farmers all over the world. In spite of the marked advancement in our knowledge about phosphorus behaviour in soil, our understanding about phosphorus dynamics in the soil plant system is still far from complete. The trend in the past few years has been to investigate the physical chemistry of the sorption of phosphorus on to simple surfaces, which will lead to ultimate comprehension on the behaviour, physical and chemical interactions and transformation, and the factors that affect the rate and direction of such transformations. Approaches and techniques of phosphorus research developed include soil test methods, soil fertilizer reaction products, Q/I measurements, adsorption isotherms, phosphorus transformation schemes, A or L values and critical phosphorus levels.

The dynamics of phosphorus transformations in the soil system and its fixation and release characteristics have been the subject of numerous research investigations, but in actual practice the most pertinent issue is to know how much phosphorus can be made available to the growing crop from the native soil pool. Lack of response on phosphorus deficient soils might be due to the general tendency to equate a given soil test value with the total pool of available phosphorus in that soil, together with the inadequate research on defining dependable critical levels of available phosphorus.

1.2 Phosphorus in soil

In soils most of the inorganic phosphorus occurs in the clay fraction as salts of orthophosphoric acid. Attempts have been made to classify inorganic soil phosphorus into different compounds according to their extractability in various reagents (Chang and Jackson, 1957). However such a classification seems to be arbitrary and does not reveal their true nature. A good deal of knowledge concerning the nature of soil phosphorus is based on solubility product principles. On the basis of available evidence, phosphorus compounds of calcium, aluminium and iron have been suggested to occur in soils.

1.2.1 Inorganic phosphates

The inorganic phosphorus compounds are mainly divided into two groups; (i) those of calcium and (ii) those of iron and aluminium. A voluminous literature is available on the distribution of various forms of inorganic phosphates.

1.2.1.1 Calcium phosphates

These exist in several forms, the most important of which are:

i) monocalcium phosphate, $Ca(H_2PO_4)_2$, water soluble.

ii) dicalcium phosphate, $CaHPO_4$ and $CaHPO_4.2H_2O$ (dehydrated and hydrated), both are slightly soluble in water.

iii) octacalcium phosphate, $Ca_8H_2(PO_4)_6.5H_2O$.

iv) tricalcium phosphate, $Ca_3(PO_4)_2$.

v) hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$.

Apatite has been reported as a common soil mineral (Shipp and Matelski, 1960). Hydroxyapatite has been reported to be a stable form over a wide range of pH (Larsen, 1967), but Wier et al. (1971) pointed out its varying ion activity products. By comparison fluorapatite appears to be more stable (Wier et al. 1972). Wild (1988) has also stated that hydroxyapatite is not stable in the presence of fluoride ion. The hydroxyapatite in soil invariably contains some carbonate ions, which makes it more chemically reactive. The presence of octacalcium phosphate has been reported in soils which have been limed and fertilized with phosphates (Webber and Mattingly, 1970).

1.2.1.2 Iron and aluminium phosphates

A number of aluminium and iron phosphate minerals have been reported to occur in soils.The most common aluminium phosphates are wavellite ($Al_3(PO_4)(OH)_3.5H_2O$) and variscite ($AlPO_4.2H_2O$). The occurrence of variscite has been postulated in soil of slight acidity (Lindsay and Moreno, 1960). At higher pH values, variscite dissolves incongruently, whereby a more basic solid phase of aluminium hydroxy phosphate is formed (Taylor and Gruney, 1964). This material, by forming a surface complex on variscite, probably controls the phosphorus concentration in solution in acid soil. However, in pure systems, only where the pH of the equilibrium solution is less than 3.1, does the solubility product of variscite control the phosphorus concentration in solution.

The most common iron phosphate is strengite ($FePO_4.2H_2O$). The evidence for the occurrence of strengite has been also obtained from solubility product studies. Chakravarti and Talibudeen (1962) concluded that a compound approximating the composition of strengite may occur in temperate soils in the pH range of 3.8 to 4.2, whereas in tropical soils strengite coexists with hydrated iron oxide from pH 3.8 to 6.7. Under reducing soil conditions, vivianite [$Fe_3(PO_4).8H_2O$] has been found to occur. This mineral is fairly soluble and may be carried down the profile and deposited at lower level. Occluded iron phosphate is believed to be present in the form of dufrenite [$Fe_2(OH)_3PO_4$].

1.2.2 Organic phosphorus compounds

Total organic phosphorus content of soil may vary from traces in arid soils to several hundred mg kg^{-1} in thick forest soils. The organic phosphorus content of soils throughout the world ranges between 7 to 1056 mg kg^{-1} soil, and by and large the soils from temperate regions have higher accumulation of organic phosphorus (Campbell and Racz, 1975). Ireland, Scotland and Finland, being cold regions, exhibited fairly high values of organic phosphorus. The values in subtropical countries with respect to organic phosphorus were very variable and ranged between 0 to 234 mg kg^{-1} soil. Virgin peat lands may contain as high as 95% of total soil phosphorus in organic form. Ipinmidun (1972) found that the parent rock had little effect on the organic phosphorus content of soils derived from gneiss, quartzite, pegmatite, alluvium, sandstone and iron pan. Soils derived from clay soils are generally higher in organic phosphorus than coarse textured soils, but lower than humus soils (Kalia, 1963). High phosphorus content was associated with high organic matter. Walker and Adams (1959) found that the phosphorus content of the organic matter decreased as the rainfall and mean temperature increased. Other factors that affect the organic phosphorus content of soils are: drainage, soil pH cultivation, inorganic phosphorus content of the parent material and sulphur content in areas of low atmospheric returns.

The chemical nature of about half of the organic phosphorus in soils is unknown (Anderson and Malcolm, 1974). Soil organic phosphorus compounds can be generally classified into three groups:

i) the inositol phosphates - the major constituent accounting for more than 50% of the total organic phosphates present.

ii) the nucleic acid, the largest amount of which is found in both plants and microorganisms. However a small proportion (3-10%) of soil organic phosphorus exists as nucleic acids or their derivatives. This group of organic phosphorus can rapidly be mineralized in soil and incorporated into microbial biomass.

iii) the phospholipids, which varies from 0.5 to 7.0% of the total soil organic phosphorus. Among the phospholipids, mainly the glycerophosphatides are present in the soil. It may be contributed by plant debris, animal wastes and microbial biomass.

The soil organic matter consists mainly of carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. All the constituents, except phosphorus and possibly sulphur, are added primarily from the atmosphere via biological tissue. Organic phosphorus owes its origin mainly to the plant and partly to the animal tissues, the ultimate source being the weathered minerals. Organic matter of mineral soils contains carbon:nitrogen:phosphorus in the ratio of about 110:9:1, but the ratio is wider in organic soils (Dalal, 1977). This variability could have been in part a consequence of limitations of the analytical procedures used. Rather arbitrary methods are usually used for the determination of total organic phosphorus by in situ conversion to inorganic forms and subsequent extraction.

Sources of soil solution phosphorus available to plants are the exchangeable phosphate from solid phase, inorganic phosphorus from plant sources and organic phosphorus mineralization. Little information is available on the nature of the organic phosphorus in the soil solution. Wild and Oke (1966) suggest that some of the organic phosphorus in the soil solution may be available to plants. The phosphatase enzyme excreted by the plant root could hydrolyze organic phosphorus in solution, thus releasing inorganic phosphorus (Martin, 1973). Since the organic phosphorus in the soil solution is more mobile than inorganic phosphorus, it would be of interest to investigate this phenomenon further, especially in soils, where organic phosphorus is of predominant form in the soil solution (Hannapel, 1964).

Though some plants have the capacity to utilize certain organic phosphorus compounds directly (Rogers et al., 1940), the bulk of organic phosphorus must be mineralized before it is available to plants. The mineralization of organic phosphorus in soil is largely due to the combined activities of the soil microorganisms and the free enzymes, phosphatases, present in the soil. It has been observed that a reduction in soil organic phosphorus content is associated with a simultaneous increase in the number of microorganisms capable of dephosphorylating these substrates (Halstead et al., 1963), showing their involvement in the mineralization process. However, the significance of the direct involvement of soil microflora in the release of inorganic phosphorus from organic phosphatic compounds in soils is not clearly understood. The factors that regulate the activity of microorganisms mainly govern the mineralization of organic phosphorus in soils. Mineralization of organic phosphorus increases with increasing temperature, particularly above 30°C (Acquaye, 1963). Eid et al. (1951) observed that the optimum temperature for soil organic phosphorus mineralization was 35°C, and that organic phosphorus was not available to plants at 20°C. Dormar (1972) observed that the soil organic phosphorus increased during winter, while decreasing during spring. Adequate moisture is essential for mineralization of organic phosphorus from soil organic matter and decomposing plant residues. Mineralization of organic phosphorus in laboratory experiments was found to be greater in flooded soils than in soils maintained at field capacity (Islam and Ahmad, 1973). It was suggested that this might have been due to anaerobic conditions, occuring in flooded soils. The effect of aeration on phosphorus mineralization is more complex (Basak and Bhattacharya, 1962). Campbell and Racz (1975) suggested that anaerobic conditions increased mineralization rate. Mineralization of soil organic phosphorus increases following liming of acid soils (Islam and Ahmad, 1973). The explanation for this phenomenon is that as the soil pH is increased, microbiological activity is markedly increased (Halstead et al., 1963), with concomitant increases in organic carbon and nitrogen mineralization. However, liming does not always increase mineralization of organic phosphorus (Dalal, 1977). Inorganic phosphorus addition to arable soil has little influence on the quantity of organic phosphorus present (Manning and Salomon, 1965).

Though a considerable amount of native inorganic phosphorus has been transformed into soil organic phosphorus over the years (Walker and Adams, 1958), few attempts have been made to study the factors governing the organic phosphorus build-up in soil and that is why the mechanism of immobilization in soil has not been completely worked out (Dalal, 1977). Van Diest (1968) suggested that increases in soil organic phosphorus might be induced either by an increase in the soil microflora following application of phosphorus fertilizer, or by accumulation of crop residues containing a fraction of organic phosphorus resistant to rapid hydrolysis, or by combination of these two processes. This is supported by the evidence that phosphate added as fertilizer is converted to organic forms in soils, where organic matter levels are increasing, as a result of increased root production and leaf decay. There might also be microbial synthesis of organic phosphorus compounds from inorganic phosphate when an energy source is added to soil, because it has been shown that addition of glucose can cause immobilization of phosphate (Wild, 1988). There is a lack of extensive critical evidence concerning the fixation of organic phosphorus by soils. A few general conclusions can be reached. Certain organic compounds, notably phytin and its derivatives, form insoluble aluminium, iron and calcium compounds in a manner similar to orthophosphate. In addition other compounds possessing a basic or cationic character, namely nucleic acids and their deritaves are adsorbed by a cation exchange mechanism (Hemwall, 1957).

1.3 Mobility of soil phosphorus

Soil phosphorus may be moved in three ways:

1.3.1 Movement by soil organisms

The activity of the larger soil animals will only cause a random redistribution, whereas higher plants will bring about a unidirectional and uneven movement. The whole of the labile soil phosphorus is involved in the latter movement. This process may result in a very uneven distribution of phosphorus in an undisturbed soil profile.

1.3.2 Movement by mass flow

The amount of movement by mass flow is the product of the concentration of phosphorus in the soil solution and the extent of the liquid flow. Movement by mass flow may be of importance in bringing soil phosphorus to the plant root and in causing leaching. Since the concentration of phosphorus in the soil solution is generally low, the amount of movement will normally be insignificant. Similarly phosphorus is not normally considered to be lost by leaching.

1.3.3 Movement by diffusion

When mass flow is unable to supply sufficient quantity of phosphate, and continued uptake occurs, the concentration of phosphate at the root surface is reduced and a concentration gradient is established. Phosphate moves by thermal motion (Brownian movement) from points of high concentration to points of low concentration when a concentration gradient exists. The diffusion of phosphate persists until equilibrium is re-established. The distance the concentration gradient exists from the root surface depends upon the rate of diffusion. The proportion of phosphorus that is supplied will depend on the size of the root system, the phosphorus adsorption characteristics of the root, the rate of water absorption by the root, and the levels of adsorbed and solution phosphorus within the soil.

Diffusion is the process by which matter is transported from one part of a system to another as a result of the thermal movement of molecules or ions. The application of diffusion theory to the supply of plant nutrients is now well established. Evidence indicating that diffusion is an important mechanism for phosphorus supply to plant roots growing in soil has been obtained by autoradiographs (Bhat and Nye, 1973), which show the depletion of phosphorus about the root and the formation of a phosphorus concentration gradient extending perpendicular to the root. The concentration gradient predicted from knowledge of the rate of phosphorus diffusion in the soil was similar to that obtained experimentally.

Diffusion of phosphorus through the soil to the root is the dominant mechanism governing the supply of phosphorus to roots growing in all soils, except those extremely high in phosphorus. Because diffusion is important, all of the factors which govern the rate of phosphorus diffusion to the root and the extent of root growth are important in determining phosphorus availability to plants growing in soil. Transport of phosphorus through the soil causes chemical changes to occur both in the liquid and solid phases, which complicate the measurement of a diffusion coefficient. This complication can be overcome by using ^{32}P which permits the measurement to be made in the absence of concentration gradient. Since diffusion occurs essentially in the liquid phase and an individual phosphorus ion spends only a relatively short time in this phase, the diffusion coefficient of phosphorus in the soil solution will be different from that in free solution. Diffusion through the solid phase is extremely slow and the phosphate ion, being negatively charged, would not likely diffuse along the negatively charged surfaces of soil particles. Phosphorus diffusion through soil is much slower than in pure water for three reasons:

i) soil water occupies only part of the soil so that the cross-sectional area for diffusion is less;

ii) the diffusion path is tortuous because the water is present as films around soil particles;

and iii) most of the diffusable phosphorus is adsorbed on soil surfaces which equilibrates with and buffers the small amount of phosphorus in soil solution that is diffusing.

The diffusion coefficient for $H_2PO_4^-$ in water at 25^oC is $0.89x10^{-5}$ cm^2 sec^{-1} (Parsons, 1959). At pH's between 4.0 and 6.5 most of the inorganic phosphorus is in this form. Assuming that phosphorus diffuses mainly through solution, Nye (1968) proposed the following equation for calcualting the effective diffusion coefficient, De, for diffusion of an ion in soil:

$$De = DQf \frac{dc_1}{dc}$$

where D is the diffusion coefficient in water

Q is the volumetric moisture content of the soil

f is the tortuosity factor and

dc_1/dc is the inverse of the differential buffer capacity of the soil.

Thus, Q,f and dc_1/dc are the three principal factors which influence the size of De.

Volumetric moisture

The percentage by volume of the soil that is occupied by soil water determines the average fraction of cross-sectional area through which phosphorus can diffuse. Hence, net diffusive flux should be directly related to the fractional volumetric moisture content. At low moisture contents, a more rigid structure of water may reduce the diffusive flux near particle surfaces. Since $H_2PO_4^-$ would be less concentrated near soil surfaces because of repulsion of like charges, the net effect for phosphorus is rather small.

Tortuosity

Tortuosity of the diffusion path depends on the thickness of water films and on the fineness of soil particles present. Tortuosity increases greatly as the moisture content of a soil decreases. Increasing tortuosity decreases f. Tortuosity is usually estimated by measuring the diffusion rate of a nonadsorbed ion such as chloride or of tritiated water. Bulk density affects tortuosity of the diffusion path.

Buffering capacity

For phosphorus, which is strongly adsorbed on the surface of soil particles, buffering capacity has a greater influence on the size of the effective diffusion coefficient than does volumetric moisture content or tortuosity. Equilibrium between phosphorus in solution and phosphorus adsorbed on soil surfaces can be determined by adsorption or desorption isotherms. The buffering capacity of a soil is usually highest when adsorbed phosphorus is low, and it decreases as more phosphorus is added to soil because a greater proportion of the added phosphorus remains in solution. Desorption curves conducted under conditions similar to those which occur near roots growing in soil would be the preferable way to measure buffering capacity. Bhat and Nye (1974) found that the amount of phosphorus desorbed was related to the logarithm of the level of phosphorus in the equilibrium solution. Increasing the buffering capacity of phosphorus on the solid phase for phosphorus in solution reduces De, and consequently also reduces the average distance for diffusion.

Temperature

The effect of temperature on diffusion coefficient is primarily due to the effect on viscosity. Increasing temperature from 20 to 30°C would increase diffusion by 30%. Changing the rate of diffusion in solution may not be the only effect of temperature. However, changes in temperature may change the amount of phosphorus found in solution as well as the amount of phosphorus on the solid phase that will equilibrate with solution phosphorus. The effect of temperature on diffusion was evaluated indirectly from data on the effect of temperature on phosphorus in solution and on isotopically exchangeable phosphorus (Sutton, 1969). Sutton (1969) reviewed this subject and reported that increasing temperature increased isotopically exchangeable phosphorus. Also increasing temperature increased phosphorus in solution 1 to 2% for each degree rise in temperature. An increase in the level of solution and adsorbed phosphorus would cause an increase in the amount of phosphorus diffusing, because an increase in concentration gradient may occur even if the diffusion coefficient was not affected; hence, increasing temperature will increase the supply of phosphorus to the root by diffusion.

As a result of these factors, the diffusion coefficient (De) for phosphate in soil is much lower than in water. Typical values measured are in the range 10^{-8}-10^{-11} $cm^3 \cdot sec^{-1}$.

1.4 Interactions of soil phosphorus

Phosphate ions are involved in major chemical reactions in soils. Consequently, phosphorus influences, or is influenced by, the availability or utilization of many other elements, both essential and nonessential.

1.4.1 Major nutrients

Nitrogen

Coprecipitation of ammonium and phosphate is one of the most obvious interactions between nitrogen and phosphorus when these two ions are applied in compound or mixed fertilizers. There is considerable evidence that nitrification causes increased solubility of phosphorus compounds in alkaline soils and is responsible for the increased availability of phosphorus when ammonium fertilizers are used. Acidic fertilizers have been found to increase soil phosphorus availability in the alkaline phosphorus deficient soils. However, there are several reports that in highly phosphorus deficient soils, nitrogen can even be ineffective (Tandon, 1987).

Potassium

Participitation of potassium in phosphorus precipitation is more pronounced in soils with high exchangeable potassium or with easily decomposed potassium bearing minerals. Potassium is probably a universal coprecipitant with phosphorus when mixed fertilizers containing both potassium and phosphorus are applied to soils (Lindsay et al., 1962).

Calcium

The general agreement that calcium and phosphorus react to form a solid-phase component has not been elucidiated to a large extent and the diverse experimental data on calcium-phosphorus interactions in soils remains elusive. However, a step toward possible unification appears in the work of Griffin and Jurinak (1973) in which calcium-phosphate adsorbed on calcite surfaces fit the Langmuir isotherm. They concluded that phosphorus was not present as an adsorbed monolayer on the calcite surface, but as adsorbed ions plus a nucleated crystalline species.

1.4.2 Micronutrients

Iron

Iron exists in soil as oxides and hydroxides as well as ionic forms and these are capable of reacting with native and applied phosphorus in soil and reducing their solubility by chemical precipitation. Hydrated iron oxides are the principal substances in acid soils that combine with soluble phosphates and render them insoluble. $H_2PO_4^-$ is either adsorbed by the iron oxide surface or stoichiometrically precipitated. Parfitt et al. (1975) used infrared and spectroscopic techniques to obtain a structural model between iron oxides and phosphorus ions. Two surface hydroxyl ions (or water molecules) are replaced by one phosphate ion. Two of the oxygen atoms of the phosphorus ions are coordinated, each to a different Fe^{3+} ion, resulting in a surface complex of the type Fe-O-P(O_2)-O-Fe. The iron phosphates, whether precipitated, adsorbed, or occluded, are not generally adequate sources of phosphorus for crops.

Aluminium

Aluminium oxides and hydroxides occur either as discrete compounds in soil or as coatings on other soil particles. They can also exist as amorphous aluminium hydroxy compounds between the layers of expandable alumino silicates. Phosphate ions not only react with soluble aluminium but also with their insoluble hydroxide, and thus causing phosphorus deficiency.

A precipitation reaction between soluble phosphorus and aluminium occurs when their soil solution activities exceed a certain solubility product. The amount of soluble phosphorus immediately removed by aluminium, however, is a function of exchangeable aluminium because of the rapid rate of cation-exchange reactions in soils (Adams, 1986).

Zinc

A large contradiction exists on zinc-phosphorus interaction. There is a possibility of an antagonistic relationship between zinc and phosphorus, inducing zinc deficiency. There are reports that the application of phosphorus or zinc had no effect on the availability of zinc or phosphorus suggesting that the depressing effect of phosphorus on the zinc content of plant was not in the soil but in the plant itself (Safaya, 1976).

Copper

Copper concentrations in soil solutions are normally very low with a large proportion being present as organic complexes. The mechanisms responsible for the reduced availability of copper as a result of phosphorus-copper interaction has been explained by considering: (i) low supply of copper on account of precipitation as copper phosphate and (ii) inhibition of copper uptake by excess $H_2PO_4^-$ in the substrate (Bingham, 1963).

Manganese

Soil solution concentrations of manganese and exchangeable manganese are governed by the solubility of manganese oxides. The dissolution and precipitation of these oxides are governed by soil pH and redox potential. Phosphorus fertilizer may have no effect, or it may increase or decrease manganese uptake by plants, depending upon the acidity produced by applied fertilizer (Bingham and Garber, 1960).

Sulphate, molybdate and boron

Application of phosphate generally appears to stimulate the uptake of micronutrient anions by plants. The phosphate-sulphate interaction is generally synergistic, with applied phosphate increasing sulphate uptake and vice versa. The effect of phosphate fertilizer added to soil is to reduce the amount of adsorbed sulphate, and thereby increase its availability (Barrow, 1970). Application of phosphate fertilizers to acid soils is generally associated with increased availability of molybdate. This might be (i) release of molybdate from the anion exchange complex by $H_2PO_4^-$ which increases the concentration of molybdate in the solution phase of soil or (ii) $H_2PO_4^-$ ions in solution phase of soil, promoting the formation of phosphomolybdate ion which is more readily adsorbed and translocated to plant tops, as compared with molybdate ion alone (Barrow, 1970).

The application of calcium phosphate generally decreases the availability of boron in acid soils (Bingham and Garber, 1960).

1.4.3 Lime

The capacity of acid soil to adsorb phosphate has been reported to increase or decrease as the pH is raised. Two opposing factors appear to influence the effect of lime on phosphate availability. On one hand, phosphate adsorption by amphoteric adsorbing surfaces decreases as the pH rises. On the other hand, in soils initially high in exchangeable aluminium, liming can result in the precipitation of hydroxy aluminium polymers which constitute a new phosphate adsorbing surface in soils. Liming, therefore, would tend to increase phosphate adsorption in soils high in exchangeable aluminium, but decrease it in soil containing low levels of exchangeable aluminium. The beneficial effects obtained in liming acid soils to pH 5.5 is attributed to the precipitation of exchangeable and hydroxy aluminium as aluminium hydroxide which fix less phosphorus, and to the lower bonding energy between sorbed phosphorus and oxide surfaces as their negative charge increases with increasing pH. In some cases liming to a pH of 6.0 is required because factors such as manganese toxicity or calcium deficiency are limiting plant growth. However, in highly weathered acid soils, liming to pH 6.5 or so can induce deficiencies of many nutrients, including magnesium, potassium, manganese, zinc, copper, boron, iron and phosphorus (Haynes, 1984).

1.5 Phosphorus requirements of plants

Phosphorus plays an important role in seed formation, cell division, flowering and fruiting, root development, strengthening straw and improving the crop quality and resistence to diseases. It plays an important role in energy transformation, in the cells of both plants and animals, as such it is necessary for normal transformation of carbohydrates in plants, the changing of starches to sugar. As a component of DNA and RNA it contributes to the genetic character of biological matter.

The phosphorus content of plant tissue is commonly ranging between 0.1 and 0.4 percent on a dry weight basis.

Phosphorus tends to concentrate in the regions of most active growth in the plant. Thus, its content in stem tips and new leaves is higher than in the older tissues produced during the vegetative stage of growth. As maturity is approached, phosphorus is transferred to the forming seed.

For most soils and plant conditions, the phosphorus supply can be broken down into four primary factors; capacity, kinetic, intensity and diffusion. The capacity factor is a measure of the phosphorus reserve in the soil and is related to the amount and forms of solid phase phosphorus, whether organic or inorganic (labile). The kinetic factor is the rate at which the solid phase is released to the solution, and the intensity factor is the amount of phosphate in the soil solution at any given time. The diffusion factor is the rate at which the absorption zone is replenished from nearby soil solution. The kinetic of phosphate uptake by plant is studied as a function of concentration of phosphate in the external solution.

The form in which phosphorus is available in the soil to be taken up by plant root systems depends primarily on the degree of dissociation of phosphate ion. The following equilibrium determines which orthophosphate anion is dominant in the soil solution. Most, possibly all crops take up $H_2PO_4^-$ more readily than HPO_4^{2-} ion, and above pH 7.0, the relative concentration of the divalent is greater than that of monovalent ion.

$$\underset{\text{(high pH)}}{HPO_4^{2-}} + H^+ \rightleftharpoons \underset{\text{(low pH)}}{H_2PO_4^-}$$

The rate of uptake also depends on the microbial environment, presence of other ions, phosphorus concentration near the root, soil temperature, texture of soil and slow phosphorus movement, mostly by diffusion.

Many rhizosphere organisms will compete with the root for phosphate, where the phosphate concentration is low. Some microorganisms will solubilize phosphate compounds of low solubility, by excreting acids. Phosphorus uptake is also affected by temperature, so the deficiency is more common in a dry, cool spring as compared to a warm, moist spring. Clay soils may need a higher level of phosphate than lighter textured soils, because they are more strongly buffered.

Since the uptake of phosphorus depends mostly on the root system, deficiency symptoms most often occur in seedlings and young plants. Phosphorus is mobile within the plant, it is removed from the older, first formed tissue and translocated to the growing points. This causes the symptons to appear on the lower leaves. Deficiency of phosphorus causes stunted growth, dark green colour associated with a purplish coloration in the seedling stage. In the absence of sufficient phosphorus, general maturity of the crop and seed formation are usually delayed.

In order to avoid wasteful practices, phosphorus fertilizer requirements should be assessed as accurately as possible. Recommendation for phosphate fertilization must take into account not only the available phosphate levels but also the phosphate sorption characteristics of soil. Bache and Williams (1971) have proposed that the phosphate requirements of soils can be estimated using quantity:intensity plots. According to Doubleday (1974), adsorption isotherms are the only sound method for assessing the phosphorus requirements of crops.

The phosphorus concentration considered to be critical for plant response varies widely. Perennial ryegrass needs a higher phosphate concentration than grass like _Festuca_ spp. and _Nardus_ _stricta_. Most agricultural crops need normally 10^{-5} M for maximum yield. Ozanne and Shaw (1968) selected 9.7 μM (0.3 ppm) for pasture plants on some Australian soils. Beckwith (1964) recommended 6.4 μM (0.2 ppm) for tropical legumes, a value later confirmed by Fox and Kamprath (1970) for millet (_Pennisetum typhoides_) on some soils of USA. White and Haydock (1968) found 2.2 μM for the maximum yield of _Macroptilium atropurpureus_ on Queensland soils, which was close to the critical value of 1.6 μM (0.05 ppm) for maize grown on Brazilian soils.

Phosphorus fertilizer requirement varies not only with the plant species but also with the buffering capacity of the soil. The value selected is important, because if it is too high more fertilizer than necessary will be applied.

1.6 Studies on phosphorus in colliery spoil

The extraction of any underground mineral deposit such as coal, metal ores, sand, gravel, clay, limestone etc. causes disruption of the land surface, both aesthetically and physically. Though mining for coal in Scotland dates back to the fourteenth century, most mining occurred during the last two centuries to meet the demands of heavy manufacturing industries for steel, gas and electricity. This resulted in a large proportion of derelict land scattered over an area of 18,000 hectares throughout Scotland (MacPherson, 1980). Of this total, about 8,000 hectares comprises mineral waste tips, mainly coal and oil shales (Luke and MacPherson, 1983).

Coal may be extracted either by removing the overburden from shallow seams or by sinking a shaft to deeper seams and hence removing the coal. Reclamation of land disturbed during opencast mining has become an integral part of the mining process and therefore poses far fewer problems compared to deep mining spoil. As most of the National Coal Board product in Great Britain comes from deep mining, reclamation theory and practice has been mainly involved with problems of waste materials resulting from deep mining of coal (Kimber, 1982).

1.6.1 Coal mine waste

Coal mine waste (hereafter referred to as coal mine soil) is the waste material that is brought to the surface when coal is mined underground. It consists of shale, sandstone and other fragments of rock, which have not been exposed to weathering and the slow process of soil formation. When brought to the surface, the discarded mine waste usually has a pH 7.0 or slightly above, suitable for plant growth, and if it has suitable texture and low pyrite content, natural colonization by grasses can occur. However, in many cases the vegetation will be short-lived due to changes in the physical and chemical properties of the spoil which take place on weathering (Costigan et al., 1981).

Most of the coal mine soils may contain spoil from a number of seams, and in many cases from more than one colliery. A single coal mine soil (bing) therefore may contain a heterogeneous mixture of materials with variable properties. Considerable variation in mine soil properties is found not only between sites, but also within each site, due to variation of time of exposure to soil forming processes. Generally, coal mine soil is composed of coal, silica, clay minerals, small amounts of other primary minerals like iron pyrites and the carbonate minerals.

Variation in properties within a bing therefore add to the problems encountered when reclaiming a coal mine soil. Despite regrading and cultivation, it is not guaranteed that the area, once revegetated, will not regress and require further treatment due to the changes which occur in these mine soils. The survival and growth of plants are hampered by a variety of physical and chemical features of such drastically disturbed material.

1.6.2 Physical properties

The physical factors that limit plant growth on coal mine soil include instability, moisture stress and high temperature. In the process of regrading a coal mine soil, heavy machines used for levelling, result in compaction of the waste material. Compaction of spoils adversely affects plant growth by increasing resistence to penetration by roots and to the emergence of seedlings and by decreasing water infiltration by the reduction of pore sizes (Smith et al., 1971). Impermeability to moisture and air is a common feature of regraded coal mine soils (Doubleday, 1974). Compaction of spoil is commonly revealed through bulk density measurements. The higher bulk density of spoil might be due to either, that spoil contains a higher percentage of rock fragments (shale and sandstone), or the rock fragments in spoil tend to be less weathered than those of a soil. Moreover, the age of most spoils is too low to have allowed any significant accumulation of organic matter and therefore the physical conditions of coal mine soils are generally very poor. Such structureless material dries up in summer and becomes waterlogged in winter because of poor water holding and drainage properties.

Slope, aspect, soil moisture and shading by plants influence temperature of spoil material with high temperature injury to plant seedlings. Moreover, the dark colour of the shale, further enhances the spoil temperature, causing drought conditions during summer.

1.6.3 Chemical properties

The chemical factors reducing plant growth on coal mine soil include: low pH (Chadwick, 1973), high levels of aluminium and manganese (Kimber et al., 1978), high concentration of salts (Doubleday, 1974) and low levels of availability of major nutrients expecially nitrogen and phosphorus (Fitter and Bradshaw, 1974; Bloomfield et al., 1982; Pulford et al., 1988).

1.6.3.1 Problems due to pyrite oxidation

Oxidation of iron pyrites (FeS_2) is the principal chemical change which takes place in the presence of air and water, resulting in severe acidity, which causes the pH of the spoil material to fall. The chemical oxidation of iron pyrites can be represented in a simple form by the following equations:

a) oxidation by oxygen

$$2FeS_2 + 2H_2O + 7O_2 \rightarrow 2Fe^{2+} + 4H^+ + 4SO_4^{2-}$$

$$4Fe^{2+} + 10H_2O + O_2 \rightarrow 4Fe(OH)_3 + 8H^+$$

b) oxidation by Fe^{3+}

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

Bacterial oxidation of pyrite also takes place, reduced sulphur compounds being used as an energy source by Thiobacillus thio-oxidans and oxidation of iron being carried out by Thiobacillus ferro-oxidans.

Apart from pyrite, shale may also contain carbonate minerals which can mitigate the effects of the acid to some extent (Caruccio, 1975; Costigan et al, 1981). If the balance is in favour of the carbonate minerals, the resulting product of weathering will be neutral or slightly alkaline. If pyrite content exceeds the carbonate content, then the acidification potential is greater than the neutralizing capacity and very low pH conditions prevail.

Apart from the direct effect of acid on plant roots, a number of spoil reactions which affect plant growth occur; that is

i) The presence of high hydrogen ion concentration results in the replacement by H^+ of the bases on the exchange sites of the spoil, leaving these free in solution and readily leached from the root zone.

ii) The breakdown of clay lattices by the action of acid reduces the spoil's cation exchange capacity, and thereby limits its ability to hold nutrients for plant uptake.

iii) High levels of trace elements, particularly Fe (from pyrite oxidation), Al (from clay lattice breakdown), Mn, Zn, Cu and other trace elements from mineral breakdown, are brought into solution as the pH falls, resulting in possibly toxic levels of these elements being available to plants. (Pulford et al., 1983). This may result in high trace element uptake, causing plant injury and can also affect the uptake of other plant nutrients eg. high Al uptake by roots causes a reduction in phosphate uptake (Clarkson, 1967).

iv) The large amounts of soluble salts produced, both from pyrite oxidation and through acid breakdown of spoil minerals, may cause plant injury by increasing the osmotic pressure of the soil solution, resulting in water being drawn from the plant roots (Bradshaw and Chadwick, 1980), the process called plasmolysis.

v) Fe and Al brought into solution at low pH, and their oxide and hydrous oxide films adsorb or complex phosphate inducing phosphate deficiency (Pulford and Duncan, 1975; Costigan et al., 1982).

Spoil pH may drop rapidly on exposure and can fall to pH 2-3 after a few years, thus discouraging plant growth and destroying any early colonizing plant species. Under such conditions the material is further attacked, exposing fresh surfaces which may be oxidized, resulting in further acid production, depending on the reservoir of oxidizable pyrite and the carbonate:pyrite ratio, until the pyrite is weathered out. The salts and trace metals will be leached from the upper layers. The pH may then begin to rise again, often very slowly until an equilibrium position of about pH 5.0 is reached (Kent, 1982). The resulting medium may be less hostile but is generally deficient in plant nutrients.

1.6.3.2 Plant nutrients

White (1987) lists sixteen elements as essential for growth and reproduction of higher plants. These elements can be divided into two groups on the basis of their concentration in plants:

1) the macronutrients, which occur at concentrations in the plant at greater than 0.1% include carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur, calcium, magnesium, potassium and chlorine and
2) the micronutrients, which are generally less than 0.01% in the plant, include iron, manganese, zinc, copper, boron and molybdenum.

The biological availability of nitrogen, phosphorus and potassium is of considerable importance because they are the major plant nutrients derived from the soil. Phosphorus deficiency is a major factor inhibiting plant growth in colliery spoil (Fitter et al., 1974; Pulford, 1976; Palmer, 1978; Bradshaw and Chadwick, 1980; Palmer and Iverson, 1983; Lister, 1987; Pulford et al., 1988)

Levels of available phosphorus are low due to the low solubility of most inorganic phosphorus compounds, giving a low concentration in solution. The situation is compounded by the fact that reactions occur in the spoil which fix soluble phosphorus and render it unavailable to plants, thus reducing the effectiveness of added soluble phosphorus fertilizers. The mechanisms by which phosphorus availability is reduced are:

a) In acid coal mine soils, the concentration of iron and aluminium ions greatly exceeds that of $H_2PO_4^-$ and so the reaction moves in favour of the formation of the stable hydroxy phosphates, which are more insoluble under acid conditions. Iron in the form of hydrolysis product of pyritic oxidation is believed to bind the phosphorus, although aluminium has also been implicated. Doubleday (1972) has suggested that the fixation of phosphate is partially correlated with amorphous iron oxide, produced by the oxidation of iron pyrites. Pulford and Duncan (1975) found that Tamm oxalate extractable aluminium has little effect on the adsorption of phosphate in coal waste material as compared to Tamm oxalate extractable iron. Liming spoil, although decreasing the soluble Fe and Al levels, results in precipitation with calcium phosphates forming above pH 6.0.

b) Adsorption on the surface of hydrous oxides of Fe and Al forming insoluble phosphates. Oxides and hydroxides of Fe and Al on clay surfaces are positively charged and react with soluble phosphate ions, $H_2PO_4^-$ and HPO_4^{2-}. Phosphate ions fixed in this way form stable complexes and lose exchangeability, with up to 75% of added phosphate being lost to plants by this process in the first year after application (Williams, 1950).

c) Isomorphous replacement in crystal lattices by which phosphate ions may substitute for a silicate or hydroxyl ions.

By these processes fertilizer phosphorus is removed from the available pool and it is often necessary to re-apply it to make good the loss and maintain a vegetation cover (Bloomfield et al., 1982).

1.6.3.3 Organic phosphorus

Colliery spoil, low in decomposable organic matter, will not contain a significant organic phosphorus fraction. However, amendment at reclamation with fertilizer and manure may result in some of the available fertilizer phosphorus being taken up into an organic form (Donald and Williams, 1954) thus reducing its plant availability until microbial breakdown releases it once more to solution. Liming, however, facilitates the release of phosphorus from the organic fraction, as the organic-phosphate bond is less stable under alkaline conditions and also microbial activity is increased by liming.

1.6.4 Biological properties

Coal mine soils exhibit reduced biological activity compared to undisturbed soils. Biological activity, however, is important for the breakdown of litter so that plant nutrients can be released and recycled within the ecosystem and for improving soil physical properties. Microbial growth and activity are also decreased as a result of mining and remain depressed in acid coal mine soils that cannot support vegetation. Respiration (Stroo and Jencks, 1982) and enzyme activities (Stroo and Jencks, 1982; Fresquez et al., 1987) were lower in acid coal mine soils compared to undisturbed soils. Lower respiration activity was attributed to high levels of soluble trace metals which occur at low pH (Sutton and Dick, 1987).

In the last several years, more specific emphasis has been given to soil enzymes in relation to reclamation management. Based on their usefulness, soil enzymatic assays were felt to have an increased potential for better understanding reclamation management effects on plant-soil system development.

1.7 Aim of thesis

As described in previous sections, coal mine soils have caused wide concern in many countries, requiring the expertise of a soil analyst to reclaim them as quickly as possible. These soils can be reclaimed successfully, provided that enough information is obtained from the material before reclamation. Additions of soil amendments such as liming materials, fertilizers, organic amendments, and covering them with soil material so that plant growth can be established, does not guarantee success, as many factors in coal mine soil make it unsuitable for plant growth.

The two nutrients most commonly lacking in these soils are nitrogen and phosphorus, which must be added at higher rates than are required in a soil for plants to grow. Nitrogen addition can be increased by the inclusion of innoculated legumes in the seed mixture. However, no such biological addition is available for phosphorus.

An understanding of the pathway of phosphorus cycling is important for successful reclamation and proper maintenance of plant growth on such drastically disturbed material. The work described in this thesis will assess different aspects of phosphorus transformation and its availability in coal mine soils.

The underlying aim of this work is to assess the value of various amendment treatments in supplying phosphorus to plants growing on coal mine soils. Particular attention will be given to the build-up of phosphorus in the soil, and to the various transformations which will ensure a continued supply of phosphorus once the influence of any reclamation treatment has declined. In addition to assessment of phosphorus status and the phosphate adsorbing ability of coal mine soils, considerable attention will be given to biologically mediated processes. In view of the low levels of phosphorus usually found in such soils, critical consideration will be given to the analytical procedures used.

CHAPTER 2

ROUTINE METHODS AND METHOD DEVELOPMENT AND ASSESSMENT

This chapter is intended to describe the various methods and procedures adopted during the present study. For some of these, routine published methods were used, while in other cases methods had to be developed to meet specific problems encountered with coal mine soils.

2.1 Standard analytical techniques

2.1.1 Measurement of coal mine soil pH

The pH of the coal mine soil was measured electrometrically by a combined glass/reference electrode using a standard 1:2.5 soil:water ratio in deionized water as well as in 0.01 M calcium chloride solution. The pH meter was first standardized with buffer solutions of pH 7.0 and 4.0. Triplicate 10 g samples of fresh coal mine soil were weighed into 4 ounce glass screw cap bottles and 25 cm^3 deionized water were added to each bottle. The suspension was shaken for 30 minutes on an end over end shaker. The pH electrode was then immersed in the suspension, stirred by swirling the electrode slightly and the pH measured.

2.1.2 Percent moisture determination

Percent moisture was determined gravimetrically, by weighing an appropriate amount of fresh soil in a clean weighed basin which was then dried overnight at 110°C, cooled and reweighed. Percent moisture was calculated on oven dry basis =

$$\frac{\text{Weight of fresh soil} - \text{Weight of oven dry soil}}{\text{Weight of oven dry soil}} \times 100$$

2.1.3 Moisture determination at -0.5 bar soil moisture potential

Determination of moisture content at -0.5 bar soil moisture potential was carried out with the pressure plate apparatus (Moisture Equipment Company, Santa Barbara, California). Fresh soil samples were placed on the plate in plastic rings, flooded with water and allowed to soak overnight. Excess water was removed from the plate, which was then placed in the pressure plate apparatus and the pressure was adjusted to 0.5 bar. Samples were allowed to equilibrate for three days or until water loss had ceased. The percent moisture content was determined as mentioned in Section 2.1.2.

2.1.4 Carbon dioxide release and measurement

2.1.4.1 Equipment and reagents

i. pH meter.

ii. Magnetic stirrer.

iii. Sodium hydroxide (1M volumetric solution).

iv. Carbon dioxide-free water, prepared by boiling deionized water to remove gases.

v. Carbonic anhydrase. 10 mg of pure enzyme (Sigma Chemical Company) was dissolved in 10 cm^3 deionized water.

vi. Hydrochloric acid (1 M volumetric standard solution).

vii. Hydrochloric acid (0.05 M volumetric standard solution).

2.1.4.2 Incubation Procedure

An amount of fresh soil equivalent to 50 g on oven dry basis was weighed into a plastic vessel 6 cm in diameter and 8 cm deep (made from a 250 cm^3 plastic bottle). The soil moisture was adjusted to -0.5 bar soil moisture potential by the addition of an appropriate weight of deionized water with the help of a pasteur pipette. The soil container was then placed in a 1.5 litre glass Kilner jar together with 25 cm^3 1 M sodium hydroxide in an open 2 oz glass bottle. The Kilner jar also contained about 20 cm^3 deionized water in order to offset the drying effect of the alkali. Blank incubations, in which the jar contained water and sodium hydroxide but no soil, were also carried through to allow correction to be made for absorption of carbon dioxide from sources other than microbial respiration in the soil. The jars were large enough to ensure that there was enough oxygen for the period of incubation. The Kilner jar lid was then screwed on over a parafilm sealing ring to make it air-tight.

The soils were incubated at 20°C for a period of 8 weeks. Carbon dioxide evolved by the soil was measured at weekly intervals. At the end of each week of incubation the bottle containing sodium hydroxide was removed and closed tightly. Fresh air was allowed to enter the Kilner jar for 10-15 minutes. Care was taken to re-adjust any loss in the weight of the soil, by adding an appropriate weight of deionized water.

Carbon dioxide absorbed by 1 M sodium hydroxide was measured by the titration method of Jenkinson and Powlson (1976).

2.1.4.3 Titration method for carbon dioxide determination

The sodium hydroxide was transferred from the bottle into a 250 cm^3 glass beaker, and the bottle was rinsed 3 times with carbon dioxide-free water into the beaker to ensure that no sodium hydroxide had been left in the bottle. Five drops of carbonic anhydrase (1 mg cm^{-3}) solution were also added to the beaker (Underwood, 1961). Immediately after adding the enzyme, the pH of the solution was brought down to 10 by slow addition of 1 M hydrochloric acid and then to 8.3 by slow addition of 0.05 M hydrochloric acid, the solution being stirred with a magnetic stirrer. The solution was finally titrated with 0.05 M hydrochloric acid to pH 3.7 and the amount of carbon dioxide evolved was calculated from the volume of the 0.05 M hydrochloric acid required to bring down the pH from 8.3 to 3.7, less that required by blanks. 1.0 cm^3 of 0.05 M hydrochloric acid is equivalent to 0.6 mg of carbon dioxide - C in the sodium hydroxide solution.

Decarbonated water was used to wash the pH electrodes between titrations.

2.1.5 Lime requirement

2.1.5.1 Equipment and reagents

i. pH meter equipped with glass and reference electrode.
ii. Standard buffers of pH 7.0 and pH 4.0.
iii. Hydrochloric acid, 0.1 M volumetric solution.
iv. Calcium hydroxide, 0.1 M. 0.37 g calcium hydroxide was dissolved in 1000 cm^3 deionized water.
v. Bromothymol blue indicator (0.04 percent).

2.1.5.2 Procedure

The lime water, prepared approximately as 0.01 N, was first standardized by titration of 10 cm^3 with 0.01 N hydrochloric acid, using bromothymol blue indicator. The end point is colorless solution.

5g oven dry equivalent, fresh samples of 4 mm sieved soil were weighed into 4 oz screw cap shaking bottles. Addition of the standardized 0.01 N calcium hydroxide and deionized water was made as shown in the Table 2.1.

Table 2.1 Additions of lime water for lime requirement

Sample No.	1	2	3	4	5	6
Vol. of 0.01 N $Ca(OH)_2$ cm^3	0	10	20	30	40	50
Vol. of deionized water cm^3	50	40	30	20	10	0

Each bottle was shaken on end over end shaker for 45 minutes, the pH of the suspension measured and a graph of pH against volume of 0.01 N $Ca(OH)_2$ added was plotted. From the graph, the volume of $Ca(OH)_2$ required to raise the pH to 6.5 was found and the lime requirement calculated.

2.1.5.3 Calculation

Lime requirement was calculated from the following relationship:

Lime Requirement in g $CaCO_3$/100 g soil

$$= v \times \frac{\text{N of } Ca(OH)_2}{2} \times f$$

where

v = volume of $Ca(OH)_2$ used

$$f = \frac{\text{mol. weight } Ca(OH)_2}{1000} \times \frac{\text{mol. weight } CaCO_3}{\text{mol. weight of } Ca(OH)_2}$$

2.1.6 Anion exchange resin method for extractable phosphorus

The analytical procedure as presented by Amer et al. (1955) and Hislop and Cooke (1968) is both time-consuming and difficult to carry out on a large scale. The major difficulties have now been overcome by placing the resin in nylon mesh bags (Sibbesen, 1977). Smith (1979), following the procedure of Sibbesen (1977), found that the resin bag procedure possesses the additional advantage of greatly facilitating the soil resin separation step and confirmed, further, that the method can be used on soils with high aluminium, iron and organic matter contents. The method followed was that of Sibbesen (1977).

2.1.6.1 Reagents

i. Nylon mesh, 0.2 mm openings (fine mesh nylon straining bag, Boots, Nottingham, England), 5 x10 cm (Sibbesen, 1977). The bags were prepared by folding the nylon netting and sewing the sides. The edges were sealed by means of an electric iron. The netting side was turned in and the bottom end was sewed up and the edge sealed.

ii. Resin, Dowex IX8. The beads of moist resin, Dowex, with a 0.5 mm diameter were obtained by sieving. The coarse fraction which would not pass through a 500 micron sieve was used. 2.0 g resin were placed in bags made of nylon netting.

iii. 1 M sodium chloride made in 0.1 M hydrochloric acid. 58.5 g analar grade sodium chloride was dissolved in 900 cm^3, 0.1 M hydrochloric acid (8.5 cm^3 concentrated hydrochloric acid, diluted to 1000 cm^3) and the volume was made up to 1000 cm^3, with 0.1 M hydrochloric acid.

iv. Working standard phosphorus solution (Section 2.2.2) made in deionized water.

2.1.6.2 Procedure

The bags of resin were placed in 4 oz screw top shaking bottles, containing 50 cm^3 1 M sodium chloride (in 0.1 M hydrochloric acid) and shaken for half an hour on an orbital shaker. The solution was discarded and the procedure repeated twice more. The excess of chloride was removed by successive shaking with deionized water (each time the decanted water was tested with silver nitrate, to ensure that excess chloride had been removed). The bags were shaken vigorously by hand for 2-3 seconds to remove excess water. The resin in the chloride form was then ready for use.

The chloride resin bags were transferred to 4 oz screw top shaking bottles, containing 50 cm^3 deionized water and 2.0 g oven dry equivalent of fresh, 4 mm sieved soil. The bottles were shaken on an orbital shaker at 50 rpm for 24 hours at 2°C. After the shaking, the soil was poured out and the bag rinsed with deionized water. Phosphate adsorbed on the resin was then eluted from the resin by shaking with 50 cm^3 1 M sodium chloride (in 0.1 M hydrochloric acid) for 30 minutes on an orbital shaker at 50 rpm at $2^{o}C$. The phosphorus concentration was determined by the method described in Section 2.2.1.2, using 0.0 and 1.0 μg P cm^{-1} as working standard solutions.

The resin bags were removed and transferred to a large bottle, where the resin in the bags could be regenerated as the chloride form.

2.1.7 Phosphatase activity

The method described here for assay of acid phosphatase activities is based on the colorimetric estimation of the p-nitrophenol released by phosphatase activity, when soil is incubated with buffered (pH 6.5), disodium paranitrophenyl phosphate (Pnpp) substrate, at controlled temperature ($20^{o}C$) for 30 minutes (Tabatabai and Bremner, 1969).

2.1.7.1 Reagents

i. Modified universal buffer (MUB) stock solution. 3.025 g of tris (hydroxymethyl) aminomethane (THAM), 2.9 g of maleic acid, 3.5 g of citric acid, and 1.57 g of boric acid (H_3BO_3) were dissolved in 122 cm^3 of 1 M sodium hydroxide. This solution was transferred quantitatively to 250 cm^3 volumetric flask and the volume made up to 250 cm^3 with deionized water and stored in the refrigerator.

ii. Toluene (Analar BDH)

iii. Modified universal buffer (MUB), pH 6.5.

50 cm^3 of MUB stock solution was placed in a beaker containing a magnetic stirring bar, and the beaker placed on a magnetic stirrer. The solution was titrated to pH 6.5 with 0.1 M hydrochloric acid and transferred to a 250 cm^3 flask and made to volume with water.

iv. p-nitrophenyl phosphate solution ($NO_2C_6H_4Na_2PO_4$), 0.115 M.

1.513 g disodium paranitrophenyl phosphate (BDH Analar) was dissolved in about 40 cm^3 of modified universal buffer (MUB) pH 6.5, and transferred to a 50 cm^3 volumetric flask and made to volume with MUB of the same pH. This solution was stored in the refrigerator.

v. Calcium chloride, 0.5 M.

73.5 g of calcium chloride was dissolved in about 700 cm^3 of decarbonated water, and diluted to 1 litre with decarbonated water.

vi. Sodium hydroxide, 0.5 M

20 g sodium hydroxide was dissolved in about 700 cm^3 of decarbonated water and diluted to 1 litre with decarbonated water.

vii. Hydrochloric acid, 0.1 M.

25 cm^3 commercially prepared 1 M hydrochloric acid was made up to 250 cm^3 in a volumetric flask, using decarbonated water.

viii. Decarbonated water.

Deionized water was heated to boiling, cooled and stored in a tight sealed flask.

ix. Standard p-nitrophenol solution.

1.0 g of p-nitrophenol was dissolved in about 70 cm^3 of decarbonated water, and diluted to 1 litre using decarbonated water. This is a solution of 1000 $\mu g\ cm^{-3}$ which was stored in the refrigerator.

x. Dilute standard p-nitrophenol was made up as follows:

1 cm^3 stock standard p-nitrophenol was diluted to 100 cm^3. This is 10 $\mu g\ cm^{-3}$ p-nitrophenol. 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 $\mu g\ cm^{-3}$ p-nitrophenol were then prepared by further dilution.

2.1.7.2 Procedure

Duplicate 1.0 g oven dry equivalent samples of fresh soil, 4 mm sieved were weighed and placed in 10 cm^3 plastic capped vials. 0.2 cm^3 of toluene, 4 cm^3 of MUB (pH 6.5) and 1.0 cm^3 of p-nitrophenyl phosphate (Pnpp) made in the same buffer were added. The bottles were swirled for a few seconds to mix the contents. Control samples were also run in the same manner, except that substrate was not added. The bottles were shaken on a multivortex shaker in an incubator for 1 hour at 37°C. After incubation, 1 cm^3 of 0.5 M calcium chloride and 4 cm^3 of 0.5 M sodium hydroxide were added. The bottles were swirled again for a few seconds. The soil suspension was filtered through a Whatman No. 1, 11.0 cm filter paper. The filtrate was diluted with water using 1 cm^3 filtrate into a 25 cm^3 volumetric flask (1:25). The diluted filtrate was transferred to a colorimeter tube and its yellow colour intensity was measured, as absorbance, with a Bausch and Lomb spectrophotometer, fitted with a blue (CE A59R) phototube at 420 nm wavelength. The p-nitrophenol content of the filtrate was calculated by reference to a calibration graph in the range 0-5 μg Pnp cm^{-3}.

Controls were run with each soil analysed to allow for colour not derived from p-nitrophenol released by phosphatase activity. For controls, the procedure described for assay of phosphatase activity was followed, except that 1 cm^3 of Pnpp was added after addition of 0.5 M calcium chloride and 4 cm^3 of 0.5 M sodium hydroxide (immediately before filtration of the soil suspension).

2.1.8 Phosphate adsorption

2.1.8.1 Reagents

i. Phosphorus stock solution I.
10.985 g of KH_2PO_4 was weighed accurately and dissolved in deionized water. The solution was diluted to 1000 cm^3 with deionized water. This solution contained 2500 μg P cm^{-3}.

ii. Phosphorus stock solution II.
100 cm^3 of the stock solution I was diluted to 1000 cm^3 with deionized water. This solution contained 250 μg P cm^{-3}.

iii. Diluted phosphorus solutions.

0.0, 1.0, 2.0, 5.0, 7.0, 10.0, 20.0, 30.0, 50.0, 70.0 and 100.0 μg P cm^{-3}. Dilute phosphorus solutions containing 0.0 to 10.0 μg P cm^{-3} and those containing 10.0 to 100.0 μg P cm^{-3} were respectively made from stock solution II and stock solution I using deionized water. These solutions were made in 250 cm^3 volumetric flasks.

2.1.8.2 Procedure

To obtain data for an adsorption isotherm, 1 g oven dry equivalent fresh soil samples, ground to pass a 4 mm sieve were weighed in 4 oz bottles. 50 cm^3 of solutions containing 0.0, 1.0, 2.0, 5.0, 7.0, 10.0, 20.0, 30.0, 50.0, 70.0 and 100.0 μg P cm^{-3} were added to these bottles. The bottles were tightly sealed and equilibrated by shaking on an end over end shaker for 24 hours at constant temperature (20^oC). Following equilibration, the soil suspensions were filtered through Whatman no. 42, 15.0 cm filter paper. A control set of phosphorus solutions having the same concentration (0-100 mg P l^{-1}) was also run, for measuring the initial phosphorus in solution. Aliquots of the clear filtrates and control solutions were analysed for phosphorus using the method described in Section 2.2.1.2 by the Technicon AutoAnalyzer. Two ranges 0-5 and 5-100 μg P cm^{-3} were used. The 0-5 range was used undiluted with sample rate of 50 per hour. The 5-100 range was diluted 21-fold with a sample flow rate of 0.1 cm^3 per minute and water flow rate of 2.0 cm^3 per minute. The sample rate was reduced to 40 per hour. The difference between the amount of phosphorus in solution after shaking and the amount initially present was taken as the amount of phosphorus adsorbed by the soil from the KH_2PO_4 solution.

2.1.9 Plant analysis

Plant material was extracted by the wet pressure digestion method adopted by Adrian (1973) and used for calcium determination by Adrian and Stevens (1977). About 0.5 g ground plant tissue was weighed into 60 cm^3 polypropylene bottles with screw caps. 4 cm^3 of concentrated nitric acid and 2 cm^3 of concentrated perchloric acid were delivered to these bottles via 100 cm^3 burettes (0.10 cm^3 division), which enhanced the speed and accuracy with which the acids could be added. The bottles were swirled to soak all the samples and kept for 24 hours for predigestion, loosely capped in the fume cupboard. Predigestion is essential as the bottle could burst if heated immediately. After predigestion the bottle caps were screwed on tightly, the bottles placed in plastic trays (twelve on each) having 1 inch depth of water to provide constant heating to each bottle. They were then placed on a water bath in a fume cupboard for 3 hours at $65^{o}C$. After cooling the bottles, 2 cm^3 deionized water was added, the bottles, loosely recapped, replaced on the water bath, and the heating continued for a further 2 hours to expel the excess of volatile acid. The bottles were then thoroughly cooled, 15 cm^3 of deionized water was then added to each bottle, and the contents were filtered through Whatman No. 540, size 11.0 cm filter paper into 100 cm^3 volumetric flasks. The volume was made to the mark with deionized water. The filtrate was shaken thoroughly and some of the solution from the flask was transferred to a clean 4 dram glass bottle and stored in the refrigerator for analysis. All analyses were carried out in duplicate. Blank digestions containing only the acid mixture were carried through all the steps along with the samples. The plant digest was analysed for phosphorus content at 880 nm based on a Technicon AutoAnalyzer method with the following modifications.

A dilution/neutralization step was incorporated with a sample flow rate 0.23 $cm^3\ min^{-1}$ and neutralization solution flow rate 2.0 $cm^3\ min^{-1}$. This gave a dilution of approximately 10:1 ratio, while also neutralizing the samples. To maintain a constant pH during the working cycle, an acid wash chamber solution was also used.

i. Wash Chamber Solution:

40 cm^3 and 20 cm^3 concentrated nitric acid and perchloric acid was mixed in deionized water and volume made to 1 litre.

ii. Neutralizing Solution:

4 g analar sodium hydroxide was dissolved in a litre of deionized water.

The standards in the range 2-25 $\mu g\ cm^{-3}$, made in 4 cm^3 concentrated nitric acid per 100 cm^{-3}, were used for phosphorus determination. All reagents used were of analytical grade.

2.2 Methods developed

2.2.1 Technicon analysis

2.2.1.1 Introduction

Accurate and rapid methods for phosphorus determination in soils and plant material are indispensable for research and diagnostic purposes. No single method has yet been reported to be suitable for the varied analysis encountered in plant nutrition studies. Murphy and Riley (1962) proposed an ascorbic acid method for phosphorus determination in water. This method has been recommended for bicarbonate soil extracts by Colwell (1965), Durge and Paliwal (1967), and Watanabe and Olsen (1965), for total phosphorus in soils by Alexander and Robertson (1968) and soil fixation studies by Ahenkorah (1968). With some modifications by John (1970), the ascorbic acid procedure of Murphy and Riley (1962) is the most versatile and commonly used method for soil and plant phosphorus determination.

Several automated techniques have been developed for the analysis of various extracts for soil phosphate using Technicon AutoAnalyzer equipment. Lacy (1965) used a method based on the ascorbic acid reduction technique of Fogg and Wilkinson (1958) for the Bray No. 2 extract (Bray and Kurtz, 1945). Ng (1970) also used a modified ascorbic acid method for Bray No. 2 extracts, and a vanadomolybdate method for phosphorus in perchloric-sulphuric acid digests of soils for total phosphorus. Colwell (1965) adopted the colorimetric method of Murphy and Riley (1962) to the automatic analysis of sodium bicarbonate (Olsen) extracts.

The automated method is labour-saving, and can handle large numbers of samples on a routine basis; it is also more precise as each sample is treated in exactly the same manner within strictly prescribed and maintained operating conditions as compared to manual methods. The technique can also tolerate slight turbidity in the extracts which are sometimes difficult to filter clean, for example in adsorption experiments where very little soil is used.

Investigations were made into the design of suitable Technicon manifolds to overcome specific problems relating to the use of various soil extractant solutions.

2.2.1.2 Basic method for phosphorus determination in water

This method is based on the formation of a phospho molybdate complex which is reduced using ascorbic acid to give a blue colour which may be measured at 660 or 880 nm. In order to speed up the formation of the complex, a small amount of antimony is also added. The intensity of the blue colour is proportional to the phosphorus concentration in the original solution.

The method is applicable to water samples and a wide range of soil extractant solutions and acid digests of plant or soil material.

2.2.1.2.1 Manifold design

The system Technicon AutoAnalyzer II consisted of sampler, proportioning pump, heating bath, chemistry manifold, single pen chart recorder, BBC microcomputer and colorimeter equipped with either 650 or 880 nm filter, 342 phototube and blue coded bulb. The manifold used for phosphorus analysis was set up as shown in Figure 2.1.

There must be no short lengths of plastic tubing in the manifold once the ascorbic acid has been added, since the blue colour is adsorbed by plastic at high phosphorus concentrations and released at low phosphorus concentrations leading to poor wash out between samples. Moreover, a slightly longer version of the glass connecting tube inside the S.C.I.C (colorimeter), is needed using silicon rubber tubing to make the shortest possible connection of tubing to the flow cell. There must be no stainless steel in the sample line, as it adsorbs and releases phosphorus depending upon its age. The sampler probe should be replaced with a glass capillary and stainless steel nipples with platinum nipples or plastic sleeved joints.

The reagents have to be relatively concentrated to remain stable, so to obtain the required total flow rate and final reagent concentrations, extra water is required if the sample flow rate is not to be very large.

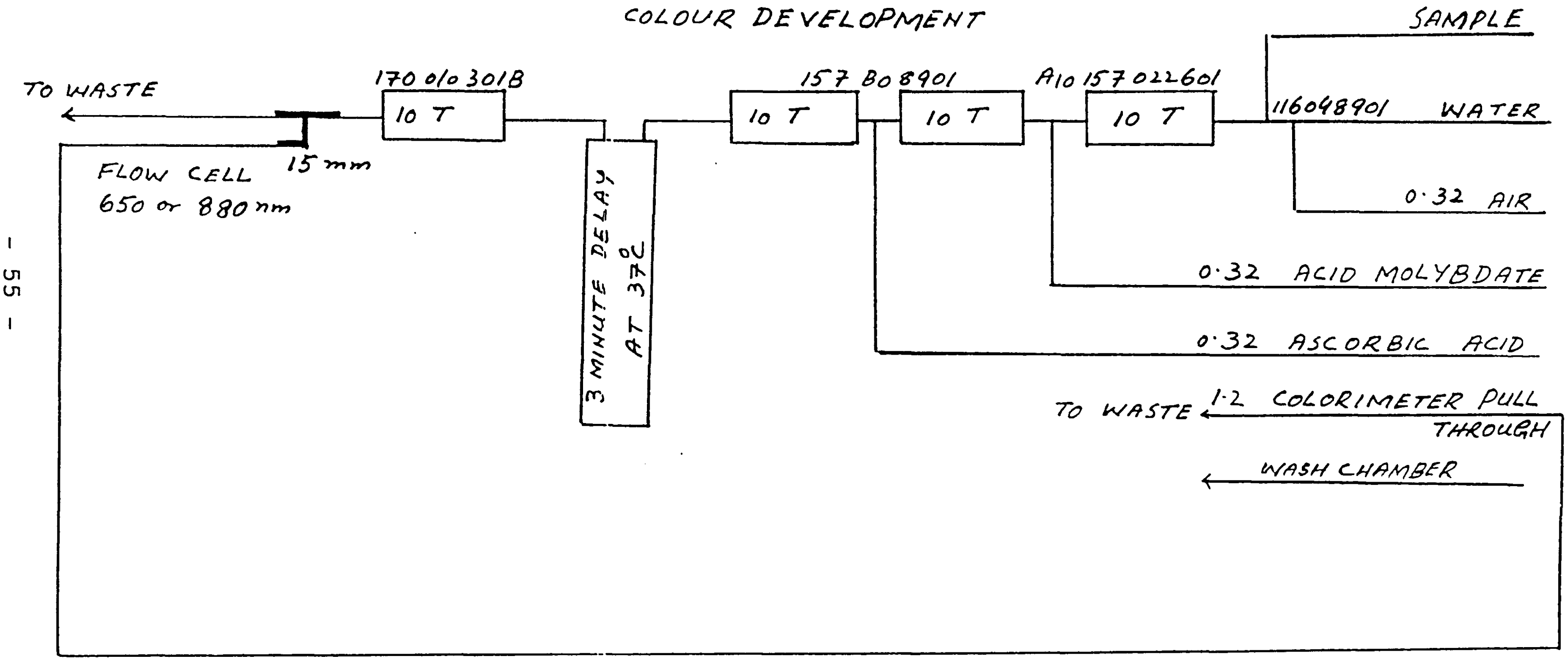


Figure 2.1 Basic Technicon manifold for determining phosphate-P using the molybdenum blue method

The water conveniently contains the wetting agent and allows a degree of flexibility at low sample phosphorus levels without the need for high scale expansions. The sample and water flow rates can be changed to suit different phosphorus ranges provided the total flow rate is approximately 1 cm^3 min^{-1}. The system gives a linear response to phosphorus up to a maximum concentration of approximately 0.8 μg P cm^{-3} in the reagent stream after addition of all reagents. The aim is to have a maximum of 0.5 μg P cm^{-3} in the reagent stream by selecting appropriate flow rates for the sample and water lines (Table 2.2).

Table 2.2 <u>Appropriate flow rates for the sample and water lines in the system</u>

Sample cm^3	Water cm^3	Sample + Water cm^3	Total Flow	Maximum Phosphorus in sample (μg P cm^{-3})
0.16	0.80	0.96	1.60	5
0.42	0.60	1.02	1.66	2
0.60	0.42	1.02	1.66	1
0.80	0.23	1.03	1.67	1

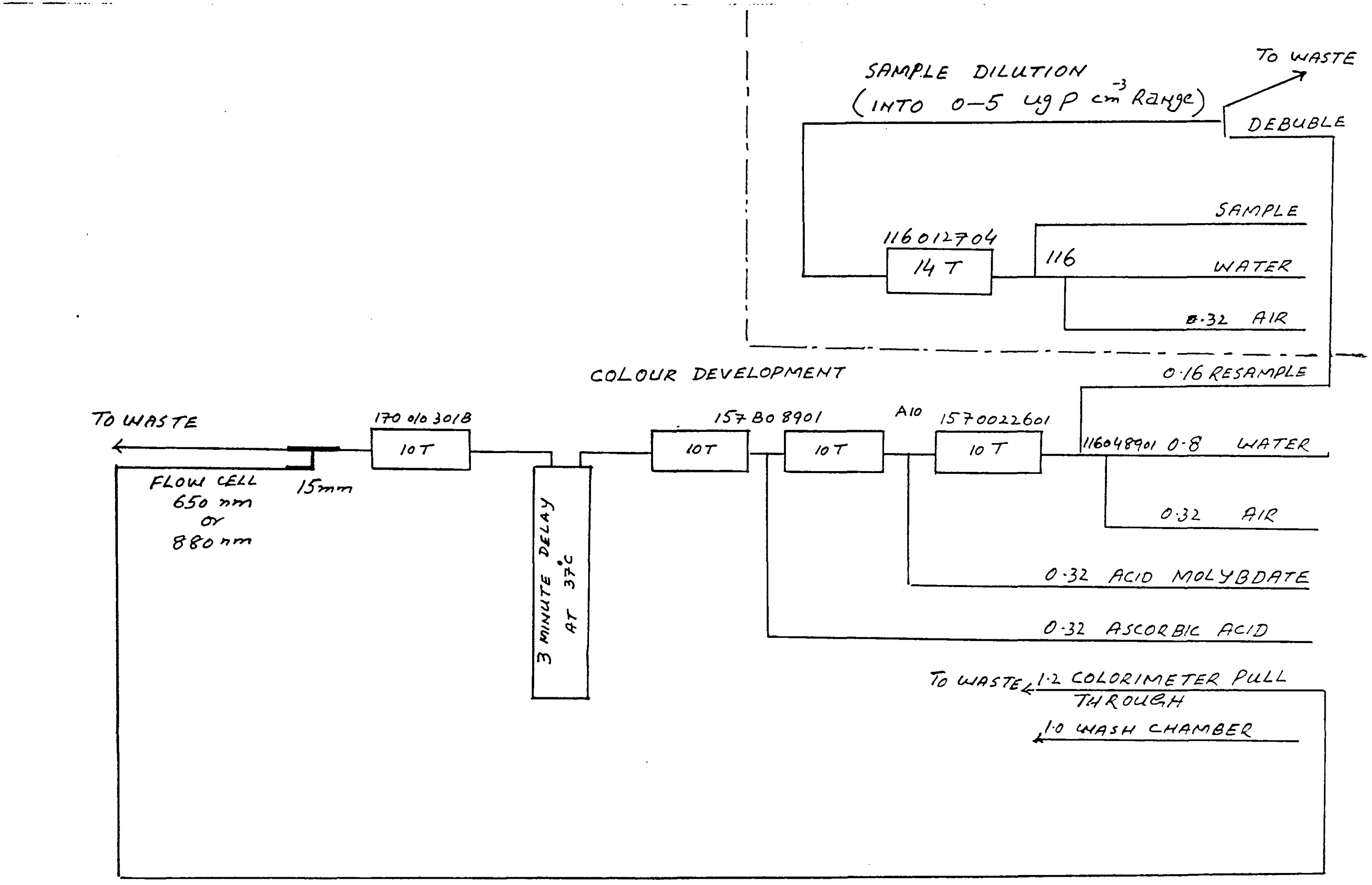


Figure 2.2 Autoanalyzer manifold for phosphate-P

With high sample flow rates there may be problems with the intersample air bubble. It is necessary to increase the concentration of wetting agent in the water as the water flow rate is decreased to maintain the final concentration. For P concentration > 5 $\mu g\ cm^{-3}$ a dilution step was incorporated into the manifold to reduce the P concentration into the 0-5 $\mu g\ cm^{-3}$ range (Figure 2.2).

2.2.1.2.2 Reagents

i. Acid molybdate.

Ammonium molybdate, 5.2 g.
Antimony potassium tartrate, 0.1 g.
Concentrated sulphuric acid, 60 cm^3.

The sulphuric acid was added to 800 cm^3 deionized water. After cooling, the ammonium molybdate was dissolved in the dilute acid. The antimony potassium tartrate was dissolved in a small volume of deionized water, added to the diluted acid and stirred. The volume was made up to 1000 cm^3 with deionized water. The solution was stored in a brown bottle away from light. The reagent was stable for one month.

ii. Ascorbic acid: 0.75 g. ascorbic acid was dissolved in 100 cm^3 deionized water. This solution was stable for one day.

iii. Dilution water.

0-1 μg P cm^{-3} range: 2 cm^3 Aerosol 22 wetting agent was diluted to 1000 cm^3 with deionized water.

0-5 μg P cm^{-3} range: 1 cm^3 Aerosol 22 wetting agent was diluted to 1000 cm^3 with deionized water.

2.2.1.2.3 Procedure

Filtered soil extracts and other test solutions were analysed directly on the Technicon AutoAnalyzer using the manifold in Figure 2.1. Sample and water flow rates were selected from Table 2.2. The samples were normally run at the rate of 50 per hour. For concentrations above 5 μg P cm^{-3}, appropriate dilution was carried out to reduce the concentration to the 0-5 range using the manifold in Figure 2.2 and the sampling rate was reduced to 40 per hour.

There are a few points to note in using the method:

- Care should be taken that individual samples of high concentration among a set of predominantly low concentration samples are not included in the same run.
- Brij-35 wetting agent used in many methods gives an intense blue colour, so no traces of brij must enter the system eg. from wash chamber where brij is used for other methods.
- There is a slight tendency for a molybdate deposit to accumulate in the flow cell and transmission tubing, causing a slight drift in base line. A daily wash out of the colour development manifold with 10 percent sodium

hydroxide solution is sufficient to remove this material. A wash out of the colour development manifold with M hydrochloric acid followed by washing with deionized water is also important when the manifold is used for alkaline extracts.

2.2.1.3 Choice of wavelength

In the normal operation of the colorimeter, several checks should be made, to ensure accurate and reliable operation. Since the spectrum of the reduced molybdenum blue complex has a peak at 880 nm and a shoulder at 660 nm, measurement at 880 nm gives a useful improvement in sensitivity over 660 nm, but this involves changing phototubes as well as filters. The standard CE 34Q phototubes in the S.C.I.C. (colorimeter) have a working range 340-660 nm. For work at 880 nm infra red phototubes CE 63V are required. These have a working range 600-900 nm.

The objectives of this study were to investigate:

i. If measurement at 800 nm gives an improvement in sensitivity compared to 650 nm.
ii. Which phototube will give best results at 880 nm.

Keeping in view these objectives, an attempt was made to compare the effect of different filters, phototube, and wavelength, in order to sort out a suitable combination of phototube and filter, which can give a useful increase in sensitivity at 880 nm.

Material and methods

i. Filters 650 nm and 880 nm.

ii. Phototube, CE 34Q, CE 63V,and CE 65V.
Operating wavelength, CE 34Q (352-700 nm), CE 63V (600-900 nm), CE 65V (340-660 nm).

iii. Yellow coded bulb and blue coded long life bulb.

iv. Working phosphorus standard solutions, 0.0 and 1.0 $\mu g\ cm^{-3}$ phosphorus, made up in water.

Phosphorus determination was made using 0.0 and 1.0 $\mu g\ P\ cm^{-3}$, using the 0 to 1 manifold (Figure 2.1). The method given in Section 2.2.1.2 was used.

The scale expansion was set to 1.0 and highest value of the chart recorder reading (20.7) was used as a theoretical maximum for the calculation of percent phosphorus recovery. The experiment was done in triplicate.

Results and discussion

Comparing the results in terms of the chart recorder reading, it was found (Table 2.3), that 880 nm filters gave the highest reading compared to 650 nm filters, in the case of both bulbs and the phototubes. No marked difference was found in the case of 650 nm filter, used with both bulbs and all phototubes. However, 880 nm filters did not give a high response in the case of the CE 65V phototube. Two pairs of CE 34Q phototubes differed markedly in their operation at 880 nm.

From the preceding discussion, it can be concluded that an 880 nm filter can be used effectively with high sensitivity, with CE 63V phototubes and either bulb. Use of CE 34Q phototubes at 880 nm depends on the performance of individual phototubes.

Table 2.3 Percent response to 1 μg P cm^{-3} standard

	Blue bulb		Yellow bulb	
	Filter		Filter	
Photo tube	650 nm	880 nm	650 nm	880 nm
CE 34Q(a)	72	98	69	92
CE 34Q(b)	70	82	ND	ND
CE 65V	72	32	73	poor
CE 63V	70	100	68	100

ND = not determined
poor = poor signal to noise ratio

2.2.1.4 Background colour correction

Coloured extracts affect colorimetric phosphate determination by giving a high background reading. The main objectives of the present study were:

A. Colour Correction System.

Various colour correction systems were tested to measure background colour under conditions where no molybdenum blue was formed. These were acid ammonium molybdate with no antimony, acid ammonium molybdate with no ascorbic acid or acid ammonium sulphate (no molybdate, no antimony with ascorbic acid.

B. Effect of Wavelength on Colour Absorption.

Since coloured extracts absorb less at high wavelength, absorption at 660 nm and 880 nm was compared.

Materials and methods

Acid ammonium molybdate and ascorbic acid were prepared as described in Section 2.2.1.2. The molybdate reagent was tested both with and without the addition of ascorbic acid.

Acid ammonium molybdate was also prepared as above but with no antimony potassium tartrate.

Acid ammonium sulphate was prepared by dissolving, 1.67 g ammonium sulphate and 60 cm^3 concentrated sulphuric acid in 1000 cm^3 deionized water.

Soil extractants were 0.5 M acetic acid (Section 3.1.3), 0.5 M ammonium fluoride (Section 2.2.1.6), Morgan's solution (3 percent acetic acid, 10 percent sodium acetate), pH 4.8, Modified Morgan's solution (0.5 M acetic acid, 0.5 M ammonium acetate pH 4.8).

Phosphate working standard solution.

0 and 5 μg P cm^3 made in each of the above extractants.

Development of the method

The manifold was configured for the 0.0 to 5.0 μg P cm^{-3} range (sample flow rate 0.16 cm^3 min^{-1}, water flow rate 0.8 cm^3 min^{-1}). 0 and 5 μg P cm^{-3} standard solutions were run under the following conditions:

(1) with ammonium molybdate (minus antimony) with ascorbic acid;

(2) with ammonium molybdate (plus antimony) but without ascorbic acid;

(3) with acid ammonium sulphate (minus molybdenum, minus antimony reagent) with ascorbic acid.

The manifold was reconfigured to the 0-1 μg P cm^{-3} range (sample flow rate 0.8 cm^3 min^{-1}, water flow rate 0.16 cm^3 min^{-1}). 0 and 1.0 μg P cm^3 standard solutions were run under the same conditions as above.

Testing of the method

The manifold was configured to the 0-1 μg P cm^{-3} range. Two soils were extracted with ammonium fluoride, acetic acid and modified Morgan's solution, to give a range of colour intensities in the extracts. The acid ammonium sulphate plus ascorbic acid colour correction system was used to measure background colour at 650 and 880 nm. The scale expansion was set to 5.0 and chart recorder readings recorded.

Results and discussion

The results in Table 2.4 indicate that acid ammonium molybdate with no antimony gave a response in water, which was 57 percent of that in the control (full reagents). This system was then discarded. There was no response in acid ammonium molybdate with no ascorbic acid in most cases. However, a small response was detected which was 0.23, 0.38 and 0.5 percent of that in the control, for modified Morgan (1 μg P cm^{-3}), modified Morgan (5.0 μg P cm^{-3}) and acetic acid (1 μg P cm^{-3}) respectively. Acid ammonium sulphate with ascorbic acid gave no response for all extractants.

Table 2.4 Blue colour development in colour correction systems expressed as percentage of that in the standard phosphate system

	acid ammonium molybdate	acid ammonium molybdate		acid ammonium sulphate
	+Sb +Asc	-Sb +Asc	+Sb -Asc	-Sb-Mo +Asc
0-5 μg P cm^{-3}				
Water	100	57.2	0.0	0.0
Acetic acid	100	ND	0.0	0.0
Morgan	100	ND	0.0	0.0
Modified Morgan	100	ND	0.23	0.0
Ammonium fluoride	100	ND	0.0	0.0
0-1 μg P cm^{-3}				
Water	100	ND	0.0	0.0
Acetic acid	100	ND	0.5	0.0
Morgan	100	ND	0.0	0.0
Modified Morgan	100	ND	0.38	0.0
Ammonium fluoride	100	ND	0.0	0.0

ND - not determined

+Sb Potassium antimony tartrate added

-Sb No potassium antimony tartrate

+Asc Ascorbic acid added

-Asc No ascorbic acid

-Mo No ammonium molybdate

From these results, it can be concluded that acid ammonium sulphate with ascorbic acid can be used for background colour correction.

The acetic acid extracts were virtually colourless and showed no difference from the blank at 650 nm or 880 nm (Table 2.5). For the modified Morgans solutions, which were slightly more coloured, a small difference relative to the blank was observed at 650 nm, but not at 880 nm. The highly coloured ammonium fluoride extracts showed differences relative to the blank at both wavelengths, but this effect was less at 880 nm than at 650 nm. Thus 880 nm is a more suitable wavelength for avoiding or reducing the need for background colour correction.

Table 2.5 Background colour intensity, measured as chart readings, in extracts of two soils and a blank extract, measured at 650 nm and 880 nm.

Extractant	Wavelength nm	Chart reading		
		Blank	Extract 1	Extract 2
acetic acid	650	5.0	5.0	5.0
	880	5.0	5.0	5.0
modified Morgan	650	5.0	5.2	5.5
	880	5.0	5.0	5.0
ammonium fluoride	650	5.0	6.2	13.4
	880	5.0	5.4	7.0

2.2.1.5 Measurement of phosphorus in sodium bicarbonate extracts

Analysis of 0.5 M sodium bicarbonate extracts of soil pose a particular problem for automated analysis as under the acid condition of the colour development, the bicarbonate reacts to produce carbon dioxide which disrupts the flow. Thus the samples must be neutralized and all carbon dioxide removed before colour development. Colwell (1965) used a separate neutralization step to remove carbon dioxide. He acidified the flow stream using 3M HCl added immediately before a short heating coil in a 95°C oil bath. The stream was cooled, debubbled using a B0 Trap and passed to the colour development manifold. Macleod (1982) used the same method successfully, omitting the cooling step. Since the method developed by both Colwell (1965) and MacLeod (1982) for the autoanalyzer, no attempt has been made in relation to the supply of carbon dioxide-free neutralized samples with a particular reference to minimum changes in phosphorus concentration. It was decided to evaluate this aspect, as it may cause serious physical interference during phosphorus determination.

The two important factors in the acidification stages are temperature and concentration of acid, because the sample is acidified at a suitable temperature to liberate the dissolved carbon dioxide before the sample is debubbled and passed through the basic manifold for colour development. Sulphuric acid was used for the neutralisation step as it was already used in the acid molybdate reagent and so should not interfere with the colour development system.

Materials and methods

i. Sulphuric acid: 1 M, 1.5 M, 2 M and 2.5 M. 55 cm^3, 83 cm^3, 110 cm^3 and 138 cm^3 concentrated analar grade sulphuric acid respectively were diluted to 1000 cm^3 with deionized water.

ii. Phosphorus standard working solution. 0.0, 1.0 and 5.0 μg P cm^{-3}, made up in 0.5 M sodium bicarbonate (Section 3.1.3).

iii. Air tube, flow rates of 0.32 and 0.23 cm^3 min^{-1}.

The flow system used is shown diagramatically (Figure 2.3). The sampling rate was set up to 40 per hour. The stream of phosphate standard solution made up in 0.5 M sodium bicarbonate was treated with acid at each of the above concentrations, and passed through a heating coil ranging in temperature from 21 to 60°C to liberate the carbon dioxide produced (1 M sulphuric acid was the calculated concentration required to neutralise 0.5 M sodium bicarbonate at a flow rate of 0.42 cm^3 per minute for the sample and 0.1 cm^3 per minute for the acid). The stream was debubbled and passed through the standard manifold for measuring phosphorus in 0-5 μg P cm^{-3} range. The effect of segmentation on the neutralization step using 0.32 and 0.23 air tubes was also investigated.

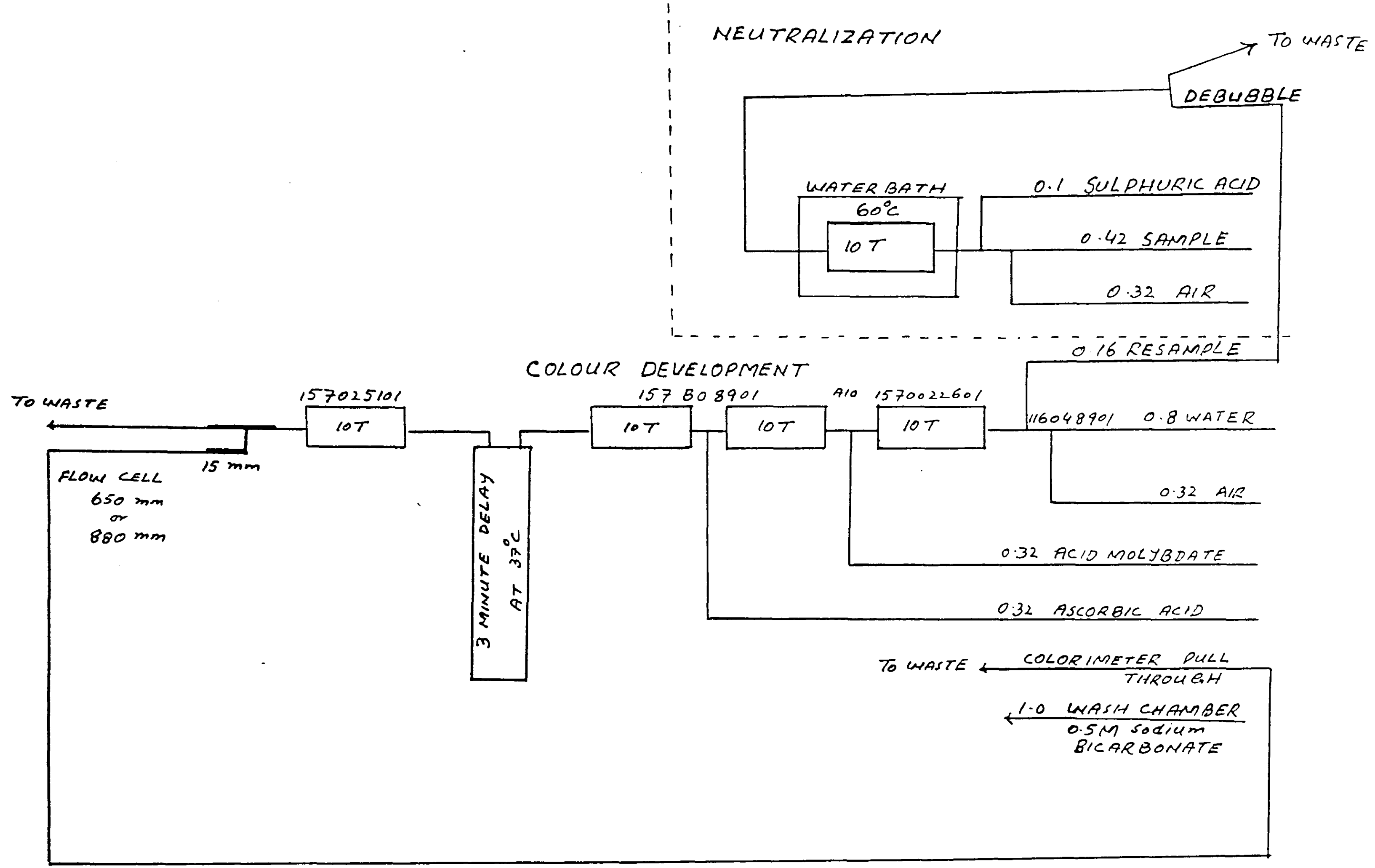


Figure 2.3 Testing Technicon manifold for 5 µg P cm^{-3} sodium bicarbonate

Results and discussion

The most important considerations in this experiment were, whether the reaction was completed and carbon dioxide was completely removed. Investigations were made on the acid concentration and temperature conditions required for neutralizing the extract and complete removal of all the carbon dioxide produced. In addition, the effect of segmentation in the neutralization step was also studied. From the results in terms of a qualitative assessment of peak development, it was found that acid concentration in the range of 1 M - 2.5 M had no effect on peak development, and thus no excess of acid is required. Peak development improved with increase of temperature from 21 to 60°C (Table 2.6). In the 21-50°C range, the flow through the main manifold was erratic due to carbon dioxide production, causing disrupted segmentation and poor peak development. This showed that the carbon dioxide removal in the neutralizing system was not complete. Above 50°C, carbon dioxide was removed completely and there was no disruption in the main manifold. Though the effect of segmentation in the neutralization step was not so apparent, it resulted in an improved base line.

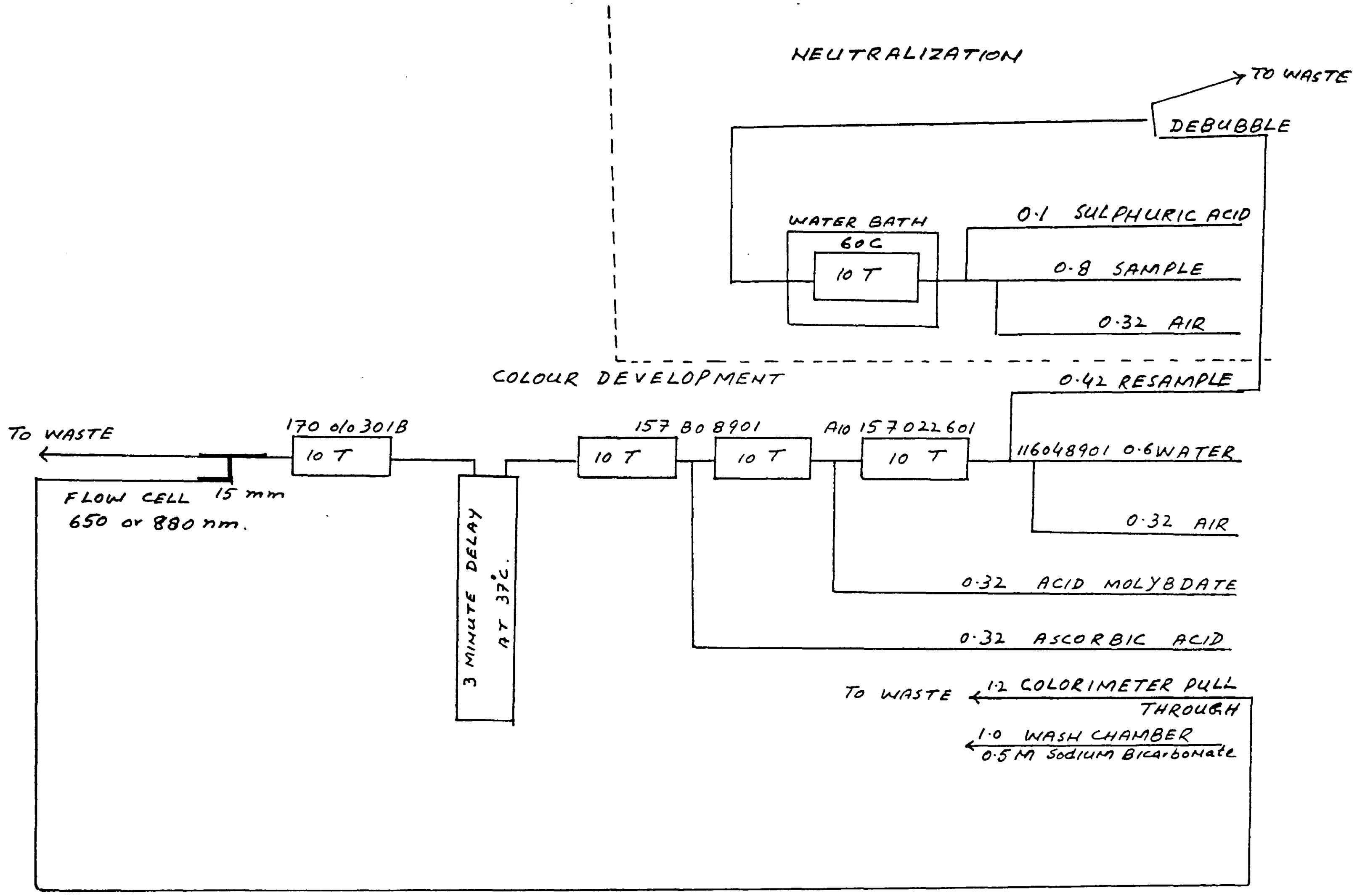


Figure 2.4 Final Technicon manifold for 1 μg P cm^{-3} sodium bicarbonate

Further modifications were made for measurements in the 0-1 μg P cm^{-3} range (Figure 2.4). Since the 1 μg P cm^{-3} system required a sample flow rate of 0.6 cm^3 per minute it was necessary to increase the total flow through the neutralization step. A flow rate of 0.8 cm^3 per minute for the sample and 0.1 cm^3 per minute for the acid was used. The acid concentration was increased to 2 M (the theoretically required concentration). However, this system did not work, due to the high gas flow as compared to liquid flow in the neutralizing step. This resulted in erratic liquid flow. The problem of erratic liquid flow can be avoided by using a maximum resample flow rate of 0.42 cm^3 per minute. Thus a modified manifold (Figure 2.4) using a resample flow rate 0.42 cm^3 per minute and water flow rate of 0.6 cm^3 per minute, was developed for 0-1 μg P cm^{-3} system. It gave satisfactory peaks.

Table 2.6 Effect of temperature on CO_2 production

Neutralization step		Main manifold	
Temperature (^oC)	air tube $cm^3\ min^{-1}$	CO_2 bubble production	Peak development
21	0.32	many	bad
	0.23	many	bad
40	0.32	few	poor
	0.23	few	poor
50	0.32	very few	fair
	0.23	very few	fair
60	0.32	none	good
	0.23	none	good

From the preceding discussion, it can be concluded that treatment with 2 M sulphuric acid at 60°C using a sample flow rate of 0.8 and acid flow rate of 0.1 cm^3 per minute, gives neutral carbon dioxide-free samples for the 0-5 and 0-1 μg P cm^{-3} manifold, provided the resample flow rate to the main colour development manifold is not greater than 0.42 cm^3 per minute. On the basis of these findings manifolds based on Figures 2.4 and 2.5 were developed to acidify the sample stream.

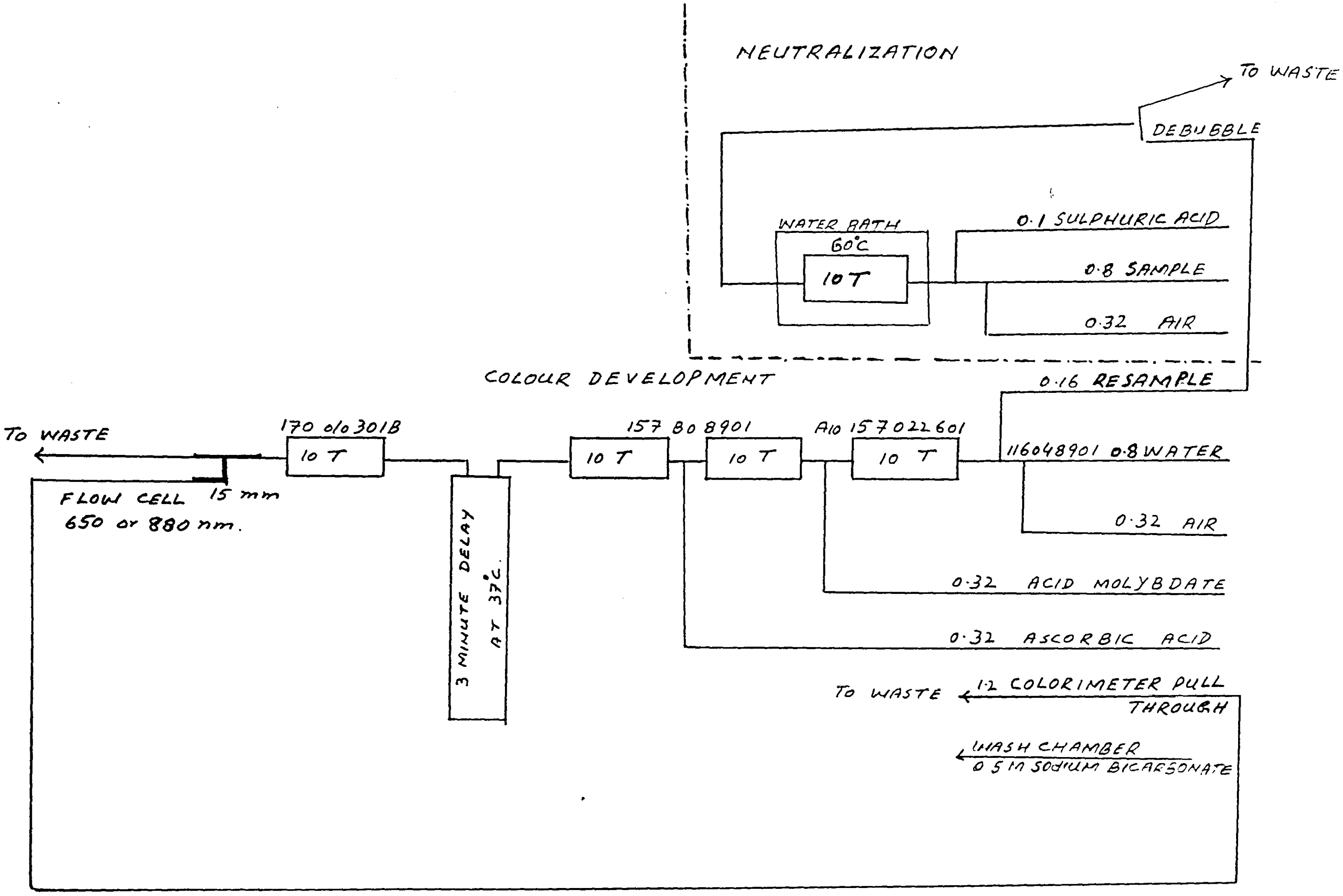


Figure 2.5 Final Technicon manifold for 5 µg P cm^{-3} sodium bicarbonate

2.2.1.6 Measurement of phosphorus in ammonium fluoride extracts

Since fluoride ions interfere in the colour development during phosphorus determination by the molybdenum blue method (Kurtz, 1942), boric acid is used to eliminate this interference. Its suitability and concentration has not been established for flow systems. Therefore it was considered necessary to determine a suitable concentration of boric acid, and also to see its effect on recovery of added phosphate.

Materials and methods

i. Diluent solution:
   0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 percent boric acid was prepared by dissolving 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g of boric acid in 100 cm^3 deionized water. Solutions were made up containing aerosol 22 at both 1 cm^3 and 2 cm^3 per 1000 cm^3.

ii. 0.5 M ammonium fluoride.
   18.52 g of analar grade ammonium fluoride was weighed and dissolved in 1000 cm^3 deionized water.

iii. Phosphorus standard working solutions:
   0 and 1.0 μg P cm^{-3} made in 0.5 M ammonium fluoride.

The percentage recovery of added phosphate (relative to a 1 μg P cm^{-3} standard in water) was determined using different tube combinations with various concentrations of boric acid and aerosol 22 (Figure 2.6). Ammonium fluoride was used as the wash chamber solution instead of deionized water.

Results and discussion

The results in Table 2.7 indicate that 100 percent phosphorus recovery was achieved by using 2 percent or higher boric acid concentrations with 0.16:0.80, 0.23:0.8 and 0.42:0.60, sample:diluent ratios. In the case of 0.6:0.42, sample:diluent ratio, 100 percent phosphorus recovery was achieved only by using 4 and 5 percent boric acid. In the case of 0.8:0.23 sample:diluent ratio, no boric acid percentage was effective in obtaining 100 percent recovery.

From the above discussion, it was concluded that 4 percent boric acid can be used effectively to eliminate interference due to F^- ions, provided that sample flow rate is maintained at not more than 0.6 cm^3 per minute. At lower sample flow rates the boric acid concentration can be reduced. Moreover, the use of 5 percent boric acid is not recommended due to the limits of solubility of boric acid.

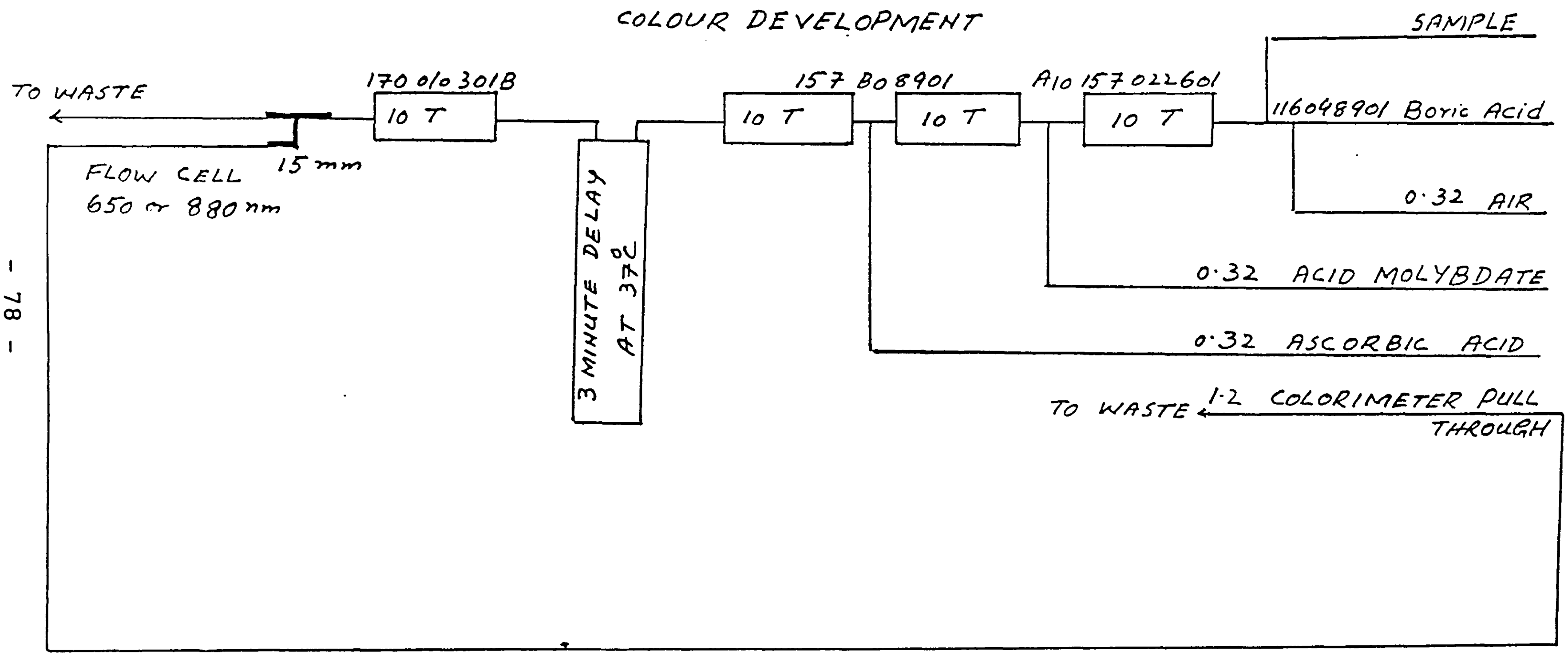


Figure 2.6 Technicon manifold for determining ammonium fluoride extractable phosphate

Table 2.7 <u>Percentage phosphorus recovery in 0.5M NH_4F solutions using different concentrations of boric acid</u>

Tube combination			Boric acid percent					
S^*	D	aerosol 22 cm^3 l-1	0.5	1.0	2.0	3.0	4.0	5.0
			% P recovered					
0.16	0.80	1	35	80	100	100	100	100
0.23	0.80	1	10	70	100	100	100	100
0.42	0.60	2	0	20	100	100	100	100
0.60	0.42	2	0	0	40	90	100	100
0.80	0.23	2	0	0	0	0	40	92

S^* sample rate cm^3/minute

D diluent rate cm^3/minute

2.2.1.7 Summary of conditions suitable for the determination of phosphorus in various soil extractants

On the basis of the assessments discussed in the previous sections, along with some other observations (such as the effect of the aerosol 22 concentration on the smoothness of flow, shape and smoothness of peak) a table of conditions for different extractants was drawn up (Table 2.8). The nature of the wash chamber solution and the amount of wetting agent were the main factors controlling the quality of peaks.

Attention was also given to:

a. the neutralization of acid or alkaline extractants.

b. use of boric acid with ammonium fluoride extracts.

c. design of the Technicon manifold for different levels of phosphorus.

Table 2.8 Suitable manifolds for different extractants and phosphorus ranges using the Technicon AutoAnalyzer

Extractant solution	Range mg P l^{-1}	Flow rate cm^3 min^{-1}		Diluent solution	Aerosol concentration cm^3 l^{-1}	Wash chamber solution	Maximum sample rate per hour
		Sample	Diluent				
Water	0-5	0.16	0.80	water	1	water	50
	0-1	0.60	0.42	water	2	water	50
	0-1	0.80	0.23	water	4	water	50
Acetic acid	0-5	0.16	0.80	water	1	water or	50
	0-1	0.60	0.42	water	2	acetic acid	50
	0-1	0.80	0.23	water	4	acetic acid	50
Morgan solution	0-5	0.16	0.80	water	1	water or	50
	0-1	0.60	0.42	0.34M H_2SO_4	2	extractant	50
	0-1	0.80	0.23	0.8M H_2SO_4	4	extractant	50
Modified Morgan solution	0-5	0.16	0.80	water	1	water or	50
	0-1	0.60	0.42	H_2SO_4	2	extractant	50
	0-1	0.80	0.23	H_2SO_4	4	extractant	50
Ammonium fluoride	0-5	0.16	0.80	2% boric acid	1	extractant	50
	0-1	0.60	0.42	4% boric acid	2	extractant	50
Sodium* Bicarbonate 0.5 M pH 8.5	0-5	0.16	0.80	water	1	extractant	40
	0-1	0.60	0.42	water	2	extractant	40

NEUTRALIZATION STEP

* 0.32 cm^3 min^{-1} air tube)
)
0.1 cm^3 min^{-1} 4.0 M H_2SO_4) at temperature 60°C
)
0.8 cm^3 min^{-1} sample)

2.2.2 Extraction of soil phosphorus

A number of soil tests, including phosphorus extraction, developed for use on neutral, calcareous and acid soils, have also proved to be useful on coal mine soil. However large differences have been found to exist between the chemical properties in individual strata associated with the coal beds (Barnhisel and Massey, 1969). Moreover, the presence of large amounts of iron and aluminium compounds in the colliery spoil together with the acid environment of the shale, further aggravate the extraction step and limit the validity and reproducibility of the results. Keeping in view these limitations, some soils from coal mine and oil shales were subjected to different extraction treatments, so that an accurate and precise method for extraction of phosphorus could be developed.

Materials and methods

Reagents.

i. 0.5 M acetic acid: 29 cm^3 glacial acetic acid were diluted to 1000 cm^3 with deionized water.

ii. 0.5 M modified Morgans solution:
29 cm^3 glacial acetic acid and 38 g ammonium acetate were dissolved in approximately 900 cm^3 deionized water and the pH adjusted to 4.8. The volume was then made up to 1 litre with deionized water.

iii. Stock phosphate standard solution:
0.4394 g of KH_2PO_4 was weighed accurately and dissolved in deionized water. The solution was

diluted to 1000cm^3 with deionized water. This solution contained 100 μg P cm^{-3}.

iv. Working phosphate standard solutions:

1 cm^3 and 5 cm^3 of the stock phosphate standard solution was diluted to 100 cm^3 with deionized water. This solution contained 1.0 and 5.0 μg P cm^{-3}, respectively.

Eight soil samples, five coal spoil samples from Baads and three oil shale samples from North Addiewell were used for the measurement of phosphorus status, both as fresh and air-dried samples. One individual sample was collected at random from the surface soil (0-14 cm) from BD_5, BD_{12}, BD_{13}, NA_1, NA_2, AND NA_3, while two individual samples, one each from the surface soil (BD_{7a}) and one from subsoil (BD_{7b}), were collected from BD_7 (for site detail see Section 2.3), making a total of eight samples. The detail of the samples are given below:

1 BD_{7a} (surface soil)
2 BD_{7b} (subsoil)
3 BD_5
4 BD_{12}
5 BD_{13}
6 NA_1
7 NA_2
8 NA_3

Four replicates were run throughout the experiment.

2.5 g soil samples, oven-dry equivalent, were weighed into 4 oz screw cap extraction bottles and 50 cm^3 0.5 M acetic acid was added. The bottles were shaken for 2 and 24 hours at temperatures of 2, 8 and 30°C, using an orbital shaker at 150 rpm. The extracts were filtered through Whatman no. 42 paper. The phosphorus concentration was measured by the method described in Section 2.2.1.2, using manifold conditions given in Table 2.8, for 0-1.0 and 0-5.0 μg P cm^{-3}. Blank (no soil) determinations were carried out with each extraction.

Phosphorus concentration was measured using the same procedure, in the modified Morgans solution extracts, but only at temperatures of 2 and 30°C.

In another experiment, two air-dried soil samples, one each from North Addiewell (NA_1) and Baads (BD_5), were extracted with 0.5 M acetic acid at four temperatures; 5, 10, 20 and 30°C, using seven shaking times; 0.5, 1.0, 1.5, 2.0, 4.0, 8.0 and 24.0 hours. The same procedure as given above was followed.

Results and discussion

Table 2.9 shows the effect of different shaking periods under different temperatures and soil moisture conditions on acetic acid extractable phosphorus. Soils 1, 4 and 5 showed no significant effects of temperature, time or moisture condition on the extraction of phosphorus. Moreover these soils contained very low levels of phosphate (<1 μg P cm^{-1}). The other soils showed variable levels of extractable phosphorus, affected by these 3 factors. These effects were not consistent within a soil or between soils.

Table 2.10 shows the effect of different shaking periods under different temperatures and soil moisture conditions on ammonium acetate extractable phosphorus. Soil 1 showed no significant effects of temperature, time or moisture condition on the extraction of phosphorus. The other soils showed significant effects of one or more of the parameters tested on the levels of extractable phosphorus.

Tables 2.11 and 2.12 show the results of the effect of both temperature and time of shaking on acetic acid extractable phosphorus, for soil 3 and soil 6 respectively, which has been further illustrated in Figures 2.7 and 2.8. Figure 2.7 illustrates the effect of different shaking periods, under the same temperature (20°C) on extractable phosphorus, while Figure 2.8 illustrates the effect of different temperatures, under the same period of shaking (2 hours) on extractable phosphorus. From these figures it is clear that the soils behaved differently and oppositely to each other. Extractable phosphorus decreased with the increase of both temperature and period of shaking in one soil, while extractable phosphorus increased with the increase of both temperature and period of shaking in the other soil. The figures further indicate that for both soils extractable phosphorus gave an approximately linear response to temperature.

Table 2.9 Effect of shaking periods under different temperatures on acetic acid contractable phosphorus ($\mu g\ P\ g^{-1}$)

Temperature (°C)	2				8				30				
Shaking period (hours)	2		24		2		24		2		24		
Soil	D	F	D	F	D	F	D	F	D	F	D	F	LSD
1	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.2
2	1.0	0.7	1.0	0.7	0.9	0.6	0.8	0.7	1.1	0.9	1.0	0.9	0.2
3	25.6	29.1	13.4	14.8	20.3	22.7	10.0	10.7	9.5	14.2	5.8	6.7	9.6
4	0.6	0.4	0.7	0.6	0.5	0.4	0.5	0.6	0.5	0.4	0.6	0.5	0.3
5	0.6	0.5	0.7	0.7	0.5	0.4	0.5	0.6	0.4	0.4	0.3	0.4	0.4
6	13.2	18.4	30.7	46.7	15.3	26.2	33.4	40.9	21.0	28.6	31.5	32.1	9.4
7	1.6	1.8	2.9	4.0	1.6	2.1	2.6	3.5	1.6	2.0	1.8	1.9	0.3
8	88.5	92.2	92.6	101.8	79.3	70.0	76.4	75.4	49.9	45.7	39.6	37.7	18.8

D - Dry F - Fresh

Each figure is the mean of 4 measurements

Table 2.10 Effect of shaking period and temperature on ammonium acetate extractable phosphorus ($\mu g\ P\ g^{-1}$)

Temperature (°C)	2				30				
Shaking period (hours)	2		24		2		24		
Soil	D	F	D	F	D	F	D	F	LSD
1	1.1	0.8	1.4	1.4	1.0	0.5	0.7	0.7	1.2
2	4.6	4.2	6.8	6.1	6.1	5.1	6.0	6.3	1.1
3	30.1	30.5	20.6	25.7	18.7	18.0	8.3	9.4	9.3
4	0.6	0.7	1.0	0.7	0.2	0.0	0.1	0.1	0.5
5	0.9	1.3	0.9	1.0	0.2	0.1	0.0	0.1	0.5
6	16.7	10.9	23.4	16.3	19.6	14.1	20.9	18.5	1.9
7	3.8	1.9	5.1	2.6	3.5	1.8	4.0	3.0	0.6
8	14.1	13.4	25.6	25.6	15.5	14.1	15.0	15.2	7.5

D - Dry F - Fresh

Each figure is the mean of 4 measurements

Table 2.11 <u>Effect of different temperatures and shaking periods on acetic acid extractable phosphorus ($\mu g\ P\ g^{-1}$) (soil 3)</u>

Temperature	Shaking period (hours)						
(°C)	0.5	1.0	1.5	2.0	4.0	8.0	24.0
5	40.3	41.2	40.0	38.7	36.6	31.8	27.8
10	36.0	34.9	32.7	32.7	33.8	26.6	19.5
20	33.5	32.4	29.6	29.3	23.9	20.0	14.0
30	30.6	23.7	22.0	19.3	17.1	13.1	10.7

Each figure is the mean of 4 measurements

Table 2.12 <u>Effect of different temperatures and shaking periods on acetic acid extractable phosphorus (μg P g^{-1}) (soil 6)</u>

Temperature	Shaking period (hours)						
(°C)	0.5	1.0	1.5	2.0	4.0	8.0	24.0
5	14.6	16.2	20.5	20.3	23.1	28.6	37.5
10	17.0	20.1	22.1	22.8	25.9	31.9	38.8
20	18.2	21.4	23.8	24.6	28.2	31.1	34.1
30	19.0	22.5	23.9	24.8	26.6	27.8	30.0

Each figure is mean of 4 measurements.

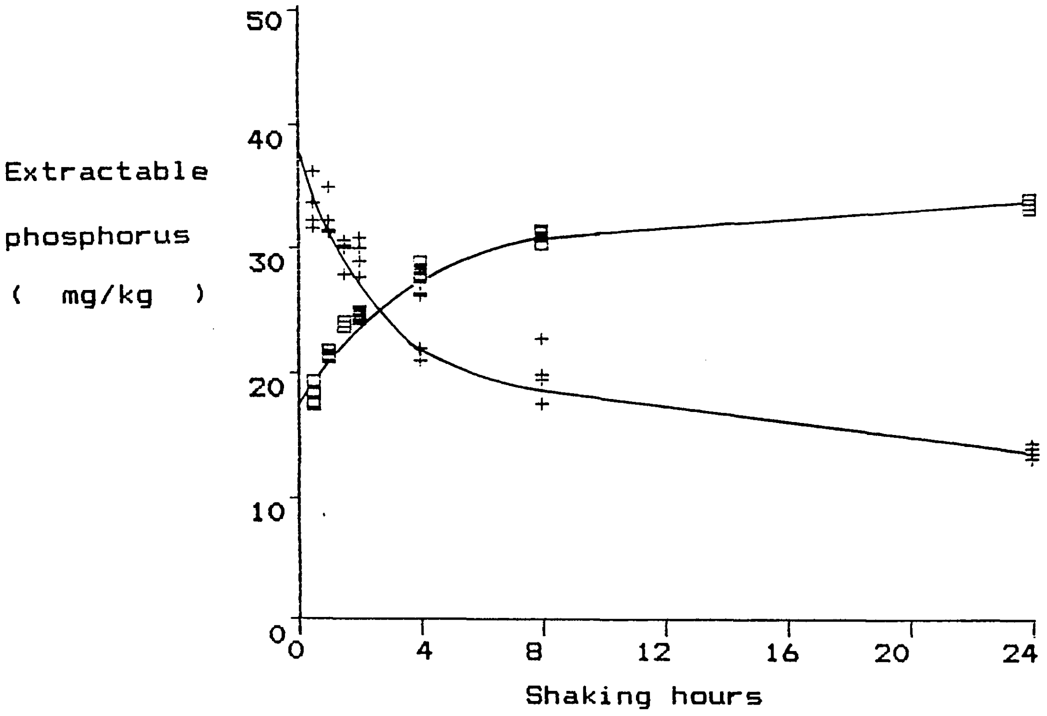


Fig. 2.7 Behaviour of two different soils, under the same temperature (20 c), and different shaking periods

Soil 3 (+)
Soil 6 (□)

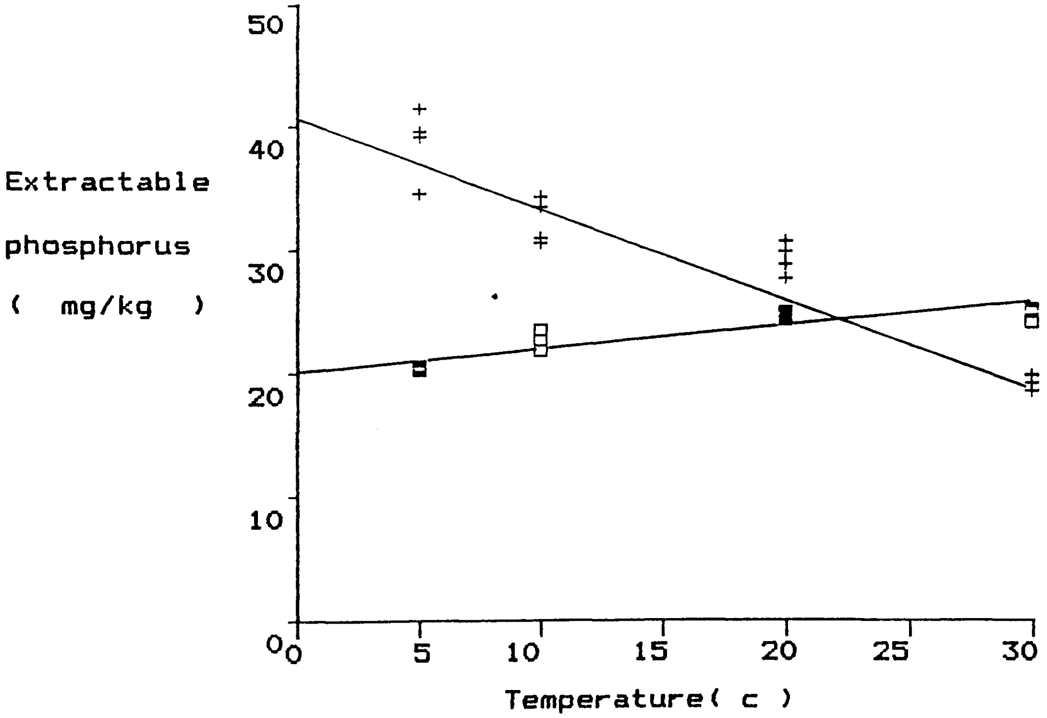


Fig. 2.8 Behaviour of two different soils, under the same shaking period (2 hours), and different temperatures.

Soil 3 (+)
Soil 6 (□)

Response to time was however non-linear. The effect of time became linear by transforming the time axis to log(time). The results of multiple regression analysis of extractable phosphorus with log(time) and temperature showed that the response of both time and temperature was significant (t test) at 0.1 percent level in the case of soil 3. Both coefficients were negative, as extractable phosphorus decreased with increasing temperature and time:

soil 3 = 43.4 - 10.4 log(time) - 0.6 temperature

In the case of soil 6, the response of extractable phosphorus to log(time) was significant at the 0.1 percent level and to temperature at the 1 percent level. Both coefficients were positive, as extractable phosphorus increased with increased temperature and time.

soil 6 = 19.2 + 10.6 log(time) + 0.06 temperature

From these results, it is clear that soil 6 had more extractable phosphorus with longer shaking periods and higher temperature, while soil 3 had low extractable phosphorus. The low level of extractable phosphorus in case of soil 3, might be due to iron and aluminium, which is being extracted and precipitating the phosphate.

General conclusions

Different responses were shown by different soils to temperature and shaking time. This leads to problems of comparison with other people's results. It is necessary to select temperature and time, and these need to be rigorously adhered to. A short shaking time is probably better, and temperature controlled conditions are necessary.

2.2.3 Use of charcoal to decolorize soil extracts

For soils containing elevated levels of organic matter, extraction with solutions such as ammonium fluoride and sodium bicarbonate gives highly coloured extracts, due to extraction of organic matter and iron, which interfere in the measurement of the inorganic phosphate. The ascorbic acid reduction procedure for phosphorus determination is less sensitive (Watanabe and Olsen, 1965) to interference by soluble organic matter than $SnCl_2$ method used by Mehta et al. (1954). However, in the case of ascorbic acid, interference by organic material has been noticed when using a Technicon AutoAnalyzer (Colwell, 1965). Such interference can be overcome by treating the extract with activated charcoal, shaking, separating the charcoal from the extract by filtration or centrifugation and analysing an aliquot of the charcoal-treated extract for inorganic phosphate.

Wood charcoal, which is used for decolorization consists of amorphous carbon, with a small proportion of hydrogen and oxygen (15%). It is prepared by a carbonization process or destructive distillation (Tedder and Nechvatal, 1975). Activated decolorizing charcoals like Darco, Nuchar and Norit (Vogel, 1978), which are prepared from wood are better to use. Since these commercially prepared charcoals are often contaminated with phosphorus, they should be washed by treating the charcoal with the extracting solution to make it free from phosphorus, before it is used as decolorizer.

Materials and methods

i. 0.5 M acetic acid (Section 2.2.2).
ii. 0.5 M modified Morgans solution (Section 2.2.2).
iii. 0.5 M sodium bicarbonate (Section 3.1.3).
iv. 0.5 M ammonium fluoride (Section 2.2.1.6).
v. 6 M hydrochloric acid, dilute 508 cm^3 concentrated hydrochloric acid in 1000 cm^3 of deionized water.
vi. Charcoal DARCO G60: activated decolorizing charcoal. high purity with low phosphate content recommended for soil analysis, BDH Limited.

Experiment 1

Two different batches of Darco charcoal (labelled I and II) were extracted with 0.5 M acetic acid, 0.5 M modified Morgan's solution, 0.5 M sodium bicarbonate and 0.5 M ammonium fluoride, at a solution:charcoal ratio of 20:1, by shaking for 2 hours on a reciprocating shaker at 20°C. Phosphorus in the extracts were measured by the method given in Section 2.2.1.2 using Technicon AutoAnalyzer.

Experiment 2

Darco charcoal was subjected to a washing cycle of the following: 0.5 M acetic acid, 0.5 M modified Morgans solution, 0.5 M sodium bicarbonate or 6 M hydrochloric acid. A washing cycle consisted of shaking for 2 hours on a reciprocating shaker at a solution:charcoal ratio of 400 cm^3:40 g, followed by filtration under reduced pressure and 5 subsequent leachings with 100 cm^3 aliquots of extractants. The charcoal was then washed with deionized water until the leachate was neutral (and also chloride free in the case of hydrochloric acid washing). The washed charcoal was dried at 110°C and then extracted as in experiment 1.

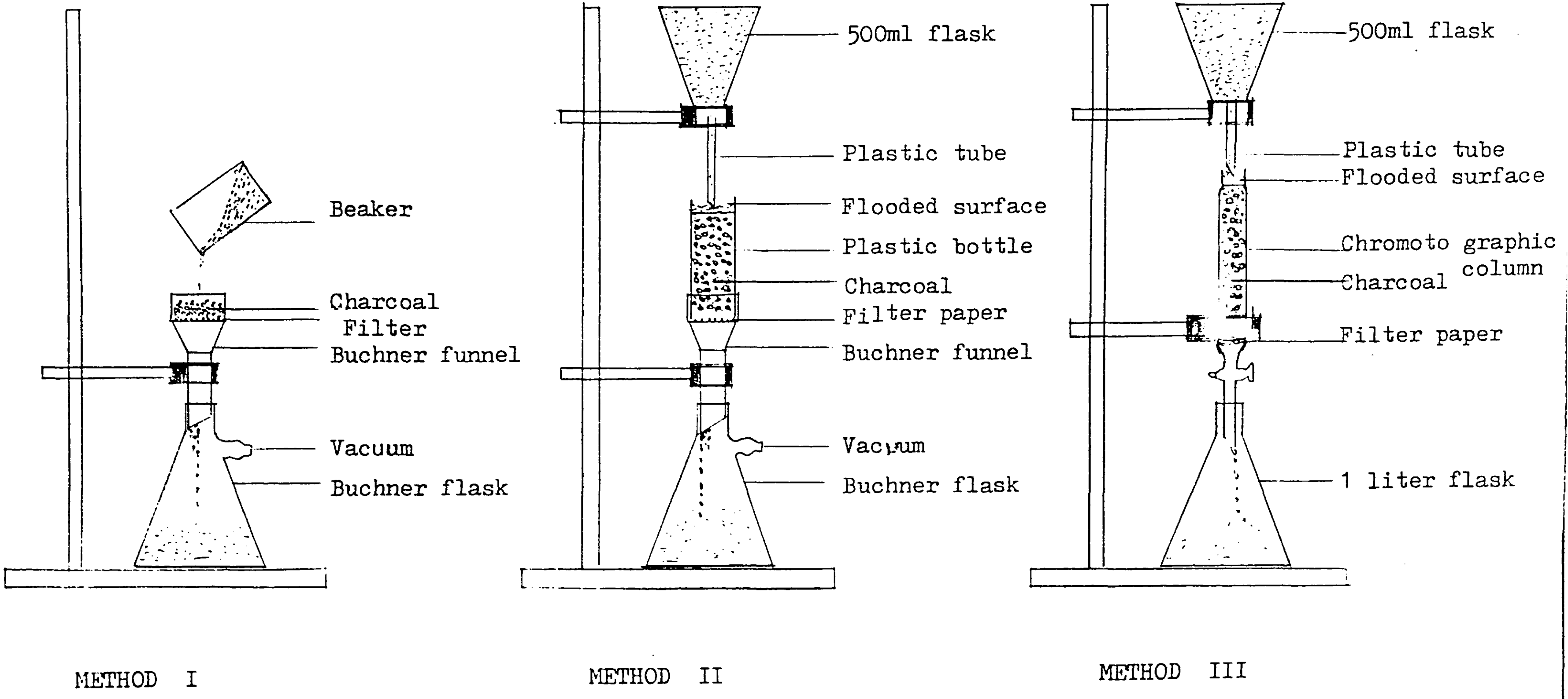
Experiment 3

Darco charcoal was subjected to 1, 2 or 3 washing cycles using 0.5 M acetic acid, then washed, dried and extracted as above.

Experiment 4

After cleaning and rinsing all apparatus thoroughly, charcoal was measured by volume (10 cm^3 = 4 g) into a measuring cylinder. A charcoal slurry made with deionized water, and transferred to a chromotographic column (40 g charcoal) (Figure 2.9, method III), and a plastic column, made from a 250 cm^3 plastic bottle cut at both ends (80 g charcoal) (Figure 2.9, Method II), and allowed to settle. Excess water was removed by reduced pressure, but the charcoal surface was kept flooded.

Figure 2.9 <u>Methods for washing of charcoal</u>



The charcoal was leached with 1 litre 6 M hydrochloric acid, followed by leaching with deionized water until the leachate was chloride free. The charcoal was allowed to drain to dryness in the column, and then transferred to a 110°C oven to complete drying. Dried clean charcoal was extracted with 0.5 M acetic acid, and phosphorus was determined in the extract as in experiment 1.

Results and discussion

The results of all the experiments are shown in Table 2.13 to Table 2.16. All results are the mean of 3 replicates.

Table 2.13 shows the mean results in μg P g^{-1} of charcoal, extracted with acetic acid, modified Morgans solution, sodium bicarbonate and ammonium fluoride.

Table 2.14 shows the results of charcoal washed with acetic acid, modified Morgans solution, sodium bicarbonate, hydrochloric acid, and extracted with acetic acid, modified Morgans solution, sodium bicarbonate and ammonium fluoride.

Table 2.15 shows the results of charcoal washed to varying degrees with acetic acid and extracted with acetic acid, modified Morgans solution, sodium bicarbonate and ammonium fluoride.

From the results it is clear that the charcoal contained 74 to 97 μg P g^{-1} of charcoal, which is very high to be used as a decolorizer. Generally, 1 to 2 μg P g^{-1} of charcoal is considered to be a reasonable level, at which charcoal can be used safely as a decolorizer.

Table 2.13 Phosphorus levels in charcoal extracted with different extractants ($\mu g\ P\ g^{-1}$)

	Acetic acid	Modified Morgans	Sodium bicarbonate	Ammonium fluoride
Darco I	86.6	94.7	85.1	96.8
Darco II	73.9	92.0	88.6	82.8

Table 2.14 Phosphorus levels in charcoal, washed with different washing solutions and then extracted with different extractants ($\mu g\ P\ g^{-1}$)

Washing	P extracted in			
solution	Acetic acid	Modified Morgans	Sodium bicarbonate	Ammonium fluoride
Acetic acid 1 wash cycle	5.2	7.7	9.1	12.3
Modified Morgan 1 wash cycle	2.3	5.0	3.1	6.1
Sodium bicarbonate 1 wash cycle	2.5	3.2	3.4	3.3
Hydrochloric acid 1 wash cycle	1.5	3.3	2.9	3.8

Table 2.15 Phosphorus levels in charcoal, washed with acetic acid and extracted with different extractants ($\mu g\ P\ g^{-1}$)

Washing solution	P extracted in			
	Acetic acid	Modified Morgans	Sodium bicarbonate	Ammonium fluoride
Acetic acid 1 wash cycle	5.2	7.7	9.1	12.3
Acetic acid 2 wash cycle	1.0	1.0	1.6	1.6
Acetic acid 3 wash cycle	0.41	0.89	1.0	3.0

Table 2.16 Phosphorus levels in charcoal, washed with HCl and extracted with acetic acid ($\mu g\ P\ g^{-1}$)

Method 1	Method 2	Method 3
1.5	2.0	0.67

The results in Table 2.14 indicate that none of the washing procedures reduced the phosphorus level sufficiently. The results in Table 2.15 indicate that acetic acid can reduce phosphorus level sufficiently, only after 3 washing cycles.

From the above results one can conclude that acetic acid can be used effectively, if charcoal is washed repeatedly. However, there are so many factors to be considered. The level of phosphorus reduced was not uniform in all replicates. This was due to cracks in the bed of charcoal produced during leaching. Time involved in this method was also too much as it needed constant watching and repeated shakings.

The leaching method should be capable of purifying large batches of charcoal, in addition to reducing the phosphorus level. Keeping in view these considerations, methods II and III (Figure 2.9) were developed and compared for their effectiveness and efficiency in reducing phosphorus level.

The results in Table 2.16 indicate that phosphorus level was reduced sufficiently in the case of both methods. Method III, though, proved to be more efficient in reducing phosphorus level, but time consumable due to very slow leaching. In the case of Method II, the phosphorus level was reduced sufficiently, as well this method does not need constant attention, leaching is uniform and takes a reasonable time.

When the experimental results are considered together with the practical advantages of method II, it can be concluded that this method is more suited on the basis that it can clean large batches of charcoal within a short time to a reasonable level of phosphorus contamination. This method can be used to decolorize extracts provided the phosphorus remaining in the charcoal is allowed for by carrying through blanks extractions with charcoal alone. Moreover, 6 M hydrochloric acid, as a washing material to remove phosphorus from the charcoal was proved to be more effective than the other extractants used as a washing material.

2.2.4 Problem due to filtration

Impurities in filter papers and adsorption due to filter papers are two common problems affecting the final phosphorus concentration determined. The levels of many impurities in filter papers are commonly stated by the manufacturer, except for phosphate content, which is not usually reported. However, on personal enquiry, it was found that Whatman no. 42 contain orthophosphate less than 10 μg per paper. According to Hughes and Macphee (1983) this content is usuallly low enough for much laboratory work. However, when filtering a solution for subsequent determination of phosphate it is unreliable at phosphate levels less than 1 μg P cm^{-1}.

Since the conclusion drawn by Hughes and MacPhee (1983) was open minded, and the soils in this study were accepted to be low in phosphate, it is necessary to investigate the possible effect of filter papers upon phosphorus determination in filtered solution. Such study will also give information about contamination related to filter papers and loss of phosphate from solution.

Materials and methods

Reagents

i. Standard phosphorus solution:0.4394 g of KH_2PO_4 as weighed accurately and dissolved in deionized water. The solution was diluted to 1 litre with deionized water. The solution contained 100 μg P cm^{-3}.

ii. 0.5 M acetic acid was prepared by diluting 29 cm^3 glacial acetic to a 1 litre with deionized water.

iii. 0.5 M potassium acetate was prepared by dissolving 49.1 g of analar grade reagent in a 1 litre of deionized water.

iv. Dilute phosphorus standard solutions:
0.0, 0.1, 0.2, 0.5 and 1.0 cm^3 of the standard phosphorus solution was diluted to 100 cm^3 with deionized water, 0.5 M acetic acid and 0.5 m potassium acetate respectively. These solutions contained 0, 0.1, 0.2, 0.5 and 1 μg P cm^{-3} in respective extractant.

Procedure

Two experiments were carried out to study the adsorption/desorption properties of filter papers on phosphate in solution.

1) Phosphate solutions in the range 0-1 μg P cm^{-3} were made up in water, 0.5 M acetic acid and 0.5 M potassium acetate. These solutions were either passed through a polypropylene funnel with no filter paper or through a polypropylene funnel with a Whatman no. 42, 12.5 cm filter paper. Solutions were collected in plastic bottles and analysed for phosphate content (see Section 2.2.1.2).

2) Phosphate solutions of 0 and 1 μg P cm^{-3} were made up as in the previous experiment. These solutions were passed through a variety of grades of filter papers in polypropylene funnels. As before, the blank control was passage of the solution through a funnel with no filter paper. Solutions were collected in plastic bottles and analysed for phosphate content (see Section 2.2.1.2).

In both experiments each treatment was replicated five times.

Results and discussion

In both experiments some very small differences in phosphate concentration in solution due to filtration were observed (Tables 2.17 and 2.18). These differences, however, were at the limit of detection of the analytical method. So, even though the apparent errors were all in the same direction (release of phosphate from filter papers) and shown to be significant in some cases by statistical analysis, such variation is not an important aspect of the measurement of phosphate in this system.

Table 2.17 Phosphorus concentration in solution after filtration through Whatman no. 42 filter paper[+]

Concentration of P $\mu g\ cm^{-3}$		water	0.5 M acetic acid	0.5 M potassium acetate
0.0	unfiltered	0.00	0.00	0.01
	filtered	0.01*	0.01*	0.01
0.1	unfiltered	0.08	0.10	0.11
	filtered	0.10*	0.11***	0.11
0.2	unfiltered	0.19	0.19	0.19
	filtered	0.20*	0.20***	0.19
0.5	unfiltered	0.49	0.49	0.51
	filtered	0.50**	0.49	0.51
1.0	unfiltered	1.00	1.02	1.00
	filtered	1.02*	1.02	0.99

[+] pooled t test of filtered and unfiltered solution at each concentration

* significant at 0.1 percent
** significant at 1.0 percent
*** significant at 5.0 percent

Table 2.18 Phosphorus concentration in solution after filtration by various grades of filters+

Grade of filter paper	Batch Number	Concentration (μg P cm^{-1})					
		Water		0.5 M acetic acid		0.5M potassium acetate	
		0	1	0	1	0	1
		μg P cm^{-3}					
1	9018	0.00	1.00**	0.00***	1.00	0.01***	1.00
2	5141/13	0.01**	1.01	0.01	1.00	0.004***	1.00*
5	656102	0.01***	1.01	0.01	1.01**	0.01	1.00
40	4621	0.01***	1.02	0.01***	1.01*	0.00	1.00
40	590452	0.01*	1.01	0.01	1.01*	0.00	1.01
42	090123	0.01	1.01	0.01	1.00	0.00	1.02
42	9549	0.00	1.02*	0.01	1.01*	0.00	1.01
50	802603	0.00	1.01	0.00	1.00	0.002	1.00
52	608907	0.01	1.01	0.01*	1.00	0.00	1.00
540	6090/09	0.01*	1.01	0.01	1.00	0.00	1.00
Blank (unfiltered solution)		0.0	1.02	0.0	1.00	0.0	1.00

+ pooled t test of each filtered solution against the corresponding blank unfiltered solution.

*** significant at 0.1 percent   ** significant at 1.0 percent   * significant at 5.0 percent

2.2.5 Problems due to storage

When soil extracts cannot be analysed immediately after extraction, they should be stored properly so that changes are minimized. No specific recommendation for storage is available, and it is possible that some determinations are more likely to be affected than others by storage prior to analysis. Microbial activity may cause some changes in the soil extract, especially if the pH is high and storage is at room temperature. Moreover, the container used for storage may also cause phosphorus adsorption or desorption. Since any change in phosphorus in the extract can affect or invalidate subsequent chemical analysis, it was decided to investigate the effect of time of storage, type of container and temperature on both phosphorus standard solutions and soil extracts.

Materials and methods

i. Effect of container and time of storage on phosphate standard solutions.

In order to see the effect of container on phosphorus concentration in solution, phosphorus standard solutions were made up in water, 0.5 M acetic acid and 0.5 M potassium acetate (Section 2.2.4) and subjected to storage for up to 45 days at 4^oC, both in glass and plastic bottles. Phosphorus was measured at 0, 2, 7, 14, 29 and 45 days. Fresh phosphorus standard solutions were made for each set of measurement.

ii. Effect of container, time of storage and temperature on phosphate in soil extracts.

Phosphate extracted from eight soils (Section 2.2.2) with 0.5 M acetic acid and 0.5 M ammonium acetate buffer, after 24 hours of shaking at 30°C were subjected to storage in plastic bottles at 4 and 20°C temperature. Phosphorus was measured at 0, 3, 5, 8 and 14 days in the case of acetic acid and at 0, 5 and 10 days in the case of ammonium acetate.

Results and discussion

The effects of storage, time and types of container of phosphate in the standard solutions are shown in Tables 2.19 to 2.21, which has been further illustrated in Figures 2.10 to 2.15.

Generally it can be concluded that there was no effect of storage and container on phosphate concentration at levels of 0 to 1 μg P cm^{-3}.

In the case of acetic acid and ammonium acetate extracts of soil, neither time of storage or temperature caused any pronounced effect on phosphate concentration in solution (Tables 2.22 and 2.23).

From the preceeding discussion it was concluded that the effect of type of container, time and temperature of storage, was very small and was within the limit of the Technicon AutoAnalyzer, so cannot affect the measurement of phosphorus concentration in soil extracts.

Table 2.19 <u>Effect of storage and container on the adsorption-desorption of standard phosphorus made in water</u>

Concentration	Days								F
(μg P cm^{-3})	0		1	2	7	14	28	45	
0	0.00	G	0.0	0.0	0.00	0.00	0.0	0.00	-
		P	0.0	0.0	0.00	0.00	0.0	0.00	-
0.1	0.09	G	0.09	0.09	0.08	0.09	0.08	0.08	2.0
		P	0.09	0.09	0.09	0.09	0.09	0.09	1.1
0.2	0.18	G	0.18	0.18	0.18	0.18	0.18	0.18	2.0
		P	0.18	0.18	0.17	0.18	0.18	0.17	0.2
0.5	0.50	G	0.50	0.49	0.49	0.51	0.49	0.50	0.8
		P	0.49	0.49	0.49	0.50	0.50	0.09	2.2
1.0	0.99	G	0.99	0.99	0.99	1.00	1.00	1.00	1.6
		P	0.99	0.99	0.99	1.00	0.99	0.99	1.7

G - Glass P - Plastic

Table 2.20 <u>Effect of storage and container on the adsorption-desorption of standard phosphorus made in acetic acid</u>

Concentration (μg P cm^{-3})	Days 0		1	2	7	14	28	45	F
0	0.00	G	0.01	0.01	0.01	0.01	0.01	0.01**	5.7
		P	0.01	0.01	0.01	0.01	0.01	0.01*	1.7
0.1	0.09	G	0.09	0.1	0.10	0.1	0.1	0.1	0.9
		P	0.10	0.1	0.10	0.1	0.1	0.11**	1.7
0.2	0.19	G	0.19	0.19	0.19	0.19	0.19	0.19	6.0
		P	0.18	0.19	0.20	0.19	0.19	0.19	4.4
0.5	0.50	G	0.49	0.49	0.51	0.50	0.50	0.50	5.5
		P	0.49	0.49	0.51	0.49	0.50	0.50**	1.9
1.0	1.00	G	0.99	0.99	1.04	0.99	1.0	1.0**	6.7
		P	0.98	1.04	1.04	1.0	1.0	0.99**	7.1

G - Glass　P - Plastic　* significant at 5 percent　** significant at 1 percent　*** significant at 0.1 percent

Table 2.21 Effect of storage and container on the adsorption-desorption of standard phosphorus made in potassium acetate

Concentration (μg P cm^{-3})	Days 0		Days 1	2	7	14	28	45	F
0	0.00	G	0.0	0.0	0.0	0.0	0.02*	0.0**	-
		P	0.0	0.0	0.0	0.0	0.0	0.0*	-
0.1	0.09	G	0.09	0.09	0.08	0.08	0.11*	0.09**	2.7
		P	0.09	0.09	0.08	0.08	0.07	0.08	3.3
0.2	0.18	G	0.19	0.18	0.18	0.17	0.20*	0.17*	1.3
		P	0.18	0.18	0.18	0.17	0.18	0.17	1.4
0.5	0.49	G	0.49	0.49	0.49	0.48	0.52	0.51***	5.5
		P	0.49	0.50	0.49	0.47	0.50	0.49	1.5
1.0	1.00	G	0.99	1.00	0.99	0.98*	1.04*	1.03***	9.4
		P	0.98	0.99	0.99	0.97	1.0	0.99	9.8

G - Glass P - Plastic * significant at 5 percent ** significant at 1 percent *** significant at 0.1 percent

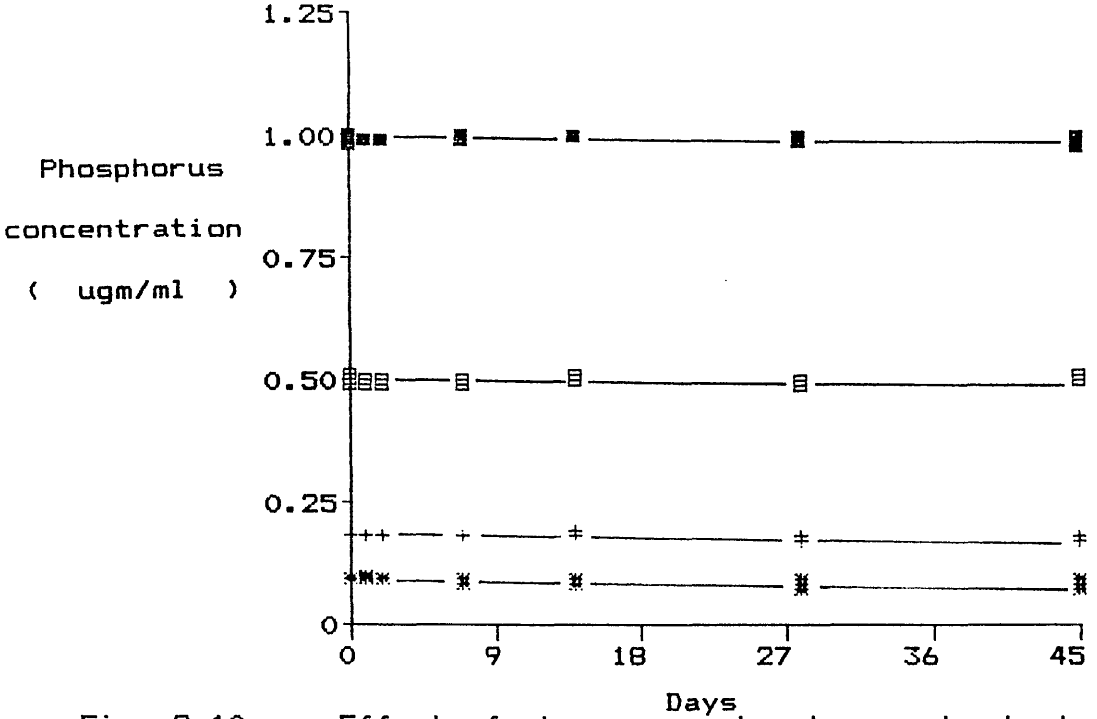


Fig. 2.10 Effect of storage on phosphorus standard solutions made in water and stored in glass bottles.
0.1ppm (*) ,0.2ppm (+)
0.5ppm (□) ,1.0ppm (■)

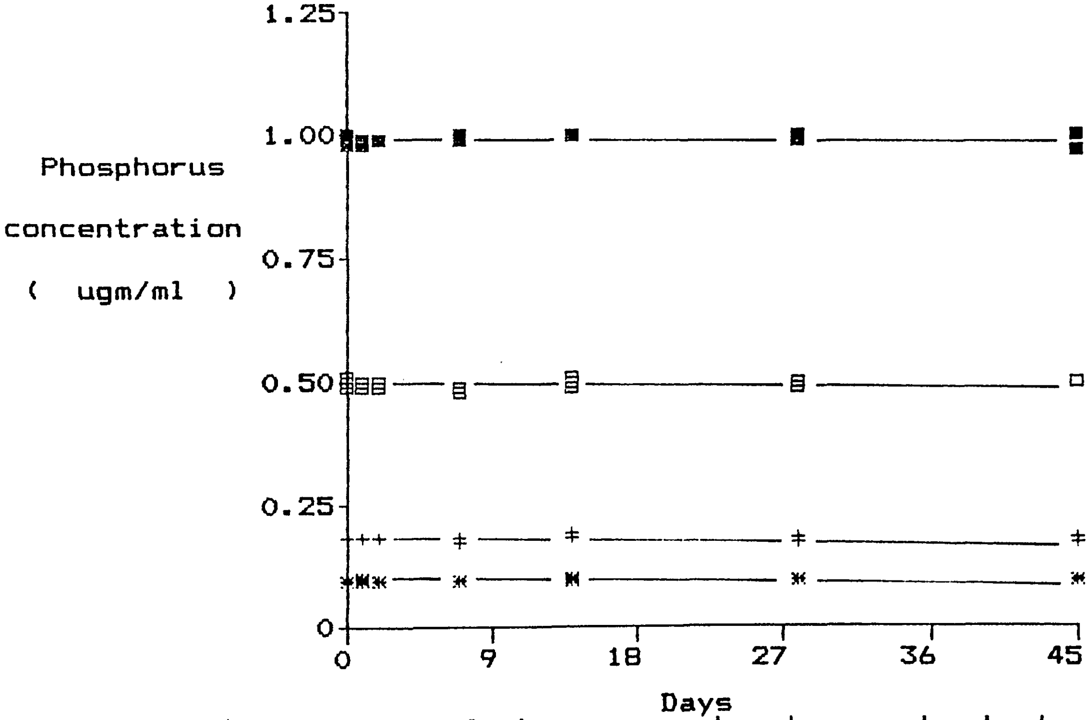


Fig. 2.11 Effect of storage on phosphorus standard solutions made in water and stored in plastic bottles.
0.1ppm (*) ,0.2ppm (+)
0.5ppm (□) ,1.0ppm (■)

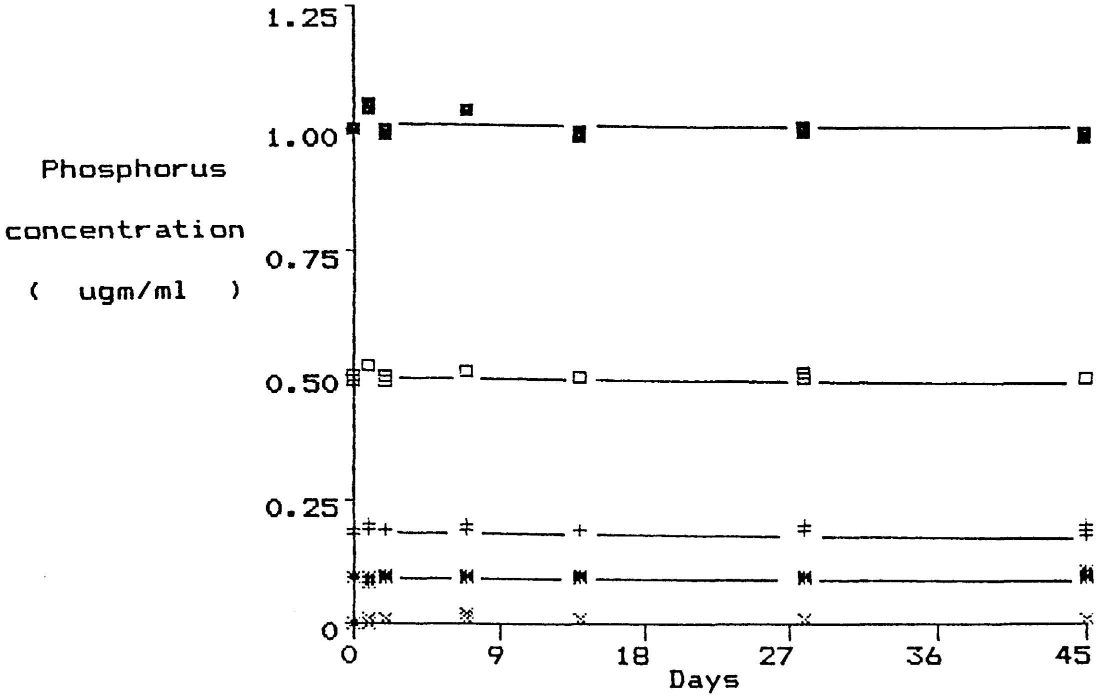


Fig. 2.12 Effect of storage on phosphorus standard solutions made in acetic acid and stored in glass bottles.
0.0ppm (×) ,0.1ppm (✱) ,0.2ppm (+)
0.5ppm (□) ,1.0ppm (■)

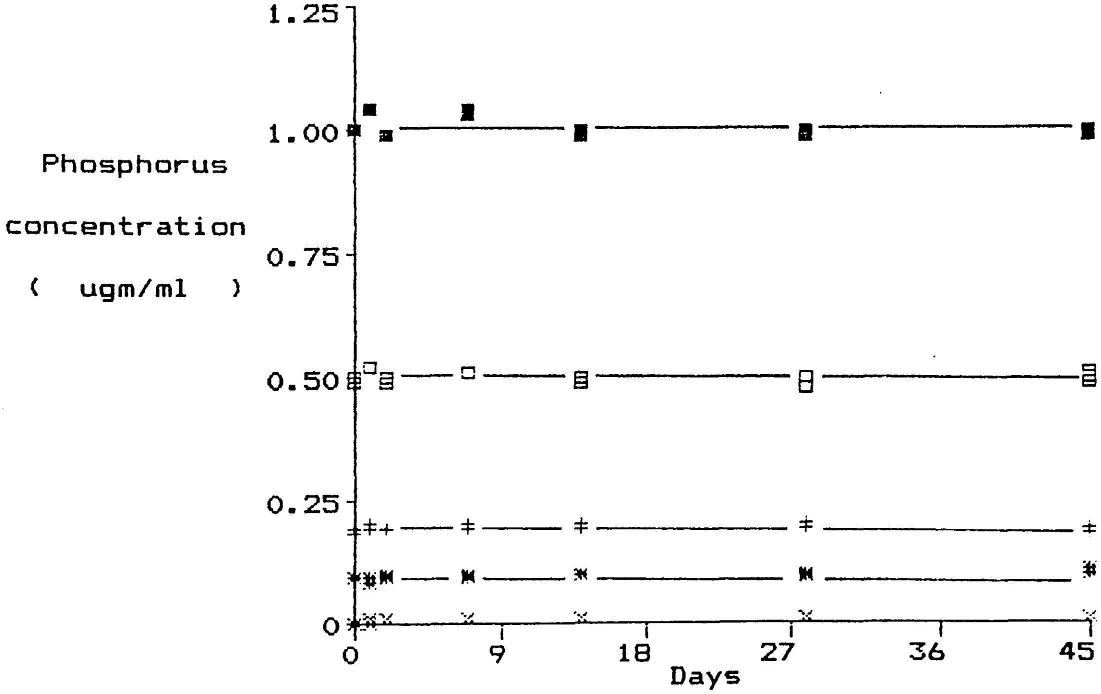


Fig. 2.13 Effect of storage on phosphorus standard solutions made in acetic acid and stored in plastic bottles.
0.0ppm (×) ,0.1ppm (✱) ,0.2ppm (+)
0.5ppm (□) ,1.0ppm (■)

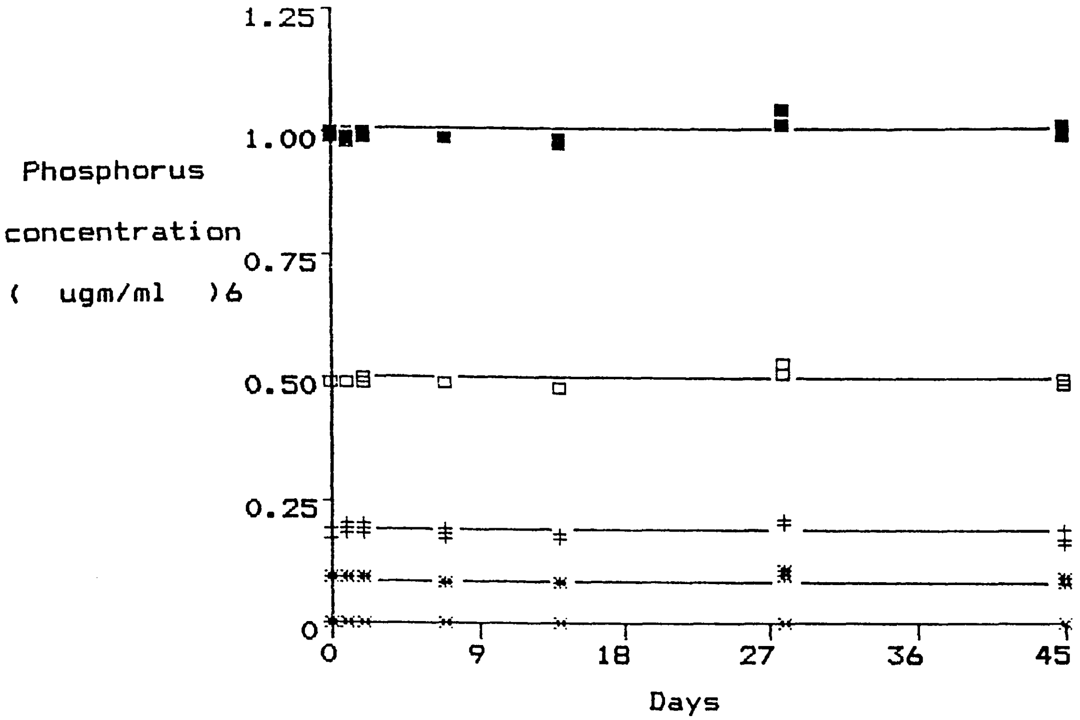


Fig. 2.14 Effect of storage on phosphorus standard solutions made in potassium acetate and stored in glass bottles.

0.0ppm (×) , 0.1ppm (✱) , 0.2ppm (+)
0.5ppm (□) , 1.0ppm (■)

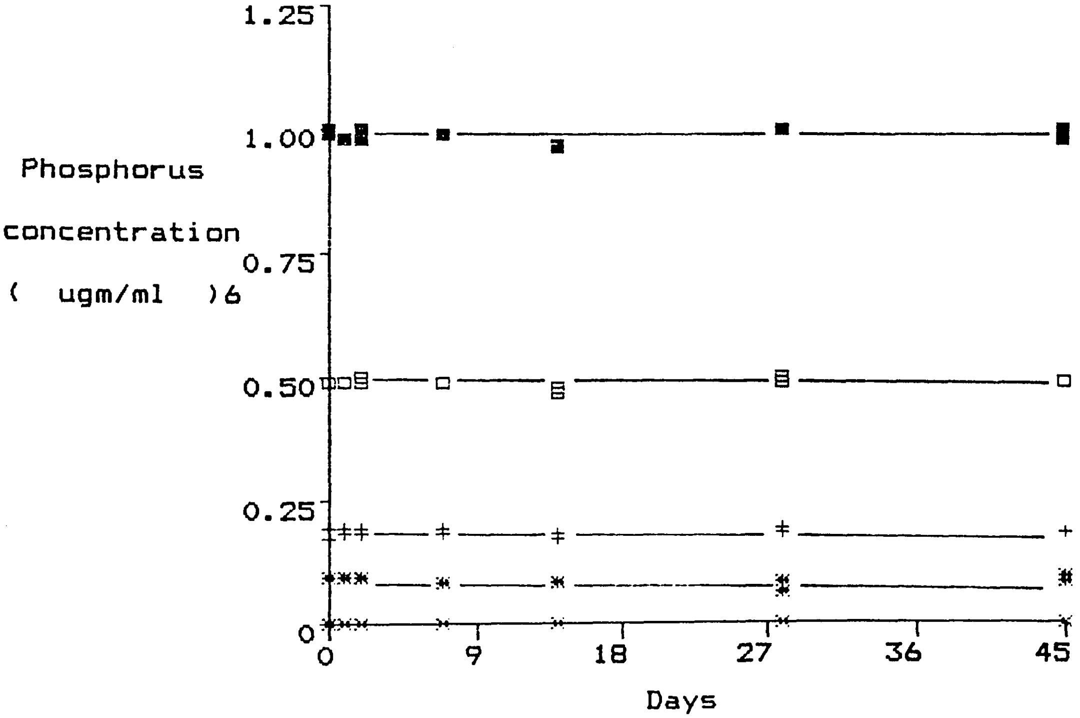


Fig. 2.15 Effect of storage on phosphorus standard solutions made in potassium acetate and stored in plastic bottles.

0.0ppm (×) , 0.1ppm (✱) , 0.2ppm (+)
0.5ppm (□) , 1.0ppm (■)

Table 2.22 Effect of temperature on soil phosphorus content

Soil	Acetic acid (ppm)					
	0 day		3 day	5 day	8 day	14 day
1	0.26	F	0.26	0.25	0.45	0.19*
		R	0.23	0.24	0.44	0.19
2	0.88	F	0.87	0.90	0.93	0.81
		R	0.87	0.87	0.90	0.84
3	6.71	R	6.59	6.51	6.59	6.35
		F	7.21	7.18	7.21	7.20
4	0.53	R	0.54	0.54	0.55	0.53
		F	0.49	0.49	0.49	0.45*
5	0.37	R	0.35	0.35	0.37	0.34
		F	0.35	0.33	0.38	0.30
6	32.1	R	31.22	30.42	30.97	31.05
		F	34.38	34.0	34.39	34.58
7	1.95	R	1.86	1.90	2.03	1.97
		F	1.89	1.95	2.14	1.96
8	37.7	R	37.98	37.88	27.76	38.16
		F	38.26	38.04	38.04	38.31*

Fresh samples extracted at 30°C, 24 hour shake.

F = 4°C R = 20°C

* significant at 5%

Table 2.23 Effect of temperature on soil phosphorus content (continued)

Soil	Ammonium acetate (ppm)			
	0 day		5 day	10 day
1	0.75	F	0.85	0.90
		R	1.01	1.11
2	6.34	F	6.20	6.24
		R	6.13	6.15
3	9.43	F	9.07	9.09
		R	9,70	9.72
4	0.14	F	0.14	0.14
		R	0.21	0.17
5	0.1	F	0.01	0.00
		R	0.17	0.23
6	18.52	F	18.12	18.18
		R	18.80	18.83
7	2.99	F	2.98	3.05
		R	3.05	3.13
8	15.23	F	14.86	14.75
		R	15.65	15.56

Fresh samples extracted at 30°C, 24 hour shake.

F = 4°C R = 20°C

2.2.6 General conclusion

i. None of the filter papers available had any contamination in respect of phosphorus, and can be used successfully for all experimental work dealing with phosphorus.

ii. Though period, temperature (4-20°C) and container have no pronounced effects on phosphorus concentration in solution, it is advisable to store soil extracts in clean plastic bottles, not above 10°C and not for long duration.

iii. Half an hour shaking at 20°C on an orbital shaker at 150 rpm was used as standardized procedure for the extraction of plant available phosphorus and the comparative study, using acetic acid, sodium bicarbonate and ammonium fluoride.

iv. In the case of sodium bicarbonate:

    a) Clean charcoal is recommended to be used as the decolorizing agent at the rate of 1 g (2.5 cm^3) per 50 cm^3 of extract.

    b) The plastic column method should be used, if needed for charcoal cleaning, before using as a decolorizer with 6 M hydrochloric acid as washing material.

    c) Before sodium bicarbonate extract is repumped through the basic manifold, for colour development, the sample stream should be passed through a double coil mounted on a bath at 55-60°C.

v. In the case of ammonium fluoride, 4 percent boric acid can be used most effectively, for the elimination of any possible interference of fluoride in the colour development.

vi. Colorimeter fitted with 880 nm filter and blue bulb is most advisable for automated determination of phosphorus.

2.3 Description of sites

Areas on a bing of uniform appearance or, as experimental plots, were considered as a representative sampling area. A visual assessment was made for selecting an area of uniform appearance over the bing, keeping in view the colour of the spoil, topography, steepness of the slope, absence or presence of vegetation, density of vegetation and any treatment in the past on each bing under study.

1. Loganlea (LL) Grid reference NS 976622

This site is situated in close proximity to the villages of Loganlea and Stoneyburn, alongside the Breich Water. The soil materials of the area are derived from the limestone coal group. The site was reclaimed in 1983. One sampling area was identified for sampling.

2. Meikle Earnock (ME) Grid reference NS 712531

The bing is situated next to the village of Meikle Earnock, outside Hamilton. The site is unreclaimed. After the mining had ceased, the spoil was washed to reclaim more coal before the bing was regraded. The site was completely barren of vegetation. One sampling area was identified for sampling.

3. North Addiewell (NA)   Grid reference NT 002630

The material on the site was derived from oil shale wastes and was under a good cover of mixed natural vegetation. Three sampling areas were identified for sampling.

NA_1 Naturally vegetated, under trees.
NA_2 Steep slope, naturally vegetated mainly heather.
NA_3 Flat, under clover.

4. Baads (BD)   Grid reference NT 003609

This bing is situated near the town of West Calder. Some parts of the bing are highly pyritic and acid patches are common. Reclamation of the site was carried out in 1980. A part of the site received 50 t/ha of lime and 30 kg/ha of a 15:10:10 NPK compound fertilizer before being seeded with a grass-white clover mixture (Lister, 1987).

Two field experiments were established. On one area various organic amendment treatments; chicken manure, sewage sludge, peat and alginure (seaweed-based soil conditioner) were compared for their ability to supply organic matter and nutrients to the spoil. On the other area a range of grass and legume species were tested for their ability to produce a self-sustaining vegetation.

Loganlea and Baads are typical of the coal mine waste tips found in West Lothian. Before reclamation, they consisted of areas of burnt and unburnt waste. The process of regrading led to some mixing of these, especially at Loganlea. Thus the sampled material comprised mainly shale, which had weathered to some extent, and some of which had been ignited burning off the organic component. Experience of these and similar sites in this area suggests that 30-50 percent of the spoil in the surface layers had broken down enough to pass a 2 mm sieve. The spoil is difficult to dig due to the clayey nature of the fine material, and the presence of a significant amount of coarse shale fragments. The waste at Meikle Earnock comprised entirely unburnt coal spoil, and was a much softer material. This was reflected by a higher percentage of less than 2 mm material (65-80%). Since this study, this site has been reclaimed and has presented a number of difficulties due to its poor texture. In a wet condition it is very sticky, while it dries to a hard mass which is difficult to dig. There are also a number of erosion gullies resulting from poor water penetration leading to surface runoff. The oil shale waste from North Addiewell was variable in terms of soil texture. This was partly because of the mixed vegetation, aspect and slope. The underlying material was very difficult to dig due to compaction. The finer material (<2 mm) was therefore quite negligible (4%), possibly due to washing of the fine material down the profile.

The detail of the treatments are as follows:

BD_1	Limed (25 t/ha), no organic amendments, and sparsely vegetated.
BD_2	Limed (25 t/ha), soil conditioner (11 t/ha) treated and well vegetated.
BD_3	Limed (25 t/ha), peat (20 t/ha) treated and well vegetated.
BD_4	Limed (25 t/ha), sewage sludge (20 t/ha) treated and well vegetated.
BD_5	Limed (25 t/ha), chicken manure (4 t/ha) treated and well vegetated.
BD_6	Control (no lime, no manure) and barren.
BD_7	Unreclaimed area of the bing. Two sampling areas were identified for sampling.
BD_{7a}	Surface soil (0-15 cm), and vegetated.
BD_{7b}	Subsoil mineral portion (15-25 cm).
BD_8	Red clover and grass mixture.
BD_9	Red clover and grass mixture.
BD_{10}	Grass mixture.
BD_{11}	Main area of the bing, not included in the experimental plot. It received 50 t/ha of lime and 300 kg/ha of 15:10:10 ratio NPK compound fertilizer during 1980, when the bing was reclaimed.
BD_{12}	Limed (25 t/ha), no organic amendment.
BD_{13}	No lime and sewage sludge (20 t/ha) treated.

Laboratory pretreatments

The freshly collected samples were brought to the laboratory as soon as possible and spread out on clean plastic sheets. The samples were kept in a cold room ($10^{o}C$) if it was not possible to sieve on the day of collection. Any vegetation, roots, stones etc. were discarded. The samples were partially air-dried to permit sieving through a 4 mm sieve with the minimum of disturbance. Care was taken to minimize any chemical and biological changes in the samples during the preparation process eg. drying of the samples at low temperatures (room temperature) and away from any chemicals containing phosphorus. The sieved samples were thoroughly mixed and refrigerated at $2^{o}C$ in tied plastic bags until they were to be used.

CHAPTER 3

BIOLOGICAL AND CHEMICAL TURNOVER OF PHOSPHORUS

3.1 Extractable phosphorus

Inorganic phosphorus can be classified as calcium-bound phosphorus (Ca-P), iron-bound phosphorus (Fe-P), aluminium-bound phosphorus (Al-P) and reductant soluble phosphorus (Rs-P). Rs-P is an adsorbed/mineral form and is often coated on or occluded by relatively insoluble oxides and hydroxides of iron and aluminium. It can be dissolved by strong reducing agents. Strong acid soils are dominated by Al-P, Fe-P and Rs-P. Neutral and slightly acid soils by Al-P, Fe-P, Rs-P and Ca-P fractions, while alkaline and calcareous soils by Ca-P only.

Methods for determining soil phosphorus, its various forms and availability to plants have been essential in developing principles and knowledge of the nature and behaviour of phosphorus in soil. During the past 50 years, soil scientists and agronomists have conducted hundreds of experiments to evaluate the phosphorus status of soils. A large number of methods have been proposed for the assessment of plant available phosphorus. Most of the methods are based on extraction procedures and differ according to the nature, concentration and pH of the extractant used, soil:extractant ratio, the extraction time and temperature. These determine the strength of the extraction and the extent to which the value can be regarded as reflecting capacity or intensity. However, the selection of a suitable method demands clear statement of objectives that require the soil phosphorus measurement.

Estimation of available phosphorus status of soils is necessary for delineating:-

i. fertilizer phosphorus recommendations.
ii. response to application of fertilizer phosphorus.
iii. the amount of phosphorus a soil can supply.

Generally, soils are grouped into, at least, three categories for the purpose of making fertilizer recommendation, low, medium and high levels of soil test phosphorus (Fitts and Nelson, 1956). SAC (1985) has classified five categories, while MAFF (1983) has ten categories of phosphorus levels from agricultural lands. Another grouping is that of deficient and adequate soil phosphorus levels. The soil phosphorus level separating the deficient soils from the adequate soils has been called the critical soil test phosphorus level (Cate and Nelson, 1965). However, with the use of computers, equations have been developed which give phosphorus fertilizer rates as a function of the specific soil phosphorus level.

Most soil phosphorus determinations have two distinct phases, the preparation of a solution containing the soil phosphorus or phosphorus fraction, and the quantitative determination of the phosphorus in this solution.

Dyer (1894) theorized that an ideal extracting solution would be one that had a pH similar to that of root sap. His studies indicated that the acidity of root sap was equivalent to a 1 percent citric acid solution. A good soil extractant should extract all or a proportionate part of the available form or forms of a nutrient from soils with specific properties. There should be a minimum of secondary precipitation and adsorption of extracted phosphorus. The amount of nutrient extracted should be measured with reasonable accuracy and speed, and finally, the amount extracted should correlate with the growth and response of each crop (Bray, 1948).

3.1.2 Common soil extractants

Soil test extractants can be placed into various categories related to the chemical nature of the extracting solutions. In certain cases the anions in the solution may have a specific effect either because of their anion replacing ability or their reaction with the cation associated with phosphorus. A list of the commonly used extractants to measure the available phosphorus is given below.

3.1.2.1 Dilute concentrations of strong acids

The common acids used for extracting phosphorus are hydrochloric acid (HCl), nitric acid (HNO_3) and sulphuric acid (H_2SO_4). The concentration of acid varies all the way from 0.002 M to 0.07 M. The pH of the solutions generally range from 2 to 3. The nitrate and chloride ions have very little effect on the extraction, but sulphate tends to reduce readsorption of the phosphorus dissolved by the acid solutions (Kamprath and Watson, 1986).

3.1.2.2 Dilute concentrations of strong acids plus a complexing ion.

The complexing ion used most often is fluoride, which forms strong complexes with the aluminium (Al) ion. The fluoride compound generally used is NH_4F. The most common extracting solutions of this kind are mixtures of HCl and NH_4F.

Neutral ammonium fluoride is also used as an extractant, which determines adsorbed phosphate. Turner and Rice (1954) found that neutral NH_4F can dissolve Al-PO_4 but not Fe-PO_4. Based on the selective solubility of phosphate in various extractants, Chang and Jackson (1956) found that NH_4F in a single extraction dissolves Al-PO_4 completely, Fe-PO_4 slightly and apatite negligibly. NH_4F in calcareous, or sometimes in acid, soil reacts with $CaCO_3$ with the formation of CaF_2 and causes over-estimation of acid extractable Ca-P, and under-estimation of non-occluded Fe and Al bound phosphates.

F^- ions are very effective in complexing Al^{3+} ions and in this manner releasing phosphorus from Al-P (Chang and Jackson, 1957). Calcium is precipitated by F^- ions and therefore the phosphorus present in soil as $CaHPO_4$ will be extracted by solution containing F^- ions (Thomas and Pealsee, 1973).

3.1.2.3 Dilute concentrations of weak acids

The most common weak acids or salts of weak acids used for extracting phosphorus are citric, lactic and acetic. The organic anions can replace adsorbed phosphorus and prevent its readsorption. 2.5 percent acetic acid is the most popular extractant used by the Advisory Service in Scotland. Acetic acid solution, which has generally a pH 2 to 3, provides sufficient hydrogen ion activity to dissolve calcium phosphate. It will also stabilize some of the aluminium and iron phosphate.

3.1.2.4 Acetate buffers (Morgan and modified Morgan) (Morgan, 1941)

Both Morgan (3% acetic acid buffered with 10% sodium acetate) and modified Morgan (3% acetic acid buffered with 4% ammonium acetate) have pH 4.8, extract very small amounts of phosphorus, as with acetic acid.

3.1.2.5 Buffered alkaline solution

Sodium bicarbonate, which originally was developed for calcareous soil (Olsen et al. 1954), is the most commonly used buffered alkaline solution for extraction of phosphorus, at a nearly constant pH of 8.5.

The HCO_3^- ion is quite effective in replacing adsorbed phosphorus, and reduces the activity of Ca^{2+} in solution by causing precipitation of Ca^{2+} as $CaCO_3$. As a result the concentration of phosphorus in solution increases. In acid soils containing Fe-PO_4 and Al-PO_4, phosphorus concentration in solution increases as the pH rises (Lindsay and Moreno, 1960). Secondary precipitation reactions in acid and calcareous soils are reduced to a minimum because the concentration of Al^{3+}, Fe^{3+} and Ca^{2+} remains at a low level in this extractant. Sodium bicarbonate extracts more phosphorus than acetic acid from the same soil, because it extracts phosphorus from soil solution, physisorbed, chemisorbed and also organic (Po) pools, plus a small amount of microbial phosphorus (Bowman and Cole, 1978). Since Olsen-phosphorus was much less dependent on soil type, it was considered as more suitable index of plant available soil phosphorus on soils differing in phosphorus retention (Smith and Gregg, 1982). Recently this extractant has been recommended by ADAS as a routine method for determining available phosphate in soils in England and Wales.

Sodium bicarbonate removes phosphorus by two mechanisms, anion replacement and hydrolysis of cation binding phosphorus. In the first mechanism, phosphorus adsorbed on surfaces of $CaCO_3$ and hydrated oxides of Fe^{3+} and Al^{3+} can be replaced by bicarbonate (Dean and Rubins, 1947; Olsen et al., 1954). When organic anions and sulphate are present in acid solution, they reduce readsorption of phosphorus. In the second mechanism, $NaHCO_3$ extracts phosphorus, from Al-P and to some extent Fe-P, due to the hydrolysis of Al^{3+} and Fe^{3+} (Tyner and

Davide, 1962).

3.1.2.6 Anion exchange resin

A wide variety of insoluble polymers that are capable of forming inorganic salts are now designed as ion exchange resins. Anion exchange resins extract inorganic phosphorus directly exchangeable with soil solution phosphorus and is a new approach to the problem of estimating soil phosphorus availability.

The skeleton of the resin is formed by co-polymerization of styrene and divinylbenzene (DVB). The DVB links the linear styrene chain together and yields an insoluble three dimensional polymer. A high content of DVB gives the resin a tight, firm, but somewhat brittle structure. Such a resin reacts more slowly than a resin with a low content of DVB, which has a more open structure. Some resins are gel types, which do not possess real pores. The ion diffuses through the gel structure. The larger the bead size of a resin, the less is the outer surface area in relation to the number of exchange sites and the more slowly it reacts.

Anion exchange resin method removes phosphorus from soils without alteration or pH changes, like that of isotopic phosphate exchange and equilibrium phosphate concentration method. However, both isotopic phosphate exchange and equilibrium phosphate concentration method are unsuitable for routine work due to time consuming and minute quantity of phosphorus determination, respectively.

The extraction of soil phosphate by resin was introduced by Amer et al. (1955) and later tested as a measure of available phosphorus in soils by many workers (Cook and Hislop, 1963; Gunary and Sutton, 1967; Dalal and Hallsworth, 1976).

The removal of phosphate from soil by plants or resin involves the following process:

P (solid) -> P (solution) -> P (plant or resin)
(Labile + nonlabile)

It is argued that the action of anion exchange resin in removing phosphorus from soil is somewhat similar to the action of plants, because it simulates the desorbing effect of plant roots better than a chemical extractant.

Actually the resin functions as a plant root with a very high capability for phosphorus uptake. Unlike the plant root the resin, however, releases chloride or bicarbonate ions for the anion extracted. Roots, however, secrete exuadates (organic acids, amino acids and sugars) which help in solubilizing phosphorus.

This method has given better correlation with phosphorus uptake by plants, than other methods, using a single extractant (Bache and Rogers, 1970; Elrashidi et al., 1975 and Kadeba and Boyle, 1978). Khalil (1981) showed that anion exchange resin is suitable for predicting phosphorus uptake, followed by acidified NH_4F,

NH_4OAc, CH_3COOH and $NaHCO_3$, in the descending order. Bache and Ireland (1980) and Khalil (1981) found that anion exchange resin in bicarbonate form extracted more phosphorus than chloride resin in acid soil. They concluded that the greater amount of phosphorus extracted by bicarbonate resin might be either due to the release of bicarbonate ions to soil solution, which resembles the natural chemical condition prevailing in the rhizosphere (Khalil, 1981) or due to lower total anion and higher desorbing anion concentration (Bache and Ireland, 1980). However, little difference between the two resins was found in the case of calcareous soil.

3.1.2.7 Choice of extractants

The experience in Britain is that extraction with sodium bicarbonate is generally the best method on which to base advice to farmers on fertilizer use. Recently, it has been recommended by MAFF as a routine method for determining available phosphorus in soils in England and Wales. The recommendation was based on the fact that this extractant was not only effective for the calcareous soils for which it was designed, but also as good as any other method for non-calcareous sands and loams. In addition, it was the best extractant for organic soils in Wales (Wild, 1988). Moreover, sodium bicarbonate extracts phosphorus from organic pools plus small amounts of microbial phosphorus, so it would be more suited for a mineralization study (Bowman and Cole, 1978).

Though extraction of colliery soils with sodium bicarbonate gives coloured extracts of varying intensity due to extraction of organic matter, a clean-up step to remove the colour component has been developed (Section 2.2.3). Also a lot of care is needed due to the fact that this method is very highly temperature sensitive.

Acetic acid extracts a small amount of phosphorus, especially in soils which fix phosphorus strongly. However, extractants which remove small amounts of phosphorus are more useful in evaluating the short-term phosphorus availability. Another advantage is that a clear extract can be obtained with colliery soil, which gives coloured extracts with other dilute acids (Lister, 1987). Work carried out in the Agricultural Chemistry Department, Glasgow Univeristy (Khalil, 1981) showed that phosphorus extracted from soils using 0.5 M acetic acid correlated significantly with plant uptake. This extractant is also commonly used by the Advisory Service in Scotland (Reith et al., 1987).

The most promising methods that attempt to simulate conditions affecting the uptake of ions by plants are those involving the use of anion exchange resins to extract phosphorus from soils. The forms of soil phosphorus which are sorbed by resins would be of great importance in identifying phosphorus potentially available to plants. Khalil (1981) showed that anion exchange resin is suitable for predicting phosphorus uptake by plants.

For these reasons, 0.5 M sodium bicarbonate, 0.5 M acetic acid and anion exchange resin were selected as test methods for studying the phosphorus status and mineralization of a large number of coal and oil shale soils.

3.1.3 Materials and methods

The measurements were made on 30 samples of coal mine soils collected from plots of established experiments at Baads Colliery situated in Central Scotland. Some of these survey samples collected were measured for the amount of phosphorus they adsorbed. The detail of these sites has been given in Section 2.3.

Ten sampling areas each representing an individual experimental plot were selected on this bing keeping in view the residual effect of various organic manures and leguminous species on the phosphorus status of coal mine soils. Three individual samples were collected at random from the surface spoil (0-15 cm) from each sampling area, making a total of thirty samples.

1. pH determination

The pH of coal mine soil samples was determined in water by the method described in Section 2.1.1.

2. Extractable phosphorus

Phosphorus was extracted with 0.5 M sodium bicarbonate, 0.5 M acetic acid and anion exchange resin (chloride form) in 30 coal mine soil samples by the methods given below and the extracts were analysed by automated colorimetric methods (Section 2.2.1.2).

a. Acetic acid extractable phosphorus

Reagents

i. 0.5 M acetic acid: 29 cm^3 of analar grade acetic acid was diluted to 1000 cm^3 with deionized water.

ii. Phosphorus stock solution 1: 4.3937 g of KH_2PO_4 was weighed accurately and dissolved in deionized water. The solution was diluted to 1000 cm^3 with deionized water. This solution contained 1000 mg P l^{-1}.

iii. Phosphorus stock solution 2: 100 cm^3 of the stock solution 1 was diluted to 1000 cm^3 with 0.5 M acetic acid. This solution contained 100 mg P l^{-1}.

iv. Phosphorus working standards: 0.0, 1.0 and 5.0 cm^3 of phosphorus, stock 2 were pipetted into 100 cm^3 volumetric flask and diluted to 100 cm^3 with 0.5 M acetic acid. These solutions contained 0, 1 and 5 mg P l^{-1} respectively.

Method

2.5 g of fresh soil samples (oven dry equivalent) ground to pass a 4 mm sieve was weighed into 4 oz screw cap extraction bottles, and 50 cm^3 of 0.5 M acetic acid added. The bottles were capped and shaken for 30 minutes, at 20°C, using an orbital shaker at 150 rpm. The extracts were filtered immediately through Whatman no.

42 paper into 4 oz plastic bottles. The phosphorus concentration was measured, using a Technicon AutoAnalyzer by the method given in Section 2.2.1.2. A blank determination was carried out with each batch of samples, using all reagents, but with no soil sample. Manifold for acetic acid given in Table 2.8 was used.

b. Sodium bicarbonate extractable phosphorus

Reagents

i. 0.5 M sodium bicarbonate: 210 g sodium bicarbonate were dissolved in deionized water, and the volume was made up to 5000 cm^3. pH was adjusted to 8.5 with 1 M sodium hydroxide. The solution was stored in a polyethylene container for a period not longer than one month. The pH of the solution was checked each time before use.

ii. 2 M sulphuric acid. 110 cm^3 concentrated analar grade sulphuric acid was diluted with deionized water to 1000 cm^3.

iii. Darco charcoal (BDH chemicals): washed by method given in Section 2.2.3.

iv. Standard phosphorus working solutions: 0.0, 1.0 and 5.0 mg P l^{-1} solutions were prepared in 0.5 M sodium bicarbonate, pH 8.5 using stock solution 2 (Section 3.1.3).

Method

The same method was followed as for acetic acid extraction (Section 3.1.3), except that 50 cm^3 of 0.5 M sodium bicarbonate was added to each bottle. 2.5 cm^3 (1g by weight) washed charcoal was also added to each bottle before shaking to obtain a clear filtrate. The phosphorus concentration was measured, using a Technicon Autoanalyser by the method given in Section 2.2.1.2. 2 M sulphuric acid was used for neutralization of bicarbonate in a heating coil at 60°C. Manifold for sodium bicarbonate given in Table 2.8 was used.

c. Anion exchange resin extractable phosphorus.

Phosphorus was extracted by anion exchange resin (chloride form) by the method given in Section 2.1.6.

3.1.4 Results and discussion

The levels of phosphorus extracted by acetic acid, sodium bicarbonate and anion exchange resin from 30 samples of coal mine soils presented in Table 3.1 indicate a great variation between sampling areas. This indicates a great deal of heterogenity and variability in coal mine soils. The levels of acetic acid, sodium bicarbonate and anion exchange resin extractable phosphorus ranged from 0.15 to 8.5 (mean = 0.97), 0.37 to 15.9 (mean = 4.17), and 0.0 to 8.1 (mean = 0.77) mg P kg^{-1} soil, respectively.

The data in Table 3.1 also indicate that all types of extractable phosphorus were very low in most of the experimental plots set up at Baads Colliery, as well as in

Table 3.1 Amount of phosphorus in coal mine soils as determined by different methods

Treatment		pH	Extractable phosphorus (mg kg^{-1}) 0.5M acetic acid	 0.5M sodium bicarbonate	 anion exchange resin
No organic amendment	A	3.0	0.42	4.78	0.85
	B	3.4	0.30	3.42	0.86
	C	4.7	0.82	2.14	0.94
Alginure	A	4.6	0.15	0.84	0.82
	B	5.0	0.37	6.29	0.81
	C	4.9	0.36	1.84	0.45
Peat	A	4.0	0.30	4.81	0.29
	B	6.8	2.68	5.85	0.70
	C	4.1	0.49	5.77	0.04
Sewage sludge	A	4.2	0.42	4.12	0.10
	B	5.4	0.80	8.87	0.39
	C	3.7	0.44	3.89	0.18
Chicken manure	A	6.8	2.95	3.93	0.32
	B	6.9	0.43	1.30	3.10
	C	4.8	8.49	15.87	8.14
Control	A	3.5	0.68	5.81	0.06
	B	2.8	0.92	7.76	0.71
	C	3.1	0.93	9.19	1.50
Native vegetation	A	3.3	0.36	0.37	0.00
	B	5.5	0.61	1.79	0.03
	C	6.8	1.38	6.26	0.33
Red clover and grass 1 mixture	A	6.9	0.46	2.35	0.12
	B	7.2	0.53	2.59	0.39
	C	7.1	1.17	4.16	0.56
Red clover and grass 2 mixture	A	6.0	1.04	2.49	0.26
	B	4.7	0.37	2.72	0.07
	C	7.0	0.28	0.55	0.25
Grass	A	6.8	0.46	0.79	0.31
	B	6.6	0.33	3.20	0.25
	C	6.6	0.33	1.37	0.15

the control and adjacent native vegetation plots, the exception being that of chicken manure treated soil. The range of acetic acid, sodium bicarbonate and anion exchange resin extractable phosphorus in this treatment ranged from 0.43 to 8.5 (mean 4.0), 1.30 to 15.9 (mean 7.0) and 0.32 to 8.1 (mean 3.9) mg P kg^{-1} soil respectively. This agrees with the general findings of previous studies that extractable phosphorus is low in colliery soils (Fitter et al., 1974; Pulford, 1976; Palmer, 1978; Lister, 1987 and Pulford et al., 1988). The high amount of phosphorus extracted in the case of chicken manure plots might be due to mineralization of phosphorus from the manure used, which contained a high level of organic phosphorus (Walker, 1988).

Pulford et al. (1988) reported that acetic acid extractable phosphorus was very low, in the experimental plots set up at Baads Colliery, except for chicken manure treated soil. In chicken manure treated soil phosphorus ranged from 17.0 (1983) to 37.9 (1984) mg P kg^{-1} and control 0.3 (1983) to 0.4 (1984) mg P kg^{-1} soil. Lister (1987) using the Bray method (acidified ammonium fluoride) also on Scottish soils (West and Central Scotland), found that extractable phosphorus ranged from zero to 19.3 mg P kg^{-1} soil, with a mean of 6.2 mg P kg^{-1} soil. Palmer (1978) used a number of standard phosphorus extractants on spoils from the Yorkshire coalfield and found that the levels of phosphorus removed were too close to zero to be of value. Fitter et al. (1974) reported a mean of 5.5 mg

P kg^{-1} soil for bicarbonate extractable phosphorus in Lancashire spoil. Using spoil from the Scottish coalfield, Pulford (1976) could find no acetic acid extractable phosphorus in acid spoil.

The data in Table 3.1, clearly indicate a preponderance of sodium bicarbonate extractable phosphorus over both acetic acid and anion exchange resin extractable phosphorus from the same soil. The former extractant extracts phosphorus from soil solution, physisorbed, chemisorbed and also organic pools together with a small amount of microbial phosphorus (Bowman and Cole, 1978). Acetic acid and anion exchange resin extract very small amounts of phosphorus because both extract soil solution and readily exchangeable phosphorus only.

Figures 3.1 to 3.3 show the relationships between the levels of phosphorus removed by the three extractants and pH. These scattergrams show no relationship between the level of extractable phosphorus and pH. Nonsignificant relationship between pH and the acetic acid extractable phosphorus content was also reported by Pulford and Duncan (1978) in these soils.

Figures 3.4 to 3.6 show the relationships between the amounts of phosphorus removed by the three extractants. Again, no obvious relationships are observed. Due to the lack of an obvious linear relationship and the presence of outlying points, correlation coefficients were not calculated.

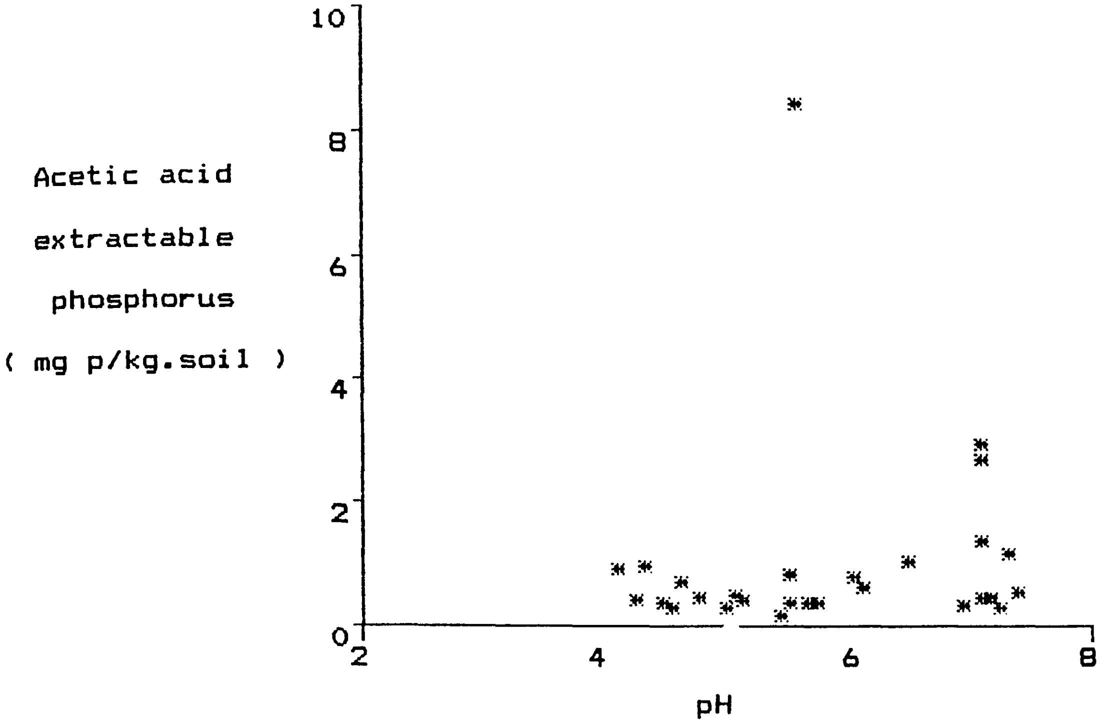


Fig. 3.1 Relationship between acetic acid extractable phosphorus and soil pH.

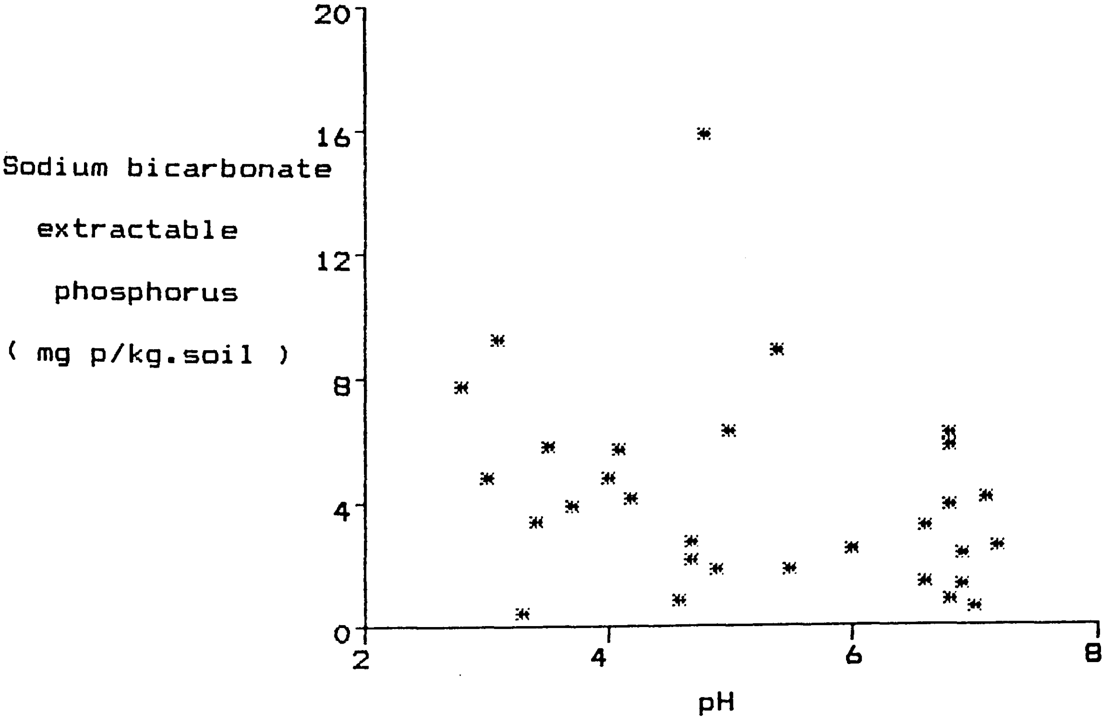


Fig. 3.2 Relationship between sodium bicarbonate extractable phosphorus and soil pH.

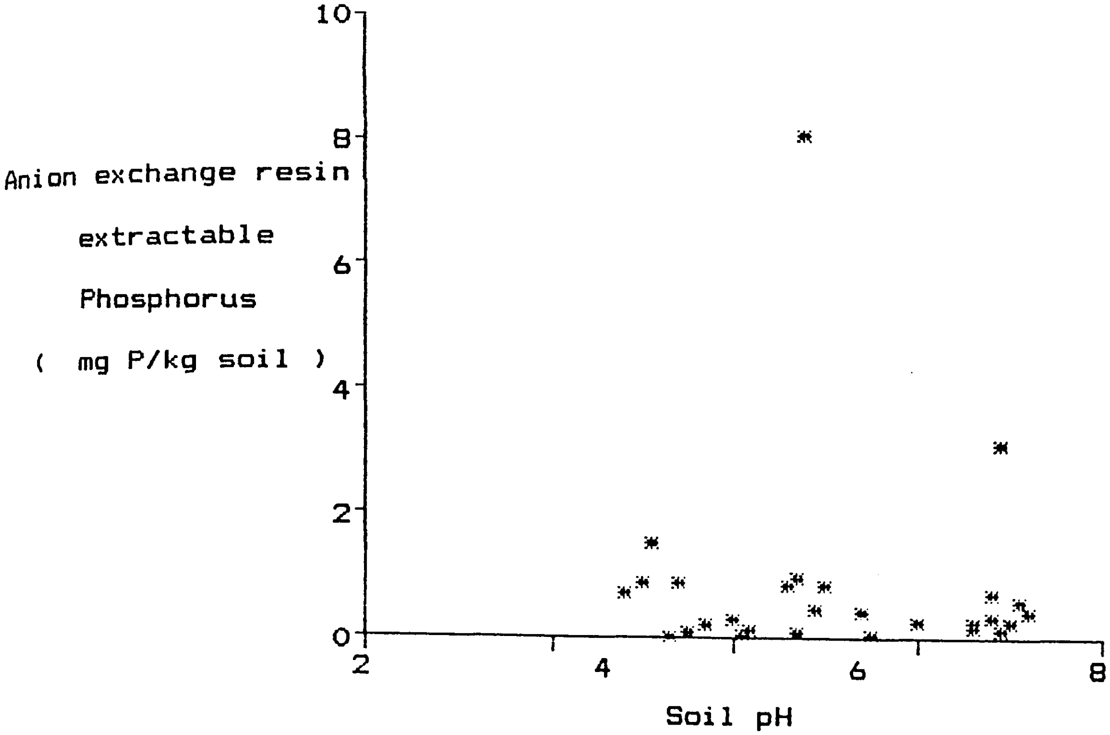


Fig. 3.3 Relationship between anion exchange resin extractable phosphorus and soil pH

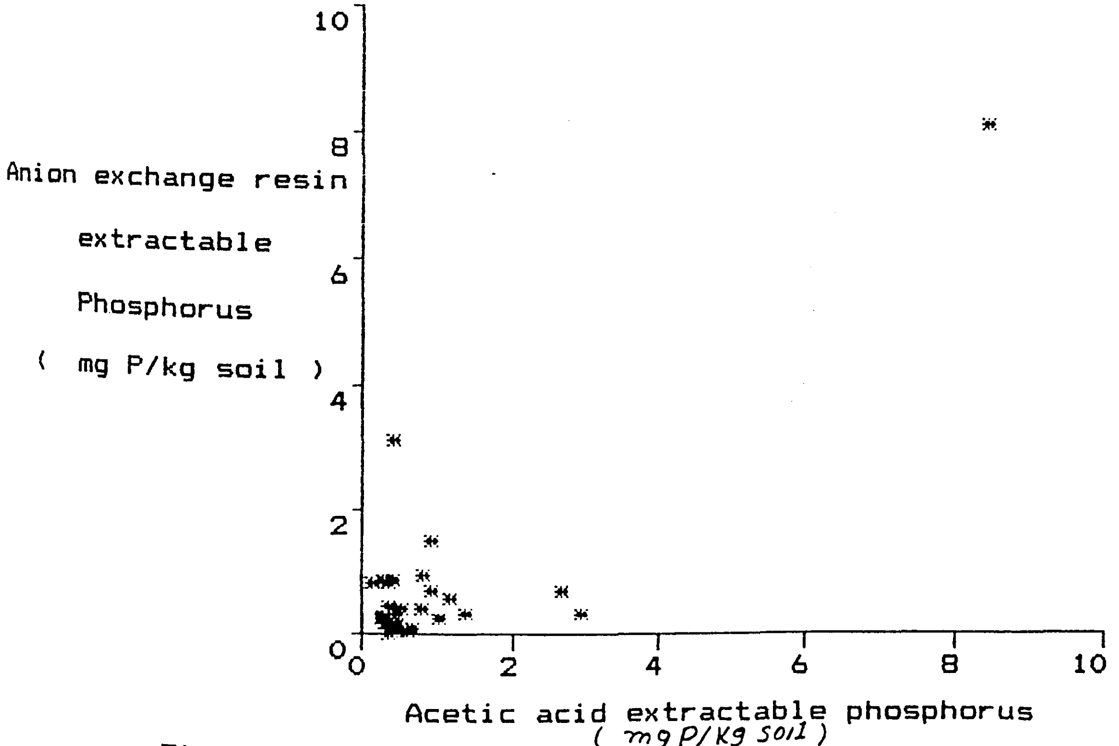


Fig. 3.4 Relationship between acetic acid and anion exchange resin extractable phosphorus

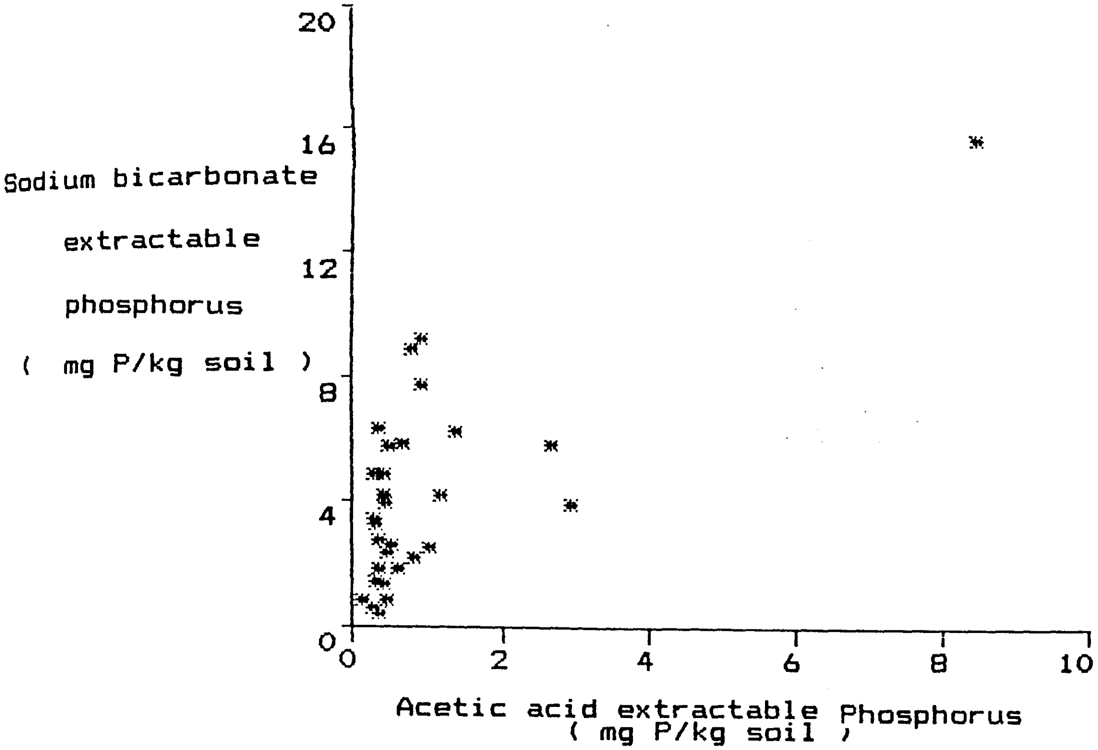


Fig. 3.5 Relationship between sodium bicarbonate and acetic acid extractable phosphorus

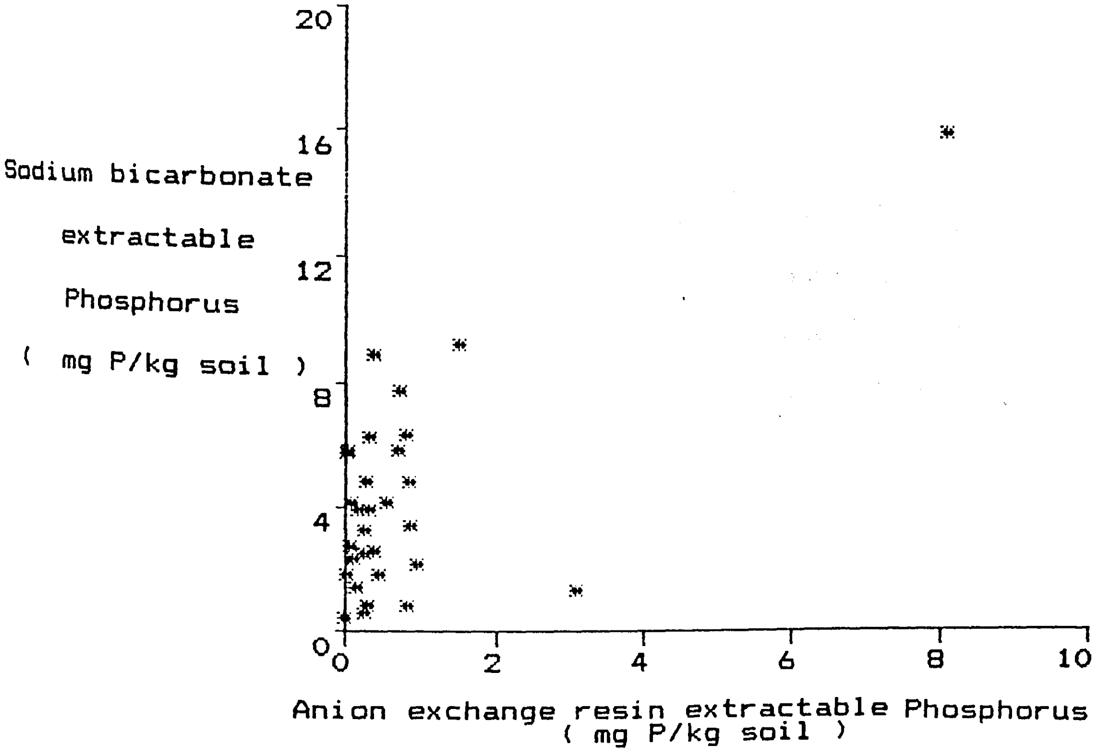


Fig. 3.6 Relationship between sodium bicarbonate and anion exchange resin extractable phosphorus

3.2 Adsorption study

3.2.1 Introduction

Adsorption is a sufficiently general term to include several kinds of surface reactions. Simply, it refers to when a chemical species passes from one bulk phase to the surface of another, where it accumulates without penetrating the surface of the second phase (Burchill et al., 1981). Sposito (1984) quoted that adsorption is the process through which a net accumulation of a substance occurs at the common boundary of two contiguous phases.

Adsorption isotherms, the determination of which is the most useful experimental procedure in the study of the interaction of anions with hydrous oxides or soils, have been used by many workers to measure the phosphate adsorption capacity of soils (Dean and Rubins, 1947; Bass and Sieling, 1950; Olsen and Watanabe, 1957; Fox et al., 1969; Barrow, 1970; Bache and Williams, 1971; Rajan and Fox, 1972; Anderson et al., 1974; Gebhardt and Coleman, 1974c). Phosphate sorption capacity is an important soil characteristic that affects the plant response to phosphate fertilizer application (Holford and Mattingly, 1976b), and the downward movement of applied phosphate in soils (Mackay et al., 1986). The phosphate sorption capacity of soils has been incorporated in the soil classification (Breauwsma et al., 1986).

Phosphate sorption isotherms have been used by Fox (1968) to estimate phosphate requirement. Kurtz and Quirk

(1965) used phosphorus sorption isotherms to evaluate the residual effects of phosphate fertilizers. Beckwith (1964) measured sorbed phosphate, to predict phosphorus fertilizer needed by plotting values of phosphate sorbed against log phosphorus remaining in the equilibrium soil solution. The straight line curves obtained gave information about phosphate buffering capacity of the soil (Baker and Woodruff, 1961). Moreover, the changes in the phosphorus concentration in solution and in the supporting pool of labile phosphate caused by the addition or depletion of phosphate, differs between soils according to the slope of the sorption isotherm (Mattingly, 1965). Fox and Kamprath (1970) reported that the nature of the soil, period and temperature of contact between soil and solution, method of shaking, soil:solution ratio, moisture content of soil, nature and concentration of supporting electrolyte, previous addition of phosphate and specifically adsorbed anions, are all factors integrated in a measurement of phosphate sorption. Barrow (1979) concluded that the reaction between soil and phosphate is a two step process, an initial rapid step followed by a slower step. Adsorption isotherm plots can characterize these steps.

An adsorption isotherm may be defined as the relation between the amount of a substance held by an adsorbent and the equilibrium concentration in solution of the substance at constant temperature (Prutton and Maron, 1953). When increasing amounts of phosphate are added to a series of soils, the phosphate sorbed per unit weight (x) can be plotted against the equilibrium phosphate concentration (c). The resulting curve flattens and appears to approach a maximum at higher concentrations, a feature typical of chemisorption reactions. For most, however, the adsorption continues to increase gradually with increasing phosphate concentration and with time and a well-defined maximum is not obtained. The data may, however, be treated according to a number of adsorption equations, but the most commonly used are the Freundlich and Langmuir equations. Adsorption isotherm equations provide a shorthand which enables the properties of a soil to be summarized using a few numbers rather than having to refer to the curve. The number so derived may then be related to some other property to find which properties are associated with adsorption (Barrow, 1978). Another alternative is to relate the measurement of adsorption to a measure of plant response, with the ultimate aim of predicting the amount of fertilizer (Barrow, 1975). A second motivation is to learn more about the nature of the adsorption process. Existing knowledge about the nature of the adsorption reaction is used to deduce a new adsorption equation, appropriate to specific problem of phosphate adsorption (Bowden et al., 1977).

Though the Freundlich equation has been frequently used in comparing the adsorption behaviour of a series of adsorbents, including soil, it has the limitation that it does not predict a maximum adsorption capacity. In contrast, the Langmuir equation (1918), which was derived for the adsorption of gases on solids, and was first used by Olsen and Watanabe (1957) and since has been widely used, does give an adsorption maximum. The equation is usually expressed as:

$$\frac{c}{x} = \frac{1}{Kb} + \frac{c}{b}$$

where c = equilibrium concentration of adsorbate (mg l^{-1})

x = mg phosphorus adsorbed/kg soil

b = adsorption maximum (mg l^{-1})

K = a constant related to bonding energy of soil for phosphorus (l mg^{-1})

In the commonly used form of the Langmuir adsorption equation, a straight line is supposed to be obtained when c/x is plotted against c, with a slope of 1/b and intercept 1/Kb. The Langmuir constant, b, is the reciprocal of the slope (1/b) and K is given by slope/intercept.

The Langmuir isotherm can often be used to give a relative measure of the energy by which phosphorus is bonded to the solid and a relative adsorption maximum. Based on this maximum, an arbitrary calculation of the degree of phosphorus saturation can be made. Such a measure has been shown to be related to plant uptake of soil phosphorus (Gunary and Sutton, 1967). Olsen and Watanabe (1957), Gunary (1970) and Bache and Williams

(1971) obtained a linear relationship between c/x and c for dilute solutions (0.5 x 10^{-4} M to 5 x 10^{-4} M). They also calculated the two constants, K and b, related to bonding energy of phosphorus to solids and maximum adsorption capacity, respectively. They have shown further that constants calculated from the Langmuir isotherm permit a sound theoretical approach to some of the problems of phosphorus retention in soils. They further stressed that agreement of the phosphorus adsorption with the Langmuir isotherm did not necessarily imply any specific adsorption or reaction mechanism. However, the Langmuir equation has been used successfully only in restricted range (1-20 μg P cm^{-1}) of equilibrium phosphorus concentration (Olsen and Watanabe, 1957). In some cases the data can best be fitted with two straight lines and this has been taken to indicate two different phosphate adsorption sites (Rajan and Fox, 1975). Some recent studies have suggested that soil adsorption data transformed according to Langmuir (1918), give a curvilinear rather than a linear isotherm (Bache and Williams, 1971). Deviations from linearity were reported at both high (Hsu and Rennie, 1962) and low (Syers et al., 1973) equilibrium phosphorus concentrations. When the development of the Langmuir adsorption equation was critically examined by Harter and Baker (1977), they concluded that the equation which soil scientists have been using is in the wrong form. The assumption that theory is obeyed either at low concentration (Olsen and

Watanabe, 1957) or considering two adsorption mechanisms (Shuman, 1975) or adding a square root term to the equation (Gunary, 1970) or underestimating the amount of adsorbate that can actually be retained, all were in error. Though the error is of no great importance when used to obtain a calculated adsorption maximum for comparison to other adsorbent properties, it does become important when used to understand adsorption dynamics and bonding strengths. Actually, the commonly reported curvilinear nature of the isotherm (c/x versus c) was simply the result of not considering the effect of adsorbed ions in the equilibrium solution. When the equation was corrected by considering the desorbed ions, the isotherm became linear. In addition, the constant K of the Langmuir equation was not simply related to the bonding energy of the adsorbed ion, but to the ratio of adsorbed and desorbed ion bonding energies. It is therefore justifiable, that the use of adsorption maximum, calculated by traditional methods should not be questioned, if used for several soils and correlated with soil properties (Harter and Baker, 1977). In short, the Langmuir isotherm has a sound theoretical derivation, is specific for smaller amounts of adsorbed phosphorus and more dilute equilibrium phosphorus concentrations. Constants calculated from it permit an explanation of some of the problems of phosphorus retention in soils.

If data do not conform to the Langmuir equation, the less demanding Freundlich equation can often be used successfully. Bohn et al. (1985) mentioned that Freundlich found that adsorption data from dilute solutions could be fitted to an equation of the form:

$$x = Kc^{1/n}$$

where K and n are empirical constants and other terms are defined previously.

The equation was originally empirical, without theoretical foundation. It implies, however, that the energy of adsorption decreases exponentially as the fraction of covered surface increases (amount of adsorption). The Langmuir equation, on the other hand, implies that the energy of adsorption on a uniform surface is independent of surface coverage. The Freundlich equation can be derived theoretically by assuming that the decrease in energy of adsorption with increasing surface coverage is due to surface heterogeneity. The linear form of the Freundlich equation is:

$$\text{Log } x = \frac{1}{n} \log c + \log K$$

The Freundlich equation has the limitation that it does not predict a maximum adsorption capacity. Despite its shortcoming, this equation is a common adsorption equation.

Over limited ranges of concentration, the Freundlich equation often describes adsorption well (Barrow and Shaw, 1975). However, some workers have reported that plots of log adsorption versus log concentration give curves rather than straight lines. Barrow (1978) has elaborated this point by explaining that a logarithmic plot involves a transformation of the data such that values of low concentration are given a high weighting. The weighting may be greater than is justified by the precision of these values. The second aspect, which he mentioned, is the difficulty of taking adequate account of the phosphate already present in the soil and taking part in the equilibration. Generally, the simple Freundlich equation often describes adsorption well, especially in phosphorus deficient soils.

3.2.2 Materials and methods

3.2.2.1 Selection of sites

The adsorption study was carried out on twelve of the soils collected from Baads Colliery. The selection of soil was based on the previous study (Section 3.1), with the main emphasis on soil pH. From treatments having a wide range of pH (eg. peat, sewage sludge, chicken manure and clover grass mixture 2), two samples, one of low and another of high pH were selected. For treatments having somewhat uniform pH (eg. alginure, control, red clover grass mixture 1 and grass) only one sample was chosen at random. Samples from no organic amendment plots and areas of natural vegetation were not considered for this study.

3.2.2.2 Procedure

1 g fresh, 4 mm sieved soil was weighed into a series of 4 ounce glass bottles, and 50 cm^3 of solutions containing all phosphorus concentrations described in the general procedure (Section 2.1.8) was added. All phosphorus solutions were made in 50 cm^3 volumetric flasks from 2500 or 1000 mg P l^{-1} stock solutions of KH_2PO_4 and volume was made with deionized water. A control set of phosphorus solutions (0-100 mg P l^{-1}) for measuring initial phosphorus in solution, was also made in 50 cm^3 volumetric flasks with deionized water. The soil-phosphorus solution suspensions were equilibrated under the procedure in Section 2.1.8, by shaking on an end over end shaker for 24 hours in a constant temperature (20^oC). After filtration, an aliquot of the clear filtrate was analysed for phosphorus by the method described in Section 2.2.1.2, using the Technicon AutoAnalyzer. The difference between the amount of phosphorus in solution after shaking and the amount initially present was taken as the amount of phosphorus adsorbed by the soil from the KH_2PO_4 solution.

3.2.3 Results and discussion

The graphical plots of the amount of phosphorus adsorbed by the soil against the equilibrium solution concentration were also plotted in the form of the Langmuir and Freundlich isotherm equations. Adsorption isotherms, Langmuir and Freundlich plots are illustrated in Figures 3.7 to 3.12, 3.13 to 3.18 and 3.19 to 3.24

respectively. Phosphorus adsorption constants calculated from Langmuir and Freundlich plots are given in Table 3.2.

Adsorption isotherms, illustrated in Figures 3.7 to 3.12, indicate that each soil exhibited different adsorption characteristics. The curves obtained were slopes smoothly rising to a plateau, over the range of phosphorus concentration used.

Adsorption data were fitted to the Langmuir adsorption equation, and the values of c/x versus c were plotted.

The Langmuir adsorption plots, as illustrated in Figures 3.13 to 3.18 were linear. Phosphorus adsorption by all twelve soils appeared to conform well to the conventional Langmuir equation and gave a good fit of the linear form of the equation. The fits (R^2) of the straight lines to the Langmuir plot are given in Table 3.2. The R^2 values indicate a good fit of the Langmuir plots. This suggested that in these soils phosphorus was adsorbed with one energy of adsorption. Soils that give single linear Langmuir isotherms do so because the adsorption process yields a constant heat of adsorption taking place on homogeneous surface sites. Soils are expected to have heterogeneous surface sites for phosphorus adsorption. But homogeneous sites may be produced when high energy sites are already saturated with phosphorus leaving mainly the lower energy sites for phosphorus adsorption (Mehadi and Taylor, 1988).

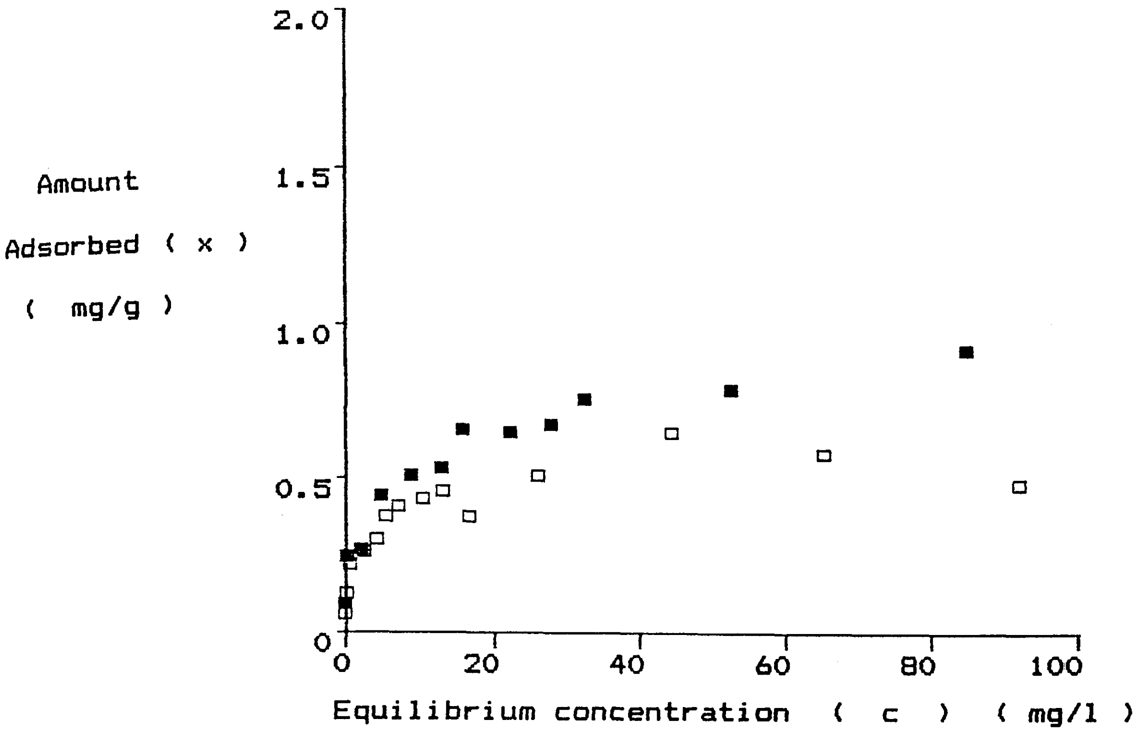


Fig. 3.7 P adsorption isotherm for Baads plot

Grass B (■)
Red clover grass 1B (□)

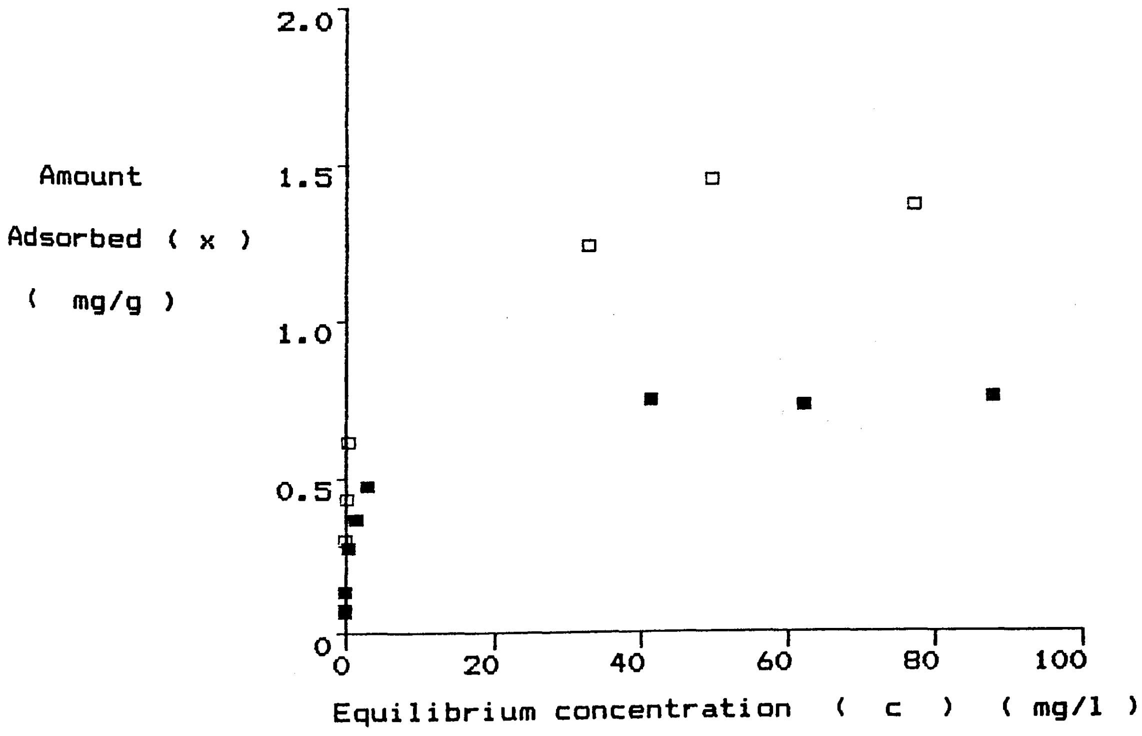


Fig. 3.8 P adsorption isotherm for Baads plot

Control B (□)
Alginure C (■)

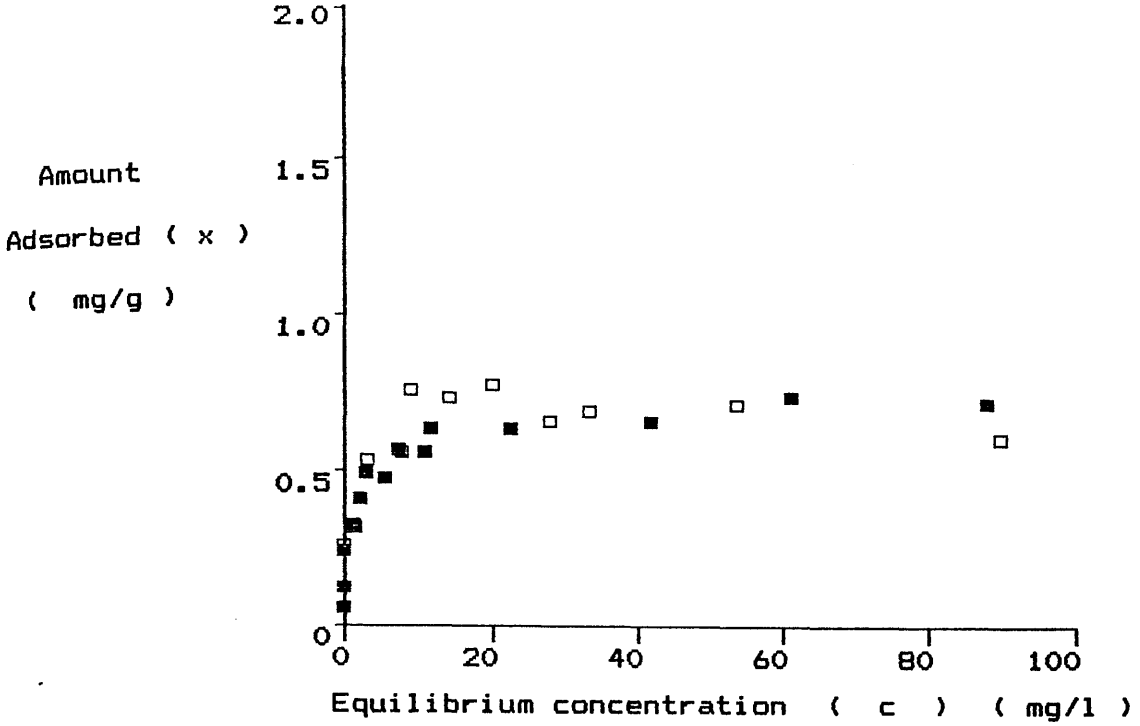


Fig. 3.9 P adsorption isotherm for Baads plot

Sewage sludge C (■)
Sewage sludge B (□)

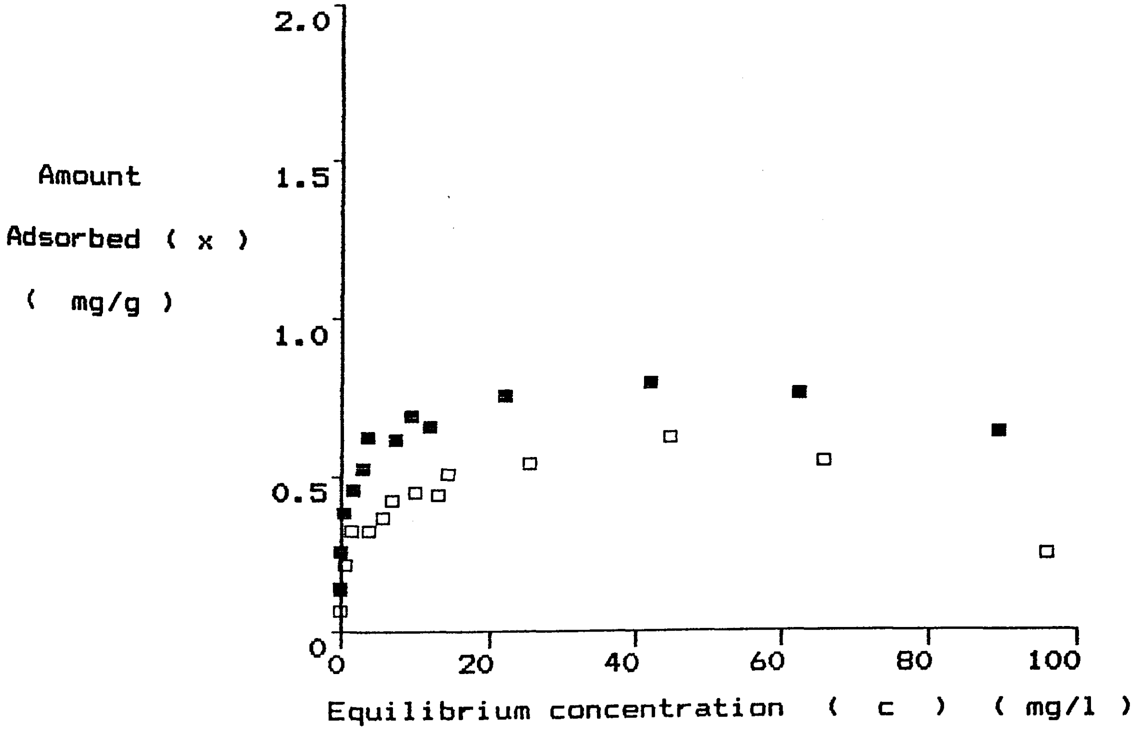


Fig. 3.10 P adsorption isotherm for Baads plot

Red clover grass mixture 2B (■)
Red clover grass mixture 2A (□)

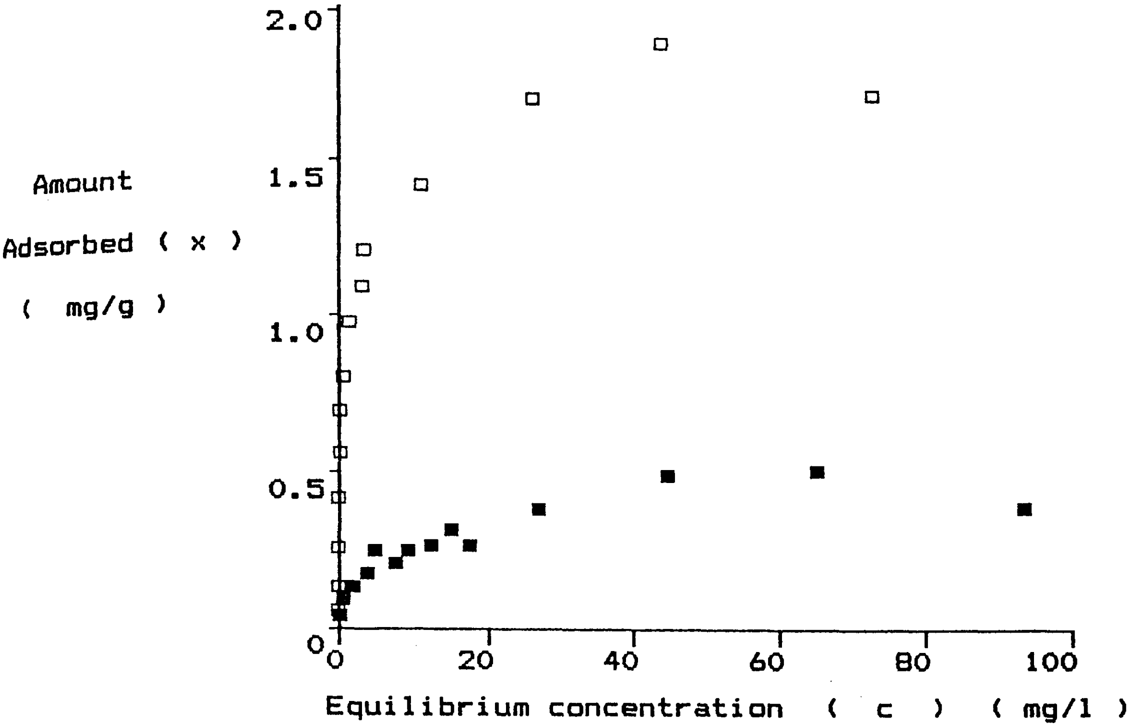


Fig. 3.11 P adsorption isotherm for Baads plot

Chicken manure C (■)
Chicken manure B (□)

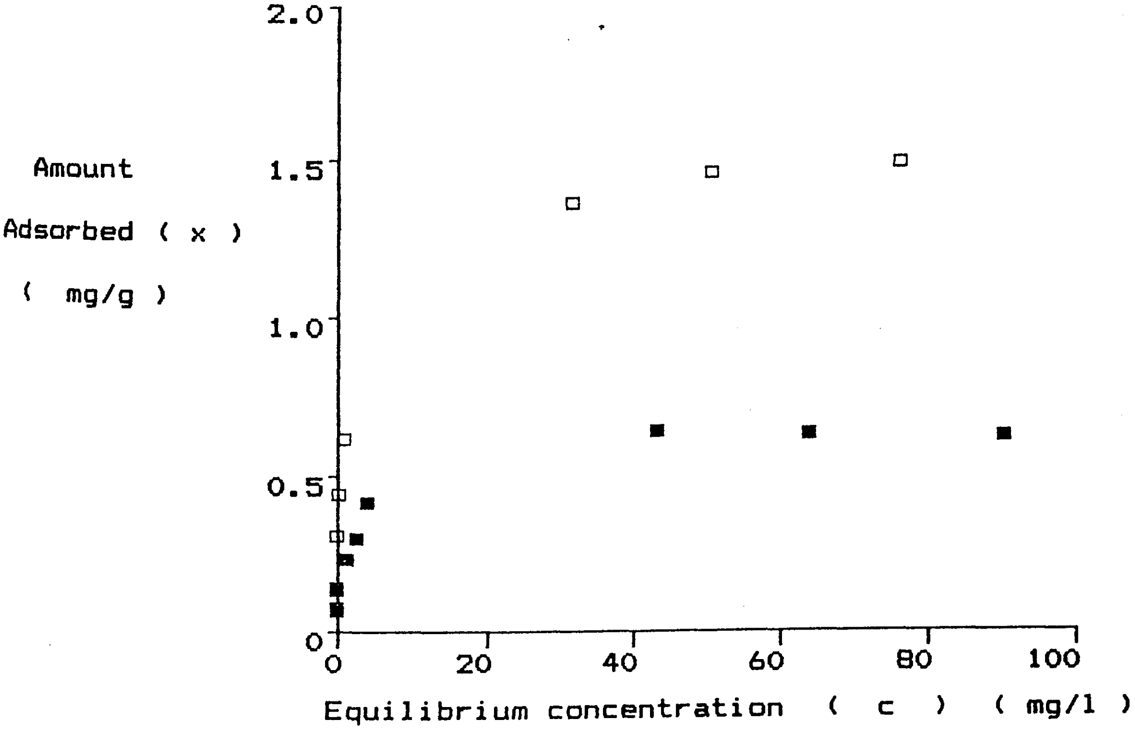


Fig. 3.12 P adsorption isotherm for Baads plot

Peat B (■)
Peat A (□)

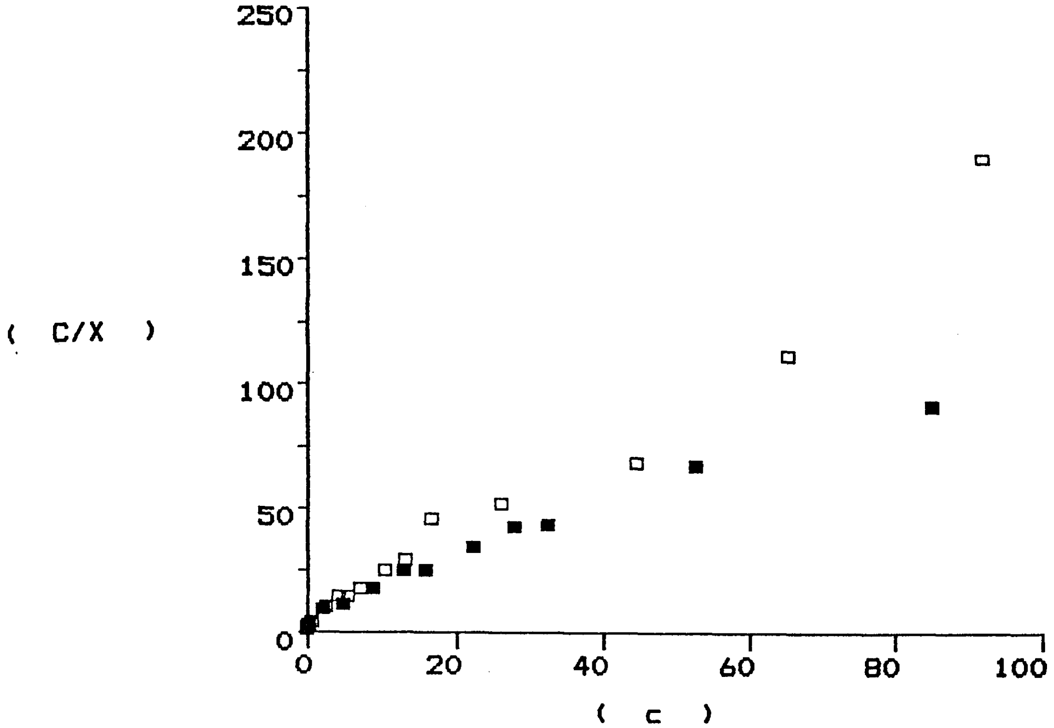


Fig. 3.13 Langmuir adsorption isotherm for Baads plot
Grass B (■)
Red clover grass 1B (□)

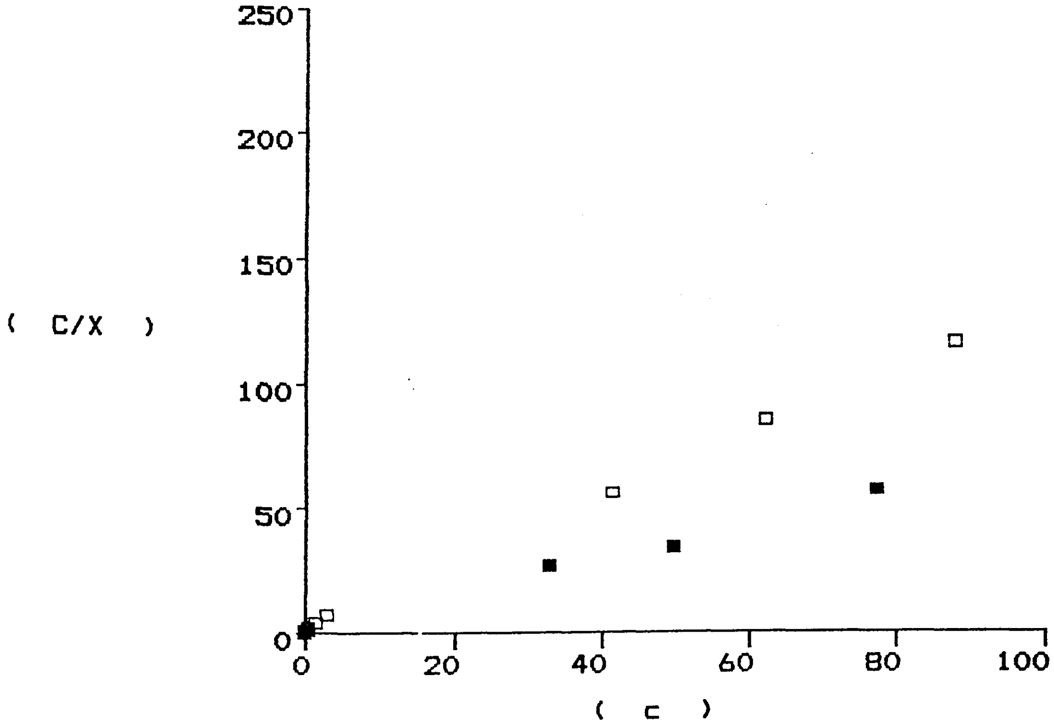


Fig. 3.14 Langmuir adsorption isotherm for Baads plot
Control B (■)
Alginure C (□)

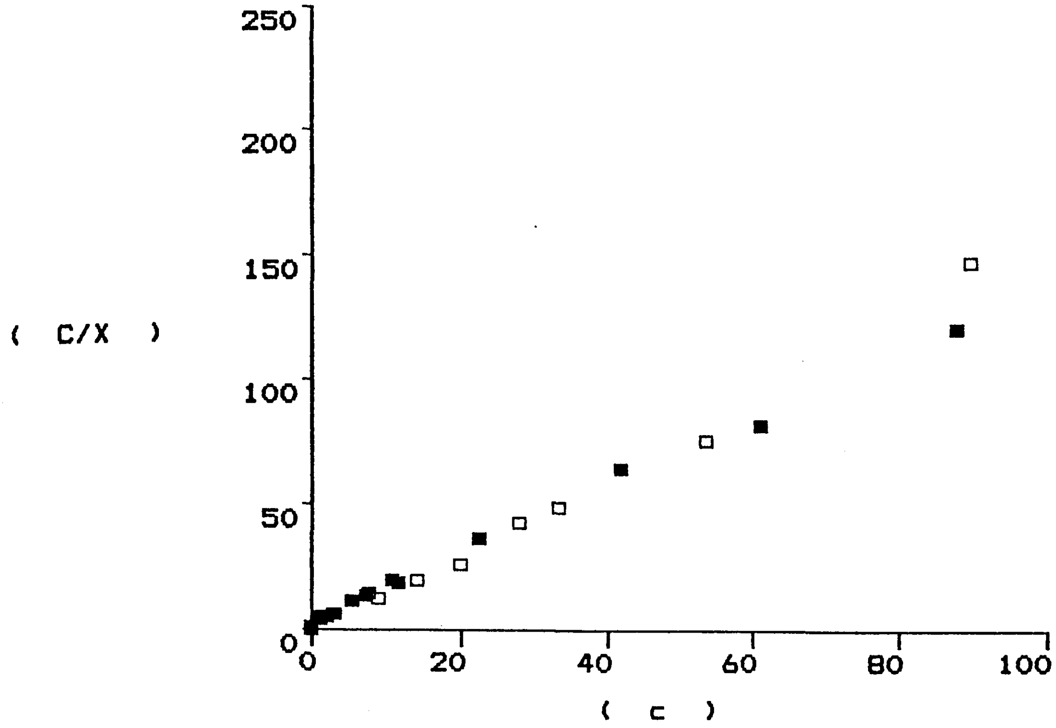


Fig. 3.15 Langmuir adsorption isotherm for Baads plot

Sewage sludge C (■)
Sewage sludge B (□)

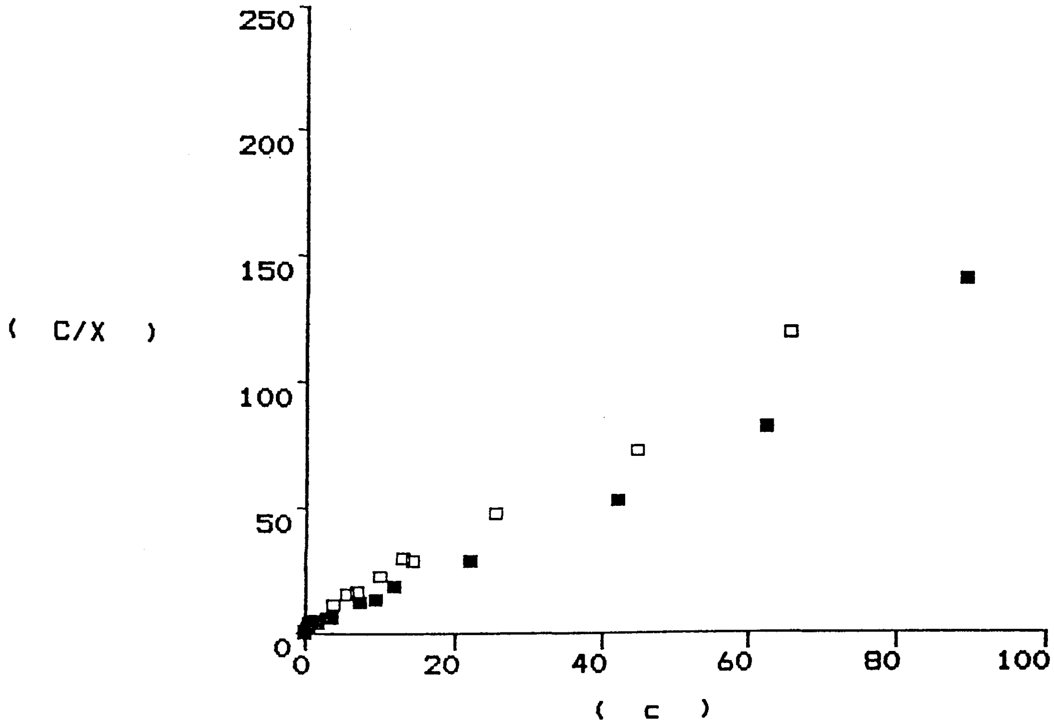


Fig. 3.16 Langmuir adsorption isotherm for Baads plot

Red clover grass mixture 2B (■)
Red clover grass mixture 2A (□)

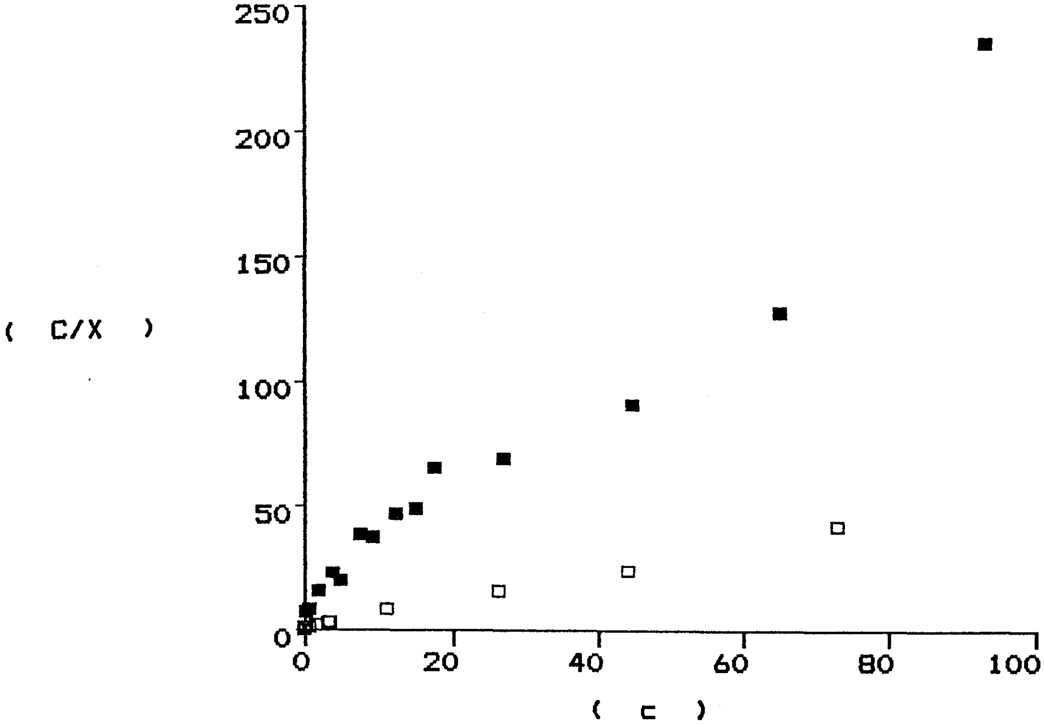


Fig. 3.17 Langmuir adsorption isotherm for Baads plot

Chicken manure C (■)
Chicken manure B (□)

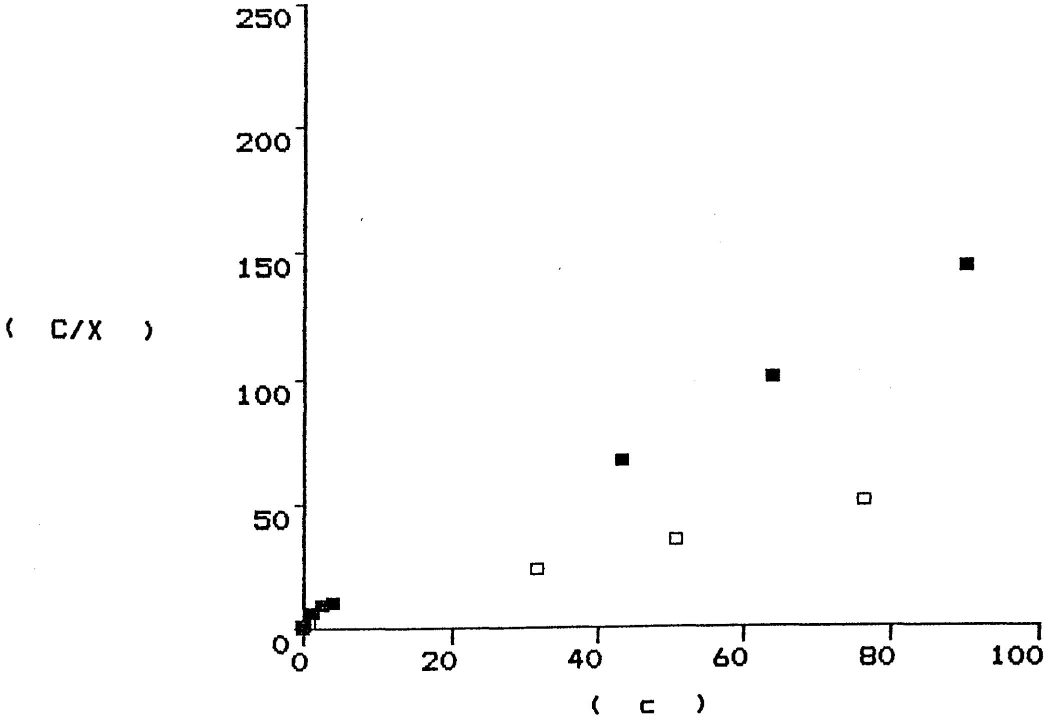


Fig. 3.18 Langmuir adsorption isotherm for Baads plot

Peat B (■)
Peat A (□)

Table 3.2 Phosphorus adsorption constants for some Baads soil calculated from Langmuir and Freundlich plots

Treatment		Langmuir plot			Freundlich plot		
		adsorption maximum mg g^{-1}	bonding energy l mg^{-1}	R^2	Slope	Intercept	R^2
Alginure	C	0.76	1.01	1.0	0.27	1.68	0.96
Peat	A	1.49	1.12	1.0	0.26	1.93	0.99
Peat	B	0.64	0.65	1.0	0.34	1.30	0.96
Sewage sludge	B	0.72	1.36	0.99	0.17	2.11	0.84
Sewage sludge	C	0.74	0.54	1.0	0.31	1.55	0.96
Chicken manure	B	1.78	1.12	1.0	0.34	1.79	0.87
Chicken manure	C	0.41	0.20	0.99	0.44	0.64	0.96
Control	B	1.39	1.83	1.0	0.27	1.88	0.80
Red clover and grass mixture	1B	0.49	0.62	1.0	0.40	1.10	0.96
Red clover and grass mixture	2A	0.58	0.50	0.99	0.28	1.48	0.95
Red clover and grass mixture	2B	0.86	0.16	0.99	0.31	1.47	0.97
Grass	B	0.86	0.16	0.99	0.31	1.47	0.97

Cole et al. (1953) amd Olsen and Watanabe (1957) obtained a single linear Langmuir relationship over the equilibrium phosphorus concentration range up to 14 mg l^{-1}. Syers et al. (1973), however, obtained two slope Langmuir plots, over this equilibrium phosphorus concentration range. They interpreted this as indicative of two populations of sites (Regions 1 and 2), on the soil surface, which have widely differing affinities for phosphorus. Gunary (1970) reported that phosphorus adsorption data for 24 soils fitted curvilinear lines. This was interpreted to signify that the soil will adsorb phosphorus with decreasing energies of bonding as adsorption increases toward a maximum. Similar curves were obtained by Mehadi and Taylor (1988), using the Langmuir adsorption equation as modified by Harter and Baker (1977), with the difference that the position of the intersection of the two lines shifted to a high equilibrium phosphorus concentration. Taylor and Ellis (1978) reported that a single linear Langmuir isotherm was obtained only at high equilibrium phosphorus concentrations (Region 2).

The bonding constants for P adsorption by Baads spoil, derived from the slope and intercept ranged from 0.16 to as high as 1.83, with a mean value of 0.84. The average value of bonding constant obtained by Rennie and McKercher (1959) was 0.14 for soils ranging in pH from 6.5 to 7.2, while 0.92 and 4.39 was reported by Olsen and Watanabe (1957) for alkaline and acidic soil respectively.

The Langmuir constant (K) which is related to the energy of adsorption of phosphorus to the soil surface, explains the ease with which the phosphorus is bound to the soil. In soils with a low adsorption constant, phosphorus is bound more loosely to the soil surfaces and as such is more available to plants. The explanation for this is that when only a small proportion of the total adsorption sites are occupied by phosphorus, these sites are more active and have greater heats of adsorption and thus higher energies of binding (Sims and Ellis, 1983). The adsorption constants for chicken manure C, grass mixture 2B and grass B, were much smaller as compared to the rest of the treatments. This suggests that in these soils phosphorus was more loosely bound to the soil surfaces, and as such should be more available to the plants. However, the constant K is markedly affected by experimental error, and little weight can be placed on the difference obtained (Rennie and McKercher, 1959).

Both adsorption maximum and bonding constant were negatively related with soil pH, but not significantly correlated (Figures 3.25 to 3.26).

Experimental data, when plotted (Figures 3.19 to 3.24) according to the Freundlich equation appeared to obey the Freundlich adsorption isotherm. The fit is observed to hold up to equilibrium concentrations of approximately 10-30 mg l^{-1} of solution. Mohapatra et al. (1979) observed that phosphorus adsorption by soil up to an equilibrium phosphate concentration of 85 mg l^{-1} could be described successfully with the help of either Langmuir or Freundlich adsorption isotherm.

The slope, intercept and R^2 values for each soil calculated from the Freundlich plots are represented in Table 3.2. The goodness of fit of the lines to the Freundlich equation is poorer than for the Langmuir.

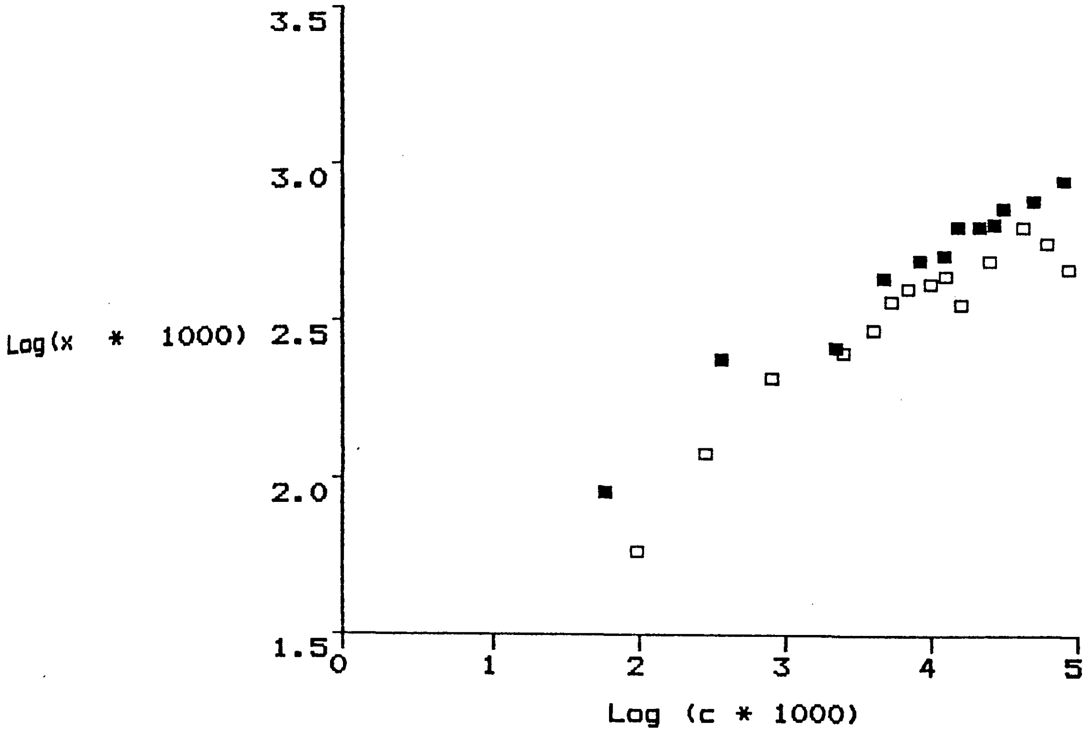


Fig. 3.19 Freundlich adsorption isotherm for Baads plot

Grass B (■)
Red clover grass 1B (□)

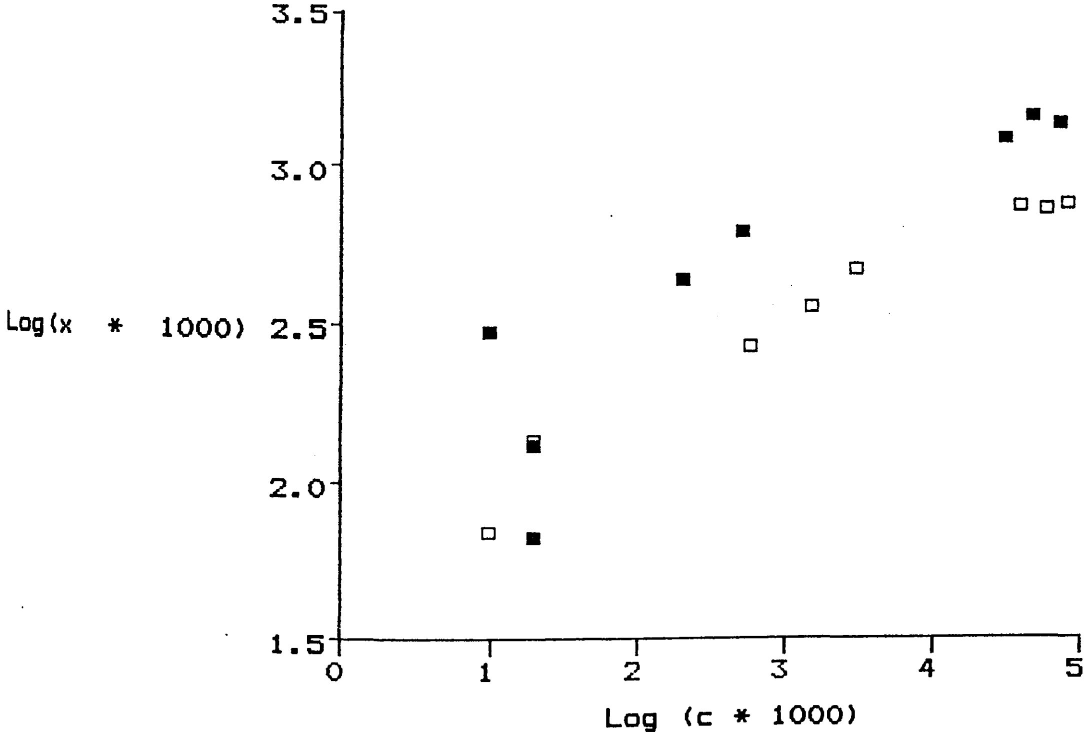


Fig. 3.20 Freundlich adsorption isotherm for Baads plot

Control B (■)
Alginure C (□)

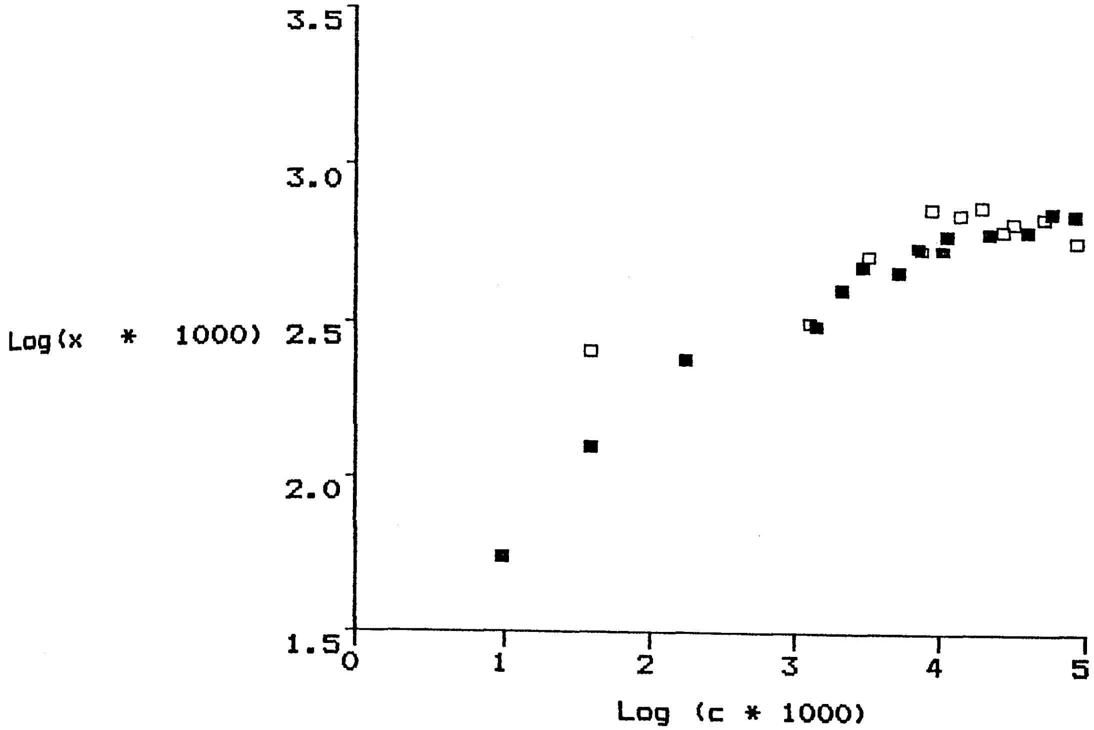


Fig. 3.21 Freundlich adsorption isotherm for Baads plot

Sewage sludge C (■)
Sewage sludge B (□)

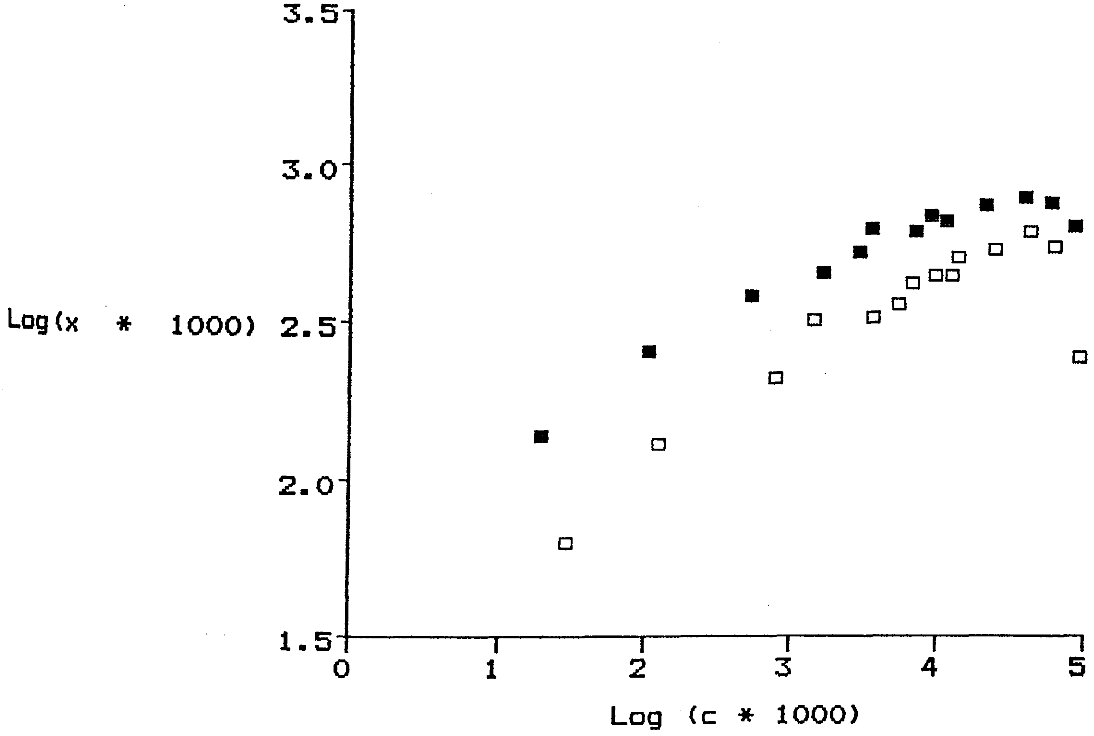


Fig. 3.22 Freundlich adsorption isotherm for Baads plot

Red clover grass mixture 2B (■)
Red clover grass mixture 2A (□)

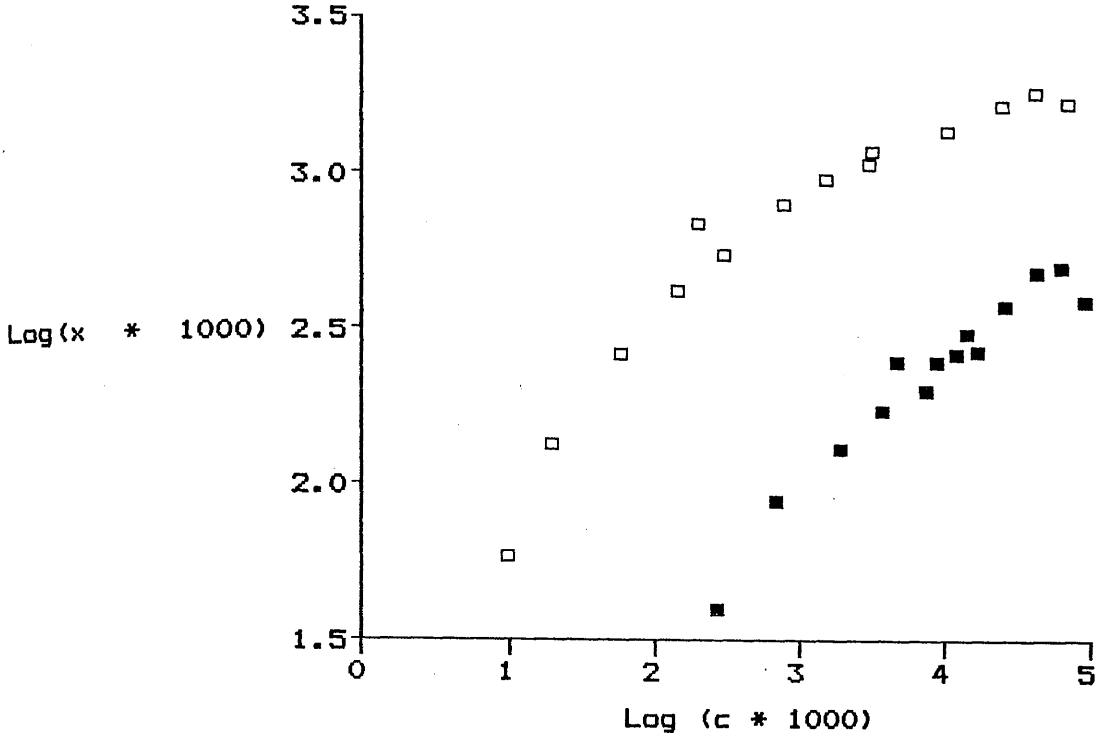


Fig. 3.23 Freundlich adsorption isotherm for Baads plot

Chicken manure C (■)
Chicken manure B (□)

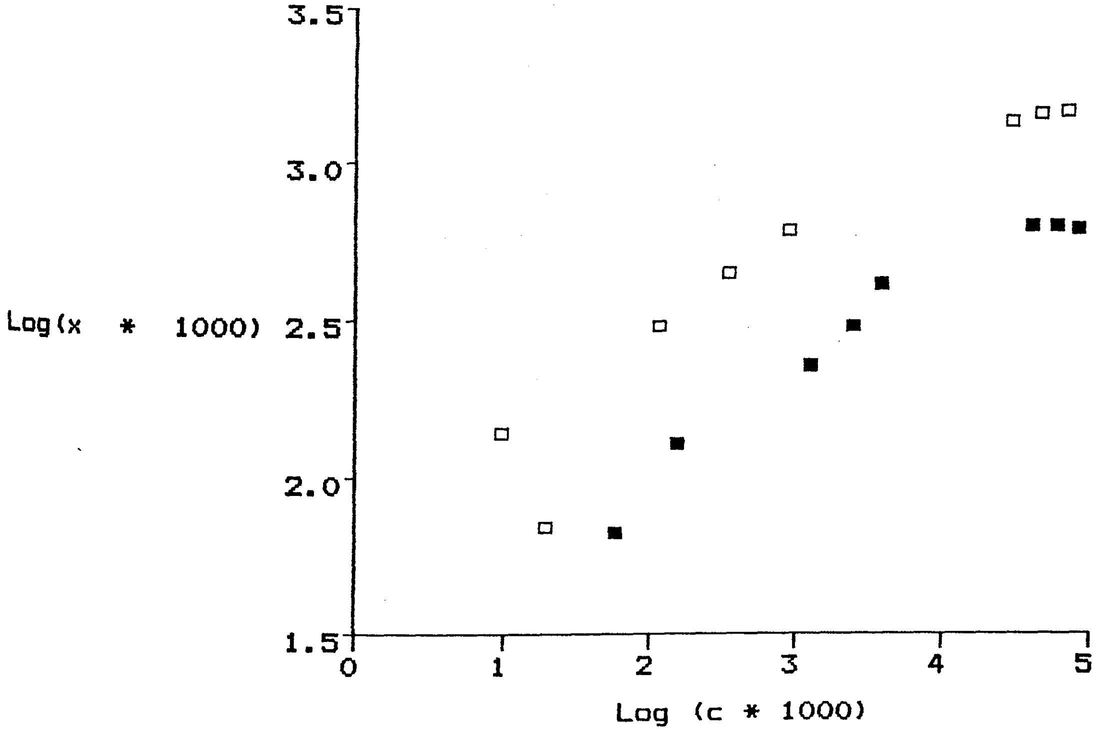


Fig. 3.24 Freundlich adsorption isotherm for Baads plot

Peat B (■)
Peat A (□)

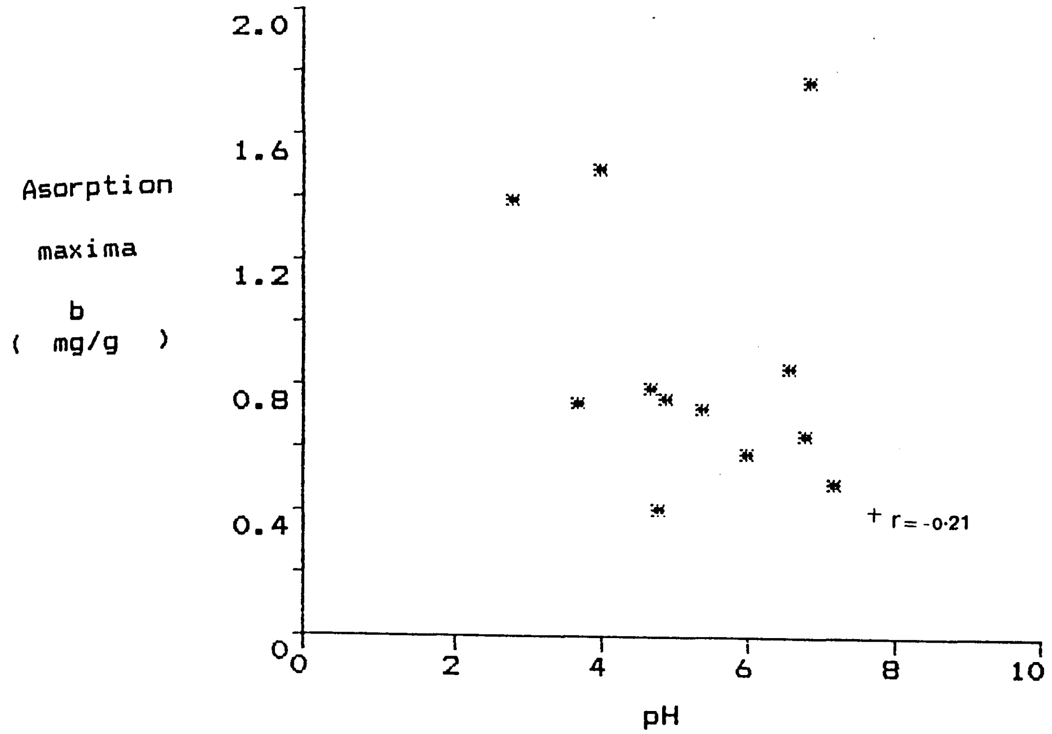


Fig. 3.25 Relationship between pH and adsorption maxima

+ r is the Pearson product-moment correlation coefficient.

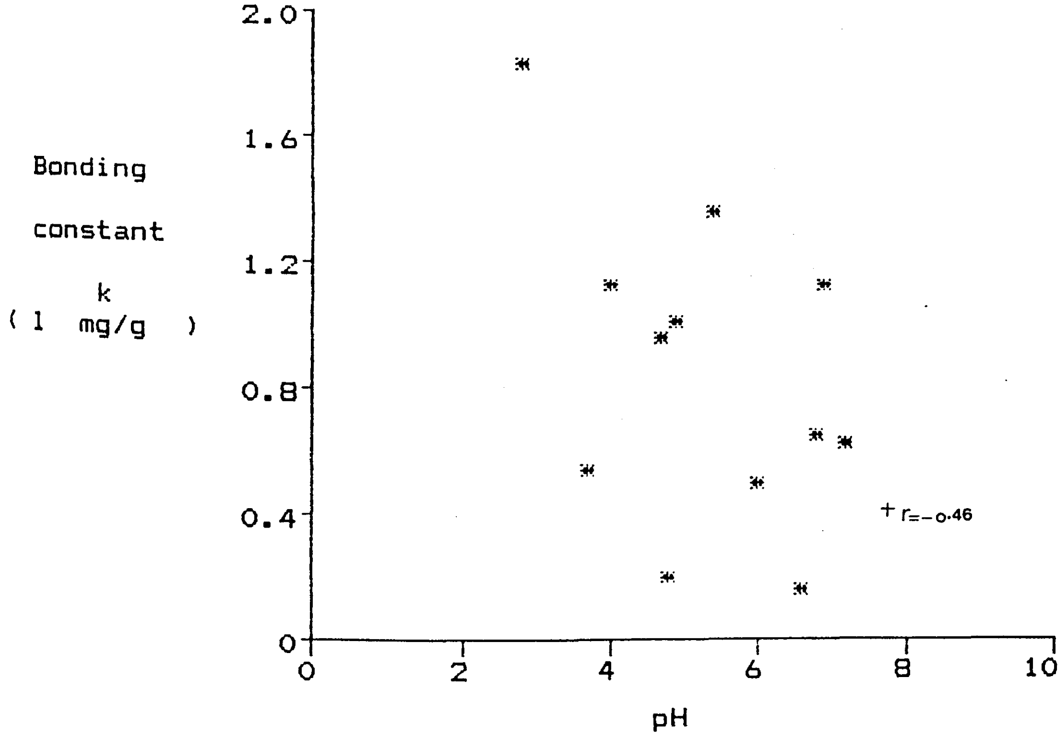


Fig. 3.26 Relationship between pH and bonding constant

+ r is the Pearson product-moment correlation coefficient.

3.3 Phosphorus mineralization

3.3.1 Introduction

Though the initial reactions of phosphorus in soil are important with respect to plant growth and efficient fertilizer use, the reactions that occur with time have received less research attention and may have even more important implications for these aspects.

Phosphorus is both mineralized and immobilized. The process of build-up of organic phosphorus may be termed immobilization, ie. inorganic phosphorus is converted biologically into organic phosphorus compounds which are unavailable to plants. The microbial conversion of soil organic phosphorus into inorganic phosphorus is termed mineralization. On mineralization, phosphorus goes into soil solution as phosphate where it may be taken up by plants, adsorbed by soil colloids, and fixed into unavailable inorganic forms, or again appropriated by microorganisms. Thus both processes, immobilization of inorganic phosphorus and mineralization of organic phosphorus, occur simultaneously in the soil and only the difference in the rates of immobilization and mineralization of organic phosphorus can be observed at any given time.

It is generally agreed that organic phosphorus is made available to plants largely after its mineralization into inorganic phosphorus, although the water soluble soil organic phosphorus may be absorbed by plants directly, or after compounds being dephosphorylated by a phosphatase enzyme near the root.

Two types of evidence indicate that soil phosphorus undergoes mineralization. The first is based on changes in soil as a result of long continued treatment, that is the content of organic phosphorus is usually lower in cultivated soil than virgin soil. The second type of evidence is based on laboratory experiments, that in incubated soil organic phosphorus decreases with a concurrent increase in extractable inorganic phosphorus (Van Diest and Black, 1959). Kalia (1961) and Halstead et al. (1963) reported a decrease in total organic, and an increase in total inorganic, phosphorus in soils incubated for 7 and 9 months respectively. Further evidence that soil organic phosphorus could be mineralized is that phosphatase activity is directly related to organic content (Gaurilova et al., 1975).

Mineralization of organic phosphorus in soil is largely due to the combined activities of soil micro-organisms and the phosphatase enzyme present in soil. In addition temperature, moisture, aeration, pH, inorganic phosphorus fertilizers, organic matter, cultivation all govern mineralization.

Different workers have used different techniques and extractants to study the changes in phosphorus induced by incubation. Sekhon and Black (1968) estimated mineralization of organic phosphorus during incubation by averaging the increase in resin extractable phosphorus and decrease in organic phosphorus. Bowman and Cole (1978) tested 0.5 M sodium bicarbonate, pH 8.5, as an extractant to measure labile phosphorus pools in soil, during the transformation of certain organic phosphorus substrates. He suggested that the sodium bicarbonate extraction method is useful to evaluate mineralization of various organic phosphorus substrates. Dalal (1979), while assessing both carbon loss and phosphorus mineralization for 10 weeks concluded that both soil solution inorganic and organic phosphorus exist in dynamic equilibrium with bicarbonate extractable phosphorus. Somani (1987) found that soil incubated with narrow (range = 52-124) C:P ratio materials showed considerable mineralization of phosphorus. Incubation of soil with wide (range = 248-249) C:P ratio material caused immobilization of phosphorus.

The importance of organic phosphorus mineralization in providing plant available phosphorus has been well established (Dormar, 1972). Accumulation of organic phosphorus in soils, whether the result of inorganic phosphorus fertilization (Sadler and Stewart, 1975) or weathering of soil parent material high in phosphorus (Walker and Adams, 1958), also has been well documented.

Information on phosphorus transformations in coal mine soils under conditions inducing the mineralization of organic phosphorus or conditions inducing the immobilization of soil inorganic phosphorus is scarce. However, a few investigations have been reported in some agricultural soils (Enwezor, 1976; Chauhan et al., 1981; Hedley et al., 1982). It was therefore decided to study the short-term changes in some fractions of soil phosphorus which may result in long-term changes in some coal mine soils from Baads. Such study will provide information on the pathway of phosphorus cycle in these soils.

3.3.2 Materials and methods

In order to assess the changes in soil phosphorus, a laboratory incubation study was carried out to evaluate phosphate availability in relation to mineralization. Carbon mineralization rates were also measured by measuring carbon dioxide evolution.

Thirty soil samples from 10 sites at Baads (Section 2.3) were used in this investigation. Samples were incubated for 9 weeks using the incubation techniques described in Section 2.1.4.2 for measuring carbon mineralization rates.

Carbon dioxide evolved was determined weekly by the method described in Section 2.1.4.3. Best-fit lines were fitted by linear regression in order to calculate the carbon mineralization rate constant. It was calculated as a linear rate which was expressed in mg C kg^{-1} $week^{-1}$.

Acetic acid and sodium bicarbonate extractable phosphorus were determined in duplicate at 0, 4 and 8 weeks of incubation using the methods described in Section 3.1. The change in phosphorus content was estimated by subtracting the initial phosphorus extracted, both by acetic acid and sodium bicarbonate, at time zero, from the final phosphorus extracted after 8 weeks of incubation. The changes in phosphorus extracted by both acetic acid and sodium bicarbonate per week were calculated by dividing the change in phosphorus by total weeks of incubation.

3.3.3 Results and discussion

The cumulative amounts of carbon dioxide mineralized in the coal mine soils during 9 weeks of incubation are shown in Figures 3.27 to 3.36. Most of these soils showed a rapid initial release in the first week, followed by a linear phase of carbon turnover extended over 7 to 9 weeks' duration. A decline in the rate of carbon dioxide released was noticed in some soils (Figures 3.27, no amendment A and C; 3.29, peat A; 3.35, clover grass mixture 2B) after 7 weeks of incubation. A very few samples (Figures 3.32, control C and 3.33, native vegetation A) showed a lag period of carbon dioxide turnover in the first week of incubation. The carbon mineralization rates calculated as a linear rate by best fit lines fitted by linear regression, are given in Table 3.3. They ranged from 10.5 to 135.7 mg C kg^{-1} $week^{-1}$, with a mean of 44.8 mg C $kg^{-1}week^{-1}$. The great variation in carbon mineralization rates confirms the large heterogenity between samples from within plots receiving the same treatment. No clear effects of treatment can be seen with such variation. The effect of vegetation on respiration rate can also be disregarded, as most of the plots under study were vegetated. If the mineralization rate is compared with the pH (Table 3.3) the large heterogenity in the carbon mineralization rate can be more easily understood. The pH which ranged from 2.8 to 7.2, also showed great variability within the same treatment, except that of the species trial (red clover grass mixture and grass). The variation in pH might be due to the variation in pyrite content in these spoils. The high pH, which generally caused an increase in carbon turnover, could result from low pyrite content as compared to calcium carbonate. Since these soils were limed in 1980, it is expected that the degree of neutralizing capacity of some plots is now decreasing, which resulted in low pH. The highly significant correlations between carbon mineralization rate and pH, and total carbon evolved with pH (Figures 3.37 and 3.38) indicate that carbon mineralization is significantly correlated with soil pH. The increase in carbon mineralization, as the soil pH increases might be the result of higher microbial activities (Halstead et al., 1963). A better understanding of the carbon turnover in the coal mine soils can be seen by splitting the data into two groups, $pH<6.0$ (19 samples) and $pH>6.0$ (11 samples), having mean mineralization constants of 29.2 and 71.9 mg C kg^{-1} $week^{-1}$ respectively, indicating low carbon mineralization in the more acidic soils.

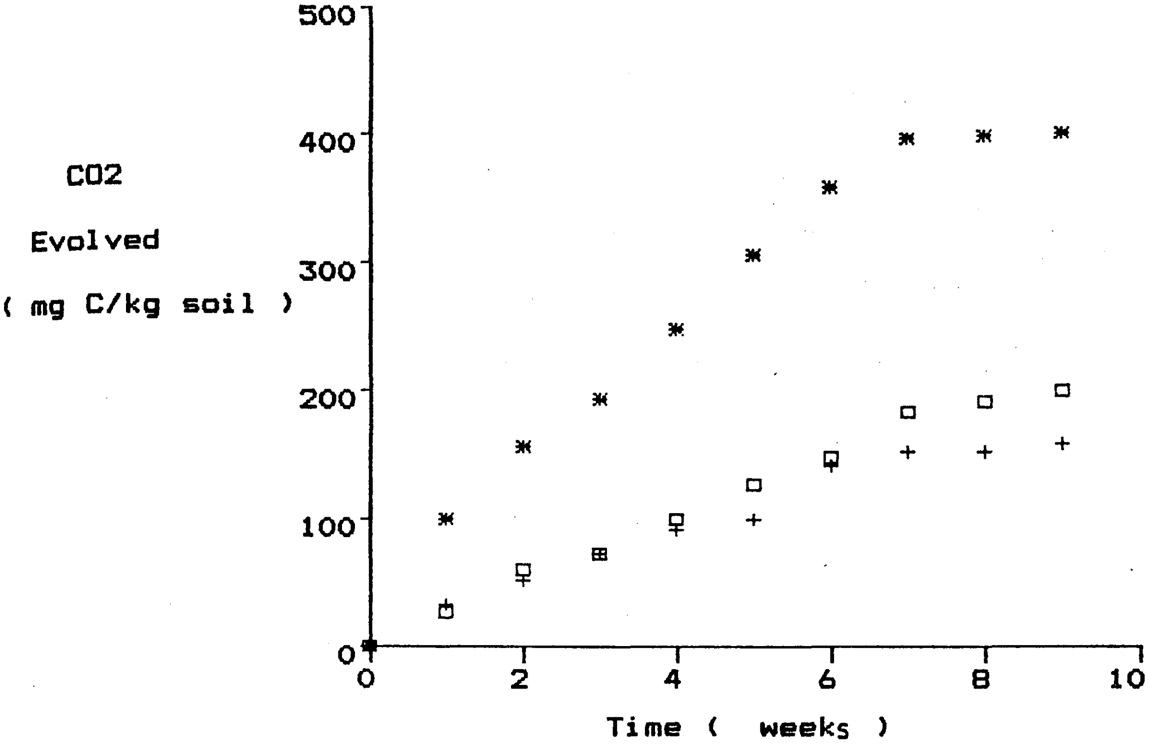


Fig. 3.27 CO2 evolved by untreated (no organic amendment)soil

A (✱) B (+) C (□)

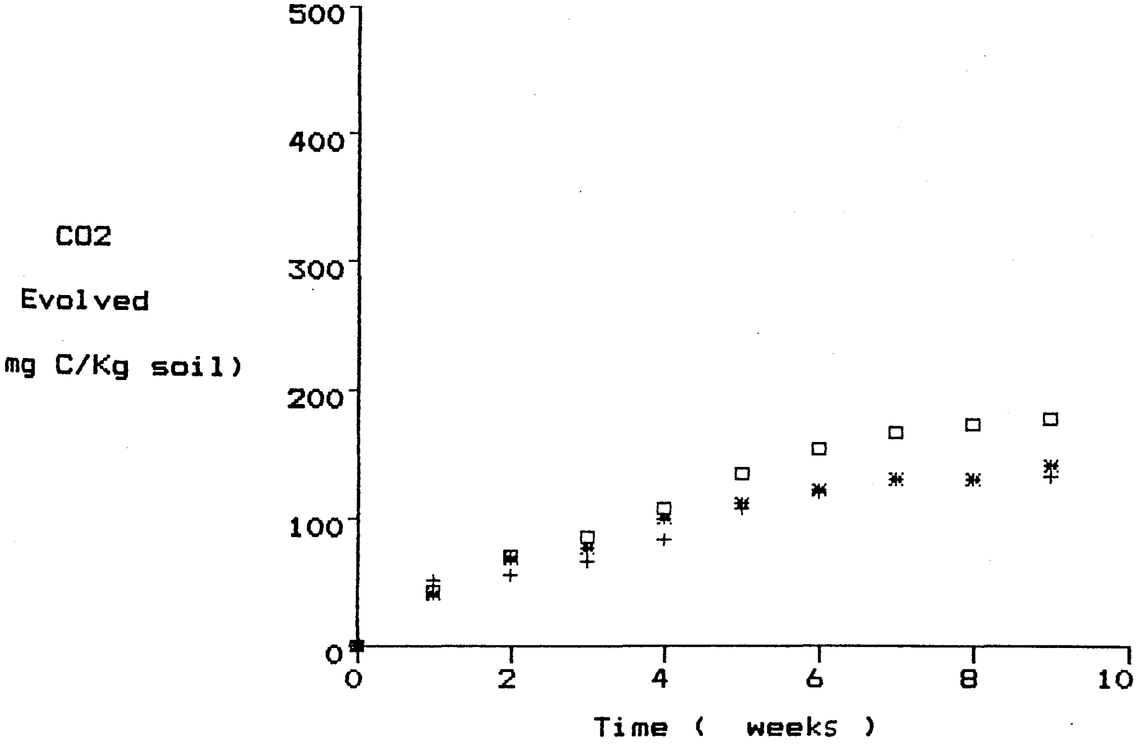


Fig. 3.28 CO2 evolved by Alginure treated soil .

A (✱) B (+) C (□)

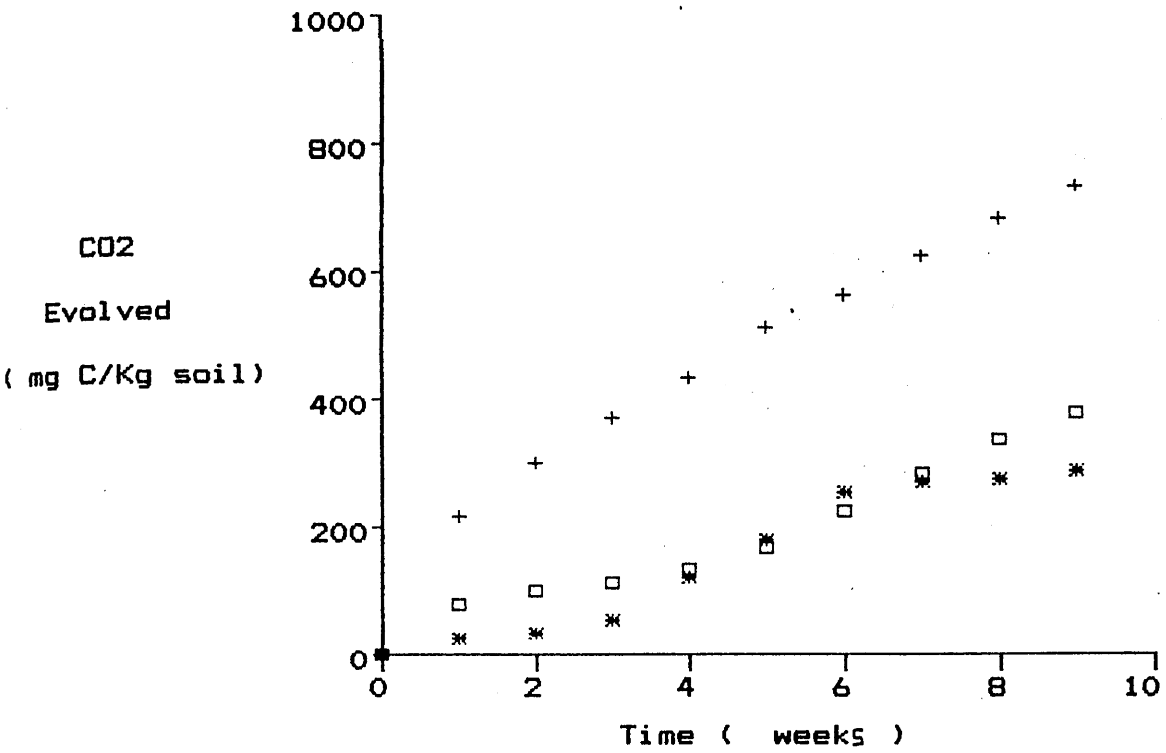


Fig. 3.29 CO2 evolved by Peat treated soil .
A (✱) B (+) C (□)

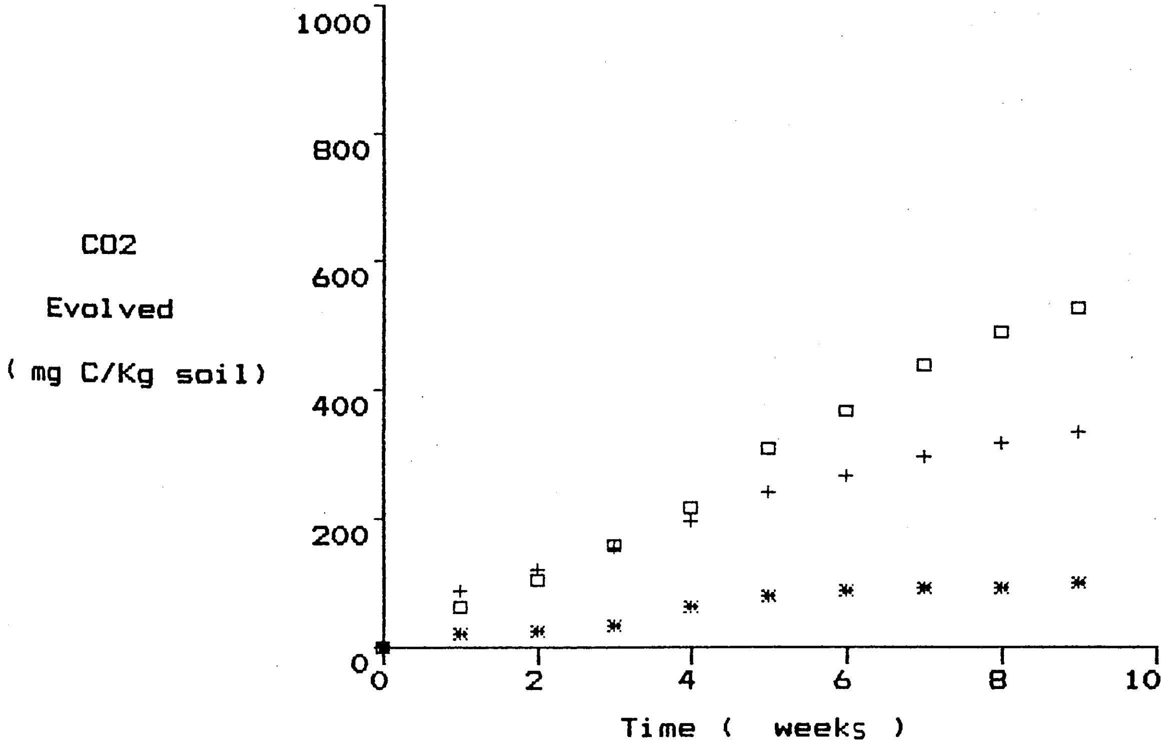


Fig. 3.30 CO2 evolved by Sewage sludge treated soil .
A (✱) B (+) C (□)

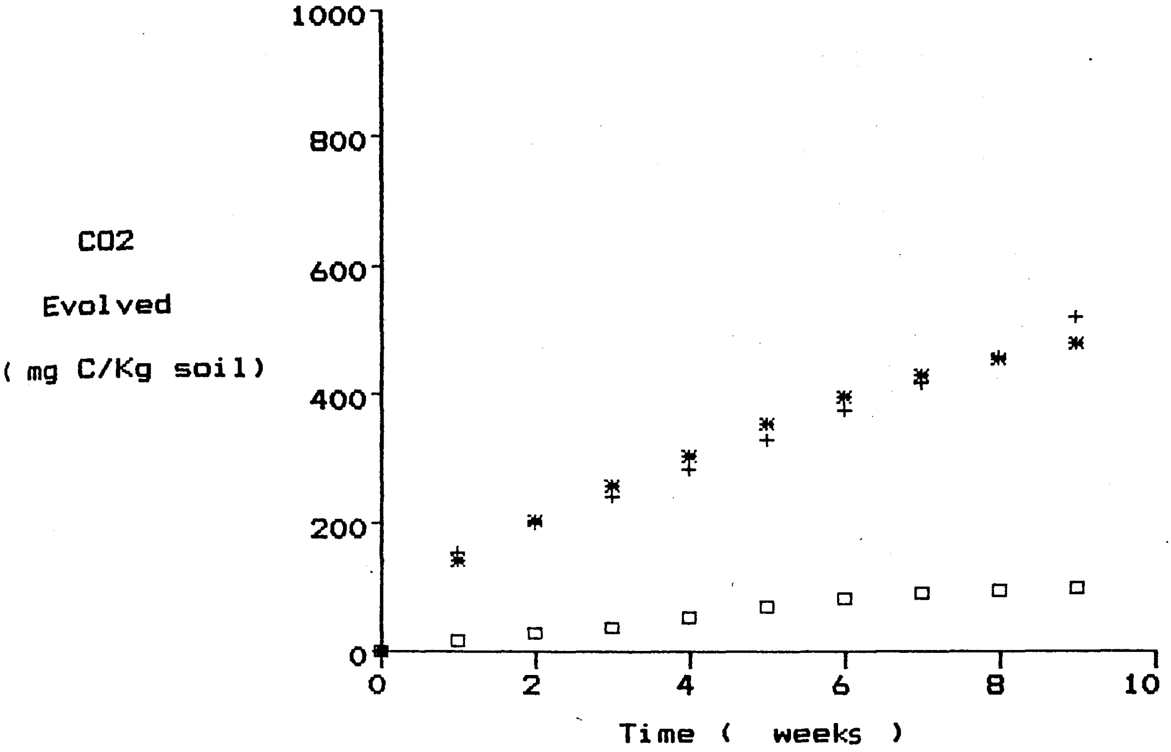


Fig. 3.31 CO2 evolved by Chicken manure treated soil .

A (*) B (+) C (□)

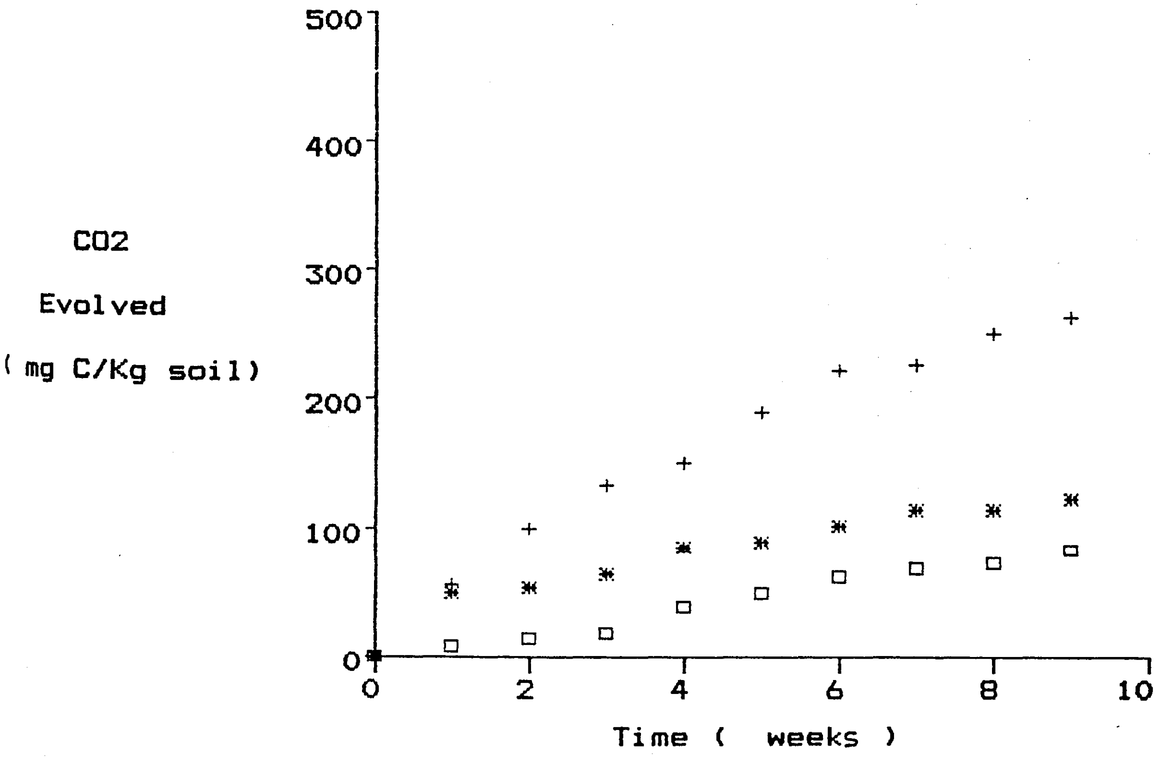


Fig. 3.32 CO2 evolved by Control soil .

A (*) B (+) C (□)

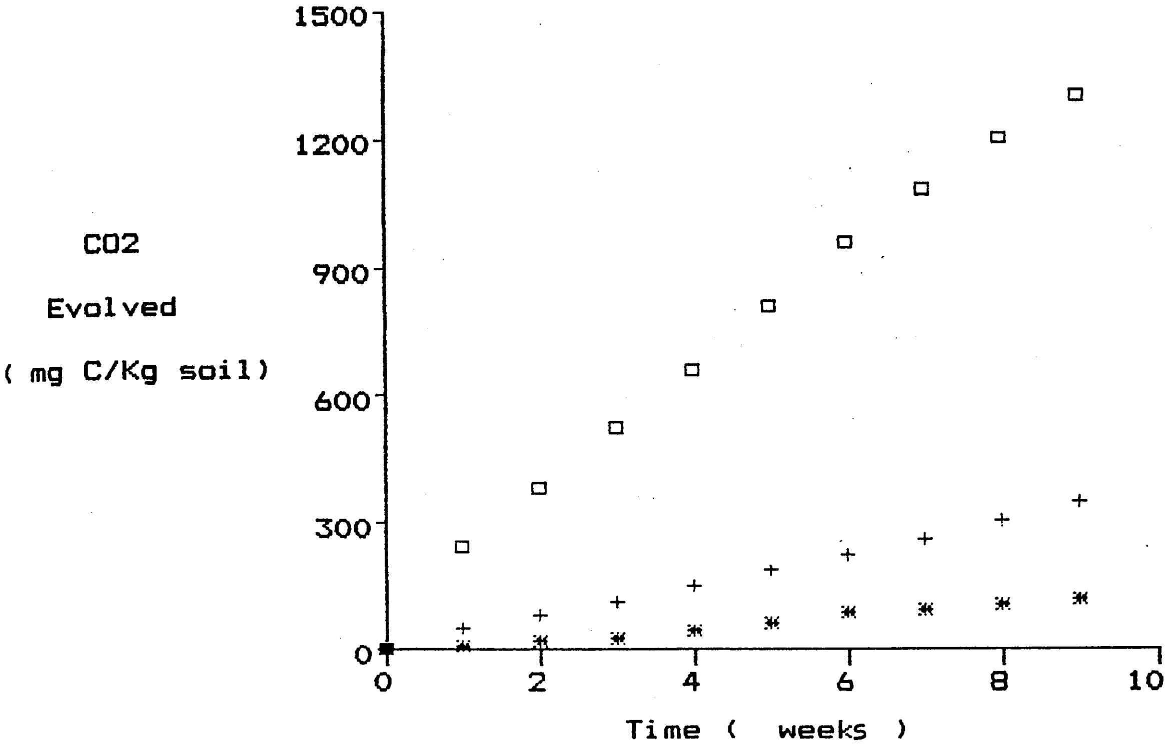


Fig. 3.33 CO2 evolved by Native vegetated soil .

A (✱) B (+) C (□)

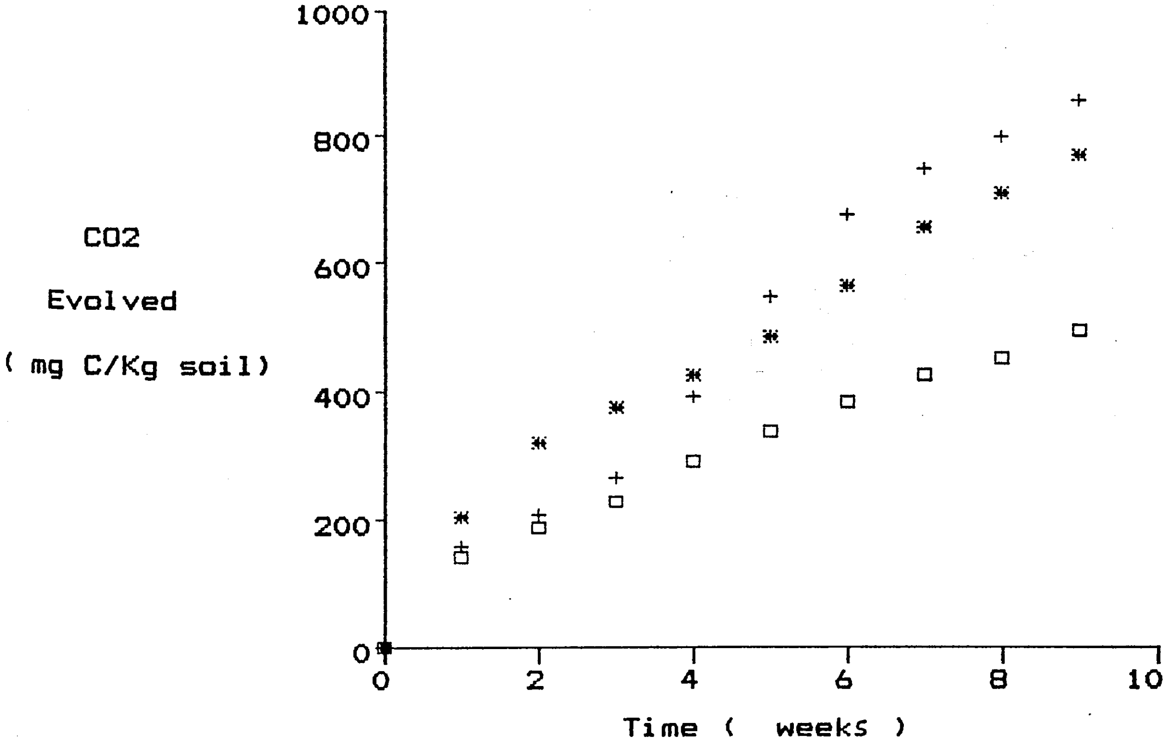


Fig. 3.34 CO2 evolved by Clover grass mixture 1 soil .

A (✱) B (+) C (□)

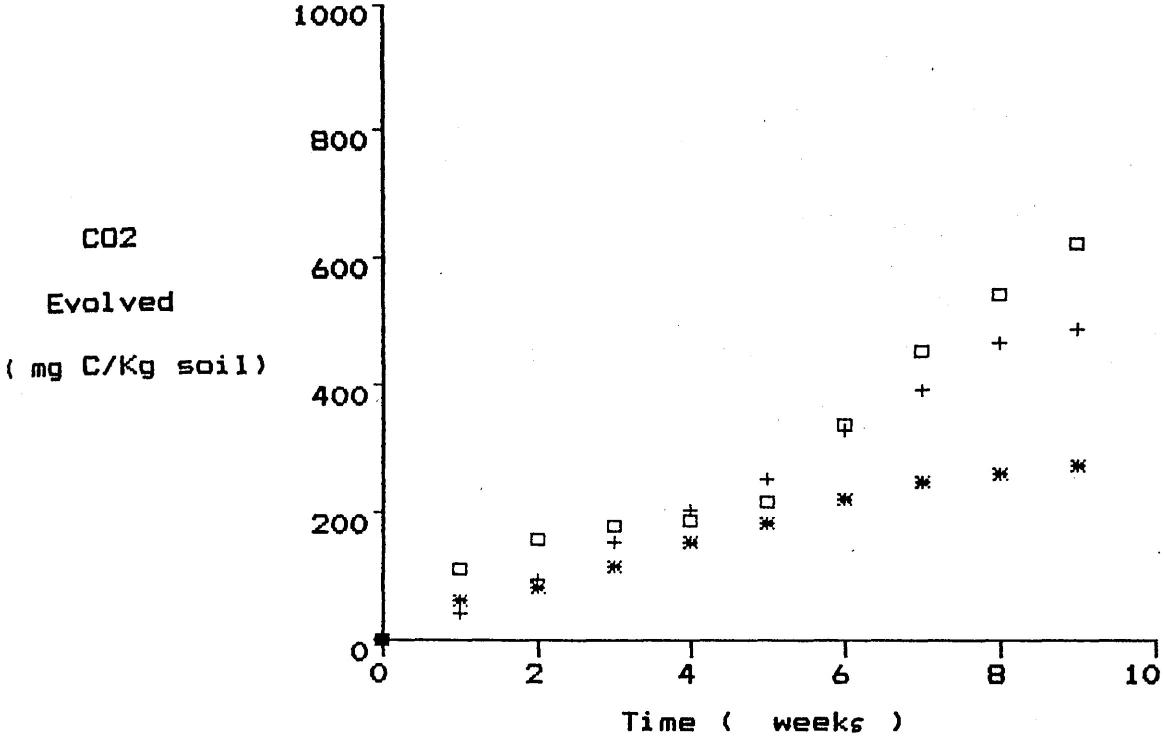


Fig. 3.35 CO2 evolved by Clover grass mixture 2 soil .
A (*) B (+) C (□)

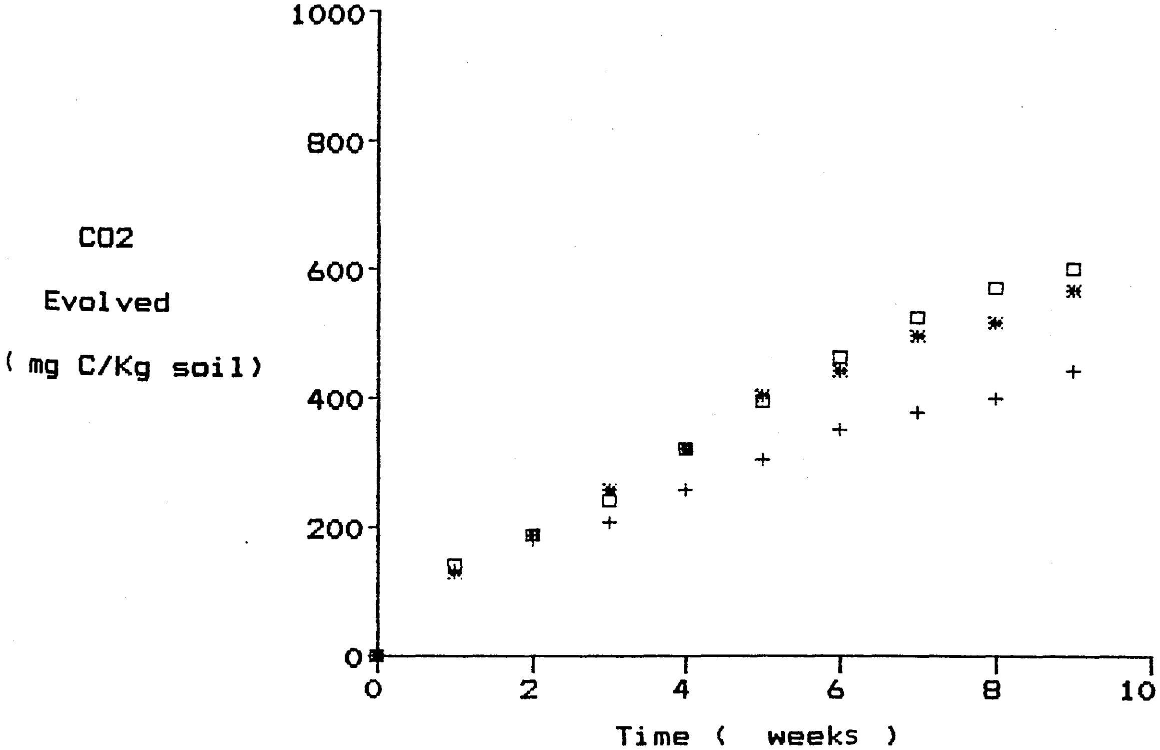


Fig. 3.36 CO2 evolved by Grass soil .
A (*) B (+) C (□)

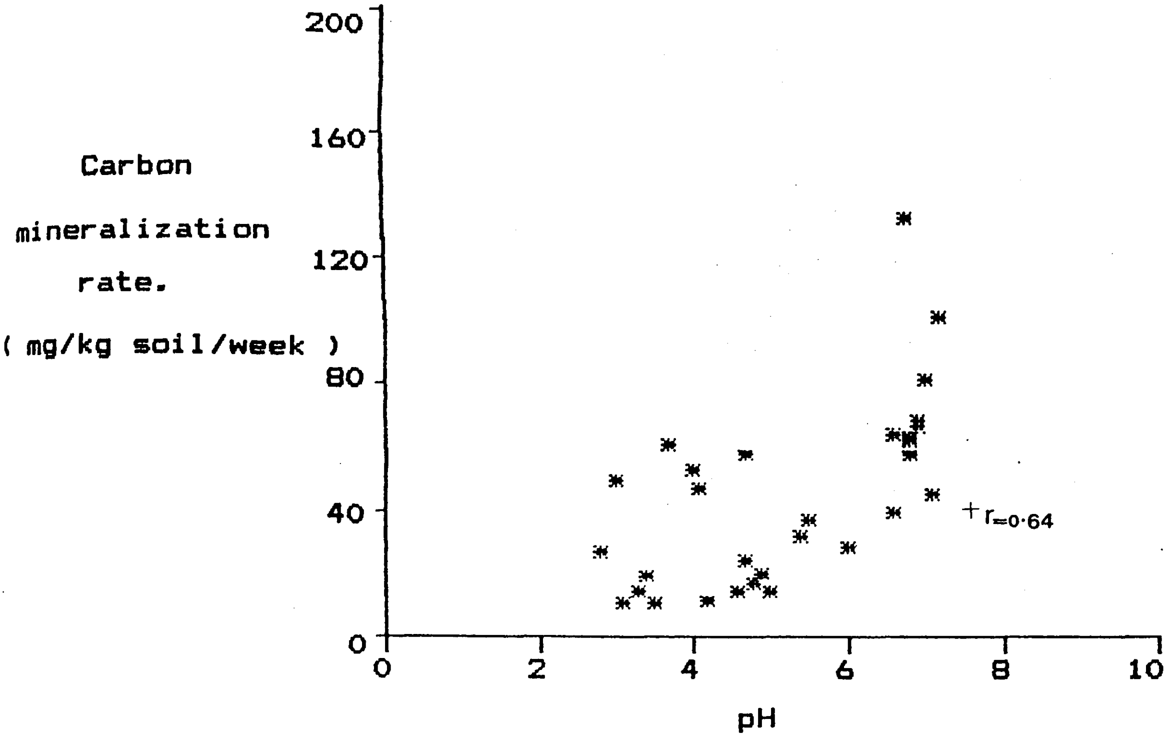


Fig. 3.37 Relationship between pH and carbon mineralization rate constant.
+ r is the Pearson product-moment correlation coefficient.

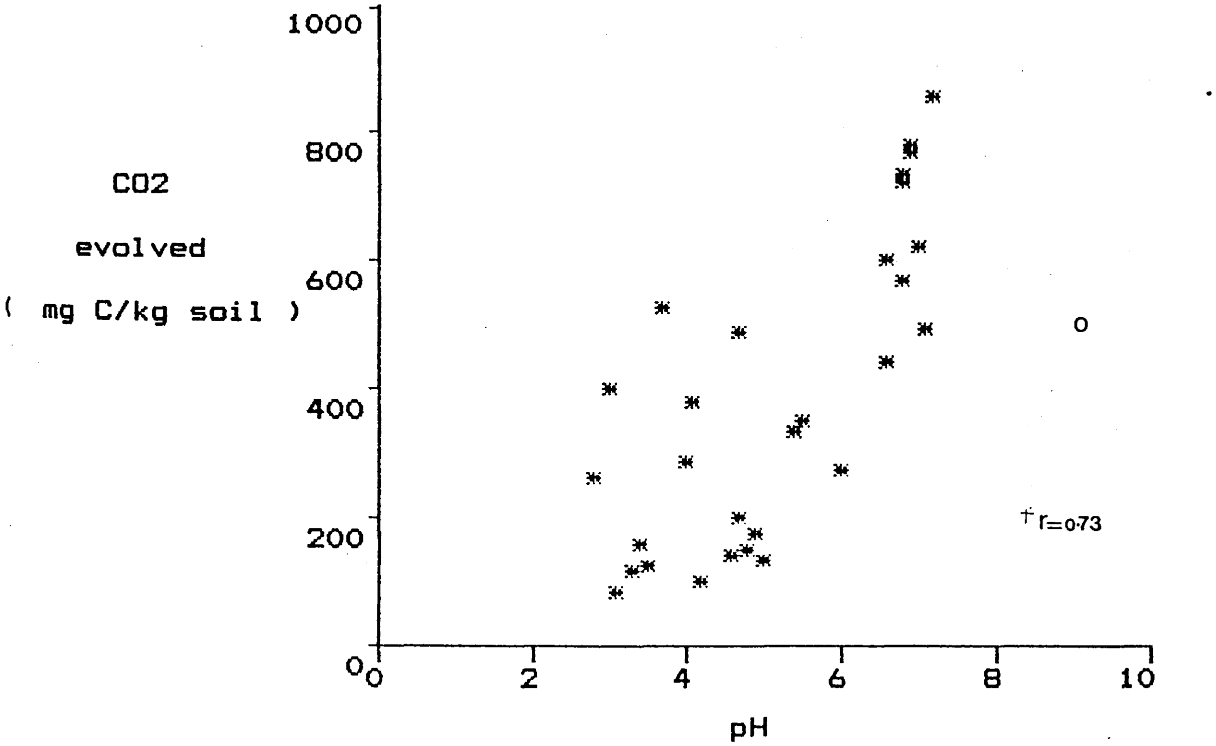


Fig. 3.38 Relationship between pH and CO2 evolved by the soil.
+ r is the Pearson product-moment correlation coefficient.

Table 3.3 Total carbon turnover and carbon mineralization rate in coal mine soil

		pH_i	Total Carbon Turnover mg kg^{-1}	Carbon Mineralization Rate mg $kg^{-1}wk^{-1}$
No organic amendment	A	3.0	398	50.0
	B	3.4	158	20.1
	C	4.7	200	24.7
Alginure	A	4.6	141	14.7
	B	5.0	133	14.6
	C	4.9	176	20.9
Peat	A	4.0	286	53.0
	B	6.8	732	64.2
	C	4.1	379	47.4
Sewage sludge	A	4.2	98	11.1
	B	5.4	333	32.1
	C	3.7	525	61.8
Chicken manure	A	6.8	718	63.3
	B	6.9	780	67.3
	C	4.8	149	17.0
Control	A	3.5	123	10.7
	B	2.8	262	27.0
	C	3.1	83	10.5
Native vegetation	A	3.3	115	15.1
	B	5.5	348	37.4
	C	6.8	1304	135.7
Red clover (1) and grass mixture	A	6.9	765	69.1
	B	7.2	854	101.6
	C	7.1	489	45.9
Red clover (2) and grass mixture	A	6.0	273	28.6
	B	4.7	487	58.3
	C	7.0	619	81.1
Grass	A	6.8	564	58.2
	B	6.6	439	39.6
	C	6.6	601	64.7

Different rates of carbon mineralization of agricultural soils have been reported by various researchers. Part of this may be due to the different temperatures of incubation used. For example Khan (1987) used 9 different agricultural soils in the fresh condition for his incubation study at 10°C, and reported carbon mineralization rates from 8.6 to 50.6 mg C kg^{-1} $week^{-1}$. Jenkinson and Powlson (1976) reported carbon mineralization rates from 4.5 to 27.5 mg C/100 g soil/10 days (31.5 to 192.5 mg C kg^{-1} $week^{-1}$), in 9 fresh soils incubated at 25°C. Shah (1988) and Pulford et al. (1988), who incubated fresh samples of coal mine spoil at 20°C, reported carbon mineralization rates from 20.0 to 181.0 and 16.5 to 146.4 mg C $kg^{-1}week^{-1}$ respectively.

The carbon mineralization rates of coal mine soils at 20°C found in the present study were comparable to those reported by Shah (1988) and Pulford et al. (1988) at 20°C, but lower than those found by Jenkinson and Powlson (1976) at 25°C, and much higher than those found by Khan (1987) at 10°C in agricultural soils.

The rates of respiration indicate that coal mine soils have a microbial population which can break down organic matter at a rate comparable to agricultural soils. Microbial respiration was enhanced in the grass species trial and native vegetation, where the pH was much higher than other treatments. In these soils, carbon turnover rate was highest in the native vegetation and grass mixture soils (135.7 and 101.6 mg C kg^{-1} $week^{-1}$).

Table 3.4 shows acetic acid and bicarbonate extractable phosphorus after 0, 4 and 8 weeks of incubation. The change in phosphorus content was estimated by subtracting the initial phosphorus extracted from a sample at time zero from the final phosphorus extracted after 8 weeks of incubation. This was expressed as a weekly rate and is shown in Table 3.5. The rate of change of extractable phosphorus ranged from -0.6 to +0.16 mg P kg^{-1} $week^{-1}$, in the case of acetic acid and -0.6 to +0.6 mg P kg^{-1} $week^{-1}$ in the case of sodium bicarbonate. More negative values in the case of acetic acid as compared to sodium bicarbonate mineralized phosphorus, clearly indicate more commonly a loss of acetic extractable phosphorus, but more often a gain in sodium bicarbonate phosphorus. Due to either loss of phosphorus or very low mineralization rate, the effect of soil pH on phosphorus turnover was not so pronounced. This can be clearly seen if we look at the correlation coefficient between phosphorus turnover and pH, shown in Figures 3.39 to 3.42 for both extractable phosphorus. These figures indicate that pH had no effect on phosphorus mineralization rate.

Table 3.4 Acetic acid and sodium bicarbonate extractable phosphorus in incubated soils at 0, 4 and 8 weeks

		Extractable phosphorus (mg/kg)					
		Acetic acid			Sodium bicarbonate		
		week 0	week 4	week 8	week 0	week 4	week 8
No organic amendment	A	0.42	0.14	0.13	4.78	4.01	4.58
	B	0.30	0.15	0.08	3.42	2.95	4.90
	C	0.82	0.90	1.02	2.14	2.23	2.93
Alginure	A	0.15	0.01	0.00	0.84	1.71	2.13
	B	0.37	0.29	0.25	6.29	5.30	6.89
	C	0.36	0.14	0.14	1.84	2.15	2.51
Peat	A	0.30	0.04	0.06	4.81	3.66	4.51
	B	2.68	3.89	4.00	5.85	9.78	10.70
	C	0.49	0.29	0.33	5.77	5.05	5.68
Sewage sludge	A	0.42	0.21	0.24	4.12	4.81	4.50
	B	0.80	0.55	0.86	8.87	6.93	8.86
	C	0.44	0.25	0.19	3.89	3.62	4.58
Chicken manure	A	2.95	3.04	4.00	3.93	4.58	5.39
	B	0.43	0.27	0.55	1.30	1.36	3.11
	C	8.49	3.91	3.72	15.87	11.81	11.21
Control	A	0.68	0.37	0.25	5.81	4.56	5.18
	B	0.92	0.71	0.69	7.76	6.71	7.94
	C	0.93	0.51	0.61	9.19	6.42	6.62
Native vegetation	A	0.36	0.11	0.13	0.37	1.10	0.71
	B	0.61	0.25	0.37	1.79	1.87	2.26
	C	1.38	4.35	1.92	6.26	5.42	5.68
Red clover and grass 1 mixture	A	0.46	0.32	0.43	2.35	1.92	2.66
	B	0.53	0.34	0.46	2.59	3.33	3.85
	C	1.17	0.76	1.28	4.16	3.21	4.44
Red clover and grass 2 mixture	A	0.15	0.01	0.00	0.84	1.71	2.13
	B	0.37	0.29	0.25	6.29	5.30	6.89
	C	0.36	0.14	0.14	1.84	2.15	2.51
Grass	A	0.46	0.30	0.60	0.79	1.40	1.82
	B	0.33	0.22	0.63	3.20	1.85	2.14
	C	0.33	0.14	0.34	1.37	1.42	1.82

Table 3.5 Phosphorus turnover and C/P ratio in coal mine soils

	Phosphorus turnover mg/kg/week		C/P	
	Acetic phosphorus	Sodium bicarbonate phosphorus	Acetic phosphorus	Sodium bicarbonate phosphorus
1A	-0.04	-0.02	-	-
B	-0.03	0.18	-	112
C	0.02	0.10	1235	247
2A	-0.02	0.15	-	92
B	-0.01	0.07	-	208
C	-0.03	0.08	-	261
3A	-0.03	-0.04	-	-
B	0.16	0.61	401	105
C	-0.02	-0.01	-	-
4A	0.02	0.05	-	222
B	0.01	0.00	3210	-
C	-0.03	0.09	-	687
5A	0.14	0.18	487	352
B	0.01	0.23	6730	293
C	-0.60	-0.58	-	-
6A	-0.05	-0.08	-	-
B	-0.03	0.02	-	1350
C	-0.04	-0.03	-	-
7A	-0.03	0.04	-	377
B	-0.03	0.06	-	623
C	0.07	-0.07	1938	-
8A	0.00	0.04	-	1727
B	-0.01	0.16	-	635
C	0.01	0.03	4590	1530
9A	0.02	0.04	1430	715
B	-0.02	0.05	-	1166
C	0.01	0.11	8110	737
10A	0.02	0.13	2910	448
B	0.04	-0.13	990	-
C	0.00	0.06	-	1078

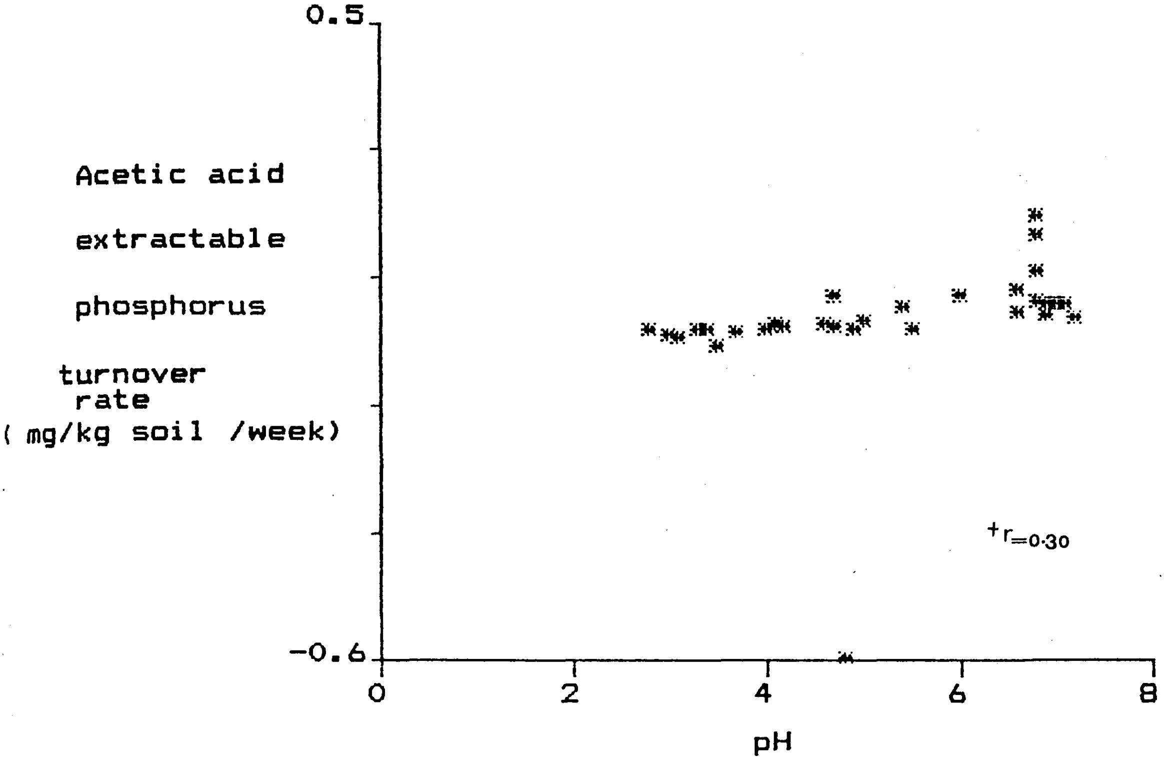


Fig. 3.39 Relationship between pH and acetic acid extractable phosphorus turnover rate.
(all values)
+ r is the Pearson product-moment correlation coefficient.

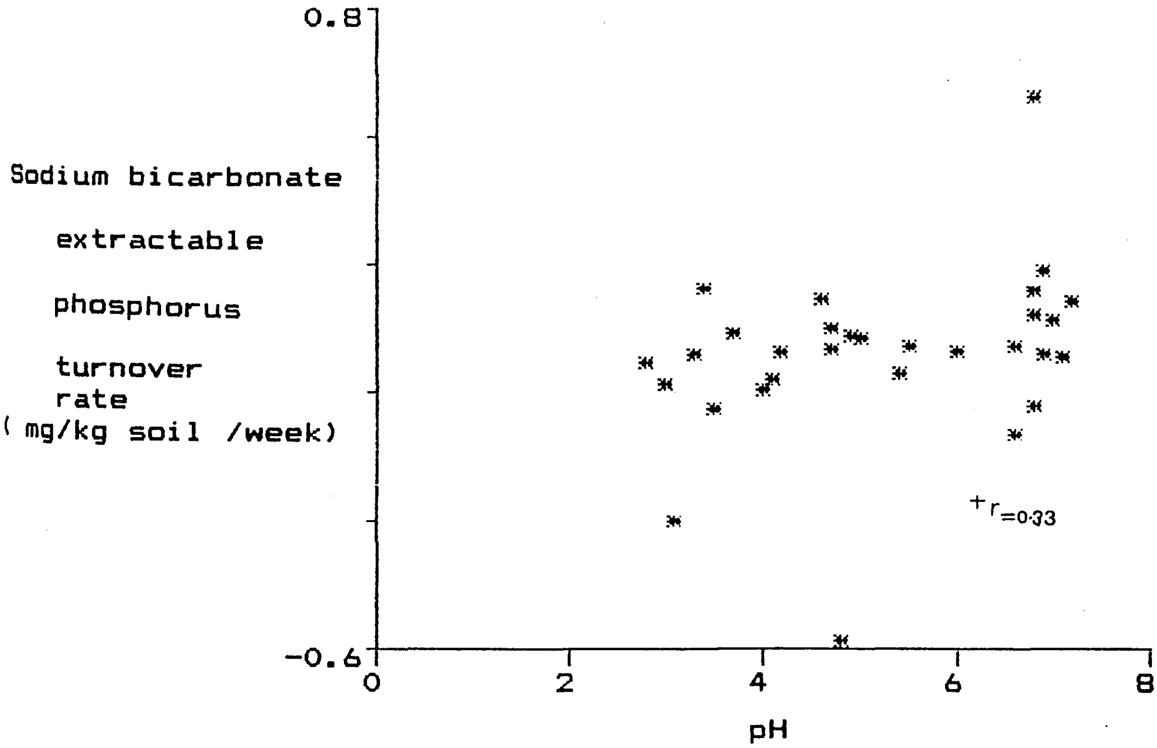


Fig. 3.40 Relationship between pH and sodium bicarbonate extractable phosphorus turnover rate.
(all values)
+ r is the Pearson product-moment correlation coefficient.

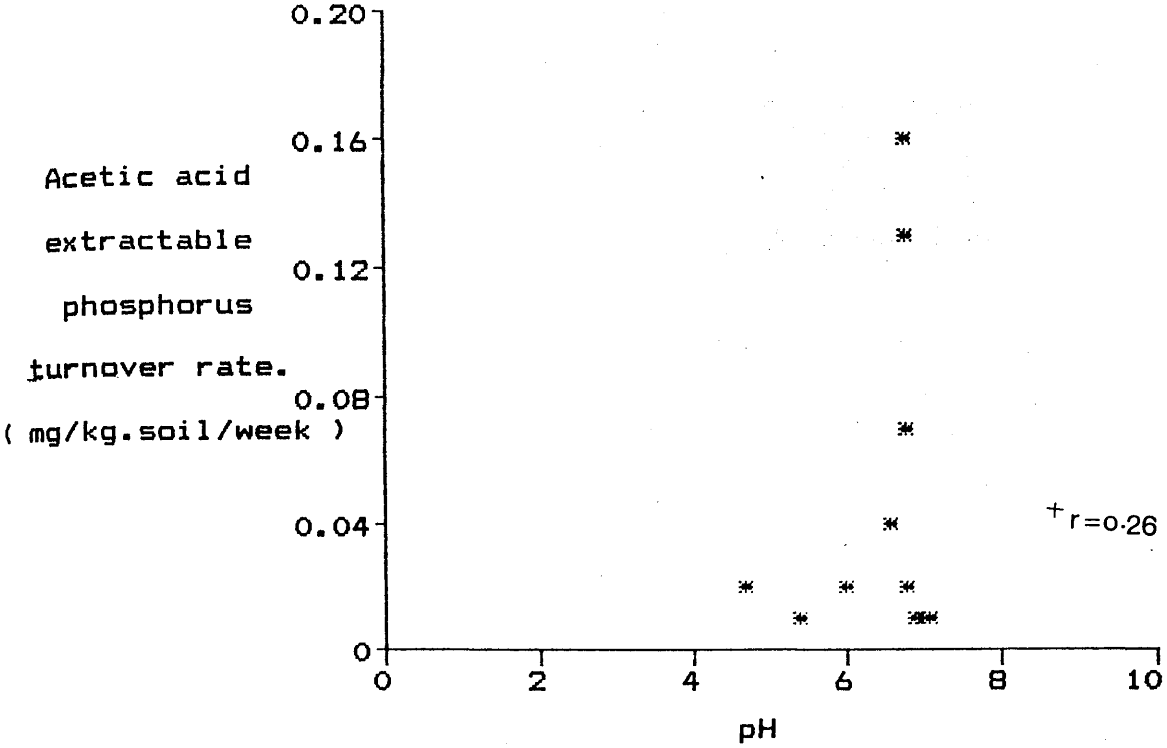


Fig. 3.41 Relationship between pH and acetic acid extractable phosphorus turnover rate . (positive values only).

+r is the pearson product-moment correlation coefficient.

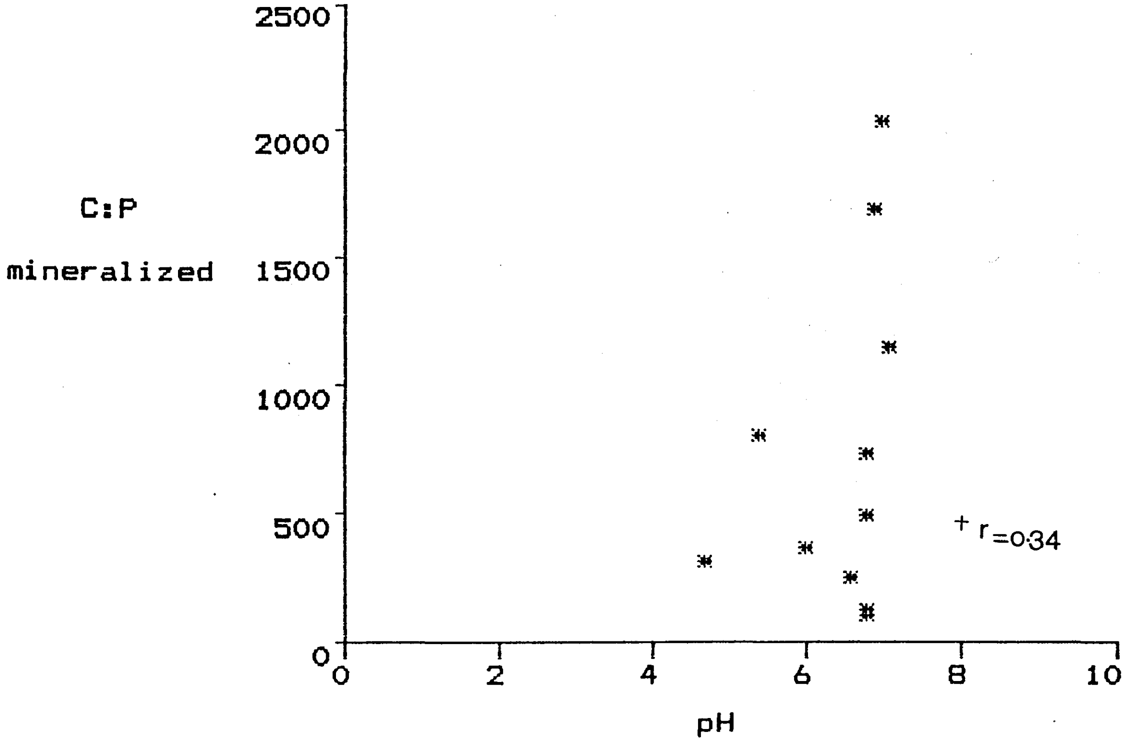


Fig. 3.42 Relationship between pH and C:P(acetic acid) mineralized.

+r is the pearson product-moment correlation coefficient.

It has been found that for soils containing small amounts of labile phosphorus, incubation alone did not alter significantly the amount of extractable phosphorus (Hedley et al., 1982). Since most of these soils also contain small amounts of labile phosphorus (Section 3.1), the phosphorus cycle in the coal mine soils is expected not to be very active.

Another possible reason might be the sorption of the organic phosphorus compounds on hydrated oxides of Fe and Al which decreases the rate of mineralization by enzymes (Greaves and Webley, 1969). The present results also support the findings of Fitter (1974) who found that chemical immobilization can be substantial, especially in colliery shales. However the biological immobilization can also not be ignored as phosphorus mineralized may be recombined into organic forms by microorganisms, and the phosphorus released may be fixed into insoluble inorganic phosphates.

From the preceding discussion, it can be concluded that the phosphorus loss in coal mine soils, resulted either from chemical changes brought about during moist aerobic incubation, which altered the extractability of soil phosphorus or from the conversion of soil phosphorus to microbial phosphorus, which was subsequently released in very few soils on cell death.

Mineralization of phosphorus has been studied very rarely in detail because of the lack of suitable analytical techniques (Wild, 1988). The comparison is also difficult because of the different methods used, for example Tam (1987), Enwezor (1976) and Dalal (1979) used temperatures ranging from 26 to 30°C, and air-dried samples for incubation study. While other workers (Somani, 1987; Hedley et al., 1982; Tam, 1987; Bowman and Cole, 1978; and Mendoza and Barrow, 1987) either used different organic substrates or added inorganic phosphorus and used different incubation periods.

Enwezor (1976) studied phosphorus mineralization in some air-dried samples at 30°C. He reported that 6.6 mg P kg^{-1} soil were mineralized after 12 weeks of incubation (0.55 mg P kg^{-1} $week^{-1}$). Hedley et al. (1982) found that 9 months' incubation caused labile phosphorus (resin plus bicarbonate) to decrease by 11 μg P g^{-1} soil, in one set of soils with high phosphorus content, and 0.2 μg P g^{-1} soil, in another set, with low phosphorus content (0.3 and 0.0 mg P kg^{-1} $week^{-1}$ respectively).

The C/P ratios for soils which mineralized phosphorus are given in Table 3.5. The overall ratios were very high. In the case of acetic acid extractable phosphorus, it varied from 401 to 8110, and ranged from 92 to 1727 in the case of sodium bicarbonate extractable phosphorus. Most of these soils mineralized negligible amounts of phosphorus, but high amounts of carbon, resulting in very high ratios of C/P mineralized. The net immobilization and mineralization of phosphorus in soil has been predicted by the use of organic carbon:organic phosphorus ratios (Alexander, 1977; Tisdale and Nelson, 1975). If the organic carbon: organic phosphorus ratio is 200:1 or less, mineralization of phosphorus occurs, and if the ratio is 300:1 or more, immobilization occurs (Alexander, 1977). The wide ratio of carbon to phosphorus indicates the path of immobilization in the phosphorus cycle in the coal mine soils. However, Enwezor (1967) observed that organic carbon:organic phosphorus ratio in soil was an unreliable index for predicting immobilization or mineralization. Birch (1961) attributed this inconsistence of C:P to predict the path of phosphorus mineralization to the variable but significant amounts of inorganic phosphate present in the organic material. The C:P is more variable because most of the organically held phosphorus is in structures peripheral to humic acid (Wild, 1988).

Changes in pH values of all the samples has been shown in Table 3.6. The pH values of almost all soils were higher at the end of the incubation period with few soils (Alginure B, Peat A, Sewage sludge A, Control B and Grass B and C) which remain unchanged. The mean increase was 0.1 and 0.33 pH units, in acid, and neutral or near neutral soil, respectively. The increase in pH might be due to the decrease in the concentration of organic acids, as decomposition proceeds (Tam, 1987). According to Cooper (1975), this increase in pH is usually temporary, as incubation proceeds further, pH values decline, presumably due to nitrification. However, nitrification is not expected in most of these soils (Shah, 1988).

Table 3.6 <u>Changes in pH values during incubation</u>

		Initial (0 week)	Final (8 week)	Change in pH
No organic amendment	A	3.0	3.2	0.2
	B	3.4	3.5	0.1
	C	4.7	4.8	0.1
Alginure	A	4.6	4.7	0.1
	B	5.0	5.0	0.0
	C	4.9	5.0	0.1
Peat	A	4.0	4.0	0.0
	B	6.8	7.4	0.6
	C	4.1	4.2	0.1
Sewage sludge	A	4.2	4.2	0.0
	B	5.4	5.8	0.4
	C	3.7	3.8	0.1
Chicken manure	A	6.8	7.2	0.4
	B	6.9	7.4	0.5
	C	4.8	4.9	0.1
Control	A	3.5	3.6	0.1
	B	2.8	2.8	0.0
	C	3.1	3.2	0.1
Native vegetation	A	3.3	3.2	0.1
	B	5.5	5.6	0.1
	C	6.8	7.2	0.4
Red clover and grass mixture	A	6.9	7.1	0.2
	B	7.2	7.6	0.4
	C	7.1	7.4	0.3
	A	6.0	6.1	0.1
	B	4.7	4.8	0.1
	C	7.0	7.4	0.4
Grass	A	6.8	7.2	0.4
	B	6.6	6.6	0.0
	C	6.6	6.6	0.0

3.4 Acid phosphatase activities

3.4.1 Introduction

The transformation of soil phosphorus is not well understood but it is generally accepted that phosphorus is taken up by the plant roots as inorganic phosphate. Since a large proportion of the phosphorus in soil is organically bound, the mineralization of these organic fractions is of major agricultural and economic importance. Organic phosphorus compounds mineralized to inorganic phosphorus in the soil close to, or on, the root surface can subsequently be absorbed by the root. Thus mineralization appears to be catalysed by root exocellular enzymes which may be either liberated into the soil solution or bound to the root epidermal cells but outside their permeability barrier (Speir and Ross, 1978).

Incubation studies in the absence of plants have shown that soil mineralizes native organic phosphorus (Kalia, 1949). Addition of an energy source containing readily assimilable carbon increased the rate of mineralization (Halm et al., 1971), indicating that microbial profileration is at least partly responsible. However, the mineralization of soil organic phosphorus is associated with the mineralization of organic matter as a whole. If the phosphorus concentration in organic matter being assimilated by microorganisms is below 0.2 to 0.3 percent, no phosphorus will be mineralized in microbial tissue (Speir and Ross, 1978).

Soil phosphatases have been accorded a role in the mineralization of phosphorus, as they catalyse the hydrolytic cleavage of ester phosphate bonds. The general name, phosphatases, has been used to describe a broad group of enzymes that catalyse the hydrolysis of esters and anhydrides of phosphoric acid. These enzymes are classified as acid or alkaline phosphatases because they show their optimum activities in acid and alkaline ranges respectively. Most of the literature, however, is related to acid phosphatase.

The first study of soil phosphatase activity using an artificial substrate was that of Kroll et al. (1955) who used disodium phenylphosphate and measured both liberated phenol and inorganic phosphate. Recent studies by Eivazi and Tabatabai (1977) and Juma and Tabatabai (1977 and 1978) show that acid phosphatase is predominant in acid soil and alkaline phosphatase in alkaline soil. The inverse relationship between soil phosphatase activity and soil pH suggests that either the rate of synthesis and release of this enzyme by soil microorganisms or the stability of this enzyme is related to soil pH. Since higher plants are devoid of alkaline phosphatase activity (Tabatabai, 1982), the alkaline phosphatase activity in soils seems to be derived totally from microorganisms.

The production of stable and productive mine soils requires microbial activities, which are essential for decomposition and humification, biochemical weathering, aggregation and proper nutrient cycling (Cundell, 1977).

However, the numbers and diversity of microorganisms have been found to be lower in mine soils than in native soils (Wilson, 1965). Moreover, levels of ATP have been found to be also lower in mine soils than native soils (Hersman, 1977). Despite all this, little attention has been paid to the study of microbial activities in general and phosphatase in particular, in coal mine soils. Acid phosphatase, which plays an important role in hydrolyzing organophosphate to release orthophosphate necessary for plant and microbial nutrition, has been studied very rarely in coal mine soils (Stroo and Jencks, 1982; Fresquezet al., 1987).

The main objective of this study was to survey the acid phosphatase activities in coal mine soils and to assess the relationship with other soil parameters, related to phosphorus behaviour. This microbial assessment will help in the overall understanding of the biochemical behaviour of phosphorus in coal mine soils of Central Scotland and can help in the reclamation of these soils.

3.4.2 Materials and methods

Acid phosphatase activity was measured in fresh coal mine spoils from Baads, using the method of Tabatabai and Bremner (1969), described in Section 2.1.7. 1 g fresh soil sample, 4 mm sieved, was weighed and incubated at $37^{o}C$ for 1 hour, using p-nitrophenyl phosphate as a substrate. The intensity of phosphatase activity was expressed as μ moles p-nitrophenyl (Pnp) liberated $g^{-1}h^{-1}$.

3.4.3 Results and discussion

Table 3.7 shows the acid phosphatase activities determined in thirty samples collected at Baads Cooliery.

This data indicate that the acid phosphatase activity in coal mine soil ranged from 1.1 to 7.6 μ moles Pnp released g^{-1} $hour^{-1}$ with a mean value of 3.9 μ moles g^{-1} $hour^{-1}$.

The values for acid phosphatase activity found in this study are similar to, or somewhat greater than, values of 0.6 to 4.8 μ moles Pnp released g^{-1} $hour^{-1}$ (with a mean value of 2.3 μ moles g^{-1} $hour^{-1}$), reported by Stroo and Jencks (1982) for coal mine soils. The comparatively higher values of acid phosphatase activity in the present study, compared to those reported by Stroo and Jencks (1982), may partly be due to the unfertilized mine soils they used for measuring acid phosphatase activity. Recent mine soils receiving high inputs of lime and fertilizers had the highest activity (Stroo and Jencks, 1982). Enzymatic activities in coal mine soils were, however, found to be lower than those reported in agricultural soils by various researchers. Stroo and Jencks (1982) studied acid phosphatase activity in some native soils, and reported 5.1 to 8.6 μ moles Pnp released g^{-1} $hour^{-1}$. Trasar-Cepda and Gil-sotres (1987) reported acid phosphate activity for a variety of soil ranging from 6.0 to as high as 21.0 μ moles g^{-1} $hour^{-1}$ at $30^{o}C$, up to a depth of 0-15 cm. Eivazi and Tabatabai (1977), studying the kinetic parameters of phosphatase in the soil, found that acid phosphatase activities ranged from 52 to 196 μg Pnp released g^{-1} $hour^{-1}$ (0.37 to 1.41 μ moles Pnp g^{-1} $hour^{-1}$).

Fresquez et al. (1987) concluded that the low phosphatase activity in coal mine soils, as compared to the adjacent undisturbed soils, may be partially attributed to the poor chemical and physical environments of these materials, as well as to the low microbial population and low diversity of microorganisms. Stroo and Jencks (1982) stated that the lower phosphatase activity in mine soils suggests that phosphorus mineralization may be slower on mine soils.

The significant negative correlation (Figure 3.43) between acid phosphatase activity and pH confirms the suggestion of Dick and Tabatabai (1984) that acid phosphatase predominates in acid soils, though both Harrison and Helliwell (1981) and Pang and Kolenko (1986) have reported neutral phosphatase activity in acid woodland soils. Inhibition of enzymatic activities has been reported to become a problem in mine soils due to higher acidities (Cundell, 1977). A negative relationship between phosphatase activity and pH was also reported by Chhonkar and Tarafdar (1985). However, Stroo and Jencks (1982) found no correlation, between any index of microbial activity and pH, in some native and coal mine soils.

Table 3.7 Acid phosphatase activity in coal mine soils from Baads (μ moles Pnp g^{-1} h^{-1})

		Pnp μ moles g^{-1} h^{-1}	pH
No organic amendment	A	6.26	3.0
	B	6.84	3.4
	C	7.06	4.7
Alginure	A	7.59	4.6
	B	6.07	5.0
	C	5.79	4.9
Peat	A	4.33	4.0
	B	2.31	6.8
	C	6.03	4.1
Sewage sludge	A	3.21	4.2
	B	3.13	5.4
	C	3.04	3.7
Chicken Manure	A	2.18	6.8
	B	2.27	6.9
	C	1.99	4.8
Control	A	7.53	3.5
	B	4.16	2.8
	C	7.17	3.1
Native vegetation	A	1.08	3.3
	B	2.93	5.5
	C	2.99	6.8
Red clover and grass mixture	A	1.72	6.9
	B	1.89	7.2
	C	1.74	7.1
	A	3.04	6.0
	B	3.38	4.7
	C	2.80	7.0
Grass	A	3.37	6.8
	B	2.66	6.6
	C	2.33	6.6

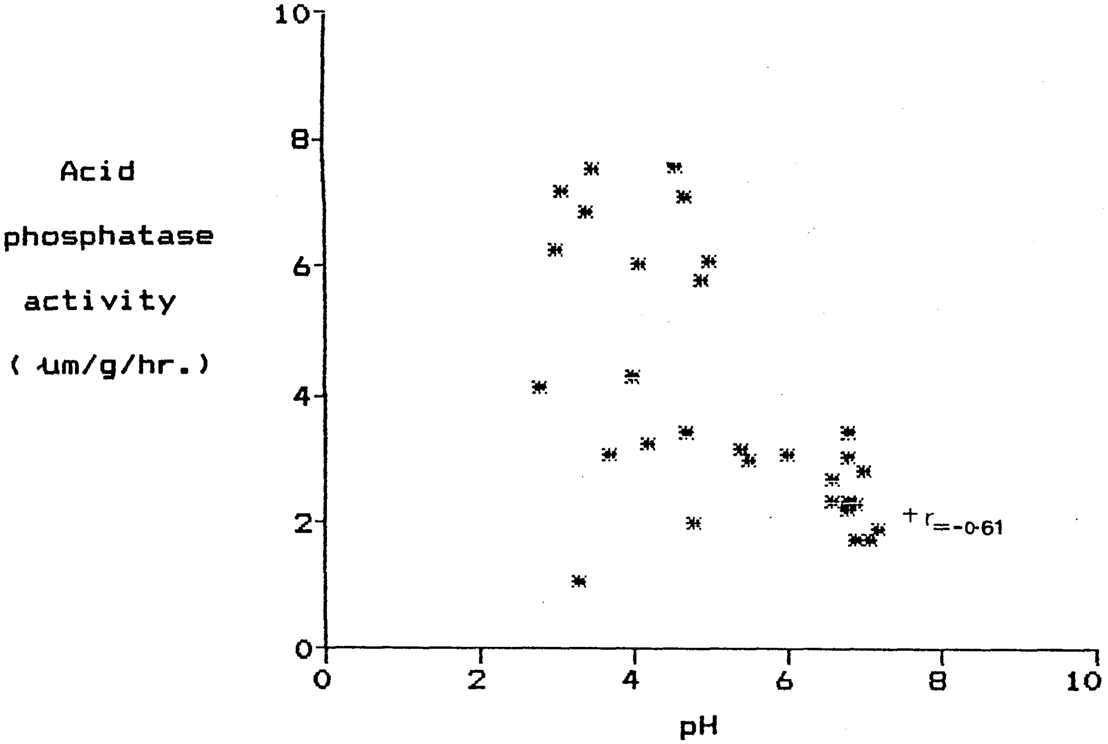


Fig. 3.43 Relationship between pH and acid phosphatase activity.
† r is the Pearson product-moment correlation coefficient.

3.5 General discussion and conclusion

The soil is not a homogeneous mass but a rather heterogeneous body of material. This is more true in the case of coal mine soils if treated as a soil in order to establish plant growth. The heterogenity of such disturbed lands make the soil sampling more intense, because of the vast variation between individual samples. The intensity with which a soil must be sampled to estimate with given accuracy some characteristics will depend on the magnitude of the variation. It is economical to perform laboratory analysis on a composite sample. However, its accuracy can be doubtful in the case of heterogeneous materials like coal mine soils, because the material tipped may have had different origins. Keeping in view the heterogenity of such material, instead of taking a composite sample (except for the assessment study, Section 2.2.2), it was decided to take 3 individual samples at random from each sampling area. Care was taken in the selection and distribution of sampling areas on a bing. Each sampling area represented a piece of land, roughly equal to 20-25 square metres, with uniform appearance in respect of vegetation, topography, colour of the soil or any treatment in the past.

Fresh samples of coal mine soils were used in the present study, because it is well established that drying of soil has an immediate and major effect on many of its biological activities (McGarity and Myers, 1967). Ladd (1978) and Roberge (1978) have documented the harmful effect of air-drying of soil on the activities of many of its enzymes. Speir and Ross (1981) found that fresh soil samples were best for preserving the original activity. Bartlet and James (1980) found that air-drying soil not only causes immediate changes in chemical properties, but if stored, these changes are continued. Lower content of extractable organic phosphorus was found in samples that had been air-dried initially and stored in that condition (Sekhan and Black, 1969). Some workers (Barrow and Shaw, 1980; Bartlet and James, 1980; Haynes and Swift, 1985) have shown that air-drying soil can markedly increase their phosphate adsorption capacity. Haynes and Swift (1985) found that at an equilibrium solution concentration of an 0.5 μg P ml^{-1}, the increase in quantity of phosphate adsorbed following drying ranged from 23% to 70% of that adsorbed by the moist samples. Air-drying of the soil samples caused a small decrease in soil pH and increase in quantities of native soil phosphate extracted by resin and sodium bicarbonate. The effect of air-drying on adsorption might be due to alteration in the number or affinity of adsorption sites and the amount of labile phosphate present (Beckett and White, 1964). Moreover, the actual field conditions can be best represented by fresh soil samples and not air-dried.

The pH of the coal mine soils, which is considered to be the major determining factor in phosphorus transformations, ranged from 2.8 to 7.2, and showed considerable variation between different sites on the same bing as well as within the three samples from the same sampling area. Similar trends of variation in extractable phosphorus (acetic-P, bicarbonate-P and anion exchange resin-P) were also found.

To assess the relationships between various properties studied, correlation coefficients for all sites (30 samples) were computed. Table 3.8 shows some of correlation coefficients of adsorption maximum and bonding constant of 12 soils with other soil characteristics related to phosphorus. Some graphs of correlation are also presented in Figures 3.44-3.51.

There was no significant correlation between carbon mineralization and phosphorus turnover. However, acid phosphatase was significantly associated with carbon dioxide evolved, carbon mineralization rate (Figures 3.44 and 3.45) and the ratio of organic carbon to mineralized bicarbonate phosphorus (Figure 3.46). Such association suggests the importance of organic matter for both microbial activity and enzyme stabilization. Chhonkar and Tarafdar (1985) reported a significantly positive correlation between phosphatase activity and organic carbon and phosphorus. Speir (1984) found that phosphatase activities were significantly correlated with organic carbon content in one group of soil, but not in another.

The relationship of adsorption maxima and bonding constant found in 12 soils with other soil properties has been shown in Table 3.9. Adsorption maxima and bonding constant showed no correlation with any soil parameters studied. In order to illustrate the relationships of adsorption maxima, some graphs have been presented in Figures 3.47 to 3.51.

The changes in phosphorus fractions in the incubation study clarify some pathways in the phosphorus cycle in coal mine soils. The low phosphorus turnover in a large number of samples from Baads colliery resulted from either chemical or biological immobilization. Though a limited number of samples were analysed for adsorption parameters, some of them had a high bonding energy as compared to agricultural soils. This might be due to the great quantity of amorphous iron oxide, which these soils contain. Pulford and Duncan (1975) found that amorphous iron oxide, which is the product of the oxidation of iron pyrite in coal mine soils, was significantly correlated with phosphate adsorption.

Table 3.8 Correlation coefficients between various components of phosphorus status of 30 coal mine soil samples collected at Baads

	Extractable phosphorus			Mineralization rate			Total		
	Acet-P	Bio-P	AER-P	Act-Pmr	Bio-Pmr	Cmr	CO_2	C/Acet-P	C/Bio-P
Bio-P	0.72^{***}	-	-	-	-	-	-	-	-
AER-P	0.84^{***}	0.64^{***}	-	-	-	-	-	-	-
Acet-Pmr	-0.70^{***}	-0.61^{***}	-0.83^{***}	-	-	-	-	-	-
Bio-Pmr	-0.44^{*}	-0.61^{***}	-0.53^{**}	0.75^{***}	-	-	-	-	-
Cmr	-0.07	-0.21	-0.18	0.34	0.26	-	-	-	-
Total CO_2	-0.01	-0.19	-0.14	0.38^{*}	0.32	0.96^{***}	-	-	-
Acid phosphatase	-0.26	0.09	-0.09	-	-0.10	-0.48^{**}	-0.54^{**}	-0.29	-0.45

Significant of correlation coefficients at 28 degree of freedom (N=30)

Values > 0.36 significant at 5%
Values > 0.46 significant at 1%
Values > 0.57 significant at 0.1%

Table 3.9 Correlation coefficient of Langmuir adsorption maximum and bonding energy with other soil properties

	Adsorption maximum	Bonding energy
Bonding energy	0.61	-
pH	-0.21	-0.46
Acid phosphatase	0.26	0.52
Acetic-P	-0.41	-0.41
Bicarbonate-P	-0.11	-0.30
AER-P	-0.28	-0.06
AP rate	0.26	0.30
Bio P rate	0.17	0.25
C rate	0.07	-0.09

Significant of correlation coefficients at 10 degree of freedom (N=12)

Value > 0.58 significant at 0.5%
> 0.71 significant at 0.1%
> 0.82 significant at 0.01%

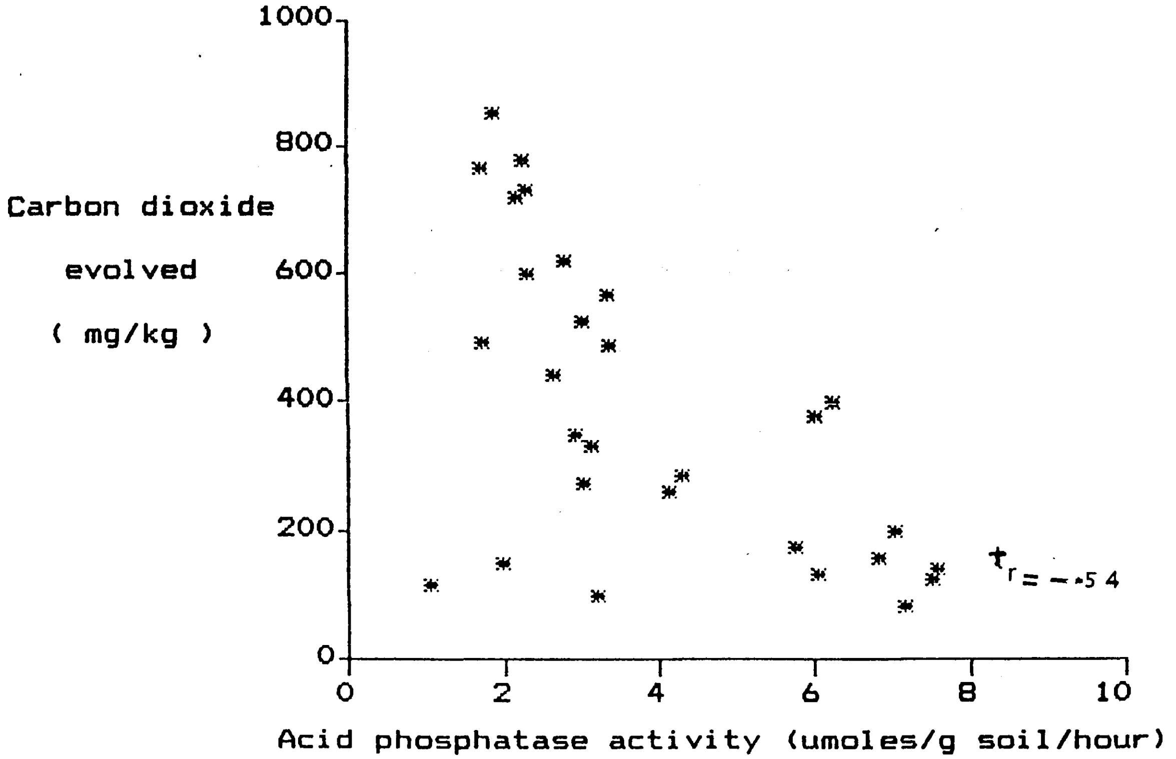


Fig. 3.44 Relationship between acid phosphatase activity and total carbon evolved by soil.

+r Pearson product-moment correlation coefficient

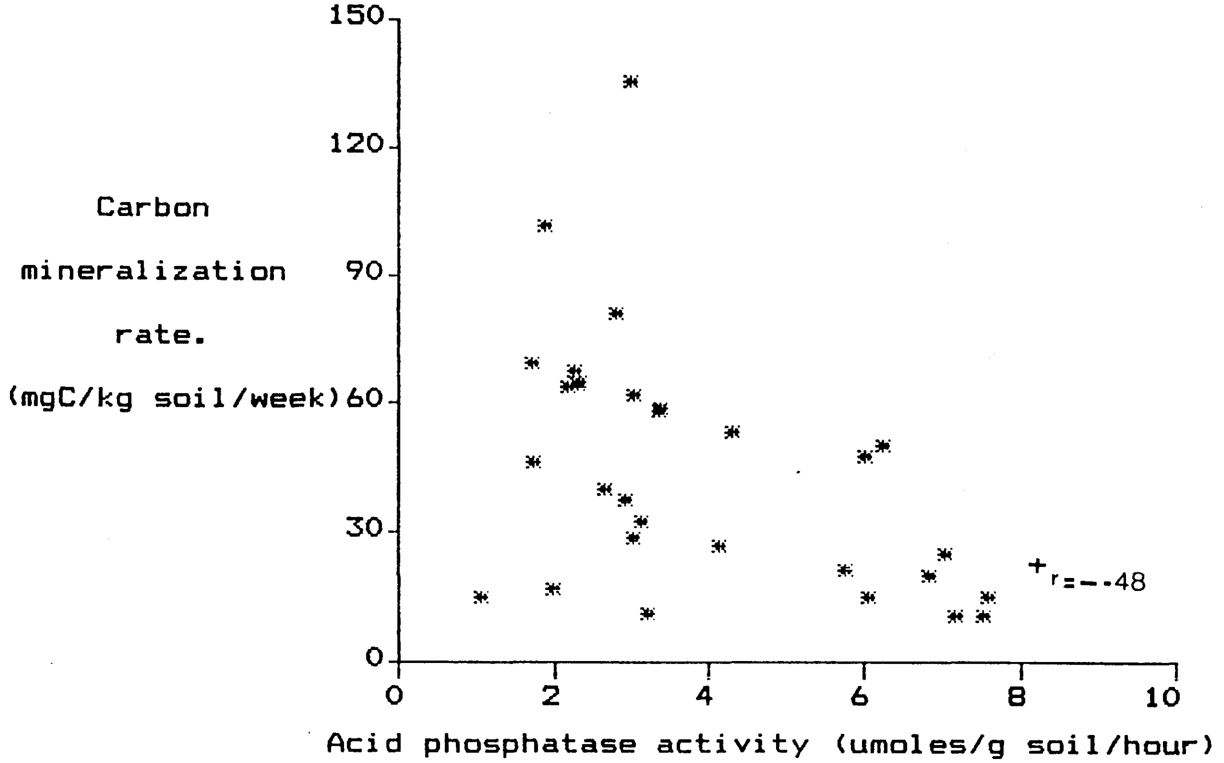


Fig. 3.45 Relationship between acid phosphatase activity and carbon mineralization rate.

+r Pearson product-moment correlation coefficient

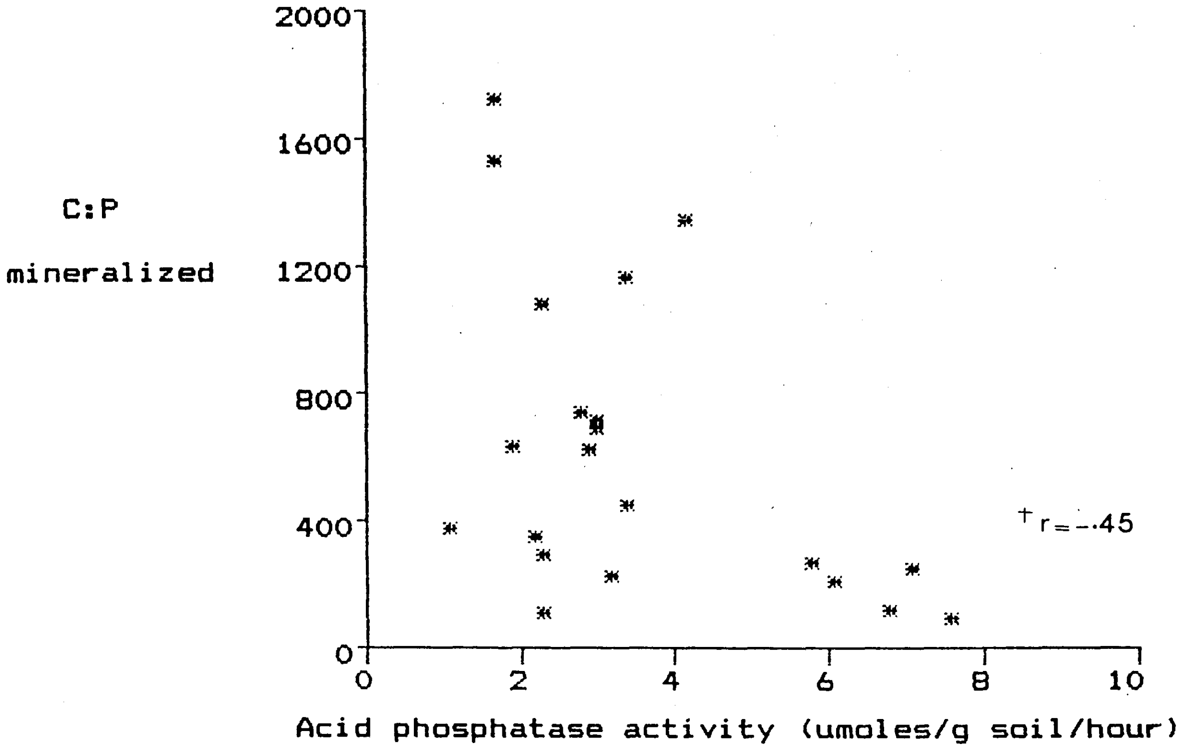


Fig. 3.46 Relationship between acid phosphatase activity and c:p (bicarbonate) mineralized.

+, Pearson product-moment correlation coefficient

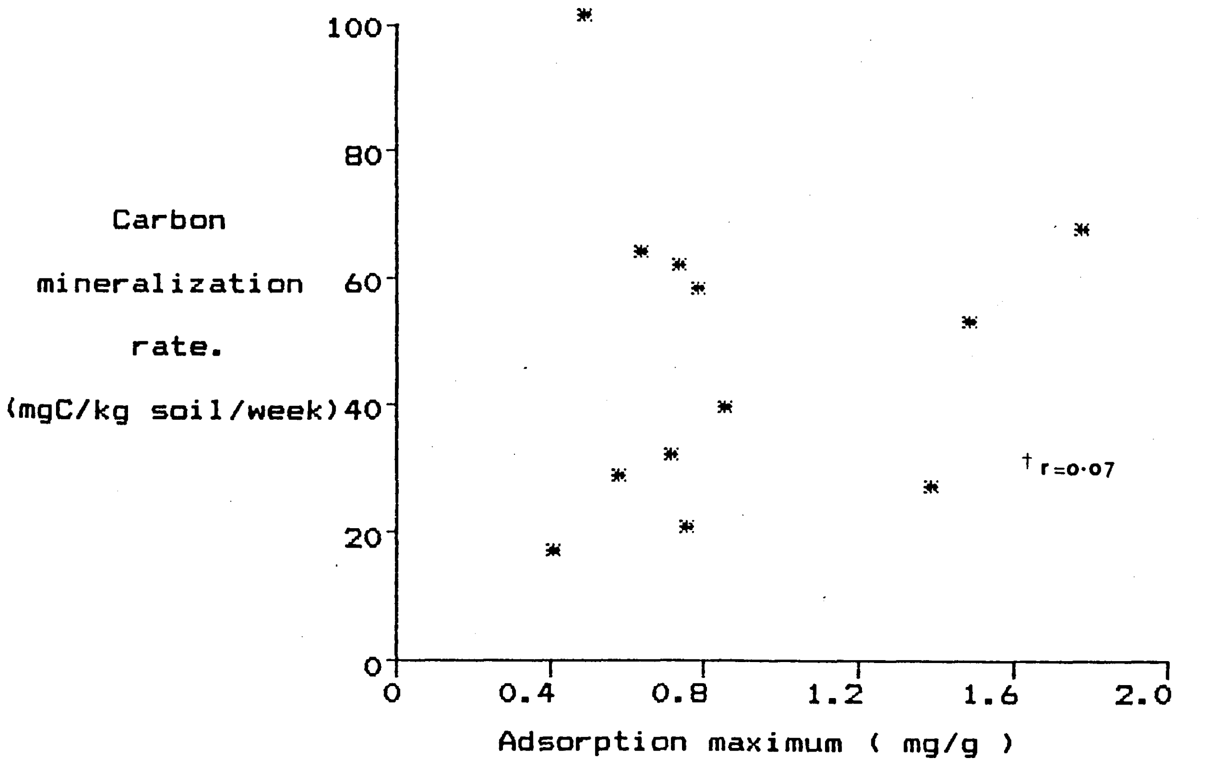


Fig. 3.47 Relationship between adsorption maximum and carbon mineralization rate.

+, Pearson product-moment correlation coefficient

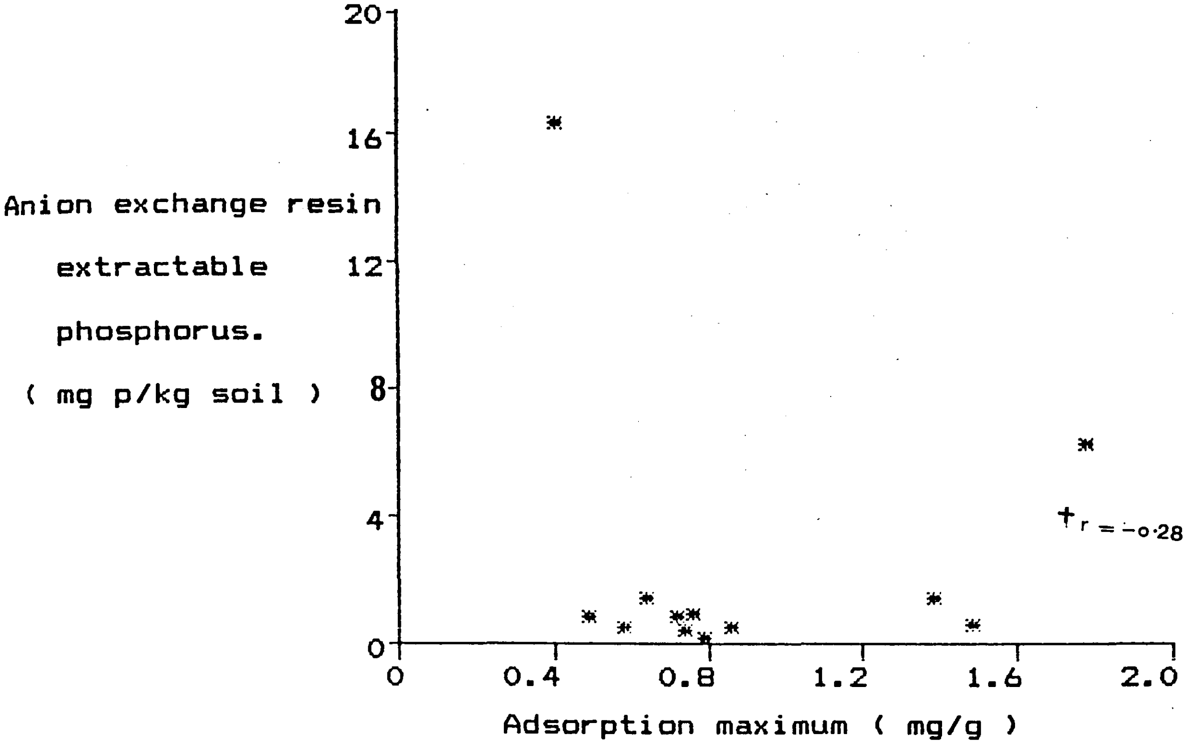


Fig. 3.48 Relationship between adsorption maximum and anion exchange resin extractable phosphorus.

$+_r$ Pearson product-moment correlation coefficient

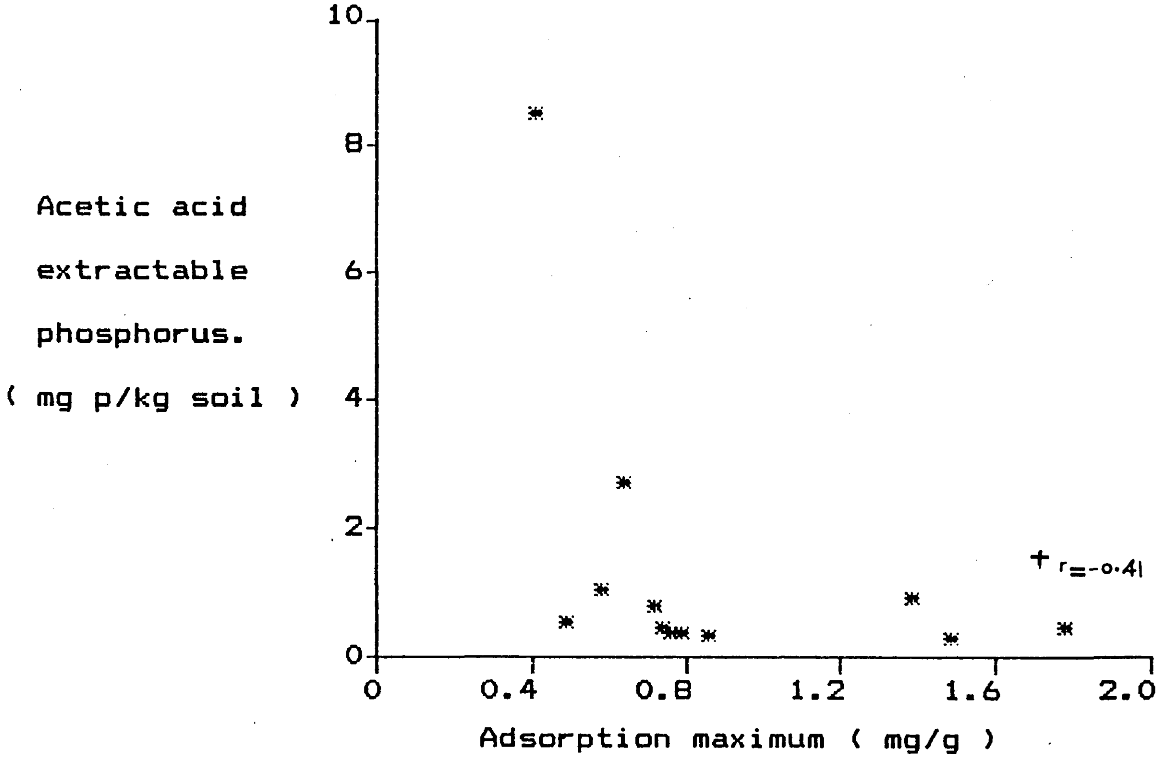


Fig. 3.49 Relationship between adsorption maximum and actic acid extractable phosphorus.

$+_r$ Pearson product-moment correlation coefficient

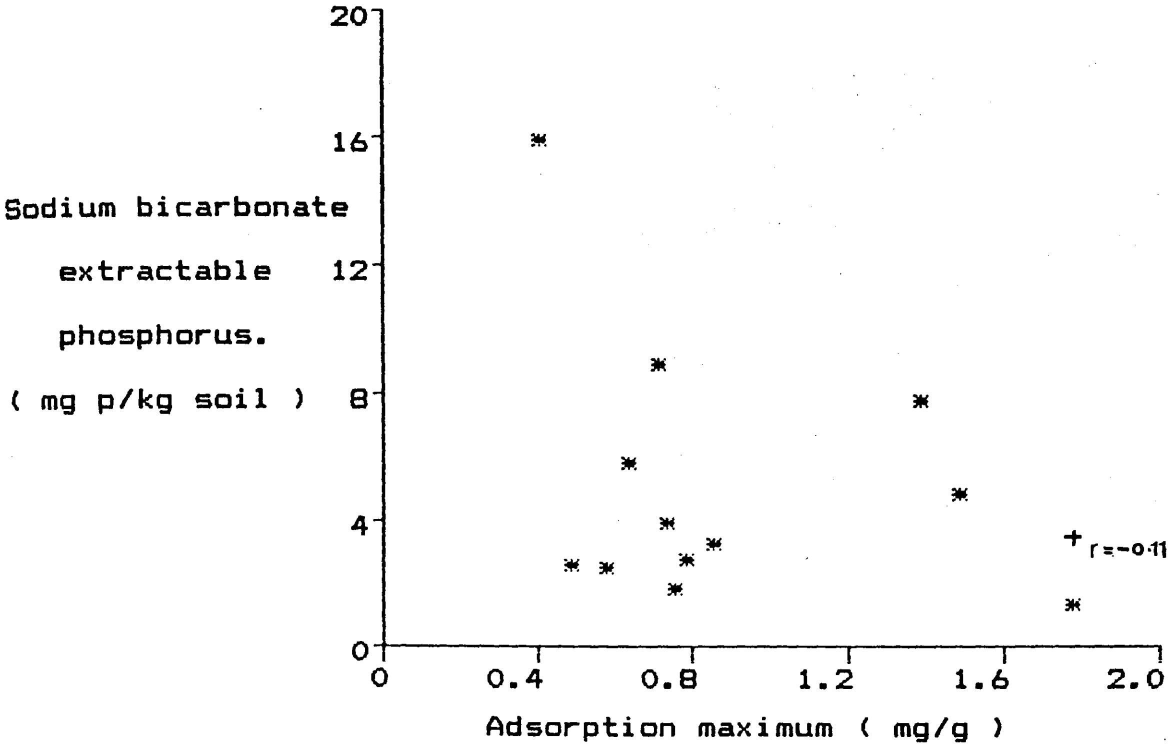


Fig. 3.50 Relationship between adsorption maximum and sodium bicarbonate extractable phosphorus.

+$_r$ Pearson product-moment correlation coefficient

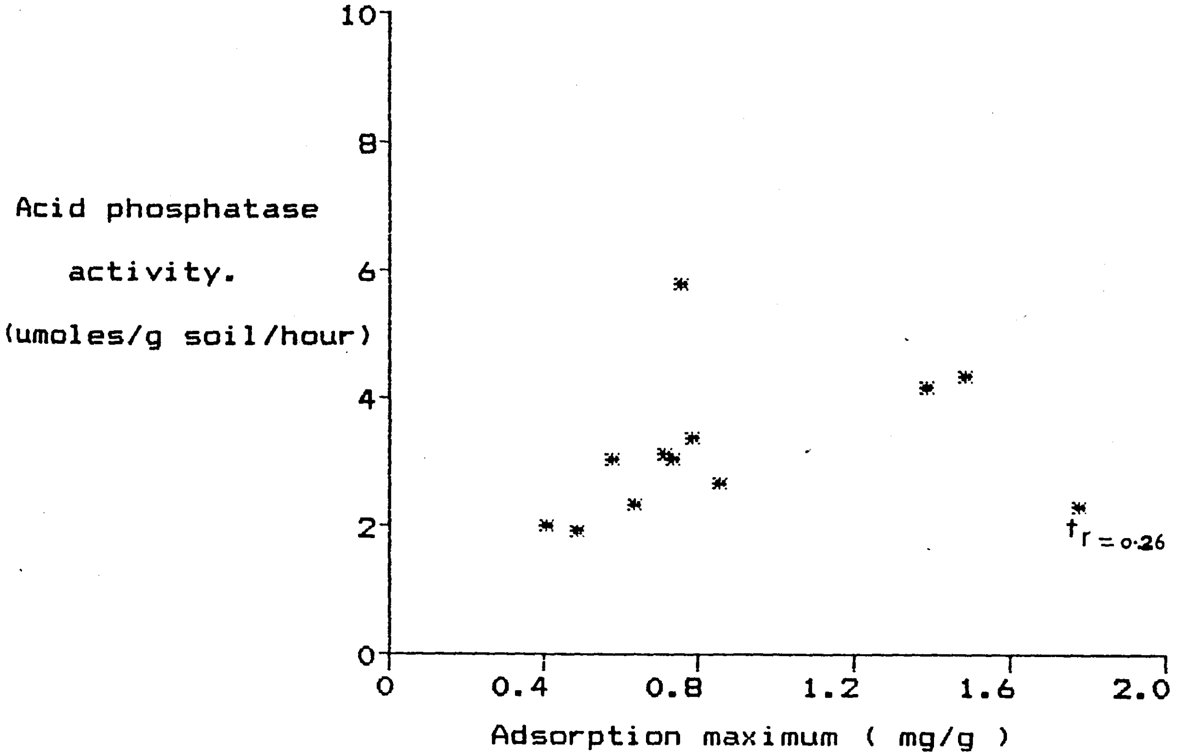


Fig. 3.51 Relationship between adsorption maximum and acid phosphatase activity.

+$_r$ Pearson product-moment correlation coefficient

Table 3.10 Summary of phosphorus status in coal mine soils

Property	Minimum	Maximum	Mean	SD
pH (in water)	2.8	7.2	5.2	1.5
Extractable phosphorus ($\mu g\ g^{-1}$)				
Sodium bicarbonate	0.37	15.87	4.17	3.3
Acetic acid	0.15	8.49	0.97	1.5
Anion exchange resin	0.0	8.14	0.77	1.5
Acid phosphatase activity (μM Pnp $g^{-1}\ h^{-1}$)	1.08	7.59	3.90	2.0
CO_2 evolution rate ($mg^{-1}\ kg^{-1}\ week^{-1}$)	10.57	135.7	44.90	29.4
Langmuir adsorption maximum ($mg\ g^{-1}$)	0.37	15.87	4.17	3.3
Langmuir bonding constant ($1\ mg^{-1}$)	0.11	1.83	0.83	0.49

CHAPTER 4

RESPONSE OF PERENNIAL RYEGRASS TO PHOSPHATE

4.1 Introduction

Colliery soils not only contain low total phosphorus, but also low available phosphorus (Palmer, 1978; Fitter et al., 1974; Pulford, 1976; Lister, 1987; Pulford et al., 1988). The problem is further aggravated by the presence of high amounts of iron pyrite and the severe acid nature of many of these soils.

The low levels of extractable phosphorus, together with the low mineralization rates of phosphorus and high ratios of C:P mineralized in the reclaimed coal mine soils of Baads, reported in Chapter 3, suggest a deficiency of plant available phosphorus. Such results indicate the need to ensure an adequate supply of phosphorus to the growing plant on such sites. There is little information on the nutritional status and response relationships between vegetation and phosphorus, except that of Fitter and Bradshaw (1974) and Pulford et al. (1988).

A great deal of work has been done on the response of grass plus clover swards to phosphorus application on managed grasslands. Such information could hardly be fully applied to coal mine soils due to the differences in the management practices and nature of the materials. Due to the significant relationship between amorphous aluminium oxides (William et al. 1958; Saunders, 1965;)

and iron oxides (Doubleday, 1971; Pulford and Duncan, 1975) with phosphate adsorption in soil and spoil, there is a great tendency for phosphorus fertilizers to be adsorbed after application.

Doubleday (1971) adapted the method of Ozanne and Shaw (1968) to asses the phosphate status of colliery spoils in Northumberland and Durham. This technique estimated the quantity of phosphate needed to raise phosphorus availability to an adequate level, and showed that some shales needed 100 to as much as 1000 kg P_2O_5 per hectare, to support an actively growing ryegrass sward. Fitter and Bradshaw (1974) also used this method of analysis to demonstrate the different levels of phosphate demand by Lolium perenne and Agrostis tenuis grown on colliery shale soils. They found that phosphorus was deficient in all cases. The response to phosphorus was correlated with various chemical estimates of soil phosphorus status, but good correlation was found only where both buffering capacity and quantity were taken into account. Lister (1987), while conducting greenhouse experiments on the response of ryegrass to phosphorus fertilization on colliery shales of Central Scotland, found that plant yield was increased by addition of 20 kg P/ha to all spoils, although no further yield increase was found resulting from the addition of phosphorus at 40 kg/ha to unlimed acid spoil or to spoil at pH 7.0 receiving lime, indicating that pH is the factor of greatest influence in spoil with pH < 4.0 and pH > 7.0.

Bloomfield et al., (1982) carried out nitrogen, phosphorus and potassium fertilizer trials on reclaimed areas of colliery spoils, and observed small effects of phosphorus application on some sites. Pulford et al., (1988) applied various combinations of nitrogen, phosphorus and potassium fertilizer treatments to vegetation on reclaimed coal mine soils at Baads and found no response to added phosphorus. Lack of response by certain crops to phosphorus, even in apparently phosphorus deficient soils, is attributed in part to mycorrhizal activity, as there is some evidence for the role of P-solubilising microorganisms in increasing phosphorus availability (Tandon, 1987).

Mays et al. (1986) reported that phosphorus concentrations in grasses range from about 0.14 to 0.51%. In most situations a concentration below about 0.2% indicates a deficiency for plant growth, while 0.3 to 0.35% is usually necessary for optimum yields. Fitter and Bradshaw (1974) suggested a critical concentration varying from 0.18 to 0.27%, or about 0.20% overall, for <u>Lolium perenne</u> grown on colliery shales. Whitehead (1966) quotes 0.3% as the optimum for pasture grasses and de Wit, Dijkshoorn and Noggle (1963) give a value equivalent to 0.25% for <u>Lolium perenne</u>. Khasawneh (1971) postulated that phosphorus uptake would be proportional to buffering capacity, whereas in soils of equal quantities of phosphorus, uptake will be inversely proportional to the buffering capacity. Data on phosphorus uptake by ryegrass from greenhouse experiments confirmed these concepts

(Holford and Mattingly, 1976a). Results on the rate of phosphorus uptake by cotton showed that a term combining the factors of intensity, quantity and buffering capacity was a better measure of soil phosphorus status than either quantity or intensity alone (Khasawneh and Copeland, 1973). Phosphorus uptake by ryegrass from a range of soils was better correlated when intensity and quantity factors were considered together (Gunary and Sutton, 1967).

The objective of the present study was to determine the response of ryegrass (*Lolium perenne*) to applications of phosphorus fertilizer, measured by dry matter yield and phosphorus content of ryegrass. Since it was expected that either biological or chemical immobilization of phosphorus, or both, might play an important role in the lack of response of crops to phosphatic fertilizer in the coal mine soils, both acid phosphatase activities (before pot experiment only) and adsorption isotherm (before and after pot experiment) were measured.

In July 1987, a pot experiment was carried out in the greenhouse using perennial ryegrass as a test crop grown in five different soils (Section 2.3) to study the above objectives.

4.2 Materials and methods

4.2.1 Soil sampling sites

Five soil samples, four of colliery soil and one oil shale from Central Scotland (Section 2.3) were collected from 0-15 cm depth. The fresh soil samples, after sieving (4 mm) were analysed for pH, acid phosphatase, phosphorus adsorption maximum (Section 2.1) and, 0.5 M acetic acid extractable phosphorus (Section 3.1.3). Some data on soil characteristics are shown in Table 4.1. Langmuir maximum was calculated from adsorption plots (Section 3.2.2).

Loganlea - The soil was moderately acidic with medium phosphatase activity and very low acetic acid extractable phosphorus.

North Addiewell - This oil shale was also moderately acidic, with medium phosphatase activity and extractable phosphorus.

Baads (chicken manure plot) - The soil was moderately acidic in nature, with a low phosphatase activity and high acetic acid extractable phosphorus.

Baads (reclaimed bing) - It was neutral in nature with low phosphatase activity and extractable phosphorus.

Meikle Earnock - The bing is alkaline in reaction, with very low acid phosphatase activity and extractable phosphorus.

Table 4.1 <u>Some chemical and biological properties of soil samples used for pot experiment</u>

Site	pH	Acid phosphatase (μ moles Pnp g^{-1} h^{-1})*	Acetic acid-P (μg g^{-1})	Langmuir adsorption maximum (mg g^{-1})
Loganlea	5.6	9.5	0.4	0.54
North Addiewell	5.6	9.9	9.1	0.54
Baads (chicken manure)	5.4	2.9	21.6	0.50
Baads (reclaimed)	7.2	2.8	5.3	0.65
Meikle Earnock	7.8	0.2	5.0	0.11

* Acid phosphatase activities given in micromoles P-nitrophenol released per gram of soil per hour.

4.2.2 Pot culture techniques

The experiment was carried out in a greenhouse at a minimum temperature, ranging from 5-16°C (mean = 10°C), and a maximum, ranging from 21-46°C (mean = 31°C), with normal day length. No supplementary heating or lighting was provided. The layout of the experiment (see next page) was a randomized block design. It consisted of five soils, to which 3 phosphorus rates were added. Each treatment was replicated four times, which makes a total of 60 pots. An extra eight border pots were placed at each end of the four blocks to counteract border effects.

The levels of nitrogen and potassium added to each pot were the same for each phosphate treatment, being equivalent to the addition of 100 kg N ha^{-1} and 50 kg K ha^{-1}. Nitrogen was added as ammonium nitrate and potassium as potassium chloride. Three rates of phosphorus addition were compared, representing the addition of 0, 50 and 100 kg P ha^{-1} as sodium dihydrogen orthophosphate. All fertilizers were added in solution form and mixed thoroughly with the soil on a plastic sheet in order to give an even distribution through the samples. To 400 g fresh sample, which was the weight of soil added to each pot, 10 ml solution containing either:-

T_0: 0 mg P, 10 mg N, 5 mg K
or T_1: 5 mg P, 10 mg N, 5 mg K
or T_2: 10 mg P, 10 mg N, 5 mg K

was added.

Layout for pot experiment

Design = randomized block

BLOCK 1	S_3T_2	S_3T_1	S_1T_0	S_5T_0	S_1T_1	S_4T_1	S_5T_1	S_3T_0	S_1T_2	S_4T_0	S_5T_0	S_2T_2	S_2T_0	S_4T_2	S_2T_1
BLOCK 2	S_1T_2	S_1T_0	S_4T_0	S_4T_1	S_5T_1	S_2T_2	S_5T_2	S_5T_0	S_3T_2	S_2T_0	S_1T_1	S_4T_2	S_3T_0	S_2T_1	S_3T_1
BLOCK 3	S_5T_0	S_2T_1	S_1T_0	S_4T_0	S_5T_2	S_1T_2	S_5T_1	S_3T_1	S_1T_1	S_4T_2	S_3T_2	S_2T_0	S_3T_0	S_4T_1	S_2T_2
BLOCK 4	S_4T_1	S_1T_1	S_4T_0	S_5T_2	S_1T_0	S_5T_1	S_2T_0	S_2T_2	S_3T_0	S_5T_0	S_3T_1	S_2T_1	S_1T_2	S_4T_2	S_3T_2

S = soil

S_1 = Loganlea

S_2 = North Addiewell

S_3 = Baads (chicken manure)

S_4 = Baads (reclaimed)

S_5 = Meikle Earnock

T = treatment

	N	P	K
T_0 =	100	0	50
T_1 =	100	50	50
T_2 =	100	100	50

Ryegrass (Lolium Perenne) seed was sown at the rate of 0.5 mg/pot. After seed sowing and fertilizer application, the pots were placed in saucers and set up on a greenhouse bench and wetted to saturation with tap water. Since water requirements varied with different soils and temperature fluctuations, there was no uniform watering schedule, but all pots were watered early and late every day. Frequent checks were made to ensure that free water was not absent from the saucers for more than a few hours during the day.

Yield was measured by cutting the grass just above the soil surface nine weeks after sowing. The total tops (leaf and stubble) were placed in sealed polythene bags. Roots were removed from soil and were washed thoroughly with deionized water to remove adhering soil material (Hargreove et al., 1985). Total tops and roots were dried separately in an oven at 80°C for 48 hours. After drying, dry matter yields of both tops and roots were recorded.

Roots and tops were ground with an electrical grinder and stored in labelled bottles for further analysis.

Soil from each pot was collected in polythene bags and stored in a cold room at 8°C for further analysis. Soil was analysed for extractable phosphorus, pH and adsorption isotherm (Section 4.2.3). Langmuir maxima were calculated from adsorption plots (Section 3.2.2).

4.2.3 Analytical procedures

pH and moisture in soil samples were measured using methods in Section 2.1.1 and 2.1.2 respectively. Phosphorus was extracted with 0.5 M acetic acid solution by following the method given in Section 3. The soil extract was analysed for phosphorus by a colorimetric method using a Technicon AutoAnalyzer II (see Section 2.2.1.2). Acid phosphatase activities and phosphorus adsorption maxima were determined by the methods described in Section 2.1.7 and 2.1.8 respectively. Plant material was extracted by the wet pressure digestion method (Section 2.1.9), and plant digest was analysed for phosphorus content at 880 nm based on a Technicon AutoAnalyzer method (Section 2.2.1.2) with some modifications (Section 2.1.9).

4.3 Results and discussion

The effect of different levels of phosphatic fertilizer on the mean yield (g/pot) of plant material, as roots and tops are presented in Table 4.2 and 4.3 respectively. Figures shown in a row with the same letter are not significantly different at the 5 percent level using a Scheffe LSD test. The results in Table 4.2 indicate that in the Loganlea and Miekle Earnock soils, root yield was significantly greater than control, at both levels of applied phosphorus, but no significant difference was found between the two levels of applied phosphorus. In North Addiewell, Baads (chicken manure) and Baads (reclaimed), no significant increase in root yield was found at either level of applied phosphorus.

The effect of applied fertilizer on tops yield (leaf and stubble) shown in Table 4.3, was similar to the effect on root yield, in the case of Loganlea, North Addiewell and both Baads soils. In Meikle Earnock soil, yield of tops was significantly greater than control, at both levels of applied phosphorus, and a significant difference was found between the two levels.

Table 4.4 shows the mean total yield, which ranged from 2.08 g/pot (control) in Loganlea soil to 5.32 g/pot in North Addiewell soil (50 kg P ha^{-1}). The results in Table 4.4 further indicate that in Loganlea soil phosphorus application at both levels significantly increased the total yield, with a significant difference found between the two levels. No response to applied phosphorus was observed in the case of North Addiewell and Baads (chicken manure) soils. In the case of Baads (reclaimed), the low level of applied phosphorus increased yield significantly over both control and high level, with no significant difference found between control and high level. In Meikle Earnock soil, both the levels of applied phosphorus significantly increased the total yield, with no significant difference between the two levels.

Table 4.2 Effect of different rates of phosphorus fertilizer on roots yield (g/pot)

Sample Location	Fertilizer rate (kg P ha^{-1}) 0	50	100
	Root yield (g/pot)		
Loganlea	0.74 a*	1.62 b	1.98 b
North Addiewell	1.98 a	2.56 a	2.37 a
Baads (chicken manure)	2.09 a	2.18 a	2.15 a
Baads (reclaimed bing)	1.84 a	1.97 a	1.74 a
Meikle Earnock	0.74 a	1.48 b	1.34 b

* Figures in a row with the same letter are not significantly different at 5% level using a Scheffe LSD test.

Table 4.3 Effect of different rates of phosphorus fertilizer on tops (leaf and stubble) yield (g/pot)

Sample Location	Fertilizer rate (kg P ha^{-1}) 0	50	100
	Yield of tops (g/pot)		
Loganlea	1.34 a*	2.36 b	2.70 b
North Addiewell	2.43 a	2.76 a	2.78 a
Baads (chicken manure)	2.40 a	2.38 a	2.44 a
Baads (reclaimed bing)	1.93 a	2.20 a	1.94 a
Meikle Earnock	1.50 a	2.22 b	2.43 c

* Figures in a row with the same letter are not significantly different at 5% level using a Scheffe LSD test.

Table 4.4 Effect of different rates of phosphorus fertilizer on total yield (g/pot)

Sample Location	Fertilizer rate (kg P ha^{-1}) 0	50	100
	Total yield (g/pot)		
Loganlea	2.08 a*	3.98 b	4.68 c
North Addiewell	4.42 a	5.32 a	5.15 a
Baads (chicken manure)	4.50 a	4.55 a	4.60 a
Baads (reclaimed bing)	3.74 a	4.20 b	3.70 a
Meikle Earnock	2.24 a	3.70 b	3.80 b

* Figures in a row with the same letter are not significantly different at 5% level using a Scheffe LSD test.

The effect of different rates of phosphatic fertilizer on root and leaf phosphorus content, expressed as mg P kg^{-1}, are shown in Tables 4.5 and 4.6 respectively. Phosphorus fertilization increased the root phosphorus content at both levels of applied phosphorus in the case of Loganlea and Meikle Earnock soils, with significant differences also between the two levels. Neither phosphorus level significantly affected the root phosphorus content in the case of Baads (chicken manure) soil. In the case of North Addiewell and Baads (reclaimed) soils, no significant effect due to the low level of applied phosphorus was found. However, phosphorus applied at the high level significantly increased root phosphorus control over control in the case of North Addiewell, and over both control and low level, in Baads (reclaimed) soils.

The results in Table 4.6 show that phosphorus fertilization increased the leaf phosphorus content at both levels of applied phosphorus in Loganlea, North Addiewell and Meikle Earnock soils, with significant differences also found between the two levels. In the case of Baads (chicken manure), no significant difference due to any level of applied phosphorus was found. Both levels of applied phosphorus increased leaf phosphorus content significantly over control, but no significant difference was found between the two levels in the case of Baads (reclaimed) soil.

Table 4.5 Effect of different rates of phosphorus fertilizer content of root (mg P kg^{-1})

Sample Location	Fertilizer rate (kg P ha^{-1}) 0	 50	 100
	P content of roots mg P kg^{-1}		
Loganlea	219 a*	482 b	741 c
North Addiewell	574 a	743 ab	832 b
Baads (chicken manure)	742 a	700 a	714 a
Baads (reclaimed bing)	660 a	651 a	790 b
Meikle Earnock	173 a	596 b	749 c

* Figures in a row with the same letter are not significantly different at 5% level using a Scheffe LSD test.

Table 4.6 Effect of different rates of phosphorus fertilizer content of leaf (mg P kg^{-1})

Sample Location	Fertilizer rate (kg ha^{-1}) 0	50	100
	P content of leaves mg P kg^{-1}		
Loganlea	265 a*	551 b	1259 c
North Addiewell	501 a	1586 ab	1382 c
Baads (chicken manure)	1469 a	1586 a	1720 a
Baads (reclaimed bing)	1105 a	1461 b	1531 b
Meikle Earnock	259 a	986 b	1511 c

* Figures in a row with the same letter are not significantly different at 5% level using a Scheffe LSD test.

Changes in soil pH, extractable phosphorus in soil, and adsorption maximum, due to phosphorus fertilization, along with their initial values, are presented in Tables 4.7, 4.8 and 4.9 respectively.

The changes in soil pH, as is clear from the results in Table 4.7, were negligible due to phosphorus fertilization. The pH was slightly decreased in Loganlea, North Addiewell, Baads (chicken manure) and Meikle Earnock soils as compared to initial values.

Comparing the results of native phosphorus with phosphorus extracted after harvesting in the control pots (no phosphorus), no significant changes were found in Loganlea, Baads (reclaimed) and Meikle Earnock soils. However, a significant increase in phosphorus level was found in North Addiewell, and significant decline occurred in Baads (chicken manure) soil, after harvesting. Studying the effect of applied phosphorus level on extractable phosphorus, both levels caused the extractable phosphorus to increase significantly over the cropped control, with significant difference found between the two levels, in all soils, except North Addiewell. In North Addiewell soil, both the applied phosphorus levels significantly increased extractable phosphorus, over the cropped control, but no significant difference was found between the two levels.

Phosphorus adsorption maxima calculated from Langmuir plots, before and after the experiment, are presented in Table 4.9.

Table 4.7 Soil pH before and after harvesting

Sample	Before	After treatment (kg P ha^{-1})		
Location	Treatment	0	50	100
Loganlea	5.6	5.4	5.4	5.4
North Addiewell	5.6	5.2	5.2	5.2
Baads (chicken manure)	5.4	5.1	5.1	5.1
Baads (reclaimed bing)	7.2	7.2	7.2	7.2
Meikle Earnock	7.8	7.6	7.6	7.6

Table 4.8 Extractable phosphorus before and after harvesting ($\mu g\ g^{-1}$)

Sample Location	Before Treatment	After treatment (kg P ha^{-1}) 0	50	100
Loganlea	0.4 a	0.6 a*	1.2 b	4.5 c
North Addiewell	9.1 a	43.2 b	53.6 c	56.9 c
Baads (chicken manure)	21.6 d	8.5 a	13.2 b	17.9 c
Baads (reclaimed bing)	5.3 a	6.3 a	13.6 b	19.3 c
Meikle Earnock	5.0 a	3.4 a	7.8 b	17.3 c

* Figures in a row with the same letter are not significantly different at 5% level using a Scheffe LSD test.

Table 4.9 Phosphorus adsorption maxima before and after grass growing, calculated from Langmuir plots (mg g^{-1})

Sample Location	Before Treatment	After treatment (kg P ha^{-1}) 0	50	100
Loganlea	0.54 b*	0.29 a	0.34 a	0.29 a
North Addiewell	0.54 b	0.27 a	0.30 a	0.38 a
Baads (chicken manure)	0.50 b	0.23 a	0.27 a	0.26 a
Baads (reclaimed bing)	0.65 b	0.46 a	0.55 ab	0.49 ab
Meikle Earnock	0.11 b	0.12 b	0.10 b	0.05 a

* Figures in a row with the same letter are not significantly different comparing the 5% level based on 95% confidence for the orders calculated as, $[\text{slope} \pm (2 \times \text{SD})]^{-1}$ where SD is standard deviation.

In Loganlea, North Addiewell and Baads (chicken manure), a clear distinction between adsorption maxima before and after grass growing was found, irrespective of phosphorus level. The distinction between adsorption maxima was not so clear in the other two soils. Generally a substantial decrease in adsorption maxima occurred after grass growing.

Total phosphorus yield calculated as both μg/pot and $\mu g\ g^{-1}$ soil is presented in Table 4.10 and 4.11 respectively. Total phosphorus yield in plant has been calculated as follows:

Total phosphorus yield (μg/pot)
= top phosphorus yield + root phosphorus yield

while top phosphorus yield
= top yield x top phosphorus concentration

and root phosphorus yield
= root yield x root phosphorus concentration

Table 4.10 Effect of different rates of phosphorus fertilizer on total phosphorus uptake by whole plant (μg/pot)

Sample	Fertilizer rate (kg P ha^{-1})		
Location	0	50	100
Loganlea	519	2071	4854
North Addiewell	2351	5144	5936
Baads (chicken manure)	5076	5305	5760
Baads (reclaimed bing)	3320	4522	4339
Meikle Earnock	575	3070	4695

Table 4.11 Effect of different rates of phosphorus fertilizer on phosphorus uptake by whole plant ($\mu g\ g^{-1}$ soil)

Sample Location	Fertilizer rate (kg P ha^{-1}) 0	50	100
Loganlea	1.3	5.2	12.1
North Addiewell	5.9	12.9	14.9
Baads (chicken manure)	12.7	13.3	14.4
Baads (reclaimed bing)	8.3	11.3	10.8
Meikle Earnock	1.3	7.6	11.8

Phosphorus yield in $\mu g\ g^{-1}$ soil was calculated by dividing the total phosphorus yield in μg/pot by weight of soil in pot, which was 400 g. The phosphorus balance presented in Table 4.12 was calculated as net mineralization by subtracting the initial available phosphorus in each soil from the final phosphorus found in the soil and grass after harvesting. It was calculated as follows:-

$$\text{Initial available phosphorus} = P_q + P_a$$

where P_q = native soluble phosphorus extracted with acetic acid

P_a = added phosphorus

$$\text{Final phosphorus found} = P_g + P_f$$

where P_g = phosphorus recovered in grass

P_f = phosphorus extracted with acetic acid at the end of the pot experiment

Percentage phosphorus recovered was looked at in two ways. Firstly, changes in the total amount of phosphorus in the system (soil + grass + fertilizer) were considered. The data presented in Table 4.13 was calculated according to the formula (Lister, 1987) given below:

$$\text{Percent phosphorus recovered} = \frac{P_g + P_f}{P_{fc} + P_a + P_{gc}} \times 100$$

where P_{gc} = phosphorus recovered in grass in the zero phosphorus treatment for each soil

P_{fc} = acetic acid extractable phosphorus in soil for zero phosphorus treatments

The second calculation considered the difference in net gain or loss of phosphorus in fertilized and unfertilized soils (Table 4.12) and expressed that as a percentage of fertilizer phosphorus added:

$$\text{Percent fertilizer phosphorus lost} = \frac{\text{Net loss of phosphorus in fertilized soil} - \text{net loss of phosphorus in unfertilized soil}}{\text{fertilizer phosphorus added}} \times 100$$

This data are presented in Table 4.14.

The results in Table 4.12 indicate that most of these soils, except North Addiewell and Baads (reclaimed) showed loss in applied phosphorus. In both Loganlea and Baads (chicken manure) the loss of phosphorus was significantly greater at both levels of applied phosphorus, compared to the control. There was also a significant difference between the two levels.

Table 4.12 Effect of applied phosphorus on net mineralization

Sample location	Native extractable phosphorus $\mu g\ g^{-1}$	pH_i	Langmuir adsorption maximum $mg\ g^{-1}$	Acid phosphatase $\mu moles\ Pnp\ g^{-1}\ h^{-1}$	Net mineralized phosphorus ($\mu g\ g^{-1}$) Fertilizer rate (kg P ha^{-1}) 0	50	100
Loganlea	0.4	5.6	0.55	9.5	1.5 a*	-6.5 b	-8.7 c
North Addiewell	9.1	5.6	0.55	9.9	40.0 a	44.8 a	37.6 a
Baads (chicken manure)	21.6	5.4	0.51	2.9	-0.4 a	-7.6 b	-14.3 c
Baads (reclaimed)	5.3	7.2	0.66	2.8	9.3 b	7.1 b	-0.2 a
Meikle Earnock	5.0	7.8	0.12	0.2	-0.3 a	-2.0 a	-1.0 a

* Figures in a row with the same letter are not significantly different at 5 percent level, using a Scheffe LSD test.

In North Addiewell soil, where substantial amounts of phosphorus was mineralized, neither level of applied phosphorus had any marked effect. In Baads (reclaimed) soil, there was net mineralization in control and low level of applied phosphorus. However,significant phosphorus loss was observed at high level of applied phosphorus. No significant changes were found in phosphorus losses, due to either level of applied phosphorus in the case of Meikle Earnock soil.

Since no loss occurs through leaching, as each pot stood in a separate saucer and watering was controlled so that no percolating water escaped, any unrecovered phosphorus must be fixed in a form which is not extractable by acetic acid, ie. adsorbed (eg. on amorphous oxides), and is not taken up by the plant after 63 days. Percent phosphorus recovered is presented in Table 4.13. These results show that the addition of higher rates (100 kg ha^{-1}) of phosphorus gave a lower recovery in North Addiewell and both Baads soils, compared to the lower (50 kg ha^{-1}) rate. In the case of Loganlea soil, the lower rate of phosphorus applied gives a lower recovery as compared to the higher rate of applied phosphorus. Percent phosphorus recovery remained unchanged in the case of Meikle Earnock soil at both levels of applied phosphorus. Lister (1987) and McConaghy and Stewart (1963) reported that the addition of higher rates (40 kg ha^{-1}) of phosphorus in general gives a lower recovery compared to the lower rate (20 kg ha^{-1}) they used.

Table 4.13 Effect of phosphorus fertilizer rates on percent phosphorus recovery

Sample Location	Fertilizer rate (kg P ha^{-1}) 50	 100
	% P recovered	
Loganlea	44	62
North Addiewell	108	97
Baads (chicken manure)	78	70
Baads (reclaimed bing)	93	76
Meikle Earnock	90	98

The percent loss in fertilizer phosphorus has been presented in Table 4.14. The results indicate a substantial amount of loss of phosphorus in the case of Loganlea and Baads (chicken manure) soils at both levels of applied phosphorus. The loss of phosphorus was very low in the case of Baads (reclaimed) soil at low levels of applied phosphorus, while in the case of Meikle Earnock, the loss of phosphorus was low at both levels of applied phosphorus. In the case of North Addiewell soil, due to substantial amount of phosphorus mineralization, there was a net gain in phosphorus at low levels of applied phosphorus, though a significant amount of phosphorus was adsorbed (Table 4.9). However, a small amount of phosphorus loss was found at high levels of applied phosphorus. The great loss of phosphorus in Loganlea and Baads (chicken manure) soils might be due to significant amounts of phosphorus adsorbed in these soils, as shown by the decrease in adsorption maxima (Table 4.9).

From the preceding results and discussion, it can be seen that in Loganlea soil (acidic with low extractable phosphorus), following fertilizer application, there was a significant increase in the level of extractable phosphorus, with a subsequent increase in plant phosphorus and crop yield. At the same time, a significant loss of phosphorus also occurred.

Table 4.14 Percent fertilizer phosphorus lost

Sample Location	Fertilizer rate ($kg\ P\ ha^{-1}$) 50	100
	% P lost	
Loganlea	64	41
North Addiewell	-38	10
Baads (chicken manure)	58	56
Baads (reclaimed bing)	18	38
Meikle Earnock	13	3

In Baads (chicken manure) soils (acidic with high extractable phosphorus) following fertilizer application, much of the phosphorus was quickly lost. Phosphorus applied did not enhance plant growth, though it increased extractable phosphorus significantly.

In North Addiewell soil (acidic with medium extractable phosphorus), applied phosphorus caused the extractable phosphorus to increase, but this did not induce any significant increase in crop yield. Though a great amount of phosphorus was mineralized, applied phosphorus had no significant effect on net mineralization.

On the Baads (reclaimed) soil (neutral with low extractable phosphorus), low levels of applied phosphorus not only increased both extractable phosphorus and yield significantly over the control, but the net loss of phosphorus was also lower at this level.

In Meikle Earnock (alkaline with low extractable phosphorus), both the levels of applied phosphorus increased both extractable phosphorus and yield significantly over the control. The percent phosphorus loss was also low at both levels of applied phosphorus.

To sum up the results, Loganlea soil seemed to be severely deficient in available phosphorus. Lolium perenne benefited from the addition of phosphorus, yield increasing with higher phosphorus fertilization, up to and including a rate of 100 kg ha^{-1}. A greater yield might be possible if higher application rates of phosphorus were applied. However, there is a danger that such increases in phosphorus addition, for the purpose of enhancing plant growth, can cause substantial phosphorus losses, as the present study indicates. Therefore it would be more justifiable to adopt some management practices to minimize such losses.

In the case of North Addiewell soil, the high phosphorus mineralization, and percent phosphorus recovery, together with no response due to applied phosphorus, indicate that this soil was not deficient in available phosphorus. The high amount of phosphorus mineralized might partly be due to high acid phosphatase activity in this soil, as compared to other soils.

Baads (chicken manure) was well supplied with available phosphorus, though it had very low acid phosphatase activity. The significant amount of phosphorus present might be due to mineralization of chicken manure, applied to this plot in 1980. This also indicates that chicken manure is a good way of increasing the phosphorus status of coal mine soil. Application of phosphorus fertilizer, for further enhancing growth, would not be economical on this soil, as it does not increase yield on one hand, and can induce great losses of fertilizer on the other hand.

In Baads (reclaimed) soil, application of phosphorus fertilizer at the rate of 50 kg ha^{-1} is considered to be economical and beneficial, as it increased the yield, and phosphorus losses were also small at this level of applied phosphorus.

In Meikle Earnock soil, one can recommend on the basis of the present study higher rates of phosphorus fertilizer, with the possibility of both getting high yield and phosphorus recovery. There is no apparent danger of phosphorus loss in this soil. This soil had very low acid phosphatase activity, together with adsorption maximum. The low adsorption maximum might be helpful in reducing phosphorus losses.

The correlation of plant phosphorus uptake and crop yield with various soil parameters is presented in Table 4.15. Most of the soil components measured, had poor correlation with phosphorus uptake and crop yield. Poor correlation between yield of ryegrass in pots, with acetic acid phosphorus was also reported by a large number of research workers (Mattingly and Pinkerton, 1961; Mattingly et al., 1963; Williams and Knight, 1963; Williams and Saunders, 1956). Mattingly and Pinkerton (1961) concluded that the limitation of acetic acid in the analysis of soils derived from different parent materials can be explained largely by the soil phosphorus dissolved, or by the new surfaces on the soil exposed, from which phosphate ions are not taken up by ryegrass; while Mattingly et al. (1963) reported the generally poor acetic acid crop uptake correlation is due to the fact that acetic acid attacks phosphorus sources not available to plants. The results of Williams and Knight (1963) support the conclusion of Mattingly et al. (1963), with the remarks that this is not the main reason for the poor correlation. According to their report, the major factor in acid soils is that the fairly low pH (5.0-6.4) and long period of extraction (2 hours) caused substantial attack on the largely unavailable acid soluble phosphorus. The full effect of any unavailable phosphorus involved should therefore be reflected in the relationship of total phosphorus involved in the extraction, but, depending on the phosphorus retention capacity of the soil, the effect on extractable phosphorus may be much smaller.

Table 4.15 * Correlation between crop yield and soil components

	Phosphorus uptake (μg/pot)	Yield (g/pot)
pH_a **	-0.34	-0.44
pH_b	-0.36	-0.43
Adsorption maximum (a)	0.40	0.35
Adsorption maximum (b)	0.49	0.51
Acid phospatase	-0.21	0.28
Native phosphorus (Pg)	0.86	0.76
Extractable phosphorus (Pf)	0.16	0.63

values > 0.80 significant at 10 percent
values > 0.88 significant at 5 percent
values > 0.96 significant at 1 percent

* Pearson product-moment correlation significant of correlation coefficient at 3 (N = 5) degree of freedom.

** a and b after and before pot experiment respectively.

CHAPTER 5

PHOSPHORUS TRANSFORMATIONS DURING INCUBATION OF COAL MINE SOILS

5.1 Introduction

From the results of the chemical and biological studies on some coal mine and oil shale soils, it was found (Chapter 3) that phosphorus turnover was either zero or negligible. The plant growth study in Chapter 4 indicated that the low turnover was due to phosphorus immobilization in these soils. Further study was undertaken to investigate these features in greater depth, with special reference to the effect of lime and incubation on phosphorus immobilization and mineralization.

Liming may affect phosphate availability and solubility through any of the following processes:

i) solubility product principles (formation of various phosphate compounds).

ii) adsorption on to surface of hydrated aluminium and iron oxides and clay minerals.

iii) mineralization and immobilization of organic phosphorus.

The beneficial effect of liming might be due to enhanced microbial activities and thus mineralization of soil organic phosphorus (Haynes, 1982). However, the extent of this transformation varies with soil conditions (McCall et al., 1956). Wild (1988) suggested that the beneficial effect of lime on phosphorus availability is due, at least in part, to the transformation of organic phosphorus to a mineral form, since liming increases the rate of decomposition of soil organic matter. A reason for the positive response of crop plants to liming of highly weathered soil (where aluminium is phytotoxic) is through the neutralization of soluble and exchangeable aluminium (Haynes, 1984). Moreover liming such soil is essential for the efficient use of soil solution phosphate, because the major effect of aluminium toxicity is the inhibition of the uptake and translocation of phosphate by plants (Haynes and Ludecke, 1981). The benefits of liming acid soil for agricultural purposes are attributed to alleviating toxicities of aluminium (Moschler et al., 1960) and manganese (Martini et al., 1974) and to increasing the availability of calcium (Schmehl et al., 1950), phosphorus (Janghorbani et al., 1975) and other nutrients.

There is a great deal of controversy surrounding the effect of liming on the phosphorus supplying power of highly weathered acid soils. When soils initially high in exchangeable aluminium are limed, the freshly precipitated amorphous polymeric hydroxy-aluminium cations can form a free oxide or coat the surface of colloidal particles and constitute a new highly active adsorbing surface. Thus liming up to a pH of around 6.5 often increases phosphate adsorption (Amarasiri and Olsen, 1973; Haynes and Ludecke, 1981). Phosphate adsorption by moist limed soil can be increased, decreased or unaffected, depending on:

a) the relative magnitude of surface charge; that is the charge on amphoteric soil surfaces which becomes more negative as the soil pH is raised.
b) precipitation of exchangeable aluminium (Haynes, 1984).

Plant uptake of phosphate in response to liming in highly weathered acid soils is often controlled by two opposing factors. Firstly, liming results in the alleviation of aluminium toxicity and therefore increases the potential for efficient use of available soil phosphate. Secondly, the precipitation of exchangeable aluminium as polymeric hydroxy-aluminium cations, following liming, creates new highly active phosphate adsorbing surfaces in soils. Thus, high lime rate may result in decreased phosphate uptake by plants due to adsorption of phosphate by the newly formed surfaces. Liming to neutralize all the initially exchangeable aluminium present may not, therefore, be desirable in terms of efficient use of fertilizer phosphate.

Conflicting views are held regarding the effect of liming on the phosphate extraction from highly weathered acid soils. It decreases or increases or may not affect the phosphate that can be extracted from such soils (Sumner, 1979) through a variety of mechanisms. The relative importance of each mechanism will depend upon individual soil properties, and on the application rate of lime and phosphate. There is ample evidence that liming can markedly decrease the extractability of applied phosphate from soils (Haynes and Ludecke, 1981). Adsorption of phosphate by the freshly precipitated aluminium and iron hydroxides, following liming, apparently explains the greater fixation of phosphate in limed soils (Haynes, 1982), although, the formation of insoluble calcium and aluminium phosphate can occur (White and Taylor, 1977).

Very little work has been done on the influence of incubation, on changes in extractable phosphate, resulting from the addition of both phosphate and lime. Winsor and Long (1963), found that acetic acid extractable phosphorus increased with the increase of liming, and levels of phosphate applied. Van diest (1963) concluded that the quantity of soil phosphate extracted was little affected by lime application. Changes in extractable phosphate resulting from the addition of lime, after 3 weeks of incubation, varied with soil type and soil extractant.

In view of the importance of phosphorus availability in the fertility of acidic derelict coal mine spoils (Pulford et al., 1984) and the uncertainty (Fitter, 1974) concerning the effects of liming on phosphate availability, in addition to that mentioned above, the present study was undertaken. In this study, changes in acetic acid and ammonium fluoride extractable phosphate, resulting from the addition of both lime and phosphate, were investigated. The effects of incubation ranging from 0 to 148 days were also considered.

5.2 Materials and methods

5.2.1 Soil sampling sites

Two soil samples, one each from colliery spoil at Baads and Loganlea were collected from 0-15 cm depth. The choice of these sites was based on extensive background research work carried out and discussed in Section 2.3.

Baads (chicken manure plot)

The soil sample was collected from a plot, which received 25 t ha^{-1} of lime and 4 t ha^{-1} of chicken manure during 1980 and was well vegetated (Section 2.3).

Loganlea

This sample was collected from a vegetated site reclaimed in 1983 (Section 2.3).

The samples were air-dried only just enough to permit sieving (4 mm) and for subsequent addition of amendment solutions to be made without exceeding the desired moisture content (-0.5 bar soil moisture potential) for incubation. After sieving, the moist samples were stored in tied plastic bags at 2°C until used.

Prior to incubation, the samples were tested for 0.5 bar water, pH and lime requirement (Section 2.1). These characteristics are given in Table 5.1.

Table 5.1 Soil pH and lime requirement of the soils at Baads and Loganlea

Soil location	pH	Lime requirement $g\ kg^{-1}$	Percent water (-0.5 bar)
Baads (chicken manure)	3.5	4.40	23.4
Loganlea	5.5	1.32	30.5

5.2.2 Liming rates

From the buffer curve (Section 2.2.5), one level of lime was calculated for each soil. The addition of lime as $CaCO_3$ was made to raise the pH to 6.5. Baads soil had a higher lime requirement (Table 5.1) than Loganlea, which might be due to greater buffering capacity and lower pH.

5.2.3 Experimental procedure

Eighteen 100 g samples (oven-dry equivalent) of each coal mine soil in the fresh condition were weighed into wide mouth 8 oz bottles. The following treatments were applied to triplicate samples of each soil:

1: no addition of lime or phosphorus

2: no addition of lime, 50 mg P/kg soil

3: no addition of lime, 100 mg P/kg soil

4: lime to pH 6.5, no phosphorus

5: lime to pH 6.5, 50 mg P/kg soil

6: lime to pH 6.5, 100 mg P/kg soil

In the case of phosphorus treatments, 1 or 2 cm^3 of a stock solution containing 5000 mg P l^{-1}, as KH_2PO_4, was applied to soil in each bottle. This is equivalent to 50 or 100 mg P kg^{-1} soil.

Lime treated coal mine soils were prepared by mixing 0.132 g $CaCO_3$ in the case of Loganlea and 0.442 g $CaCO_3$ in the case of Baads with 100 g soil.

The required amount of deionized water was added for the desired moisture content. The contents in the bottles were then thoroughly mixed.

A 16 litre plastic bin lined with water soaked filter paper was used as an incubation chamber for a set of twelve bottles. One replicate of each treatment was placed in each of 3 bins. The bottles containing soils were left open to prevent anaerobic conditions developing. The bins were closed (to prevent evaporation) and stored in a growth room maintained at 25^{o}C. The bottles were weighed before each sampling time and deionized water was added to compensate for moisture loss. This was thoroughly mixed in before removing the samples. In the case of long intervals between sampling dates, the plastic bins were opened each week and the samples allowed to aerate for about 10 minutes. They were then weighed and brought to the required moisture content, if needed, before closing the bin.

Phosphate, extractable in acetic acid and ammonium fluoride, was measured at 0, 3, 7, 28, 58, 88 and 148 days of incubation. The pH of the incubated soil samples was also determined at the end of the experiment.

5.2.4 Analytical methods

Measurements of pH were made with a soil deionized water ratio of 1:2.5 using the method given in Section 2.1.

Phosphate was extracted by shaking 2.5 g of fresh soil with 50 cm^3 of 0.5 M acetic acid (Section 3.1.3) or 0.5 M ammonium fluoride solution for 30 minutes at 20°C. The suspensions were filtered using Whatman filter paper no. 42. The filtrates were analysed by colorimetric methods using a Technicon Autoanalyser II (Section 2.2.1.2). In the case of ammonium fluoride (Section 2.2.1.6), 4 percent boric acid was used to eliminate interference due to fluoride ions.

5.3 Results and discussion

Changes in the acetic acid and ammonium fluoride extractable soil phosphate of both treated and untreated soils are shown in Figures 5.1 to 5.8, for both Loganlea and Baads soils.

In the case of untreated (no phosphorus and no lime) soils, both soils showed a constant level of extractable soil phosphate in each extractant, irrespective of incubation time (Figures 5.1, 5.3, 5.5 and 5.7). The constant level of phosphate was continued up to 148 days, indicating no net mineralization of organic phosphate, or immobilization of inorganic phosphate by chemical or biological processes in these soils at any stage of incubation.

So far as phosphate transformations in treated coal mine soils were concerned, the two levels of added phosphate without lime behaved similarly in both soils. There was a gradual decline of ammonium fluoride extractable phosphate which was continued up to 148 days (Figures 5.1 and 5.3). In the case of acetic acid extractable phosphorus, after a rapid initial decline in the first 30 days (Figures 5.5 and 5.7), there was a constant level of phosphate up to 148 days indicating no further changes in the availability of added phosphate.

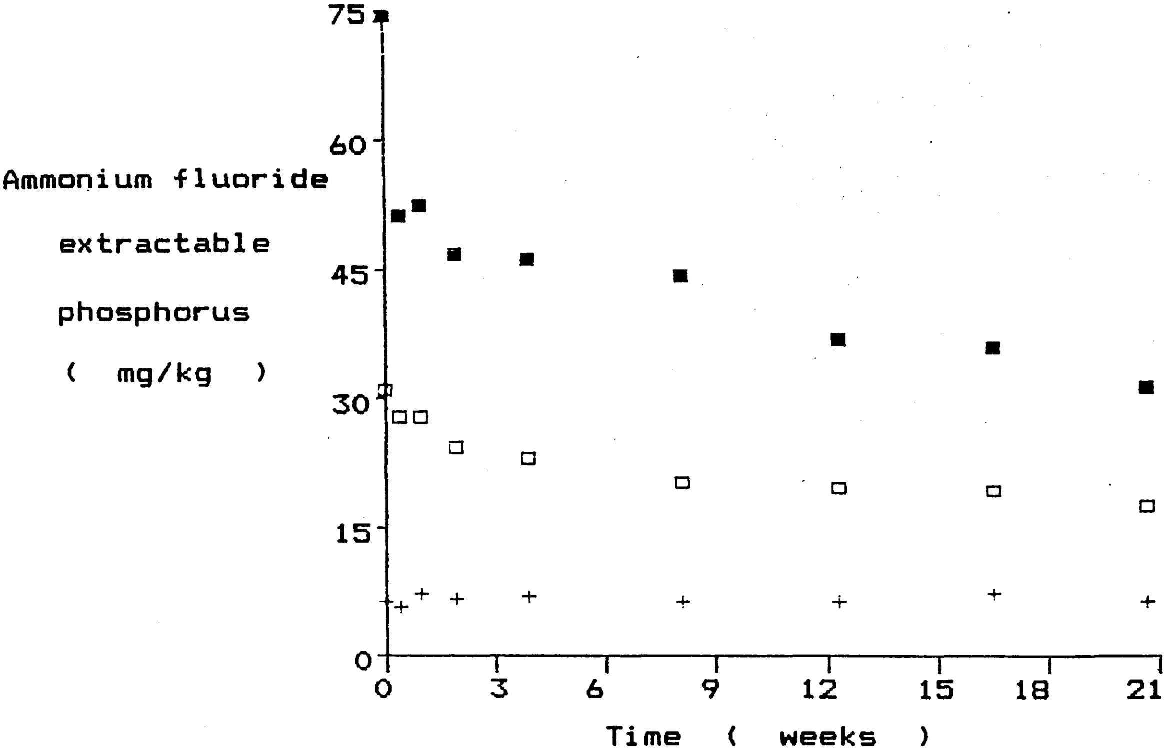


Fig. 5.1 Changes in ammonium fluoride extractable phosphorus in unlimed Loganlea soil.
no P (+), low P (□), high P (■)

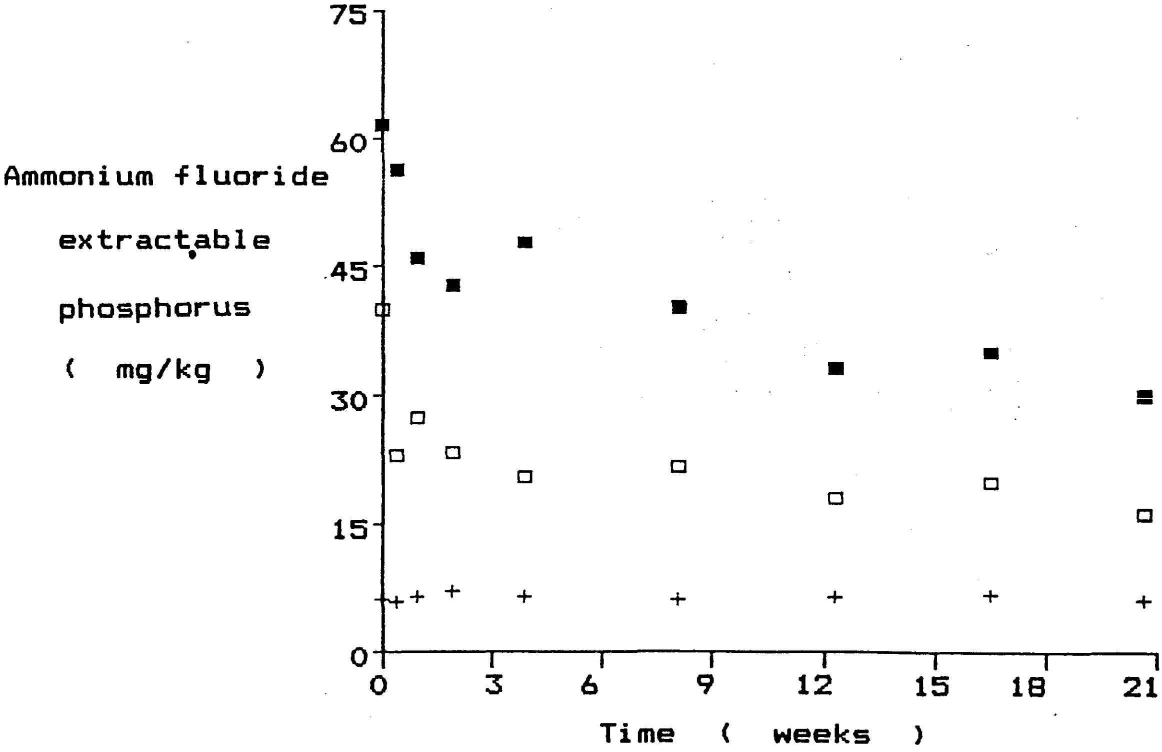


Fig. 5.2 Changes in ammonium fluoride extractable phosphorus in limed Loganlea soil.
no P (+), low P (□), high P (■)

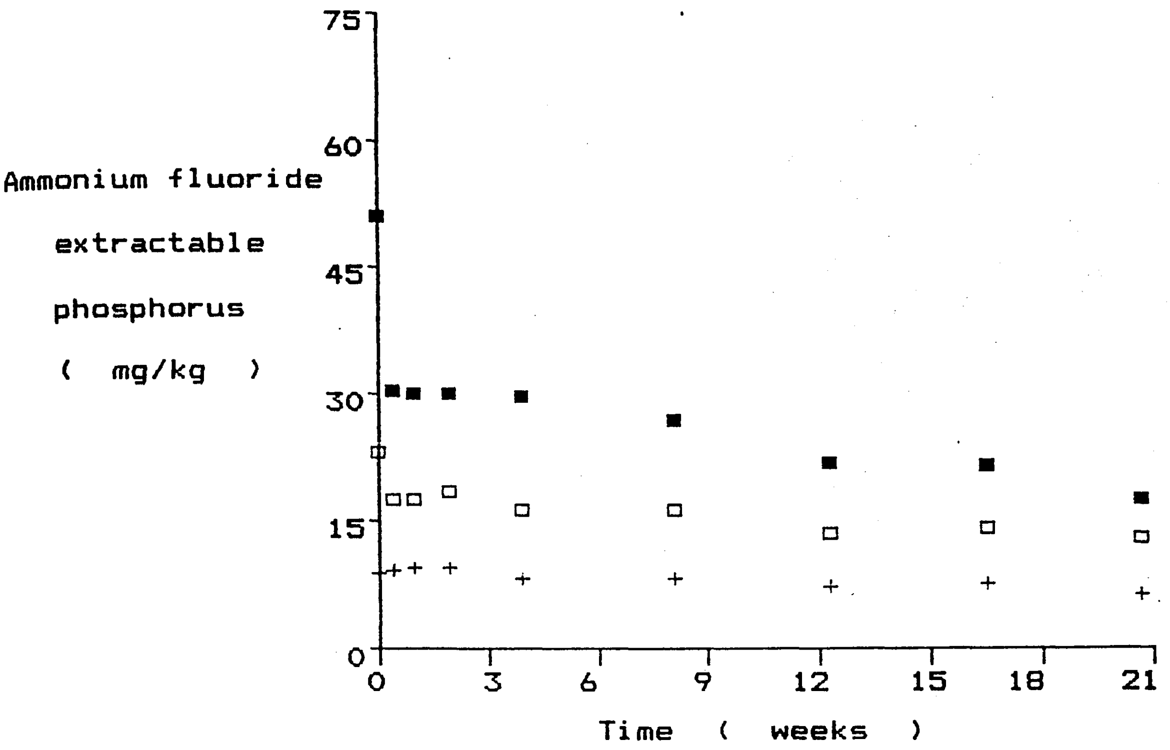


Fig. 5.3 Changes in ammonium fluoride extractable phosphorus in unlimed Baads soil.

no P (+), low P (□), high P (■)

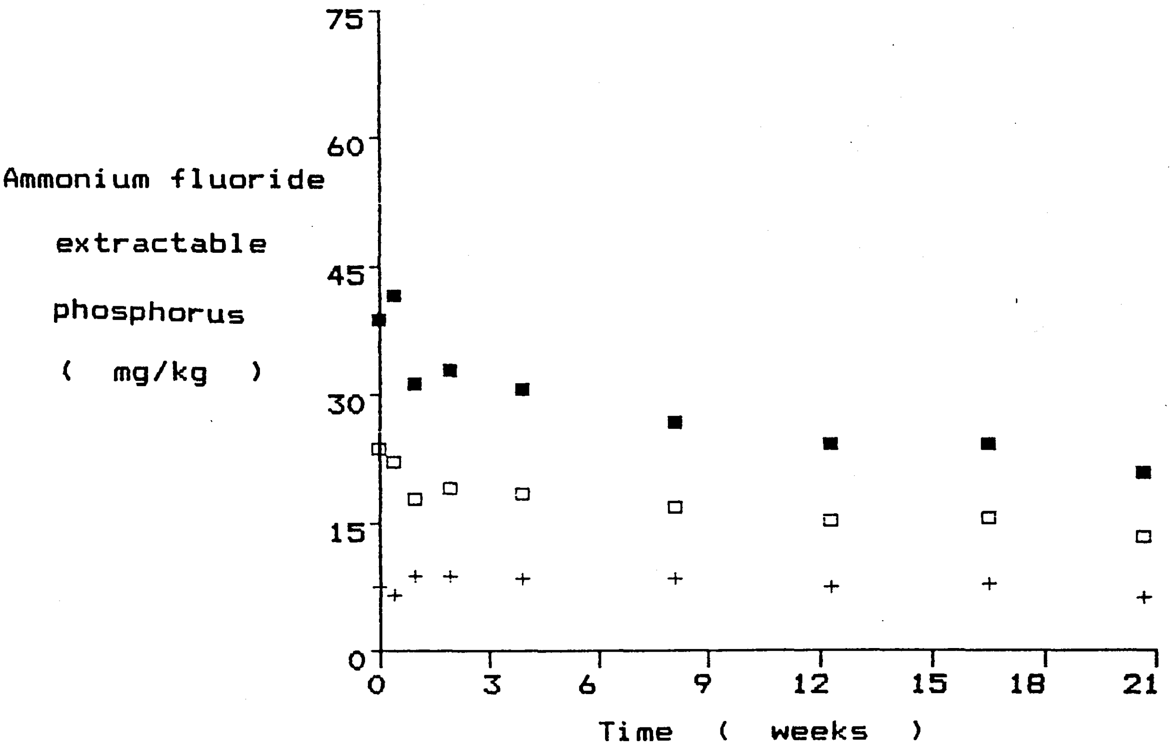


Fig. 5.4 Changes in ammonium fluoride extractable phosphorus in limed Baads soil.

no P (+), low P (□), high P (■)

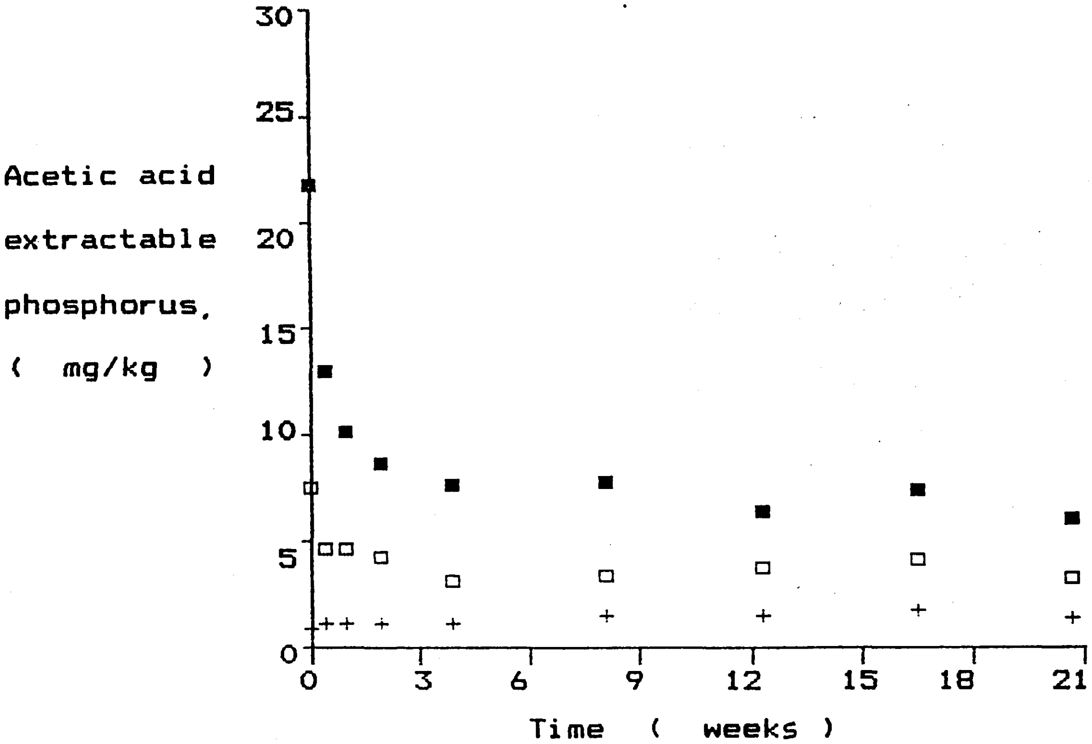


Fig. 5.5 Changes in acetic acid extractable phosphorus in unlimed Loganlea soil.
no P (+), low P (□), high P (■)

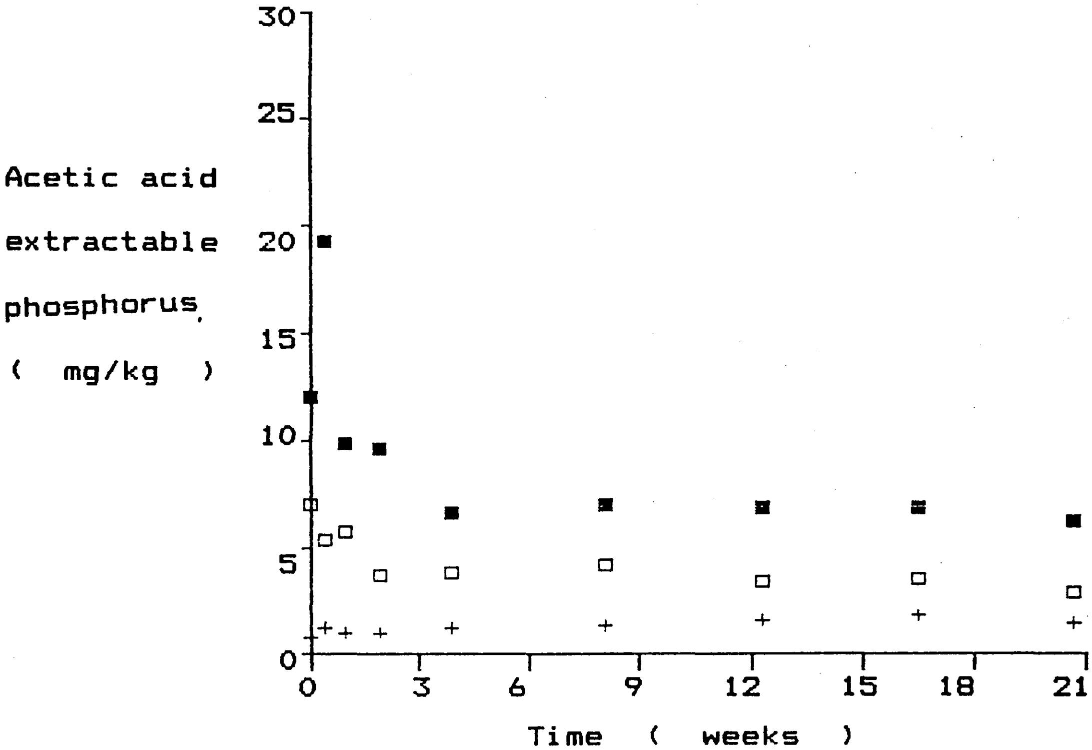


Fig. 5.6 Changes in acetic acid extractable phosphorus in limed Loganlea soil.
no P (+), low P (□), high P (■)

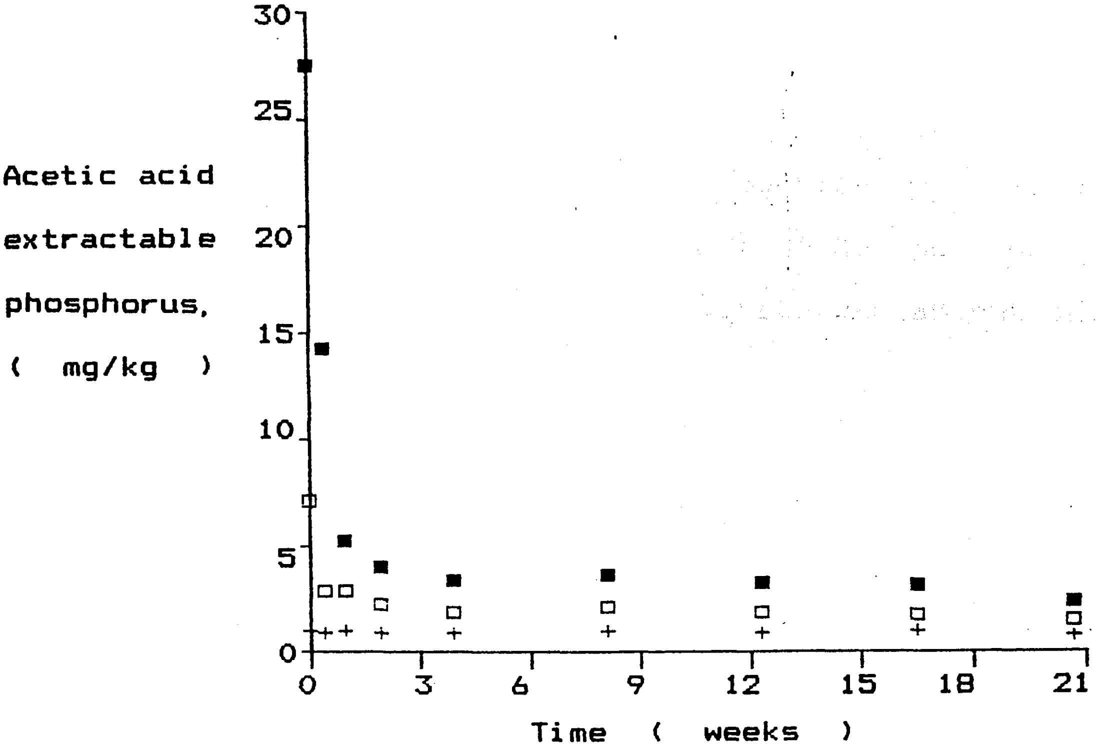


Fig. 5.7 Changes in acetic acid extractable phosphorus in unlimed Baads soil.

no P (+), low P (□), high P (■)

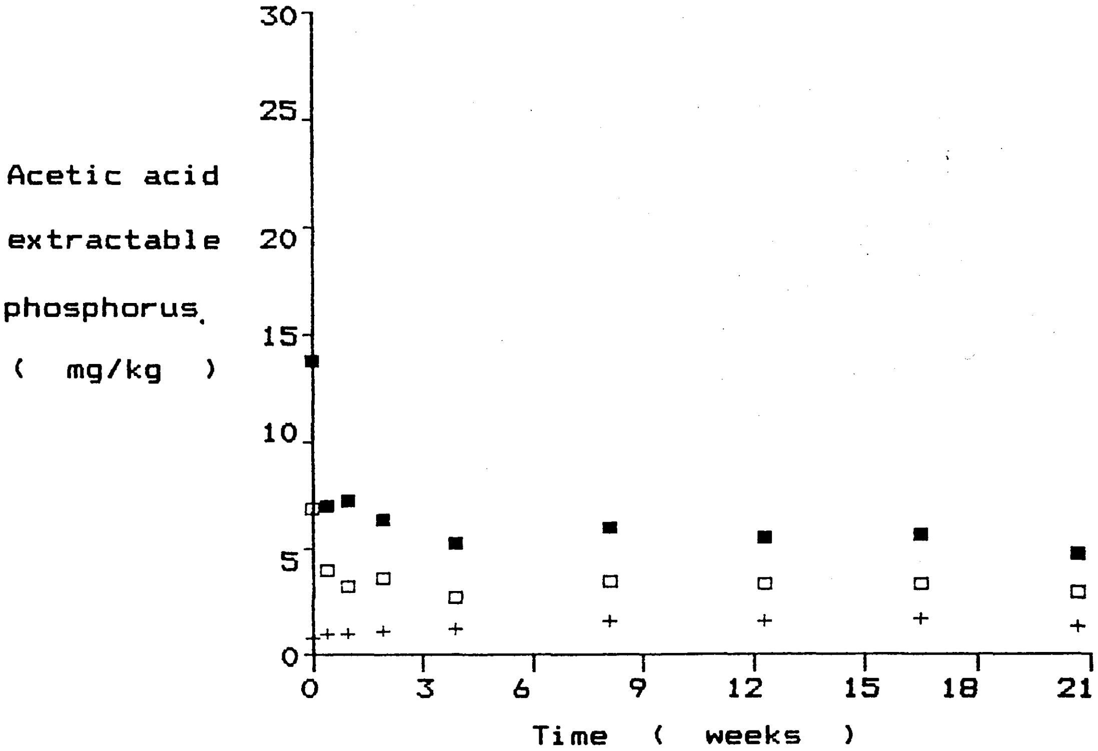


Fig. 5.8 Changes in acetic acid extractable phosphorus in limed Baads soil.

no P (+), low P (□), high P (■)

The effect of lime on phosphorus transformation over time has been presented in Figures 5.2, 5.4, 5.6 and 5.8. The figures indicate that, in Loganlea soil, lime had no effect on the transformation of either pool of extractable phosphate both when phosphate was absent and when it was added. Similarly, lime induced no changes in ammonium fluoride extractable phosphate in the case of Baads soil with phosphate absent or present. However, liming markedly increased the acetic acid extractable soil phosphate in the case of Baads soil both when phosphate was absent and present.

To look for lime, phosphate and time main effects, and the appropriate interactions, a repeated measures (over time) analysis of variance was carried out on the data. As the variance of the data increased with the level of extractable phosphate, the log of extractable phosphate was used to reduce this effect. The two soils and the two extractants were treated independently as four experiments. Each experiment was a 2 by 3 factorial (lime x phosphate) across time (9 repeated measurements). The conclusions drawn from this analysis have been presented in Table 5.2.

In Loganlea soil, both extractants showed significant effects ($P<0.001$) of time and phosphate as main effects, and a time x phosphate interaction as well. No significant effect of lime was found.

Table 5.2 Effect of lime, phosphate, time and their interaction on extractable phosphate (logged data)+

Effects	Locality			
	Loganlea		Baads	
	Ammonium fluoride	Acetic acid	Ammonium fluoride	Acetic acid
Lime				***
Phosphate	***	***	***	***
Lime x phosphate interaction				
Time	***	***	***	***
Time x lime interaction			*	***
Time x phosphate interaction	***	***	***	***
Time x lime x phosphate interaction			*	

* denotes $P<0.05$
** denotes $P<0.01$
*** denotes $P<0.001$

\+ all P-values calculated used Greenhouse-Geisser correction

For Baads soil, ammonium fluoride extractable phosphate showed significant effects ($P<0.001$) of time and phosphate as the main effects, and time x phosphate interaction as well. Some evidence of a time x lime and time x lime x phosphate interaction ($P<0.05$) was also found. No significant main effect of lime was found. In the case of acetic acid extractable phosphate, both time and phosphate showed these effects as main effects ($P<0.001$) as well as lime, time x lime and time x phosphate interactions. This was the only case where a clear cut effect of lime was found.

The phosphate extracted at the end of the incubation seemed to be more representative of the effect of lime, phosphate and lime-phosphate interaction over time. Therefore a separate analysis of variance (Table 5.3) was carried out to see the effect of lime, phosphate and their interaction on extractable phosphate. The two soils and the two extractants were again treated independently as four experiments . Each experiment was treated as 2x3 factorial (lime x phosphate), on the extractable phosphate level at the final sampling date.

The results in Table 5.3 indicated again that both the extractants showed main effects of phosphate in both soils. The effect of lime and lime x phosphate interaction were significant only in the case of acetic acid extractable phosphate in Baads soil.

Table 5.3 <u>Effect of lime and phosphate and their interaction on the extractable phosphate at day 148</u>

Effects	Loganlea		Baads	
	Ammonium fluoride	Acetic acid	Ammonium fluoride	Acetic acid
Lime	-	-	-	***
Phosphate	***	***	***	***
Lime/phosphate interaction	-	-	-	**

Locality

* denotes $P<0.05$
** denotes $P<0.01$
*** denotes $P<0.001$

The results obtained in this investigation support the contention that the quantity of soil phosphorus extracted was little affected by lime application (Martini et al., 1974). For example, lime addition caused no changes in both acetic acid and ammonium fluoride extractable phosphate in Loganlea soil and ammonium fluoride extractable phosphate in Baads soils for both no added phosphate and when it has been added. However, the effect of lime was different in Baads soils where acetic acid extractable phosphate was increased significantly by liming. This may result from different mechanisms of phosphorus extraction by different extractants under the same set of conditions. Van diest (1963) reported that changes in extractable phosphate resulting from addition of lime, after 3 weeks of incubation, varied with soil type and extractant.

The effects of liming on the availability of soil phosphorus has been widely investigated by many workers, but the results reported are not always consistent. The presence of phyto-toxic levels of soluble and exchangeable aluminium usually complicate the effect of liming on phosphate availability (Haynes, 1982). Haynes (1984) reported that liming would tend to increase phosphate adsorption in soils initially high in exchangeable aluminium but decrease it where soils initially contain low levels of exchangeable aluminium. Since the mineralization-immobilization balance depends to a large extent on the relative sizes of the easily mineralizable pools of carbon and phosphorus in soils (Chauhan et al., 1981), lime may not stimulate net mineralization of phosphorus, as these soils contain generally low available phosphorus. Significant effect of added phosphate suggests that additions of lime may not be necessary in all cases. A more important factor is the supply of macronutrients (Pulford et al., 1984).

The results obtained in the case of acetic acid extractable phosphorus in Baads soil, however, partly support the contention that part of the beneficial effect of lime on phosphorus availability is due to mineralization of organic phosphorus (Halstead et al., 1963).

The effect of lime on soil pH is presented in Table 5.4. The results indicate that the pH, which was raised to 6.5 by lime application, dropped to its original or near original pH values, in both the soils, after incubation. However, the drop in the pH was greater in the case of Baads soil, as compared to Loganlea soil. It can explain partly the beneficial effect of liming on acetic acid extractable phosphorus in Baads soil.

Generally, liming did not induce any significant changes in the soil pH, which may explain up to some extent the nonsignificant effect of lime on phosphorus mineralization. The fall in pH indicates that there is still a problem of acid regeneration in these soils. A problem of acid regeneration arises when there is any pyrite present, as it can be a source of potential acidity

Table 5.4 <u>Effect of lime on soil pH after 148 days incubation</u>

	Loganlea		Baads	
Treatment	pH_b^*	pH_a^*	pH_b	pH_a
L_0P_0	5.5	5.3	3.5	3.4
L_0P_1	5.5	5.3	3.5	3.4
L_0P_2	5.5	5.3	3.5	3.4
L_1P_0	5.5	5.7	3.5	4.2
L_1P_1	5.5	5.8	3.5	4.3
L_1P_2	5.5	5.7	3.5	4.4

pH_b: soil pH before lime application and incubation

pH_a: soil pH after lime application and incubation

(Walker, 1988). Pulford et al. (1976) has reported pyrite contents of up to 5% in spoils found in Central Scotland. When this is oxidised, one of the products is a colloidal amorphous ferric hydroxide which precipitates as a thin coat over the spoil mineral particles. The production of this coatings causes a correlation between falling pH and increasing phosphate adsorption (Walker, 1988) in the spoil. Most acid spoils fixed phosphate more rapidly than spoils with original pH values near neutrality. Therefore when a lime requirement is calculated, the source of potential acidity must be taken into account. Lime should be applied at the rate which not only counteract the existing acidity, but also to maintain pH if regeneration occurs (Walker, 1988). Data reported by Evans and Kamprath (1970) showed that to reach neutrality in acid soils, liming should theoretically neutralize about 200% of the exchangeable acidity. According to Pulford et al. (1984), lime should be applied according to the total lime requirements, which is the sum of lime required for neutralizing both existing and potential acidity. Liming to bring soil pH to neutrality requires very high rates because of the high buffering capacity provided by the exchangeable aluminium present in acid soils (Martini et al., 1974). The lack of the beneficial effect of lime on phosphorus mineralization might be due to not considering the potential acidity, while assessing the lime requirement. Potential acidity is assessed by measuring the pyrite content, as theoretically 1% pyrite produces acid requiring 40 tonnes lime per hectare to neutralize (Pulford et al., 1984). The present study suggests that lime effect on phosphorus availability is greatly influenced by relative changes in soil pH.

CHAPTER 6

GENERAL CONCLUSIONS

The investigations carried out have been concerned with the study of some aspects of phosphorus cycling and its availability to plants in some coal mine and oil shale soils of Central Scotland.

6.1 Analytical methods

During the present investigation, in addition to routine published methods used as such, some methods were modified to meet specific problems encountered with coal mine soils.

The choice of wavelength, phototube and background colour correction were investigated and methods were developed for phosphorus analysis on a Technicon AutoAnalyzer. Measurement of phosphorus in sodium bicarbonate and ammonium fluoride extracts were also investigated and some modifications were made. Investigation on phosphorus extraction method, use of charcoal and effect of filter papers, storage and temperature on phosphorus determination in filtered solution were also carried out. Measurement at 880 nm gave a useful improvement in sensitivity over 650 nm, but this involved changing phototube as well as filters. A suitable combination of phototube and filter was decided upon by comparing the effect of different filters, phototubes and wavelengths. It was found that an 880 nm filter can be used effectively with high sensitivity with a CE 63 V phototube and either bulb (blue or yellow) (Table 2.3).

Coloured extracts affect colorimetric phosphate determination by a high background reading. Therefore various colour correction systems were tested and it was found that acid ammonium sulphate with ascorbic acid can be used for background colour correction. Comparing the absorbance of coloured extracts at different wavelengths, it was found that 880 nm is a more suitable wavelength for avoiding the need for background colour correction (Tables 2.4 and 2.5).

The production of carbon dioxide during the automated analysis of sodium bicarbonate extracts of soil disrupts the flow and therefore the samples must be neutralized. Treatment with 4 M sulphuric acid at 60°C gave a neutral, carbon dioxide-free sample using the manifold developed during the present study (Table 2.6). Since fluoride ions interfere in the colour development during phosphorus determination, a study was made to find out a suitable concentration of boric acid on the basis of phosphorus recovery. 4% boric acid can be used effectively to eliminate fluoride interference (Table 2.7). On the basis of these assessments, and in addition consideration of the effect of aerosol 22 concentration on the smoothness of flow, shape and smoothness of peak, a table of conditions for different extractants was drawn up

(Table 2.8).

Activated charcoal was used to eliminate possible interference of any soluble organic material during the phosphorus analysis. Since most of the commercially prepared charcoals are contaminated with phosphorus, they should be washed free of phosphorus, before being used as a decolorizer. Different batches of charcoal were washed with acetic acid and extracted with different extractants using a buncher funnel, chromotographic glass column and plastic column methods. Plastic column made from a plastic bottle, cut at both ends, was found to be more effective for the removal of phosphorus contamination, using 6 M hydrochloric acid as washing material (Figure 2.9, Tables 2.13-2.16).

Since impurities in filter papers and adsorption due to filter papers are two common problems affecting the final phosphorus concentration determined, it was necessary to investigate the possible effect of filter paper upon phosphorus determination in filtered solution. None of the filter papers available had any contamination in respect of phosphorus and can be used successfully for all types of experimental work dealing with phosphorus (Tables 2.17 and 2.18).

No specific recommendation for the storage of soil extracts is available, and it is possible that some determinations are more likely to be affected than others by storage prior to analysis. The container used for the storage may also cause phosphorus adsorption and desorption. The effect of time of storage, type of container and temperature on both phosphorus standard solutions and soil extracts was found to be very small and was within the limit of the Technicon AutoAnalyzer, so did not affect the measurement of phosphorus concentration in soil extracts (Tables 2.19-2.21).

The extraction of coal mine soils can be limited due to the large heterogenity which exists between the chemical properties in individual strata associated with coal beds, in addition to the presence of large amounts of iron and aluminium compounds, together with their acid environment. On the basis of different responses by the same soils to different temperatures and shaking periods, in respect of extractable phosphorus, and by comparison with other people's results, a short time of extraction under controlled conditions was considered to be better. Half an hour shaking at 20°C on an orbital shaker at 150 rpm was used as standardized procedure for the extraction of plant available phosphorus.

6.2 Chemical and biological transformations of phosphorus

A detailed study was carried out to study various chemical and biological transformations in the phosphorus cycle on 30 coal mine soil samples from Baads, Central Scotland, representing a wide range of properties. It is important to evaluate such properties of coal mine soils to determine which parts of the phosphorus cycle are functioning. The problem in these soils is not that there is no phosphorus, but that so little of the phosphorus present is available for plant uptake. The present study also confirmed that all types of extractable phosphorus (acetic acid, sodium bicarbonate and anion exchange resin) were very low in most of these soils. Sodium bicarbonate extracted more phosphate than both acetic acid and anion exchange resin, because the former extractant extracts phosphate from soil solution, physisorbed, chemisorbed and organic pools together with a small amount of microbial phosphate (Table 3.1).

The rate of carbon dioxide evolution was high compared to the very low rate of phosphorus turnover. Only 40% of the sites studied showed phosphorus turnover in the case of acetic acid extractable phosphorus and 70% in the case of sodium bicarbonate extractable phosphorus. The rest of the sites exhibited phosphate immobilization. Most of these soils contained small amounts of extractable phosphate and that is why the phosphorus cycle in these soils is not very active. These soils contain amorphous iron oxide (Pulford and Duncan, 1975), as the product of the oxidation of iron pyrite, which might sorb organic phosphorus, thus decreasing the rate of mineralization of phosphorus by enzymes (Greaves and Webley, 1969). Acid phosphatase enzyme plays an important role in releasing orthophosphate by hydrolyzing organophosphate. This may also contribute to the low mineralization rate, as most of these soils had low acid phosphatase activities. Biological immobilization can also not be ignored as phosphate mineralized may be recombined into organic forms by microorganisms, and the phosphate released may be fixed into insoluble inorganic phosphate. However, chemical immobilization is generally substantial in such soils (Fitter, 1974) (Tables 3.3-3.7).

An encouraging result from this study was the reasonably high values of carbon mineralization rates. These rates indicate that coal mine soils have a microbial population which can break down organic matter, especially on the grass species and vegetated plots, where the pH was much higher than other treatments. The rate of carbon dioxide evolution from soils measured under laboratory conditions could be used as an index of organic matter availability for microbial activity and breakdown. This value indicates a sufficient amount of degradable organic material which is the portion of importance to phosphorus cycling. Though the rate of carbon dioxide evolved was substantially high, it exerted little effect on phosphorus mineralization, due to low pH of these soils.

The adsorption maximum values of some of these soils were very high. If it is assumed that one hectare contains 1800 tonnes of soil, then an equivalent value of adsorption capacity of 3.15 t P ha^{-1} can be expected. The values of bonding energy constants also confirm that phosphate is bound more strongly to the soil surfaces and as such would be not available to plants. Adsorption maxima and bonding energy both were negatively related with soil pH, but not significantly correlated (Tables 3.2 and 3.4).

6.3 Plant uptake of phosphorus

In order to study the response of ryegrass (Lolium perenne) to added phosphatic fertilizer, a pot experiment was carried out with three levels of phosphatic fertilizer, ranging from 0 to 100 kg P/ha. Five different soils, ranging in extractable phosphorus from 0.4 to 21.6 mg P/kg soil were used for this study. Response of ryegrass to applied phosphate in these five soils was not the same, because of the wide variation in phosphorus supplying power of these soils. For example Loganlea, Baads (reclaimed) and Meikle Earnock soils with small extractable phosphate gave response to applied phosphate. While North Addiewell and Baads (chicken manure) which contained medium to high phosphate, did not show any response. These soils also differ in other chemical and biological properties (pH, adsorption and acid phosphatase activity), which might also influence the reaction and availability of the added phosphate. Since the other chemical and biological properties had no direct influence on crop response to applied phosphate, it is considered that the initial phosphate level of the soil is the most important factor influencing crop response to added phosphate. This can be seen by the response given by ryegrass up to 100 kg P ha^{-1} in the case of Loganlea soil, which has very low extractable phosphorus, and up to 50 kg P ha^{-1}, in the case of Baads (reclaimed) soil, which has low extractable phosphorus. The present study indicated that crop response to applied phosphate is expected to be high if the soil is deficient in available phosphate. This study further showed that all the coal mine soils are not deficient in phosphate. Therefore any recommendation of phosphate fertilizer for a given area cannot be applied to all coal mine soils (Tables 4.2-4.11).

The phenomenon of phosphate fixation and the great variation that the different soils showed in their phosphate fixing capacity have an important bearing in soil test crop response correlation studies. It has been found that the response of crops is lower to high phosphatic fertilizer dose on soils of high phosphate fixation capacity. The adsorption maxima of most of these soils did not show any relation with the crop yield. However, a significant decrease occurred in the adsorption maxima of Loganlea, North Addiewell and Baads (chicken manure) irrespective of phosphate applied after grass cutting (Table 4.9).

It is evident from the results that Loganlea, Meikle Earnock and most of the Baads soils are generally lower in extractable phosphate. The rate of carbon turnover (only studied in Baads soil) is higher compared to the very low rate of phosphate turnover. Such results suggest a deficiency of plant available phosphate in these soils. This was further confirmed by the results obtained from the crop response study. These findings emphasize the need for the addition of an adequate level of phosphate for the maintenance of a reasonable plant cover on such sites. A good plant cover on coal mine waste material is not only important from the agricultural point of view, but also needed to make them aesthetically attractive, and to minimize pollution of streams due to erosion and leaching (Tables 4.12-4.15).

North Addiewell soil gave the highest crop yield indicating that this soil is not deficient in plant available phosphate. The high amount of both extractable and mineralized phosphate might partly be due to the high rate of acid phosphatase activity in this soils (Stroo and Jencks, 1982).

Baads (chicken manure) soil is well supplied with available phosphate. Sufficient amount of phosphate present might be due to mineralization of chicken manure applied to this plot in 1980. This also indicates that chicken manure, which is a waste material and contains a reasonably high amount of organic phosphorus, is a good way of increasing the phosphate status of those coal mine soils which show phosphate deficiency. Chicken manure will not only supply an initial input of phosphate, but it can also supply reasonable amounts of nitrogen and organic matter. However, good yields of herbage were obtained for the first few years using such types of manures, but after 7 years there was no noticeable effect due to any of the treatments and the yields were almost equal to the control plots (Pulford et al., 1988).

6.4 Management strategies for phosphorus supply

Over the years, two main management strategies have emerged. The first, or higher option, consists of a heavy initial investment followed by substantial residual effects for several years. In addition to solving the problem immediately, heavy phosphate applications also serve to act as a soil amendment, producing beneficial changes in soil physical and chemical properties in highly weathered acidic soils. The second strategy involves a low input option. It has been traditionally based on applying phosphate in bands to satisfy the fixation capacity in small soil volume. However, on coal mine soils with very high phosphate fixation, the application of inorganic phosphatic fertilizer becomes a major management problem. A phosphate deficient soil which can be corrected by an application of 20 to 50 kg P/ha is not a problem soil (eg. Baads reclaimed and Meikle Earnock), but a soil which requires more phosphatic fertilizer (Loganlea) needs a different management for profitable crop production (Sanchez and Uehara, 1986). The problem becomes more serious when such soils are scattered over a large area and have a higher degree of phosphate fixation. For example in Loganlea soil, in addition to the large phosphate requirement, the loss of phosphate fertilizer was substantially greater than in the other soils studied.

Recently two other management strategies have emerged. These are selecting cultivars tolerant to a low available phosphate level, or decreasing the soil's capacity to fix phosphate. To decrease the soil's phosphate fixation capacity, less expensive amendments such as lime and silicates are applied. Considerable controversy exists in the literature regarding whether or not liming decreases phosphate fixation. Liming has little or no influence in decreasing phosphate fixation in soils with pH values ranging from 5.0 to 6.0 (Reeve and Sumner, 1970). This is true in the case of Loganlea soil with a pH 5.5, where liming has not influenced either acetic acid or ammonium fluoride extractable phosphorus, both native and added.

More so, the nonsignificant effect of lime might be also due to the fact that these soils are deficient in both phosphorus and nitrogen, and the important factor is the supply of macronutrients rather than liming. The beneficial effects obtained in liming acid soils to a pH of about 5.5 or less is attributed to the lower bonding energy between sorbed phosphate and oxide surfaces as their negative charge increases with increasing pH (Kamprath and Foy, 1971). This is true in the case of Baads soil with a pH 3.5, where liming increased significantly the acetic acid extractable phosphate, both native and added. The reason that ammonium fluoride extractable phosphate was not influenced by liming in the same soil, is explained by the different mechanism of the different extractants, as ammonium fluoride generally extracts only aluminium phosphate and not iron phosphate. The poor correlation of acetic acid extractable phosphate with crop yield as compared to plant phosphorus uptake by crop, makes doubtful the suitability of acetic acid to be used for crop response studies, especially on coal mine soils, which are derived from different parent materials. Phosphate ions are not taken up by crop grown on such material, because of the new surfaces being exposed by weathering.

6.5 Further development of this work

Although phosphorus is a thoroughly studied plant nutrient in agriculture, much remains to be studied about its behaviour in coal mine soils. The present results, as well as the reports in the literature about the behaviour of phosphate in coal mine soils, are conflicting. The main reason for this is firstly phosphate itself and secondly the heterogeneous nature of the coal mine soil. Therefore much work is needed to be done, especially the laboratory findings should be oriented toward the applied aspects.

It is common knowledge that crop response to phosphate is higher on soils of low phosphate status. But a finding which has gone unannounced, is that there are cases where, in spite of optimum phosphate application, crop yields on low phosphate soils remain lower than the yield obtained on high phosphate status soils. There could be some other deficiencies or imbalances in phosphate deficient soil or it could be that phosphate depleted soil becomes less productive in more than one way. During the present study such findings were observed in two soils, North Addiewell oil shale and Baads soil which had been treated with chicken manure in 1980. The high yield obtained in these two soils, without phosphate application as compared to other soils, which had lower yield in spite of the fact that they had been treated with 50 and 100 kg P/hectare, focusses attention toward the role of soil enzymes and organic wastes especially chicken manure.

The study of soil enzymes could play a vital role in the production of biologically stable coal mine soils. There is an obvious need for research to investigate phosphatase activities in freshly exposed coal mine soils as well as in agricultural soils, for the purpose of comparison, under the same conditions.

Contrary to the chemical methods for the coal mine reclamation, such as applications of fertilizer, the soil should be treated as a living system. Restoration of biological activities of these soils is important for their amelioration and productivity. One of the methods for increasing the biological activity is to add organic wastes. One of the major advantages of organic wastes over inorganic fertilizers in improving soil properties is their slow release characteristics and their long-term benefit in supplying plant nutrients. The high yield obtained on Baads, chicken manure treated soil, indicated that 8 years after the application of chicken manure, this soil still contained a sufficient amount of mineralized phosphate (>20 mg P/kg soil). Since chicken manure also contains nitrogen, in addition to organic phosphate, a further more comprehensive study is needed on the effect of chicken manure on mineralization, enzyme activity and other plant nutrients and soil physical and chemical characteristics.

During the incubation study, it was noticed that a large number of coal mine soils did not show mineralization of organic phosphorus, but exhibited a considerable loss of phosphate due to fixation. The significant effect of pH on organic carbon mineralization, emphasizes the need of liming in the reclamation of these soils. Lime addition was valuable on some soils with very low pH in increasing phosphate availability. Further work is needed on its suitability by testing a variety of acidic soils.

The nonsignificant effect of lime on the phosphate availability suggests that either these soils are in severe shortage of other nutrients, which hinders the effect of lime, or the lime requirement method does not fulfil the actual lime requirement. Therefore further work is needed to incorporate additional parameters in a lime requirement test, which may hold promise for improving its predictive ability. Another approach would be to neutralize existing acidity and identify areas of potential acidity which could be monitored and limed as part of a maintenance programme.

It would be worthwhile to study and compare the effect of chicken manure, liming and silicate on the availability of both native and added phosphate. Such a study would be certainly helpful in sorting out the reasons for the lack of mineralization.

Inorganic phosphate fertilizers, which are usually applied for getting an immediate effect, are usually fixed, especially in coal mine soils, which have high fixation capacity. Heavy applications of phosphatic fertilizers also serve to act as soil amendments, producing beneficial changes in soil physical and chemical properties. More long-term studies on the residual effect of phosphate applications are needed. Since the amount of phosphate necessary to be added for maximum growth is directly related to the sorbing power of the soil, it would be more justifiable to conduct such experiments on well characterized sites, where phosphate fixation is a problem.

Incorporating tolerance to phosphate stress as a breeding objective is also needed. Plant breeders and soil scientists working together can make a major contribution to this effect.

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