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INFLUENCE OF SOIL PHOSPHORUS ON ENZYME ACTIVITY, RYEGRASS YIELD AND EXTRACTABLE PHOSPHATE

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ABSTRACT

To investigate the effect of soil Phosphate on enzymatic activity and plant availability under controlled conditions, five Scottish soil series viz. Dreghorn, Darvel, Caprington, Dunlop and Midelney having different physico – chemical characteristics were studied. The experiment was laid out in a Latin square design, with two blocks, with and without ryegrass. The enzymic activities showed a significant difference in all the soils except Dreghorn soil for alkaline phosphatases and phosphodiesterase. The enzyme activities generally increased in grassed soils as compared to non grassed soil with the variable differences to original soils. The yield of roots and tops of ryegrass and P uptake was significantly different amongst the different soils. The highest yield of tops was recorded 4.36 and 4.07 g pot^{-1} in Darvel and Midelney soil series respectively. The dry matter yield of roots was significantly highest in Darvel soil. The total P uptake by the leaves and roots also showed a significant difference between the soils. The highest P concentration of 0.276 and 0.209 % P in leaves and roots was found in Midelney soil. Positive correlation (r = 0.7955) was recorded between the dry matter yield and P uptake by the ryegrass. The soil phosphate extracted by using different extractants also showed significant differences amongst the soils before planting and after harvesting. The acetic acid extractant in grassed pot was lower which suggest that 0.5 M acetic acid is a good measure of P utilized by the grass.

Key words: Phosphate, Ryegrass, Phosphatases Enzymes, Phosphate uptake.

INTRODUCTION

The limitation of availability of phosphorus to plants arises from various processes and interactions of nutrient elements with both soil constituents and plants. The interaction in soil system is of more significance since it determines how much nutrients are accessible to plants. Soil minerals can immobilize most of the nutrients especially phosphorus when added to the soil, thereby reducing its availability to growing plants. The most important mechanism involved in the movement of phosphate to absorbing roots is diffusion. Many researchers agreed that phosphate moves to the roots of plant by diffusion (Mason *et al*., 2011). Mass flow contributes only a small proportion of P (Comerford, 2005), about 5% of the actual uptake of crops, as estimated for field grown maize (Barber, 1995). Thus the concentration of phosphate in the soil solution is vital, since the soil solution must contain sufficient phosphorus to provide the concentration gradient necessary for net movement to the roots. The required concentration of phosphorus in soil solution necessary for plant growth depends primarily on the crop species being grown. Phosphatase production is correlated with reduced P in the soil solution (Comerford, 2005). Soil microbial properties have a greater correlation with soil health (Mohammadi, 2011). Soil enzymes play major role in, transformation of organically bound nutrients into inorganic plant available nutrients. Soil enzymes have significant role in numerous reactions essential for life processes of soil microorganisms as decomposition of organic residues, cycling of nutrients and formation of organic matter and soil structure (Kandeler *et al*., 1999). Saha *et al*. (2008) reported that the nature and amount of applied organic fertilizers to the soil significantly influence the activity of phosphatase enzymes and available phosphorus. Among the soil phosphatases, phosphomonoesterases and to lesser extent phosphodiesterases, have been the most widely studied. The phosphomonoesterases are further classified as acid and alkaline phosphatases (Dodor and Tabatabai, 2003).

Phosphorus extractants are used in different laboratories for determining the Phosphate content of soil depending on the soil type. The extractants being used have certain problems and shortcomings which may lead to a difficulty in interpretation of the test result (Myers *et al*., 2005). Many workers have employed these extractant for determining available P including Bray and Kurtz (1945); Watanabe and Olsen (1965); Fox and Kamprath (1970); Barrow (1979); Mehlich (1984). Bray-1 and Mehlich-3 extractants are designed to extract P for used in acid soils; whereas Olsen extraction method is meant for soils characterized alkaline calcareous soils.

The present study investigated the effect of soil P on enzymes activities, P uptake and plant growth of five soil series. Also the study focused on the comparison of various extractants used in different soils.

MATERIALS AND METHODS

To study the availability of phosphate to ryegrass and its influence on the activity of phosphatic enzymes, a pot experiment was carried out under controlled condition at Department of Analytical and Environmental Chemistry, University of Glasgow, Scotland. Five different Scottish soil series were used in the study viz. Dreghorn, Darvel, Caprington, Dunlop and Midelney. The experiment was laid out in a Latin square design, with two blocks. In one block ryegrass was sown, while the other block was kept fallow. Each soil was replicated five times in a block. The physico – chemical properties of the original soils are given in Table 1.

Ryegrass seed was sown at the rate of 0.5 g per pot (10 cm diameter). After sowing the pots were covered with a black polythene sheet to enhance germination. After complete germination the pots were placed in saucers on bench. Deionized water was applied regularly to each pot from the saucers according to requirement of each soil. Nitrogen, Potassium and magnesium were applied to each pot one week after emergence of the seedlings at the rate of 100, 50 and 5 mg per pot respectively. The sources of nutrients were Ammonium nitrate for N, muirate of potash for K and magnesium sulphate for Mg. After 12 weeks of sowing the watering was stopped, the grass was cut just above the soil surface.

Table 1. Physico – chemical properties of the soil series

Soil Series	Soil Texture	pH	Organic Carbon ($g\ kg^{-1}$)	Fe (%)	Al (%)
Dreghorn	Sandy Loam	5.2	18.4	0.29	0.33
Darvel	Sandy Clay loam	5.7	29.5	0.85	0.42
Caprington	Sandy Clay loam	5.9	34.1	0.52	0.17
Dunlop	Clay loam	4.9	66.9	1.08	0.58
Midelney	Clay	6.3	61.8	0.74	0.11

The fresh soil samples were assayed for acid phosphatases, alkaline phosphatases and phosphodiestrases enzymes activities based on the colorimetric estimation of the nitrophenyl released by these enzymes when soil was incubated with relevant buffered p – nitrophenyl phosphate solution (Tabatabai, 1994). The fresh yields of tops (leaves + stubbles) were obtained by weighing. Roots were obtained through washing to remove adhering soil materials. The plant materials were chemically analysed for various parameters. Soil samples before sowing and after harvesting of ryegrass and non grassed were analysed for extractable Phosphate using three different extractants including 0.5 M Acetic acid, 0.5 M $NaHCO_3$ and acidified NH_4F (Bray I). The statistical analysis was carried out using the method given by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Enzyme activities in grassed and non - grassed Pots: The activities of three enzymes i.e. acid phosphatases, alkaline phosphatases and diestrases were measured in all the pots (Table 2). The result showed significant differences in the enzymes activities in all the soils except in Dreghorn soil for alkaline phosphatases and phosphodiesterases. The enzymes activities generally increased in grassed soils as compared to non grassed pot soil with the variable differences to original soils. However, the results revealed an effect of plant growth on enzyme activities.

Sarapatka *et al*. (2004) reported phosphatase activity in the rhizosphere is greater due to increased microbial numbers in the rhizosphere and the excretion of plant root enzymes. Plant roots are known to secrete acid phosphatases which have the ability to induce hydrolysis of certain organic phosphates (George *et al*., 2005; Tran *et al*., 2010). By comparing the activities of different enzymes it was observed that the activity of acid phosphomonoesterase was higher than the other two enzymes. It supports the findings that acid phosphatases is predominant in acid soils and alkaline phosphatase is predominant in alkaline soils (Wang *et al*., 2011). Phosphodiestrase activity was considerably less in all soils examined than the both phosphomonoestrases.

Crop Yield and Phosphate uptake: Dry matter yield and P uptake were used to evaluate the availability of residual soil P. It was recognized that there should be differences in dry matter production due to inherent differences between soils. The results showed that there were significant differences in dry matter yield of tops and roots of plants (Table 3). The greatest yields of tops were 4.36 and 4.07 g pot^{-1} in Darvel and Midelney soils respectively, followed by Caprington.

The Dreghorn and Dunlop produced the lowest dry matter yield of tops. The dry matter yield of roots was significantly highest in Darvel soil. The total P uptake by the leaves and roots also varied between the soils significantly. The highest concentration of 0.276 and 0.209 % P in leaves and roots was found in Midelney soil, while Dunlop had the lowest P uptake of 0.112% in leaves. Pathiram and Prasad (1990) reported the highly significant correlation between P uptake and dry matter yield of maize. The correlation studies of dry matter yield and P uptake by ryegrass showed positive correlation (r = 0.7955) (Fig. 1).

Table 2: Enzymic activities of soils used in pot experiment before planting and after harvesting.

Soil Enzymes	Soil Series				
	Dreghorn	Caprington	Dunlop	Darvel	Midelney
	Enzymic activities (µ moles pnp g^{-1} soil per hour)				
Acid phosphatases					
Before	2.42 b	3.84 b	4.84 a	2.84 ab	2.90 b
Non grassed	2.94 a	3.42 b	4.07 b	2.68 b	3.56 a
Grassed	2.80 ab	3.96 a	5.31 a	3.05 a	3.60 a
Alkaline Phosphatses					
Before	0.30 a	2.15 a	1.31 b	0.94 a	4.15 b
Non Grassed	0.34 a	1.49 b	1.33 b	0.78 ab	3.85 b
Grassed	0.21 a	1.97 a	1.60 a	0.76 b	4.70 a
Phosphodiesterase					
Before	0.11 a	0.75 a	0.29 b	0.22 b	1.35 a
Non Grassed	0.22 a	0.34 b	0.23 c	0.16 c	0.72 c
Grassed	0.18 a	0.83 a	0.39 a	0.25 a	1.14 b

Means in a column of an enzyme followed by similar letter(s) did not differ significantly at 0.05 level of significance

Table: 3 Average yield of ryegrass obtained in pot experiment and P uptake

Soils	Dry matter yield in g pot^{-1}			P uptake in %	
	Tops	Roots	Total	Leaves	Roots
Dreghorn	1.84 c	0.804 bc	2.64 d	0.140 c	0.162 bc
Caprington	3.01 b	0.874 bc	3.89 c	0.188 b	0.185 ab
Dunlop	1.32 c	0.626 c	1.95 d	0.122 c	0.116 d
Darvel	4.36 a	1.804 a	6.16 a	0.216 b	0.160 c
Midelney	4.07 a	1.144 b	5.22 b	0.276 a	0.209 a

Means in a column followed by similar letter(s) did not differ significantly at 5 % level of significance

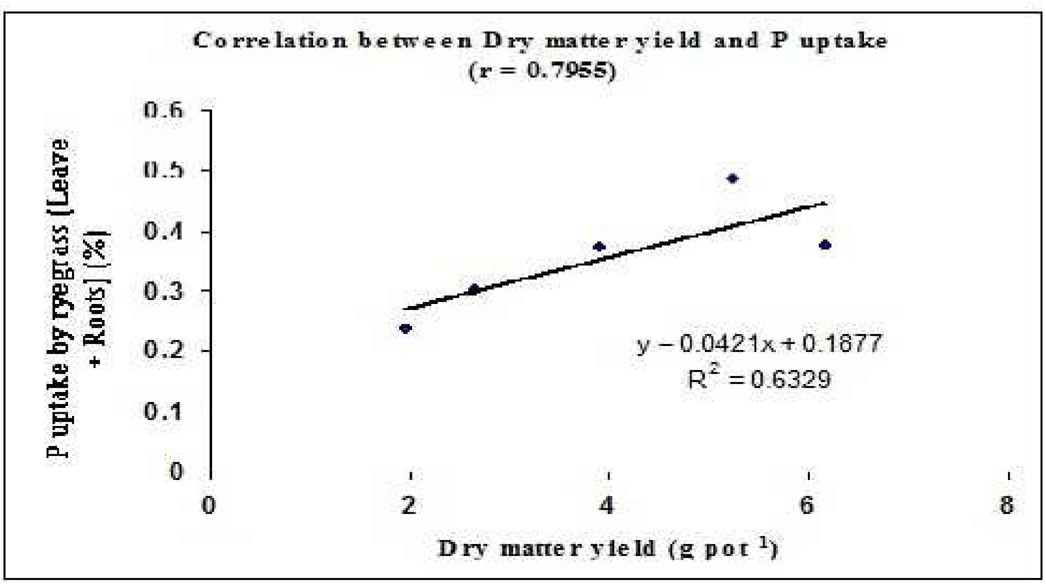

Fig. 1 Correlation between the Dry matter yield and P uptake

Sarir *et al*. (2009) reported a poor correlation between P uptake and crop yield of ryegrass and described that it might be due to the limitation of Acetic acid used in the soil analysis as acetic acid activates P sources not available to plants.

Effect of plant growth on soil phosphate content using three extractants: The Phosphate extracted before planting was compared with the after harvest soil samples both with the grassed and non grassed using three different extractants (Table 4). The results showed that acetic acid extractable P in non grassed was comparable in all soils except Darvel.

Table 4: Phosphate extracted from soil using three extractants before and after planting of grass and non grassed pots.

Soils	Extractable P in mg kg^{-1}		
	Before Planting	After Harvesting	
		Grassed pots	Non–grassed pots
	0.5 Acetic acid extractant		
Dreghorn	5.8 a	4.7 b	5.9 a
Caprington	12.6 a	10.5 b	12.9 a
Dunlop	1.7 ab	1.5 b	1.8 a
Darvel	9.0 a	5.9 c	8.3 b
Midelney	107.8 a	84.0 b	104.5 a
	0.5 Sodium Bicarbonate extractant		
Dreghorn	21.2 b	20.5 b	23.0 a
Caprington	16.5 b	13.7 c	19.1 a
Dunlop	5.1 a	4.7 a	4.9 a
Darvel	40.7 a	33.0 c	39.2 b
Midelney	45.0 a	32.4 b	46.3 a
	Bray I (NH_4F) extractant		
Dreghorn	57.0 b	57.9 b	64.1 a
Caprington	26.7 b	24.7 b	31.7 a
Dunlop	23.7 b	23.1 b	25.2 a
Darvel	107.6 b	98.9 c	119.5 a
Midelney	92.6 a	64.3 b	91.1 a

Means in a row followed by similar letter(s) did not differ significantly at 5 % level of significance.

The acetic acid in grassed pot was lower which suggest that 0.5 M acetic acid is a good measure of P utilized by the grass. The results are supported by the finding of Jansson and Tuhkanen (2003), they used Acetic acid as P extraction and found it as better estimate for the P loading potential of pasture. In case of Bray I extractant, higher P in all non – grassed soils than the original soils suggest changes in this fraction of P. Bray I extractable P in the grassed soils was same as or less than the P extracted from the soils before planting. It may be result of the competitive processes reflected by the extractants. Sodium bicarbonate extractable P was lower in grassed soil than non grassed soils except Dunlop. There was variable effect in the extractable P of soils before planting.

REFERENCES

Barber, S. A. (1995). Soil nutrient bioavailability: a mechanistic approach. 2nd Ed. John Wiley: New York, (USA). 414 p.

Bray, R. H, L. T. Kurtz (1945). Determination of total, organic, and available forms of soil phosphorus in soils. J. of Soil Sci. 59: 39 – 45.

Barrow, N. J. (1979). The description of desorption of phosphate from soils. J. Soil Sci. 31: 259 – 270.

Comerford, N. B. (2005). Soil factors affecting nutrients availability. In: H. Bassiri Rad (Ed.) Nutrient Acquisition by Plants an Ecological Perspective. 1 – 14 p.

Dodor, D. E and M. A. Tabatabai (2003). Effect of cropping systems on phosphatases in soils. J. Plant Nut. Soil Sci. 166: 7 – 13.

Fox, F. J. and E. J. Kamprath (1970). Phosphate sorption isotherm for evaluating the phosphate requirement of soils. Soil Sci. Soc. America proceedings 34. 902 – 907.

George, T. S., A. E., Richardson and R. J. Simpson (2005). Behaviour of plant-derived extracellular phytase upon addition to soil. Soil Biol. Biochem. 37: 977–988.

Gomez, K. A. and A. A. Gomez (1984). Statistical Procedure for agricultural Research. 2nd edition. John Wiley and Sons inc., New York (USA).

Jansson, H. and H. R. Tuhkanen. (2003). Reducing nutrient load from dairy farm pastures. In: Uusi-Kamppa, J., M. Yli-Halla and K. Grek (eds.). Reducing environmental loading from dairy farming. 40–47 p. (In Finnish)

Kandeler, E., D. Tscherko and H. Spiegel (1999). Long-term monitoring of microbial biomass, N mineralization and enzyme activities of a Chernozem under different tillage management. Biology and Fertility of Soils. 28: 343-351.

Mason, S., K. Ferrier and T. McBeath (2011). Phosphorus management following high yields Mallee Sustainable Farming. (2010). Results Compendium. 86–92 p.

Mehlich, A. (1984). Mehlich 3 soil extractants: A modification of Mehlich 2 extractants. Commun. Soil Sci. Plant Anal. 15: 1409 – 1415.

Mohammadi, K. (2011). Soil Microbial Activity and Biomass as Influenced by Tillage and Fertilization in Wheat Production. American-Eurasian J. Agric. and Environ. Sci. 10 (3): 330-337.

Myers, R. G., A. N. Sharpley, S. J. Thien and G. M. Pierzynski (2005). Ion-Sink phosphorus extraction methods applied on 24 soils from the continental USA. Soil Sci. Soc. Am. J. 69: 511-521.

Pathiram, R. N. R and R. N. Prasad (1990). Forms of soil phosphorus and suitable extractants for available phosphorus in acid soils of Sikkim. J. Indian Soc. Soil Sci. 38: 237 – 242.

Saha, S., B. L. Mina, K .A. Gopinath, S. Kundu and H. S. Gupta (2008). Relative changes in phosphatase activities as influenced by source and application rate of organic compost in field crop. Bioresource technology. 99: 1750-1757.

Sarapatka, B., L. Dudova and M. Krskova (2004). Effect of pH and phosphate supply on acid phosphatase activity in cereal roots. Biologia Bratislava. 59(1): 127 - 131.

Sarir, M. S., M. Sharif, I. D. Pulford, T.H. Flower and I. Ahmad (2009). Response of Ryegrass to Phosphate in the reclamation of coal mine soil. Sarhad J. Agric. 25 (2): 203 – 207.

Tabatabai, M. A. (1994). Soil enzymes. In: Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties.) R. W. Weaver, S. Angle, P. Bottomley, D. Bezdieck, S. Smith, A. Tabatabai and A. Wollum eds. (Soil Science Society of America: Madison, Wisconsin), Book Series No. 5. 775 – 833 p.

Tran, H. T., B. A. Hurley, W. C. Plaxtona (2010). Feeding hungry plants: the role of purple acid phosphatases in phosphate nutrition. Plant Sci. 179: 14 – 27.

Wang, J. B., Z. H. Chen, L. J. Chen, A. N. Zhu and Z. J. Wu (2011). Surface soil phosphorus and phosphatases activities affected by tillage and crop residue input amounts. Plant Soil Environ. 57 (6): 251–257.

Watanabe, F.S and S. R. Olsen (1965). Test of ascorbic acid method for determining phosphorus in water and NaHCO3 extract from soil. Soil Sci. Soc. of America Proceedings 29. 677 – 678.