

A modified technique for measurement of phosphorus concentration gradient in the flooded soil around rice root

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ABSTRACT

The phosphorus (P) concentration gradients at rice rhizosphere helps to study the root induced changes in the rice soil. For measurement of vertical concentration gradients rice seeds were sown in open ended plastic cylindrical pots consisting of two chambers. The upper chamber was filled with P free sand where the rice seeds were sown, while lower chamber was filled with 500 g soils. Soils and plant roots were separated by a horizontally placed stainless steel sieve (300 meshes) in such a way that only root hair could penetrate through the sieve. In another set to obtain the radial concentration gradient of P from plant roots, stainless steel mesh made cylinder was placed vertically in pot and plant was grown in soils placed inside the central cylinder. P concentration of rice rhizosphere was measured up to a distance of 45 mm from the vicinity of roots both in the vertical and radial directions. In Vertisol the P concentration was 5.48 mg kg⁻¹ at 9 mm and attained 6.48 mg kg⁻¹ at a distance of 27 mm from root surface in vertical direction. Where as, in Inceptisol P concentration was 33.78 mg kg⁻¹ in first soil section and it was increased to 38.24 mg kg⁻¹ at 27 mm away from root surface in radial direction. The concentration gradients were found up to a distance of 27 mm from root surface when the crop was 50 days old. This rhizosphere sampling technique could be effectively used under flooded rice condition considering no inter-root competition. But it has to be validated in field condition considering inter-root competition.

Key words: Phosphorus concentration gradient, technique rice, rhizosphere, vertisol, inceptisol

Rice roots can greatly modify the soils near them. As a result the rhizosphere soil is entirely different from bulk soil although it is the latter whose properties are normally measured. The boundary between the root and soil changes constantly because roots continually modify the nearby soil by their mechanical and metabolic activities. Physical changes induced by roots include, compression of the soil of root surface to create a zone of minimal voids and root surface has a high negative potential caused by evaporative demand of the aerial parts of the plants. Rice roots also bring about chemical changes like, precipitation of dissolved salts at root surface, pH change (Hinsinger, 2001), change of oxygen and carbon dioxide levels in rhizosphere due to root respiration. Moreover, some root exudates, may stimulate microbial growth and also modify nutrient availability.

Considerable efforts have been devoted in recent years to develop techniques for sampling the rhizosphere soil. However, most methods still suffer from technical and or conceptual insufficiency. A classical widely applied method for sampling involves careful removal of the soils from the roots after adjusting the soil moisture to a suitable level (40-50%). But, Helal and Sauerbeck (1991) questioned the validity of the method and attributed its inadequacy mainly for two problems. Firstly, it is almost impossible to remove an intact root system with the adhering soils. By this method mainly old thick root axes and some primary branches withstand the isolation procedure. Fine roots branches of higher orders which represent the functionally most active part of the root system are lost during the isolation. The soil fraction in contact with them is therefore left with bulk non-rhizosphere soil. Secondly, the separation method

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does not recognize the dynamic nature of the root system. Studying the rhizosphere soil by separating it from the roots by some water permeable membrane seemed to offer a solution to above mentioned problems. Harmaen and Jager (1963) used a technique by which the roots were restricted to a chamber made from a nickel sheet perforated with holes 60 μm in diameter and they considered the whole chamber as rhizosphere. However, it is clear that the soil directly outside the box, receiving diffusible exudates and metabolites through pores must be by definition also considered as rhizosphere. Bhat and Nye (1973) stated P concentration gradient in vicinity of plant roots may range over a distance of only a few mm. They have shown that the distribution of phosphate and rubidium in the soil near plant roots can be determined by an auto-radiographic technique. However, this method was only applicable to those elements which have radioisotopes with suitable characteristics. Boero and Thien (1979) used a system of concentric cylinders made of nylon netting and confined the root system to the central core. This model accounted for root-soil interactions and made possible the preparation of rhizosphere soil in a reproducible way. The main problem with this system, however, was the difficulties of preparing regular cylinders from nylon gauge and breakthrough of roots at their joints. Helal and Sauerbeck (1991) described containers with several vertical screens in order to divide the soil into layers of different distances from the roots. The possible minimum thickness of these layers, however, exceeded that is necessary for the purpose.

Root activity can modify the chemistry of the rhizosphere and alter phosphorus availability and uptake (Bhattacharyya and Data, 2004). The chemical conditions of the rhizosphere considerably differ from bulk soil, as a consequence of a range of processes that are induced either directly by root activities or by the activities of rhizosphere microflora. Among these, the uptake of plant roots must be taken into account in the first place. A second group of activities which is of major concern with respect to P availability are those processes that can affect soil pH, proton/bicarbonate release (anion/cation balance) and gaseous (O_2/CO_2) exchange. Thirdly the release of root exudates such as organic ligand is another process. However, relatively little was known about the dynamics of soil solution P at the root surface because of the inability to measure

in situ changes in solution P concentration at the plant root (Wang, et al. 2004). The main problem associated with determination of nutrients concentration gradient around root is the dynamic nature of roots, which continually change the boundary between soil-root interfaces. All these experiments were carried out for very shorter period of crop growth and only few millimeters around the roots. In the present study, it was tried to see the P concentration gradient in soil at 50 days of plant growth and a relatively larger area around roots.

MATERIALS AND METHODS

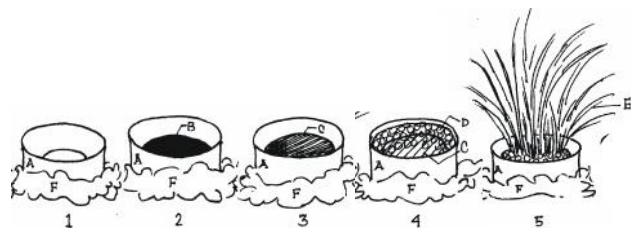
Each sample contained five soil sections of 9 mm thickness. The section in which maximum P concentration was found noted as C_0 . Phosphorus concentration (C) of rest of the remaining soil sections expressed as a ratio of the maximum concentration (C/C_0) to nullify the inherent variability of soils in each pots. The C/C_0 values were plotted against the distances from root surface in each soil. All the roots accumulated on sieve surface and the interface between soil and sieve was considered as root surface. And the measurement of distance of the soil section was started from this interface.

Rice variety, Pusa Basmati 1 (135 days maturity) was grown in two types of soils, Vertisol (Typic Chromostert) and Inceptisol (Typic Usocrypt) in specialized plastic pot (150mm diameter and 150mm height) under greenhouse condition in three replications. Single plant was planted at the centre of the open ended pots. One end of these pots was covered by plastic sheets for restricting the leaching loss. Optimum doses of N-P-K were applied to the crops in all types of soils. Nitrogen (53.33 mg kg^{-1}), phosphorus (19.50 mg kg^{-1}) and potassium (37.63 mg kg^{-1}) were applied as basal and another 53.33 mg kg^{-1} N was top dressed at 30 days after sowing. Potassium dihydrogen phosphate (KH_2PO_4) and potassium chloride (KCl) were used as sources for P and K.

The pots were made up of two parts (Fig. 1). The plants were grown on P free sand (500 gm) in the upper part and the lower part contained soil (500 gm). Soil and plant roots were separated by 300 mesh stainless steel sieve (fitting to the inner diameter of the plastic pots) in such a way that only root hairs can penetrate through the sieve. After 50 days of sowing

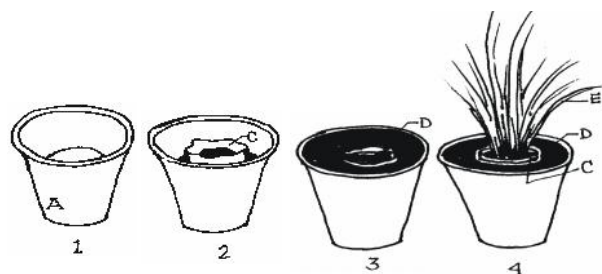
the plants in the pots were removed without disturbing the soil, separated by stainless steel sieve. After that sieves were removed and thin soil sections were cut. The soils were pushed from below by a piston rod and as the soil surface slightly protruded from the edge of the pot, a thin section was cut by a sharp knife and kept immediately in a polythene bag in deep freezer. The subsequent sections (9 mm thick) were cut one by one following the same procedure as in the case of first section. This set up was used to determine vertical P concentration gradient from the vicinity of plant roots.

In another set, cylinders made of 300 mesh stainless steel sieve (100 mm height and 5mm diameter) were placed vertically and plants were grown in soils placed inside the cylinder to measure the radial P concentration gradient from plant roots (Fig. 2). After removing the 50 days old plants from central cylinder, thin cylindrical sections were cut by pushing sharp edge metallic cylinders of different radii and the soil samples kept in polythene bag and preserved in deep freezer. Olsen's P (0.5 N NaHCO₃ extracted) in the soils of



A: Open ended 150mm diameter plastic pots ; B: 500g Soil; C: Stainless steel made sieve (300 mesh); D: Sand; E: Plants; F: Perforated plastic sheets

Fig. 1. Set up for Vertical P concentration gradient measurement



A: Plastic pots ; B: 500g soil; C: Stainless steel made sieve(300 mesh); D: Soil; E: Plants

Fig. 2. Set up for radial P concentration gradient measurement

five sections each of 9 mm thickness were analysed in wet condition by adjusting the concentration of Olsen reagent according to the moisture content of the soil samples (Gahoonia *et al.*, 2000).

RESULTS AND DISCUSSION

The two soils used in this investigation were slightly alkaline in reaction. The soils were having varying amount of organic carbon, clay contents, cation exchange capacities and Olsen P contents. Vertisol was silty clay in texture where as the texture of Inceptisol was silty loam. Amorphous Ferri Alumino-Silicate (AFAS) in clay was maximum in Vertisol (32%) followed by Inceptisol (23%). Semi-quantitative analysis of clays showed that smectite and illite were the dominant clays in Vertisol and Inceptisol, respectively (Table 1).

In Vertisol, rhizosphere soils depicted lower C/C₀ up to 27 mm away from root (Fig. 3). The rhizosphere P concentration gradually increased with distances both in vertical (up to 27 mm) and radial directions (up to 36 mm) away from root. The P concentration was 5.48 mg kg⁻¹ at 9 mm and attained 6.48 mg kg⁻¹ at a distance of 27 mm from root surface in vertical direction (Table 2).

Table 1. Characteristic properties of soils under study

Property	Vertisol (Typic Chromostert)	Inceptisol (Typic Ustocrept)
pH (1:2.5; soil:water)	7.75	7.65
EC (dS m ⁻¹)	0.627	0.449
C.E.C [cmol (p ⁺)kg ⁻¹]	65.11	17.56
Organic carbon (%)	0.92	0.76
Available P (mg kg ⁻¹)	12.62	46.25
Bulk density (g cm ⁻³)	1.1	1.3
Size fraction (%)		
Clay	56.5	15.2
Silty	30.0	36.1
Sand	13.5	48.7
Texture	Silty clay	Silty loam
Clay fractions (%)		
Smectite	44	19
Illite	13	44
Kaolinite	11	14
Amorphous clay (AFAS)	32	23

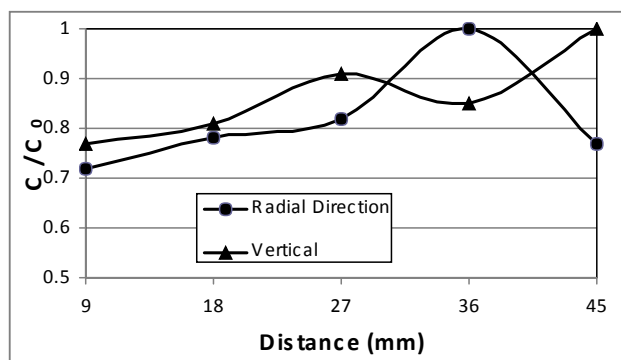
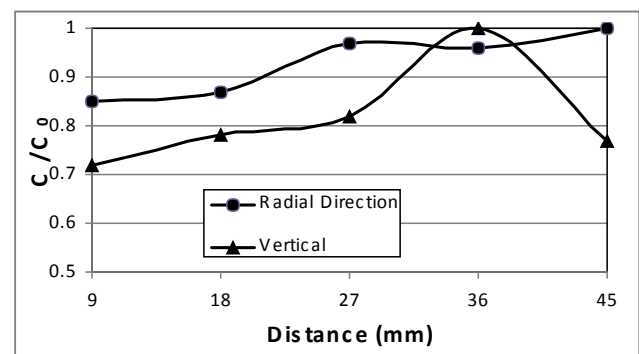
Table 2. Phosphorus concentration (mg kg^{-1}) in different soil section in Vertisol and Inceptisol.

In Vertisol Distance	In vertical direction		In radial direction	
	Rhizosphere	$\pm\text{SD}$	Rhizosphere	$\pm\text{SD}$
9 mm	5.48	0.11	8.56	0.12
18 mm	5.73	0.06	9.29	0.09
27 mm	6.48	0.03	9.74	0.11
36 mm	6.04	0.12	11.93	0.28
45 mm	7.10	0.09	9.23	0.04
In Inceptisol				
9 mm	26.06	0.06	33.78	0.20
18 mm	27.89	0.31	34.52	0.06
27 mm	29.09	0.08	38.24	0.07
36 mm	33.19	0.09	38.16	0.17
45 mm	34.46	0.13	39.62	0.12

There was a sharp increase in the ratio of P concentration (higher value of C/C_0) at 36 mm away from the root surface in rhizosphere with increase in vertical distances from root surface in Inceptisol. C/C_0 value increased from 0.76 at vicinity of root to 0.96 at 36 mm away from root surface (Fig. 3). P concentration was 26.06 mg kg^{-1} in first soil section and increased to 34.46 mg kg^{-1} at 45 mm away from root surface. Concentration gradient was found up to 27 mm in radial direction from root surface in Inceptisol. C/C_0 value in rhizosphere soil was 0.85 at 9 mm distance and sharply increased to 0.97 at 27 mm but after that distance it was almost unchanged (Fig. 3).

Phosphorus occurs in very low concentration in soil solution and their uptake results in a further

decrease of its concentration in the soil solution near plant roots (Bhat *et al.*, 1976). A severe decrease in soil P in the rhizosphere may cause a shift in the adsorption-desorption and dissolution precipitation equilibrium involved in the dynamics of soil P. However, due to poor reversibility of P sorption onto soil constituents (Parfitt, 1978) and to the low solubility of the various phosphate minerals occurring in soil (Lindsay *et al.*, 1989), very small solution P concentrations must be reached for these phenomena to proceed to a significant extent. Such critical P concentrations might then be too low for sustaining adequate growth of plants. In addition to such considerations, the rate of desorption of soil P or the rate of dissolution of P bearing soil constituents would need to be larger than fluxes of P uptake to prevent any growth restriction. As pointed out by Darrah (1993), we still lack knowledge about the kinetics of these reactions involved in dynamics, so the amount of P mobilized by plant roots from the rhizosphere is hardly predictable. Kirk and Saleque (1995) estimated that, whereas total soil P was depleted to about 50% of its initial value in the vicinity of rice roots growing in a flooded soil at various rates of P fertilization, a 5 - 20 fold increase in solution P concentration occurred at 2-4 mm from root surface. Phosphorus concentration in soil solution is closely related to soil pH and this relation varies with soil. The direct effect of root induced pH reduction on P uptake is due to rhizosphere soil acidification which usually increases solution P concentration in the rhizosphere and changes the proportion of various forms of P. Li and Barber (1991) calculated that legume reduced rhizosphere soil pH by 0.39 to 0.77 units and increase P availability of 20.8 to 241.7 per cent. Nevertheless,

**Vertisol****Inceptisol**

= P concentration in a section; C_0 = Highest P concentration in a particular section

Fig. 3. Phosphorus concentration gradients of the rice rhizosphere in Vertisol and Inceptisol

above findings suggest that the uptake of mineral nutrients not only is the ultimate stage of the acquisition process at the root-soil interface but also results itself in severe changes in ionic concentration that can then shift the equilibria of adsorption desorption or dissolution precipitation involved in the dynamics of the P in the soil. Because of the lack of quantification of the different processes involved here, it is difficult to state if these phenomena may be regarded as efficient strategies of mineral nutrition (Darrah 1993). Certainly these root induced changes of ionic concentration deserve further consideration as major components of the whole process of nutrient acquisition by higher plants.

Kuchenbuch and Junk (1982) used nylon sieve for separating roots and rhizosphere soil but an important limitation of that the occasional penetration of roots occurred through nylon sieve. To overcome this limitation, in the present study stronger stainless steel mesh sieve was used. This helped to nullify the problem faced by Boero and Thien (1979) for preparing regular cylinders of nylon gauze to specify zones away from plant roots. Helal and Sauerbeck (1991) concluded from their study that the rhizosphere area is not restricted to the immediate root soil interface but involves a much larger soil volume particularly when plants were grown in for longer duration. In the present study, P concentration gradients were measured up to 45mm around the root. The present technique could be effectively used in flooded condition and as the roots were restricted in a chamber it represent only a situation where no inter-root competition exists, which is very much required for development of P uptake models. But the technique needs to be validated in field condition considering inter-root competition.

Nutrients commonly occurring at low concentration in the soil solution, such as K and P, are transferred by the mass flow process in amounts insufficient to meet the requirement of plant. Their uptake thus results in a decrease in their concentration in soil solution near plant roots; this depletion then generates a concentration gradient and diffusion of ions towards roots (Kraus *et al.*, 1987). Diffusion of immobile nutrients particularly P, further creates concentration difference in rhizosphere. The diffusion co-efficient of P in soil (saturated condition) is very low; nearly in range of 10^{-9} cm² sec⁻¹. So, obviously its movement is restricted to a few millimetres near root

surfaces (Hinsinger and Gilkes, 1996). However, Kuchenbuch and Jungk (1982) found P concentration gradient around root zone were only up to 9 and 10 mm away from root surfaces, but Shaviv *et al.* (1992) reported a distinguishing P concentration gradient up to 20 mm away from roots after 50 hours.

Earlier the investigation was done in short time and gradient was found only up to 10 mm (Hinsinger and Gilkes, 1996). In the present investigation the duration of crops in special pots was extended up to 50 days, to see whether the extent of P concentration gradient could be extended due to long duration of cropping. The concentration gradient was observed up to 27 mm. This might be due to the fact that there was minimum inter-root competition in the present set up, where all the roots were accumulated in the same plane and acting as common sink surface. But when roots are allowed to grow in normal condition in soils, the concentration gradient extended up to a distance of r_1 , where r_1 is equal to $1/(L)^{1/2}$, where, L is the root length density (RLD). The principle behind this hypothesis is that each root is allowed to take nutrient from the soil volume presented in a cylinder of radius $1/L$. Because of root competition the nutrients from adjacent cylinders is not allowed to pass through it. That is why the boundary condition for inter-root competition is kept as

$$de/dr = 0 \text{ at } r_1 = r/(L)^{1/2}, t > 0$$

So, in the present set up the extension of concentration gradient is possible.

In rice rhizosphere P concentration gradients were prominent. Gradual increase of P concentration from vicinity of roots both in vertical and radial direction occurred in Inceptisol and Vertisol. The modified technique could be used in all types of soils. Changes in concentration at vertical direction found upto 27 mm and 45 mm, around root surface in Vertisol and Inceptisol, respectively, might be due to extensive root growth and long duration to which crop was grown. This study could provide valuable information in studying P dynamics in flooded rice rhizosphere and modifying P uptake models which required P concentration gradients with definite boundary conditions *i.e* without inter-root competition.

REFERENCES

- Bhat KKS and Nye PH 1973. Diffusion of phosphate to plant roots in soil. I. Quantative autoradiography of the depletion zone. *Plant Soil* 38 : 161-175
- Bhat KS, Nye PH and Baldwin JP 1976. Diffusion of phosphate to plant roots in soil. IV. The concentration distance profile in the rhizosphere of root with root hairs in a low P soil. *Plant and Soil*. 44 : 63-72
- Bhattacharyya P, Datta SC 2004. Modification of phosphate uptake model considering buffering capacity of soil and release threshold limit of phosphorus in soil due to rhizosphere effect. *Communications in soil science and plant analysis*. 35 (13 &14) : 1773-1792
- Boero G and Thien S 1979. Phosphate activity and phosphorus availability in the rhizosphere corn. *In : The Soil root interface*. J.L. Harley and R.S. Russell (ed.) Academic Press. London pp 231-242
- Darrah PR 1993. The rhizosphere and plant nutrition : A quantitative approach. *Plant and Soil*. 155 : 1-20
- Gahoonia TS, Asmar F, Giese H, Nielsen GG and Nielsen NE 2000. Root-released organic acids and phosphorus uptake of two barley cultivars in laboratory and field experiment. *European J. of Agronomy*.12:281-289
- Harman GW and Jager G 1963. Determination of the quantity of carbon and nitrogen in rhizosphere of young plants. *In : Soil organicm*. J Doeksen and J rander Driff (ed.) North Holland. Amsterdam. pp 245-251
- *Helal HM and Sauerbeck D 1981. Fin. Verfahren Zur Trennung von Bodenzonen untorschiedlicher Wurzelnane. *Z. Pflanzonernahr. Bodenk* 144 : 524-527
- *Helal HM and Sauerbeck D 1991. Phosphatumsetzungen Wurzelraum von pflanzen. *Milteilgn. Ptsch. Bodenkundl. Gesellsch.* 32 : 295-304
- Hinsinger P 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil*. 237:173-195
- Hinsinger P and Gilkes RJ 1996. Dissolution of phosphate rock in the rhizosphere of the five plant species grown in an acid. P-fixing mineral substrate. *Geoderma*. 75 : 231-249
- Kirk GLD and Saleque MA 1995. Solubilization of phosphate by rice plants growing in reduced soil : Prediction of the amount solubilized and the resultant increase in uptake. *Eur. J. Soil Sci.* 46 : 247-255
- Kraus M, Fussedder A and Beek E 1987. *In situ* determination of phosphate gradient around a root by radiography of frozen soil section. *Plant Soil*. 97 : 407-418
- Kuchenbuch R and Jungk A 1982. A method for determining concentration profiles at the soil root interface by thin slicing rhizospheric soil. *Plant Soil*. 68 : 391-394
- Li Y and Barber SA 1991. Calculating changes of legume rhizosphere soil pH and soil solution phosphorus from phosphorus uptake. *Commun. Soil Sci. Plant Anal.* 22 (9) : 955-973
- Lindsay WL, Vlek PLG and Chien SH 1989. Phosphate minerals. *In : Minerals in Soils Environment*. 2nd ed. Soil Sci. Soc. of Am. Madison W.I. pp 1089-1130
- Olsen SR 1953. *Soil and Fertilizer phosphorus in crop nutrition* W.H. Pierse and A.G. Norman (eds.) *Agron. Monogr.* 4 : Acad. Press. New York, pp 80
- Parfitt RL 1978. Anion adsorption by soil and soil materials. *Adv. Agron.* 30 : 1-50
- Shaviv A, Shnek M, Shavit U and Ravina I 1992. Modelling nutrient uptake considering sorption kinetics : Phosphorus uptake from one dimentional rhizosphere. 15 (7) : 1099-1114
- Wang ZY, Kelly JM and Kovar JL 2004. In situ dynamics of phosphorus in rhizosphere solution of five species. *J Environ. Quality*. 33:1387-1392