Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 3, p. 220-227, 2015
http://www.innspub.net

INNSPUB
RESEARCH PAPER
OPEN ACCESS

Potential of phosphorus solubilizing microorganisms to transform soil P fractions in sub-tropical Udic Haplustalfs soil

Ahmad Ali Khan[1*], Ghulam Jilani[2], Muhammad Saleem Akhtar[2], Muhammad Islam[1], S.M. Saqlan Naqvi[2]

[1]*National Fertilizer Development Centre, Ministry of Planning, Development and Reform, Islamabad, Pakistan*

[2] *PMASArid Agriculture University, Rawalpindi, Pakistan*

Article published on September 28, 2015

Key words: Soil Ptransformation, Phosphate solubilizingbacteria/fungi,Calcium phosphate, Maize, Bio-available P.

Abstract

The objective of the present study was to investigate the effect of phosphorus solubilizing microorganisms (PSMs) for their potential to transform soil phosphorus fractions. For the purpose, non-calcareous sieved subtropical Udic Haplustalfs soil (Kahuta soil series) was thoroughly mixed with PSM strains, two each from bacteria and fungi and was filled with plastic pots. Maize (*Zea mays*) was grown in all pots till 90 days after sowing. Inorganic phosphorus fertilizer was not applied in any pot. Soil samples were taken at 30 day interval till 90th day and were analyzed for different phosphorus fractions sequentially. The results revealed that there was significant increase in bio-available P fractions, *viz.*, Olsen and di-calcium bound phosphorus (Ca_2-P) with inoculation of PSM. Concomitantly, there was significant decline in apatite (Ca_{10}-P) and octa-calcium phosphate (Ca_8-P) with the passage of time. However, occluded soil P fraction remained unaffected by PSM inoculation. Moreover, fungal isolates were more effective in transforming unavailable P fraction into bio-available forms as compared to their bacterial counterparts. Phosphorus solubilizing microorganisms transform soil aluminum and iron bound P fractions to bio-available phosphorus fractions such as di-calcium phosphate and Olsen phosphorus with the passage of time.

***Corresponding Author:** Ahmad Ali Khan ✉ awan.aak@gmail.com

Introduction

Phosphorus nutrition is critical in soil fertility management and crop production under subtropical climatic conditions and calcareous soil regimes. There is no biological source to replenish the soil P pool unlike nitrogen. Phosphorus orthophosphates predominately occurs in soil inorganic fractions either adsorbed to soil mineral surfaces or assparingly available precipitates and is accounted for up to 70 percent of total soil P (Richardson, 2011). Its availability to crop plants remained restricted despite very high total P contents in many cropping environments. The P deficiency is mainly attributed to the strong sorption of PO_4^{3-} to oxides of Al, Fe and Ca which in turn transform large applied available P pool to unavailable P forms. Agriculturally important soils accumulate large reserve of insoluble P*viz.*, Ortho-phosphates or oxides/ hydrated oxides of Al, Fe, Mn etc. due to regular P fertilization.Only small fraction i.e. 1.0 mg kg^{-1} out of total soil P (1200 mg kg^{-1}) may be available to the crop plants due to the comparatively low content of P in the parent material, but also due to the high reactivity of inorganic P that results in strong retention by the soil's mineral matrix (Jones and Oburger, 2011).

Use of phosphate solubilizing microorganisms have vital role in solubilizing the insoluble forms of phosphorus. Soil microbes, *viz.*, bacteria and fungi, solubilize hardly soluble mineral phosphate in soil which indeed is an important aspect in agricultural ecosystems. They solubilize the native or applied soil P through lowering of pH either by carboxylic acid production or proton extrusion that convert the insoluble phosphates into soluble monobasic and dibasic ions to help chelation and exchange reaction (Rodriguez *et al.,* 2004). Moreover, P bio-availability enhancement by these microorganisms includes mineralization of organic forms of P by phosphatases that transform P from non-available, organically bound forms into bio-available phosphate ions (Scervino *et al.*, 2010).Therefore utilizing potential PSM strains is inevitable with an aim of reducing our reliance on mineral fertilizers. Encompassing above discussion, a study was designed to investigate the phosphorus solubilising microbes for their potential to transform the soil P fractions under non-calcareous soil regime.

Material and methods

Soil samples (in triplicate) were taken from three different locations of Kahuta soil series in the pothwar region (Rawalpindi division). All the sampling sites were fallow with moisture contents below field capacityat the time of sampling. The mean annual rainfall varies from 750 mm; of which more than 70 percent received in the months of July to September (monsoon season). Average temperature of thee xperimental soil was 24.5^{o}C. Major crops of the area are groundnut, wheat, millet and maize. Soil management practices are poor, mostly without involving modern technology due to lack of awareness and economic constraints faced by of the farmers. Tillage is carried out usually with cultivators up to 12-15 cm soil depth whereas some farmers occasionally use mould board plough down to20 cm depth.

The soil series developed from underlying tertiary siwalik sandstone parent material, very deep well drained, moderately fine and medium textured, viz., sandy loam to sandy clay loam with argillic B horizon, dark brown (10YR 4/3) surface and are slightly to calcareous in nature. The soils are Udic Haplustalfs according to USDA system of soil taxonomy with average surface pH of 7.09, average surface $CaCO_3$ contents of 7.33 g kg^{-1} and initial average total P of 327 mg kg^{-1}. Basic information about sampling sites has been presented in Table 1.

Two genetically distinct species each from fungi or bacteria and isolated from the same soil series in another experiment were grown afresh on Pikovskaya's broth (Pikovskaya, 1948). Pure cells were suspended in sterilized saline solution of 0.6% NaCl for final cell suspension of 0.5 at OD600 to give 10^8 (bacteria) or 10^5 (fungi) CFU g^{-1} of sterilized peat soil (1:3 v/w). Sieved autoclaved soil (500 g) was thoroughly mixed with above mentioned fungi or bacteria. Treatments were applied in triplicate, comprising of control without any inocula (P0), bacteria isolate B1 *(Burkholderiacepacia)*, bacteria isolate B2 *(Bacillus subtilis)*, fungal isolate F1 (*Penicilliumpinophilum)* and fungal isolate F2 (*Aspergillusniger)*. Phosphorus fertilizer was not applied in any treatment.

Soil samples from soil filled pots and sown with maize (*Zea mays*) were taken at 30 day interval till 90thdayafter sowing. Crops related parameters and data has not been given here. The samples wereanalyzed for soil P forms such as di-calcium phosphate (Ca_2-P), Octa-calcium phosphate (Ca_8-P), aluminium phosphate (Al-P), iron phosphate (Fe-P), occluded phosphorus (O-P) and apatite (Ca_{10}-P). Soil inorganic P fractions were determined according to a modified fractionation scheme of Gu and Jiang(1989) based on methods described by Hedley *et al.* (1982). In the Pi fractionation scheme, soil P_i was divided into aforesaid six fractions using a sequential extraction procedure with a) 0.25 mol L^{-1} $NaHCO_3$ solution at pH 7.5 to remove Ca_2-P, b) 0.5 mol L^{-1} NH_4Ac at pH 4.2 to remove Ca_8-P, c) 0.5 mol L^{-1} NH_4F at pH 8.2 to remove Al-P, d) 0.1 mol L^{-1} NaOH-0.1 mol L^{-1} Na_2CO_3 to remove Fe-P, e) 0.3 mol L^{-1} Na_3(citrate)-$Na_2S_2O_4$-0.5 mol L^{-1}NaOH solution to remove occluded P, and f) 0.25 mol L^{-1} H_2SO_4 to remove Ca_{10}-P.

Total phosphorus (TP) in soil was assayed through digestion with perchloric acid and optical density measurement of aliquot at wavelength of 882 nm as described by Watanabe and Olsen (1965). Olsen-P was determined by extraction of air-dry soil with 0.5 mol L^{-1} $NaHCO_3$ at pH 8.5 (Olsen *et al.,* 1954). Titrimetric procedure based on dissolution of lime and subsequent absorption of CO_2 by aqueous KOH was used for $CaCO_3$ determination (Loeppertand Suarez,1996).

The data collected were analysed using variance technique in complete randomized design with factors followed by mean comparison with Duncan's multiple range test for least significant difference (Steel*et al.,* 1997). Moreover, computer software "MSExcel" and "MSTAT-C" was employed for the purpose.

Results and discussion

Ca_2-P contents in soil

The Ca_2-P represents monocalcium phosphate [$Ca(H_2PO_4)_2$] and di-calcium phosphate ($CaHPO_4 \cdot 2H_2O$) equivalents. The Ca_2-P includes water-soluble P, citrate soluble P and partial surface-adsorbed P, and can be readily taken up by plants. Data revealed that inoculation of soil with bacteria or fungi resulted in significant increase in Ca_2-P contents of soil (Table 2).

Table 1. Basic information regarding soil sampling site.

Soil series	Site Description						
	Location	Elevation (feet)	Latitude	Longitude	$CaCO_3$ g kg^{-1}	pH	Total P mg kg^{-1}
Kahuta	ThandaPani	2071±18	33° 36´	73° 25´	5.91	6.94	343
	Morian	2130±16	33° 31´	73° 23´	6.39	7.12	315
	ChakDaulat	1744±14	33° 18´	73° 10´	9.70	7.21	322
Average					7.33	7.09	327

Sato *et al.*(2005) reported that organic acids produced by PSMs might delay the crystallization and transformation to stable Ca–P forms such as hydroxyapatite by chemical bonding or adsorption onto the crystalline surfaces, thus favouring existence of soluble P form. Significant increase in Ca_2-P contents of soil was recorded as duration after sowing increased from 30th to 90th day. The solubility of P is mostly controlled by the sorption of P on calcite or by formation of di-calcium phosphate (Ma *et al.,* 2009). Phosphorus solubilising microorganism secrete organic acid or/ and proton that help release insoluble P by proton substitution or complexing acid anions with Ca^{2+}(Takeda and Knight, 2006).

Ca_8-P contents in soil

The Ca_8-P represents a group of phosphates with chemical structure similar to $Ca_8H_2(PO_4)_6{\cdot}5H_2O$. The Ca_8-P fraction belongs to the sparingly soluble P, but can be partly used by plants. Different microbial inocula had significant declining effect on soil Ca_8-P contents (Table 3). The lowest value of Ca_8-P content was recorded in F2(*Aspergillusniger) viz.*,95.5 mg $kg^{-1}$and F1 *(Penicilliumpinophilum) viz.*,96.4 mg kg^{-1} followed by 101 mg kg^{-1}in B1 *(Burkholderiacepacia)* and highest (114 mg kg^{-1} soil) in non-inoculated control. There was gradual decline in soil Ca_8-P content from 105.7 mg kg^{-1} on 30th day to 97.6 mg kg^{-1} on 90th day. Oxmann and Schwendenmann(2015) reported that microbial mediated pH decline solubilized the metastable Ca_8–P form.

Table 2. Ca_2-P contents (mg kg^{-1} soil) as affected by microbial inocula.

Treatments	Time (days after sowing)			Average
	30	60	90	
P0	7.25f	7.31f	6.28f	6.95 D
B1	7.80ef	9.69c-f	11.9bcd	9.78 BC
B2	6.64 f	8.11def	9.34c-f	8.03 CD
F1	9.45c-f	11.5b-e	14.3ab	11.8 B
F2	12.1bc	14.2ab	16.0a	14.1 A
Average	8.64 A	10.2 AB	11.6 A	

Means with different letter (s) within the column, differ significantly at5%levelofprobability.

Table 3. Ca_8-P contents (mg kg^{-1} soil) as affected by microbial inocula.

Treatments	Time (days after sowing)			Average
	30	60	90	
P0	114 a	116 a	113ab	114 A
B1	105bcd	101cde	96.1ef	101 BC
B2	108abc	102cde	97.4def	102 B
F1	102cde	96.4def	91.0 f	96.4 CD
F2	99.6 de	96.2ef	90.9 f	95.5 D
Average	105.7 A	102 B	97.6 C	

Means with different letter (s) within the column, differ significantly at5%levelofprobability.

Al-P content in soil

The Al-P (Al phosphates) has very low availability to plants under calcareous soil conditions, but can be constrainedly utilized by plants when available soil P is severely depleted. It is mostly dominant form of P in acidic soils.Application of different inocula resulted in significant decrease in Al-P contents (Table 4). Fungal isolates were more effective in decreasing soil Al-P contents as compared to bacterial inocula. Oliveira *et al.*(2009) advocated that fungal population was more effective than bacteria in solubilizing P sources of aluminium. Decrease in soil Al-P content with the passage of time after sowing from 45.7 mg kg^{-1} on 30th day to 42.3 mg kg^{-1} on 90th day was recorded. Reduction in Al-P and Fe-P fractions of P was due to the fact that Al and Fe bound P is chemically mobilized by production of carboxylic acids by PSMs(Henri*et al.*, 2008). These acids solubilized P fixed with Fe or Al either through direct dissociation of mineral phosphate by anion exchange of PO_4^{3-} with acid anion, or by chelation of both Fe and Al ions associated with phosphate (Omar, 1998). Moreover, acid production by PSM decrease soil pH that favours the carbonate substitution in the apatite mineral in calcareous soils and is generally associated with the increase of free Al– and Fe–oxides instead of their complexes with phosphates (van Straaten, 2002)

Table 4. Al-P contents (mg kg^{-1} soil) as affected by microbial inocula.

Treatments	Time (days after sowing)			Average
	30	60	90	
P0	47.5ab	49.1 a	48.0ab	48.2 A
B1	46.2abc	43.6abc	42.6 a-d	44.1 BC
B2	48.0ab	46.2abc	45.0abc	46.4 AB
F1	44.4abc	40.6bcd	39.7 cd	41.6 CD
F2	42.5 a-d	39.0 cd	36.1 d	39.2 D
Average	45.7 A	43.7 AB	42.3 B	

Means with different letter (s) within the column, differ significantly at5%levelofprobability.

Fe-P contents in soil

The Fe-P (Fe phosphates) has low solubility in calcareous soil regime in addition to its limited plant bioavailability. The Fe-P can be constrainedly utilized by plants for their survival when available soil P is severely depleted. Soil treatment by PSM resulted in significant decrease in Fe-P contents as compared to control (Table 5). Liptzin and Silver (2009) reported that microbial activity and corresponding fluctuations in redox potential released Fe–bound soil P. Henri *et al.*(2008) and Jha*et al.*(2013) reported that carboxylic acids mainly solubilize Al–P and Fe–P through direct dissolution of mineral P because of anion exchange of PO_4^{3-} by acid anion or by chelation of both Fe and Al ions associated with phosphate.

Table 5. Fe-P contents (mg kg^{-1} soil) as affected by microbial inocula.

Treatments	Time (days after sowing)			Average
	30	60	90	
P0	54.8 a	51.2ab	49.2abc	51.7 A
B1	51.4ab	47.4abc	39.4cde	46.1 BC
B2	53.4 a	48.5abc	42.4 b-e	48.1 AB
F1	48.4abc	45.4 a-d	36.4 de	43.4 BC
F2	46.5 a-d	42.1 b-e	34.2 e	40.9 C
Average	50.9 A	46.9 A	40.3 B	

Means with different letter (s) within the column, differ significantly at5%levelofprobability.

Occluded P content in soil

There was non-significant effect of PSM inoculation on Occluded-P contentsof soil (data not shown). This may be due to fact thatoccluded phosphorus refers to phosphorus present within the mineral matrix of discrete mineral phases and remains unaffected by chemical changes occurring in the surroundings(Schrijver*et al.*, 2012).

Ca_{10}-P contents in soil

The Ca_{10}-P represents a group of phosphates with chemical structure similar to $Ca_{10}(PO_4)_6{\cdot}(OH)_2$, which is difficult to be used by plants.Soil inoculation with fungi resulted in decline of Ca_{10}-P contents as compared to bacteria and un-inoculated control (Table 6). Sahoo and Gupta (2014) reported that PSM through secretion of organic acids / pH induced apatite dissociation or cation complex formation through microbial carboxylic anions production help solublizedCa bound P. Different microbial inocula had significant declining effect on soil Ca_{10}- P contents which is in line with the findings of Yousefi*et al.*(2012). Bacteria produce biological materials such as auxin, gibbrelic acids, hormones and vitamins which cause dissolution of phosphate from inorganic P compounds (Mahdi*et al.*, 2012).

Table 6. Ca_{10}-P contents (mg kg^{-1} soil) as affected by microbial inocula.

Treatments	Time (days after sowing)			Average
	30	60	90	
P0	107ab	100abc	103abc	104 A
B1	95.2bcd	79.4 d-h	77.3 e-h	84.0 B
B2	113 a	93.6 b-f	94.6 b-e	101 A
F1	88.1 c-g	75.3gh	74.3gh	79.2 B
F2	76.7fgh	65.6 h	64.4 h	68.9 C
Average	96.1 A	82.8 B	82.8 B	

Means with different letter (s) within the column, differ significantly at5%levelofprobability.

Olsen – P contents in soil

Different microbial inocula had significant effect on soil Olsen -P contents (Table 7). The highest Olsen P content (15.6 mg kg^{-1}) were recorded in F2 (*Aspergillusniger)*, followed by F1 (*Penicilliumpinophilum)* and the lowest (8.67 mg kg^{-1}) in un-inoculated control. Acevedo *et al.*(2014) found that isolates of *Aspergillus* and *Penicillium* showed higher solubilization activities (82 and 80%, respectively) when compared with *Klebsiella*and *Burkholderia*genus of bacteria. Soil Olsen-P increased with the passage of time after sowing from 9.04 mg kg^{-1} on 30^{th} day to 13.1 mg kg^{-1} on 90^{th} day. These results are in conformity with the findings of Yousefi*et al.*(2012) who observed 126 percent increase in Olsen P with application of PSM as compared to control. The PSM produce carboxylic and other organic acids reducing soil pH to a level optimum for solubilization of inorganic fixed P (Reena*et al.*, 2014).

Table 7. Olsen P contents (mg kg^{-1} soil) as affected by microbial inocula.

Treatments	Time (days after sowing)			Average
	30	60	90	
P0	8.59fgh	9.11 e-h	8.30gh	8.67 D
B1	8.08gh	13.1bcd	12.8 b-e	11.3 BC
B2	6.90 h	10.0 d-h	11.4 c-g	9.42 CD
F1	9.26 e-h	13.5bcd	14.6bc	12.4 B
F2	12.4 c-f	16.3ab	18.2 a	15.6 A
Average	9.04 B	12.4 A	13.1 A	

Means with different letter (s) within the column, differ significantlyat5%levelofprobability.

Conclusion

Various phosphate solubilising bacteria and fungi have similar pattern to transform the P forms in non-calcareous soil. Furthermore, fungal isolates have greater P solubilization potential than bacteria. Calcium bound P forms are more prone to transformation than Al-P or Fe-P while Occluded P fraction is not affected by PSM inoculants.

Acknowledgement

Higher Education Commission of Pakistan is thankfully acknowledged for sponsoring the study.

References

Acevedo E, Galindo–Castaneda T, Prada F, Navia M, Romero HM. 2014. Phosphate–solubilizing microorganisms associated with the rhizosphere of oil palm (*Elaeisguineensis*Jacq.) in Colombia. Applied Soil Ecology **80,** 26–33.

Gu Y, Jiang B. 1989. A suggested fractionation scheme of inorganic phosphorus in calcareous soils. Fert. Res. 20, 159–165.

Hedley MJ, Stewart JWB, Chauhan BS. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. **46,** 970–976.

Henri F, Laurette NN, Annette D, John Q, Wolfgang M, François-Xavier E, Dieudonné N. 2008. Solubilization of inorganic phosphates and plant growth promotion by strains of *Pseudomonas fluorescens*isolated from acidic soils of Cameroon. African Journal of Microbiological Research **2,** 171-178.

Jha A, Saxena J, Sharma V. 2013. An investigation on phosphate solubilization potential of agricultural soil bacteria as affected by different phosphorus sources, temperature, salt and pH. Communication in Soil Science and Plant Analysis **44,** 2443–2458.

Jones, DL, Oburger E. 2011. Solubilization of Phosphorus by Soil Microorganisms. In: BünemannEK*et al.,*Eds. Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling. Springer-Verlag Berlin Heidelberg 169-198 p.

Liptzin D, Silver WL. 2009. Effects of carbon addition on iron reduction and phosphorus availability in a humid tropical forest soil. Soil Biology and Biochemistry **41,** 1696–1702.

Loeppert RH, Suarez DL. 1996. Carbonate and gypsum. In: SparksDL, Ed. Methods of Soil Analysis, Chemical Methods – part 3, 3rd ed. SSSA Series No. **5.** Soil Sci. Soc. Am. Madison, WI, USA, 437–474 p.

Ma B, Zhou ZY, Zhang CP, Zhang G, Hu YJ. 2009. Inorganic phosphorus fractions in the rhizosphere of xerophytic shrubs in the Alxa Desert. Journal of Arid Environment **73,** 55–61.

Mahdi, SS, Talat MA, Dar MH, Hamid A, Ahmad L. 2012. Soil phosphorus fixation chemistry and role of phosphate solubilizing bacteria in enhancing its efficiency for sustainable cropping-A review. Journal of Pure and Applied Microbiology **6(4),** 7-9.

Oliveira CA, Alves VMC, Marriel IE, Gomes EA, Scotti MR, Carneiro NP, Guimaraes CT, Schaffert RE, Sa NMH. 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biology and Biochemistry **41(9),** 1782–1787.

Olsen SR, Cole CV, Watanabe FS, Dean LA. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular No. 939, US Government Print Office, Washington, DC.

Omar SA. 1998. The role of rock phosphate solubilizing fungi and vesicular arbuscularmycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World Journal of Microbiology and Biotechnology **14,** 211–219.

Oxmann JF, Schwendenmann L. 2015. Authigenic apatite and octacalcium phosphate formation due to adsorption–precipitation switching across estuarine salinity gradients. Biogeoscience **12,** 723–738.

Pikovskaya RI. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiology **17,** 362-370.

Reena T, Dhanya H, Deepthi MS, Pravitha D. 2013. Isolation of phosphate solubilizing bacteria and fungi from rhizospheres soil from banana plants and its effect on the growth of *Amaranthuscruentus* L. IOSR Journal of Pharmacy and Biological Science **5,** 6–11.

Richardson AE, Simpson RJ. 2011. Soil microorganisms mediating phosphorus availability. Plant Physiology **156,** 989–996.

Rodriguez H, Gonzalez T, Goireand I, Bashan Y. 2004. Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturewissenschaften **91,** 552-555.

Sahoo HR, Gupta N. 2014. Phosphate–solubilizing fungi: Impact on growth and development of economically important plants. In: Khan MS, ZaidiA, JavedM, Eds. Phosphate Solubilizing Microorganisms. Springer Switzerland 87–112 p.

Sato S, Solomon D, Hyland C, Ketterings QM, Lehmann J. 2005. Phosphorus speciation in manure and manure–amended soils using XANES spectroscopy. Environmental Science and Technology **39,** 7485–7491.

Scervino JM, Mesa MP, Mónica ID, RecchiM, Moreno NS, Godeas A. 2010.Soil fungal isolates produce different organic acid patterns involved inphosphate salts solubilization. Biol. Fertil. Soils **46,** 755–763.

Schrijver AD, Vesterdal L, Hansen K, Frenne PD, Augusto L, Achat DL, Staelens J, Baeten L, Keersmaeker LD, Neve SD, Verheyen K. 2012. Four decades of post–agricultural forest development have caused major redistributions of soil phosphorus fractions. Oecologia, Springer Switzerland **169(1),** 221–234.

Steel RGD, Torrie JH, Dickie DA. 1997. Principles and procedures of statistics – a biometric approach, 3rd ed. Mc Graw Hill Publishing Company, Toronto.

Takeda M, Knight JD. 2006. Enhanced solubilization of rock phosphate by *Penicilliumbilaiae* in pH-buffered solution culture. Canadian Journal of Microbiology **52,** 1121-1129.

Van Straaten P. 2002. Rocks for Crops: Agrominerals of Sub–Saharan Africa. ICRAF, Nairobi, Kenya, 338 p.

Watanabe FS, Olsen SR. 1965. Test of ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Science Society of America Proceedings **29,** 677–678.

Yousefi AA, Barzegar AR, Sadeghi M. 2012. Effects of Phosphate Solubilizing Bacteria and ArbuscularMycorrizal Fungi on contribution of inorganic phosphorus fractions. Advances in Environmental Biology **6,** 2323– 2328.