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Acidulated activation of phosphate rock enhances release, lateral transport and uptake of phosphorus and trace metals upon direct-soil application

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ABSTRACT

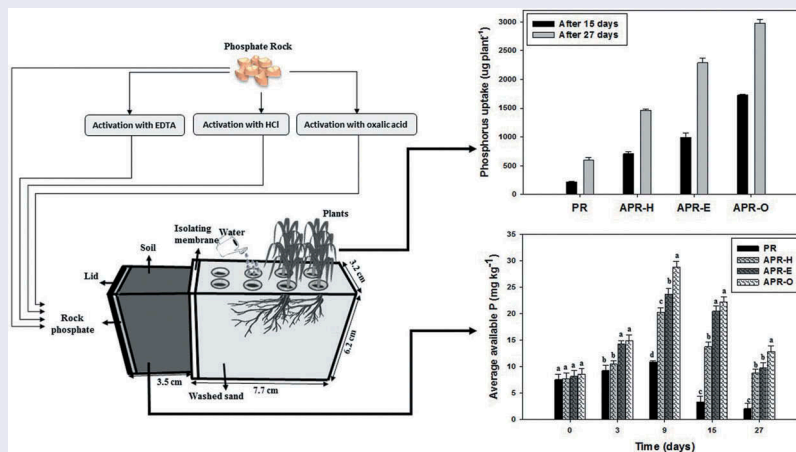
Phosphate rock (PR) was activated via acidulation with HCl, EDTA, and oxalic acid to enhance its reactivity. The release, lateral transport, and uptake of phosphorus (P) along with trace metals from pristine and activated PRs were investigated in a soil micro-block system over a period of 27 days, using wheat (*Triticum aestivum* L.) plants. Significantly ($p < 0.05$) higher amounts of available soil P, Fe, Mn, and Zn were released from all the PRs after application to soil within first 9 days of seedling transplantation, while the release of other trace metals (Cd, Co, Cr, Cu, Ni, and Pb) was minimal ($<1.2 \text{ mg kg}^{-1}$). On cumulative basis, APR-O (oxalic acid activated PR) was the most efficient amendment releasing 164% more available P, followed by APR-E (EDTA activated PR) releasing 130% more available P, compared to the pristine PR. Similar results were also observed in the release of available Fe, Mn, Zn, and other trace metals. The highest diffusive mass fluxes for available P, Mn, Fe, and Zn in soil were observed after 3 days of seedling transplantation, which reduced subsequently. The uptake of P, Fe, Mn, and Zn by wheat plants was increased by 394%, 715%, 92%, and 91%, respectively, in APR-O application compared to the pristine PR, while it was increased by 280%, 188%, 16%, and 27%, respectively, in APR-E application compared to the pristine PR. Subsequently, APR-O and APR-E amendments resulted in enhanced shoot lengths, root lengths, shoot dry matter, and root dry matter contents of wheat plants. Hence, it was concluded that activation of PR with oxalic acid and EDTA prior to direct soil application may enhance the reactivity of PR and could serve as a cost-effect fertilization strategy for higher wheat crop production.

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Diffusive mass flux; plant growth; release; activation; uptake



1. Introduction

Phosphorus (P) is the second most common growth-limiting macronutrient responsible for lower crop production. It is associated with many metabolic components and enzymatic reactions within the plant. Unavailability of P to plants during early growth stages results in reduced root growth and seed formation (Ahmad *et al.* 2018a, 2018b). The availability of P to plants depends on its concentration in soil solution which subsequently depends on several factors such as soil mineralogy, pH, concentration of

soluble Ca, Fe, and Al, organic matter, and microbial activities (Guppy *et al.* 2005; Stewart and Tiessen 1987). Despite the fact that many agricultural soils contain higher amounts of total P, only a small proportion of it could become available to the plant. It has been reported that more than 80% of the soil added P immediately converts to unavailable forms due to a series of reactions such as complexation of P with Fe, Al (acid soils), and Ca (alkaline soils), adsorption, precipitation, and/or oxidation-reduction (Holford 1997; Al-Rohily *et al.* 2013). Therefore, the researchers are in

continuous surge to develop new strategies to improve P availability to the plants after P fertilization.

One of the most important sources of available P is water soluble P fertilizers; nonetheless, their application has many concerns such as higher prices, conversion of soil applied P into nonavailable forms, and presence of certain toxic trace metals in phosphate fertilizers (Mehmood *et al.* 2009). Therefore, utilization of low-cost local P sources such as phosphate rock (PR) to supply P and other essential nutrients to the plants for a sustainable crop production has gained much attention of the researchers (Rafael *et al.* 2018). The PR is composed of apatite group in addition to other minerals such as carbonates, fluorides, silicates, quartz, and metal oxides (Harris 2002). The PR is used in manufacturing the commercial P fertilizers and can also be used as a direct source of P; however, its direct application is considered inefficient due to lower solubility and reactivity. The efficiency of PR is predicted by determining the rate of dissolution and its reactivity (Ghosal and Chakraborty 2012). Only few PRs are reported to be efficient in releasing P when applied directly to the soil, while majority of them are known as ineffective. A number of constraints associated with the direct application of PR has been reported such as the limited solubility of the most of the phosphate compounds in PR, relative inconvenience in handling the dusty material, and the presence of varying amounts of trace metals (As, Sb, Cr, Pb, etc.), radioactive elements and rare earth metals (Hurst *et al.* 1989; Kpombekou and Tabatabai 2003). The direct application of PR containing higher levels of aforementioned contaminants could pollute soil, environment, and water sources.

Despite the abovementioned concerns, the direct use of PR is still a possible option if it is applied after certain treatments for activation such as partial acidulation, blending with water-soluble P fertilizers (single superphosphate and triple superphosphate), and microbial inoculations (Chien and Hammond 1988). For instance, it has been reported that lower soil pH can increase the effectiveness of PR by releasing more P (Chien *et al.* 2010; Prochnow *et al.* 2006). Van Straaten (2002), Savini *et al.* (2006), and Ivanova *et al.* (2006) reported that P release efficiency of the PRs can be increased by acidulating them prior to direct applications to soils. However, the application of strong inorganic acids is discouraged due to their harsh impact on soil properties and soil microbial communities (Li *et al.* 2017). Conversely, acidulation with organic acids may result in the formation of complexes and chelates with polyvalent cations such as Ca and Mg ions and enhance the solubility of PR.

In real soil-plant system, plant fulfils a larger part of its water requirements by drawing water from the soil. A suction force developed by evapotranspiration activities causes soil solution along with dissolved elements (nutrients) to move toward roots where it is absorbed by the plant roots (Nye and Tinker 1977). In sloppy and waterbodies (river, lakes, etc.) adjacent areas, a major portion of the applied P is lost due to leaching, surface runoff, and lateral movement, subsequently reducing the P availability to plants on one hand and polluting the nearby waterbodies on the other. However, in plain areas, P leaching is considered to be less important (Gächter *et al.* 1998); therefore, lateral transport and surface

runoff are the major contributors for P movement, resulting in contaminating the waterbodies especially in areas with less fertile soils having less adsorption capacities. Hence, it is of great importance to understand the lateral transport of P along with other metals in the soil following P fertilization. Therefore, the objectives of the current study were to investigate the release, lateral transport, and diffusion of P and trace metals from pristine PR and the PRs activated with HCl (hydrochloric acid), EDTA (ethylenediaminetetraacetic acid), and C₂H₂O₄: (oxalic acid). Additionally, the uptake of P and trace metals by wheat (*Triticum aestivum* L.) plants and subsequent changes on wheat growth were investigated in soil micro-block system.

2. Materials and methods

2.1. Soil sampling and characterization and activation

Surface soil samples (0–15 cm) were collected from the Experimental Station of King Saud University in Dirab, Saudi Arabia, by using a wooden spade to avoid contamination with metals. The sample was collected from different spots and a composite soil sample was prepared thereafter, which was brought to the laboratory for further analysis. The sample was dried, grinded, and sieved through a 2-mm screen and subjected to basic soil characterization. Electrical conductivity (EC), pH, and soluble cations and anions were analyzed in saturated soil paste (Richard 1954). Particle size distribution was determined according to method reported by Bouyoucos (1962) by using a hydrometer. Calcium carbonate was determined by following the procedure of Allison and Moodie (1965), while organic matter contents were assessed using Walkley-Black method (Walkley and Black 1934). The soil porosity was analyzed by following the method of Matko (2003). Available P and trace metals in soil were extracted with AB-DTPA solution (Ammonium Bicarbonate-diethylenetriamine-pentaacetic acid) as described by Soltanpour and Schwab (1977), while the total soil P and trace metals were analyzed after microwave (MARS, CEM Corporation, USA) digestion (USEPA 1998). Total and available P in the extracts were determined by molybdate-ascorbic acid method using UV/VIS spectrophotometer (Lambda EZ 150, PerkinElmer, USA), while trace metals were analyzed using ICP-OES (PerkinElmer Optima 4300 DV, USA).

2.2. PR preparation and activation

PR was collected from eastern part of Turaif town, Saudi Arabia. A composite sample of PR was brought to laboratory, grinded, passed through 2-mm screen to remove large size particles, and stored in airtight containers. Particle size distribution of the PR was analyzed using Long Bench Mastersizer (Malvern Instruments, Malvern, England), while pH and EC were determined using 1:5 (w/v ratio) suspension with deionized water. The mineralogical composition of PR was investigated using X-ray diffractometer (MAXima_X XRD-7000, Shimadzu, Japan) using 30 mA Cu K α radiation in continuous scanning mode at the scan speed of 2 degree min⁻¹. The total P was determined after microwave digestion (as mentioned in

previous section), while available P and trace metals in the PR were extracted using 2% formic acid (Rajan *et al.* 1992). Briefly, 100 mL of the extractant was added in 1 g of PR, shaken for 30 min, and filtered. The concentrations of P and metals in the filtrates were analyzed by molybdate-ascorbic acid method using UV/VIS spectrophotometer.

The PR was activated via acidulation with different chemical agents such as HCl, EDTA, and oxalic acid ($C_2H_2O_4$) at a solid to liquid ratio of 1:5. Briefly, 2 g of fine powdered PR was added to 10 mL of 0.5 M HCl, 0.5 M EDTA, or 0.5 M oxalic acid, mixed thoroughly and kept at room temperature ($23 \pm 1^\circ C$) for 5 days to obtain equilibrium. Thereafter, the suspensions were dried in a water bath at $85^\circ C$, and the resultant activated PRs (APRs) with HCl, EDTA, and oxalic acid were labeled as APR-H, APR-E, and APR-O, respectively.

2.3. Soil micro-block experiment

Soil micro-block system was used to study the lateral transport of released P and trace metals from pristine and APRs (Fig. 1A). The micro-block was made up of plexiglass comprising of two blocks, i.e., a sand block with dimensions of 7.70 cm length, 6.20 cm height, and 3.20 cm width containing eight holes on top, and a soil block with dimensions of 3.50 cm length, 6.20 cm height, and 3.20 cm width. The opened end of the soil block was covered with detachable lid. The white sand was washed with 30% H_2O_2 , 10% HCl, and deionized water for several times to remove organic matter, carbonates, and other impurities. The sand and soil were dried and filled in sand and soil blocks, respectively, by gently tapping the blocks. The density of sand and soil after filling were noted as 1.57 (about 240 g in each block) and 1.40 g cm^{-3} (about 98 g in each block), respectively. Soil and sand in the blocks were moistened to their field capacities. Both the blocks were joined together using petroleum jelly. To avoid mixing of sand with soil, two media were separated with an isolating membrane, which allows solute to pass through it, while

blocks free movement of soil and sand particles across it. A uniform layer of each PR (2 g) was developed on the open end of the soil block and moistened with a few drops of deionized water. The lid was joined with petroleum jelly to the open end of the soil block. The seeds of wheat cultivar Kawmy-1 were germinated on petri plates on cotton swabs to 5 cm length. Four wheat seedlings were transplanted to the holes of sand block (one seedling per hole), while other four holes were kept without seeds to refill deionized water to maintain field capacity in the sand block (Fig. 1A). The whole unit was covered with adhesive taps from all sides except top to prevent leakage of water as well as to elude light. The micro-block units were arranged in completely randomized design with three replications and kept in a growth chamber at $25^\circ C \pm 1^\circ C$. The moisture was maintained in sand block at field capacity using deionized water. After 0, 3, 9, 15, and 27 days of seedling transplantation, triplicates blocks of each PR were taken, dismantled, soil in the blocks was taken out smoothly as a block of soil (without breakage) and divided into five equal sections each having 5 mm width. The section immediate to the PR layer was labeled as L_1 (0–5 mm), followed by L_2 (5–10 mm), L_3 (10–15 mm), L_4 (15–20 mm), and L_5 (20–25 mm). These sections were dried in air for 3 days, ground, and subjected to further analyses. The available contents of P and trace metals in each soil layer were analyzed through UV/VIS spectrophotometer and ICP-OES, respectively, after extraction with AB-DTPA. Wheat plants (including roots) were separated from sand smoothly washed with tap water to remove sand particles, with EDTA to remove possibly entrapped metals and finally with deionized water. Extra water from the plants was wiped out smoothly with a tissue paper and plant height and fresh weight were recorded. Plants samples were kept in paper bags for dryness at $65^\circ C$ in an oven. After completely dried, dry weight was recorded and samples were digested according to Wolf's (1982) procedure and subjected to P and trace metal analyses. As the plants were grown in washed sand (free of nutrients, carbonates, and organic matter) and

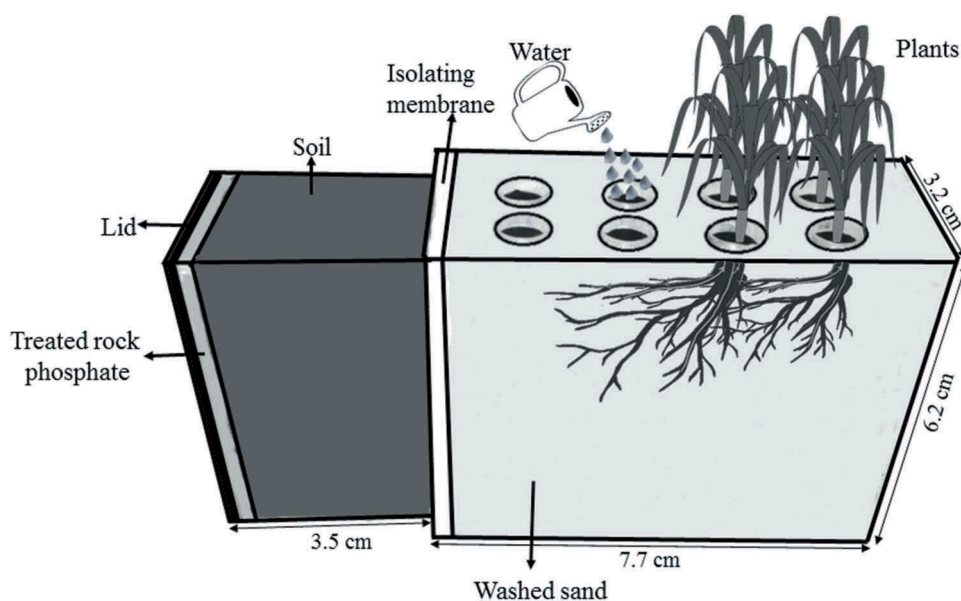


Figure 1. Soil micro-block system showing the mechanism it involves to study lateral transport of phosphorus and trace metals in soil under unsaturated conditions.

no exogenous nitrogen and potassium fertilization was applied, therefore the uptake of these nutrients was not analyzed in plant tissues.

2.4. Lateral transport in soil in micro-block system

One-dimensional diffusive flux of elements (P and trace metals) in soil under unsaturated conditions (at field capacity) were calculated by using an equation given by Porter *et al.* (1960) based on Fick's first law (Equation (1)).

$$J_d = -\theta_w D_e \frac{dC}{dz} \quad (1)$$

where

J_d is diffusive mass flux ($ML^{-2} T^{-1}$)

θ_w is volumetric water content ($L^3 L^{-3}$)

D_e is effective diffusion coefficient ($cm^2 sec^{-1}$)

$\frac{dC}{dz}$ is the initial concentration gradient ($ML^{-3} L^{-1}$)

M is the amount of P or metals diffused in time t

L is the distance through which diffusion has took place

T is the time for diffusion in seconds

Effective diffusion coefficient D_e was calculated from the equation (Equation (2)) given by Jost (1952) as follows:

$$D = \frac{Mt^2\pi}{A^2(C_2 - C_1)^2t} \quad (2)$$

where Mt is the amount of P or trace metal diffused in time t , A is the cross sectional area of the diffusion, $(C_2 - C_1)$ is concentration gradient of the extractable elements between fertilizer layer and unfertilized soil in block.

Soil structure is an important factor to influence the mobility of ions in the soil. To take in account, the restrictive effect of soil structure, effective diffusion coefficients were divided by square of soil tortuosity Θ . Soil tortuosity was calculated from Sweerts *et al.*'s (1991) relationship (Equation (3)).

$$\Theta^2 = 0.47\Phi + 1.91 \quad (3)$$

where Φ is the porosity of the soil.

2.5. Statistical analysis

Statistix software package 8.1, SPSS and Microsoft Excel programs were used for statistical analysis. Means were compared by using Tukey test with a 5% probability level.

3. Results and discussion

3.1. Characteristics of soil and PR used in the experiment

Physiochemical characteristics of the soil used in the experiment are presented in Table 1. The soil used in the experiment was sandy loam in texture, with water holding capacity of 21.68%, and $CaCO_3$ content of 6.48%. The soil was severely

deficient in organic matter contents with <1.0% (0.73%) with pH 7.67 and EC 0.28 $dS m^{-1}$. The concentrations of cations such as Ca^{2+} , Mg^{2+} , Na^+ , and K^+ in the soil were 2.46, 1.94, 0.5, and 0.11 $cmol_c kg^{-1}$, respectively, while anions such as HCO_3^- , Cl^- and SO_4^{2-} were 0.38, 2.1, and 0.58 $cmol_c kg^{-1}$, respectively, whereas CO_3^- was not detected. Total and plant available P and trace metals in the soil and PR are presented in Table 2. It was noticed that total P in the soil was significantly higher (650.1 $mg kg^{-1}$) than the plant available P (7.36 $mg kg^{-1}$). Concentrations of total trace metals in soil revealed that the contents of Fe were highest (854 $mg kg^{-1}$) among the analyzed metals, followed by Mn, Cr, Zn, and Ni (207.9, 18.8, 18.2, and 14.7 $mg kg^{-1}$, respectively), while Cd was not detected. Available concentrations of Fe and Zn in soil were higher (2.41 and 1.19 $mg kg^{-1}$, respectively) among all the analyzed metals. Contents of total P in the PR were 43,700 $mg kg^{-1}$, out of which only 6.90 $mg kg^{-1}$ was plant available (AB-DTPA-extractable) which corresponds to 0.016% of the total P. Total Fe content in PR was found to be highest (341.05 $mg kg^{-1}$) among the analyzed metals, followed by Zn (37.30 $mg kg^{-1}$), Cr (32.5 $mg kg^{-1}$), and Mn (11.8 $mg kg^{-1}$), while plant available Fe and Zn concentrations were higher (2.82 and 2.39 $mg kg^{-1}$, respectively) compared to other analyzed metals in PR. Results of pH, EC, mineralogical composition, and particle size distribution of the PR are shown in Table 3. The PR was slightly alkaline with pH 7.39, while the EC was found to be 0.80 $dS m^{-1}$. Particle size distribution analyses showed that maximum portion of the PR possessed particles of larger than 0.05 mm (92.96%), while only 3.02% particles were in the size range of 0.002–0.05 mm, and 4.02% were below 0.002 mm. The mineralogical composition of the PR as assessed through XRD indicated that hydroxyapatite (HAp) was the most dominant mineral in the PR, followed by, carbonate fluorapatite (CFAp), apatite (Ap), quartz (Q), and carbonate hydroxyapatite (CHAp). The mineralogical composition of the PR was therefore found containing various minerals such as Ap ($Ca_5(PO_4)_3(OH)$), HAp ($Ca_{10}(PO_4)_6(OH)_2$), CFAP ($Ca_5(PO_4)_3F$), CHAP ($Ca_5(PO_4)_3(OH)$), and Q (SiO_2).

3.2. Temporal P release in soil

The release of plant available P from pristine and APRs after soil application at various time intervals has been represented in Fig. 2. Significant ($p < 0.05$) variations in available P release with different PR amendments and time intervals were observed. The efficiency of P release at each time interval followed the order of: APR-O > APR-E > APR-H > PR. On the basis of cumulative available P release, application of APR-O, APR-E, and APR-H released about 164%, 130%, and 84% more P, respectively, compared to PR. Most significant ($p < 0.05$) changes were observed after 9 days of seedling transplantation, where the highest amount of available P was recorded. After 15 days, P release decreased sharply in PR treatment,

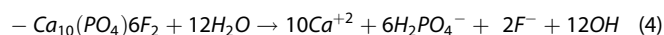
Table 1. Selected physicochemical properties of soil used in the experiment.

Particle size distribution (%)							Soluble cations ($cmol_c kg^{-1}$)				Soluble anions ($cmol_c kg^{-1}$)			
Sand	Silt	Clay	pH	EC ($dS m^{-1}$)	OM (%)	$CaCO_3$ (%)	Ca^{2+}	Mg^{2+}	Na^+	K^+	CO_3^-	HCO_3^-	Cl^-	SO_4^{2-}
67.47	25.15	7.38	7.67	0.28	0.73	6.48	2.46	1.94	0.5	0.11	0.00	0.38	2.10	0.58

Table 2. Total and available heavy metal and phosphorus contents (mg kg⁻¹) in soil and phosphate rock.

	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	P
P and heavy metal contents in soil										
Total	ND	4.95 ± 0.7	18.8 ± 3.1	5.60 ± 1.1	854 ± 31	207.9 ± 17	14.7 ± 1.5	7.53 ± 0.9	18.2 ± 2.1	650.1 ± 28
Available	ND	0.04 ± 0.0	0.02 ± 0.0	0.10 ± 0.0	2.41 ± 0.4	0.48 ± 0.2	0.08 ± 0.0	0.34 ± 0.1	1.19 ± 0.3	7.36 ± 0.6
P and heavy metal contents in phosphate rock										
Total	2.5 ± 0.1	0.45 ± 0.2	32.5 ± 3.4	2.25 ± 0.8	341.05 ± 25	11.8 ± 1.4	3.05 ± 0.8	2.4 ± 0.7	37.3 ± 3.8	43,700 ± 42
Available	0.06 ± 0.0	0.04 ± 0.0	0.03 ± 0.0	0.23 ± 0.1	2.82 ± 0.8	0.72 ± 0.9	0.14 ± 0.0	0.22 ± 0.1	2.39 ± 0.3	6.90 ± 1.0

while interestingly other treatments (APR-H, APR-E, and APR-O) continued to release available P. Our results are in agreement with the findings reported by Kpomblekou and Tabatabai (2003), where they observed that low-molecular weight organic acid treated PR can release available P for longer periods of time. The highest P release with APR-O and APR-E application could be due to acid-induced inactivation of Ca²⁺ during the activation process. The oxalic acid and EDTA might have resulted in chelation of Ca²⁺ subsequently resulting in its unavailability to bind with P in the solution, which eventually resulted in enhanced P release (Reddy *et al.* 1999). Additionally, acidulation process might have reduced the pH and increased the solubility of the PR. Marschner *et al.* (1995) reported in a study that acidulation of soil solution with organic acids (citric, oxalic, and tartaric acid) has enhanced P solubility and availability in soil. The hydrolysis of phosphate ions due to acidulation may result in release of OH⁻ ions in the solution (Equation (4), Rajan *et al.* 1996). The soil tries to neutralize the higher amount of OH⁻ ions consequently increasing the dissolution of PR (Ghosal and Chakraborty 2012).



The higher P release after 9 days could be due to the presence of water soluble and extractable P which reduced subsequently. It has been reported that the maximum P release from PR can be achieved within 8 weeks, and thereafter P release ceases due to its insoluble properties (Barnes and Kamprath 1975; Singh *et al.* 1976; Al-Oud 2011). If accompanied with acidulation, the duration of P release from PR can be extended. Hence, time lag is very critical to be observed from acidulation of PR to the maximum P release (Sinclair *et al.* 1993). Thus, it was suggested that activation of PR with oxalic acid or EDTA prior to direct application in soil could significantly increase the available P release and duration.

3.3. Lateral transport of P in soil

The calculated P diffusive mass fluxes for different PRs over time are presented in Fig. 3. The APR-O showed maximum P diffusive mass fluxes at all the time intervals (except 27 days), followed by APR-E and APR-H, while PR exhibited the minimum P diffusive mass flux. Lowest P diffusive flux in PR could be due to its limited solubility compared to the activated PRs. The diffusive mass fluxes calculated in current study are comparable with the P diffusive mass fluxes reported by Bhadoria *et al.* (1991). The results of P diffusive mass flux indicated maximum diffusion in L₁ soil layer (adjacent to source) after 3 days of seedlings transplantation, compared to other layers. After 9 days of seedling transplantation, the maximum diffusive flux was observed in L₂ suggesting that P has released from the PRs and moving laterally from L₁ to L₂ and so on. It was very interesting to notice that after 9 days of seedling transplantation, P diffusive flux in L₁ reduced by 56%, 38%, 44%, and 41%, in PR, APR-H, APR-E, and APR-O amended soil, respectively, compared to L₁ after 3 days of seedling transplantation. Contrarily, P diffusive flux after 9 days in L₂ increased by 347%, 440%, 480%, and 347% in PR, APR-H,

Table 3. Selected physiochemical characteristics and mineralogy of the phosphate rock.

Property	pH	EC (dS m ⁻¹)	Minerals					Particle size distribution (%)		
			CFAp	HAp	CHAp	Q	Ap	<0.002 mm	0.002–0.05 mm	>0.05 mm
	7.39	0.80						4.02	3.02	92.96

CFAp: Carbonate fluorapatite, HAp: Hydroxyapatite, CHAp: Carbonate hydroxyapatite, Q: Quartz, Ap: apatite

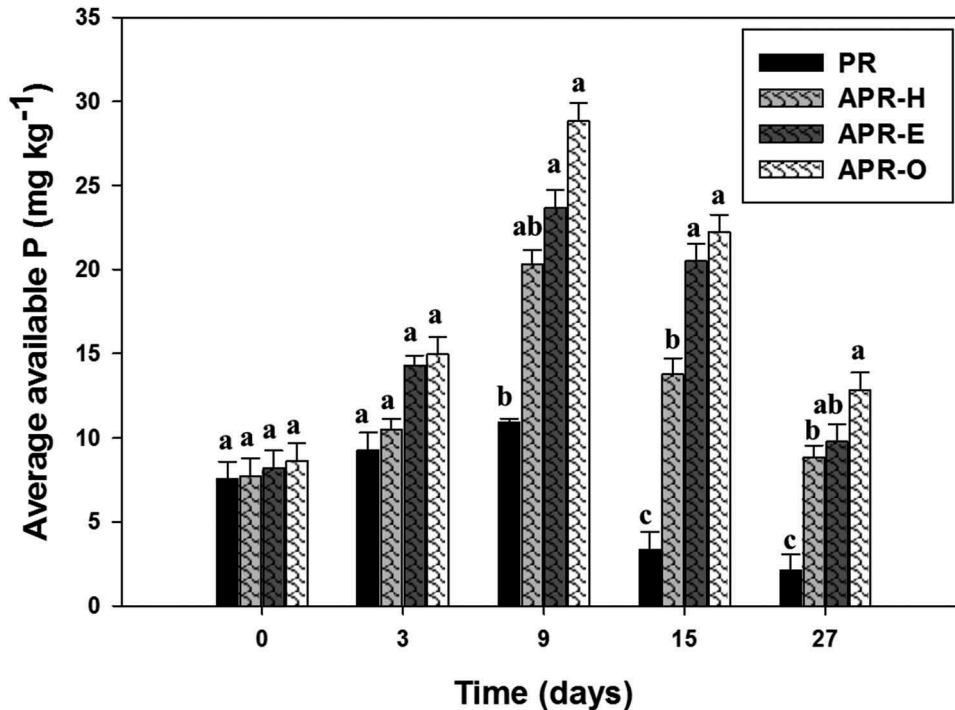


Figure 2. Temporal dynamic of phosphorus (P) released from various phosphate rocks on cumulative basis (PR: phosphate rock, APR-H: HCl activated phosphate rock, APR-E: EDTA activated phosphate rock, and APR-O: oxalic acid activated phosphate rock). (Different letters above bars represent significant difference at $p < 0.05$ among various treatments and bars with same letters represent no significant difference).

APR-E, and APR-O amended soil, respectively, compared to 3 days of seedling transplantation. Reduction in diffusive mass flux in L₁ and increment in L₂ after 9 days of seedling transplantation compared with 3 days after seedling transplantation was due to transport of P from L₁ to L₂ and subsequently to the next layers (L₃, L₄, and L₅). Similar trend was also observed after 15 days of seedling transplantation, where P diffusive flux reduced in L₁ and L₂, while increased in L₃, L₄, and L₅ in all PRs, compared to that after 9 days of seedling transplantation. It was interesting to notice that P diffusive mass fluxes were lowest in pristine PR amended soil in all layers after 15 days, compared to APR-O, APR-E, and APR-H, suggesting that activated PR were more efficient to release P over longer period of time. After 27 days of seedling transplantation, P diffusive mass fluxes were reduced significantly in all the layers, in pristine and APRs (with exception of L₂ of APR-E) compared to 3, 9, and 15 days after seedling transplantation. The higher P releasing capacity of APRs might be attributed to their higher dissolution tendency due to acidulation releasing higher P in soil solution.

Application of oxalic acid and EDTA might have released H⁺ ions to the soil solution, which might have adsorbed on functional groups of surfaces of layer silicate hydroxylated – e.g., silanol, aluminol – and/or to the hydroxyl groups of Al or Fe oxides, resulting in reduced P adsorption in soil (Essington

2015; Nishanth and Biswas 2008). Moreover, organic acid anions have potential to chelate with Ca²⁺, Fe²⁺, Fe³⁺, and Al³⁺, resulting in higher phosphate solubility (Sagoe *et al.* 1998; Vassilev *et al.* 2006). Yan *et al.* (2016) reported that application of organic amendments could increase mobile P fraction resulting in enhanced P transportation. The results of the current study suggested that activation of the PR with oxalic acid and EDTA prior to direct application could significantly enhance the P releasing efficiency. Activation of P with organic acid does not only enhance maximum P release capacity of PR, but also extends the P releasing period, maintaining a continuous P supply to the plants. However, it is very critical to calculate the capacity, kinetics, intensity, and diffusivity of P released from a particular PR prior to direct application to soil. Higher amounts of P released beyond plant needs could possibly transport laterally to nearby waterbodies. The same can also happen with P releasing from PR for longer period of times where the surplus P might end up in adjacent waterbodies, resulting in water contamination.

3.4. Temporal release of trace metals in soil

Temporal release of AB-DTPA-extracted trace metal contents at each time interval is shown in Table 4. The results revealed that release of Cd, Co, Cu, and Cr from different PRs was lowest

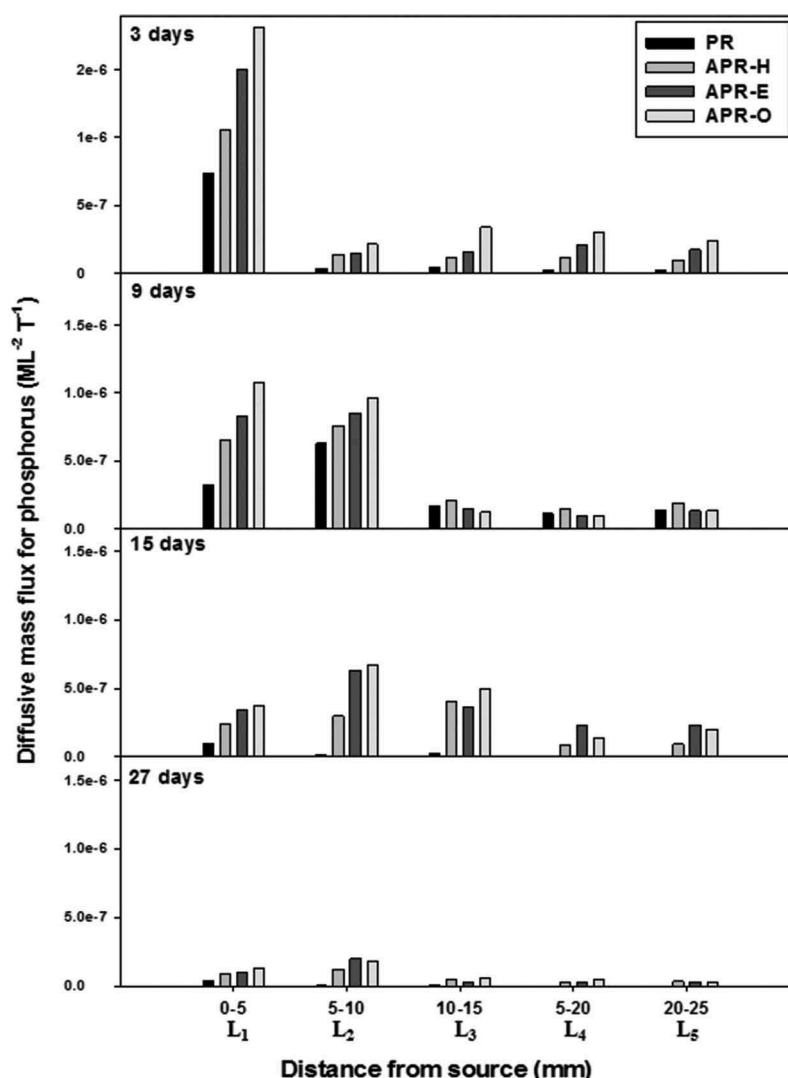


Figure 3. Temporal changes of diffusive mass flux of P in unsaturated soil in micro-block system after amending with various phosphate rocks (PR: phosphate rock, APR-H: HCl activated phosphate rock, APR-E: EDTA activated phosphate rock, and APR-O: oxalic acid activated phosphate rock. M is amount of metal diffused in mg, L is distance in cm, and T is time in seconds).

(<0.8 mg kg⁻¹), Pb and Ni was medium (0.002–1.10 mg kg⁻¹), and Fe, Mn, and Zn was higher (0.4–12 mg kg⁻¹) in all the studied metals. The release of available Fe was higher (2.41, 6.19, 3.13, 5.12, and 5.80 mg kg⁻¹, after 0, 3, 9, 15, and 27 days, respectively) among all the trace metals, followed by Mn (0.48, 6.10, 1.52, 2.02, and 2.99 mg kg⁻¹, after 0, 3, 9, 15, and 27 days, respectively) and Zn (0.90, 1.26, 1.01, 1.24, and 1.15 mg kg⁻¹, after 0, 3, 9, 15, and 27 days, respectively). It was very interesting to notice that the maximum concentrations of Fe, Mn, and Zn were released during initial 3 days after seedling transplantation, and decreased subsequently. The Fe release was 2.6, 1.3, 2.1, and 2.4 fold higher after 3, 9, 15, and 27 days, respectively, compared to release at 0 days. Likewise, Mn release was 12.8, 3.2, 4.2, and 6.2 fold, and Zn release was 1.4, 1.1, 1.4, and 1.3 fold higher after 3, 9, 15, and 27 days, respectively, compared to their release at 0 days. Due to higher release of Fe, Mn, and Zn among the studied trace metals, these metals were selected for diffusion mass flux calculations. The release of Fe, Mn, and Zn followed different trends under pristine and APRs application. On cumulative basis, the APRs released higher amounts of Fe, Mn, and Zn compared to pristine PR. The availability and solubility

of metals in the soil depends on various factors such as pH, organic matter, cation exchange capacity, and mineralogy of the soil (Schmidt 2003; Li and Shuman 1996). A negative correlation has been established between pH and soil metals mobility; however, amending the soil with EDTA or acidulation with organic acids has been reported to enhance metal mobility and solubility (Sukreeyapongse *et al.* 2002; Bang and Hesterberg 2004). The EDTA has been used in various studies as chelating agent to enhance phytoextraction of trace metals (Evangelou *et al.* 2007). The mechanism involved in increasing metal phytoavailability due to EDTA is not well known yet due to its dependence on metal type and speciation; however, a generally accepted hypothesis is the formation of highly stable EDTA-metal complexes (Wenzel *et al.* 2003; Sarret *et al.* 2001). Likewise, low molecular weight organic acids such as oxalic acid due to their complexing characteristics play a vital role in solubilizing the trace metals in soil via influencing microbial activities, chelation, acidulation, and oxidation reduction reactions (Marschner *et al.* 1995; Uren and Reisenauer 1988; Krishnamurti *et al.* 1997; Nigam *et al.* 2001). Similar results have also been reported by a number of scientists suggesting that EDTA

Table 4. Plant available heavy metal concentration (mg kg^{-1}) in soil released after amending with activated and non-activated phosphate rock (PR: phosphate rock, APR-H: HCl activated phosphate rock, APR-E: EDTA activated phosphate rock and APR-O: oxalic acid activated phosphate rock).

	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
After 0 days									
PR	ND	0.008 ± 0.00	0.004 ± 0.00	0.004 ± 0.01	2.408 ± 0.1	0.478 ± 0.2	0.002 ± 0.00	0.138 ± 0.00	0.900 ± 0.02
APR-H	ND	0.008 ± 0.01	ND	0.040 ± 0.01	2.408 ± 0.1	0.478 ± 0.2	0.020 ± 0.00	0.138 ± 0.00	0.900 ± 0.02
APR-E	ND	0.008 ± 0.02	ND	0.040 ± 0.01	2.408 ± 0.1	0.478 ± 0.2	0.020 ± 0.00	0.138 ± 0.00	0.900 ± 0.02
APR-O	ND	0.008 ± 0.03	ND	0.040 ± 0.01	2.408 ± 0.1	0.478 ± 0.2	0.020 ± 0.00	0.138 ± 0.00	0.900 ± 0.02
After 3 days									
PR	0.031 ± 0.05	0.066 ± 0.03	0.003 ± 0.00	0.756 ± 0.28	6.320 ± 0.75	5.427 ± 1.42	0.790 ± 0.31	0.439 ± 0.07	1.429 ± 0.31
APR-H	0.013 ± 0.02	0.084 ± 0.05	0.002 ± 0.00	0.570 ± 0.22	5.904 ± 0.48	6.227 ± 1.90	0.478 ± 1.57	0.421 ± 0.06	1.273 ± 0.18
APR-E	0.15 ± 0.02	0.106 ± 0.03	0.005 ± 0.01	0.618 ± 0.24	5.514 ± 0.70	8.312 ± 1.55	0.340 ± 0.09	0.388 ± 0.07	1.036 ± 0.22
APR-O	0.003 ± 0.00	0.046 ± 0.03	0.010 ± 0.00	0.573 ± 0.22	7.029 ± 2.46	4.444 ± 1.49	0.552 ± 0.51	0.550 ± 0.06	1.290 ± 0.26
After 9 days									
PR	ND	0.016 ± 0.05	0.002 ± 0.00	0.264 ± 0.18	2.642 ± 0.77	1.333 ± 1.45	0.620 ± 0.11	0.353 ± 0.07	0.975 ± 1.00
APR-H	ND	0.034 ± 0.02	0.011 ± 0.00	0.412 ± 0.07	2.813 ± 0.63	1.792 ± 0.74	0.536 ± 0.06	0.426 ± 0.06	1.056 ± 0.161
APR-E	ND	0.222 ± 0.06	0.009 ± 0.03	0.299 ± 0.10	4.181 ± 0.70	1.208 ± 0.19	0.635 ± 0.05	0.463 ± 0.04	1.009 ± 0.22
APR-O	ND	0.042 ± 0.05	0.005 ± 0.00	0.350 ± 0.06	2.904 ± 0.36	1.750 ± 0.13	0.406 ±	0.432 ± 0.10	0.999 ± 0.29
After 15 days									
PR	ND	0.061 ± 0.02	0.005 ± 0.00	0.381 ± 0.03	3.605 ± 0.11	1.982 ± 0.56	0.484 ± 0.25	0.420 ± 0.02	2.253 ± 0.16
APR-H	ND	0.046 ± 0.02	0.007 ± 0.02	0.264 ± 0.15	2.930 ± 0.40	1.334 ± 0.61	0.274 ± 0.15	0.349 ± 0.05	0.841 ± 0.17
APR-E	ND	0.159 ± 0.02	0.030 ± 0.00	0.170 ± 0.03	2.728 ± 1.00	1.072 ± 0.23	0.223 ± 0.26	0.297 ± 0.04	0.465 ± 0.25
APR-O	ND	0.086 ± 0.01	ND	0.242 ± 0.05	11.21 ± 0.06	3.691 ± 0.39	0.427 ± 0.09	0.521 ± 0.03	1.397 ± 0.10
After 27 days									
PR	ND	0.040 ± 0.04	ND	0.160 ± 0.04	8.307 ± 0.62	3.276 ± 0.23	1.025 ± 0.43	0.556 ± 0.05	1.559 ± 0.16
APR-H	ND	0.066 ± 0.07	ND	0.162 ± 0.03	9.155 ± 0.51	4.801 ± 0.64	0.673 ± 0.26	0.551 ± 0.07	1.497 ± 0.34
APR-E	ND	0.149 ± 0.06	0.001 ± 0.00	0.261 ± 0.07	2.961 ± 0.26	1.548 ± 0.63	0.310 ± 0.08	0.340 ± 0.04	0.572 ± 0.14
APR-O	ND	0.098 ± 0.05	0.003 ± 0.00	0.387 ± 0.07	2.760 ± 0.59	2.363 ± 1.20	0.445 ± 0.16	0.435 ± 0.04	0.952 ± 0.24

and organic acids have potential to mobilize trace metals in soil by chelation and complexation (Heil *et al.* 1996; Blaylock *et al.* 1997; Huang *et al.* 1997).

3.5. Lateral transport of Fe, Mn, and Zn in soil

The diffusive mass fluxes calculated for the lateral transport of Fe, Mn, and Zn are presented in Fig. 4. The APR-E and APR-O were found to diffuse higher amounts of Fe, Mn, and Zn contents at all the times compared to APR-H and PR. After 3 days of seedling transplantation, the maximum concentrations of the diffusive mass fluxes of Zn were observed in L₁ (Fig. 4A), which transported to other layers (L₂, L₃, L₄, and L₅) within next 9 days. It was very interesting to notice that after 15 and 27 days of seedling transplantation, Zn diffusive mass flux was reduced in all the layers compared to the diffusive mass fluxes after 9 days. The reduction in Zn diffusion mass fluxes from 15 to 27 days was might be due to transportation toward root of wheat plants, which have grown up enough and have taken up the Zn. Another possible reason for reduced Zn release after 15 days could be the adsorption of Zn onto soil particles. After 27 days, the ability of PR and APR-H to release Zn was reduced significantly, while that of APR-E and APR-O still continued to release Zn (Fig. 4A). A similar trend was also observed in case of Mn and Fe diffusive mass fluxes with a little variation (Fig. 4B,C). After 3 days, the maximum amounts of Mn and Fe diffusive mass fluxes were observed in L₁, which were further moved to other layers with passage of time. The highest diffusive mass fluxes of Mn and Fe were observed after 9 days in all the layers, which was reduced significantly to the minimum after 15 and 27 days because of plant uptake. Similar to the Zn, APR-O and APR-E were observed to release significant amount of Fe and Mn throughout the study period. The higher release of Zn, Mn, and Fe in APR-O and APR-E application could be due to complexation and chelation properties of oxalic acid and EDTA with toxic metals (Heil *et al.* 1996; Blaylock *et al.* 1997; Huang *et al.* 1997). Role of organic acids and chelating agents on the desorption, solubility, and mobility of trace metals in

soil has also been studied by Khodadoust *et al.* (2005) and Schwab *et al.* (2005); however, the lateral transport of these metals to nearby waterbodies or groundwater is still unclear, because metal transportation depends on metal speciation, organic acid type, and the environmental conditions.

3.6. Effect of PR application on plant growth and nutrient uptake

The variations in length and dry matter contents of root and shoot of wheat plants are presented in Fig. 5. The results revealed that plant growth was significantly ($p < 0.05$) influenced by the application of APRs compared to the pristine PR. The highest shoot and root lengths were exhibited under the application of APR-O (31.32 and 16.51 cm, respectively), followed by APR-E (25.74 and 14.55 cm, respectively) and APR-H (18.64 and 8.51 cm, respectively), while the minimum in PR (15.44 and 6.98 cm, respectively), after 27 days of seedling transplantation (Fig. 5A). Amending the soil with APR-O, APR-E and APR-H increased shoot length by 102.88%, 66.76%, and 20.75%, respectively, and root lengths by 136.59%, 108.45%, and 21.92%, respectively, compared to PR after 27 days. A similar trend was observed in case of dry matter production (Fig. 5B), resulting in highest shoot and root dry matter production under APR-O application (0.56 and 0.37 g, respectively), followed by APR-E (0.49 and 0.31 g, respectively) and APR-H (0.38 and 0.24 g, respectively), while minimum under PR application (0.32 and 0.19 g, respectively) after 27 days. Shoot dry matter was increased by 76.25%, 53.91%, and 17.34%, while root dry matter was increased by 92.39%, 61.68%, and 25.98% in APR-O, APR-E, and APR-H, respectively, compared to PR after 27 days. The enhanced plant growth (shoot and root lengths and dry matter contents) could be attributed to higher P and trace metal release and transportation to the root of plants. The P and trace metals such as Fe, Mn, and Zn were taken up by the wheat plants and served as nutrients for the wheat, resulting in increased plant growth. The uptake of these nutrient elements is presented in Fig. 6. It is clear from the results that application APRs has improved the uptake of P, Fe, Mn, and Zn significantly

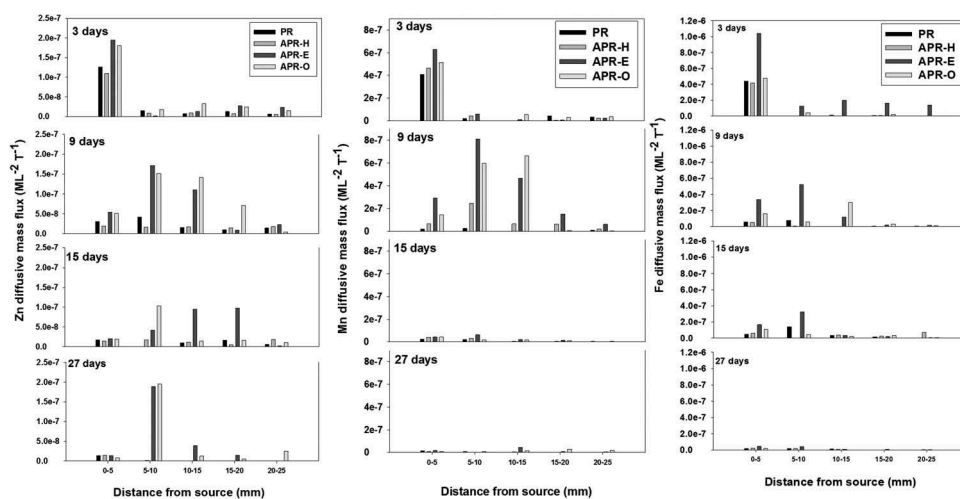


Figure 4. Temporal changes of diffusive mass flux of heavy metals in unsaturated soil in micro-block system after amending with various phosphate rocks (PR: phosphate rock, APR-H: HCl activated phosphate rock, APR-E: EDTA activated phosphate rock, and APR-O: oxalic acid activated phosphate rock. M is amount of P diffused in mg, L is distance in cm, and T is time in seconds).

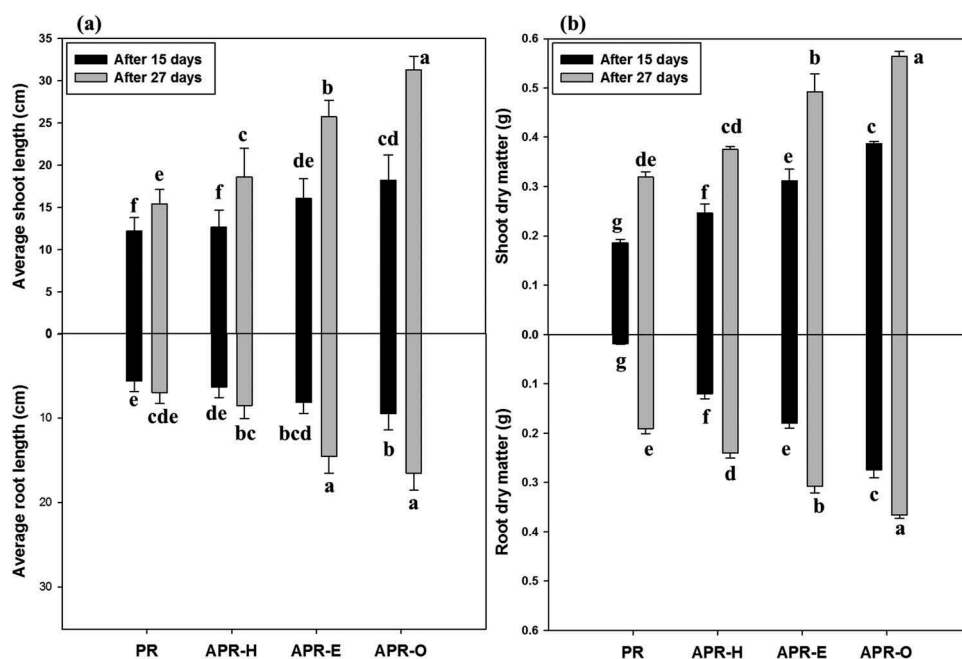


Figure 5. Amendments (PR: phosphate rock, APR-H: HCl activated phosphate rock, APR-E: EDTA activated phosphate rock, and APR-O: oxalic acid activated phosphate rock) effects on average length and dry matter of root and shoot of wheat plant grown in a soil micro-block system. (Different letters above bars represent significant difference at $p < 0.05$ among various treatments and bars with same letters represent no significant difference).

($p < 0.05$) by plant biomass. The APR-O application showed the maximum uptake of P ($2973.98 \mu\text{g plant}^{-1}$), Fe ($652.51 \mu\text{g plant}^{-1}$), Mn ($79.67 \mu\text{g plant}^{-1}$), and Zn ($76.77 \mu\text{g plant}^{-1}$) after 27 days of seedling transplantation. The application of APR-O, APR-E, and APR-H increased the uptake of P by 394.33%, 280.06%, and 143.53% and uptake of Fe by 714.78%, 188.00%, and 83.00%, respectively, as compared to PR (Fig. 6A,B).

notice that Mn uptake was increased by 92.09% and 15.80% under APR-O and APR-E application, respectively, while it decreased by 10.04% under APR-H, compared to PR application (Fig. 6C). This trend was even different in case of Zn uptake, where it increased by 14.53% under APR-O application, while decreased by 28.37% and 23.44% under PAR-H and APR-E application as compared to PR (Fig. 6D). The higher uptake of P and

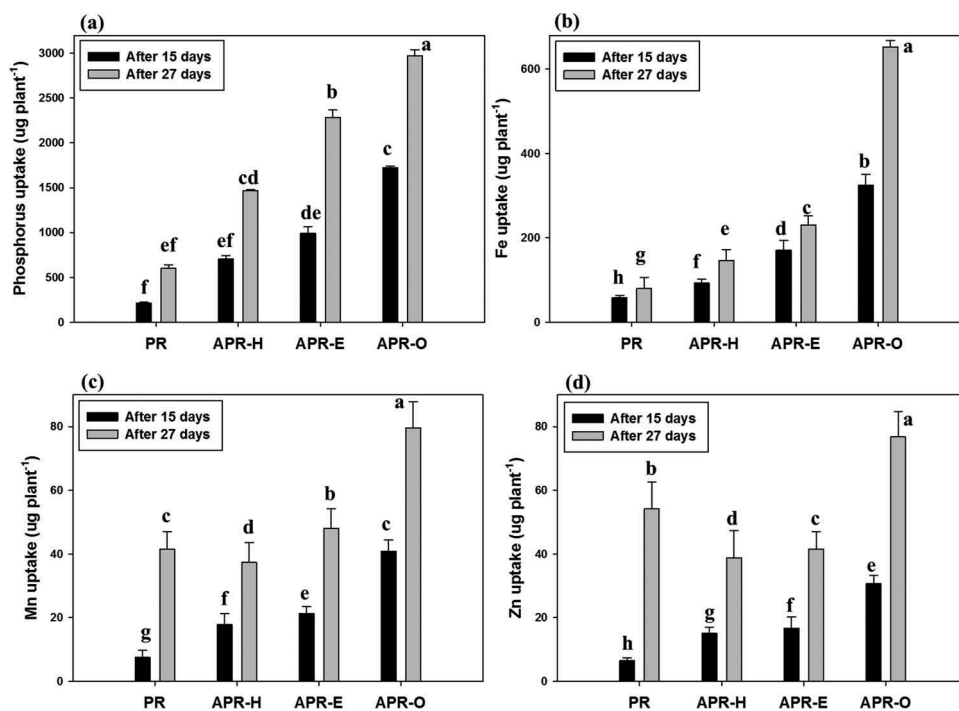


Figure 6. Amendments (PR: phosphate rock, APR-H: HCl activated phosphate rock, APR-E: EDTA activated phosphate rock, and APR-O: oxalic acid activated phosphate rock) effects on uptake of phosphorus, Fe, Mn, and Zn to the wheat plants grown in a soil micro-block system. (Different letters above bars represent significant difference at $p < 0.05$ among various treatments and bars with same letters represent no significant difference).

trace metals due to EDTA and oxalic acid could be due to the complexation and chelation of these elements into bioavailable forms. Another possible reason for higher nutrients uptake could be due to the possible reduction in soil pH after amending with acidulated PRs resulting in enhanced bioavailability of the P, Fe, Mn, and Zn (Kayser *et al.* 2000; Ghoneim *et al.* 2017). The increase in metal uptake after the application of low molecular weight organic acids and EDTA has already been reported by many researchers (Alkorta *et al.* 2004; Usman and Mohamed 2009; Evangelou *et al.* 2007; Komarek *et al.* 2008). An increase in metal uptake by plants after EDTA and oxalic acid APRs (APR-O) could be due to solubilization, mobilization, and chelation (Baldantoni *et al.* 2011; Chen *et al.* 2004; Grcman *et al.* 2003; Gheju and Stelescu 2013). Therefore, it can be stated that oxalic acid and EDTA APRs (APR-E) are more efficient in releasing nutrients from PR subsequently resulting in higher nutrient uptake and growth of the wheat plants. The direct application of activated PR to soil can enhance nutrient release and mobility resulting in improved crop growth. Moreover, it is very critical to estimate the maximum nutrient release capacity and duration for a particular PR when activated with a specific organic acid or EDTA to enhance crop production and to avoid lateral transport and buildup of toxic metals in the soils.

4. Conclusion

The PR was activated with HCl (APR-H), EDTA (APR-E) and oxalic acid (APR-O), and tested for their capabilities to release soil available P and trace metals in a soil micro-block system. Additionally, the lateral transport of P and trace metals (Fe, Zn, and Mn) in soil under unsaturated conditions was investigated. The effects of the amendments on the uptake of P, Fe, Mn, and Zn, and wheat plant growth were explored over a period of 27 days. Results revealed that APR-O and APR-E were the most efficient amendments in releasing and transporting higher amounts of P, Fe, Mn, and Zn in the soil for longer time periods compared to pristine PR. The similar treatments resulted in improved plant growth by enhancing lengths and dry matter production of shoot and root of wheat plants. The enhanced plant growth under APR-O and APR-E applications resulted in higher uptake of P, Fe, Mn, and Zn to the wheat plants. Therefore, it was concluded that activation of raw PR with EDTA or low molecular organic acids such as oxalic acid could release higher amounts of P and some trace metals over a longer period of time compared to raw PR, resulting in higher crop production when applied directly to soil as amendments.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

- Ahmad M, Ahmad M, El-Naggar AH, Usman AR, Abduljabbar A, Vithanage M, Elfaki J, Abdulelah AF, Al-Wabel MI 2018a: Aging effects of organic and inorganic fertilizers on phosphorus fractionation in a calcareous sandy loam soil. *Pedosphere*, **28**, 873–883.
- Ahmad M, Usman AR, Al-Faraj AS, Ahmad M, Sallam A, Al-Wabel MI 2018b: Phosphorus-loaded biochar changes soil heavy metals availability and uptake potential of maize (*Zea mays* L.) plants. *Chemosphere*, **194**, 327–339.
- Alkorta I, Hernandez-Allica J, Becerril JM, Amezcaga I, Albizu I, Onaindia M, Garbisu C 2004: Chelate-enhanced phytoremediation of soils polluted with heavy metals. *Rev. Environ. Sci. Biotechnol.*, **3**, 55–70. doi:10.1023/B:RESB.0000040057.45006.34
- Allison LE, Moodie CD 1965: Carbonate. In *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*, Ed. Black CA, pp. 1379–1396. ASA, Madison, WI, USA.
- Al-Oud SS 2011: Improving phosphorus availability from phosphate rock in calcareous soils by amending with: organic acid, sulfur, and/or organic manure. *Ozean J. App. Sci.*, **4**, 227–235.
- Al-Rohily KM, Ghoneim AM, Modaihsh AS, Mahjoub MO 2013: Phosphorus availability in calcareous soil amend with chemical P fertilizer, cattle manure compost and sludge manure. *Int. J. Soil Sci.*, **8**, 17–24. doi:10.3923/ijss.2013.17.24
- Baldantoni D, Bellino A, Ciatelli A, Castiglione S 2011: Artificial mycorrhization does not influence the effects of iron availability on Fe, Zn, Cu, Pb and Cd accumulation in leaves of a heavy metal tolerant white poplar clone. *Plant Biosyst.*, **145**, 236–240. doi:10.1080/11263504.2010.509941
- Bang J, Hesterberg D 2004: Dissolution of trace element contaminants from two coastal plain soils as affected by pH. *J. Environ. Qual.*, **33**, 891–901.
- Barnes JS, Kamprath EJ 1975: Availability of North Carolina Rock Phosphate Applied to Soils. North Carolina Agricultural Station Technical Bulletin, pp. 229.
- Bhadoria P, Kaselowsky J, Claassen N, Jungk A 1991: Soil phosphate diffusion coefficients: their dependence on phosphorus concentration and buffer power. *Soil Sci. Soc. Amer. J.*, **55**, 56–60. doi:10.2136/sssaj1991.03615995005500010009x
- Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I 1997: Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents. *Environ. Sci. Technol.*, **31**, 860–865. doi:10.1021/es960552a
- Bouyoucos GJ 1962: Hydrometer method improved for making particle size analysis of soils. *J. Argon.*, **53**, 464–465.
- Chen YH, Li XD, Shen ZG 2004: Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process. *Chemosphere*, **57**, 187–196. doi:10.1016/j.chemosphere.2004.05.044
- Chien SH, Hammond LL 1988: Agronomic Evaluation of Partially Acidulated Phosphate Rocks in the Tropics. IFDS's Experience. Paper Series. International Fertilizer Development Center IDFC-P-7, Muscle Shoals, AL, USA.
- Chien SH, Prochnow LI, Mikkelsen R 2010: Agronomic use of phosphate rock for direct application. *Better Crops*, **94**, 21–23.
- Essington ME 2015: *Soil and Water Chemistry: An Integrative Approach*. CRC press.
- Evangelou MW, Ebel M, Schaeffer A 2007: Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. *Chemosphere*, **68**, 989–1003. doi:10.1016/j.chemosphere.2007.01.062
- Gächter R, Ngatiah JM, Stamm C 1998: Transport of phosphate from soil to surface waters by preferential flow. *Environ. Sci. Technol.*, **32**, 1865–1869. doi:10.1021/es9707825
- Gheju M, Stelescu I 2013: Chelant-assisted phytoextraction and accumulation of Zn by *Zea mays*. *J. Environ. Manage.*, **128**, 631–636. doi:10.1016/j.jenvman.2013.06.017

- Ghoneim AM, Elbassir OI, Modahish AS, Mahjoub MO 2017: Compost production from olive tree pruning wastes enriched with phosphate rock. *Compost. Sci. Util.*, **25**, 13–21. doi:10.1080/1065657X.2016.1171737
- Ghosal PK, Chakraborty T 2012: Comparative solubility study of four phosphatic fertilizers in different solvents and the effect of soil. *Resour. Environ.*, **2**, 175–179.
- Grcman H, Vodnik D, Velikonja-Bolta S, Lestan D 2003: Ethylenediaminedisuccinate as a new chelate for environmentally safe enhanced: lead phytoextraction. *J. Environ. Qual.*, **32**, 500–506. doi:10.2134/jeq2003.5000
- Guppy CN, Menzies NW, Moody PW, Blamey FP 2005: Competitive sorption reactions between phosphorus and organic matter in soil: a review. *Soil Res.*, **43**, 189–202. doi:10.1071/SR04049
- Harris WG 2002: Phosphate minerals. In *Soil Mineralogy with Environmental Applications*, Eds. Dixon JB, Schulze BG, pp. 637–665. Soil Sci. Soc. Amer, Madison.
- Heil D, Hanson A, Samani Z 1996: The competitive binding of lead by EDTA in soils and implications for heap leaching remediation. *Radioact. Waste Manage. Environ. Restor.*, **20**, 111–127.
- Holford ICR 1997: Soil phosphorus: its measurement, and its uptake by plants. *Aust. J. Soil Res.*, **35**, 227–239. doi:10.1071/S96047
- Huang JW, Chen JJ, Berti WR, Cunningham SD 1997: Phytoremediation of lead-contaminated soils: role of synthetic chelating in lead phytoextraction. *Environ. Sci. Technol.*, **31**, 800–805. doi:10.1021/es9604828
- Hurst FJ 1989: *The Recovery of Uranium from Phosphoric Acid*, pp. 553. IAEA, IAEA/TECDOC, Vienna.
- Ivanova RP, Bojinova DY, Gruncharov IN, Damgaliev DL 2006: The solubilization of rock phosphate by organic acids. *Phosphorus Sulfur Silicon*, **181**, 2541–2554. doi:10.1080/10426500600758399
- Jost W 1952: *Diffusion in Solids, Liquids, Gases*. Academic press Inc, New York.
- Kayser A, Wenger K, Keller A, Attinger W, Felix HR, Gupta SK, Schulin R 2000: Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: the use of NTA and sulfur amendments. *Environ. Sci. Technol.*, **34**, 1778–1783. doi:10.1021/es990697s
- Khodadoust AP, Reddy KR, Maturi K 2005: Effect of different extraction agents on metal and organic contaminant removal from a field soil. *J. Hazard. Mater.*, **117**, 15–24. doi:10.1016/j.jhazmat.2004.05.021
- Komarek M, Tlustos P, Szakora J, Chrastny V 2008: The use of poplar during a two-year induced phytoextraction of metals from contaminated agricultural soils. *Environ. Pollut.*, **151**, 27–38. doi:10.1016/j.envpol.2007.03.010
- Kpombekou AK, Tabatabai MA 2003: Effect of low-molecular weight organic acids on phosphorus release and phytoavailability of phosphorus in phosphate rocks added to soils. *Agri. Ecosys. Environ.*, **100**, 275–284. doi:10.1016/S0167-8809(03)00185-3
- Krishnamurti GSR, Cielinski G, Huang PM, vanRees KCJ 1997: Kinetics of cadmium release from soils as influenced by organic acids: implementation in cadmium availability. *J. Environ. Qual.*, **26**, 271–277. doi:10.2134/jeq1997.00472425002600010038x
- Li S, Liu Y, Wang J, Yang L, Zhang S, Xu C, Ding W 2017: Soil acidification aggravates the occurrence of bacterial wilt in South China. *Front. Microbiol.*, **8**, 703. doi:10.3389/fmicb.2017.00703
- Li Z, Shuman LM 1996: Heavy metal movement in metal contaminated soil profiles. *Soil Sci.*, **161**, 656–666. doi:10.1097/00010694-199610000-00003
- Marschner B, Henke U, Wessolek G 1995: Effects of meliorative additives on the adsorption and binding forms of heavy metals in a contaminated topsoil from a former sewage farm. *J. Plant Nutr. Soil Sci.*, **158**, 9–14.
- Matko V 2003: Porosity determination by using stochastic method. *Automatika: casopis za automatiku, mjerenje, elektroniku, racunarstvo i komunikacije. ATKAAF*, **44**, 155–162.
- Mehmood T, Chaudhry MM, Tufail M, Irfan N 2009: Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan. *Microchem. J.*, **91**, 94–99. doi:10.1016/j.microc.2008.08.009
- Nigam R, Srivastava S, Prakash S, Srivastava MM 2001: Cadmium mobilisation and plant availability – the impact of organic acids commonly exuded from roots. *Plant Soil*, **230**, 107–113.
- Nishanth D, Biswas DR 2008: Kinetics of phosphorus and potassium release from rock phosphate and waste mica enriched compost and their effect on yield and nutrient uptake by wheat (*Triticum aestivum*). *Bioresour. Technol.*, **99**, 3342–3353. doi:10.1016/j.biortech.2007.08.025
- Nye PH, Tinker PB 1977: *Solute Movement in the Soil-Root System*. Studies in Ecology, Vol. 4 University of California Press, Berkeley and Los Angeles, California, USA.
- Porter LK, Kemper WD, Jackson RD, Sterart BA 1960: Chloride diffusion in soils as influenced by moisture content. *Proc. Soil Sci. Soc. Amer.*, **24**, 460–463. doi:10.2136/sssaj1960.03615995002400060014x
- Prochnow LI, Quispe JFS, Francisco EAB, Braga G 2006: Effectiveness of phosphate fertilizers of different water solubilities in relation to soil phosphorus adsorption. *Scientia Agricola*, **63**, 333–340. doi:10.1590/S0103-90162006000400004
- Rafael RBA, Fern´andez-Marcos ML, Cocco S, Ruello ML, Weindorf DC, Cardelli V, Corti G 2018: Assessment of potential nutrient release from phosphate rock and dolostone for application in acid soils. *Pedosphere*, **28**, 44–58. doi:10.1016/S1002-0160(17)60437-5
- Rajan SSS, Brown MW, Boyes MK, Upsdell MP 1992: Extractable phosphorus to predict agronomic effectiveness of ground and unground phosphate rocks. *Fertilizer Res*, **32**, 291–302. doi:10.1007/BF01050366
- Rajan SSS, Watkinson JH, Sinclair AG 1996: Phosphate rocks for direct application to soils. *Adv. Agron.*, **57**, 77–159.
- Reddy DD, Rao AS, Takkar PN 1999: Effects of repeated manure and fertilizer phosphorus additions on soil phosphorus dynamic under soybean wheat rotation. *Biol. Fertil. Soil.*, **28**, 150–155. doi:10.1007/s003740050477
- Richard LA 1954: *Diagnosis and improvements of saline and alkali soils*, pp. 60–160. U.S. Salinity Laboratory DA. U.S Department of Agriculture. Hand Book No. 60.
- Sagoe CI, Ando T, Kouno K, Nagaoka T 1998: Relative importance of protons and solution calcium concentration in phosphate rock dissolution by organic acids. *Soil Sci. Plant Nutr.*, **44**, 617–625. doi:10.1080/00380768.1998.10414485
- Sarret G, Vangronsveld J, Manceau A, Musso M, D'Haen J, Menthonnex JJ, Hazemann JL 2001: Accumulation forms of Zn and Pb in *Phaseolus vulgaris* in the presence and absence of EDTA. *Environ. Sci. Technol.*, **35**, 2854–2859.
- Savini I, Smithson PC, Karanja NK 2006: Effects of added biomass, soil pH and calcium on the solubility of Minjingu phosphate rock in a Kenyan Oxisol [Einfluss von zugeführter Biomasse, Boden pH und Calcium-Löslichkeit von Minjingu Phosphatgestein in einem Keniatischen Oxisol]. *Arch. Agron. Soil Sci.*, **52**, 19–36. doi:10.1080/03650340500471922
- Schmidt U 2003: Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. *J. Environ. Qual.*, **32**, 1939–1954. doi:10.2134/jeq2003.1939
- Schwab AP, He YH, Banks MK 2005: The influence of organic ligands on the retention of lead in soil. *Chemosphere*, **61**, 856–866. doi:10.1016/j.chemosphere.2005.04.098
- Sinclair AG, Johnstone PD, Smith LC, O'Connor MB, Nguyen L 1993: Agronomy, modeling and economics of reactive phosphate rocks as slow release phosphate fertilizers for grasslands. *Fert. Res.*, **36**, 229–238. doi:10.1007/BF00748701
- Singh D, Mannika ND, Srivas NC 1976: Fertilizer value of indigenous rock phosphates compared with single super phosphate: laboratory incubation studies with farm yard manure. *J. Indian Soc. Soil Sci.*, **24**, 78–80.
- Soltanpour PN, Schwab AP 1977: A new soil test for simultaneous extraction of macro- and micronutrients in alkaline soils. *Comm. Soil Sci. Plant. Anal.*, **8**, 195–207. doi:10.1080/00103627709366714
- Stewart JWB, Tiessen H 1987: Dynamics of soil organic phosphorus. *Biogeochemistry*, **4.1**, 41–60. doi:10.1007/BF02187361
- Sukreaypongse O, Holme PE, Strobel BW, Panichsakpatana S, Magid J, Hansen HCB 2002: pH-dependent release of cadmium, copper, and lead from natural and sludge-amended soils. *J. Environ. Qual.*, **31**, 1901–1909. doi:10.2134/jeq2002.1901
- Sweerts JR, Kelly C, Rudd J, Hesslein R, Cappenberg T 1991: Similarity of whole sediment diffusion coefficient in freshwater sediments of low and high porosity. *Limnol. Oceanogr.*, **36**, 335–342. doi:10.4319/lo.1991.36.2.0335
- Uren NC, Reisenauer HM 1988: The role of root exudates in nutrient acquisition. *Adv. Plant Nutr.*, **3**, 79–114.
- USEPA. 3051a. 1998: *Microwave assisted acid digestion of sediments, sludges, soils, and oils – reference to*. Revised 1998. www.epa.gov/epaoswer/hazwaste/test/pdfs/3051a.pdf

- Usman ARA, Mohamed HM 2009: Effect of microbial inoculation and EDTA on the uptake and translocation of heavy metals by corn and sunflower. *Chemosphere*, **76**, 893–899. doi:10.1016/j.chemosphere.2009.05.025
- Van Straaten P 2002: Rocks for Crops: agrominerals of Sub-Saharan Africa, pp. 338. ICRAF, Nairobi, Kenya.
- Vassilev N, Medina A, Azcon R, Vassileva M 2006: Microbial solubilization of rock phosphate on media containing agro-industrial wastes and effect of the resulting products on plant growth and P uptake. *Plant Soil*, **287**, 77–84. doi:10.1007/s11104-006-9054-y
- Walkley A, Black IA 1934: An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.*, **37**, 29–38. doi:10.1097/00010694-193401000-00003
- Wenzel WW, Unterbrunner R, Sommer P, Pasqualina S 2003: Chelate-assisted phytoextraction using canola (*Brassica napus* L.) in outdoors pot and lysimeter experiments. *Plant Soil*, **249**, 83–96. doi:10.1023/A:1022516929239
- Wolf B 1982: A comprehensive system of leaf analyses and it's uses for diagnostic crop nutrient status. *Comm. Soil Sci. Plant. Anal.*, **13**, 1035–1059. doi:10.1080/00103628209367332
- Yan Z, Chen S, Li J, Alva A, Chen Q 2016: Manure and nitrogen application enhances soil phosphorus mobility in calcareous soil in greenhouses. *J. Environ. Manage.*, **181**, 26–35. doi:10.1016/j.jenvman.2016.05.081