The Comparison of Olsen, DMT-HFO and DGT Methods for Assessment of Plant Available Phosphorus in Soils

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Abstract— Phosphorus (P) is one of the most important limiting nutrients for crop production in the world soils. Excessive and deficiencies of P in calcareous soils has been reported. Several methods can be used to recognize soil bioavailable phosphorus. Soil P tests involve chemical extraction and ion-sink extractants. The diffusive gradient in thin-films (DGT) is a new technique for measuring available soil phosphorus. This technique attempts to simulate physico-chemical processes influencing the uptake of solutes by plant roots. Also, the dialysis membrane tubes filled with hydrous ferric oxide solution (DMT-HFO) has recently been used to assess P desorption over long-term laboratory studies. The DMT-HFO method acts like the DGT method. Both systems are based on Fick’s law and try to imitate physicochemical uptake of P by plant roots in soils. The aim of this research was to use Fick’s law in the interpretation of DMT measurements and compare it with the Olsen and DGT methods for assessment of corn available phosphorus in some calcareous soils. For this purpose, the diffusion coefficients of P in the DMT membrane and DGT gel were calculated. The DMT-HFOs were located in soil suspensions at 24, 72, 240, and 500 hours and the concentration of DMT-HFO (C_{DMT-HFO}) for P in the mentioned times were compared with those of Olsen and DGT methods on assessing of corn P uptake. Corn (SC 704) were grown in a greenhouse on ten different calcareous soils. Based on the results, the diffusion coefficient of P (H_{DMT-HFO}) in DMT (5.54*10⁻⁸ cm².s⁻¹) was lower than that of DGT gel (5.8*10⁻⁶ cm².s⁻¹). The linear correlation coefficient (r) between C_{DMT-HFO} and corn P content was increased by the time (24- 500 hours). Based on the r² values, the lowest and highest correlations were related to C_{DMT-HFO} in 24 hours (r²=0.22, p<0.05) and 500 hours (r²=0.9, p<0.05), respectively. The DMT-HFO provides a more precise correlation with P uptake by corn (r²=0.9 in 500 hours) than Olsen P (r²=0.86) and C_{DGT} (DGT) (r²=0.76). In roots, the best correlation between C_{DMT-HFO} and corn P uptake was obtained in 250 hours. However, in 24 to 250 hours, the statistical correlations based on r² values were very similar.

Keywords— DGT; DMT-HFO; Olsen-P; Corn; Phosphorus

I. INTRODUCTION

This Phosphorus is an essential nutrient for all life forms and is involved in vital plant processes. Also phosphorus is one of the most important limiting plant nutrient [1]. So it’s needed to add for crop growth. The diagnosis of phosphorus deficiency in agricultural ecosystems is important in order to avoid environmental pollution due to over-applied in the form of fertilizers and to reduce effects on crop yields [2]. Many methods have been used to determinate nutrient deficiencies on plants including plant tissue analysis and soil tests. Plant tissue analysis is expensive and usually done when the crop have been become deficient but soil test is used prior to plant cultivation so fertilizers could be added [3]. Several methods can be used to recognize bioavailable phosphorus on soils. Soil P tests involve chemical extraction and ion-sink extractants. The most of these methods are based on chemical extractants, for example NaHCO₃ solution [4], solution of acetate, ammonium nitrate, ammonium fluoride, nitric acid and EDTA (Mehlich extraction) [5] and HCl and ammonium fluoride solution [6]. But there is no agreement on which extractants are more acceptable for any type of soils [7]. Different extractants used for different soil types with different soil pH, for instance Bray and Mehlich are acidic extractants and Olsen have been developed for alkaline soils [8].

The ion-sink extractants includes ionic exchange resin membranes, resin bags and iron oxides coated filter papers (Pi-test) [9]. The dialysis membrane tubes filled with hydrous ferric oxide (DMT-HFO) was a method that has been done on the long-term desorption kinetics study of soil P [10]. DMT-HFO acts as an infinite sink for P desorbed from soil. In contrast to FeO-impregnated filter paper (Pi-test) the DMT-HFO can be separated from soil suspension without contamination of the sink. Adhering soil particles may cause FeO paper to overestimate P desorption. Relatively, little information is available in the literature in relation to the use of this method [11].
Diffusive gradients in thin films (DGT) is a new technique for measuring soil available P. This technique has been developed to assess element availability in natural water [12]. It has been proven this technique is suitable for evaluation of the metal bioavailability to plant, later on, DGT has been used for several years to assess bioavailable elements in waters and soils [13-15]. This technique is made up of a device that engage two layers of hydrogel, a diffusive layer and a binding layer. The DGT system basically is designed according to Fick’s first law that ions diffuses through the diffusive gel layer and accumulate on binding gel layer [12]. The binding layer is made from polyacrylamide hydrogel impregnated with Fe oxide that acts as zero sink for phosphate. DGT employment on soils leads to decrease P in the soil solution and resupply from solid phase. Mason et al. [2] was compared dry matter or grain yield response to P fertilizer under field conditions versus available P that measured by three soil testing methods (DGT, anion exchange resin membrane, and Colwell P method) for wheat. Their results showed that DGT could successfully predict P content and can be used to improve prediction of fertilizer requirements and different factors like climate, wheat variety and soil type didn’t have affected on DGT measurement while conventional method was a poor predictor of dry matter responses over a wide range of soil types. Six et al, [16] was declared that in tropical P deficient soils, DGT and CaCl₂ measurement P have shown a better correlation with maize P content than Olsen, Colwell, Bray, Mehlich, and Ammonium oxalate methods. In this studies, DGT was a better indication for P requirements in plant than the extraction techniques. DMT-HFO method acts like DGT. The both system are based on Fick’s law and it seems that they try to imitate physicochemical uptake of P by plant roots in soil. Also, it seems that the theoretical interpretation of DGT measurements could be used on DMT-HFO measurements. So, the aim of this study was to use Fick’s law in the interpretation of DMT measurements and to compare that with P content by corn plant, Olsen-P and DGT measurements in 10 calcareous soils.

A. Olsen method Theory

The Olsen method test is the most used method in world’s calcareous soils and also in Iran. Olsen’s method [4] is mainly based on phosphate solubility in calcareous soils though this method has been used in acid and neutral soils. In this method, it is believed that phosphate is replaced by bicarbonate, carbonate and hydroxide ions [17]. High pH of NaHCO₃ solution decreases the activity of soil solution Ca²⁺ by precipitating as calcium carbonate, resulting in dissolution of Ca-P and also Fe and Al-P compounds in calcareous and acid soils, respectively [18]. As an extractant, NaHCO₃ acts through pH and ion effect to remove soil solution inorganic P, plus some organic P and some labile solid-phase P compounds such as phosphate adsorbed to free lime, slightly soluble calcium phosphate precipitates, and phosphate loosely adsorbed to Fe and Al oxides and clay minerals. NaHCO₃ also removes labile organic P forms that may be readily hydrolyzed to inorganic forms and contribute to plant-available P or be re- assimilated by microorganisms [18].

B. DGT and DMT-HFO method Theory

As shown in Fig. 1, a DGT device contains a binding layer (ferrihydrite impregnated gel for P) overlain by diffusive gel and filter membrane (as a diffusive layer) that contacts the solution [19].

![Fig. 1 Cross-section of a DGT device with binding and diffusive layer](image)

Also, DMT-HFO device consists on DMT strips as diffusive layer and hydrous ferric oxide solution as binding layer (Fig. 2).

![Fig. 2 Illustration of DMT-HFO device A: 3D and B: close up of the binding and diffusive layer on DMT-HFO](image)

When DGT or DMT-HFO is deployed in solution, P diffused through the diffusive layer and immobilized at the binding layer. A concentration gradient is established within the diffusive layer (Fig. 3). Therefore, the flux of P through the diffusive layer is controlled by Fick’s first law of diffusion (Eq. 1).

\[
F = \frac{D \, dC}{dX} \quad (1)
\]

Where, \(F\) is the flux of P per unit time and area, \(D\) is the diffusion coefficient in diffusive layer and \(dC/dx\) is the concentration gradient in diffusive layer. The Eq. 1 could be written as follow in finite differences:

\[
F = \frac{D \, (C-C_0)}{\Delta g} \quad (2)
\]

Where, \(C\) is the concentration of P at the solution and diffusive layer interface (\(C_{DGT}\) or \(C_{DMT-HFO}\)), \(C_0\) is the concentration of P at the binding layer interface and \(\Delta g\) is the thickness of the diffusive layer. According to the immobilizing of P on binding layer, \(C_0\) is zero. Also, the flux of P can be explained as \(F = \frac{M}{A \, t}\). So, Eq. 2 becomes:

\[
C = \frac{M \, \Delta g}{(D \, A \, t)} \quad (3)
\]
Where, \( M \) is the mass of P accumulated by the binding layer, \( A \) is the area of exposure diffusive layer (3.14 cm\(^2\) for DGT and 41.1 cm\(^2\) for DMT-HFO) and \( t \) (s) is deployment time.

\[ C = \frac{C_{\text{DGT}}}{R_{\text{diff}}} \]  

\( R_{\text{diff}} \) is the extent of soil solution depletion at the DGT interface. For calculating \( R_{\text{diff}} \), a 2D numerical model of the DGT-soil system was used; this is called 2D-DIFS (DGT Induced Fluxes in Sediments and Soils). The input parameters of DIFS in the calculation of \( R_{\text{diff}} \) were based on the “diffusion only” case that was described by Sochaczewski et al. [21]. Therefore, the calculation of CE from CDGT based on the input parameters (\( T_c = 10^{10} \) s, \( K_d = 10^{-10} \) cm\(^3\) g\(^{-1}\)) and the diffusion case only depends on the % WHC (Water holding capacity) of soils.

II. EXPERIMENTAL

A. Soil properties

Ten different soil samples (0-30 cm) were collected from different fields in Azerbaijan, a province of Iran. Soils were primarily chosen based on the difference in soil textural class and available phosphorus content. All soils were air dried at room temperature. Soils used for analysis were sieved to <2 mm and for pot trial were sieved to <8 mm. Some soil chemical and physical characteristics including pH in water at a soil/solution ratio of 1:2 (w/v) [22], soil texture [23], calcium carbonate equivalent [24] and organic carbon by dichromate oxidation [25] were determined and presented in table 1. Soil P analyses were performed by 0.5 M NaHCO\(_3\) (pH 8.5) solution as the extractant solution [4].

B. Preparation of Dialysis Membrane Tubes filled with hydrous ferric oxide solution

Fifty grams of ferric nitrate nonahydrate (Fe(NO\(_3\))\(_3\)-9H\(_2\)O) were added to a 500 mL of deionized water. The pH of suspension was adjusted to 7 – 8 using 1 M NaOH solution. Then, the suspension was centrifuged, elutriated, and resuspended in deionized water at least two times. Finally, the HFO suspension was brought to a volume of 1 liter and its pH was adjusted to 7 that is close to the pH of our soil suspension. Eleven centimeter length DMT strips (Scientific Laboratory Co., London, dialysis tubing: Visking, approximate pore size 1.5-2.0 nm, membrane thickness 0.075 mm) were boiled for 5 minutes in deionized water and filled with 20 mL HFO suspension [26].

1) Diffusion coefficient of P in DMT membrane: Diffusion coefficient of P in the DMT membrane was calculated using a diffusion cell in the pseudo steady state mode which has been described by Zhang and Davison [27] (Fig. 4). This cell was comprised two separate parts (A and B) that were linked together with a 3 cm diameter window that filled with a DMT membrane. One part was filled with deionized water (B) and another part with 100 mg.kg\(^{-1}\) P solution (KH\(_2\)PO\(_4\)) (A). Finally, by measuring changes in the concentration of the KH\(_2\)PO\(_4\) solution, we were able to calculate the diffusion coefficient of KH\(_2\)PO\(_4\) according to Zhang et al. procedure [28]. Using Eq. 6, the diffusion coefficient of P (H\(_2\)PO\(_4\)-) (\( D \)) was obtained from the salt diffusion coefficient (KH\(_2\)PO\(_4\)) (\( D_s \)).

\[ D_s = 2D_k(D + D_k) \]  

where \( D_k \) is the diffusion coefficient of the potassium and assumed to be same as for water [28].

C. DGT method

The DGT devices are consist of a two plastic plate (a backing plate and a front plate with a 2 cm diameter window) that holds two gels. The binding layer is placed at the back of diffusive layer so that the diffusive layer (ion-permeable gel) is exposed to the soil. A binding gel layer of 0.6 mm thickness and a 0.7 mm thick diffusive gel layer were used on the experiments. To prevent diffusive gel from being in touch with soil particles, a 100-µm-thick, 0.45-µm pore size cellulose nitrate membrane was placed on top of the two gel layers [29].

1) Gel preparation: Acrylamide, agarose, ammonium persulfate, TEMED, Allylglycidyl ether, sodium borohydride, methanol and NaOH were purchased from Merck Millipore.

As a cross-linker, we were used allylagarose that was synthesized according to published procedure [30]. 33 mg sodium borohydride and 1.6 mL allylglycidyl ether were
added to a suspension of 1 g agarose in NaOH solution (33 mL, 0.3M), under 12 hours stirring. After stirring periods, the derivative agarose was recovered by filtration, washed with distilled water to neutral pH and dehydrated with methanol and dried in an oven at 35°C [30].

Diffusive gel solution was prepared with 15% w/v acrylamide and 0.3% w/v allyl agarose (dissolving allyl agarose at distilled water at 95°C and cooling to 40°C) [30].

70 µl ammonium persulphate (10% w/v) and 20 µl of TEMED catalyst were added to 10 mL of the gel solution. The gel was hydrated in a plastic spacer of known thickness and allowed to polymerize at 42–46°C for 1 hours. The gel was hydrated in deionized water and analyzed for P colorimetrically using the vanadomolybdate-yellow method [26].

The volume of dissolved HFO was adjusted to 100 mL with deionized water. In this research, we used the value of 5.8*10⁻⁶ cm².s⁻¹ in calculating of Cₐ in DMT-HFO [7].

3) Pot experiment: Three kilograms of air-dry soil was sieved to <4.75 mm and filled into plastic-dry pots. Soil moisture was maintained at approximately 80% FC before planting. Then six seeds of corn plant (Zea mays L.) single cross 704 variety were cultivated in the pots. After one week, seeds were thinned to three plants per pot. Soil water content maintained close-to-filled capacity, weighed every second day during plant growth. All required nutrients except P were used according to soil testing and customary fertilizer recommendations. For this, 120 mg urea.kg⁻¹, 10 mg Zn.kg⁻¹ as zinc sulfate (ZnSO₄.7H₂O) and 5 mg Fe.kg⁻¹ of soil as FeEDDHA were applied. Since soils have enough available potassium, the potassium fertilizer was not used. Plants were grown in a condition of 14 hours’ day, 10 hours’ night and in a temperature of 28°C.

After 60 days, the corn plant shoot and roots were harvested from soils, rinsed with deionized water, dried at 80°C to constant weight and finally powdered. Digestion of plant samples was done by wet oxidation method [33]. The concentration and content of P in plant samples were determined by the vanadomolybdate-yellow method [34]. Plant P content was calculating by P concentration in plant*plant dry matter. The three replicates were used for all experiments.

III. RESULT AND DISCUSSION

A. Diffusion in gels and DMT

The mass of phosphorus with time during a diffusion experiment in acrylamide gel and DMT at 25 °C are provided in figure 5. The diffusion coefficient of P (H₃PO₄) at 25 °C using an acrylamide gel (15% by volume acrylamide and 0.3% by volume allylagarose cross-linker) was 5.8*10⁻⁶ cm².s⁻¹. According to fig. 5, the diffusion coefficient of KH₂PO₄ in DMT was 1.1*10⁻⁷ cm².s⁻¹ that referring to Eq. 6, the diffusion coefficient of P (H₃PO₄) was 5.54*10⁻⁸ cm².s⁻¹. Polyacrylamide gels are nonrestrictive and commonly used to separate proteins between 2000 to 200000Da. In which case the corresponding pore size will be about 2-9 nm diameter [35]. Polyacrylamide gels when immersed in water swelling to 95% total volume of gel depending on the amount and type of cross-linker. This condition lets hydrated cations with radius of 0.2-0.3 nm to move freely through the gel [12]. Therefore, the diffusion coefficient of P in acrylamide gel was very closely to the diffusion coefficient of P in water (about 68%). In DMT, diffusional properties were not known. The molecular weight cut-off of DMT is 12,000 to 14,000 Dalton that approximate pore size will be about 1.5-2.0 nm. Also, its structure doesn’t have water like acrylamide gel. Thus, the diffusion coefficient of P in DMT was much lower than that of water. In this research, we used the value of 5.8*10⁻⁶ cm².s⁻¹ in calculating of Cₐ for DGT and 5.54*10⁻⁸ cm².s⁻¹ in calculating Cₐ for DMT-HFO.
Table 1

CHARACTERISTICS OF 10 CALCAREOUS SOILS USED IN THIS STUDY

| Soil | pH   | Olsen-P (mg kg⁻¹) | CCE  | OC (g kg⁻¹) | Clay (g kg⁻¹) | Silt (g kg⁻¹) | Sand (g kg⁻¹) | Soil Textural Class |
|------|------|------------------|------|-------------|--------------|--------------|--------------|-------------------|
| 1    | 8.2  | 16.6             | 4.8  | 8.1         | 70           | 150          | 780          | loamy sand        |
| 2    | 8.3  | 10.1             | 17.9 | 4.6         | 200          | 270          | 530          | sandy loam        |
| 3    | 8.3  | 5.2              | 13.5 | 8.5         | 540          | 300          | 160          | clay              |
| 4    | 8.3  | 11.1             | 9.7  | 20          | 220          | 340          | 440          | loam              |
| 5    | 8.4  | 21.8             | 6.7  | 8.3         | 320          | 360          | 320          | clay loam         |
| 6    | 8.2  | 9                | 6    | 12.7        | 490          | 360          | 150          | clay              |
| 7    | 7.8  | 12               | 5.7  | 9.5         | 180          | 310          | 510          | loam              |
| 8    | 8.2  | 17.3             | 23.8 | 15.6        | 340          | 500          | 160          | silty clay loam   |
| 9    | 8    | 3.1              | 24   | 8.1         | 280          | 380          | 340          | clay loam         |
| 10   | 8.1  | 24.6             | 22.1 | 9.5         | 180          | 510          | 310          | silt loam         |

pH of a 1:2 extract (soil: distilled water), Olsen-P, CCE, SP and OC are soil available P, calcium carbonate equivalent, saturation percentage and soil organic carbon, respectively.

B. Field study

By measuring and correlating of plant P content (P concentration in plant*plant dry matter), $C_{DMT-HFO}$, $C_E$ and P extracted by Olsen method or test, we obtain a prediction of the potential P supply for plants by studied soils. We tested DMT-HFO and calculated $C_{DMT-HFO}$ in times 24, 72, 240 and 500 hours on studied soils to evaluate optimum deployment time of DMT-HFO (Fig. 6, 7). The results showed that based on $r^2$ concept, the correlations between $C_{DMT-HFO}$ and plant P content on shoot increased with deployment time. According to the $r^2$ value, the lowest and highest correlation were related to 24 hours ($r^2=0.22$) and 500 hours ($r^2=0.9$), respectively (Fig. 6). Therefore, in 500 hours, we were able to explain 90% of the variation ($r^2=0.90$) in shoot P content. The P pool that is easily available to plants in a short time was referred to fast release kinetics and are comprised P bound to the reactive surfaces and are in direct contact with the aqueous phase [36]. Also, Koopmans et al. [37] have described that the P pool with slow release kinetics was related to sesquioxide aggregates and available only over a long period of time. The P extracted by DMT-HFO in short times was probably related to P pool with fast release kinetics and that is probably why the correlation was weaker but the P extracted by DMT-HFO in long period of time was probably related to the both of P pools and showed a strongly significant correlation with P content of shoots.

![Fig. 5 Mass of phosphorus for different times during a diffusion experiment at 25 °C. The fitted line corresponds diffusion coefficient for KH₂PO₄. A) 6.9*10⁻⁶ cm².s⁻¹ in DGT B) 1.1*10⁻⁷ cm².s⁻¹ in DMT](image-url)
Fig. 6 The relationship of P content on corn shoots with P extracted by DMT-HFO in a) 24 hours b) 72 hours c) 240 hours and d) 500 hours

$r^2 = 0.22$

$r^2 = 0.31$

$r^2 = 0.52$

$r^2 = 0.9$

Fig. 7 The relationship between corn root P content with P extracted by DMT-HFO method) on times a) 24 hours b) 72 hours c) 240 hours d) 500 hours

$r^2 = 0.75$

$r^2 = 0.79$

$r^2 = 0.87$

$r^2 = 0.65$
The results showed that the relation between CDMT-HFO and plant P content of roots increased with deployment time to 250 hours and decreased to 500 hours based on $r^2$ value (Fig. 7). The close correlation was in 250 hours. However, in 24 to 250 hours, the statistical correlations based on $r^2$ concept were very similar. Taddesse et al. [38] was declared that fast release of P, approximately within the first two weeks and after that a slower release were happened. The correlations between CDMT-HFO and plant roots P content based on $r^2$ value were showed that probably the plant uptake on roots was related to fast release kinetics. By increasing the deployment time, the release of P was controlled by slow diffusion and the correlation was decreased.

The relationship between $C_E$ and Olsen-P with shoot P content is shown in Fig. 8. In this study, based on $r^2$ concept, the parameters of $C_E$ and Olsen-P were able to explain 76% and 86% of the variation ($r^2=0.76$ and $r^2=0.86$) in shoot P content, respectively. $C_E$ showed the close correlation with P content of roots ($r^2=0.84$) (Fig. 9). Also, the correlations between Olsen-P and concentrations in soil by the DGT ($C_T$) were significant ($p<0.05$, $r^2=0.84$) (Fig. 10). Many researchers have found a good relationship between Olsen P and plant P content for individual soils, especially in calcareous soils [39-41]. Unlike our study, Tandy et al. [7] observed no relationship between Olsen P and P content of barley leaf but $C_E$ showed the best correlations ($r^2=0.72$) in their experiment. This may possibly related to soil pH value. They used soils with neutral or acidic pH (5-7). Menzies et al. [14] have described that the bicarbonate extraction including Olsen P and Colwell P advance for calcareous soils. P transports form soil particle to roots are controlled mainly by desorption kinetics. DGT like plant roots(regardless of biological reactions) act as a sink for P which causes lowering of P concentration in soil solution and re-supply from the solid phase [42]. In comparison to extraction methods, DGT uses soil moisture conditions close to reality. Therefore, it could better imitate the P diffusion restriction to plant roots [2].

**IV. CONCLUSIONS**

This study was carried out to evaluate the performance of DGT and DMT-HFO devices (as new and relatively new techniques, respectively) as well as Olsen method (as a conventional method in calcareous soils) and comparing them to assess corn P content. This research showed that the diffusion through the DMT might be the rate-limiting step and this condition might simulate P release kinetics in soils with slow release kinetics. The amount of P extracted by DMT-HFO showed high correlations with corn P content. Although, soil test results in shorter times were more
appropriate like extraction or DGT methods but the DMT-HFO method can use to assess the availability of residual P in soils. This research would have benefitted by inclusion of more plant species and more soil types and could also be conducted in field scales which better simulates the conditions of plant P absorption in real situation.

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