

## Article

# Sensitivity of Three Phosphate Extraction Methods to the Application of Phosphate Species Differing in Immediate Plant Availability

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**Abstract:** Extractive tests for determining the plant-availability of soil phosphorus (P) give varying results due to the inherently different characteristics of the extraction solution. Generally, classical soil P tests such as the Olsen or calcium acetate/lactate (CAL) method do not give an indication on the total amount of plant available P, but merely give an indication of the equilibrium between soil and extraction solution. It is also not entirely clear which fractions of P are directly determined through the various methods of extraction, i.e., determined P must not be immediately plant available, as is the case for rock phosphate. It is therefore possible that extraction methods either over or under estimate the amount of P available for plant consumption. In this research, we compared three methods of soil P determination (CAL, Olsen and diffusive gradients in thin films (DGT)) with regards to their ability to determine P species ( $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ,  $\text{CaHPO}_4$ ,  $\text{Ca}_3(\text{PO}_4)_2$  and Inositol-6-hexakisphosphate) added to soils of high sorption capacity, immediately after as well as two weeks after application. For each of the methods, it could be shown that sorption processes in the soil immediately (0 days incubation) fix P to a point where it is not extractable through any of the described methods. These sorption processes continue over time, leading to a further decrease of determined P. The acidic CAL extraction method gives higher results of extractable P compared to the Olsen method. Due to the extraction of  $\text{Ca}_3(\text{PO}_4)_2$ , the CAL method may overestimate immediately plant-available P. The most suitable methods for the determination of immediately plant available P may therefore be the Olsen and DGT methods. Organic IP6 is not determined by any of the extraction methods. At low concentrations of soil P, the DGT method may fail to give results.

**Keywords:** diffusive gradients in thin films; phosphate availability; soil chemistry; plant nutrition; phosphate aging; phytate

## 1. Introduction

In recent years, attention has been brought to the depleting reserves of phosphate rock and the necessity of improving agronomic approaches to make the best use of available phosphorus (P) in agricultural soils, thereby reducing the need for frequent applications of P by farmers [1]. In order to improve agronomic practices that allow a reduction of P application to soil, it is necessary to relate P application to the P status of the soil, which can be determined through wet extraction of soils with acidic or alkaline extractants. In contrast to the rather straightforward method for analyzing the soil mineral nitrogen (N) status, the interpretation of results regarding available P is made difficult through the complex binding of P in natural soils [2,3].

In Europe, the extraction methods for the determination of soil P classification vary. In Germany and Austria, the main methods for extraction are the double-lactate method (DL) and the

calcium-acetate-lactate method (CAL) [4,5]. As with other extraction methods, there is often confusion about the fraction of P determined by these methods, as the determined concentration of extracted P is related to “plant-available” P, even though the relatively aggressive, acidic extraction also releases P from sorbed and precipitated fractions that are not immediately plant available. Rather, the methods more likely describe an equilibrium between the soil and extracting solution, which is helpful only for classifying soils according to their respective  $P_{\text{CAL}}$  or  $P_{\text{DL}}$  concentrations in combination with long-term analyses of yield in dependency of soil P status. Even then, depending on soil-pH and other physical and chemical properties, the classification of soils may differ dramatically, leading to false assumptions on P availability of soils [6]. In some cases, soils classified as low in  $P_{\text{CAL}}$  nevertheless lead to high yields despite the omission of P application, while high P soils may respond to the application of mineral P.

In contrast to the acidic extraction methods, the Olsen method uses an alkaline extractant ( $\text{NaHCO}_3$ ), thereby causing principally different desorption and dissolution processes in the soil-extractant mixture [3] and possibly causing a higher contribution of labile organic P fractions [6–8].

For basic purposes, such as the evaluation of soil P status as a guideline for P fertilization to agricultural systems, the method of extraction is suitable as long as the differences in P extraction from soil are taken into account [3]. Problems arise when the P status of the soil does not correlate to the observations of yield after P fertilization events, i.e., low P soils do not respond to P fertilization or high P soils respond to fertilization as mentioned above. In these cases, the extraction methods fail to give information on the underlying sorption and release processes. These observations, as well as recent developments of the global P market, have pointed out the need for suitable soil extraction methods that depict the availability of P in agricultural soils for plants, with the overall aim of increasing P use efficiency in crop production. As the classical extraction methods are not always reliable and must be evaluated according to their capacity for solubilizing bound, precipitated and sorbed P fractions, alternatives that better depict plant available P are being sought [9]. Further, it is necessary to identify analytical tools that allow sensitive observations of short-term changes to soil P fractions, thereby increasing our understanding of soil’s physical and chemical processes determining P sorption and desorption [2].

Various analytical approaches, such as the Resin-P approach, using cationic absorption materials, and the diffusion method, using iron-oxide ( $\text{FeO}$ ) impregnated filter papers [3,10], aim to imitate P uptake through plant roots, thereby determining both the soluble P fraction, as well as easily desorbed P fractions. A comparable approach is the diffusive gradients in thin films (DGT) method, which uses a ferrihydrite-containing hydrogel that allows the quantitative estimation of P concentrations in solutions/soil solutions by creating a concentration gradient of P near the DGT device [11–14].

In a recent paper, Christel et al. [14] described the sensitivity of the DGT method for the prediction of P availability in soils recently fertilized with pig slurry, stating that, while the capacity of the binding layer (ferrihydrite containing layer) of the DGT device was a limiting factor, the method could generally be used to depict increases in P availability after the application of organic fertilizers.

Yet it remains unclear which fractions of P are sensitive to DGT analysis and whether P extracted from agricultural soils is indeed only in the form of plant available P, or whether other, non- plant available fractions are extracted through the DGT method.

In this study, we determine the sensitivity of DGT devices and wet extraction methods (CAL and Olsen) in applications of different P species (calcium dihydrogen phosphate ( $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ); calcium hydrogen phosphate ( $\text{CaHPO}_4$ ); Tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ) and Inositol-6-hexakisphosphate (IP6)) to soils showing a high sorption capacity of P and an inherently low  $P_{\text{CAL}}$  fraction.

The aim of this paper is to determine which P fractions are sensitive to different extraction methods and whether or not CAL, Olsen and DGT are suitable methods for determining short-term changes in P availability driven by sorption/desorption processes of agricultural soils.

The hypotheses for this research were that (1) the determination of P from soils with recently added P forms depends on (a) the chemical characteristics of the extractant and (b) the water-solubility of the added P form and that (2) short-term changes of P availability in recently fertilized soils are best described through extraction methods that do not change the chemical equilibrium in soil/solution mixtures.

## 2. Materials and Methods

### 2.1. Test Soils

We collected soil from the uppermost 20 cm of two fields (Baimiskreuz, Hundsbrunnen) in the municipality of Hirrlingen, Baden-Württemberg, Germany (48°24' N 8°53' E). The soils were characterized by a relatively heavy texture and inherently low  $P_{\text{CAL}}$  and  $P_{\text{Total}}$  concentrations, for which they were primarily chosen, and differed in pH (Table 1), thereby making them suitable for principle investigations on P sorption after fertilization. In preparation of the experiment, the soils were air-dried and passed through a 5 mm mesh sieve. Soil pH was determined using the electrometric determination of  $H^+$  activity in a 0.01 M CaCl solution (1:2.5 soil (m) to solution (V)). Soil texture was analyzed through combined wet sieving (2 to 0.063 mm) and sedimentation (particles < 0.063 mm) after dispersion of soil samples and pre-treatment with  $H_2O_2$  (pipette method according to Köhn) [5].  $P_{\text{CAL}}$ ,  $P_{\text{Olsen}}$  and  $P_{\text{Total}}$  were analyzed through standard procedures. All analyses were carried out according to VdLUFA standard methods [5].

**Table 1.** Important basic characteristics of experimental soils.

Field	pH	Sand (%)	Silt (%)	Clay (%)	Texture	$P_{\text{CAL}}$ (mg kg <sup>-1</sup> )	$P_{\text{Olsen}}$ (mg kg <sup>-1</sup> )	$P_{\text{Total}}$ (mg kg <sup>-1</sup> )
Baimiskreuz	6.4	7	61	32	silty clay loam	16.5	11.6	510
Hundsbrunnen	7.4	5	66	29	silty clay loam	8.2	13.9	500

### 2.2. Experimental Treatments

In order to compare the sensitivity of 3 different P extraction methods, soil from the 2 fields described above was fertilized with 4 different P species, representing the primary ( $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ), secondary ( $\text{CaHPO}_4$ ) and tertiary ( $\text{Ca}_3(\text{PO}_4)_2$ ) calcium-salts of phosphoric acid ( $\text{H}_3\text{PO}_4$ ) as well as an organic phosphate species, inositol hexakisphosphate (IP6). P was added at a dose of 100 mg kg (soil)<sup>-1</sup>. An unfertilized control was included in the experimental design. With the two factors of soil and fertilization, the experiment comprised of 10 treatments with 4 laboratory replications each. The water content of the soil was adjusted to 18% (*w/w*). The soil was kept in glass jars at ambient temperatures for the duration of the incubation period (14 days). The soil was analyzed for P content using the CAL, Olsen and DGT methods at the beginning of the experiment (day 1) and at the end of the incubation period (day 14).

### 2.3. CAL Analysis

P was extracted from soil using CAL solution (0.05 M calcium-acetate, 0.05 M calcium lactate, 0.05 M acetic acid adjusted to pH 4.1) at 180 rpm for a duration of 90 min. As an adjustment to the original method, a measure of activated charcoal (1 tsp) was added to the soil and extractant solution before shaking to prevent the coloration of the extractant through humic substances in the soil. After shaking, the extract was filtered (150 mm MN 619 G filter paper) and prepared for the colorimetric determination (ammoniumvanadat/molybdat) of P concentrations at 436 nm wavelength.

#### 2.4. Olsen Analyses

With the Olsen method, P is extracted from soil using a 0.5 M sodium bicarbonate ( $\text{NaHCO}_3$ ) solution by shaking at 180 rpm for 30 min. The extractant was filtered (150 mm MN 619 G filter paper), after which P concentration of the extract was determined using inductively coupled plasma optical emission spectrometry (ICP-OES).

#### 2.5. DGT in Standard Solution

DGT devices were deployed to solutions ranging from 1 to 100 mg (P)  $\text{l}^{-1}$  and an additional solution of purified water for 24 h at ambient room temperature (20 °C), after which the DGT devices were removed, rinsed with purified water and opened. Both the diffusive layer of the DGT device, as well as the ferrihydrite binding layer of the device were eluted separately in 4 mL 1 M  $\text{HNO}_3$  for a period of 24 h, again at ambient room temperature. The P concentration of the eluant was analyzed using inductively coupled plasma mass spectrometry (ICP-MS).

#### 2.6. DGT Analysis in Soil

For the DGT analysis soil was prepared by creating a saturated paste of 50 g (soil) and 30 mL deionized  $\text{H}_2\text{O}$ . The saturation paste was left to settle for a period of 24 h, after which the ferrihydrite containing DGT devices (DGT Research, Lancaster, UK) were applied. After 24 h, the devices were removed and the ferrihydrite gel component of the DGT device was eluted in 4 mL 1 M  $\text{HNO}_3$  for a further 24 h. The eluant was analyzed for P concentration using inductively coupled plasma mass spectrometry (ICP-MS). Data are displayed as P eluted per disc.

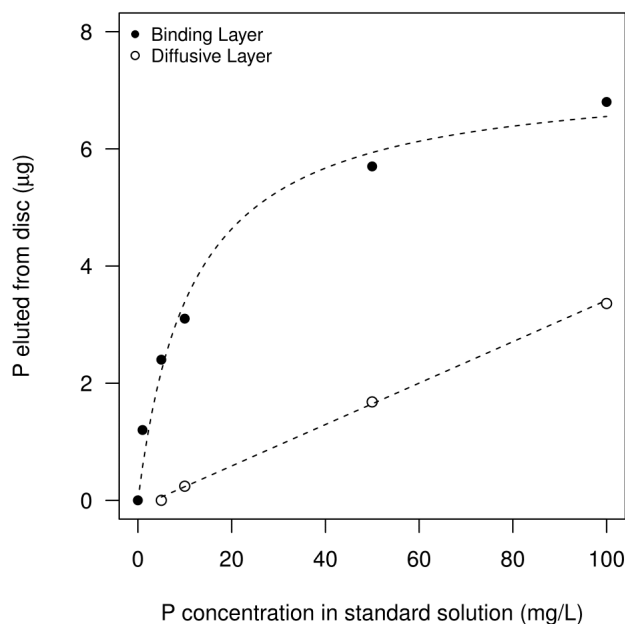
#### 2.7. Statistical Analysis

The results were analyzed using the R environment for statistical computing [15] and the drc package for bioanalysis [16]. Data were analyzed for variance and differences between factors and their interactions were determined using the appropriate post-hoc test, as indicated in the results section.

### 3. Results and Discussion

#### 3.1. DGT-Standard Curve

The uptake behavior of P from standard solutions ranging from 1 to 100 mg (P)  $\text{l}^{-1}$  corresponds with results previously described by Christel et al. [14] (Figure 1). The results of the standard curve indicate a sharp increase of P uptake into the binding-gel following a Michaelis-Menten curve with a calculated maximum uptake of 7.3  $\mu\text{g}$  (P)  $\text{disc}^{-1}$ . The results indicate that the DGT method is sensitive to low concentrations of P and is a suitable method for determining the plant available fraction of P in agricultural soils.



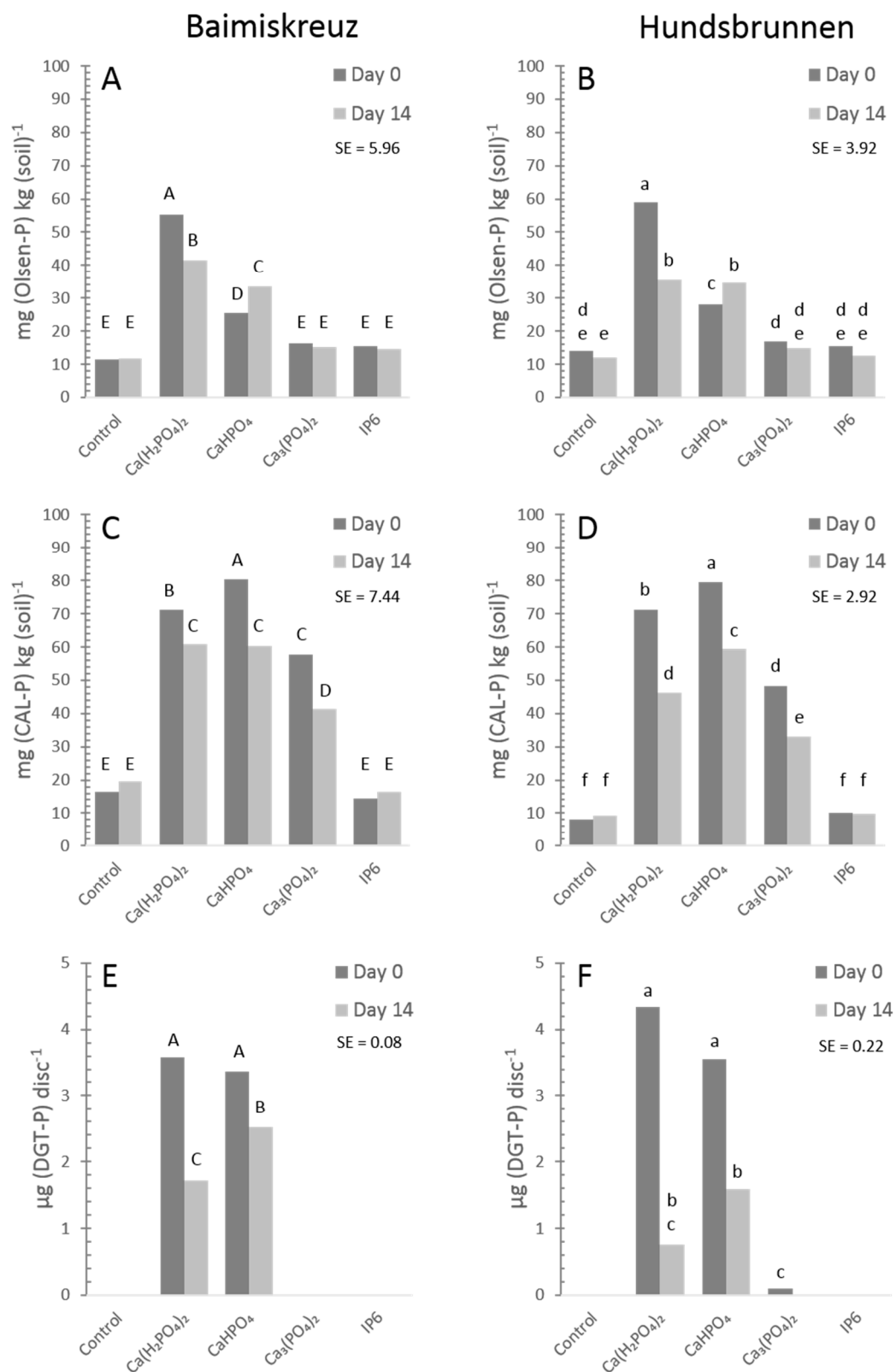
**Figure 1.** P eluted from a binding layer and a diffusive layer of diffusive gradients in thin films (DGT) devices following an exposure of 24 h to standard solutions with increasing phosphorus (P) concentrations and after the elution of the layers in 1 M HNO<sub>3</sub>. The dotted lines indicate the Michaelis-Menten-like P uptake of the binding gel (with a calculated maximum capacity of 7.3 µg (P)) and the linear relation of the diffusion of P into the diffusive layer at increasing P concentrations.

### 3.2. Comparison of Extraction Methods

Due to negligible differences between the two soils, a two-factorial ANOVA was conducted for each soil separately, followed by Tukey's test for honestly significant difference (TukeyHSD) at a confidence level of  $\alpha = 0.05$ .

The results of our analysis show that, generally, the extraction methods used give highly contrasting results when trying to determine the availability of P for plant production systems. Differences in P determined through the extraction method follow a decreasing order of CAL > Olsen > DGT, which is consistent with results described by Wünscher et al. [3], even though the DGT method was not part of their investigation.

Of the P species used in our experiment, only Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and CaHPO<sub>4</sub> may be considered to be immediately available for plant uptake, due to dissolution of the ions in soils. The remaining mineral P species, Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> with its strong similarities to rock phosphate, as well as the organic molecule IP6 are not immediately available for plant uptake as chemical [17] and biological processes are needed to dissolve P over time [18,19]. Many of these processes may be plant-mediated. Regarding these basic observations of P availability for plants, the Olsen method returns the most understandable picture, as the P species is determined in a decreasing order Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> > CaHPO<sub>4</sub> > Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> (Figure 2A,B). In contrast, the CAL method determines P species in a decreasing order of CaHPO<sub>4</sub> > Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> > Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>. The DGT method determines only the immediately available Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and CaHPO<sub>4</sub> (Figure 2E,F).



**Figure 2.** Olsen (A,B), calcium acetate/lactate (CAL) (C,D) and DGT (E,F) extracted P from the experimental soils Baimiskreuz (pH 6.4) (A,C,E) and Hundsbrunnen (pH 7.4) (B,D,F) after the addition of 100 mg (P) kg (soil)<sup>-1</sup>. Where no bars are depicted, P concentrations were below the analytical detection limit. Letters above bars indicate significant differences between observations (P source × time, TukeyHSD,  $\alpha = 0.05$ ) evaluated for each soil separately, SE indicates the pooled standard error.

The results of our experiment further show that, irrespective of the extraction method, soil processes restrict the determination of added P immediately after application. The amount of P not extracted from soils increases after an incubation period of 14 days after application, except for the determination of  $\text{CaHPO}_4$  through Olsen analysis, where P concentrations increase after incubation. Previous observations showed that, especially in low P soils, P sorption increases with the duration of incubation, especially at low rates of application [20,21].

The DGT method only determines P added through highly water-soluble P species. P species of higher molecular weight were not determined through the DGT method, thereby supporting the method as suitable for the determination of immediately plant available P.

As the acidic extraction of the CAL method may give false information on immediate P availability through the dissolution of unavailable  $\text{Ca}_3(\text{PO}_4)_2$  species, only the DGT and Olsen method are suitable if immediate plant availability of P is to be determined. Of these two, only the Olsen method was able to determine changes to the immediately available P pool at low soil-P concentrations. Further, the Olsen method may be more reliable compared to the DGT method when the aim is to predict plant availability of P or when changes to the immediately plant-available P fraction are to be determined, as described by Burkitt et al. [22].

It has further been stated that, while the DGT method may accurately depict the amount of P immediately in solution, it may not always correlate well to plant performance as plant-mediated dissolution processes are not taken into account [23,24]. While the DGT method is described as an accurate method for the determination of plant-available P even at low concentrations in various soil types [9,25], our study only determined DGT available P after the addition of highly soluble P species, which may indicate the limits of the method when applied to soils that show both low P concentrations as well as a high sorption capacity, as indicated through the CAL and Olsen methods.

#### 4. Conclusions

Our work identifies to what extent different P species are extracted from soils of high sorption capacity through common methods for the determination of P concentrations. It shows that processes of P sorption may lead to an immediate fixation of added P to a point where portions of added P are not determinable through extraction methods. When immediately plant available fractions and short-term changes of these fractions should be determined, researchers should choose extraction methods that only determine immediately plant available P forms (Olsen, DGT). Further, our research shows that, especially when the chemical equilibrium of soil/solution mixtures is changed through acidic extraction solutions an overestimation of plant available P is possible, especially when insoluble tricalciumphosphate-like P forms are present in the soil environment. Future research should focus on combined approaches to soil P analysis in order to identify the extracted P species, thereby allowing conclusions on soil chemical processes determining the plant availability of P in soils.

**Author Contributions:** T.E.H. conceived and planned the experiment. T.E.H., I.W. and Y.Y. carried out the experiments and performed the experimental analyses and calculations. T.E.H. took the lead in writing the manuscript. All authors discussed the results and contributed to the final manuscript. T.M. supervised the project.

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