Biogeochemistry of phosphorus in soil:

Optimization criteria for an efficient and environmentally acceptable agronomic use of a nonrenewable resource

PhD Student : Ramiro Recena Garrido
Director : Antonio Delgado García

Universidad de Sevilla

Departamento de Ciencias Agroforestales

TESIS DOCTORAL

Biogeoquímica del fósforo en el suelo:

Optimización de criterios para un uso agronómico eficiente y ambientalmente aceptable de un recurso no renovable

Biogeochemistry of phosphorus in soil:

Optimization criteria for an efficient and environmentally acceptable agronomic use of a nonrenewable resource

Ramiro Recena Garrido

Universidad de Sevilla

Departamento de Ciencias Agroforestales

Biogeoquímica del fósforo en el suelo:

Optimización de criterios para un uso agronómico eficiente y ambientalmente aceptable de un recurso no renovable

Biogeochemistry of phosphorus in soil:

Optimization criteria for an efficient and environmentally acceptable agronomic use of a nonrenewable resource

Memoria presentada para aspirar al Título de Doctor con mención internacional por la Universidad de Sevilla

Director:

Fdo. Antonio Delgado García

Catedrático de Universidad
Dep. de Ciencias Agroforestales

Fdo. Ramiro Recena Garrido

Ing. Superior de Montes

Agradecimientos

Me gustaría expresar mi más sincero agradecimiento a las personas que han participado en la presente tesis doctoral, así como a las instituciones; CONSEJERÍA DE ECONOMÍA, INNOVACIÓN, CIENCIA Y EMPLEO DE ANDALUCÍA, Secretaría General de Universidades, Investigación y Tecnología, con Código del proyecto AGR-6385, de Investigación de Excelencia, INCENTIVOS A PROYECTOS DE INVESTIGACIÓN DE EXCELENCIA, a la Universidad de Sevilla, al Centro Escuela Técnica Superior de Ingeniería Agronómica, así como al personal de los grupos de Investigación AGR-188 y AGR-165 y a la Universidad de Almería, personalizadndo en Dr. D. Miguel Guzmán y Dr. D. Agustín Sánchez.

A mi director de tesis, Dr. D. Antonio Delgado García, por su lucidez intelectual, pasión por el trabajo y su contagiosa motivación. Además, por el trato pesonal inmejorable y por haberme guiado y apoyado durante estos intensos años. Gracias, a un jefe que sonríe.

A Ana García, mi compañera con la que tanto disfruto trabajando, sin la que nada habría sido igual, a mi amiga.

Gracias Oliva Polvillo, por tus consejos, por tu cariño, pero más aún si cabe, por estar ahí…siempre. Así como a Purificación Pajuelo, por cuatro años disfrutándote como compañera. Gracias a las dos.

A Dra. Dña. Isabel Díaz de la Torre, por poner su corazón y facultades, pase lo que pase, en su claro camino del trabajo noble y eficiente, por su respaldo y ánimo personal y emocional, gracias Isa. Al Dr. D. José Torrent Castellet y Dra. Dña. Mª del Carmen del Campillo García, ya que hay conocimientos y experiencia de valor incalculable que uno no sabe como agradecer haber contado con ellos.

A mis compañeras de despacho al empezar esta etapa, que tan bien y con tanto cariño me han tratado siempre, Celia Borrero, Paloma, Rocío López y Rocio Álvarez, Mari Luz, Aurora2 y Silvia.

A los profesores de la Universidad de Sevilla Dr. Eusebio Carmona Chiara, Dr. José Manuel Quintero Ariza, Dra. María Mercedes Valera Córdoba, Dra. María José Martín-Palomo Grarcía, Dr. Manuel Avilés Guerrero, Dr. Luis Andreu Cáceres, Dra.

María Teresa Moreno Aguirre, Dr. José María Urbano Funtes-Guerra, Dr. Victor Fernandez Cabanas, Dr. Alberto Horcada, Dra. Rocío Giménez, por haberme apoyado tanto técnica como anímicamente, por su familiaridad y camaradería, ya que tanto en docencia, colaboraciones o de manera personal, me ha hecho sentir uno más.

A quienes después llegaron, Esther y María José, Manuel Ángel y Aurora Moreno. A Marcos Carrasco, Jorge Pino, Rocío Brenes y Aurora Moreno, por dejarme disfrutar en la "Interacción con malas hierbas".

A Dra. Bárbara Cade-Menun (SPARC, Agriculture and Agri-Food Canada, AAFC), por su dedicación técnica personalizada, por el extraordinario acogimiento personal del que me hizo disfrutar, así como por el intenso y motivante trabajo y aprendizaje. Gracias por poner su valia personal e intelectual así como su experiencia, en esta colaboración. Al Director del centro "The Semiarid Prairie Agricultural Research Centre (SPARC)" Bruce McArthur, por todo lo compartido, bebido y hablado.

A mis amigos, que más que amigos son hermanos. A los que están cerca, a los que están lejos y a los que ya no están. Muy especialmente a Javier Marquez, porque cada vez que la vida me rompe, aparece tu presencia silenciosa, a Ernesto Ruz Pérez…no te olvido ni te olvidaré un solo dia, a la gente de Baños de la Encina y Cazorla, porque yo soy vosotros. A Lourdes Pérez Aguilera, porque contigo empezó todo estoy a Emilio Peña.

A pesar de todo, a mi familia. En especial a mi "Tita Juanita" y mi hermana Paula.

Al trabajo en sí mismo, porque levantarte motivado cada día y que la fatiga física sea el único limitante para su disfrute, no tiene precio.

"Without mentors in live, you will never be nothing"

A mi madre...con toda mi alma.

General Index

Page

Acknowledgment 1

General Index 4

Index of Figures 5

Index of Tables 8

Summary 9

Chapter I Introduction and Objectives 19

Chapter II Accuracy of Olsen P to assess plant P uptake in relation to soil properties and P forms 43

Chapter III Estimation of threshold Olsen P values for fertilizer response in soils of Mediterranean areas 66

Chapter IV Estimation of total plant available phosphorus in representative Mediterranean soils 90

Chapter V Organic phosphorus forms in representative Mediterranean soils as related to soil properties and fertilization 125

Chapter VI General Discussion 158

Chapter VII Conclusions 173

Chapter VIII General References (Introduction and Discussion) 177

Annexes 196

Index of figures

Page

2.1. Experiment with cucumber in growing chamber. a Plant after approximately 20 days after planting, without symptoms of P deficiency. b Clear symptoms of P deficiency in basal leaves at the end of the experiment (50 days after planting). Plants in growing chamber at 30 days after planting 57

2.2. a Relationship of P uptake by plants in high- (empty symbols) and low-P (solid symbols) samples with the affinity factor (b) in the Freundlich equation ($Y=A X^{1/b}$); $R^2=0.76$, $P<0.001$ for high-P samples ($R^2=0.4$, $P<0.01$ if marked point is not included), and $R^2=0.46$, $P<0.01$ for low-P samples. b Relationship of P uptake by plants in high-P samples and the sum of Fe extracted by citrate-ascorbate (ca) and citratebicarbonate- dithionite (d) [the point marked with an arrow was not included in the regression since it was clearly influential according to the Leverage test]. $R^2=0.57$, $P<0.001$. The experiment was performed with one plant per pot; in each pot the amount of Olsen P was the same (1 mg pot^{-1}). Since P deficiency symptoms were evident in all soils, P uptake by the plant in each pot provides a measure of the ratio of total available P to Olsen P...58

2.3. P uptake by plants in low-P samples as a function of the following. a The amount of molybdate reactive P (inorganic P) extracted by NaOH and citrate-bicarbonate (cb) in the sequential fractionation scheme ($R^2=0.52$, $P<0.001$). b As a function of the amount of total organic P (TOP) in pots and alkaline phosphatase activity (Phosp) in the rhizosphere soil ($R^2=0.37$, $P<0.01$). c As a function of this enzymatic activity and soil pH ($R^2=0.5$, $P<0.001$). It should be noted that the sign of multiple linear regression in (b) does not provide real information about the sense of the contribution of each independent variable since phosphatase and TOP were significantly correlated...........................59

3.1. Pot starvation experiment: (a) sunflower in growing chamber with incipient P deficiency symptoms (b) and clear deficiency symptoms (c); and wheat (d) with P deficiency symptoms (e). 75

3.2. Estimation threshold Olsen P values for fertilizer response as a function of: **a)** clay, pH, and mean phosphatase activity (Phosp) in the rhizosphere (for the four crops in high-P samples); $Y = 32 - 0.015\ clay - 3.26\ pH + 0.05\ Phosp$; $R^2 = 0.87$, $P < 0.001$, and coefficients for each explicative variable significant at $P < 0.05$ in the least significant case; and **b)** clay, soil organic carbon (SOC) and phytase hydrolysable P in CB extracts of high-P samples (CB_h); $Y = 14 + 2.05\ SOC - 0.22\ CBh - 0.02\ clay$; $R^2 = 0.75$, $P < 0.001$, and coefficients for each explicative variable significant at $P < 0.1$ in the least significant case. Threshold value can be estimated also as a function of clay and $pH: 36 - 0.012\ clay - 3.02\ pH$; $R^2 = 0.81$; $P < 0.001$; and coefficients for each explicative variable significant at $P < 0.01$ in the least significant case and mean absolute error = 1.12...80

3.3. Estimation of the logarithm of the threshold P_{CaCl2} values for fertilizer response as a function of: a) clay, and the ratio of Fe_{ca} to Fe_d; $Y = -2.02 - 0.0018\ clay + 2.92\ Fe_{ca}/Fe_d$; $R^2 = 0.57$, $P < 0.001$; coefficients for each explicative variable significant at $P < 0.05$ in both cases; and b) pH and the sum of phytase hydrolysable in NaOH and CB extracts, $Y = 2.38 - 0.62\ pH + 0.034\ (NaOH_h + CB_h)$; coefficients for each explicative variable significant at $P < 0.05$ in both cases. Threshold value can be also estimated as a function of clay and active Ca carbonate equivalent (ACCE): $Y = -1.13 - 0.039\ ACCE - 0.002\ clay$; $R^2 = 0.56$, $P < 0.01$, and coefficients for each explicative variable significant at $P < 0.05$ 82

4.1. Example of estimation of P_{ClCa2} for null P uptake (threshold value for P uptake) and for estimation of total plant available P from the cumulative uptake curve in one of the soils studied. Relative P uptake for sunflower is represented, and it was estimated as the ratio of P uptake in each step to P extraction in the first depletion step (a). Cumulative P uptake for the soils in the 8 depletion steps considered (4 for high-P and 4 for low-P samples) (b). P_{CaCl2} is the concentration of P in 0.01 M $CaCl_2$ 101

4.2. Estimation of the total plant available P in soil (TAP, mean value for wheat and sunflower) as a function of Olsen P (Olsen et al. 1954) and the ratio of citrate ascorbate (Fe_{ca}) to citrate-bicarbonate-dithionite (Fe_{cbd}) extractable Fe (a), as a function of Olsen P and the estimate of the buffer capacity from the sorption isotherms at a concentration in the solution of 1 mg P L^{-1} (BC) (b), and as a function of Olsen P, clay and Cacarbonate equivalent (CCE) in soil (c). When Olsen P was taken as the only predictive variable, $R^2 = 0.53$ and $P < 0.001$. 109

4.3. Estimation of the total plant available P in soil (TAP, mean value for wheat and sunflower) as a function of extractable P by resin in Cl^- form after 1 h (Cl-AER) and the phytase hydrolysable P in NaOH and CB extracts of the fractionation scheme proposed by Ruíz et al. (1997) (a), and as a function of Cl-resin extractable P at 1 h and the ratio of citrate ascorbate (Fe_{ca}) to citrate-bicarbonate-dithionite (Fe_{cbd}) extractable Fe (b). When Cl-resin at 1 h was taken as the only predictive variable, $R^2 = 0.77$, $P < 0.001$ (the same with a logarithmic transformation of both variables).. 110

4.4. Estimation of the total plant available P in soil (TAP, mean value for wheat and sunflower) as a function of extractable P by resin membranes in HCO_3^- form after 24 h extraction ($P_{HCO3\text{-}AER}$). Model does not change significantly when points above 5 times the mean influence were not considered in the regression ($Y = 14.1 + 2.1\ X$ with all points, and $Y = 13 + 2.3\ X$, $R^2 = 0.8$; $P < 0.001$ when influential points were not taken into account). Regression was also highly significant ($R^2 = 0.67$, $P < 0.001$) with normalized data (logarithmic transformation for TAP and $data^{-0.3}$ for $P_{HCO3\text{-}AER}$). 111

5.1. ^{31}P NMR spectra of NaOH-EDTA extract of soils PLP and ZMB for low-P and high-P samples 136

5.2. Relationship between the difference in monoester phosphates concentration between high- and low-P samples (using C2 correction for diester degradation), and the difference in Olsen P between the same samples 143

5.3. Relationship between the mono to diester phosphates ratio (C2 correction for diester degradation) and Olsen P in studied soils (all the samples, high- and low-P); data marked with an arrow was excluded from the regression..................... 144

5.4. Relationship between: a) the ratio of monoester phosphates to total organic P and Olsen P; b) the ratio of chiro-IP6 to total organic P and Olsen P, and c) the ratio of myo-IP_6 to total organic P and Olsen P. Total organic P determined by the combustion method.. 145

Index of tables

Page

2.1. Main inorganic and organic P fractions according to the fractionation scheme of Saavedra et al. (2007) and availability index according to Olsen et al. (1954) in the studied soils before crop experiment 53

2.2. Sorption curve parameters, estimated buffer capacity (BC) at different P concentration in the soil solution, dry mass (DM) yield, P concentration in shoot and roots, and P uptake by plants from pots and alkaline phosphatase activity in rhizosphere with a mixture of siliceous sand and soil to encompass 1 mg of Olsen P supplied per plant. 56

3.1. Mean, standard deviation (SD) and range of the properties of studied soils (n = 18) 72

3.2. Correlation coefficients between different properties of studied soils 73

4.1. .Mean, standard deviation (SD) and range of the properties of studied soils (n = 18) 104

4.2. Mean, standard deviation (SD) and range of the quantity and intensity indexes of studied soils (n = 18) 105

4.3. Correlation between total available P to plants in soil (TAP), different P extractions used as soil availability index, and different soil properties 107

5.1. General soil properties, affinity parameter (b), and Olsen P of studied soils 134

5.2. Chemical shifts of peaks detected in ^{31}P-NMR spectra 135

5.3. ^{31}P NMR forms expressed as % of extracted P in the NaOH-EDTA extracts 138

5.4. Groups of main P forms in NaOH-EDTA extracts 140

Summary

A relevant issue for accurate phosphorus fertilization management is to estimate phytoavailable phosphorus (P), which is defined as the amount of P present in soil that can be used by plants in successive crops until evident P deficiency symptoms appear in crop. It can be supposed that there is a starvation of this available pool when P release from solid phase does not maintain a high enough P concentration to maintain P adsorption by plants. This plant available P is normally assessed by "P availability indexes" or “soil P test” (SPT). However, the accuracy of these different methods, over a wide range of soils, may be limited and poor relationships are frequently observed between phosphorus availability indexes, such as Olsen P, and phosphorus absorption by plants. An efficient use of phosphorus in agriculture should be based on an accurate estimationof bio-available phosphorus in soils. In this thesis, focused on soils from Mediterranean environments, the Olsen P will be taken as a reference for SPT.

In the first chapter of this PhD thesis, a general introduction to the problem of P in agriculture, with information regarding its relevance as non-renewable and strategic resource necessary in agriculture, was done. In this chapter, objectives of this thesis were defined, all focused on the sustainable use of P in agriculture.

In the second chapter, factors affecting the accuracy of Olsen phosphorus index (Olsen-P) were studied. To this end, a P depletion experiment in 17 Mediterranean soils was carried out. Two samples differing widely in Olsen-P were used for each soil, named "low-P" and "high-P". The soil was mixed with silica sand to achieve 1 mg of P as Olsen-P per pot. Thus, the proportion of unavailable P to Olsen-P, either inorganic or organic P in the pot, was higher for "low- P" samples than for "high-P" samples. In "high-P" samples, P uptake by crop (cucumber) increased with increased affinity factor of soil phase, estimated from the P sorption curves (R^2 = 0.76; $P < 0.001$) and with increased P adsorption capacity of soil, estimated as the concentration of Fe in oxides in soils (R^2 = 0.56; $P < 0.001$). On the other hand, in "low-P" soil samples, total organic P and phosphatase activity in the rhizosphere contributed to explain P absorption by crops(R^2 = 0.37, $P < 0.01$). These results revealed that both organic P and the hydrolytic activity in rhizosphere have implications in explaining P availability to plants when the ratio of organic P to Olsen P is high (low-P samples). On the other hand, the physical and chemical properties controlling the dynamics of inorganic P played an important role in the uptake of P from high-P samples.

The third chapter of this of this PhD Thesis was focused on the identification of soil properties affecting threshold values of Olsen P, below which a response by crop to P fertilization can be expected. There are recent evidences that, even in soils where a given SPT such as Olsen P is recommended, threshold values for SPT may range widely. This significantly affects the practical implications of SPT. Based on the soil properties affecting threshold values for Olsen P, models were proposed for the accurate estimation of threshold values in the interpretation of Olsen-P as SPT in these soils. This is basic for the identification of P responsive sites in geographical areas where this SPT is recommended. Threshold values for Olsen P and P in soil solution estimated by an extraction with 0.01 M$CaCl_2$ varied widely between soils. Both indexes were positively correlated with each other ($P < 0.001$).

Clay content was the soil property affecting to a greater extend threshold values for Olsen P, explaining a 60 % of variation ($P < 0.001$). Overall, Olsen P threshold values decreased with increased buffer capacity (estimated with adsorption isotherms at 1 mg P L^{-1}) and P sorption capacity (estimated as Fe in Fe oxides or clay). Threshold values for Olsen P can be predicted in studied soils by multiple regressions involving clay content, pH, and phosphatase activity in the rhizosphere (R^2 = 0.87, $P <$ 0.001); when only pH and clay were taken into account, it was explained 81 % of

variation ($P < 0.001$) with an average of the absolute error of 1.12. This means that a single model based on pH and clay content can accurately estimate threshold values in the studied soils. In the case of P_{CaCl2} threshold values can be estimated as a function of clay and the ratio of Fe in poorly crystalline to that in crystalline Fe oxides($R^2 = 0.57$, $P < 0.001$). In addition, accurate predictions of P_{CaCl2} threshold values can be also achieved based on multiple regressions with pH and phytase hydrolysable P in some P fractions. It can be concluded that, in the group of soils studied, soil properties related with P buffering capacity of soils are the most relevant explaining threshold values for both SPT tested. However, it should be highlighted the contribution of factors related to organic P dynamics affecting threshold values.

The fourth chapter of this PhD Thesis was aimed to define more precise methods to estimate total plant available P (TAP).To this end, different P extraction methods were tested: (i) Olsen P (ii) P concentration in 0.01 M $CaCl_2$, (iii) 0.27 M Na citrate + 0.11 M $NaHCO_3$ (CB), (iv) the two first sequential extractions in the Ruíz et al. (1997) fractionation scheme (NaOH +CB), (v) extraction with anion exchange resins (AER) in Cl^- and HCO^-_3 forms. Total available P (TAP) values in soils were obtained by P depletion experiment by growing plants successively in soils. TAP was significantly related to HCO_3^-AER but also with Cl^- AER (R^2 = 87% and 77% respectively). In addition, variance of TAP explained by Cl-AER increased till 86% if phytase hydrolysable P in NaOH and CB extracts was taken into account. AERs have proved to be sensitive not only to *Q*, but also to buffer capacity (BC). When Olsen P was considered as the only predictive variable, it only explained a 53 % of the TAP variation (mean value for wheat and sunflower, $P < 0.001$). When the ratio Fe_{ca}/Fe_{cbd} and the buffer capacity (BC) were also included as predictive variables in the model, variance explained increased to 69 and 61 %, respectively. When clay and CCE were included in the predictive model with Olsen P, variance explained increased till 80 %. Overall, better results obtained with AERs than with Olsen P or other single chemical extraction and were explained by their better relationship with most of the soil properties conditioning TAP.

In the fifth chapter of this PhD Thesis, organic P forms and their potential contribution to available P to plants in Mediterranean soils were studied. Organic P in soil is the less understood aspect of the P cycle in soils, particularly in those from semiarid and arid lands. Organic P forms and how may be affected by P status and soil properties was dealt in this chapter. The study was carried out by NMR spectroscopy of ^{31}P (^{31}P NMR) in NaOH-EDTA extracts. From the soil collection used in the study described in chapter 1, eight representative Mediterranean soils were selected, and for each one, a "low-P" and "high-P" sample was studied. Organic P concentration in “high-P” and “low-P” samples was not significantly different. Beside this, the concentration of orthophosphate monoesters and their relative contribution to extracted P or OP according to the dry combustion method did not significantly differ between low- and high-P samples. Monoesters were the dominant fraction of OP accounting for half of the total organic P. The differences in monoesters phosphates between low- and high-P soil samples increased with increasing differences in Olsen P ($R^2 = 0.61$, $P < 0.05$). The mono- to diesters ratio increased with increased Olsen P in soil ($R^2 = 0.49$; $P < 0.01$), also monoesters and IP_6, as OP proportion, increased as increased Olsen P in soil in vineyards and olive orchards, where almost no crop residue accumulation occurs. All these results reveal that, under P starvations conditions, some hydrolysis of these OP forms and potential contribution to P supply to plants may be expected. *Myo*-IP_6 seems to stabilize by adsorption, as revealed by its positive relationship with the ratio of Fe in oxide to clay content and its decreased concentration in soil with increasing pH. On the other hand, precipitation of Ca phosphates may explain the retention of other IP_6 stereoisomers in these soils.

A general discussion of results is done in the sixth chapter, and finally, exposition of main conclusions of the present work is presented in the seventh chapter.

Resumen

Una cuestión relevante para gestionar de manera precisa la fertilización fosfatada es estimar el fósforo (P) disponible para las plantas en el suelo, que se define como la cantidad de P presente en el suelo que puede ser utilizado por las plantas en cultivos sucesivos hasta que aparecen evidentes síntomas de deficiencia de P en el cultivo. Se puede suponer que hay una necesidad de aplicar este nutriente al suelo cuando la liberación de P desde la fase sólida no mantiene una concentración de P lo suficientemente alta para mantener la adsorción de P por las plantas. Este P disponible para la planta se evalúa normalmente utilizando los denominados "índices de disponibilidad de P" o "Tests de P en suelo" (SPT). Sin embargo, la exactitud de estos diferentes métodos aplicados a suelos con propiedades muy diferentes puede ser limitada y con frecuencia se observan malas relaciones entre los índices de disponibilidad de fósforo, tales como P Olsen, y la absorción de fósforo por las plantas. Un uso eficiente de fósforo en la agricultura se debe basar en una estimación precisa del fósforo biodisponible en los suelos. En esta Tesis Doctoral, centradaen suelos de ambientes mediterráneos, el P Olsen se tomará como referencia para el SPT.

En el primer capítulo de esta Tesis Doctoral, se hizo una introducción general al problema de P en la agricultura, con información sobre su importancia como recurso no renovable y estratégico necesario en la agricultura. En este capítulo, se definieron los objetivos de esta Tesis Doctoral, todo enfocado a un uso sostenible de P en la agricultura.

En el segundo capítulo, se estudiaron los factores que afectan a la precisión del P Olsen como índice de disponibilidad del nutriente. Con este fin, se llevó a cabo un experimento de agotamiento de P en 17 suelos representativos de ámbito Mediterráneo. Se utilizaron para cada suelodos muestras que diferíanampliamente en el valor de P , llamadas "high -P" y "low -P". El suelo se mezcló con arena de sílice

para tener1 mg de P como P-Olsenpor maceta. Por lo tanto, la proporción de P no disponible (inorgánico u orgánico) con respecto al P-Olsenen maceta fue mayor para las muestras "low-P" que para las muestras "high-P".En las muestras "high-P", la absorción de P por los cultivos (pepino) aumentó con el aumento del factor de afinidad de la fase sólida, estimado desde las curvas de absorción de P ($R^2 = 0{,}76$; $P <0{,}001$) y con el incremento de la capacidad de adsorción de P del suelo, estimada como concentración de Fe en óxidos en los suelos ($R^2 = 0{,}56$; $p <0{,}001$). Por otro lado, en las muestras "low -P", la actividad fosfatasa orgánico total en la rizosfera y el P orgánico contribuyó a explicar la absorción de P por los cultivos ($R^2 = 0{,}37$, $P <0{,}01$). Estos resultados revelaron que ambos factores, el P orgánico y la actividad hidrolítica en rizosfera tienen implicaciones en la explicación de la disponibilidad de P para las plantas cuando la proporción de P orgánico con respecto al Olsen P es alto (muestras “low-P”). Por otra parte, las propiedades físicas y químicas que controlan la dinámica del P inorgánico juegan un papel importante en la absorción de P en las muestras ”high-P”.

El tercer capítulo de la presente Tesis Doctoral se centró en la identificación de las propiedades del suelo que afectan a los valores críticosdel P-Olsen por debajo del cual se puede esperar una respuesta por parte de los cultivos a la fertilización fosfatada. Hay evidencias recientes de que, incluso en los suelos donde se recomienda un SPT dado como el P-Olsen, los valores umbral para dicho SPT pueden variar ampliamente. Esto afecta de manera significativa a la precisión del método. Sobre la base de las propiedades del suelo que afectan a los valores críticos de P Olsen, se han propuesto modelos para la estimación precisa de los valores de umbral en la interpretación del Olsen como SPT para suelos representativos de ámbito mediterráneo. Esto es básico para la identificación de los suelos que pueden responder a la aplicación de P en zonas geográficas donde se recomienda este SPT. Los valores críticospara P Olsen y P en la solución del suelo estimada por una extracción con $CaCl_2$ 0,01 M variaron ampliamente entre los suelos. Ambos índices se correlacionaron positivamente entre sí ($P < 0{,}001$).

El contenido de arcilla fue la propiedad del suelo que afectó en mayor grado a los valores críticosde P Olsen, explicando un 60% de la variación ($P < 0.001$). En general, los valores umbral de P Olsen disminuyeron con el aumento de la capacidad tampón (estimado con las isotermas de adsorción a 1 mg P L^{-1}) y la capacidad de sorción de P (estimado como Fe en óxidos de Fe o arcilla). Los valores umbral para el P Olsen se pueden predecir en los suelos estudiados con regresiones múltiples que contemplan el contenido de arcilla, pH y actividad de la fosfatasa en la rizosfera (R^2 = 0.87, $P < 0.001$); cuando sólo el pH y la arcilla se tuvieron en cuenta, se explicó el 81% de variación ($P < 0{,}001$), con una media del error absoluto de 1.12. Esto significa que un solo modelo basado en el pH y el contenido de arcilla pueden estimar con precisión los valores de críticosen los suelos estudiados. En el caso de valores de umbral de P_{CaCl2} puede estimarse como una función de la arcilla y la proporción de Fe en forma cristalina con respecto a la de los óxidos de Fe cristalinos (R^2 = 0.57, $P < 0.001$). Además, las predicciones exactas de valores de umbral de P_{CaCl2} pueden ser también obtenidos basándose en múltiples regresiones con el pH y el P hidrolizable por fitasa de algunas fracciones de P. Se puede concluir que, en el grupo de los suelos estudiados, las propiedades del suelo relacionadas con la capacidad tampón de P de los suelos son las más relevantes a la hora de explicar los valores de umbral para ambos SPT analizados. Sin embargo, cabe destacar la contribución de factores relacionados con el P orgánico que afectan a los valores umbral.

El cuarto capítulo de esta tesis doctoral tenía como objetivo definir los métodos más precisos para estimar el P disponible total para la planta (TAP). Para ello, se estudiarondiferentes métodos de extracción de P: (i) P Olsen, (ii) concentración de P en 0.01 M$CaCl_2$, (iii) 0.27 MNa citrato + 0.11 M $NaHCO_3$ (CB), (iv) las dos primeras extracciones del fraccionamiento secuencial de Ruiz el al., (1997) (NaOH +CB), (v) extracción con resinas de intercambio aniónico (AER) en forma de Cl^- y HCO^-_3 . Los valores para el P total disponible (TAP) en suelo se obtuvieron de un ensayo de agotamiento de P en suelo mediante cultivos sucesivos. El TAP se relacionó significativamente con HCO_3^-AER pero también con Cl^- AER (R^2 = 87% y 77%, respectivamente). Además, la varianza del TAP explicada por Cl-AER aumentó hasta el 86% cuando el P hidrolizable por fitasa en extractos NaOH y CB se tuvo en cuenta. Las AERs han demostrado ser sensibles no sólo al factor cantidad en el suelo (Q), sino también la capacidad tampón (BC) .Cuando el P Olsen se consideró como la única variable predictiva, explicó solamente un 53% de la variación del TAP (valor medio para el trigo y girasol, $P < 0.001$). Cuando la relación de Fe_{ca}/Fe_{cbd}y la capacidad tampón (BC) también se incluyeron como variables de predicción en el modelo, la varianza explicada por el P Olsen aumentó a 69 y 61%, respectivamente ($P < 0.001$). Cuando la arcilla y CCE se incluyeron en el modelo predictivo con P Olsen, la varianza explicada aumentó hasta el 80%. En general, mejores resultados se obtuvieron con AERs que con P Olsen u otra simple extracción química, y se explicó por su mejor relación con la mayoría de las propiedades del suelo que condicionan el TAP.

En el quinto capítulo de la presente Tesis doctoral, se estudiaron las formas de P orgánico y su potencial contribución al P disponible para las plantas en suelos mediterráneos. El P orgánico es el aspecto menos comprendido del ciclo P en los suelos, sobre todo en tierras áridas y semiáridas. El estudio se llevó a cabo por espectroscopia de resonancia magnética nuclear de ^{31}P (^{31}P NMR) en extractos de NaOH-EDTA. De la colección del suelo utilizado en el estudio descrito en el capítulo 1, se seleccionaron ocho suelos mediterráneos representativos, y para cada suelo, se estudió una muestra "low-P" y "high-P". La concentración de P orgánico en muestras "high -P" y las "low -P" no fue significativamente diferente. Además, la concentración de monoésteresortofosfato y su contribución relativa al P extraído o al OP con respecto al método de combustión seca, no fue significativamente distinto entre las muestras "high-P" y las "low-P". Los monoésteres fueron la fracción dominante del OP, representandola mitad del total del OP. Las diferencias en monoésteres fosfatos entre las muestras "high-P" y las "low-P" de suelo, aumentó con el aumento de las diferencias en P Olsenentre ambos tipos de muestra (R^2 = 0.61, P <0.05). El ratio mono/diésteres tendió a aumentar con el aumento de P Olsen en el suelo (R^2 = 0,49; P <0,01). Losmonoésteresy los inositoles-6-P (IP_6), expresados como proporción del OP, aumentó con el aumento del P Olsen en el suelo en viñedos y olivares, donde casi no se produce la acumulación de residuos del cultivo. Todos estos resultados revelan que, en condiciones de deficiencia de P, se puede esperar hidrólisis de estas formas de OP y potencial contribución al suministro de P para las plantas. *Myo*-IP_6 parece que se estabiliza por adsorción, tal como se vislumbra por su relación positiva con la proporción de Fe en óxido con respecto al contenido de arcilla y su decreciente concentración en el suelo con el aumento del pH. Por otro lado, la precipitación de los fosfatos de Ca puede explicar la retención de otros estereoisómeros de IP_6 en estos suelos.

Una discusión general de los resultados se realiza en el sexto capítulo, y, por último, la exposición de las principales conclusiones del presente trabajo se presentan en el capítulo siete.

Chapter I. Introduction and Objetives

In the first chapter of this PhD Thesis, Introduction of the P issues and main objectives are displayed.

Introduction

Phosphorus as a non-renewable resource

Phosphorus, as phosphate and its esters, is involved in many biological processes. These processes include structural functions as part of the nucleic acids or phospholipids in membranes of living organisms (Ryan et al., 2012). The phosphoesters have a key role in metabolic reactions, particularly those involving transfer of energy (Deevey, 1970). Thus, P is an essential element for live and therefore for agriculture, but it is a non-renewable resource whose reserves are known to be limited (Keyzer, M., 2010; Cordell et al., 2009; van Vuuren et al., 2010). Future scarcity is expected as a consequence of depletion of reserves of phosphate rock and increasing needs of food of a growing population. In spite of this expected scarcity, P loss from agricultural land is nowadays considered to be a major underlying and persistent cause of eutrophication all over the world accunting for a key environmental problem (Carpenter, 2005; Haygarth et al., 2013; Cordell and Neset, 2014). In fact, till the “Phosphorus crisis” in 2008 (Vaccari, 2009; Cordell et al. 2009), most of the literature release about P was focused on its environmental impact (Delgado and Scalenghe, 2008).

Around 170 million tons of phosphate rock (PR) are extracted annually worldwide, and the main producers are United States, China, and Morocco. These countries produce around 3 quarters of the global PR production, thus revealing that P is not only a non-renewable resource, but also a strategic resource (Ryan et al., 2012). The “P crisis” in 2008 was in fact more related to this concentrated offer than to the scarcity of the commodity. Most of P resources are consumed in agriculture; this means around 19 Mt y^{-1} of P for fertilizer producton. However, only 15 % of this P applied in agricultural production reaches the final consumer through the food chain

(Schröder et al., 2011). This reveals a very low efficiency in both fertilizers applied and chains of production and consumption (Cordell et al., 2009; Schröder et al., 2011; Withers et al., 2014).

Phosphorus needs for fertilizer production is expected to increase in the next future as the consequence of the need of producing more food. The demand of P is expected to grow from 50 to 100 % by 2050 (EFMA, 2000; Cordell et al., 2009). This means an increase by 3 million tons over the 30 next years (United Nations, 2007; Schröder et al, 2011). The most pessimistic predictions reveal that PR rock reserves may be exhausted in 50-150 years. In addition, the critical point is believed to be reached with the peak of production, expected on 2035, where demand will outstrip supply (Cordell et al, 2009; Schröder et al., 2011).

Phosphorus is relatively abundant in the Earth crust, it being the 11th most abundant (Donatello et al, 2010). However, it is mostly concentrated in deposits of phosphate rock, essentially Ca phosphates of reduced solubility (Delgado and Scalenghe, 2008). Agriculture, in fact, implies a redistribution of P present in the crust of the planet. In spite of its relatively high global abundance, natural soils are inherently deficient in P, not only by its natural concentration, but also by chemical reactions that determine an usual low P availability to plants in soils, even in fertilized agricultural soils. This reactions also means a low P concentration in soil solution, which usually ranges between 0.1 and 10 μM (Mengel and Kirkby, 2001), less than optimal for proper plant development, which usually requires P concentrations higher than 10 μM (Föhse et al., 1988).

Balance of phosphorus in soil and of its efficient use in agriculture

Only a small fraction of applied P fertilizer is known to remain available for crops due to its reactions in soil (Delgado et al., 2002; Saavedera et al., 2007). This uncertainty ascribed to the biogeochemical cycle of the nutrient justified in the recent past the application of large amounts of P fertilizers, with excessive P balances in agricultural soils (Delgado and Scalenghe, 2008). This imbalance turns the phosphorus present in the agricultural soil into an environmental problem when it is loss to water reservoirs. Therefore, this situation was identified as a more pressing concern than their agronomic problems in economically developed countries (Bennet et al., 2001; Carpenter, 2008; Delgado and Scalenghe, 2008).

Improving efficiency of P in agriculture may contribute to substantially reduce the overall demand for this nutrient. Efficiency, at present still very low, is a key issue in chains of production and consumption, which can be improved thus helping to postpone the depletion of mineral reserves. A key issue in handling such essentials inputs is to balance the supply-demand equation (Ryan, 2008).

An efficient use of P in agriculture should rely on the estimation of available P to plants in soil, and particularly in the identification of sites where a response of crops to P supply can be expected. This identification has been normally assessed by using "P availability indexes" or “soil P test” (SPT) which are usually based on chemical extractants as bicarbonate (e.g. the widely used Olsen P; Olsen et al, 1954.). For each SPT, threshold values below which an increase in crop yields can be expected when P fertilizer is applied should be defined (Recena et al., 2015). Other more accurate methods to estimate the potential response to P application has been proposed; however, this higher precision usually implies a more complex laboratory procedure, unsuitable for routinely soil analysis, such as anion exchange resins, diffusive gradients through think films, or exchange isotopic ^{32}P (Delgado and Torrent, 1997; Delgado and

Scalenghe, 2008; Delgado et al., 2010; Tandy et al., 2011). Overall, SPT accurately assess the P available status in the soil, i.e. the probability of response to the application of phosphate fertilizer, for soils within a range of properties. However, their accuracy can be limited when the properties of soils range widely (Delgado and Torrent, 1997; Delgado and Scalenghe, 2008). This would lead to misleading interpretations for the same crop grown in different soils and it also explains that different countries and different areas in a country assess P fertilization based on different SPT. There is not a universal recommendable SPT due to the different effect that a given chemical extractant can have on very different P forms with very different availability to plants depending on the soil type (Delgado and Scalenghe, 2008; Jordan-Meille et al., 2012).

Geochemistry of phosphorus in soils

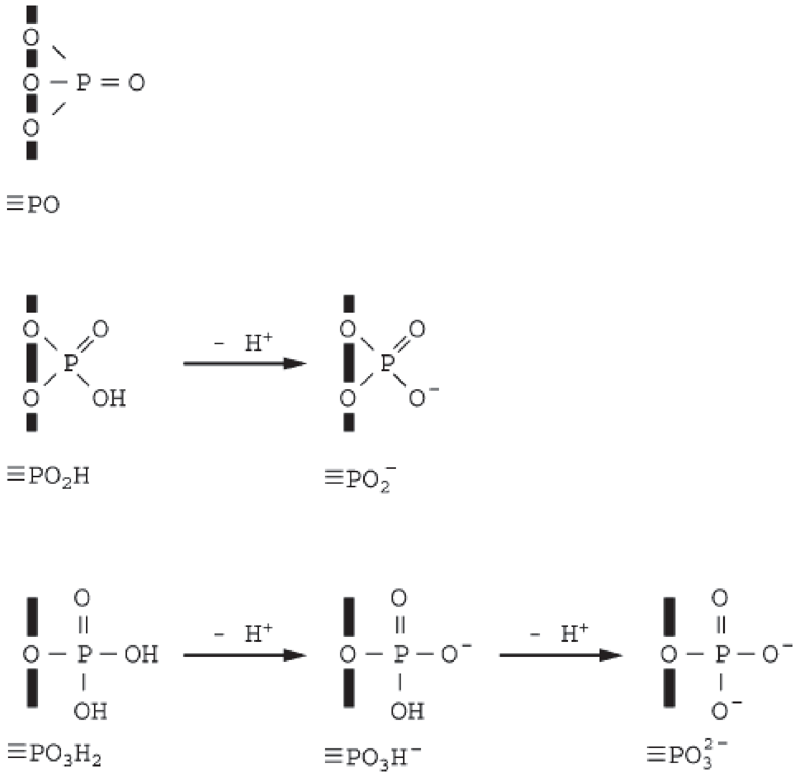
Total phosphorus content in soils is much higher than the amounts extracted by crops. Fixing reactions of phosphorus in soils are responsible of loss of availability of the phosphoric fertilizer applied. Chemical reactions of P in soils involve physical adsorption, chemisorptions, anion exchange, surface precipitation and precipitation of solid phase. Each reaction may have different implications on the availability of P. However, in practice, it is difficult to distinguish and identify dominant P reactions in soil involved in its binding to solid phase and constraining availability to plants (Delgado and Scalenghe, 2008).

The main source of phosphate for life in soils is originated by disintegration and decomposition of rocks containing the mineral apatite. Apatite is the 10th most abundant mineral under each geological and geochemical condition (Hughes and Rakovan, 2002). Its dissolution is enhanced under acidic conditions as follows:

Ca_{l0} $(PO_4)6F$ 2 + 12 $H^+ \rightarrow 10$ Ca^{2+} + 6 $H_2PO_4^-$ + 2F

This apatite originates fluorapatite (Ca_{10} (PO_4) $6F_2$), chlorapatite (Ca_{10} (PO_4) $6Cl_2$), and hydroxyapatite (Ca_{10} (PO_4) $6OH_2$) (Bolan and Hedley, 1990). Also, P can be combined with Fe and Al precipitating as other metal phosphates like $PO_4Al.2H_2O$ (variscite) or $PO_4Fe.2H_2O$ (estrengite).

Phosphorus at the mineral surface may possibly have either one, two, or three oxygen atoms enclosed in the mineral structure. This gives rise to a number of possible protonated (H PO_2, PO_3H^-, H_2PO_3) and unprotonated phosphorus sites (PO, PO_2^- , and PO_3^{2-}) at the mineral surface (Jarlbring et al., 2006).



(Jarlbring et al., 2006)

In a study of the surface reactions of fluorapatite, Wu et al.,(1991) suggested that the mineral surface holds active sites including a calcium hydroxide group (CaOH) and a phosphate group (POH). It is reasonable to assume that a combination of hydrated, protonated, and deprotonated surface sites exists at the mineral particles at different pH (Jarlbring et al., 2006).

Soil pH is a key factor controlling the type of mineral or compound which reacts with P and its forms in the soil solution. At low pH values, monovalents forms are the most abundant. As pH increases, trivalents forms became predominant. Monovalents forms are more easily absorbed by plants. This implies that optimums pH for P uptake is close to neutral pH (6 - 6,5). At pH 7, the relationship $H_2PO_4^-$ / HPO_4^{2-} is approximately equal. Above this pH, the concentration of HPO_4^{2-} increases. This latter form can be also absorbed by crops (Spivakov et al., 1999).

In addition to precipitation reactions, phosphate present in soil solution can be specifically adsorbed by many surfaces. Other anions are non-specifically adsorbed. These other anions respond only to positive charges of colloids in soil. However, phosphate is absorbed regardless of the charge of soil surface (White, 1981). The inorganic P fraction is adsorbed by exchange sites, associated with Fe, Al and Mn oxides; also associated with carbonates and with Ca as apatite or bound to crystalline minerals (Frossard et al., 1995; Mckelvie, 2005).

The surface of oxides consists of OH^- ions and water molecules that are mono-, di- and tri-coordinated to metallic ions. The most important sites for the adsorption of phosphate are monocoordinated under acidic conditions. This adsorption capacity depends on the specific surface which is higher in poorly crystalline oxides (Colombo et al., 1994; Barrow, 2015). The phosphates adsorption mechanism by oxides is dominated by ligand exchange. In this reaction, two hydroxides groups or water molecules are replaced by a phosphate anion (Torrent, 1997). This reaction leads to strong phosphate complexes that in practice mean that a portion of adsorbed P is slowly exchangeable (hysteresis behaviour). Thus, this strength of the complexes promotes that a portion of adsorbed P cannot be released to the solution, thus remaining unavailable to plants (Reddy et al., 1999; Goldberg and Sposito, 1985). Usually, it has been considered that P binds to Al and Fe oxides by fast or slow phase reactions (Chen al et., 1973). The fast phase involves ligand exchange reactions (e.g. monocoordinated, bicoordinated), while the slower phase is stronger and less reversible and likely involves ligand exchange (e.g. binuclear) or precipitation reactions (Chen et al., 1973; Torrent et al., 1990).

Clay minerals can adsorbe P by changing the ligand groups (OH) H coordinated at the edges of the structure. The adsorption capacity is lower than that of Fe or Al oxides and depends, among on other factors, on the area occupied by the reactive groups of the faces (Afif, 2005). As clay minerals, carbonates constitute a secondary P adsorption element. The adsorption on the surface of calcite is produced by substitution of water molecules, HCO_3^- or OH^-. In calcareous soils, P applied can be adsorbed to high affinity sites (Fe and Al oxides) but also on calcite surface (Castro and Torrent, 1998).

As mentioned above, phosphate precipitation results, in practice, difficult to distinguish from adsorption. At high P concentration in soil solution, precipitation is enhanced over adsorption (Afif, 2005). At basic pH and appreciable Ca activity in soil solution, precipitation of Ca phosphates is enhanced because these are the most thermodynamically stable forms (Lindsay, 1979; Delgado et al., 2002). This is very relevant in soils typical from Mediterranean climate areas, and particularly, in calcareous soils. Under these conditions, after the application of a soluble P fertilizer, dicalcium phosphate and octocalcium phosphate may be formed as precipitation products. Even more soluble forms such as monocalcium phosphates can precipitate initially. The precipitation of Ca phosphates is a dynamic process, in which there is a slow evolution from more soluble and less stable forms to a more thermodynamically stable phase which is hydroxyapatite (Delgado et al., 2002). Hydrolysis from dicalcium to octocalcium phosphate occurs, with higher rates when dicalcium phosphate is in poorly crystalline form (Von Wandruszka, 2006). Dicalcium phosphate, octocalcium phosphate and tricalcium phosphate, in solubility decreasing order, are present in calcareous soils (Delgado and Torrent, 2000). In the usual term of a crop cycle, most of the P applied to a calcareous soil can be supposed to be precipitated as hydroxyapatite and thus not available to plants (Delgado et al. 2002).

The role of organic matter in the geochemistry of P in soils is relevant. Soil organic matter (SOM) only adsorbed P when associated with metal cations. On the contrary, when SOM is not associated with metals, it can compete for adsorption sites with P (Ryden et al., 1987). Competitive adsorption between low molecular weight SOM is known to delay P adsorption on Fe oxides (Geelhoed et al., 1999; Staunton and Leprince, 1996), thus increasing P fertilizer efficiency in soils amended with organic matter. Organic compounds may also affect the rate and form in which P precipitates in calcareous soils (Delgado et al., 2002). Organic acids inhibit the precipitation of hydroxyapatite (HAP) (Inskeep and Silverthooth, 1988) and favour the formation of dicalcium phosphate dihydrate (DCPD) over other more thermodynamically stable and less soluble phosphates (Grossl and Inskeep, 1991). High molecular weight organic acids are more effective inhibitors of HAP precipitation than low molecular weight acids (Inskeep and Silverthooth, 1988).

Organic phosphorus in soils

Organic and inorganic phosphorus compounds interact with soil´s components. Both are subjected to various chemical transformations affecting the retention of the element (Turner et al., 2005). Organic forms dominate both soils and aquatic systems, but are part of the more unknown global phosphorus dynamics (Delgado and Schalenghe, 2008). The organic phosphorus can be defined as those forms that are part of compounds with carbon-hydrogen (Turner et al., 2005) and encompasses many different compounds including: nucleic acids, phospholipids, inositol phosphate, fosfoamidas, phosphoproteins, organophosphate pesticides, humic acids associated with phosphorus compounds by metal cations, condensed organic phosphates, colloids and associated forms (Turner et al., 2003).

Phosphate monoesters have been described as the most abundant organic P form in soils. Between monoesters, the predominant form of organic phosphorus are Inositols, 1,2,3,4,5,6 hexaphosphate (IP_6). These compounds are a formed by six esterified phosphate groups and their salts are known with the term phytate. IP_6 can be present in four stereosomic forms (*myo, scyllo, chiro, neo*) (Turner et al., 2002). They are very stable forms in soils due to its interactions with soil components by adsorption or precipitation (sorption). In addition, plants do not produce extracellular phytases activity, the enzyme group that catalyzes the hydrolysis of phytate (Hayes et al, 1999). All this explain why IP_6 is considered a non-available P form to plants and why it accounts for the largest proportion of the organic P found in soils (Turner al., 2002; George et al., 2006).

Monoesters are selectively stabilized by processes that keep off enzymatic degradation (Condron et al., 1990; Celi and Barberis, 2005). To be hydrolysed, organic P should be in soil solution. Thus, sorption and precipitation in stable forms prevent this degradation, which is only feasible when organic P is desorbed to solution (Berg and Joern, 2006; Gianvenno et al., 2008). Iron and Al oxides can strongly adsorb monoesters. In acid soils, it was proved that IP_6 concentration in soils depends on the Al and Fe amorphous oxides content (Richardson, 2001). The humic substances can incorporate them into. Monoesters different from IP_6 and diesters are less stabilized through adsorption or precipitation than IP_6, thus being more accessible to enzymatic degradation (Celi and Barberis, 2005). Redox reactions affect organic P dynamics: if Fe(III) reduction to Fe(II) (dissolution of Fe oxides) occurs, sorbed organic P may be available for enzymatic hydrolysis. If after this reduction, aerobic conditions prevail again, precipitation of oxyhydroxides can increase stabilization of organic P through adsorption process (Zhang et al., 1994).

Soil reaction (pH) is an important factor affecting inositol phosphates dynamics in soil. These compounds have a high charge density at normal soil pH range and desorption can increases with pH (Frossard et al.,2000). Desoprtion rates may depend on mineral composition of soil, mainly on their oxide content, and also on oxide affinity for organic P (Celi et al., 2003). A pH higher than 6, the precipitation of Ca salts of IP_6 is enhanced, which is another reaction explaining its stabilization in soil (Celi et al., 2005)

Phosphorus in Mediterranean soils

In Mediterranean soils, the main P adsorbents are iron Fe and Al oxides, clay and $CaCO_3$ (Matar et al. 1992; Torrent, 1997; Horta et al., 2007). Prediction of P adsorption in calcareous soils has been less successful than in other types of soils in this area. The high energy adsorption surface is related to the dithionite-soluble iron (free iron) in calcareous soils, indicating that the oxyhydroxides are also important in the P adsorption in these soils (Peña and Torrent, 1990).

Saavedra et al., (2005) in a representative collection of soils from south Spain described that the dominant inorganic P fraction was ascribed to hydroxiapatite. Dominguez et al. (2001) in soils from the Guadalquivir Valley found the same relevance of this insoluble Ca phosphate. This reveals the crucial role of precipitation as low soluble Ca phosphates in the dynamics of P and explains the low recovery rates of applied fertilizer by crops in these soils (Castro and Torrent, 1998; Domínguez et al., 2001).

Regarding organic P, in general, a relevant fraction of total P in soils from the Mediterranean area is associated with very stable forms of organic P (Saavedra and Delgado, 2005). In typical Mediterranean soils, the organic P has been shown to account for a significant portion of total P in soil (even more than 50%), even though the organic matter content was lower than 2% (Saavedra and Delgado, 2005; Saavedra et al., 2007). However, the agronomic significance of this organic P is assumed to be reduced. The reason why is that most of this organic P is probably related to non-labile forms such as inositol phosphates or humic compounds (Condron et al., 1985).

There is a lack of information about the chemical structure of the main organic P compounds in typical Mediterranean soils. There are evidences of its significant contribution to the P nutrition of plants in soils from other geographical areas (e.g. Ohel et al., 2001). This evidences that organic P can be important from an agronomic point of view. Thus, characterization and quantification of organic P compounds is a relevant issue due to the fact that chemical forms determine the bioavailability and environmental reactivity of organic P (Cade-Menun and Liu, 2013).

Methods to the study of the forms of phosphorus in the soil

Knowledge on P forms can provide relevant information to understand it dynamics and bioavailability in soils and sediments. Overall, there two forms of studying P forms: direct spectroscopic methods, such as FT-IR or ^{31}P-NMR, which can provide information on specific P compounds, or operational indirect approaches such as solubility equilibria (Pierzynsky et al. 1990a) and sequential chemical fractionation

(Delgado and Scalenghe, 2008). These latter methods use sequentially different extractants, each one being able to selectively dissolve some specific P forms. This solubilisation by an specific extractant is determined by the interaction between P and other inorganic and organic components in soils which determines the solubility of P bound to some specific compounds of the solid phase; e.g., the use of reductants as extractants promote the reduction of Fe(III) and subsequent release of Fe-bound P.

Sequential chemical fractionation schemes do not provide information on specific P compounds, but allows an “operational” distinction of P fractions in soils or sediments, e.g. Ca- or Fe-bound P with different abilities to be released to the surrounding solution in soils or in water reservoirs, and thus, to be used by plants or aquatic organisms (Barbanti et al., 1994; Saavedra and Delgado, 2005; Delgado and Scalenghe, 2008; Condron and Newman, 2011). Overall, sequential chemical fractionation has been more used to study inorganic P fractions than organic P fractions. In general, fractionation methods do not quantify the organic fraction using a specific extractant; usually the fraction remaining after inorganic constituents have been removed is taken as organic P (Golterman, 1976; Barbanti et al., 1994).

In soils from Mediterranean areas, where calcareous soils are frequent and where Ca-bound P is usually relevant between P fractions, the classical schemes by Chang and Jackson (1957, 1958) has been widely used (e.g. Delgado and Torrent, 2000) and adapted, such as the modification proposed by Ruiz et al. (1997). This latter scheme is not only focused on the distinction of Ca- and Fe-bound P, but also on the estimation of Ca-bound P of different solubility and on the distinction of occluded P in poorly crystalline and crystalline Fe oxides (Saavedra and Delgado, 2005; 2006). It has been also adapted to study organic P fractions in Mediterranean soils (Saavedra et al. 2007).

Also, the information obtained through these sequential extractions allows settling down trace of metals bound to the solid phases. Of great interest is also the mobilization and characterization of the biogeochemical processes that occur. Actually, fractionation schemes values are kind of an estimation of solubility and content, which is far from easy (Delgado and Torrent, 2000).

Delgado and Scalenghe (2008), and Condron and Newman (2011) provided an extensive review on the most usual sequential fractionation methods used over the last 50 years, As a summary, it can be mentioned depending on the main objective of the fractionation scheme:

- Specific schemes for determining the inorganic phosphorus: Chang and Jackson, 1957, with 5 fractions, and its modification by Ruiz et al. (1997) with 7 fractions; Hieltjes and Lijklema, 1980, with 3 fractions

- Schemes focused on organic P: Summers et al 1972, with 7 fractions; Bowman and Cole 1978, with 5 fractions, and Ivanoff et al, 1998, with 6 fractions including microbial P

- Schemes intended to study organic and inorganic P: van Eck, 1982, with 6 fractions; Psenner et al, 1988, with 5 fractions;. Ruttenberg, 1992, with 5 fractions; Golterman et al, 1998, with nine fractions; Chen et al, 2000, with 9 fractions; Pardo et al, 2003, with five fractions; Saavedra et al. (2007) with 9 fractions; Tiessen and Moir, 2008, with 9 fractions).

- Schemes for studying inorganic, organic and microbial P, such as that proposed by Hedley et al. (1982), with 10 fractions, and the inclusion of microbial P in the scheme through the chloroform fumigation of duplicate samples (Condron and Newman, 2011).

Contrasting with sequential chemical fractionation, spectroscopic methods provide information on specific P forms in soil. Due to the low concentration of total P in soil, usually less than 1 g kg^{-1}, it is difficult to achieve useful information on specific P compounds with these techniques without methods to concentrate P in soil (e.g. chemical extraction). X-ray diffraction (Pierzynsky et al. 1990b), FT-IR (Pierzynsky et al. 1990c; He et al. 2006; 2007), and X-ray absorption near-edge structure (XANES) spectroscopy (Negassa and Leinweber, 2009; Negassa et al. 2010) has been used to identify specific organic and inorganic P compounds in soil, but they are useful in P-rich materials or in separated P-rich particles.

Nuclear magnetic resonance spectroscopy of ^{31}P (^{31}P NMR) has been widely used in the study of specific P compounds and it has been considered has the most powerful direct spectroscopic method to assess P forms in soils (Cade-Menun, 2005). It use has been expanded rapidly in soil and environmental research since the first time it was used by Newman and Tate (1980). Ths use of ^{31}PNMR has improved and accelerated studies in this field and it has been mainly used in many studies to characterize organic P in soils and sediments (e.g. Gil-Sotres et al. 1990, which was the first study focused on Spanish soils), and less in the identification of inorganic P compounds in soil (e.g. Lookman et al. 1995). Delgado et al. (2002) used solid state ^{31}P NMR to study specific Ca phosphates formed in the soils after P fertilizer application.

Nuclear magnetic resonance (NMR) has also limitations when analyzing organic P due to its low sensitivity and the presence of paramagnetic ions (Turner et al., 2005). Therefore, the organic P must be extracted and concentrated before being identified and quantified by ^{31}P NMR. There are numerous extraction methods and each one may lead to different results (Turner et al., 2005). The choice of the extractant not only affects the recovery of organic soil P, but also on the composition of extracted components (Cade-Menun and Preston, 1996). Bowman and Moir (1993) proposed NaOH + EDTA in a single extraction step to determine total organic P. NaOH solubilized organic P, while EDTA chelates metal cations to increase the efficiency of extraction of P. Cade-Menun (1995), as proposed by Bowman and Moir (1993), described that NaOH + EDTA as a very effective method in extracting organic P, which solubilize at different concentrations cations and different P forms (Cade-Menun et al., 2002). This single stage NaOH-EDTA extraction procedure is especially useful in ^{31}P NMR spectroscopy due to the complexation of paramagnetic ions (Cade-Menun and Preston, 1996; Cade-Menun, 2002).

The NMR technique can be used as liquid or solid state spectroscopy. The ^{31}P NMR spectroscopy in solution provides high quality spectra. This technique avoids problems with humic acids (complexation of P by humic acid) and allows one to quantify the organic P independently to inorganic forms (Turner et al., 2005). It has also the advantage that every species of P in samples can be observed due to its minimal alteration (Condron et al., 2005). The main restrictions in its use are: (i) the low P concentration in soil samples: 1 g P kg^{-1} is needed for accurate signal detection, and (ii) the presence of paramagnetic ions which causes low ratios of signal to noise (Magid et al., 1996; Condron et al., 2005).

All P species within a sample can be potentially detected by NMR spectroscopy. Therefore, NMR spectroscopy quantitatively identifies different forms in a sample or soil extract. With ^{31}P-NMR it has been usually identified: phosphonates, orthophosphate, orthophosphate monoesters, orthophosphate diesters, pyrophosphate and polyphosphate (Hill and Cade-Menun, 2009; Liu et al., 2009).

Study of P availability to plants.

The fraction of total P in soils that is available to plants is not a constant value, it ranging widely according to the environmental conditions. As an example, Delgado and Torrent (1997), working with representative soils from Europe, observed that total available P to plants accounted for between 10 and 70 % of total P in soils. The estimation of available P by chemical extraction is basically an empirical measurement which is necessary to be correlated with actual measurement values of P absorption by crops (Fixen and Grove, 1990). The amount of P removed by any single extractant is determined not only by the nature of the extractant, but also for existing forms of P in soil (Delgado and Scalenghe, 2008; Jordan-Meille et al., 2012). These P forms are determined by soils properties. Thus, depending on soil properties, the same chemical extraction method can extract very different amounts of P for the same amount of available P in soil. This is the reason why different extraction methods to assess P availability to plants has been proposed in different geographical areas. Thus, there is not an universal method to assess this availability, and only in Europe 17 methods have been proposed (Delgado and Scalenghe, 2008).

A good correlation between amounts chemically extracted and P uptake by plants is the fist step in assessing accurate fertilization of P (Sánchez-Alcalá et al., 2014). For a given extraction method, research is necessary to define threshold values below which a response in crop yield can be expected to the application of fertilizer. The definition of these threshold values enables a given P extraction method to distinguish responsive and non-responsive sites to P fertilization. These chemical extraction methods to assess P fertilization are usually named as “Soil P index” or “Soil P test” (SPT). Between these usual STP, it can be mentioned:

- Mehlich-3 test was developed by Adolph Mehlich in 1984. The Mehlich-3 extracting solution consists of multiple chemical solutions: acetic acid, ammonium nitrate, ammonium fluoride, nitric acid and the chelate, EDTA. (Mehlich, A. 1984)

- Bray-Kurtz P1 test (often referred to as the Bray-P1 test) was developed in 1945 at the University of Illinois by Dr. Bray and Dr. Kurtz. The extractant is a diluted hydrochloric acid and ammonium fluoride solution. It is recommended for neutral and acid soils (pH <7.0), but not for alkaline soils (pH > 7.0) (Bary and Kurtz, 1945)

- Olsen test was developed for the alkaline soils of Colorado by Drs. Olsen, Cole, Watanabe, and Dean in 1954. It extract P using 0.5 M sodium bicarbonate (buffered at pH = 8.5). It is likely is the most simple chemical extraction. P Olsen has been giving the best correlations, usually with phosphate uptake by crops and the relative yield. Also, it can be sensitive in some cases to soil´s P buffer capacity (Holford, 1980). The method is based on the action of OH^- and CO_3^2- ions that make decrease the concentration or activity of Ca^{2+} and Al^{3+} ions. Then, this causes an increase in phosphorus solubility. Although the extractant can be used in acid and calcareous soils, Delgado and Torrent (1997) observed that the ratio of P available to plants to amounts extracted with the Olsen method was near 3 times higher in acid than in calcareous soils. In calcareous soils, the calcium phosphate solubility increased due to a decreased Ca concentration promoted by the high concentration of CO_3^2-, which causes its precipitation as $CaCO_3$. In acidic or neutral soils, the solubility of iron and aluminium phosphates increases due to an increase in the concentration of OH. In addition, Al^{3+} concentration decreases by forming aluminates (Afif, 2005). The increase of negative surface charges and or the reduction of the number of sorption sites on oxides at high pH may also be responsible for the desorption of P sorbed by action of an alkaline reagent (Kamprath and Watson 1980; Afif, 2005).

Other more complex soil P extractions has been developed and studied to assess P availability to plants in soil. Between these methods, resins extraction (Delgado and Torrent, 1997; Delgado et al. 2010), isotopic dilution (Delgado and Torrent, 1997), or difussive gradient thin films, DGT, (Tandy et al., 2011) have been proposed. However, the complexity in the extraction makes difficult their use for practical assessing P fertilization.

Phosphorus concentration in the soil solution (intensity, *I*), the amount of P in the solid phase in equilibrium with the solution (quantity, *Q*) and P buffer capacity (*dQ/dI*, *BC*) are soil characteristics involved in the availability of P to crops. This buffer capacity is related with the other two factors (Q, I) (Sánchez et al., 2015) and it is defined as the soil ability to keep in constant P concentration in solution (Frossard et al., 2000). This ability to maintain constant P activity in solution when is crucial defining the ability of a soil providing P to plants (Tisdale and Nelson, 1996). Changes in intensity may occur due to P absorption by plants (decrease) or to the application of P fertilizers (increase). Changes in intensity promote displacement of the equilibrium between the solid phase and soil solution, which should result in a replacement of P in solution from P in solid phase when low concentrations in solution occurs.

These factors (concentration in soil solution, amount of P in solid phase and buffer capacity) depend on the physicochemical characteristics of each soil (Holford, 1997). Parameters affecting the adsorption-desorption dynamics are clay, pH, $CaCO_3$, Al, Ca and Fe and organic matter. Thus, these properties can affect the capacity of a soil to replenish P in soil solution. Thus, soil physical and chemical properties when related to *I*, *Q* or *BC,* may enhance the prediction potential of tests to study crops yields at a given P fertilizer rate applied (Moody and Bolland, 1999; Sanchez et al., 2015). In this regard, SPT can be usually considered as an estimate of the quantity factor, but they provide more accurate estimates of plant available P if they are sensitive to soil buffer capacity (Delgado et al. 2010).

It is usually considered that a soil have a capacity to supply P to plants until a critical value of P concentration in solution is reached; usually 0.01 to 0.03 mg P L^{-1} depending on the crop (Sánchez-Alcalá et al. 2014). However, even for group of soils with relatively homogeneous properties, the value of Olsen P, or other SPT, in equilibrium with this critical concentration may range widely (Delgado et al. 2010; Sánchez-Alcalá et al. 2014). This has explained the lack of accuracy of SPT and the wide range of threshold values observed for soils where a given SPT is recommended (e.g. Delgado et al. 2010). The correlation between Olsen P or other SPT and the concentration of P in the soil solution is not well known (Sánchez et al., 2014) but it is crucial to provide more accurate P fertilization schemes.

Objetives

The main objective of this PhD is to contribute to the **optimization of the use of phosphorus in agriculture (P)**.

To achieve this general objective, different specific objectives were defined, as follows:

(1) The **identification of the factors affecting the accuracy of a widely soil P test (SPT) such as P-Olsen** (Chapter II). Overall, the accuracy of different soil P availability indexes (SPT) in groups of soils ranging widely in properties is poor. Even for soil where a given SPT is recommended, this test may not be accurate enough for precise P fertilization schemes. This objective is focused on the understanding of soil properties affecting the accuracy of Olsen P on the estimation of the amount of P in soil that can be taken by plants with a view of defining more accurate models based on this SPT for assessing P fertilization.

(2) The use of a single soil P extraction as SPT depend on the definition of precise threshold values below which response to P fertilization can be expected. This allows the identification of P-responsive and non-responsive sites. However, threshold values for a given SPT can range widely even in groups of soils with similar properties. To contribute to the advance in the accurate use of Olsen P as SPT, **the soil properties affecting the threshold values of Olsen-P for crop response to P fertilization in representative Mediterranean soils** were studied in the Chapter III of this PhD. On the basis of soil properties affecting Olsen-P threshold values, models based on these properties can be proposed for a more accurate estimation of threshold values. This will allow more precise P fertilization schemes.

(3) Olsen P has proved to be limited for accurate estimations of available P to plants. **Deeper knowledge on factors affecting this lack of accuracy**, allowing the

performance of models based on this extraction to estimate plant available P, or alternative and **more accurate single P extractions methods in assessing available P in soils** are required for achieving more efficient P fertilization. These are the main objectives defined in the Chapter IV.

(4) There are evidences of contribution of organic P to P uptake by plants. To assess the contribution of organic P to the potential available P pool to plants, it is a need a deeper knowledge of organic P forms within the P cycle in soil. In Mediterranean environments, the information of dominant organic P compounds and how these compounds can contribute to P supply to plants is particularly scarce. There are evidences for this supply, such as those described in Chapter II. To this end, **main organic P compounds in representative Mediterranean soils were studied** by nuclear magnetic resonance spectroscopy focusing on the isotope ^{31}P (^{31}P- NMR) with the aim of elucidate how are affected by soil properties, uses of soil and soil P status. This study is the main target defined in Chapter V.

Objetivos

El principal objetivo de la presente Tesis Doctoral es contribuir a la **optimización del uso del fósforo (P) en la agricultura**. Para lograr este objetivo general, se definieron diferentes objetivos específicos, como sigue:

(1) La **identificación de los factores que afectan la precisión de un test de P para una amplia gama de suelos (SPT), tal como Olsen P** (Capítulo II). En general, la exactitud de diferentes índices de disponibilidad de P en suelo (SPT) para grupos de suelos que ampliamente varían en sus propiedades, es pobre. Incluso para suelos, donde se recomienda un determinado SPT, esta prueba no puede ser lo suficientemente exacta para establecer esquemas de fertilización fosfórica precisos. Este objetivo, se centra en la comprensión de las propiedades del suelo que afectan a la exactitud del Olsen P en la estimación de la cantidad de P en el suelo que puede ser tomada por las plantas, con el fin de definir modelos más precisos basados en este SPT para la evaluación de fertilización fosfórica

(2) El uso de una única extracción de P en suelo, como SPT, depende de la definición de valores umbral precisos por debajo de los cuales se puede esperar una respuesta a la fertilización fosfatada. Esto permite la identificación de sitios sensibles y no sensibles a la fertilización fosfórica. Sin embargo, los valores umbral para un determinado SPT pueden variar ampliamente, incluso en grupos de suelos con propiedades similares. Para contribuir al avance en el uso preciso del Olsen-P como SPT, **las propiedades del suelo que afectan a los valores umbral de Olsen P en la respuesta del cultivo a la fertilización fosfatada en suelos Mediterráneos representativos** fueron estudiadas en el Capítulo III de la presente Tesis Doctoral. Sobre la base de las propiedades del suelo que afectan a los valores umbral del Olsen

P, los modelos basados en estas propiedades puede ser propuestos para una estimación más precisa de los valores umbral. Esto permitirá esquemas de fertilización fósfórica más precisos.

(3) El Olsen P ha demostrado ser limitado para la estimación precisa del P disponible para las plantas. **Se requiere un conocimiento más profundo de los factores que afectan a esta falta de precisión, lo que permite la realización de modelos basados en esta extracción para estimar el P disponible**, o métodos más precisos en la evaluación del P disponible en los suelos para lograr mayor eficiencia en la fertilización P. Estos son los principales objetivos definidos en el Capítulo IV.

(4) Hay evidencias de la contribución del P orgánico en la absorción de P por las plantas. Para evaluar la contribución del P orgánico a la reserva potencial de P disponible para las plantas, es necesario un conocimiento más profundo de las formas de P orgánico dentro del ciclo de P en el suelo. En ambientes mediterráneos, la información de compuestos orgánicos P dominantes y cómo estos compuestos pueden contribuir al suministro de P para las plantas es particularmente escasa. Hay evidencias de este suministro, tales como los descritos en el capítulo II. Con este fin, **los principales compuestos de P orgánico en suelos mediterráneos representativos se estudiaron** por espectroscopia de resonancia magnética nuclear, que se centra en el isótopo ^{31}P (^{31}P- RMN), con el objetivo de racionalizar cómo se ve afectado por las propiedades del suelo, usos del suelo y el nivel de P en el suelo. Este estudio es el principal objetivo definido en el Capítulo V.

Chapter II

In the second chapter of this PhD Thesis, the identification of **factors affecting the accuracy of Olsen phosphorus availability index (Olsen-P**) was studied. One of the resons for an unefficient use or P in agriculture is due to a non-accurate evaluation of bioavailable phosphorus in soil. In fact, bad relationships are frequently observed between P availability indexes such as Olsen-P and P uptake by plants.

Accuracy of Olsen P to assess plant P uptake in relation to soil properties and P forms

Accuracy of Olsen P to assess plant P uptake in relation to soil properties and P forms

Abstract

Efficient use of phosphorus (P) by plants in agriculture relies on accurate estimation of the phytoavailable soil P. However, poor relationships are frequently observed between P availability indices, such as Olsen P, and P uptake by plants. We therefore studied which soil properties modify the accuracy of Olsen P as a P availability index.We conducted a soil P depletion experiment in pots with 17 soils. Two samples differing widely in Olsen P were used for each soil and named “low-P” and “high-P.” Soil was mixed with siliceous sand to achieve 1 mg of Olsen P per pot, so that the ratio of non-readily available P, either inorganic P or organic P, to Olsen P in the pot was higher for low-P than for high-P samples. Results show that, in high-P samples, P uptake by cucumber is positively correlated with the affinity of the soil solid phase for P, with R^2 of 0.76. In high-P samples, P uptake by cucumber is positively correlated with total P adsorption capacity as estimated by Fe in the form of Fe oxides, with R^2= 0.56. P uptake is positively correlated with the inorganic P released by NaOH and citrate-bicarbonate in low-P samples, with R^2 of 0.52. In low-P samples, total organic P and phosphatase activity in the rhizosphere explained 37 % of plant P uptake. In low-P samples, phosphatase activity and pH explained 50 % of plant P uptake. These findings reveal that organic P forms and hydrolytic activity by roots are explaining P availability to plants. Whereas physico-chemical processes controlling inorganic P dynamics play a major role for low organic P/Olsen P ratios.

Key words: Olsen P, availability index, threshold values, buffer capacity, organic P

Introduction

Phosphorus is a non-renewable and strategic resource that is essential for life—and thus for agriculture. Limited known reserves and increased demand to match rising food needs of a growingWorld population make feasible future scarcity and rising prices of this commodity (Cordell et al. 2009; Keyzer 2010; Van Vuuren et al. 2010; Ryan et al. 2012). This future P scarcity is emerging as one of the key global environmental challenges (Cordell and Neset 2014). Society depends on inorganic P, but its use is inefficient (Withers et al. 2014).

More than 80 % of mined phosphate rock is used in fertilizer industry (Cordell et al. 2009; Ryan et al. 2012), and it is well known that only a fraction of applied P remains available to crops (Delgado et al. 2002; Delgado and Scalenghe 2008). More efficient use of P in agriculture requires more accurate P fertilizer recommendations, which should rely on accurate estimation of the phytoavailable P in soil. This estimation is based on chemical extractions as a proxy for the P potentially releasable from the solid phase and thus available to plants (Kpomblekou and Tabatabai 1997; Delgado et al. 2010). Depending on soil properties and dominant P forms, different relationships between extractable and plant-available P can be expected (Mallarino 1997; Delgado and Torrent 1997; 2001), thus making it impossible to adopt a universal method to assess available P in soil (Delgado and Scalenghe 2008; Tandy et al. 2011). This justifies that, only in Europe, 16 P extraction methods to assess plant-available P have been proposed (Neyroud and Lischer 2003; Tóth et al. 2014).

The primary target of any “available P index” is to identify responsive sites to P fertilization via defining a precise threshold value above which the probability of a crop to respond to the soil application of a P fertilizer is low (Neyroud and Lischer 2003; Tandy et al. 2011). Different factors can affect the relationship between P in solid and liquid phases thus affecting the accuracy of estimated threshold levels for each index even in groups of soils of homogeneous properties (Delgado et al. 2010). It is generally accepted that the P buffer capacity (PBC), which is the soil solution resistance to a change in P concentration, is a crucial factor affecting equilibrium between solid and liquid phases (Holford 1980; Ehlert et al. 2003; Celardin 2003). Factors such as soil P status (Barrow and Debnath 2014), the affinity of sorbent surfaces for P, or the proportion of exchangeable cations can also affect this equilibrium (Delgado et al. 2010). All these factors contribute to explain the poor relationship frequently observed between a widely used index such as Olsen P (bicarbonateextractable P; Olsen et al.

1954) and P uptake by plants (Kulhánek et al. 2007), even in soils with similar properties (Delgado et al. 2010). To overcome this limitation, other methods theoretically sensitive to soil PBC such as the use of near infinite sinks (i.e., Fe oxides, Delgado and Torrent 1997; or resins, Delgado et al. 2010) or to the study of diffusive gradients in thin films (DGT; Mason et al. 2010; Tandy et al. 2011) have been proposed. Also, corrections of traditional indexes based on single soil properties could lead to more accurate assessing of available soil P thus to more accurate P fertilization recommendations (Delgado et al. 2010).

A better understanding of how a given available P index is related to the plant-available P in soil and how this relationship is affected by soil properties will provide new insight into the correct use of Olsen's and other P availability indices by defining more precise threshold values. In this regard, the aim of this work was to identify the factors that affect the accuracy of Olsen P, a widely used test in many areas of the world. To this end, the relationship between plant P uptake and Olsen P was assessed for a widely ranging group of soils from Mediterranean areas in order to assess how this relation can be affected by soil properties and, particularly, by the soil status.

Material and methods

Soils

A soil P depletion experiment was conducted in a growth chamber with 17 agricultural soils that encompassed the most representative soil types of the Mediterranean part of Spain. According to Soil Taxonomy (Soil Survey Staff 2010), the soils were classed as Mollisols, Entisols, Inceptisols, Alfisols, and Vertisols and most of them were in the typical rainfed crop rotation in Spain, i.e., wheat-sunflower. Soil samples were taken at least 1 year after P fertilizer had been applied to soil and, in rainfed rotations, mostly after sunflower (i.e., 2 years after the last P application). A set of samples of the surface layer (0–20 cm) in each location where a homogeneous soil was identified were taken in order to obtain a collection of samples widely ranging in Olsen P. The samples with the two extreme values were selected for this study and named “low-P” and “high-P” samples according to their P status. Soil properties, including Fe in Fe oxides, were checked to ensure that they differed by less than 5 % between “high-P” and “low-P” samples.

Soil samples (5–7 kg) from each soil were homogenized, dried, and ground to <6 mm. A representative subsample was taken for physical and chemical analysis. This subsample was ground to pass a 2-mm sieve. Particle size analyses were carried out using the densimeter method (Gee and Bauder 1986), organic matter (SOM) was determined by dichromate oxidation (Walkley and Black 1934), and the cation exchange capacity (CEC) by using 1 M NH4OAc buffered at pH 7 (Sumner and Miller 1996). The total CaCO3 equivalent (CCE) was determined by the calcimeter method, and pH was measured in water at a soil:extractant ratio of 1:2.5. Olsen P was determined according to Olsen et al. (1954), replacing filtration through active carbon by centrifugation at 1000 g during 10 min. Molybdate-reactive P (Murphy and Riley 1962) and total P after persulphate digestion in autoclave (Díaz-Espejo et al. 1999) were determined in the supernatant.

Experimental design

An experiment was performed in 350-mL cylindrical polystyrene pots (5.5 cm diameter, 15 cm high) filled with a mixture of washed siliceous sand and soil. The amount of soil in the pot was that which contained 1 mg of Olsen P and ranged from 21 to 109 g for high-P samples and from 100 to 170 for low-P samples. Concentration of Olsen P and Fe oxides in the washed sand was negligible. The amount of Olsen P in each pot was intended to provide an amount of available P in each pot similar to the amount expected to be extracted by crops during the experiment, in such a way that some symptoms of P deficiency could be expected at the end of the experiment. In each pot, a cucumber plant (Cucumis sativus L. cv Serena; hybrid F1; Nunhems, Almería, Spain) was grown for 50 days. Previously, seeds were germinated on alveolated trays containing perlite as substrate and transplanted to the pots after 16 days.

Plants were grown under controlled conditions in a growth chamber with 14-h photoperiod, a relative humidity of 45 (day period) and 60 % (night period), and an active photosynthetic radiation of 22 W m^{-2}. The experimental design was a randomized complete block with two treatments (high-P and low- P samples) and two replications. Pots were irrigated with 20 mL of P-free Hoagland-type nutrient solution at pH 6– 6.5 four times a week, enough to result in a modest drainage, and with the same volume of deionized water once a week to prevent salt accumulation in soil. The composition of the solution applied was (all concentrations in mmol L−1) $MgSO_4$ (2), $Ca(NO_3)_2$ (5), KNO_3 (5), KH_2PO_4 (1), KCl (0.05), H_3BO_3 (0.009), $MnCl_2$ (0.0023), $CuSO_4$ (0.0005), $ZnSO_4$ (0.002), and H_2MoO_4 (0.0005).

Study of P forms and P sorption capacity in soils

Phosphorus forms in soil before the experiment were studied by means of an operational method based on sequential chemical fractionation, which provides information about the P fractions in the soil solid phase with different ability to be released to the soil solution. The sequential fractionation scheme used was that of Ruiz et al. (1997) modified according to Saavedra et al. (2007), which includes two additional steps to extract “hot” alkali-extractable organic P and residual organic P. Extraction steps include:

(i) 0.1 *M* NaOH + 1 *M* NaCl (NaOH fraction), which releases adsorbed P, P precipitated as Fe- and Al-phosphates, and P bound by Fe and Al organic complexes;

(ii) 0.27 *M* Na citrate + 0.11 *M* $NaHCO_3$ (citrate–bicarbonate, cb fraction), which extracts remaining adsorbed P and soluble Ca phosphates, partly precipitated or adsorbed on calcite after NaOH extraction in calcareous soils;

(iii) 0.25 *M* Na–citrate at pH 6, and

(iv) 0.2 *M* Na–citrate pH 6, being the combination of P extracted by these two consecutive extractions (citrate, c fraction) ascribed to P precipitated as pedogenic Ca phosphates not dissolved in the previous step (mostly hydroxyapatite);

(v) 0.2 *M* Na citrate + 0.05 *M* ascorbate at pH 6 (citrate–ascorbate, “mild reductant soluble P”, ca fraction), which releases P occluded in poorly crystalline Fe oxides mainly;

(vi) 0.27 *M* Na citrate + 0.11 *M* $NaHCO_3$ + 2 % Na dithionite (citrate–bicarbonate–dithionite, “reductant soluble P”, cbd fraction), which releases P occluded in crystalline Fe oxides;

(vii) 1 *M* NaOAc buffered at pH 4 (OAc), which releases residual pedogenic Ca phosphates previously not dissolved by citrate;

(viii) 1 *M* HCl, which dissolves most lithogenic apatite;
(ix) 2 *M* NaOH, which releases alkali extractable organic P; and
(x) 0.5 *M* H_2SO_4 + 0.37 *M* $K_2S_2O_8$ for 1 h which releases residual organic P.

All extractions were performed in triplicate using polyethylene flasks at 25 ℃, except the two last steps, which were performed at 90 ℃ ("hot" alkali extractable) and 130 ℃ in an autoclave. After centrifugation at 100 0 g during 10 min, all extracts were analyzed for molybdate-reactive P (Murphy and Riley 1962) and total P following sulphuric–persulphate digestion (Díaz-Espejo et al. 1999), except for "hot NaOH-P," which was analyzed for total P only, and the last step, where only molybdate-reactive P was determined. Differences between molybdate-reactive P and total P in the extracts can be assigned mainly to organic P (Golterman et al. 1998), so a distinction could be made between organic and inorganic (molybdate-reactive, MRP) P in each fractionation step. The P extracted in the last two steps was taken as "recalcitrant" organic P. In the citrate-ascorbate and citrate-bicarbonatedithionite extracts from steps v and vi, Fe was also determined by atomic absorption spectrometry (Feca and Fed, respectively). The citrate-ascorbate extractable Fe was ascribed to poorly crystalline Fe oxides and the citrate-bicarbonate-dithionite extractable to crystalline Fe oxides. Inorganic P in soils was estimated as the sum of molybdate-reactive P in each fraction (except fractions ix and x, ascribed to recalcitrant organic P), and organic P as the sum of organic P in each fraction (considering as organic, total P in the "hot NaOH" extraction, and MRP in the last step).

Sorption capacity of soils was assessed by performing sorption curves at 6 days according to the method of Fox and Kamprath (1970). Sorption curves were fitted to the Freundlich equation:

$$Y=AX^{1/b} \tag{1}$$

Where Y is the sorbed P in soil, X is the remaining P concentration in solution, and b can be considered the affinity factor, in such a way that higher values correspond to higher affinity of soil particles for P. The PBC was estimated as the slope of the sorption curve at equilibrium concentration (BCe) and at 0.2 (BC0.2) and 1 mg L^{-1} (BC1).

Plant and soil analysis at the end of the experiment

After cropping, plants were harvested and shoots and roots separated. Plant material was washed and then dried in a forced-air oven until constant weight at 65 ℃ for at least 48 h. After determining dry matter (DM), plant tissues were ground to pass a 1-mm screen and analyzed for P. To this end, plant material was mineralized by igniting a portion of 0.25 g in porcelain crucibles in a furnace at 550 ℃ during 8 h. After that, 10 mL of 1 M HCl was used to dissolve ashes, heated at 100 ℃ for 15 min, and P determined by the colorimetric method of Murphy and Riley (1962) in the resulting digest. Certified plant material (1547 Peach Leaves, Sugerlabor, S.A., National Institute of Standard and Technology, Gaithersburg) was also analyzed to assess complete recovery of P by this procedure, confirming that the recovery of P in this certified material was always 90–110 %. After harvest, alkaline phosphatase activity in rhizosphere soil was determined according to the method of Tabatabai (1994) as the amount of p-nitrophenol released from p-nitrophenol phosphate at 37 ℃ and at pH of 9. Until phosphatase determination, the soil was stored at 5 ℃ for no more than 10 days.

Statistical analysis

The effect of soil and treatment (high-P vs low-P) on different variables was assessed by means of a two-way ANOVA using the general linear model procedure in Statgraphics Plus 5.1 (StatPoint, 2000). Previously, data were checked for normal distribution and homoscedasticity by using the Kolmogorov–Smirnov test and Levenne test, respectively, as implemented with the previous software. Differences between means were assessed by the LSD test ($P<0.05$) Regressions and correlations were also calculated using the same software; influential points in regressions were assessed by the Leverage test.

Results and discussion

Soils ranged widely in properties, particularly in carbonates and Fe oxides, which are relevant properties affecting P dynamics in soil. Clay content ranged from 48 to 640 g kg^{-1}, organic matter (SOM) between 5.5 and 20 g kg^{-1}, calcium carbonate equivalent (CCE) between 34 and 723 g kg^{-1}, citrate-ascorbate- and citrate-bicarbonate-dithionite extractable Fe according to Ruiz et al. (1997) between 0.17–2.44 and 2.2–21 g kg^{-1}, respectively, pH from 6.5 to 8.3, and cation exchange capacity from 9.1 to 52.3 cmol(+) kg^{-1}.

Phosphorus enrichment by fertilization only increased inorganic P as revealed by the significant difference between high- and low-P samples (Table 2.1). Only inorganic P in the cb and ca fractions differed significantly (Table 2.1). From the excess of 200 mg P kg^{-1} in the sum of inorganic P fractions of high-P relative to low-P samples, only 63 mg kg^{-1} were recovered in the fractions corresponding to the most readily desorbable P (NaOH+cb; Table 2.2) and only 15 mg kg^{-1} by bicarbonate (Olsen's method). According to the ratio of total plant-available P to Olsen P defined by Delgado and Torrent (1997) in similar soils (total available P was roughly equivalent to 1.5 times Olsen P), this means that approximately a 10 % of applied P remained readily available to plants. It should be noted that non-significant differences were observed in total organic P or organic fractions between highand low-P samples (Table 2.1). Organic P, estimated as the sum of organic P fractions, accounted on average for 50 % of total P estimated as sum of inorganic and organic P fractions in low-P samples (Table 2.1). This means that, in spite of being poorly understood P forms in these soils, they can be dominant (Saavedra and Delgado 2005; Delgado and Scalenghe 2008). On average, the ratio of organic P to Olsen P was 2.4 times higher in pots with low- than in pots with high-P samples, meanwhile the ratio of inorganic P to Olsen P was 1.7 times higher. Also, total and organic P in pots was roughly twice in low- (83 and 42 mg kg^{-1}, respectively) than in high-P samples (41 and 19 mg kg−^{1}, respectively). Thus, the ratio of non-readily phytoavailable P to that assumed to be available was greater in pots with low- than in those with high-P samples. Significant differences were neither observed in sorption parameters nor in PBC estimate (Table 2.2).

Table 2.1 Main inorganic and organic P fractions according to the fractionation scheme of Saavedra et al. (2007) and availability index according to Olsen et al. (1954) in the studied soils before crop experiment

			Inorganic fractions							
		Olsen P	**PNaOH**	**Pcb**[a]	**Pc**	**Pca**[a]	**Pcbd**	**P OAc**	**PHCl**	
		mg kg-1	mg kg-1							
Low-P samples	Mean±SD	9±3	13±21	41 ± 15,	102±94	56±47.9	47±17	37±50	25±13	
	Range	5–16	1–74	17–68	9–337	13–179	21–86	3–190	8–60	
High-P samples	Mean±SD	24±10	20±32	97±69	158±164	107±178	56±35	53±66	30±21	
	Range	9–49	1–100	26–302	12–684	19–778	28–185	4–262	5–79	
			Organic fractions							
		Olsen OP	**NaOH**	**cb**	**c**	**ca**	**cbd**	**OAc**	**HCl**	**Recalcitrant OP**
		mg kg-1	mg kg-1							
Low-P samples	Mean±SD	3±2	21±12	42±15	78±32	43±25	30±13	41±18	19±5	78±29
	Range	0–8	5–56	21–77	1–132	1–114	0–51	24–107	9–31	32–137
High-P samples	Mean±SD	2±2	23±15	41±18	79±22	61±84	33±24	54±50	21±7	80±24
	Range	0–7	10–68	7–83	40–112	21–392	0–70	30–244	9–33	48–126

IP inorganic P (molybdate reactive), OP organic P; subindexes: NaOH NaOH extractable, cb citrate-bicarbonate, c citrate, ca citrate-ascorbate, cbd citrate-bicarbonate-dithionite, OAc acetate, Olsen OP, organic P in bicarbonate extracts according to Olsen et al. (1954), SD standard deviation a Differences between low- and high-P samples were only significant for cb, ca, and sum of IP; P=0.005, P=0.0166, and P=0.0141, respectively

Phosphorus concentration in shoots and roots did not differ significantly between high- and low-P samples. Dry matter yield in shoots was significantly greater in high- than in low-P samples (Table 2.2). In spite of the same amount of Olsen P in the pots (1 mg per pot), P extraction by plants ranged from 1.05 to 3.33mg pot^{-1} (mg plant^{-1}) in high-P samples and from 1.02 to 2.19 mg pot−1 in low-P samples, and P extraction by plants differed between samples with different P status, it being on average 11 % greater in high- (mean 1.65 mg pot^{-1}) than in low-P samples (mean 1.49 mg pot^{-1}; Table 2.2). These evidences reveal that the actual amount of available P in pots was not accurately predicted by this soil P availability index. Previous evidences in a group of calcareous clayey soil with similar properties revealed that threshold values of Olsen P for fertilizer response ranged widely (from 2 to 15) revealing that this method was not a precise index for fertilizer recommendations (Delgado et al. 2010). This was also in agreement with results by Tandy et al. (2011) who did not observe significant relationship between Olsen P and P in plant in a group of soils ranging widely in pH. On average, P extracted by plants accounted for the expected total available P in soil according to Delgado and Torrent (1997), which was 1.5 times Olsen P, thus explaining the evident P deficiency symptoms observed at the end of the experiment (Fig. 2.1). Thus, as expected, the experimental setup was efficient to deplete the total plant available P in soil.

Phosphorus uptake did not depend on the amount of P in soil that was potentially available to plants, i.e., the classical “quantity” factor, which basically relates to Olsen P (Sánchez-Alcalá et al. 2014). Only the affinity factor of the Freundlich equation (b) was a significant explanatory variable for both high- and low-P samples (Fig. 2.2). This reveals, as expected, that factors affecting the equilibrium between inorganic P in the solid and liquid phases, such as soil affinity for P, affect the uptake of P by plants much more that the amount of potentially desorbable P as estimated from chemical extraction. Affinity for P is known to be a crucial factor explaining the release P from the solid phase (e.g., to subsurface flow, McDowell et al. 2002). The relevance of affinity for P affecting P availability to plants was also described by Delgado et al. (2010) using the Langmuir model in a group of soils with a narrow range of properties. In contrast, our results do not reveal a very clear effect of the PBC as estimated from the sorption curves: only a slight negative correlation ($r=-0.48$; $P<0.05$) between plant P uptake and PBC at the equilibrium concentration in high-P samples was found. This lack of relevant explicative value of PBC contradicts previous works (Ehlert et al. 2003; Celardin 2003) which highlighted the relevance of this factor for the availability of P to plants. Since our experiment likely promoted a relevant starvation of available P in soil disposed in pots, with a significant change in the amount of sorbed P in equilibrium with the soil solution, a factor related to this equilibrium across a wide range of P concentrations in solution, such as the affinity factor (b), was likely to explain P phytoavailability better than PBC at a given solution P concentration. The affinity factor (b) depends on the nature of the P sorbing surfaces in soil. Crystalline Fe oxides show a higher affinity for P (higher b values) than their poorly crystalline counterparts (Colombo et al. 1994; Guzman et al. 1994), as revealed in our high-P samples by the significant negative correlation between Fe_{ca}/Fe_d and b ($r=-0.63$; $P<0.01$). This correlation contributes to explain the positive correlation between Fe_{ca}/Fe_d and plant P uptake ($r=0.51$; $P<0.05$) for these samples. Not only the nature but also the amount of sorbents seems to affect P uptake as concluded from the correlation between P uptake and $Fe_{ca} + Fe_d$ in high-P samples (Fig. 2.2). This can be partially explained by the negative correlation found between this sum and b ($r=-0.5$; $P<0.05$).

Table 2.2 Sorption curve parameters, estimated buffer capacity (BC) at different P concentration in the soil solution, dry mass (DM) yield, P concentration in shoot and roots, and P uptake by plants from pots and alkaline phosphatase activity in rhizosphere with a mixture of siliceous sand and soil to encompass 1 mg of Olsen P supplied per plant.

		A	**b**	**BCe**	**BC0,2**	**BC1**	**DM**	**DM roots**	**P concentration shoots** [a]	**P concentration roots**	**P uptake** [b]	**Phosphatase**[c]
Low-P samples	**Mean±SD**	72 ± 32	2.2±0.31	348±413	78±35	32±14	1.45±0.31	0.22±0.09	0. 97±0.14	1.31±0.30	1.49±0.39	56±21
	Range	16–140	1.5–2.6	9–1480	18–151	9–67	1.07–2.15	0.12–0.46	0.73–1.46	0.67–2.33	1.02–2.19	29–102
High-P samples	**Mean±SD**	75 ± 36	2.2±0.61	201±167	80±40	33±17	1.65±0.32	0.22±0.06	0.93±0.20	1.34±0.31	1.65±0.56	34±18
	Range	0.4–135	0.3–3.3	4–540	0.05–151	1–67	0.80–2.34	0.09–0.35	0.55–1.47	0.88–2.38	1.05–3.33	12–96
LSD		2.91	0.07	44	3	1.16	0.02	0	0.01	0.02	0.02	0.5

A and b according to the Freundlich equation $Y=A\cdot X^{1/b}$, BC buffer capacity estimated from the derivate of the Freundlich equation at e=equilibrium; 0.2 mg P L^{-1} , and 1 mg P L^{-1} , SD standard deviation, LSD least significant difference (P<0.05)

[a] Significant at *P*=0.0002

[b] Significant at *P*=0.0105

[c] Significant at *P*=0.0001

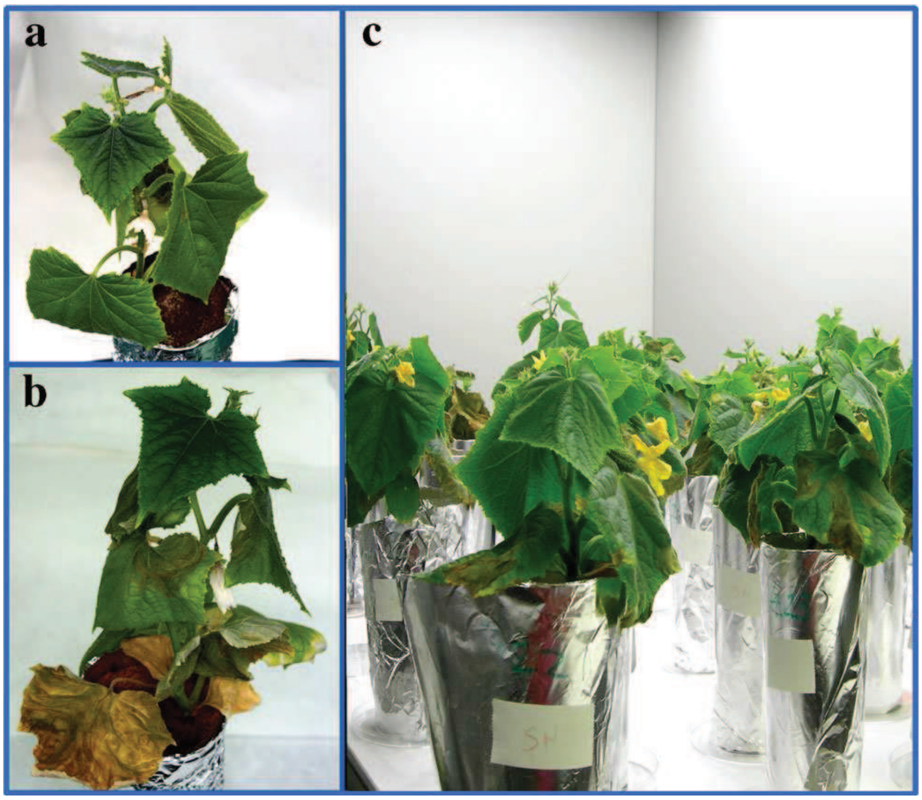


Fig. 2.1 Experiment with cucumber in growing chamber. a Plant after approximately 20 days after planting, without symptoms of P deficiency. b Clear symptoms of P deficiency in basal leaves at the end of the experiment (50 days after planting). c Plants in growing chamber at 30 days after planting

Besides this, a high content in adsorbing surfaces, as those of Fe oxides (Peña and Torrent 1990), could imply a dominant role of P adsorption relative to P precipitation. In Mediterranean soils, adsorbed P appears to be more available than precipitated P in the form of insoluble metal phosphates (Delgado and Torrent 1997; Saavedra and Delgado 2005), thus contributing to explain the positive correlation between crop P uptake and Fe in oxides.

In contrast to high-P samples, plant P uptake from low-P samples related to the sum of inorganic P extracted by NaOH and cb, which explained 52%of plant P uptake (Fig. 2.3). This is consistent with correlations described between the sum of inorganic P in both fractions and the P availability index (Saavedra and Delgado 2005; Saavedra et al. 2007). However, in spite of these correlations, a fraction of adsorbed and precipitated P in these fractions cannot be considered readily available, because it likely includes P adsorbed on high energy sites or in metal phosphates (mainly Ca phosphates in these soils) not soluble at the usual P concentration in the rhizosphere (Delgado and Torrent 1997; Delgado et al. 2010). The relationship between P extracted by plants and NaOH-P + cb-P indicated that the inorganic P in these fractions that was not extracted with the Olsen reagent contributed to plant uptake in low-P pots but not in high-P pots. Mobilization of these non-bicarbonate-extractable inorganic P forms can be probably ascribed to organic acid exudation by roots (Jones 1998; Ström et al. 2005; Richardson et al. 2011). Extraction with cb,which is able to dissolve Ca phosphates (Saavedra and Delgado 2005), mimics to some extent the effect of the exudation of acids in rhizosphere soils buffered at basic pH.

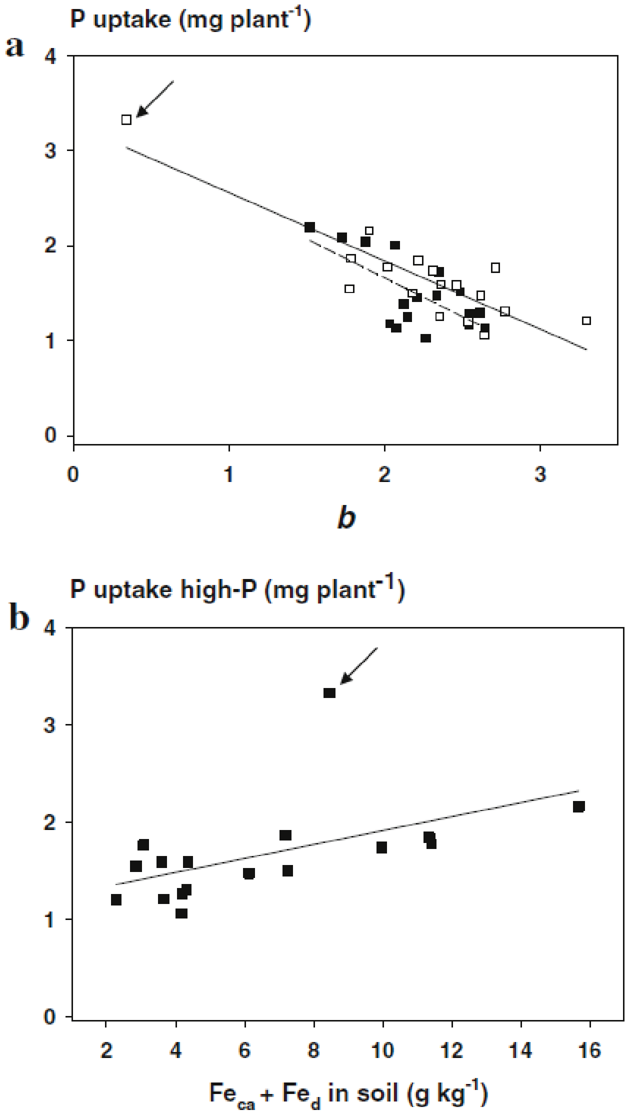


Fig. 2.2 a Relationship of P uptake by plants in high- (empty symbols) and low-P (solid symbols) samples with the affinity factor (b) in the Freundlich equation ($Y=A\ X^{1/b}$); $R^2=0.76$, $P<0.001$ for high-P samples ($R^2=0.4$, $P<0.01$ if marked point is not included), and $R^2=0.46$, $P<0.01$ for low-P samples. b Relationship of P uptake by plants in high-P samples and the sum of Fe extracted by citrate-ascorbate (ca) and citratebicarbonate- dithionite (d) [the point marked with an arrow was not included in the regression since it was clearly influential according to the Leverage test]. R2=0.57, P<0.001. The experiment was performed with one plant per pot; in each pot the amount of Olsen P was the same (1 mg pot-1). Since P deficiency symptoms were evident in all soils, P uptake by the plant in each pot provides a measure of the ratio of total available P to Olsen P

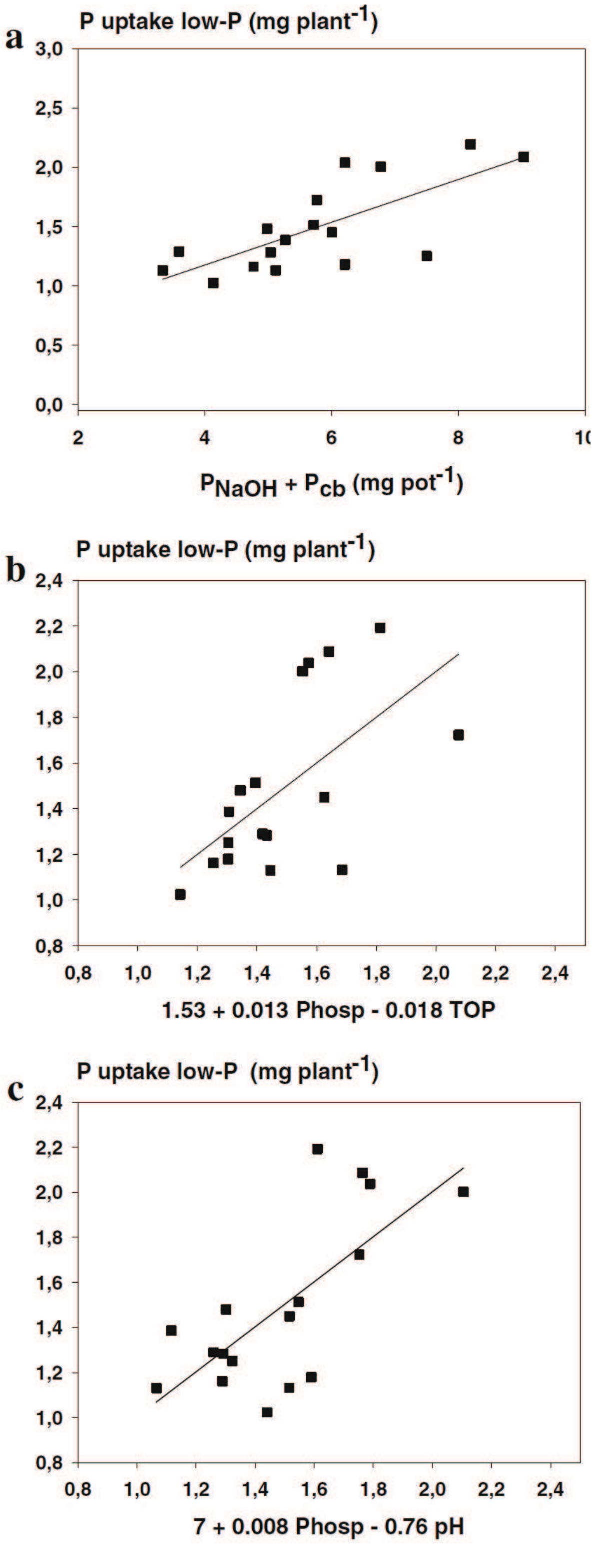


Fig. 2.3 P uptake by plants in low-P samples as a function of the following. a The amount of molybdate reactive P (inorganic P) extracted by NaOH and citrate-bicarbonate (cb) in the sequential fractionation scheme (R2=0.52, P<0.001). b As a function of the amount of total organic P (TOP) in pots and alkaline phosphatase activity (Phosp) in the rhizosphere soil (R2=0.37, P<0.01). c As a function of this enzymatic activity and soil pH (R2=0.5, P<0.001). It should be noted that the sign of multiple linear regression in (b) does not provide real information about the sense of the contribution of each independent variable since phosphatase and TOP were significantly correlated

The phosphatase activity in the rhizosphere soil was positively correlated with the sum of organic P fractions present in pots with both high- and low-P samples ($r=0.75$; $P<0.001$, and $r=0.7$; $P<0.01$, respectively). This explains this activity being on average a 65 % higher in pots with low-P than in those with high-P samples (Table 2.2) since the amount of organic P was much higher in the former pots. Thus, hydrolysable organic P can be expected to contribute to P uptake by plants. However, P extraction by plants was not correlated with the total organic P in the pot (as sum of OP in all the fractions) or with the phosphatase activity in the rhizospheric soil in both types of samples. When the total organic P in pot and phosphatase activity were considered in the model, a 37% of P uptake by plants growing on low-P samples was explained (Fig. 2.3). Also, a linear model including phosphatase and pH as independent variables explained 50 % of P extracted by plants on low-P samples (Fig. 2.3). This reveals that not only the total amount of organic P in the growing medium but also factors related to plant ability to hydrolyse these P forms or to affect dynamics of organic and inorganic P forms and hydrolytic activity (pH) can be relevant with a view to explaining P uptake by plants. However, this contribution of organic forms and hydrolytic activity was evident with large amounts of organic P present in the growing media (low-P samples).

These results stress in summary that the accuracy of a soil P index, Olsen P in this study, is affected by: (i) the affinity of soil surfaces for P; (ii) the P forms considered non-readily available to plants such as non-readily soluble inorganic P or organic P forms; and (iii) root activity (acid and hydrolytic enzyme exudation). However, although these factors are known to affect the availability P to plants depending on particular soil conditions, an integrated view of their effects on the accurate estimation of plant-available P has been rarely addressed, thus explaining why they are not considered in the usual availability P indices. To overcome this limitation, a more accurate definition of threshold values for soil P indices on the basis of the aforementioned factors is required. A simple model was proposed for Olsen P by Sánchez-Alcalá et al. (2014), which was however limited since it only considered the short-term equilibrium of inorganic P in soil. Also, our results are of practical interest for designing new P indices which should rely on chemical P extractions sensitive to these factors. Although P mobilization processes linked to the rhizosphere activity are widely known (Mollier et al. 2008; Hinsinger et al. 2011), our results revealed which are the targeted P fractions in this mobilization and under which circumstances this mobilization can contribute to plant P uptake (P starvation in soil). They also provided additional support for the need to consider this mobilization in models to estimate P availability to plants from P indices and threshold values for these indices. As mentioned above, pots with low-P samples contained much more total and organic P than those with high- P samples, but both had the same amount of readily available P as that estimated by Olsen P. However, results evidenced an increased use of non-readily available forms in low-P samples. This indicates a likely decreased use of inorganic P related to Olsen P in low-P samples when compared with high-P samples and some compensation in the P supply to the plant from other sources. This could reveal that not only P starvation in soil but also the ratio of non-readily available to available P forms in soil can determine the final contribution of non readily available P in rhizosphere to the final P uptake by the plant. However, the P extracted by plants in pots with low-P samples was even slightly lower than in those with high-P samples. All these results highlight the limitations of the accuracy of P availability indices. In low-P samples, mobilization of non-readily available forms did not contribute to completely overcome the nutritional consequences of a P deficient growing medium. In fact, in pots with low-P samples, an increased scarcity of the nutrient, in spite of the same theoretical amount of available P to plants, contributes to explain the triggering of plant root strategies to mobilize P, such increased hydrolytic activity (Richardson et al. 2011). On the other hand, the physico-chemical factors affecting the dynamics of inorganic P play a crucial role in high-P samples.

Conclusion

Olsen P could not accurately predict plant P uptake from the studied soils. This uptake was explained by factors affecting the equilibrium between P in solid and liquid phases in high-P samples, mainly the affinity of soil particles for P and the total adsorption capacity estimated from the total Fe bound in Fe oxides. On the other hand, in pots with low-P samples, inorganic P released by NaOH and citrate/bicarbonate and the combination of total organic P, phosphatase activity in the rhizosphere, and pH contributed to explain plant P uptake. In spite of the theoretically same amount of readily available P and the potential increased use of non-readily available forms (poorly soluble inorganic P and organic P), P extracted by plants in pots with low-P samples was even slightly lower than in those with high-P samples thus suggesting that the Olsen method likely overestimates available P in samples with low-P status. These results are of practical interest for the correction of classical P availability indices by defining more accurate threshold values based on properties affecting the relationship between plant P uptake and P index values or for the design of new ones sensitive to these properties and leading thus to a more precise estimation of the soil P status.

Acknowledgments

This study was funded by the Spanish Ministry of Science and Innovation and the European Regional Development Fund of the European Union through the National Research, Development and Innovation Program(PlanNacional I+d+i,AGL2011-29893-CO2-01 and AGL2011-29893-CO2-02) and by the Regional Government of Andalucia (Project AGR 63–85). Mr. Ramiro Recena was PhD student with a grant from the Regional Government. Fertiberia S.A. and OCA Lebrija collaborated in the collection of soil samples for the present study.

References

Barrow NJ, Debnath A (2014) Effect of phosphate status on the sorption and desorption properties of some soils of northern India. Plant Soil 378:383–395. doi:10.1007/s11104-014-2042-8

Celardin F (2003) Evaluation of soil P-test values of canton Geneva/ Switzerland in relation to P loss risks. J Plant Nutr Soil Sci 166: 416–421. doi:10.1002/jpln.200321142

Colombo C, Barrón V, Torrent J (1994) Phosphate adsorption and desorption in relation to morphology and crystal properties of synthetic hematites. Geochim Cosmochim Acta 58:1261–1269

Cordell D, Neset T (2014) Phosphorus vulnerability: a qualitative framework for assessing the vulnerability of national and regional food systems to the multi-dimensional stressors of phosphorus scarcity. Global Environ Change 24:108–122. doi:10.1016/j.gloenvcha. 2013.11.005

Cordell D, Drangert J-O,White S (2009) The story of phosphorus: global food security and food for thought. Global Environ Change 19:293– 305

Delgado A, Scalenghe R (2008) Aspects of phosphorus transfer in Europe. J Plant Nutr Soil Sci 171:552–575. doi:10.1002/jpln. 200625052

Delgado A, Torrent J (1997) Phosphate rich soils in the European Union: estimating total plant-available phosphorus. Eur J Agron 6:205–214

Delgado A, Torrent J (2001) A comparison of soil extraction procedures for estimating the phosphorus release potential of agricultural soils. Commun Soil Sci Plant Anal 32:87–105. doi:10.1081/CSS- 100102995

Delgado A, del Campillo MC, Torrent J (2010) Limitations of the Olsen method to assess available phosphorus in reclaimed marsh soils. Soil Use Manage 26:133–140. doi:10.1111/j.1475-2743.2010.00264.x

Delgado A, Madrid A, Kasem S, Andreu L, del Campillo MC (2002) Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. Plant Soil 245:277–286

Díaz-Espejo A, Serrano L, Toja J (1999) Changes in sediment phosphate composition of seasonal ponds during filling. Hydrobiologia 392: 21–28. doi:10.1023/A:1003557408640

Ehlert P, Morel C, Fotyma M, Destain JP (2003) Potential role of phosphate buffering capacity of soils in fertilizer management strategies fitted to environmental goals. J Plant Nutr Soil Sci 166:409–415 Fox RL, Kamprath EJ (1970) Phosphate sorption isotherms for evaluating the phosphate requirements of soils. Soil Sci Soc Am Proc 34:902–907

Gee GW, Bauder JW (1986) Particle-size analysis. In Klute A (ed) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI pp 383–411

Golterman HL, Paing J, Serrano L, Gómez E (1998) Presence of and phosphate release from polyphosphates or phytate phosphate in lake sediments. Hydrobiologia 364:99–104. doi:10.1023/ A:1003212908511

Guzman G, Alcántara E, Barrón V, Torrent J (1994) Phytoavailability of phosphate adsorbed on ferrihydrite, hematite, and goethite. Plant Soil 159:219–225

Hinsinger P, Brauman A, Devau N, Gérard F, Jourdan C, Laclau JP, Le Cadre E, Jaillard B, Plassard C (2011) Acquisition of phosphorus and other poorly mobile nutrients by roots.Where do plant nutrition models fail? Plant Soil 348(1–2):29–61. doi:10.1007/s11104-011-0903-y

Holford ICR (1980) Effects of phosphate buffer capacity on critical levels and relationships between soil tests and labile phosphate in wheat growing soils. Aust J Soil Res 18:405–414

Jones DL (1998) Organic acids in the rhizosphere –a critical review. Plant Soil 205:25–44

Keyzer M (2010) Towards a closed phosphorus cycle. DE Economist 158:411–425

Kpomblekou K, Tabatabai MA (1997) Effect of cropping systems on quantity/intensity relationships of soil phosphorus. Soil Science 162:56–68

Kulhánek M, Balík J, Černý J, Nedvědv KB (2007) The influence of different intensities of phosphorus fertilizing on available phosphorus contents in soils and uptake by plants. Plant Soil Environ 53: 382–387

Mallarino AP (1997) Interpretation of soil phosphorus test for corn in soils with varying pH and calcium carbonate content. J Prod Agric 10:163–167

Mason S, McNeill A, McLaughlin MJ, Zhang H (2010) Prediction o wheat response to an application of phosphorus under field condition using diffusive gradients in thin-films (DGT) and extraction methods. Plant Soil 337:243–258

McDowell R, Sharpley A, Withers P (2002) Indicator to predict th movement of phosphorus from soil to subsurface flow. Enviro Sci Technol 36:1505–1509

Chapter III

In the third chapter of this PhD is to **propose single models able to estimate precisely threshold values for Olsen P, depending on soil properties, as a tool for more accurate P fertilization schemes..**

Estimation of threshold Olsen P values for fertilizer response in soils of Mediterranean areas

Estimation of threshold Olsen P values for fertilizer response in soils of Mediterranean areas

Abstract

The main objectives of this work were to elucidate which soil properties affect threshold Olsen P values for fertilizer response, and to develop models to estimate accurate threshold values for a more precise use of this soil P test (SPT). To this end, threshold values for Olsen P and 0.01 M $CaCl_2$-extractable P were estimated in 18 representative agricultural soils of Mediterranean areas by means of P starvation pot experiments. Threshold values for Olsen P were negatively correlated with clay content, pH, phosphate buffer capacity (PBC), and Fe in oxides. Clay content, pH, and phosphatase activity in the rhizosphere explained near 90 % of variation in Olsen P threshold values. Factors related with the PBC of the soils studied were the most relevant explaining threshold values for both SPTs. However, the contribution of factors related to the dynamics of organic P (hydrolysable forms and phosphatase activity) was significant. The clay content and pH of the soils explained alone 81 % of the variance of threshold values. In summary, all these results will be useful for defining more precise P fertilization recommendation schemes in the future.

Key words: Olsen P, availability index, threshold values, buffer capacity, organic P

Introduction

Agriculture depends on the use of P fertilizers obtained from phosphate rock (PR) which is a non-renewable and strategic resource (Keyzer, 2010; Van Vuuren et al. 2010). The demand of P fertilizers is expected to grow as the logical result of the growing food needs of an increasing population, whereas the production of PR is expected to peak in the next decades (Schröder et al., 2011; Ryan et al., 2012). Expected future P scarcity is thus emerging as a global challenge for humankind (Cordell and Neset 2014). In addition, the use of P in agriculture is globally very inefficient, in such a way that only 15% of applied P to agricultural soil goes into the food chain (Cordell et al. 2009; Withers et al. 2014).

The former considerations point to the need of a more efficient and sustainable use of P in agriculture. In particular, fertilization schemes should rely, among other factors, on accurate distinction of P-responsive sites (Recena et al. 2015) in order to concentrate this resource on soils in which the highest return of P fertilization is to be expected. This distinction is usually assessed with the use of an “available P test” or “soil P test” (SPT) that is correlated with plant P uptake. The commonly used SPTs are based on extraction with a chemical reagent, as is the case of the worldwideused Olsen P test (Olsen et al. 1954).The key issue for their practical use is the definition of threshold values below which yield response to fertilizer application can be expected (Delgado and Scalenghe 2008; Tang et al. 2009). However, although SPTs are deemed to be useful for assessing P fertilizer needs, they can be inaccurate because the definition of their threshold values is not always precise. In fact, actual threshold values for a given SPT can range widely among those soils for which its use is recommended (e.g. for Olsen P; Delgado et al. 2010; Sánchez-Alcalá et al. 2015). Thus, Olsen P is not always well related to plant P uptake (Delgado and Torrent 1997; Kulhanek et al. 2007; Tandy et al. 2011). In soils with very similar properties, Delgado et al. (2010) observed a wide range in threshold values for Olsen P, which clearly depended on the soil PBC. Sánchez-Alcalá et al. (2014) reported that the values of Olsen P for a critical P concentration in solution for plant P uptake were affected by properties related to the P sorption capacity of the soil such as carbonate and Fe oxides contents. Carbonate content and pH were the soil properties affecting Olsen P threshold values in plant growth experiments (Sánchez-Alcalá et al. 2015). Recena et al. (2015) demonstrated that the Olsen P predictive value for plant P availability is affected not only by those soil properties influencing the equilibrium between inorganic phosphate in solution and that in solid phase but also by factors related to organic P dynamics. Thus, it can be hypothesized that there is a need of more precise methods to estimate P-responsive sites. One possibility is the development of SPTs based on more complex P extraction methods such as those using resins (Delgado et al. 2010) or diffusive gradients thin films (Tandy et al. 2011; Santner et al. 2015), which are more sensitive to soil P buffer capacity. Another option is the development of single models that are based on traditional SPTs such as Olsen P in combination with soil properties related to the dynamics of soil P. These models should rely on a deeper knowledge of whichsoil properties affectthe relationship between plant P uptake and chemically extractable P.

The objectives of the present study were as follows:(i) identification of soil properties affecting threshold Olsen P values for fertilizer response in a set of representative Mediterranean soils where this SPT is usually recommended, and (ii) to develop simple models based on soil properties usually determined in routine soil analysis to estimate accurate threshold values for use of Olsen P as SPT in these soils.

Materials and Methods

Soils

The set of soils used in this study was the same one used by Recena et al. (2015), with one additional soil included (18 soils in total). The soils were classed as Mollisols, Entisols, Inceptisols, Alfisols, and Vertisols according to Soil Taxonomy (Soil Survey Staff, 2010). The agricultural use of these soils was detailed in Recena et al. (2015), and the additional soil considered was used for the typical biannual rainfed crop rotation in Spain, i.e. wheat–sunflower. The main properties of the 0–20 cm soil layer, from which samples were taken, are described in Table 3.1.

Methods for soil sampling, processing, analysis of basic properties, study of P and Fe forms, and P sorption capacity (from P sorption isotherms) are described in Recena et al. (2015). In addition, the concentration of phytase hydrolysable P in the alkali and citrate-bicarbonate (CB) extracts of the sequential fractionation scheme described in that work to study P forms was also determined. These extracts in the sequential fractionation scheme are supposed to contain inorganic and organic P mostly adsorbed or precipitated as soluble metal phosphates (Saavedra et al. 2007). The phytase-hydrolysable P was estimated as the increase in molybdate reactive P

(Murphy and Riley 1962) after 30 min of incubation of the extract with phytase(Bio-Feed phytase L, Novozimes, Bagsvaerd, Denmark) at 37 ^{o}C.To this end, the enzyme was added to the soil extract in 0.2 ml of buffer solution (0.4M $C_2H_4O_2$, 10mM EDTA, pH 5.5,) to a final activity of 10nKat ml^{-1} and the reaction was stopped by adding 15 % TCA.

Table 3.1. Mean, standard deviation (SD) and range of the properties of studied soils (n = 18)

General soil properties								
	Clay	**SOC**	**CCE**	**ACCE**	**Fe_{ca}**	**Fe_d**	**pH**	**Olsen P**
	——— g kg^{-1} ———							mg kg^{-1}
Mean ± SD	288 ± 155	10.6 ± 3.8	270 ± 234	81 ± 67	1.02 ± 0.74	5.52 ± 3.05	7.8 ±0.5	24 ± 10
Range	48 - 640	5.5 - 20	0 - 723	0 - 178	0.17 - 2.44	2.04 - 13.24	6.5 - 8.3	9.1 - 49.1
Organic P related properties								
	$NaOH_h$	**CB_h**	**Phosp**					
	———kg^{-1} ———		mg PNP kg^{-1} h^{-1}					
Mean ± SD	5.21 ± 5.25	7.0 ± 4.6	142 ± 18					
Range	0.48 – 24.02	0 - 13.5	105 - 172					
Properties related to P adsorption								
	A	***b***	**BC_e**	**$BC_{0.2}$**	**BC_1**			
Mean ± SD	72 ± 32	2.22 ± 0.31	348 ± 413	78 ± 35	32 ± 14			
Range	16 - 140	1.52 - 2.64	9 - 1480	18 - 151	8.5 - 67			
Threshold values for Olsen P and P concentration in CaCl2 extracts (P_{CaCl2}) and P values and R^2 for the Cate-Nelson model								
	P_{CaCl2}	P values	R^2		**Olsen-P**	P values	R^2	
	mg L^{-1}				mg kg^{-1}			
Mean ± SD	0.025 ± 0.043	0.0203 ± 0.0240	0.72 ± 0.14		9.2 ± 3.2	0.0326 ± 0.038	0.68 ± 0.19	
Range	0.002 – 0.175	0 – 0.0868	0.47 – 0.96		4.9 – 16.5	0 – 0.1065	0.38 – 0.96	
Median	0.006	0.0117	0.74		8.9	0.0164	0.69	

Olsen P, initial Olsen P in high-P samples; SOC, soil organic carbon; CCE, calcium carbonate equivalent; ACCE, active calcium carbona equivalent,subindexca, citrate ascorbate extractable; subindex d, citrate-bicarbonate-dithionite extractable; subindex h, phytase hydrolysable P in th corresponding extracts of high-P samples; Olsen OP, organic P in bicarbonate extracts of low-P samples, Phosp, mean of the phosphatase activity in the fo crops in high-P samples; A and b according to the Freundlich equation,
$Y = A \cdot X^{1/b}$ in high-P samples; BC, P buffer capacity estimated from the derivate of the Freundlich equation at e = equilibrium; 0,2 mg P L^{-1}, and 1 mg P L^{-1}

Table 3.2. Correlation coefficients between different properties of studied soils

	P_{CaCl2} TV	**Clay**	**pH**	**Phosp**	**SOC**	**ACCE**	**Feca + Fed**	**Feca/Fed**	**BC_1**	**$NaOH_h$+CB_h**
Olsen P TV	0.48*	–0.80***	– 0.73***	0.25ns	–0.04ns	– 0.23ns	– 0.39ns	0.42 ns	– 0.7**	0.30ns
P_{CaCl2} TV		–0.6**	– 0.5*	–0.3ns	0.17ns	– 0.47*	–0.17ns	0.68 **	– 0.62**	0.77***
Clay			0.52*	0.52*	0.23ns	0.27ns	0.52*	–0.3 ns	0.84***	– 0.52*
pH				0.33ns	0.07ns	0.61**	0.19ns	– 0.73***	0.47*	0.2ns
Phosp					0.31ns	0.19ns	0.23ns	0.29ns	0.52*	– 0.41 ns
SOC						0.03ns	–0.01ns	–0.02ns	0.12ns	0.17ns
ACCE							– 0.58*	– 0.70**	0.17ns	– 0.26ns
Feca + Fed								0.2ns	0.50*	– 0.32ns
Feca/Fed									– 0.35ns	0.55*
BC_1										– 0.42ns

TV, threshold value; P_{CaCl2}, P concentration in $CaCl_2$ soil extracts; SOC, soil organic carbon; ACCE, active calcium carbonate equivalent; subindexca, citrate ascorbate extractable; subindex d, citrate-bicarbonate-dithionite extractable; subindex h, phytase hydrolysable P in the corresponding extracts; Olsen OP, organic P in bicarbonate extracts in low-P samples;Phosp, mean of the phosphatase activity in the four crops in high-P samples; BC_1, P buffer capacity estimated from the derivate of the Freundlich equation at 1 mg P L^{-1}

*, **. ***, significant at $P < 0.05$, 0.01, and 0.001, respectively; ns, not significant

Phosphorus concentration in 0.01 M $CaCl_2$ extracts (P_{CaCl2}) was used as a proxy forthe concentration of P in the soil solution. This extraction was performed in duplicate and at a soil:extractant ratio of 1:10 (2 g of soil in 20 ml of extractant) in polyethylene falcon tubes that wereend-over-end shaken at 2.5 s^{-1} for 30 min. The extract was centrifuged at 1000 g for 10 min, and then a portion of 2 ml centrifuged in Eppendorf tubes at 19000 g to remove particles with diameter >0.05 microns (Sánchez-Alcalá et al. 2015). In the remaining solution, molybdate reactive P was determined according to Murphy and Riley (1962).Molybdate reactive P and organic P after persulphate digestion in autoclave in bicarbonate extracts (Olsen et al. 1954) were also determined.

As described by Recena et al. (2015), from a collection of soil sites in which the individual samples ranged widely in Olsen P, those soil samples with the two extreme Olsen P values were selected from each site and named “low-P” and “high-P” samples according to their P status. The “high P” and “low P” samples were examined in order to check that they differedby less than 5% in the main soil properties. For the experiment described below, soil was ground to <6 mm. Olsen P levels in high-P samples were all well above threshold values for fertilizer response according to the current stage of knowledge, even in samples with the lowest values (9 mg kg^{-1}) which were samples with a very high P buffer capacity (PBC) and thus with expectedthreshold values below 5 mg kg^{-1}(Delgado et al. 2010). For most low-P samples, Olsen P was assumed to be close to threshold values (data not shown; means and range in Recena et al. 2015).

Experimental setup

An experiment intended to deplete soil P was conducted in a growth chamber where durum wheat and sunflower were grown till anthesis twice (wherebysimulating a typical rainfed crop rotation) for both “high-P” and “low-P” samples. Thus, for each type of sample of each soil four successive crops were grown. Polystyrene pots (5.5 cm-diameter, 15 cm-high) filled with 300 g of soil were used. Seeds were pre-germinated on a moistened Petri plate for 14 days and, after that,germinated in trays with perlite as substrate and irrigated with deionized water. After 16 days seedlings were transplanted. For each soil and sample, the number of pots per depletion stage decreased from five in the first stage to two in the last onebecause300gwas retired after each stage for further laboratory analysis. After transplanting, pots were watered daily with 20 ml of a Hoagland type nutrient solution without phosphorus at pH 6–6.5, sandwiching between three irrigations one with the same volume of deionized water to avoid salinization of the soil. Plants were grown with a 14 h photoperiod, a relative humidity of 45% (day period) and 60% (night period), and an active photosynthetic radiation of 22 W m^{-2}. The composition of the solution applied was (all concentrations in mmol L^{-1}): $MgSO_4$ (2), $Ca(NO_3)_2$ (5), KNO_3 (5), KH_2PO_4 (1), KCl (0.05), Fe-EDDHA (0.01), H_3BO_3 (0.009), $MnCl_2$ (0.0023), $CuSO_4$ (0.0005), $ZnSO_4$ (0.002), and H_2MoO_4 (0.0005).

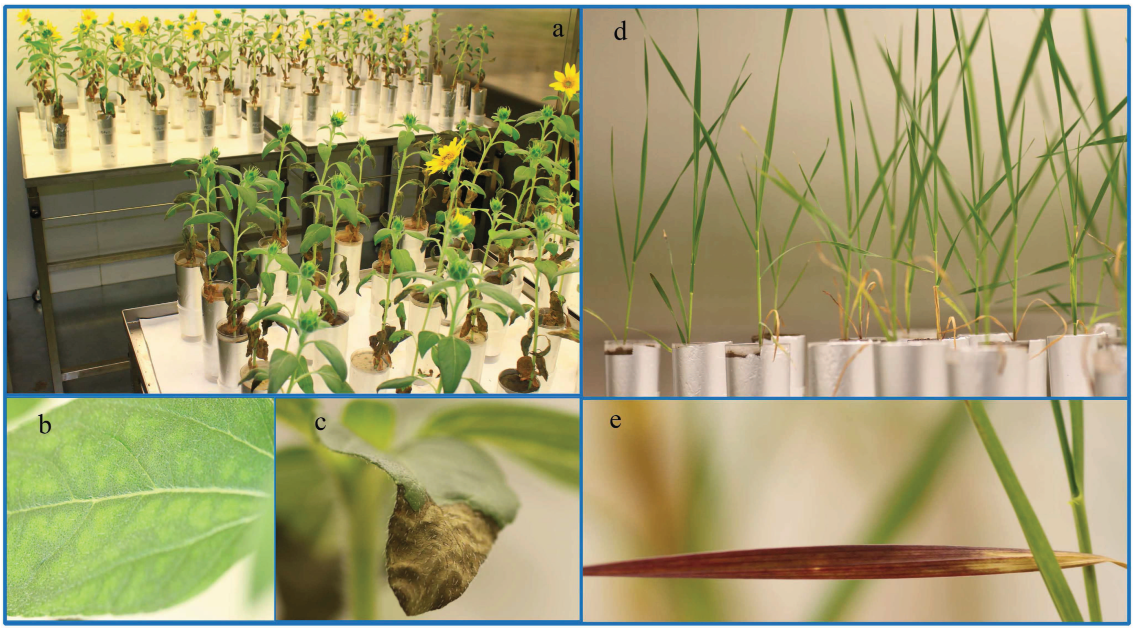


Figure 3.1 Pot starvation experiment: (a) sunflower in growing chamber with incipient P deficiency symptoms (b) and clear deficiency symptoms (c); and wheat (d) with P deficiency symptoms (e).

Plant and soil analysis after crop

After each cropping, plant shoots and roots were carefully separated, washed and finally dried in a forced-air oven until constant weight at 65°C for at least 48 h. Root and shoot dry matter (DM) was then measured. After each cropping step, soil from all the replications for each soil and type of sample was mixed and processed by drying and grounding to 6 mm; 300 g of soil was retired for chemical analysis and ground to pass a 2 mm sieve. In these samples, molybdate reactive P in bicarbonate and 0.01 M $CaCl_2$ extracts was determined as described above.

Data analysis

Data from high- and low-P samples were analyzed as an unique set of data for each soil. Dry matter yield (DM) for wheat and sunflower was expressed on a relative basis considering the maximum yield the average DM obtained in high-P samples for wheat and sunflower in their first crop. Because Olsen P values were generally above 12 mg kg^{-1} before the first sunflower crop (data not shown), and sunflower is very efficient in extracting P (Delgado and Torrent, 1997), sunflower DM was not restricted; only two soils showed lower Olsen P values before sunflower (6 and 9) which were considered non-limiting due to the high PBC of the soil (Delgado et al. 2010). Threshold values for Olsen P and P_{CaCl2} were estimated according to the Cate and Nelson method (1971). This method provides threshold values for each SPT tested below which the yield significantly decreased from the maximum relative yield defined for each soil, and seems more accurate to define SPT threshold values than other methods such as the linear- or quadratic- plateau fittings or the Mitscherlich-type equations (Mallarino and Blackmer 1992). The critical level for the Cate-Nelson model was estimated as that value of the SPT (P_{CaCl2} or Olsen P) that maximized the sum of squares between two populations of soil SPT (Geng et al. 2013) usingthe ANOVA option of Statgraphics 5.1.

Although estimates of threshold values for P in soil solution (P_{CaCl2}) have been considered unique for one given crop (Delgado et al. 2010; Sánchez-Alcalá et al. 2014), recent studies have revealed that they also differ from one soil to another (Sánchez-Alcalá et al. 2015).

Results and Discussion

The set of soils used in this study ranged widely in properties related to P dynamics (Table 3.1). Regarding phytase hydrolysable P, this can be assumed to be mainly monoesters which can be potentially hydrolyzed by the action of rhizosphere enzymes and can potentially contribute to P uptake by plants. On average, amounts of these monoesters adsorbed or precipitated as soluble metal phosphates were not negligible since they were equivalent to half of the bicarbonate extractable inorganic P in soil (Olsen P, Table 3.1). As expected, the soil PBC was correlated to clay content in soil (Shivani et al. 2005) and the P adsorption capacity (estimated from Fe related to Fe oxides) and negatively correlated with the portion of Fe in oxides ascribed to poorly crystalline oxides (Fe_{ca}/Fe_{d}). Table 3.2 show results for PBC at high solution P concentration, 1 mg l^{-1}). This negative correlation is explained by the usual lower affinity for P of poorly crystalline Fe oxides relative to crystalline oxides (Colombo et al. 1994). Iron in Fe oxides (Fe_{ca} + Fe_{d}) and the Fe_{ca}/Fe_{d} ratio were correlated with clay content (Table 3.2), thus revealing that oxides are part of the clay fraction but also that the ratio of poorly crystalline to crystalline Fe oxides increased with decreased clay content in soil. On the other hand, the Fe in Fe oxides was negatively correlated with the active calcium carbonate equivalent (ACCE), evidencing that mineral weathering and subsequent precipitation of oxides during soil genesis is inhibited when soil pH is buffered by carbonate.

Overall, the Cate Nelson model provides reasonable estimates of threshold values for both STPs (median values for the portion of variance explained was 69 % for Olsen P and 74% for P_{CaCl2} (Table 3.1).Threshold values for Olsen P were in the range usually observed in field experiments (Colomb et al. 2007; Bai et al. 2013) similar to those described by Delgado et al. (2010) in pot experiments using similar amount of soil. Observed threshold values for this SPT ranged widely between soils (Table 3.1). Such a degree of variability in threshold values for Olsen P was observed by Delgado et al. (2010) in a group of clay soils with a narrow range of variation in soil properties. Sánchez-Alcalá et al. (2015) also observed a wide range for Olsen P and P_{CaCl2}threshold values in a group of soils which, in that case, ranged widely in their properties.

Threshold values for both indices were correlated between them; however, that for Olsen P was negatively correlated with clay content, whereas that for P_{CaCl2} was positively correlated with clay (Table 3.2). In addition to clay, soil properties with which Olsen P threshold values were correlated were: pH, PBC, and Fe_{ca} + Fe_d, these correlations being also negative (Table 3.2). Negative correlation with clay and Fe_{ca} + Fe_d reveals that threshold values decreased with increased P sorption capacity of the soil. As in the group of soils studied, clay and Fe oxide content were correlated (Table 3.2), the relative contribution of clay and Fe oxides to the P dynamics is difficult to assess.

P_{CaCl} threshold values were affected, in addition to those properties affecting those for Olsen P, positively by ACCE, Fe_{ca}/Fe_d, and hydrolysable OP in NaOH and CB extracts (Table 3.2). This reveals the potential contribution of OP to P supply to plants as evidenced previously for a similar group of soils by Recena et al. (2015).The effect of the ratio Fe_{ca}/Fe_d on threshold P_{CaCl} can be explained by the difference in affinity for P between poorly crystalline and crystalline Fe oxides (Colombo et al. 1994).

Phosphorus buffer capacity (PBC) seems a crucial property explaining variability in threshold values for Olsen P and P_{CaCl2}. This is in agreement with the current stage of knowledge (Holdford 1980; Bolland et al. 1994; Ehlert et al. 2003). Indeed, the correlation of threshold values with other soil properties such clay or Fe oxide content can be partly explained by the correlation of these properties with PBC (Table 3.2). It should be noted in this respect that the P adsorption capacity of soils depends, between other factors, on clay and Fe in oxides (Fe_{ca} + Fe_d), and that for the same P concentration in solution PBCusually increaseswith increasing P sorption capacity.

As reported by Delgado et al. (2010), PBC seems to account for more variation of the Olsen P threshold values when it is measured at relatively high P concentration in solution (1 mg L^{-1}) rather than at lowor equilibrium concentration (data not shown). Although the correlation of pH withPBC and clay makes it difficult to extract clear-cut conclusions, the effect of pH can be also ascribed not only to its influence on soil P dynamicsbut also by its correlation with other mineralogical properties, such as sorbent surface type, also affecting availability to plants (Delgado and Torrent, 1997). Threshold values for Olsen P can be accurately predicted in our soils from multiple regressions involving different soil properties. Clay content, pH and phosphatase activity in the rhizosphere explained near 90 % of variation in Olsen P threshold values (Figure 3.2a). In addition, clay, soil organic carbon, and the phytase hydrolysable P in the CB fraction explained 75 % of variation in threshold values for Olsen P (Figure 3.2b).

These relationships reveal that not only those factors affecting the equilibrium of inorganic phosphate between the solid and liquid phases of soil, such as P buffer capacity, but also factors related to organic P dynamics, such as the amount of hydrolysable organic P or the phosphatase activity in the rhizosphere,influence the threshold values for a given SPT. Overall, lower threshold values can be expected with increased contents of hydrolysable organic P (Figure 3.2b) due to the contribution of the organic P to plant supply which is not taken into account in usual SPT which are based on the determination of inorganic P (usually molybdate reactive) in soil extracts.

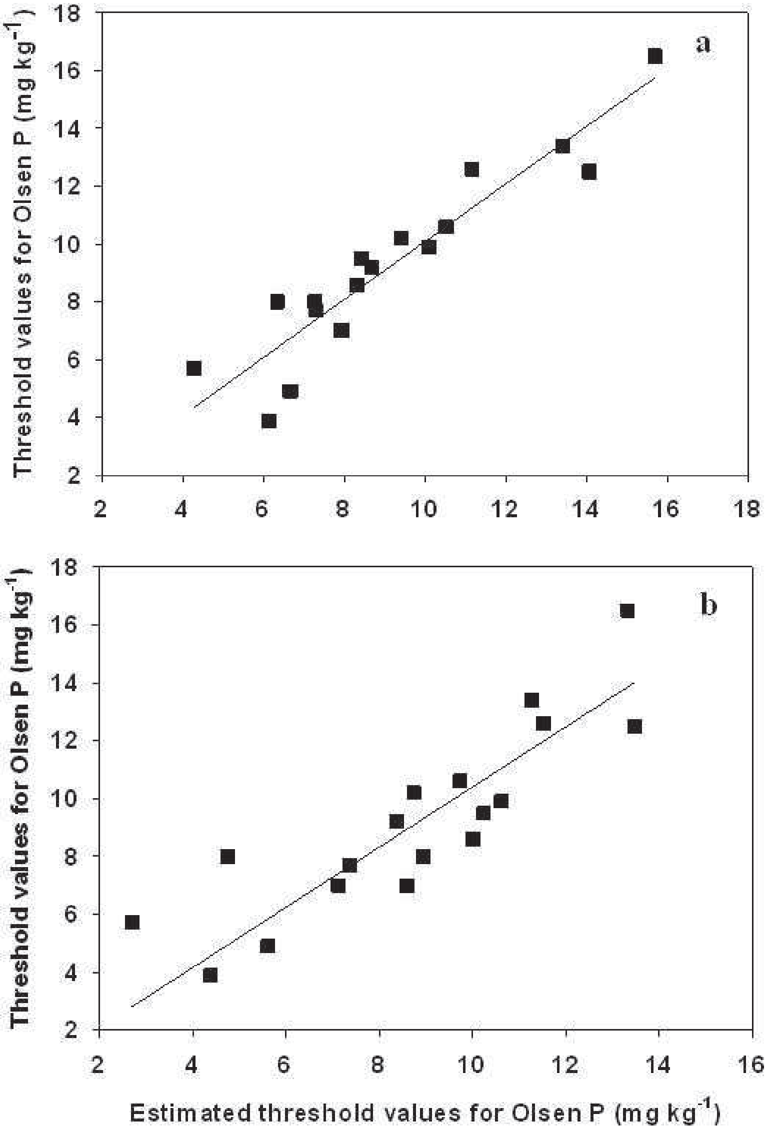


Figure 3.2. Estimation threshold Olsen P values for fertilizer response as a function of: **a)** clay, pH, and mean phosphatase activity (Phosp) in the rhizosphere (for the four crops in high-P samples); *Y = 32 – 0.015 clay – 3.26 pH + 0.05 Phosp;* R^2 *= 0.87, P < 0.001*, and coefficients for each explicative variable significant at P < 0.05 in the least significant case; and **b)** clay, soil organic carbon (SOC) and phytase hydrolysable P in CB extracts of high-P samples (CB_h); *Y = 14 + 2.05 SOC – 0.22 CBh – 0.02 clay*; R^2 *= 0.75, P < 0.001*, and coefficients for each explicative variable significant at P < 0.1 in the least significant case. Threshold value can be estimated also as a function of clay and *pH: 36 – 0.012 clay – 3.02 pH;* R^2 *= 0.81; P < 0.001*; and coefficients for each explicative variable significant at P < 0.01 in the least significant case and mean absolute error = 1.12.

.

In the case of P_{CaCl2}explicative variables not only included clay and pH or pH-related properties such as ACCE, but also the amount of hydrolysable P in NaOH and CB extracts and the Fe_{ca}/Fe_{d} ratio (Figure 3.3). This signals the potential contribution of organic P to the supply of P to plants. In addition, the type of sorbent surfaces was also a relevant property affecting threshold values since the soil affinity for P decreased with increasing content of the poorly crystalline relative to crystalline Fe oxides, consistent with the negative correlation between PBC and Fe_{ca}/Fe_{d}.

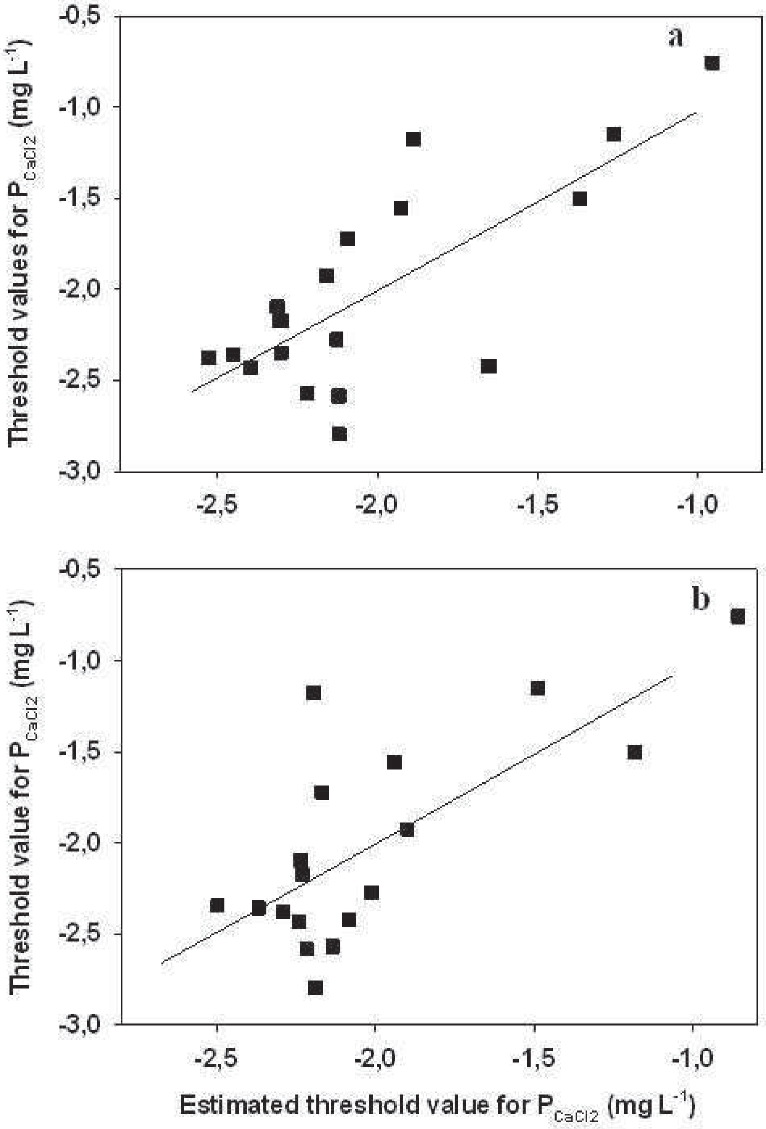


Figure 3.3 Estimation of the logarithm of the threshold P_{CaCl2} values for fertilizer response as a function of: a) clay, and the ratio of Fe_{ca} to Fe_{d}; $Y = -2.02 - 0.0018\ clay + 2.92\ Fe_{ca}/Fe_{d}$; $R^2 = 0.57$, $P < 0.001$; coefficients for each explicative variable significant at $P < 0.05$ in both cases; and b) pH and the sum of phytase hydrolysable in NaOH and CB extracts, $Y = 2.38 - 0.62\ pH + 0.034\ (NaOH_h + CB_h)$; coefficients for each explicative variable significant at $P < 0.05$ in both cases. Threshold value can be also estimated as a function of clay and active Ca carbonate equivalent (ACCE): $Y = -1.13 - 0.039\ ACCE - 0.002\ clay$; $R^2 = 0.56$, $P < 0.01$, and coefficients for each explicative variable significant at $P < 0.05$

The contribution of organic P-related properties to explain threshold values of both SPTs is significant when these properties are the initial (phytase hydrolysable P in NaOH and CB extracts) or mean values (phosphatase) for the for high-P samples but not for the low-P samples (data not shown). Thus, the contribution of organic P to P supply to plants is not necessarily related to starvation in inorganic available P; it seems to be more related to the availability of organic P forms to enzymatic hydrolysis in the rhizosphere as suggested by Recena et al. (2015).

Organic carbon in soil (SOC) also seems to contribute to explain Olsen P threshold values (Figure 3.2b). It is well known that dissolved organic matter affects the equilibrium of P in soil through competition with phosphate for the sorbent surfaces and inhibition of metal phosphate precipitation (Delgado et al. 2002; Saavedra et al. 2007). In addition, increased organic carbon in soils resulting from organic matter addition can promote a decrease in soil P buffer capacity (Sui and Thompson 2000) which also contribute to explain potential effects of SOC on SPT threshold values.

From a practical standpoint,clay content and pH in soil explained 81 % of the variation in the threshold values for Olsen P (Figure 3.2a). This means that single models based on these two soil properties may provide a fairly accurate estimation of Olsen P threshold values in soils from Mediterranean regions. In our study, the estimated mean absolute error in the relationship between threshold values for Olsen P and these two soil properties was 1.1, which in practical terms does not have a relevant economical impact in assessing P fertilization. In practice, the easiest way to translate this type of model is to recommend different threshold values depending on clay content and pH.Clay alone explained 60 % of variation in threshold Olsen P (data not shown). Development of these models should be performed, for practical recommendations, at field scale and for different crops because factors affecting root development and the potential contribution of subsurface horizons are relevant for the supply of P to crops. It should be noted here that the size of the pot may explain why the average threshold value estimated for Olsen P in our study was less than half of the average estimated by Sánchez-Alcalá et al. (2015) using soils with similar properties but micropots of 15 mL.

In summary, factors related with the soil PBCstand as the most relevant to explain the threshold values for both SPTs. However, factors related to organic P dynamics are also influential. In practice, definition of accurate threshold values for Olsen P in soils of Mediterranean regions should profit from two basic soil properties: clay content and pH.

Acknowledgements

This study was funded by the Spanish Ministry of Science and Innovation and the European Regional Development Fund of the European Union through the National Research, Development and Innovation Program (Plan Nacional I+d+i, AGL2011-29893-CO2-01 and AGL2011-29893-CO2-02) and by the Regional Government of Andalucia (Project AGR 63-85). Mr. Ramiro Recena was PhD student with a grant from the Regional Government. Fertiberia S.A. and OCA Lebrija collaborated in the collection of soil samples for the present study.

References

Bai, Z, H Li, X Yang, B m, X Shi, B Wang, D Li, J Shen, Q Chen, and W Qin (2013a) The critical soil P levels for crop yield, soil fertility, and environmental safety in different soil types. Plant Soil 372:27-37. DOI 10.1007/s11104-013-1696-y

Bolland MDA, Wilson IR, Allen DG (1994) Effect of P buffer capacity and P retention index of soils on soil test P, soil test P calibrations and yield response curvature. Aust J Soil Res 32: 503–517. DOI:10.1071/SR9940503

Cate, R B, and Nelson, L A (1971) A simple statistical procedure for partitioning soil test correlation data into two classes. Soil Sci Soc Am J 35(4): 658-660. DOI :10.2136/sssaj1971.03615995003500040048x

Colombo, C, Barrón, V, Torrent, J (1994) Phosphate adsorption and desorption in relation to morphology and crystal properties of synthetic hematites. Geochim Cosmochim Acta 58:1261–1269. DOI: 10.1016/0016-7037(94)90380-8

Colomb, B, Debaeke, P, Jouany, C, and Nolot, J M (2007). Phosphorus management in low input stockless cropping systems: Crop and soil responses to contrasting P regimes in a 36-year experiment in southern France. Eur J Agron 26(2): 154-165. DOI : 10.1016/j.eja.2006.09.004

Cordell, D, and Neset, T S (2014) Phosphorus vulnerability: a qualitative framework for assessing the vulnerability of national and regional food systems to the multi-dimensional stressors of phosphorus scarcity. Glob Environ Chang: 24, 108-122. DOI: 10.1016/j.gloenvcha.2013.11.005

Cordell, D, Drangert, J O, and White, S (2009) The story of phosphorus: global food security and food for thought. Glob Environ Chang: 19(2), 292-305. DOI: 10.1016/j.gloenvcha.2008.10.009

Delgado, A, Torrent, J (1997) Phosphate rich soils in the European Union: estimating total plant-available phosphorus. Eur J Agron 6:205–214. DOI: 10.1016/S1161-0301(96)02048-5

Delgado, A, Madrid, A, Kasem, S, Andreu, L, del Campillo, MC (2002) Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. Plant Soil 245:277–286. DOI: 10.1023/A:1020445710584

Delgado, A, and Scalenghe, R (2008) Aspects of phosphorus transfer from soils in Europe. J. Plan Nutr Soil Sci:171(4), 552-575. DOI: 10.1002/jpln.200625052

Delgado A, del Campillo MC and Torrent J (2010) Limitations of the Olsen method to assess available phosphorus in reclaimed marsh soils. Soil Use Manage 26:133–140. DOI: 10.1111/j.1475-2743.2010.00264.x

Ehlert, P, Morel, C, Fotyma, M, and Destain, J P (2003) Potential role of phosphate buffering capacity of soils in fertilizer management strategies fitted to environmental goals. Plan Nutr Soil Sci: 166(4), 409-415. DOI: 10.1002/jpln.200321182 · Source: OAI

Geng, X, Guillard, K, and Morris, T F (2014) Relating Turfgrass Growth and Quality to Frequently Measured Soil Nitrate. Crop Science: 54(1), 366-382. DOI: 10.2135/cropsci2013.03.0145

Holford, ICR (1980) Effects of phosphate buffer capacity on critical levels and relationships between soil tests and labile phosphate in wheat growing soils. Aust J Soil Res 18:405–414. DOI: 10.1071/SR9800405

Keyzer, M (2010) Towards a closed phosphorus cycle. De Economist: 158(4), 411-425. DOI: 10.1007/s10645-010-9150-5

Kulhánek, M, Balík, J, Černý, J and Nedvědv, KB (2007) The influence of different intensities of phosphorus fertilizing on available phosphorus contents in soils and uptake by plants. Plant Soil Environ 53: 382–387.

Mallarino, A P, and Blackmer, A M (1992) Comparison of methods for determining critical concentrations of soil test phosphorus for corn. Agronomy Journal: 84(5), 850-856. DOI: 10.2134/agronj1992.00021962008400050017x

Murphy, J, Riley, JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27: 31–36. DOI:10.1016/S0003-2670(00)88444-5

Olsen, SR, Cole, CV, Watanabe, FS, Dean, LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. US Government Printing Office, Washington DC.

Ryan J, Ibrikci H, Delgado A, Torrent J, Sommer R, Rashid A (2012) Significance of Phosphorus for Agriculture and the Environment in the West Asia and North Africa Region. Adv Agron 114:91–153. DOI: 10.1016/B978-0-12-394275-3.00004-3

Recena, R, Torrent, J, del Campillo, M C, and Delgado, A (2015) Accuracy of Olsen P to assess plant P uptake in relation to soil properties and P forms. Agron Sustainable Dev: 35(4), 1571-1579. DOI: 10.1007/s13593-015-0332-z

Saavedra, C, Velasco, J, Pajuelo, P, Perea, F, Delgado, A (2007) Effects oftillage on phosphorus release potential in a SpanishVertisol. Soil Sci Soc Am J 71:56–63. DOI: 10.2136/sssaj2006.0162

Sánchez-Alcalá, I, del Campillo, MC, Barrón, V, Torrent, J (2014) The Olsen P/solution P relationship as affected by soil properties. Soil Use Manage 30:454–462. DOI: 10.1111/sum.12141

Sánchez-Alcalá, I, Campillo, MC, and Torrent, J (2015) Critical Olsen P and CaCl2-P levels related to soil properties results rommicropot experiments. Soil Use Manage. DOI: 10.1111/sum.12184

Santner, J, Mannel, M, Burrell, L D, Hoefer, C, Kreuzeder, A, and Wenzel, W W (2015)

Phosphorus uptake by Zea mays L. is quantitatively predicted by infinite sink extraction of soil P. Plant soil :386(1-2), 371-383. DOI: 10.1007/s11104-014-2271-x

Schröder, J J, Smit, A L, Cordell, D, and Rosemarin, A (2011) Improved phosphorus use efficiency in agriculture: A key requirement for its sustainable use. Chemosphere: 84(6), 822-831. DOI: 10.1016/j.chemosphere.2011.01.065

Shirvani, M, Shariatmadari, H, and Kalbasi, M (2005) Phosphorus buffering capacity indices as related to soil properties and plant uptake. J. Plant Nutr: 28(3), 537-550. DOI: 10.1081/PLN-200049235

Soil Survey Staff (2010) Keys to soil taxonomy, 11th edn. USDA-Natural Resources Conservation Service, Washington, DC.

Sui, Y, and Thompson, M L (2000) Phosphorus sorption, desorption, and buffering capacity in a biosolids-amended mollisol. Soil Sci Soc Am J. 64(1), 164-169. DOI: 10.2136/sssaj2000.641164x

Tandy, S, Mundus, S, Yngvesson, J, de Bang, TC, Lombi, E, Schjoerring, JK and Husted, S (2011) The use of DGT for prediction of plant available copper, zinc and phosphorus in agricultural soils. Plant Soil 346: 167–180. DOI: 10.1007/s11104-011-0806-y

Tang, X, Ma, Y, Hao, X, Li, X, Li, J, Huang, S, and Yang, X (2009) Determining critical values of soil Olsen-P for maize and winter wheat from long-term experiments in China. Plant Soil: 323(1-2), 143-151. DOI: 10.1007/s11104-009-9919-y

Van Vuuren, D P, Bouwman, A F, and Beusen, A H W (2010) Phosphorus demand for the 1970–2100 period: a scenario analysis of resource depletion. Glob Environ Chang: 20(3), 428-439. DOI: 10.1016/j.gloenvcha.2010.04.004

Withers, P J, Neal, C, Jarvie, H P, and Doody, D G (2014) Agriculture and eutrophication: where do we go from here?. Sustainability: 6(9), 5853-5875. DOI: 10.3390/su6095853

Figure captions

Figure 3.1 Pot starvation experiment: (a) sunflower in growing chamber with incipient P deficiency symptoms (b) and clear deficiency symptoms (c); and wheat (d) with P deficiency symptoms (e).

Figure 3.2. Estimation threshold Olsen P values for fertilizer response as a function of: a) clay, pH, and mean phosphatase activity (Phosp) in the rhizosphere (for the four crops in high-P samples); $Y = 32 – 0.015\ clay – 3.26\ pH + 0.05\ Phosp; R^2 = 0.87, P < 0.001$, and coefficients for each explicative variable significant at $P < 0.05$ in the least significant case; and b) clay, soil organic carbon (SOC) and phytase hydrolysable P in CB extracts of high-P samples (CB_h); $Y = 14 + 2.05\ SOC – 0.22\ CBh – 0.02\ clay$; $R^2 = 0.75, P < 0.001$, and coefficients for each explicative variable significant at $P < 0.1$ in the least significant case. Threshold value can be estimated also as a function of clay and $pH: 36 – 0.012\ clay – 3.02\ pH; R^2 = 0.81; P < 0.001$; and coefficients for each explicative variable significant at $P < 0.01$ in the least significant case and mean absolute error = 1.12.

Figure 3.3. Estimation of the logarithm of the threshold P_{CaCl2} values for fertilizer response as a function of: a) clay, and the ratio of Fe_{ca} to Fe_d; $Y = – 2.02 – 0.0018\ clay + 2.92\ Fe_{ca}/Fe_d; R^2 = 0.57, P < 0.001$; coefficients for each explicative variable significant at $P < 0.05$ in both cases; and b) pH and the sum of phytase hydrolysable in NaOH and CB extracts, $Y = 2.38 – 0.62\ pH + 0.034\ (NaOH_h + CB_h)$; coefficients for each explicative variable significant at $P < 0.05$ in both cases. Threshold value can be also estimated as a function of clay and active Ca carbonate equivalent (ACCE): $Y = – 1.13 – 0.039\ ACCE – 0.002\ clay; R^2 = 0.56, P < 0.01$, and coefficients for each explicative variable significant at $P < 0.05$.

Chapter IV

In the fourth chapter of this PhD different methods to **accurately estimate plant available P in soi**l. P sinks (anion exchange resins) and single chemical extractions, including Olsen P, will be use to this end.

Estimation of total plant available phosphorus in representative Mediterranean soils

Estimation of total plant available phosphorus in representative Mediterranean soils

Abstract

Usual soil P tests, as the widely used Olsen P, may not accurately assess plant-available P in soils ranging widely in properties. The objectives of this work were: (i) the study the accuracy of Olsen P in assessing available P to plants; (ii) the development of more precise estimations based on Olsen P, and (iii) testing other extraction methods to assess phytoavailable P, in a representative group of soils from Mediterranean areas. To achieve these objectives, a P depletion experiment was conducted using 18 soils to estimate total available P to plants in soil (TAP). Also, different P extractions were performed to check their accuracy in predicting TAP: Olsen P, 0.27 M Na citrate + 0.11 M $NaHCO_3$ (CB), 0.1 M NaOH and CB sequentially (NaOH + CB), anion exchange resins (AER) in Cl^- and HCO^-_3 forms, and 0.01 M $CaCl_2$.

Poor predictions of TAP with Olsen P ($R^2 = 0.53$, $P < 0.001$) were improved by including P buffer capacity (PBC at 1 mg L^{-1}) and the portion of Fe in oxides ascribed to poorly crystalline ones in the regressions. Best predictions of TAP based on Olsen P were achieved when clay and carbonates were taken into account in a predictive model ($R^2 = 0.8$, $P < 0.001$). The best estimations of TAP were obtained with AERs, AER membrane in HCO_3^- form ($R^2 = 0.87$, $P < 0.001$) performing better than in Cl^- form ($R^2 = 0.77$, $P < 0.001$). When potential organic P inputs to available P, estimated as phytase hydrolysable P in NaOH and CB extracts, were taken into account, variance of TAP explained by Cl-AER increased to 86 %. Better results obtained with HCO_3-AER can be explained because this extraction seems to be better related to most of the factors affecting the equilibrium of inorganic P between solid and liquid phases in soils and consequently affecting P availability to plants. Accurate estimations of TAP are feasible with Olsen P if clay and carbonates are included in the model.

Key words: soil P test, Olsen P, resin extractable, organic P, buffer capacity, Fe oxides

Abbreviations: AER, anion exchange resin; ca, citrate-ascorbate; cbd, citrate-bicarbonate-dithionite; SOM, soil organic matter; CCE, calcium carbonate equivalent; PBC, P buffer capacity of soil; SPT, soil P test

Introduction

Phosphorus is a needful nutrient for crops, and thus crucial for food production (Carpenter and Bennet, 2011), which has been excessively applied during almost the latter half of the past century in many agricultural lands (Lemercier et al., 2008; Bouwman et al., 2009; Bai et al., 2013). The consequent P enrichment of agricultural soils led to relevant environmental problems in many areas of the World (Carpenter, 2008; Vadas et al., 2009). On the other hand, P is a non-renewable and strategic resource whose production is expected to peak by middle of the century (Cordell et al. 2009) and which is inefficiently used in agriculture (Schröder et al., 2011). A more efficient use of P in agriculture is mandatory not only to overcome the consequences of progressive depletion of P rock reserves on agricultural production, but also to reduce the environmental implications of imbalanced P applications to agricultural land (Colomb et al., 2007; Jordan-Meille et al., 2012; Ryan et al., 2012). This efficient and rational P management should rely on more precise prediction of available P to plants in soil (Menzies et al., 2005; Tandy et al., 2011; Jordan-Meille et al., 2012).

Soils retain applied P through adsorption and precipitation processes and slowly release only a fraction of the accumulated P as P available to plants (Toth et al., 2014). In a group of representative European soils, Delgado and Torrent (1997) observed that available P pool ranged between 10 and 70 % of the total soil P, the lowest percentages being observed for highly P-fixing calcareous soils. Plants may use inorganic P from the solid phase and P mineralized from organic P compounds (Zaidi et al., 2013; Recena et al., 2015). The fraction of total soil P available to the plants depends mainly on texture, pH, mineralogy and soil organic matter, which are properties affecting P reactions and dynamics in soil (Cross and Schlesinger, 1995; Green et al., 2005; Messiga et al., 2011). In addition, biological processes in rhizosphere such as organic acids and hydrolytic enzyme exudates by plants and microorganisms affect P mobilization and uptake by plants (Richardson et al., 2009; Lambers et al., 2010; García-López et al., 2015). Thus, soil P availability should be considered the result, not only of P accumulation, but also the consequence of complex physical, chemical, and biological interactions at the rhizosphere.

Available P to plants is usually assessed by means of P-availability tests or soil P tests (SPTs), i.e., chemical extractions able to predict when a response to P fertilization can be expected rather than provide a precise estimation of the quantity of soil P available to plants (Tang et al., 2009). To this end, good correlations between chemical extractions and P uptake by plants are necessary.

Phosphorus availability to plants is mainly ruled by the concentration of P in the soil solution (‘intensity’ factor, *I*), the amount of P in the solid phase that can be easily made available to plants (‘quantity’ factor, *Q*), and the capacity of soil to keep P concentration in soil solution (P buffer capacity, PBC) (Sánchez et al., 2014). Usual SPTs extract amounts of P from soils which are deemed estimates of either *I* (e.g. P concentration in soil solution or in a low molarity salt soil extracts; Schoenau and Huang, 1991; Van Raij, 1998) or *Q*, or a *Q*-related variable (e.g. classical chemical extractions such as Olsen P; Olsen et al. ,1954), depending on the particular test (Sánchez et al., 2015). However, the usefulness of SPT in routine soil analyses is limited by the fact that a single measurement cannot encompass all P-related factors potentially affecting plant performance (Sánchez-Alcalá et al., 2015). Thus, P-availability estimates based on estimates of PBC and *Q* (Delgado et al., 2010), or *I* and *Q* (van Rotterdam et al., 2012) have proved more effective in assessing available P to plants in soil (Sánchez et al., 2015).

In addition, SPTs related to the *Q* factor may not extract rapidly all the available P compounds in soil (Menon et al. 1989; Six et al., 2012), the amounts extracted depending on the chemical nature of the extractant, the dominant P forms, and soil properties (Delgado and Scalenghe, 2008). This latter point constrains the use of one given SPT in different types of soils explaining the wide variety of official SPTs used for assessing P fertilization in different countries (Neyroud and Lischer, 2003; Delgado and Scalenghe, 2008; Jordan-Meille et al. 2012). Only the use of near-infinite sinks, such as Fe oxides or anion-exchange resins, has proved to estimate total available P to plants in soil ranging widely in soil properties (Delgado and Torrent, 1997). This may be explained because sinks remove P from the solution displacing the equilibrium from soil solid phase into the soil solution (Fernandes and Coutinho, 1997) avoiding chemical reactions that solubilise P (e.g., by use of acids) and mimicking the functioning of plant roots (Abdu, 2006). In addition, these extractions have proved to be sensitive not only to *Q*, but also to PBC, which is crucial affecting P availability to plants (Ehlert et al. 2003; Celardin 2003), explaining their better results when compared with classical test such as Olsen P (Delgado et al. 2010).

Although widely used in many areas of the World, such as in Mediterranean countries, Olsen P has limited usefulness in precise assessment of plant available P (Delgado et al., 2010; Tandy et al., 2011; Sánchez-Alcalá et al. 2014; 2015). Deeper knowledge on the reasons affecting its lack of accuracy, and the consequent proposal of models based on this STP to accurately estimate plant available P, and more accurate single P extractions methods in assessing available P in soils are required to achieve more efficient P fertilization practices (Recena et al. 2015). This work is thus aimed at: (i) the study of soil properties affecting the accuracy of Olsen P in assessing available P to plants; (ii) development of more precise estimations based on Olsen P, and (iii) testing other single and easily performable extraction methods to assess phytoavailable P in a representative group of soils from Mediterranean areas.

Materials and Methods

Soils

The group of soils selected for the present study is the same as that used by Recena et al., (2016). The soils came from different agricultural areas of Spain, encompassing the most representative orders under Mediterranean climate according to Soil Taxonomy (Soil Survey Staff, 2010): Mollisols, Entisols, Inceptisols, Alfisols, and Vertisols. Most of these soils were under the typical rainfed crop rotation in Spain, i.e., wheat-sunflower. In each selected location, soils were sampled at 20 cm depth, obtaining a collection of soil samples ranging widely in Olsen P values. From this collection, those soil samples with the two extreme Olsen P values from each site were selected and named “low-P” and “high-P” samples according to their P status. For the P starvation experiment described below, soil was ground to < 6 mm, and for soil analysis, part of the soil was ground to < 2 mm. The “high P” and “low P” samples were examined in order to check that they differed by less than 5% in the basic soil properties.

Soil analysis

Soils were analysed for particle size distribution according to Gee and Bauder (1986), organic matter (SOM) by the oxidation method of Walkley and Black (1934), total $CaCO_3$ equivalent (CCE) by the calcimeter method, and pH in water at a soil:extractant ratio of 1:2.5. The sequential chemical fractionation of de Santiago and Delgado (2006) was used to measure the Fe present in Fe oxides: citrate-ascorbate to release Fe in poorly crystalline Fe oxides (Fe_{ca}), and citrate-bicarbonate-dithionite to release Fe in crystalline oxides (Fe_{cbd}). In these extracts, Fe was determined by atomic absorption spectrometry.

Total inorganic P (Pi) was determined as that extractable with 0.5 M H_2SO_4 (Kuo 1996). Total P (TP) was determined following dry combustion at 550 ^oC for 2 h and extraction with 0.5 M H_2SO_4 (Kuo 1996). In both acid extracts for Pi and TP determination, P was determined using the molybdenum blue colorimetric method of Murphy and Riley (1962). Total organic P (OP) was estimated as the difference between TP and Pi (Kuo 1996).

Sorption curves at 6 d in 0.01 M $CaCl_2$ were performed according to the method of Fox and Kamprath (1970). Sorption curves were fitted to the Freundlich equation:

$$Y = A\,X^{1/b} \qquad [1]$$

Where the sorbed P by soil (*Y*) is expressed as a multiplicative function of the remaining P concentration in solution (*X*), and *b* can be considered the affinity factor, in such a way that soil affinity P increases with increasing *b* values. The P buffer capacity (PBC) was estimated as the slope of the sorption curve at equilibrium concentration, and at 0.01, 0.02, 0.2 and 1 mg L^{-1} (PBC_1). The Langmuir model was used to estimate the maximum P sorption capacity (*Xm*).

Phosphorus extractions in soil

Different P extraction methods were used to assess their ability to estimate total available P to plants (TAP). Olsen P was determined according to Olsen et al. (1954), replacing filtration through active carbon by centrifugation at 1000 *g* for 10 min. An extraction with 0.27 M Na citrate + 0.11 M $NaHCO_3$ (CB) was performed using 1 g of soil in 40 mL of extractant at 1, 3 and 24 hours in polyethylene centrifuge tubes. The two first sequential extractions in the Ruíz et al. (1997) fractionation scheme were performed, involving first 0.1 M NaOH + 1 M NaCl, and second citrate-bicarbonate, as described above, as second step, both during 17 hours. In both steps, the phytase hydrolysable P was determined as described by Recena et al. (2016). In all these chemical extractions, P was determined by the colorimetric method of Murphy and Riley (1962).

Extraction by near-infinite sinks was performed by using two types of anion exchange resins (AER). Extraction with AER in Cl^- form ($P_{Cl\text{-}AER}$) was performed as described by Saavedra et al. (2007) using 2.2 g of Dowex 1 x 4-50 (Sigma, Barcelona, Spain) in nylon bags settled in 50 mL flask with 40 mL of deionized water and 1 g of soil. Resin bags were removed at 1, 3, 6 and 24 h, and replaced by a regenerated one.

Before use, resins were regenerated with a 30 min wash in 50 mL of 0.5 M NaCl at pH 6 shaking at 60 rpm in a reciprocating shaker and then washed twice in deionized water to remove the excess of NaCl. Elution of P adsorbed by resins was done with two washes with 50 mL of 0.5 M HCl during 30 min. Both eluates were collected in 100 mL volumetric flask from where an aliquot was taken to determine P concentration by the method of Murphy and Riley (1962).

Another anion exchange adsorption extraction was carried out following a modification of the method of Fernandez and Coutinho (1997) using AER membranes in HCO_3^- form ($P_{HCO3\text{-}AER}$). This AER membrane (size 125x125 mm, 551642S, BDH-Prolabo, VWR International, Barcelona, Spain) used in this study has been widely used in other previous work (Fernández and Coutiño, 1997; Turner and Romero, 2009; Cheesman et al. 2010). AER membranes are polystyrene–divinylbenzene copolymer base doped with quaternary ammonium as the ionogenic group and preloaded with Cl^- counterions (Cheesman et al. 2010). Membranes were cut to 7.75 cm^2 of surface, and, in order to use in bicarbonate form, regenerated with two 30 min washes in 50 mL of 0.5 M $NaHCO_3$ at 1 s^{-1} in an end-over-end shaker. Water washing was done as for Cl-AER and pH was not adjusted. Extraction was performed placing 2 g of soil in 40 mL deionized water during 16 hours in 50 mL polyethylene centrifuge tubes. Dimension of membranes used did not allow its movement in the centrifuge tube. The elution of P adsorbed by resin was done with two washes with 40 mL 0.5 M HCl during 30 min and collecting both eluates in 100 mL volumetric flasks. P concentration was determined as for Cl-AER.

As an estimate of the P in soil solution (factor *I*), extraction with 0.01 M $CaCl_2$ according to Sanchez-Alcalá et al. (2015) was performed at a soil:extractant ratio of 1:10 (2 g of soil in 20 ml of extractant) in polyethylene falcon tubes that were end-over-end shaken at 2.5 s^{-1} for 30 min. The extract was centrifuged at 1000 g for 10 min, and then a portion of 2 mL centrifuged in Eppendorf tubes at 19000 g to remove particles with diameter > 0.05 microns. In the remaining solution, molybdate reactive P was determined according to Murphy and Riley (1962).

All the extractions were performed in duplicate, and data from high-P samples considered for assessing TAP.

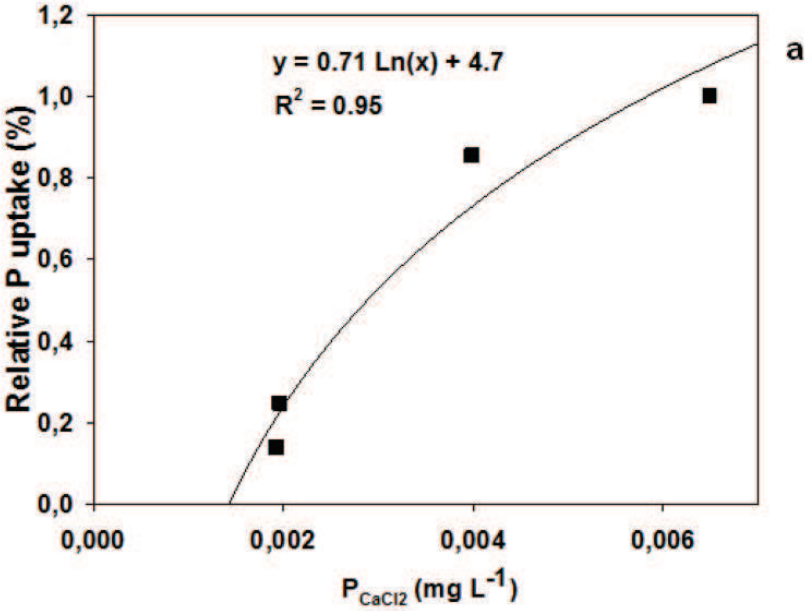
Phosphorus depletion experiment

The experiment was conducted in a growth chamber by growing successively durum wheat and sunflower on both high- and low-P samples. This rotation, typical in rainfed areas of Spain, was repeated twice, so four soil depletion stages were achieved for each low-P and high-P sample. Seeds were pre-germinated on moistened Petri plate in refrigerator for 14 days in trays with perlite as a substrate. Seedlings were transplanted after cleaning the roots with water in 350 mL (5.5 cm diameter, 15 cm-high) filled with 300 g of soil (< 6 mm). For each soil, the number of replications was from five in the first depletion step to two replications in the fourth, since 300 g of soil was removed after each stage for laboratory analysis. Irrigation was done daily with 20 mL of a Hoagland type nutrient solution without phosphorus at pH 6-6.5. After three irrigations with the nutrient solution, one irrigation with the same volume of deionized water was applied to avoid soil salinization. Nutrient concentration (mmol L^{-1}) in the fertigation solution was: $MgSO_4$ (2), $Ca(NO_3)_2$ (5), KNO_3 (5), KH_2PO_4 (1), KCl (0.05), Fe-EDDHA (0.01), H_3BO_3 (0.009), $MnCl_2$ (0.0023), $CuSO_4$ (0.0005), $ZnSO_4$ (0.002), and H_2MoO_4 (0.0005). Plants were grown with a 14 h photoperiod, a relative humidity of 45% (day period) and 60% (night period), and an active photosynthetic radiation of 22 W m^{-2}.

After each depletion step, P in 0.01 M $CaCl_2$ extracts and Olsen P was determined in soil as described above. Plants were harvested and roots carefully separated from soil. Aerial parts and roots were separated, washed, and dried at 65 ^{o}C in forced air oven during 48 h. After drying, biomass in aerial parts and roots was determined. Phosphorus concentration in the dried plant material was determined colorimetrically according to Murphy and Riley (1962) after combustion at 550 ^{o}C during 8 h and dissolving ashes with 1 M HCl. P uptake by plants in each depletion step was estimated as the total P present in aerial parts and roots minus the amount present in seeds.

Estimation of total available P to plants (TAP) in soil

Data from high- and low-P samples were analyzed as an unique set of data for each soil. Cumulative P uptake for each soil was related to P concentration in 0.01 M $CaCl_2$ extracts after each depletion step by means of a second-order polynomic function (an example is given in Figure 4.1). To this end, data of a low-P sample was considered as a continuation of the dataset of the high-P sample for the same soil. This was acceptable because P in $CaCl_2$ extracts after the first depletion step in low-P samples was always lower than P concentration in the same extract for the last (fourth) depletion step in the high-P sample. The use of high- and low-P samples in the same dataset allowed us to have a depletion range from a P level clearly above threshold values for fertilizer response to a P level clearly insufficient for proper plant development, and to have enough data to estimate total plant available P for the two crops involved in the study.



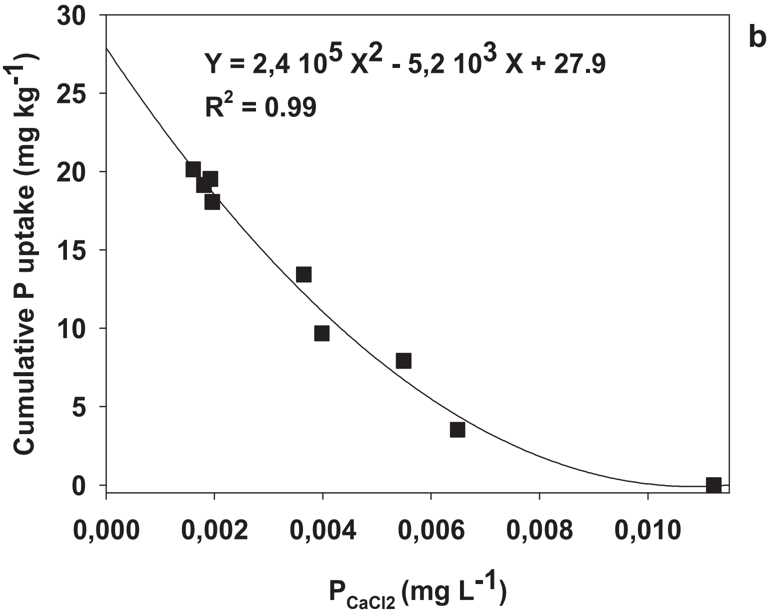


Figure 4.1. Example of estimation of P_{ClCa2} for null P uptake (threshold value for P uptake) and for estimation of total plant available P from the cumulative uptake curve in one of the soils studied. Relative P uptake for sunflower is represented, and it was estimated as the ratio of P uptake in each step to P extraction in the first depletion step (a). Cumulative P uptake for the soils in the 8 depletion steps considered (4 for high-P and 4 for low-P samples) (b). P_{CaCl2} is the concentration of P in 0.01 M $CaCl_2$

In addition, for each soil and crop, using both low- and high-P data, P uptake in each depletion step was logarithmically related to P concentration in 0.01 M $CaCl_2$ extracts. The interception of this function with X-axis provides an estimate of the concentration in the mentioned extracts at which 0 uptake can be expected (example in Figure 4.1). Extrapolation of P uptake to this concentration in the cumulative P uptake curve was considered the estimate total available P to plants in soil (TAP).

The cumulative P uptake till a given final P concentration below which response to P fertilization can be expected (e.g. at 0.02 mg L^{-1}, Delgado et al. 2010) has been considered in several works as the amount of available P in soil (Delgado and Torrent 1997; Delgado et al. 2010). However, in our case, the critical P concentration in the proxy of solution P (P in $CaCl_2$ extracts) was not an unique value for different soils, and depended on the particular soil.

Statistics analysis

Correlation and regressions were performed to assess relationships between variables studied. In the regressions, the Durbin-Watson test was performed to assess correlations of residuals depending on the order of data. Non-typical residuals were assessed by student transformation of residuals, in order to check differences between observed values and estimated values. Influential points were assessed by the Laverage test, in such a way that in case of values with 5 times the mean influence, the regression model was estimated without this value to assess if there is a significant change in the model when the point was avoided. In multiple regressions, all the variables included were significant at $P < 0.05$. In the case of non-normal distribution of data, regressions were also performed with transformed data. All statistical tests were performed with the software Statgraphics 5.1. (Statpoint 2000).

Results

The set of soils used in this study widely varied in properties (Table 4.1), especially in carbonates, Fe in oxides, either poorly crystalline Fe oxides (Fe_{ca}) or highly crystalline oxides (Fe_{cbd}), and clay content. Also, a wide range of parameters related to P sorption dynamics, such as affinity factor (*b*) and P maximum sorption capacity (*Xm*), was observed. Soils also showed very different concentrations of total organic P (OP) and total inorganic P (Pi), the latter reflecting the different fertilization history in soils which explained the wide range of Olsen P values observed, which varied from 9 to 49 mg kg^{-1} in high-P soil samples, with an average of 24 mg kg^{-1}. Amounts extracted by other chemical extractants were usually higher than Olsen P. Single extraction with citrate-bicarbonate (P_{CB}) yielded extracted amounts from 22 to 318 mg kg^{-1}, with an average of 67 (Table 4.2). Sequentially extracted amounts with NaOH and CB ($P_{NaOH+CB}$) according to the fractionation scheme proposed by Ruiz et al. (1997) were greater than the single CB extraction, ranging from 53 to 376 mg kg^{-1}, with an average of 118 mg kg^{-1}. Phosphorus extracted with Cl-AER at 1 hour and HCO_3-AER amounted to similar values (respective mean values of 7.3 and 6.8 mg kg^{-1}), overall smaller than the values for other chemical extractions (Table 4.2). Among all the potential P indexes studied, P concentration in $CaCl_2$ was, in relative terms, the most widely ranging (0.002 to 0.771 mg L^{-1}; median = 0.019 mg L^{-1}).

Table 4.1. Mean, standard deviation (SD) and range of the properties of studied soils (n = 18)

	Clay	**SOM**	**CCE**	**Fe$_{ca}$**	**Fe$_d$**	**pH**	**Organic P**	**Inorg. P**	**Olsen P**	***A***	***b***	**BC$_1$**	**Xm**
	g kg^{-1}						mg kg^{-1}						mg kg^{-1}
Mean	288	10.6	270	1.02	5.52	7.8	131	340	24	72	2.22	32	204
SD	155	3.8	234	0.74	3.05	0.5	75	172	10	32	0.31	14	137
Range	48 - 640	5.5 - 20	0 - 723	0.17 - 2.44	2.04 - 13.24	6.5 – 8.3	43 – 319	122 – 753	9 – 49	16 – 140	1.52 -2.64	8.5-67	33 – 455
Median	260	10	240	0.82	4.7	7.9	116	299	21	73	2.24	31.8	154

Subindex ca, citrate-ascorbate extractable; d, citrate-bicarbonate-dithionite extractable
SOM, soil organic matter, CCE, calcium carbonate equivalent
Organig P, inorganiP, Olsen P values, *A* and *b*, parameters of the Freundlich sorption equation; BC$_1$; P buffer capacity estimated using Freundlich fitting at 1 mg
maximum sorption capacity according to the Langmuir equation. All these values reflect the initial values in high-P samples

Table 4.2. Mean, standard deviation (SD) and range of the quantity and intensity indexes of studied soils (n = 18)

	P_{ow}	P_{os}	TAPw	TAPs	P_{CaCl2}	$P_{HCO3\text{-}res}$	$P_{Cl\text{-}res}$	$P_{h(NaOH\ +\ CB)}$	$P_{NaOH\ +\ CB}$	P_{CB}
	——— μg L^{-1}———		———— mg kg^{-1} ———		mg L^{-1}	———————— mg kg^{-1} ————————				
Mean	7.3	4.6	29.4	31.3	0.103	7.3	6.8	12.3	117	67
SD	12	7	19	20	0.2	8.5	5.9	7.1	78	66
Range	0.032 - 44.7	0.05 – 27	7.5 - 80	9.9 – 83.3	0.002 – 0.771	1.7 – 33.5	2.3 – 27.5	3.3 – 35.2	53 – 376	22 – 318
Median	1.9	1.6	26.7	27.3	0.019	3.5	4.7	12.4	91	47

P_{0w} and P_{0s}, estimated P concentration in $CaCl_2$ extracts for which the P uptake by plants is zero. TAP, total available P to plants estimated by extrapolation of the relationship of the cumulative P uptake as a function of the P in $CaCl_2$ extracts to P exhaustion levels (P_0 values for each crop)

Ssubindex w and s, data for wheat and sunflower, respectively; Cl-res, P extracted with resins in chloride form duirng 1 h; HCO_3-res, P extracted with resin membranes in bicarbonate form; $CaCl_2$-P, P extracted in 0.01 M $CaCl_2$; NaOH+CB, P extracted with NaOH + P extracted with citrabe-bicarbonate according to the fractionation scheme of Ruiz et al. (1998); h(NaOH+CB), P hydrolysable with phytases in the former extracts; CB, P extracted with citrate-bicarbonate during 1 h

Phosphorus concentration in the soil solution proxy (i.e., the 0.01 M $CaCl_2$ extract) at which a null P uptake by the plants is expected (threshold values for P uptake) also ranged widely between soils (Table 4.2). These values were lower for sunflower than for wheat, thus evidencing the higher P efficiency of the former crop (Table 4.2). This estimate of the threshold P concentration in solution for P uptake was negatively correlated with the buffer capacity of soils at 1 mg L^{-1}; $r = -0.56$ and -0.5 for wheat and sunflower, respectively; $P < 0.05$ in both cases) and clay ($r = -0.53$ and -0.49 for wheat and sunflower, respectively; $P < 0.05$ in both cases). However, this estimate of the threshold P concentration in solution for P uptake was not correlated with PBC at lower concentrations than 1 mg L^{-1} (data not shown). In addition, it was , and positively correlated with the Fe_{ca} to Fe_{cbd} ratio ($r = 0.62$ and 0.61 for wheat and sunflower, respectively; $P < 0.01$ in both cases). The best estimate of these threshold values of P_{CaCl2} (mean value for both crops) were achieved by a multiple regression using soil organic matter (SOM), clay and Fe_{ca}/Fe_{cbd} ($Y = 0.01\ SOM - 4 \cdot 10^{-5}\ Clay + 0.07\ Fe_{ca}/Fe_{cbd}$; $R^2 = 0.71$; $P < 0.001$).

The estimation of total available P to plants (TAP) ranged widely between soils, the average and the range being similar for both crops, but with greater values for sunflower in soils with low TAP (Table 4.2). Mean TAP for both crops accounted for around 6 % of total P in soils, and for 10 % of inorganic P. Overall, mean TAP for both plants was significantly correlated with all the tests for extractable P (Table 4.3), the best correlations being found for the near-infinite sink extractants, and particularly for resin membranes in bicarbonate form ($r = 0.93$, $P < 0.001$), and the worst for P_{CB} (Table 4.3). Estimation of the intensity factor (P_{CaCl2}) performed worse than estimations of quantity factors in predicting TAP. Regarding soil properties, TAP was negatively correlated with PBC_1, clay, carbonates and pH, and positively correlated with Fe_{ca}/Fe_{cbd}. The only estimation of PBC to which TAP was correlated (negatively) was that at 1 mg L^{-1} (Table 4.3; data for other estimates of BC not shown). The P adsorption capacity, estimated by the sum of Fe in oxides, or by *Xm* according to the Langmuir model was not correlated with TAP. However, TAP was negatively correlated with the adsorption at 1 mg L^{-1} according to the Freundlich model (*A*; Table 4.3). Although similar trends in correlations were observed for all the P extraction methods tested as SPT, P_{CaCl2} and P extracted with HCO_3-AER were the only P extractions correlated with the same soil properties than TAP (Table 4.3).

Table 4.3. Correlation between total available P to plants in soil (TAP), different P extractions used as soil availability index, and different soil properties

	Olsen-P	$P_{Cl\text{-}res}$	Inc Cl-res	$P_{HCO3\text{-}res}$	P_{CaCl2}	P_{CB}	$P_{NaOH+CB}$	$P_{(NaOHh+CBh)}$	PBC_1	Fe_{ca}/Fe_{cbd}	$Fe_{ca}+Fe_{cbd}$	clay	A	CCE	pH	Xm
TAP	0.73***	0.88***	0.76***	0.93***	0.65**	0.49*	0.71**	0.47ns	– 0.67**	0.68**	0.07ns	– 0.59*	– 0.51*	– 0.59*	– 0.68**	0.1ns
Olsen P		0.79***	0.72***	0.78***	0.65**	0.79***	0.81***	0.45ns	– 0.53*	0.43ns	0.03ns	– 0.48*	– 0.65**	– 0.29ns	– 0.53*	0.02ns
$P_{Cl\text{-}res}$			0.94***	0.93***	0.73**	0.92***	0.89***	0.21ns	– 0.61**	0.53*	0.17ns	– 0.54*	– 0.54*	– 0.54*	– 0.67**	0.24ns
Inc Cl-res				0.86***	0.76***	0.96***	0.86***	0.03ns	– 0.52*	0.72**	0.21ns	–0.41ns	– 0.45ns	– 0.68**	– 0.57*	0.46ns
$P_{HCO3\text{-}res}$					0.88***	0.78***	0.75***	0.48*	– 0.65**	0.77***	0.08ns	– 0.58*	– 0.55*	– 0.58*	–0.75***	0.65**
P_{CaCl2}						0.48*	0.46ns	0.43ns	– 0.58*	0.76***	– 0.08ns	– 0.55*	– 0.48*	– 0.49*	– 0.62**	0.57*
P_{CB}							0.93***	0.02ns	– 0.56*	0.32ns	0.04ns	– 0.44ns	– 0.55*	– 0.28ns	– 0.52*	–0.2ns
$P_{NaOH+CB}$								0.00ns	– 0.54*	0.34ns	0.10ns	– 0.43ns	– 0.53*	– 0.35ns	– 0.57*	0.05ns
$P_{NaOH+CBh}$									– 0.42ns	0.55*	– 0.32ns	– 0.53*	– 0.43ns	– 0.18ns	– 0.22ns	0.1ns
PBC_1										– 0.35ns	0.50*	0.84***	0.66**	0.14ns	0.47*	0.03ns
Fe_{ca}/Fe_{cbd}											0.18ns	– 0.26ns	– 0.24ns	– 0.77***	– 0.73***	0.74***
$Fe_{ca}+Fe_{cbd}$												0.52*	0.21ns	– 0.61**	– 0.10ns	0.34ns
clay													0.44ns	– 0.01ns	0.44ns	–0.09ns
A														0.14ns	0.06ns	0.44ns
CCE															0.61**	– 0.57*
pH																–0.74**

Subindexes: Cl-res, P extracted with resins in chloride form duirng 1 h; HCO_3-res, P extracted with resin membranes in bicarbonate form; $CaCl_2$-P, P extracted in 0.01 M $CaCl_2$; CB, P extracted with citrate-bicarbonate during 1 h; NaOH+CB, P extracted with NaOH + P extracted with citrabe-bicarbonate according to the fractionation scheme of Ruiz et al. (1998); NaOHh+CBh, P hydrolysable with phytases in the former extracts; ca, citrate-ascorbate extractable Fe; cbd, citrate-bicarbonate-dithionite extractable Fe

Inc Cl-res, increment in the P extraction with resins in chloride form from 1 to 24 h; Xm, maximum sorption capacity according to the Langmuir equation; CCE, calcium carbonate equivalent

*, **. ***, significant at $P < 0.05$, 0.01, and 0.001, respectively; ns, not significant

When Olsen P was considered as the only predictive variable, it only explained a 53 % TAP (mean value for wheat and sunflower, $P < 0.001$). When the ratio Fe_{ca}/Fe_{cbd} and PBC estimated at 1 mg P L^{-1} were also included as predictive variables in the model, the variance accounted for 69 and 61 %, respectively (Figure 4.2). When clay and CCE were included in the predictive model with Olsen P, variance explained increased till 80 % (Figure 4.2). Overall, TAP was better related to P in sinks than Olsen P. $P_{Cl\text{-}AER}$ at 1 hour explained 77 % of the variance in TAP (linearly, $P < 0.001$); this percentage of variance explained was similar for all the extraction times using Cl-AER (data not shown). When phytase hydrolysable P in NaOH and CB extracts of the fractionation scheme used was considered in the model along with Cl-AER extractable P at 1 hour, explained variance of TAP raised to 86%, and when the ratio of Fe_{ca} to Fe_{cbd} was included as explicative variable, variance explained was 84 % (Figure 4.3). Without including any additional explicative variable, $P_{HCO3\text{-}res}$ itself provided the highest explanation of the variation in TAP (87 %, Figure 4.4).

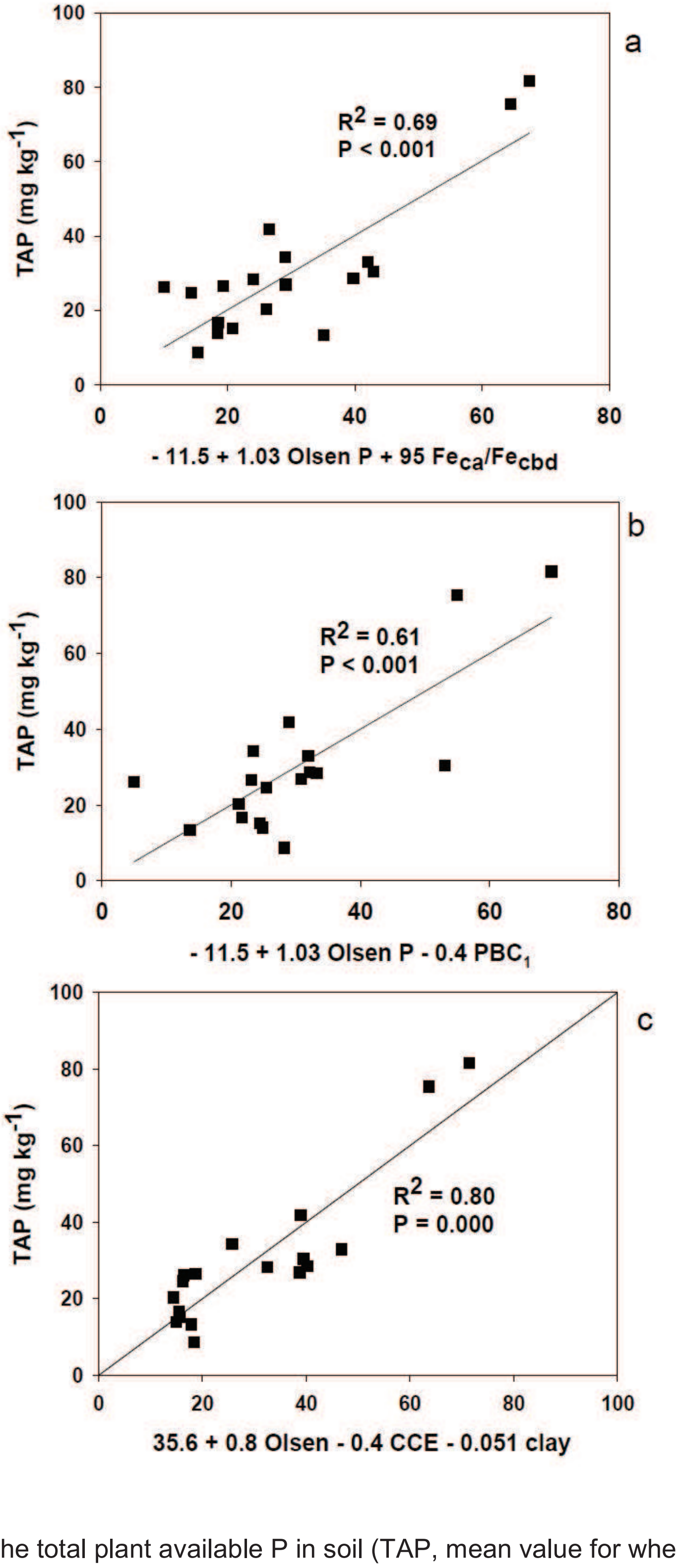


Figure 4.2. Estimation of the total plant available P in soil (TAP, mean value for wheat and sunflower) as a function of Olsen P (Olsen et al. 1954) and the ratio of citrate ascorbate (Fe_{ca}) to citrate-bicarbonate-dithionite (Fe_{cbd}) extractable Fe (a), as a function of Olsen P and the estimate of the buffer capacity from the sorption isotherms at a concentration in the solution of 1 mg P L^{-1} (BC) (b), and as a function of Olsen P, clay and Cacarbonate equivalent (CCE) in soil (c). When Olsen P was taken as the only predictive variable, R^2 = 0.53 and P < 0.001.

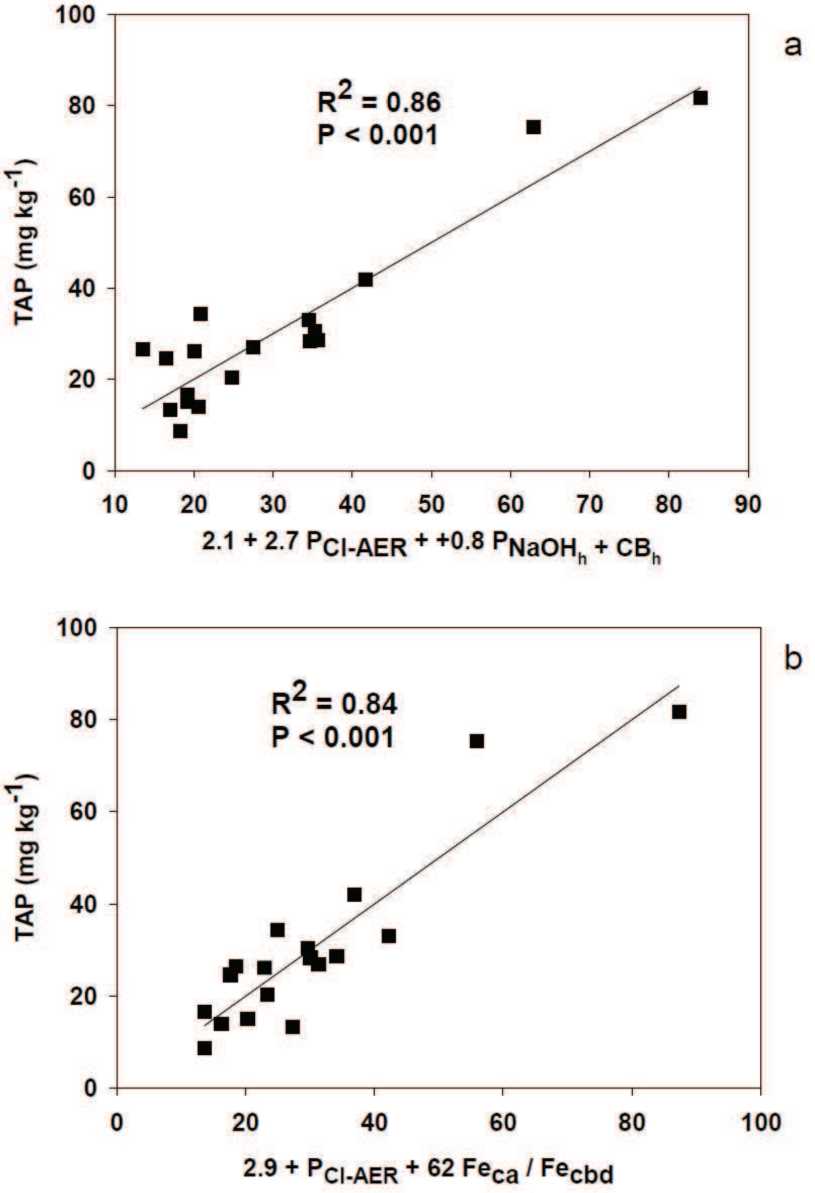


Figure 4.3. Estimation of the total plant available P in soil (TAP, mean value for wheat and sunflower) as a function of extractable P by resin in Cl^- form after 1 h (Cl-AER) and the phytase hydrolysable P in NaOH and CB extracts of the fractionation scheme proposed by Ruíz et al. (1997) (a), and as a function of Cl-resin extractable P at 1 h and the ratio of citrate ascorbate (Fe_{ca}) to citrate-bicarbonate-dithionite (Fe_{cbd}) extractable Fe (b). When Cl-resin at 1 h was taken as the only predictive variable, $R^2 = 0.77$, $P < 0.001$ (the same with a logarithmic transformation of both variables).

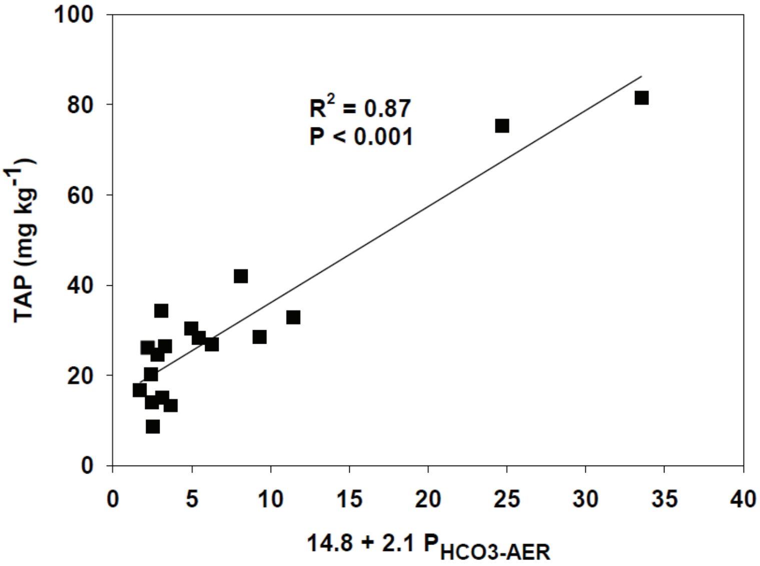


Figure 4.4. Estimation of the total plant available P in soil (TAP, mean value for wheat and sunflower) as a function of extractable P by resin membranes in HCO_3^- form after 24 h extraction ($P_{HCO3\text{-}AER}$). Model does not change significantly when points above 5 times the mean influence were not considered in the regression (Y = 14.1 + 2.1 X with all points, and Y = 13 + 2.3 X, R^2 = 0.8; $P < 0.001$ when influential points were not taken into account). Regression was also highly significant (R^2 = 0.67, $P < 0.001$) with normalized data (logarithmic transformation for TAP and data$^{-0.3}$ for $P_{HCO3\text{-}AER}$).

Discussion

Estimated threshold values of P_{CaCl2} for P uptake depended on soil properties, and not only on the ability of a particular crop to absorb P. The ratio of Fe ascribed to poorly crystalline oxides to crystalline oxides (Fe_{ca}/Fe_{cbd}) and SOM were positively related to threshold values of P_{CaCl2}, whereas PBC_1 and clay content were negatively related to these threshold values. Overall, poorly crystalline Fe oxides have a lower affinity for P than crystalline oxides, and thus, for the same degree of saturation of these sorbent surfaces, a greater concentration in soil solution can be expected at increased ratios of poorly crystalline to crystalline Fe oxides (Recena et al. 2015). Soil organic matter competes for sorption sites with P and interferes in the precipitation of poorly soluble metal phosphates (Delgado et al. 2002). Thus, for the same degree of P enrichment in soil, and for the same amount of available P sorbed (*Q* factor), greater P concentration in soil solution can be expected in soils with higher portion of Fe oxides ascribed to poorly crystalline ones and with a greater concentration of SOM, but the capacity of replenish P in solution is finished at higher P concentration in solution. On the other hand, and in agreement with Sánchez-Alcalá et al. (2015), PBC_1 and properties strongly correlated with it (e.g. clay content), negatively affected P_{CaCl2} threshold values. This means that, in soils with high PBC_1, P extraction by plants is conducted till lower P concentration in solution when compared with soils with low PBC_1 because there is still capacity of replenish P in solution

Overall, a much more accurate estimation of TAP was obtained with P sinks than with single chemical extractions such as Olsen P. Both resins tested were able to explain more than 75 % of TAP (Figures 4.2 and 4.3). In particular, it should be highlighted that extraction with AER membranes in bicarbonate form, which is easy to perform, explained near 90 % of variation in TAP. This is in agreement with previous evidences with AER in Cl^- form (Delgado et al. 2010) or with similar techniques such as DGT (Tandy et al. 2011). Interaction of resins with soil solution chemistry and pH can be modified by charging the resins with different anions, either HCO^-_3 or Cl^- (Agbenin and Raij, 2001). The use of HCO_3-AER has usually provided better results than Cl^-AER (Delgado and Torrent, 1997), since plant roots accumulate bicarbonate in the rhizosphere, leading to an increase in rhizosphere pH in acid to neutral soils and a decrease in rhizosphere pH in calcareous soils (Sibbesen, 1978). In Cl^- form, the Cl^- accumulated in solution inhibits the exchange reaction (Myers et al., 2005) and not simulates effects of rizhosphere on soil.

Regarding Cl-AER, when a potential organic P input to P uptake by plants, which was the phytase hydrolysable P in NaOH and CB extracts, was taken into account, this extraction explained near 90 % of TAP (Figure 4.3). Organic P in NaOH extracts remains as a fraction controlling immobilization-mineralization of P (McGill and Cole, 1981). Thus, this reflects potential contribution of organic P to P supply to plants. In addition, the portion of Fe in oxides ascribed to poorly crystalline oxides (Fe_{ca}/Fe_{cbd}) also increased variation explained by Cl-AER up to 84%. As mentioned above, increased P concentration in soil solution can be expected with increased poorly crystalline to crystalline Fe oxides ratio. Since AER are not really near-infinite P sinks, and relative high P concentration in solution is required for effective P adsorption by AER (Delgado et al. 2010), an increased ratio Fe_{ca}/Fe_{cbd} is expected to enhance P adsorption by Cl-AER.

Overall, none of the single chemical extraction tested (CB or NaOH+CB) that can be deemed as estimates of the *Q* factor, improved the results of Olsen P. This latter index is supposed to release P mainly from the NaOH+CB fraction (Saavedra and Delgado, 2005). Worse prediction of TAP with Olsen P and other chemical extractions when compared with AERs seems to be related to their lower correlation with PBC_1. For bicarbonate-based SPT, Holford (1980) obtained similar results. Phosphorus buffer capacity seems a crucial property explaining variability in P availability indexes (Bolland et al. 1994; Ehlert et al. 2003), and the correlation of P availability indexes values with soil properties such clay, pH or Fe oxide content and portion of Fe in oxides ascribed to poorly crystalline oxides can be explained, at least in part, by the correlation of these properties with PBC (Table 4.3). In fact, estimation of TAP with Olsen P significantly improved with the inclusion of PBC_1 in the model and Fe_{ca}/Fe_{cbd} (Figure 4.1), thus reflecting the limitation of this chemical extraction when compared with AERs, whose predictions were not improved with PBC or PBC-related soil properties, such as clay and pH (Table 4.3). In fact, extractions with both AERs were better correlated with PBC than chemical extractions (Table 4.3). When physicochemical soil properties related to PBC and Fe_{ca}/Fe_{cbd} were included in a multiple regression with Olsen P (Figure 4.1), i.e. clay and carbonates (Table 4.3), 80 % of the variance in TAP was explained (Figure 4.2). This means that single models which take into account these properties can be effective in using Olsen P in assessing TAP.

Sensitiveness of AERs to PBC has been usually explained because their efficiency in P extraction depends on the capacity of soil to replenish P in solution (Delgado et al. 2010). The negative correlation between PBC and AER-extractable P on can be explained, in our case, because soils with high PBC were soils which maintained low P concentration in solution, which negatively affected the amounts of P extracted by the AER. Overall, our results confirm the classical results of Holford (1980) revealing that the larger the negative effect of PBC on the extraction of labile phosphate by a SPT, the higher is the correlation between P uptake by plants and the SPT. Other properties contributing to explain equilibrium between P in soil solution and P in solid phase, such as Fe_{ca}/Fe_{cbd}, improve predictions of different SPT tested, by except HCO_3-AER.

Kinetics factors in P release can be also relevant explaining TAP. The increase in the amount extracted with Cl-AER between 1 and 24 h was significantly correlated with TAP (Table 4.3). Overall, all the SPT tested were significantly correlated with this measure of the slow P release. Ca carbonate (CCE) in soil was not related to PBC, but it was negatively correlated with TAP. Carbonate is a relevant factor explaining P availability and efficiency of P fertilization in soils (Afif et al. 1993). The SPTs better correlated with TAP, were also negatively correlated with CCE. In this regard, it should be mentioned that Olsen

P was not correlated with CCE (Table 4.3), and this can contribute to explain its lack of accuracy in estimating TAP.

Overall, the best results in estimating TAP obtained with HCO_3-AER can be explained not only by it being an estimate of *Q* (correlated with estimates of *Q* by chemical extractions), but also by its more significant correlation with PBC and PBC-related properties than other indices tested, and also with the Fe_{ca}/Fe_{cbd}, which has been shown to be relevant in explaining the relationship of SPTs and TAP. In addition, it was the extraction more significantly correlated with the estimate of the *I* factor (P_{CaCl2}). Thus, it seems to better correlate with most of the factors affecting the equilibrium of inorganic P between solid and liquid phases in soils and consequently affecting P availability to plants than other SPT

Conclusions

Anion exchange resins (AER) performed better, as estimators of TAP, than Olsen P. This can be explained because P sinks are better related to soil properties such as PBC and F_{eca}/Fe_{cbd}, affecting the equilibrium of inorganic P between solid phase and soil solution. Olsen P can be suitable for estimating TAP if PBC and Fe_{ca}/Fe_{cbd}, or other soil properties such as clay and carbonates related to them, are included in the models. AER membranes in bicarbonate form explained near 90 % of variation in TAP. This procedure is easy to be standardized and applied. This accuracy is explained because it is the P extraction better correlated with all the factors affecting P availability to plants.

Acknowledgements

This study was funded by the Spanish Ministry of Science and Innovation and the European Regional Development Fund of the European Union through the National Research, Development and Innovation Program (Plan Nacional I+d+i, AGL2011-29893-CO2-01) and by the Regional Government of Andalucia (Project AGR 63-85). Mr. Ramiro Recena was PhD student with a grant from the Regional Government. Authors wish to thank Prof. José Torrent his valuable suggestions during the performance of the experiments and prior revision of the manuscript. Fertiberia S.A. and OCA Lebrija collaborated in the collection of soil samples for the present study. Authors also will to thanks the Agricultural Research Service of the University of Seville by the technical support and access to infrastructure facilities.

References

Abdu, N., 2006. Soil-phosphorus extraction methodologies: A review. Afr. J. Agric. Res. 1, 159-161.

Afif E, Matar A, Torrent J., 1993. Availability of phosphate applied to calcareous soils of West Asia and North Africa. Soil Sci. Soc. Am. J., 57, 756-760. doi:10.2136/sssaj1993.03615995005700030022x

Agbenin, J.O, B.V. Raij., 2001. Kinetics and energetics of phosphate release from tropical soils determined by mixed ion-exchange resin. Soil Sci. Soc. Am. J. 65, 1108-1114. doi:10.2136/sssaj2001.6541108x

Bai, Z., Li, H., Yang, X., Zhou, B., Shi, X., Wang, B., Oenema, O., 2013. The critical soil P levels for crop yield, soil fertility and environmental safety in different soil types. Plant Soil, 372(1-2), 27-37. doi: 10.1007/s11104-013-1696-y

Bolland, M.D.A., Wilson, I.R., Allen, D.G., 1994. Effect of P buffer capacity and P retention index on soil test P, soil test calibrations and yield response curvature. Aust. J. Soil Res. 32, 503–517.doi: 10.1071/SR9940503

Bouwman, A. F., Beusen, A. H. W., Billen, G., 2009. Human alteration of the global nitrogen and phosphorus soil balances for the period 1970–2050. Glob. Biogeochem. Cycles, 23 (4). doi: 10.1029/2009GB003576

Carpenter, S. R., 2008. Phosphorus control is critical to mitigating eutrophication. Proc. Natl. Acad. Sci., 105 (32), 11039-11040. doi: 10.1073/pnas.0806112105

Carpenter, S. R., Bennett, E. M., 2011. Reconsideration of the planetary boundary for phosphorus. Environ. Res. Lett., 6 (1), 014009. doi: 10.1088/1748-9326/6/1/014009

Celardin, F., 2003. Evaluation of soil P□test values of canton Geneva/Switzerland in relation to P loss risks. J. Plant Nutr. Soil Sci., 166 (4), 416-421.doi: 10.1002/jpln.200321142.

Cheesman, A. W., Dunne, E. J., Turner, B. L., Reddy, K. R., 2010. Soil phosphorus forms in hydrologically isolated wetlands and surrounding pasture uplands. J. Environ. Qual., 39(4), 1517-1525. doi: 10.2134/jeq2009.0398.

Colomb, B., Debaeke, P., Jouany, C., Nolot, J. M., 2007. Phosphorus management in low input stockless cropping systems: Crop and soil responses to contrasting P regimes in a 36-year experiment in southern France. Eur. J. Agron., 26 (2), 154-165. doi: 10.1016/j.eja.2006.09.004

Cordell, D., Drangert, J. O., White, S., 2009. The story of phosphorus: global food security and food for thought. Glob. Environ. Chang., 19(2), 292-305. doi:10.1016/j.gloenvcha.2008.10.009

Cross, A. F., Schlesinger, W. H., 1995. A literature review and evaluation of the. Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma, 64(3), 197-214. doi: 10.1016/0016-7061(94)00023-4

de Santiago, A., Delgado, A., 2006. Predicting iron chlorosis of lupin in calcareous Spanish soils from iron extracts. Soil Sci. Soc. Am. J, 70(6), 1945-1950. doi:10.2136/sssaj2005.0343

Delgado, A., Torrent, J., 1997. Phosphate rich soils in the European Union: estimating total plant-available phosphorus. Eur J Agron 6, 205–214. doi:10.1016/S1161-0301(96)02048-5

Delgado, A., Madrid, A., Kasem, S., Andreu, L., del Campillo, M.C., 2002. Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. Plant Soil 245, 277–286. doi :10.1023/A:1020445710584

Delgado, A., Scalenghe, R., 2008. Aspects of phosphorus transfer in Europe. J Plant Nutr Soil Sci 171, 552–575. doi. 10.1002/jpln.200625052

Delgado, A., del Campillo, M.C. and Torrent, J. 2010. Limitations of the Olsen method to assess available phosphorus in reclaimed marsh soils. Soil Use Manage., 26, 133–140. doi: 10.1111/j.1475-2743.2010.00264.x

Ehlert, P., Morel, C., Fotyma, M., Destain, J.P., 2003. Potential role of phosphate buffering capacity of soils in fertilizer management strategies fitted to environmental goals. J. Plant Nutr. Soil Sci. 166, 409–415. doi: 10.1002/jpln.200321182

Fernandes, M. L., Coutinho, J., 1997. Anion and cation exchange resin membranes to assess the phosphorus status of some Portuguese soils. Commun. Soil Sci. Plant Anal., 28(6-8), 483-495. doi: 10.1080/00103629709369805

Fox, R.L., Kamprath, E.J., 1970. Phosphate sorption isotherms for evaluating the phosphate requirements of soils. Soil Sci. Soc. Am. Proc., 34:902–907. doi: 10.1007/BF02182893

Garcia-Lopez, A. M., Aviles, M., Delgado, A., 2015. Plant uptake of phosphorus from sparingly available P-sources as affected by Trichoderma asperellum T34. Agric. Food Sci., 24(3), 249-260.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In Klute A (ed) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI pp 383–411. doi: 10.2136/sssabookser5.1.2ed

Green, V.S., Cavigelli, M.A., Dao, T.H., Flanagan, D.C., 2005. Soil physical properties and aggregate-associated C, N, and P distributions in organic and conventional cropping systems. Soil Sci. 170, 822–831. doi: 10.1097/01.ss.0000190509.18428.fe

Jordan-Meille, L., Rubæk, G. H., Ehlert, P. A. I., Genot, V., Hofman, G., Goulding, K., ... Barraclough, P., 2012. An overview of fertilizer P recommendations in Europe: soil testing, calibration and fertilizer recommendations. Soil Use Maneg., 28(4), 419-435. doi: 10.1111/j.1475-2743.2012.00453.x

Kuo, S., 1996. Phosphorus. In: Sparks DL (ed) Methods of soil analysis, Part 2, Chemical properties, 3rd edn. ASA, SSSA, CSSA, Madison, WI, pp 869–919. doi:10.2136/sssabookser5.3.frontmatter

Lemercier, B., Gaudin, L., Walter, C., Aurousseau, P., Arrouays, D., Schvartz, C., ... Abrassart, J., 2008. Soil phosphorus monitoring at the regional level by means of a soil test database. Soil Use Maneg, 24(2), 131-138. doi: 10.1111/j.1475-2743.2008.00146.x

Lambers, H., Brundett, M.C., Raven, J.A., Hopper, S.D., 2010. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. Plant Soil 334, 11–31. doi. 10.1007/s11104-010-0444-9

McGill, W.B., and C.V. Cole., 1981. Comparative aspects of C, N, S and P cycling through soil organic matter during pedogenesis. Geoderma 26,267-286. doi:10.1016/0016-7061(81)90024-0

Menon, R.G., Hammond, L.L., Sissingh, H.A., 1989. Determination of plant-available phosphorus by the iron hydroxide impregnated filter-paper (Pi) soil test. Soil Sci Soc Am J, 53,110–115. doi:10.2136/sssaj1989.03615995005300010020x

Menzies, N.W., Kusumo, B., Moody, P.W., 2005. Assessment of P availability in heavily fertilized soils using the diffusive gradient in thin films (DGT) technique. Plant Soil 269(1–2),1–9. doi: 10.1007/s11104-004-1725-y

Messiga, A.J., Ziadi, N., Angers, D.A., Morel, C., Parent, L.E., 2011. Tillage practices of a clay loam soil affect soil aggregation and associated C and P concentrations. Geoderma 164, 225–231. doi: 10.1016/j.geoderma.2011.06.014

Myers, R.G., A.N. Sharpley, S.J. Thien, G.M. Pierzynski., 2005. Ion-sink phosphorus extraction methods applied on 24 soils from the continental USA. Soil Sci. Soc. Am. J. 69, 511-521. doi: 10.2136/sssaj2005.0511

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27, 31–36. doi:10.1016/S0003-2670(00)88444-5.

Neyroud, J. A., Lischer, P., 2003. Do different methods used to estimate soil phosphorus availability across Europe give comparable results?. J. Plant Nutr. Soil Sci., 166 (4), 422-431. doi: 10.1002/jpln.200321152

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. US Government Printing Office, Washington DC

Recena, R., Torrent, J., del Campillo, M. C., and Delgado, A., 2015. Accuracy of Olsen P to assess plant P uptake in relation to soil properties and P forms. Agron. Sustainable Dev., 35 (4), 1571-1579. doi: 10.1007/s13593-015-0332-z

Richardson, A.E., Barea, J.M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321, 305–339. doi: DOI: 10.1007/s11104-009-9895-2

Ryan, J., Ibrikci, H., Delgado, A., Torrent, J., Sommer, R., Rashid, A., 2012. Significance of phosphorus for agriculture and the environment in the West Asia and North Africa region. Adv Agron 114, 91–153. doi: 10.1016/B978-0-12-394275-3.00004-3

Ruiz, J.M., Delgado, A., Torrent, J., 1997. Iron-related phosphorus in overfertilized European soils. J. Environ Qual. 26, 1548–1554. doi: 10.2134/jeq1997.00472425002600060014x

Saavedra, C., Delgado, A., 2005. Phosphorus fractions and release patterns in typical Mediterranean soils. Soil Sci Soc Am J 69, 607–615. doi: 10.2136/sssaj2004.0156

Saavedra, C., Velasco, J., Pajuelo, P., Perea, F., Delgado, A., 2007. Effects of tillage on phosphorus release potential in a Spanish Vertisol. Soil Sci. Soc. Am. J. 71, 56–63. doi: 10.2136/sssaj2006.0162

Sánchez Alcalá, I., Campillo, M. C., Torrent, J., 2014. Extraction with 0.01 M CaCl2 underestimates the concentration of phosphorus in the soil solution. Soil Use Manage., 30 (2), 297-302. doi: 10.1111/sum.12116

Sánchez Alcalá, I., Campillo, M. C., Torrent, J., 2015. Critical Olsen P and CaCl2 P levels as related to soil properties: results from micropot experiments. Soil Use Manage. 31(2), 233-240. doi: 10.1111/sum.12184

Schoenau, J.J., Huang, W.Z., 1991. Anion-exchange membrane, water, and sodium-bicarbonate extractions as soil tests for phosphorus. Commun Soil Sci Plant Anal 22, 465–492. doi: 10.1080/00103629109368432

Sibbesen, E., 1978. An investigation of the anion-exchange resin method for soil phosphate extraction. Plant Soil 50, 305-321. doi: 10.1007/BF02107180

Six, L., Pypers, P., Degryse, F., Smolders, E., Merckx, R., 2012. The performance of DGT versus conventional soil phosphorus tests in tropical soils—an isotope dilution study. Plant Soil. 359(1-2), 267-279. doi: 10.1007/s11104-012-1192-9

StatPoint. 2000. Statgraphic plus 5.1. Rockville, Maryland, USA.

Schröder, J. J., Smit, A. L., Cordell, D., Rosemarin, A., 2011. Improved phosphorus use efficiency in agriculture: a key requirement for its sustainable use. Chemosphere, 84(6), 822-831. doi: 10.1016/j.chemosphere.2011.01.065

Soil Survey Staff, 2010. Keys to soil taxonomy, 11th edn. USDA-Natural Resources Conservation Service, Washington, DC

Tandy, S., Mundus, S., Yngvesson, J., de Bang, T.C., Lombi, E., Schjoerring, J.K., Husted, S., 2011. The use of DGT for prediction of plant available copper, zinc and phosphorus in agricultural soils. Plant Soil 346, 167–180. doi: 10.1007/s11104-011-0806-y

Tang, X., Ma, Y., Hao, X., Li, X., Li, J., Huang, S., Yang, X., 2009. Determining critical values of soil Olsen-P for maize and winter wheat from long-term experiments in China. Plant Soil 323:143–151. doi: 10.1007/s11104-009-9919-y

Tóth, G., Guicharnaud, R.A., Tóth, B., Hermann, T., 2014. Phosphorus levels in croplands of the European Union withimplications for P fertilizer use. Eur J Agron 55, I42–52. doi:10.1016/j.eja.2013.12.008

Turner, B. L., Romero, T. E., 2009. Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils. Soil Sci. Soc. Am. J., 73(6), 1972-1979. doi:10.2136/sssaj2008.0407

Vadas, P. A., Good, L. W., Moore, P. A., Widman, N., 2009. Estimating phosphorus loss in runoff from manure and fertilizer for a phosphorus loss quantification tool. J. Environ. Qual., 38(4), 1645-1653. doi: 10.2134/jeq2008.0337

Van Raij, B., 1998. Bioavailable tests: Alternatives to standard soil extractions. Commun Soil Sci Plant Anal 29, 1553–1570. doi: 10.1080/00103629809370049

Van Rotterdam, A. M. D., Bussink, D. W., Temminghoff, E. J. M., Van Riemsdijk, W. H. , 2012. Predicting the potential of soils to supply phosphorus by integrating soil chemical processes and standard soil tests. Geoderma, 189, 617-626. doi:10.1016/j.geoderma.2012.07.003

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci 63, 251–263. doi.org/10.1097/00010694-194704000-00001

Ziadi, N., Whalen, J. K., Messiga, A. J., and Morel, C., 2013. Assessment and modeling of soil available phosphorus in sustainable cropping systems. Adv Agron, 122, 85-126. doi: 10.1016/B978-0-12-417187-9.00002-4

Figure Captions

Figure 4.1. Example of estimation of P_{ClCa2} for null P uptake (threshold value for P uptake) and for estimation of total plant available P from the cumulative uptake curve in one of the soils studied. Relative P uptake for sunflower is represented, and it was estimated as the ratio of P uptake in each step to P extraction in the first depletion step (a). Cumulative P uptake for the soils in the 8 depletion steps considered (4 for high-P and 4 for low-P samples) (b). P_{CaCl2} is the concentration of P in 0.01 M $CaCl_2$

Figure 4.2. Estimation of the total plant available P in soil (TAP, mean value for wheat and sunflower) as a function of Olsen P (Olsen et al. 1954) and the ratio of citrate ascorbate (Fe_{ca}) to citrate-bicarbonate-dithionite (Fe_{cbd}) extractable Fe (a), as a function of Olsen P and the estimate of the buffer capacity from the sorption isotherms at a concentration in the solution of 1 mg P L^{-1} (BC) (b), and as a function of Olsen P, clay and Ca carbonate equivalent (CCE) in soil (c). When Olsen P was taken as the only predictive variable, R^2 = 0.53 and $P < 0.001$.

Figure 4.3. Estimation of the total plant available P in soil (TAP, mean value for wheat and sunflower) as a function of extractable P by resin in Cl^- form after 1 h (Cl-AER) and the phytase hydrolysable P in NaOH and CB extracts of the fractionation scheme proposed by Ruíz et al. (1997) (a), and as a function of Cl-resin extractable P at 1 h and the ratio of citrate ascorbate (Fe_{ca}) to citrate-bicarbonate-dithionite (Fe_{cbd}) extractable Fe (b). When Cl-resin at 1 h was taken as the only predictive variable, R^2 = 0.77, $P < 0.001$ (the same with a logarithmic transformation of both variables).

Figure 4.4. Estimation of the total plant available P in soil (TAP, mean value for wheat and sunflower) as a function of extractable P by resin membranes in HCO_3^- form after 24 h extraction ($P_{HCO3\text{-}AER}$). Model does not change significantly when points above 5 times the mean influence were not considered in the regression (Y = 14.1 + 2.1 X with all points, and Y = 13 + 2.3 X, R^2 = 0.8; $P < 0.001$ when influential points were not taken into account). Regression was also highly significant (R^2 = 0.67, $P < 0.001$) with normalized data (logarithmic transformation for TAP and $data^{-0.3}$ for $P_{HCO3\text{-}AER}$).

Chapter V

The fifth chapter of this PhD deal with the study of **organic P forms in representative Mediterranean soils**. Besides, **how they may be affected by P status and soil properties** is also studied. This will provide information on the potential contribution of organic P to P supply to plants.

Organic phosphorus forms in representative Mediterranean soils as related to soil properties and fertilization

Organic phosphorus forms in representative Mediterranean soils as related to soil properties and fertilization

Abstract

To effectively manage soil phosphorus (P) for adequate crop growth with minimal impacts on water quality, the dynamics and plant availability of soil organic P (OP) must be understood. The objective of this study was to obtain precise information about the effect of soil P status and properties on OP compounds in representative Mediterranean soils. To this end, ^{31}P NMR spectroscopy was used to characterize P in NaOH-EDTA extracts from eight soils, using two samples per soil with very different P status (“low” and “high” P samples).

Orthophosphates monoesters dominated OP, with a relative contribution to OP (around 50 %) in the range described in the literature. M*yo*- and *chiro*-inositol-6-phosphate (IP_6) were the dominant IP_6 stereoisomers and contributed almost equally to total IP_6. The differences in monoesters concentration between high- and low-P samples increased with increasing differences in Olsen P between both types of samples ($R^2 = 0.61$, $P < 0.05$). The mono- to diesters ratio increased with increased Olsen P in soil ($R^2 = 0.49$; $P < 0.01$). The contribution of monoesters and most of the IP_6 isomers to OP increased as increased Olsen P in soil, but only where the crop residue accumulation was scarce (vineyards and olive orchards). From these results, degradation of monoesters including IP_6, under conditions of P limitation can be supposed, allowing us to expect some contribution of these OP forms to plant P supply. The positive relationship of *myo*-IP_6 with the ratio Fe_{ca}+Fe_d/clay, (where Fe_{ca} (poorly crystalline iron oxide) and Fe_d (highly crystalline iron oxide) are extracted with citrate-ascorbate and citrate-bicarbonate-dithionite respectively) and its decreased concentration in soil with increasing pH suggest that adsorption on Fe oxides stabilized *myo*-IP_6 in these soil. None of the other IP_6 steroisomers was significantly related to the ratio of Fe oxides to clay or to pH in soils. This suggests that adsorption on clay minerals and precipitation of Ca phosphates may explain the retention of other IP_6 stereoisomers in these soils.

Key words: organic phosphorus, inositol-6-phosphate, monoesters, diesters, iron oxides

Introduction

Despite the importance of P for agricultural production, its chemical nature and dynamics in soils are still not fully understood (Condron et al. 2005; Delgado and Scalenghe 2008). Organic P (OP) is the most poorly understood aspect of the P cycle, despite it being the dominant P form in many soils (Saavedra and Delgado 2005a; Delgado and Scalenghe 2008). The enhancement in the use of residual soil P by plants, which encompasses a range of different chemical forms, is crucial to provide a sustainable cycle between agricultural inputs and plant uptake (Stutter et al. 2012). In this regard, mobilization of residual OP is a pivotal issue in achieving a lower dependence on mined P and thus for achieving the future sustainability of agricultural systems (Stutter et al. 2012). To this end, a deeper knowledge of OP forms in soil is required because these chemical forms determine their bioavailability and environmental reactivity (Condron et al. 2005; Cade-Menun and Liu 2014).

The main OP forms in soils are orthophosphate esters, divided into the orthophosphate monoesters (sugar phosphates, mononucleotides, inositol phosphates) and orthophosphate diesters (DNA, RNA, phospholipids). In many soils, the predominant OP forms are the inositol hexaphosphates (IP_6), which can be present in four stereoisomeric forms (*myo, scyllo, chiro, neo*; Giles et al. 2011). It was believed that IP_6 do not contribute significantly to plant P uptake because they are strongly bound to hydroxylated surfaces, which makes them unavailable for enzymatic hydrolysis (Berg and Joern 2006; Gianveno et al. 2008). However, this may not be true for all soils (Leytem et al. 2006; Doolette et al. 2010). The most effective method to identify specific OP forms in soil extracts is ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR; Cade-Menun 2005), and extraction with NaOH-EDTA has been proposed by many authors as the most suitable for analysis of soil OP by NMR spectroscopy (Cade-Menun and Preston 1996; Turner et al. 2005; Cade-Menun and Liu 2014).

Organic P can be dominant even in agricultural Mediterranean soils with scarce organic matter content (Saavedra et al. 2007) and in eroded sediments from these soils (Saavedra and Delgado 2005b; Díaz et al. 2013). Recena et al. (2015) observed that OP (determined as the difference between total P and molybdate-reactive P) accounted for between 25 and 75 % of total extracted P using sequential fractionation in a group of representative Mediterranean soils. Some studies on OP have been performed in soils from Mediterranean areas using sequential chemical fractionation (e.g. Saavedra et al. 2007; Recena et al. 2015). However these methods do not provide information on specific

P forms. Although ^{31}P NMR can provide more precise information on specific organic compounds, there have been few published studies of Mediterranean soils. In early solution ^{31}P NMR studies (Trasar-Cepada et al. 1989; Gil-Sotres et al. 1990; Turrión et al. 2001), low-resolution spectra did not permit the identification of specific P forms. More recent studies have investigated changes in OP after fire in forest soils (Turrión et al. 2010) or OP transformations in soils irrigated with reclaimed wastewater (Zohar et al. 2013), while a study using solid-state ^{31}P NMR focused on the residual inorganic P (Pi) resulting from fertilization (Delgado et al., 2002).

Given the importance of agriculture in the Mediterranean region, effective P management requires a full understanding of P cycling, including OP dynamics, in soils of this region. As such, the objectives of this study were 1) to use ^{31}P NMR to obtain precise information about OP compounds and dynamics in representative Mediterranean soils; and 2) to assess the effect of soil P status and properties on these OP forms.

Materials and Methods

Soil selection

Eight locations in different areas of Mediterranean soils of Spain were selected for soil sampling. These soils encompassed Entisols, Inceptisols, Mollisols, Alfisols, and Vertisols according to Soil Taxonomy (Soil Survey Staff, 2010) and included tree orchards (vineyards or olive) and field crops (wheat/sunflower rotation or cereals). At each location, samples of the surface layer (0–20 cm) of the soil were taken to obtain a set of samples widely ranging in Olsen P. Within this broader sample set, samples with the two extreme values with respect to their P status for each location were picked for this study and named “low-P” and “high-P” samples (Recena et al. 2015). Soil properties other than P were checked to ensure that they differed by less than 5% between “high P” and “low P” samples for each location.

Soil analysis

Soil samples were dried and ground to pass a 2 mm sieve. As described in Recena et al. (2015), particle size distribution was assessed by the Gee and Bauder (1986) procedure, organic matter (SOM) was determined by dichromate oxidation (Walkley and Black 1934), and the cation exchange capacity (CEC) was measured using 1 *M* NH_4OAc buffered at pH 7 (Sumner and Miller 1996). The calcimeter method was used to determine the total $CaCO_3$ equivalent (CCE), and pH was measured in water at a soil:extractant ratio of 1:2.5. Soil test P was determined according to Olsen et al. (1954), replacing filtration through active carbon by centrifugation at 1000 g for 10 min. Total Pi was determined as that extractable with 0.5 M H_2SO_4 (Kuo 1996). Total P was determined using the molybdenum blue colorimetric method (Murphy and Riley 1962) following dry combustion at 550 ^{o}C for 2 h and extraction with 0.5 M H_2SO_4, and OP was estimated as the difference between total P and total Pi (Kuo 1996). Iron (Fe) was sequentially extracted by citrate-ascorbate (Fe_{ca}) and citrate-bicarbonate-dithionite (Fe_d) according to de Santiago et al. (2006), the former being ascribed to poorly crystalline Fe oxides, and the later to crystalline oxides.

Soils were extracted for ^{31}P NMR with a modification of the Cade-Menun and Preston (1996) 0.25 M NaOH + 0.05 M Na-EDTA extraction (3 g of soil, 30 mL extractant) in 50 ml polyethylene flasks at 25ºC temperature, shaking for 16 hours on a reciprocating shaker at 3 Hz. After extraction, suspensions were centrifuged at 1000 x *g* for15 min. An aliquot of the supernatant was analyzed for total extracted P (extr P) as molybdate-reactive P following sulfuric–persulphate digestion (Diaz-Espejo et al., 1999); the remainder was frozen at –80 ^{o}C and lyophilized.

^{31}P NMR spectra

The lyophilized extracts were dissolved in 0.8 mL D_2O, and 0.6 mL each of deionized H_2O, 10 M NaOH and the NaOH-EDTA extraction solution; after 10 min with occasional vortex mixing, samples were centrifuged (20 min, ~1500 x *g*), and then filtered through 0.2 µm syringe filters into 10-mm NMR tubes. Dissolved extracts were maintained at 4 ^{o}C before analysis. Spectra were recorded within 12 h after dissolution.

Solution ^{31}P NMR spectra were collected on a 500 MHz Bruker Avance NMR spectrometer (Bruker Biospin Corporation, Fremont, CA) with a 10-mm broadband probe at the Saskatchewan Structural Science Center (SSSC, University of Saskatchewan, Canada). The NMR acquisition parameters were: 202.45 M Hz frequency; 13 µs (45 °) pulse; 1.32 s acquisition time; 4.5 s relaxation delay; 3176 to 8066 scans (6 to 12 h); no proton decoupling. Based on the ratio of P/(Fe+Mn) in the extracts, the total delay time of 5.82 s was estimated to satisfy at least 3 x T1 for all samples (McDowell et al 2006; Cade-Menun and Liu 2014).

Phosphorus compounds were identified by their chemical shifts, relative to an external orthophosphoric acid standard (85%). Spectra were processed with 7 and 2 Hz line-broadening using NUTS software (2000 edition: Acorn NMR, Livermore, CA). The orthophosphate peak in each sample was standardized at δ = 6 ppm (Cade-Menun et al. 2010). Peak assignments were based on spiking experiments [*myo*-hexakisphosphate (myo-IP_6), glucose-6-P (g6P), glucose-1-P (g1P), α and β-glycerol-P (α-gly, β-gly) and P-choline (Pcho)] and previous references (Cade-Menun 2005; Cade-Menun et al. 2010; Turner et al. 2012; Cade-Menun 2015a).

Statistical analysis

In order to assess differences between high- and low-P samples, an ANOVA was performed considering two factors, namely: soils (8) and P status (low or high). Since only one ^{31}P NMR spectrum was acquired for each sample, the error term was considered the interaction between both factors. The ANOVA and regressions were performed using the Statgraphics 5.1 software (Statpoint 2000). Centered log-ratio transformation was used to conduct ANOVA analyses (Aitchison 1986; Abdi et al. 2014, 2015).

Results and Discussion

Properties and phosphorus content of soils

The studied soils ranged widely in properties (Table 5.1). The greatest variations were detected in carbonate and clay content and in the poorly crystalline and crystalline Fe oxides (Fe_{ca} and Fe_d values). The different fertilization histories are reflected in the total Pi content (mean 188 and 353 mg kg^{-1} in low- and high-P samples, respectively) and in the Olsen P values (mean 10 and 26 mg kg^{-1} in low- and high-P samples, respectively; Table 5.1). Non-significant differences in OP concentration according to the dry combustion method were observed between high- and low-P samples (Table 5.1). Total OP was higher in soils used for field crops than in those used for vineyards and olive orchards (178 and 93 mg kg^{-1}, respectively; $P < 0.05$), which can be ascribed to a greater residue incorporation to soil through roots and stubble under field crop management. The affinity factor for orthophosphate in the Freudlich equation (*b*) did not differed between high- and low-P samples (Table 5.1), and it increased linearly with clay content ($R^2 = 0.42$; $P < 0.01$). This was expected because clay content is an indicator of adsorption capacity, as revealed by correlation with Fe oxides ($Fe_{ca} + Fe_d$, $r = 0.56$, $P < 0.05$), and Recena et al (2015) observed that *b* increased with increasing adsorption capacity (Fe oxides).

Table 5.1. General soil properties, affinity parameter (b), and Olsen P of studied soils

											calcination					**Olsen P**	
Soil	**Soil taxonomy**[1]	**Use**	**SOM**	**Clay**	**pH**	**CCE**	**ACCE**	**Fed**	**Feca**	**CEC**	**OP Low**	**Pi Low**	**OP High**	**Pi High**	***b***	**Low**	**High**
	(Suborder)		g kg^{-1}							cmol(+) kg^{-1}	mg kg^{-1}					mg kg^{-1}	
ECO	Xererts	W/S (FC)	10	492	7.9	75	25.5	10.4	1.2	41.0	71,1	346,5	109,7	447,2	3.6	7	9
ZMB	Fluvents	C (FC)	20	190	7.8	168	38	6.2	0.9	16.0	216,1	255,0	237,7	513,4	1.8	8	23
PZB	Xeralfs	W/S (FC)	15	48	7.2	0	0	3.1	0.9	9.1	119,9	112,0	121,6	169,7	1.3	15	38
LCV	Xeralfs	O	8.7	412	8.1	32	0	12.3	2.2	13.6	102,8	82,9	104,1	212,7	1.1	7	33
ADM	Xerepts	O	7.8	67	6.8	0	0	6.7	1.8	10.5	86,4	142,8	52,6	561,3	0.1	16	49
PLP	Xeralfs	O	12.5	640	7.9	77	35	9.0	2.2	52.3	126,0	102,6	150,3	155,9	1.6	6	21
OCL	Xerepts	C (FC)	14	360	7.9	244	86	7.5	1.5	18.6	262,6	191,2	285,7	236,9	2.8	13	21
TRB	Xerolls	V (O)	8.4	400	8.1	400	156	3.5	0.8	36.3	82,2	273,8	43,2	526,5	2.6	5	17
Mean			14,8	326,1	7,7	124,5	45,0	7,3	1,4	24,7	133,4	188,4	138,1	353,0	1.9	10b	26a

SOM, soil organic matter; CCE, calcium carbonate equivalent; ACCE, active calcium carbonate equivalent; CEC, cation exchange capacity; ca and d subscript refer to citrate-ascorbate and citrate-bicarbonate-dithionite Fe sequential extractions; *b* affinity parameter for P according to Freundlich model to describe sorption curves: $Y = A X^{1/b}$

Use: W/S, wheat/sunflower rotation; C, cereals; O, olive orchards; V, vineyards

[1] According to Soil Survey Staff (2010)

Means followed by different letters in the same row are significantly different at P < 0.05

Table 5.2 Chemical shifts of peaks detected in ^{31}P-NMR spectra.

Category	P Form or Compound Class	Chemical Shift (ppm)
Inorganic P		
	Orthophosphate	6.00 ± 0.00[a]
	Pyrophosphate	-4.10 ± 0.10
	Polyphosphates	-3.98 ± 0.10, -4.88 ± 0.40, -6.96 ± 0.63, -8.54 ± 0.45, -9.83 ± 0.82, -12.51 ± 0.43, -13.51 ± 0.63, -16.47 ± 0.83,-19.20 ± 0.78, -20.96 ± 0.87, -22.36 ± 0.80, -23.79 ± 0.36
Organic P		
	Phosphonates	25.28 ± 0.59, 23.21± 0.73, 21.54± 0.37, 20.11± 0.46, 18.63 ± 0.28, 16.73 ± 0.60, 15.44 ± 0.38, 12.82 ± 0.62, 10.89 ± 0.71, 7.86 ± 0.60
Orthophosphate Monoesters		
	myo-IHP	5.40 ± 0.05, 4.46 ± 0.04, 4.09 ± 0.05, 4.03 ± 0.05
	scyllo-IHP	3.68 ± 0.05
	neo-IHP	6.33 ± 0.02, 4.25 ± 0.02
	D-*chiro*-IHP 4e/2a[b]	6.48 ± 0.04, 5.25 ± 0.02, 3.95 ± 0.04
	D-*chiro*-IHP 4a/2e	6.19 ± 0.02, 4.74 ± 0.01, 4.32 ± 0.01
	Glucose 6-phosphate	5.15 ± 0.04
	α-glycerophosphate	4.79 ± 0.03
	ß-glycerophosphate	4.42 ± 0.03
	Mononucleotides	4.39 ± 0.01, 4.35 ± 0.01, 4.30 ± 0.01, 4.23 ± 0.02, 4.19 ± 0.01, 4.14 ± 0.03
	Choline phosphate	3.79 ± 0.06
	Unknown 1	4.56 ± 0.03
	Unknown 2	4.92 ± 0.03
	Monoester 1	6.96 ± 0.03, 6.85 ± 0.04, 6.68 ± 0.07, 6.53 ± 0.09, 6.26 ± 0.02
	Monoester 2	5.85 ± 0.03, 5.76 ± 0.02, 5.66 ± 0.03, 5.57 ± 0.03, 5.07 ± 0.05, 4.92 ± 0.07, 4.65 ± 0.03
	Monoester 3	3.57 ± 0.04, 3.43 ± 0.04, 3.30 ± 0.06, 3.20 ± 0.05, 3.11 ± 0.07, 2.97 ± 0.05, 2.84 ± 0.07, 2.62 ± 0.06
Orthophosphate Diesters		
	Other Diester 1	2.42 ± 0.06, 2.09 ± 0.12, 1.82 ± 0.14, 1.47 ± 0.13, 1.18 ± 0.15, 0.89 ± 0.11, 0.55 ± 0.12, 0.30 ± 0.13, 0.07 ± 0.05, -0.24 ± 0.13, -0.428 ± 0.12
	DNA	-0.75 ± 0.06, -0.85 ± 0.08
	Other Diester 2	-1.02 ± 0.07, -1.29 ± 0.07, -1.59 ± 0.113, -1.98± 0.11, -2.37 ± 0.07, -2.68 ± 0.10, -3.18 ± 0.13, -3.52 ± 0.05

[a] Average chemical shifts. Peaks had to be present in at least six of the sixteen samples to be listed here

[b] 4e/2a: 4 equatorial, 2 axial configuration; 4a/2e: 4 axial, 2 equatorial configuration

Phosphorus compounds in soils determined by ^{31}P NMR

Representative ^{31}P NMR spectra are shown in Fig. 5.1. The ^{31}P NMR results are in agreement with the well-known fact that orthophosphates are the main extractable P form in soils with very different properties and under very different management (e.g. Zhang et al. 2012; Cade-Menun et al., 2015b; Schneider et al. 2016).

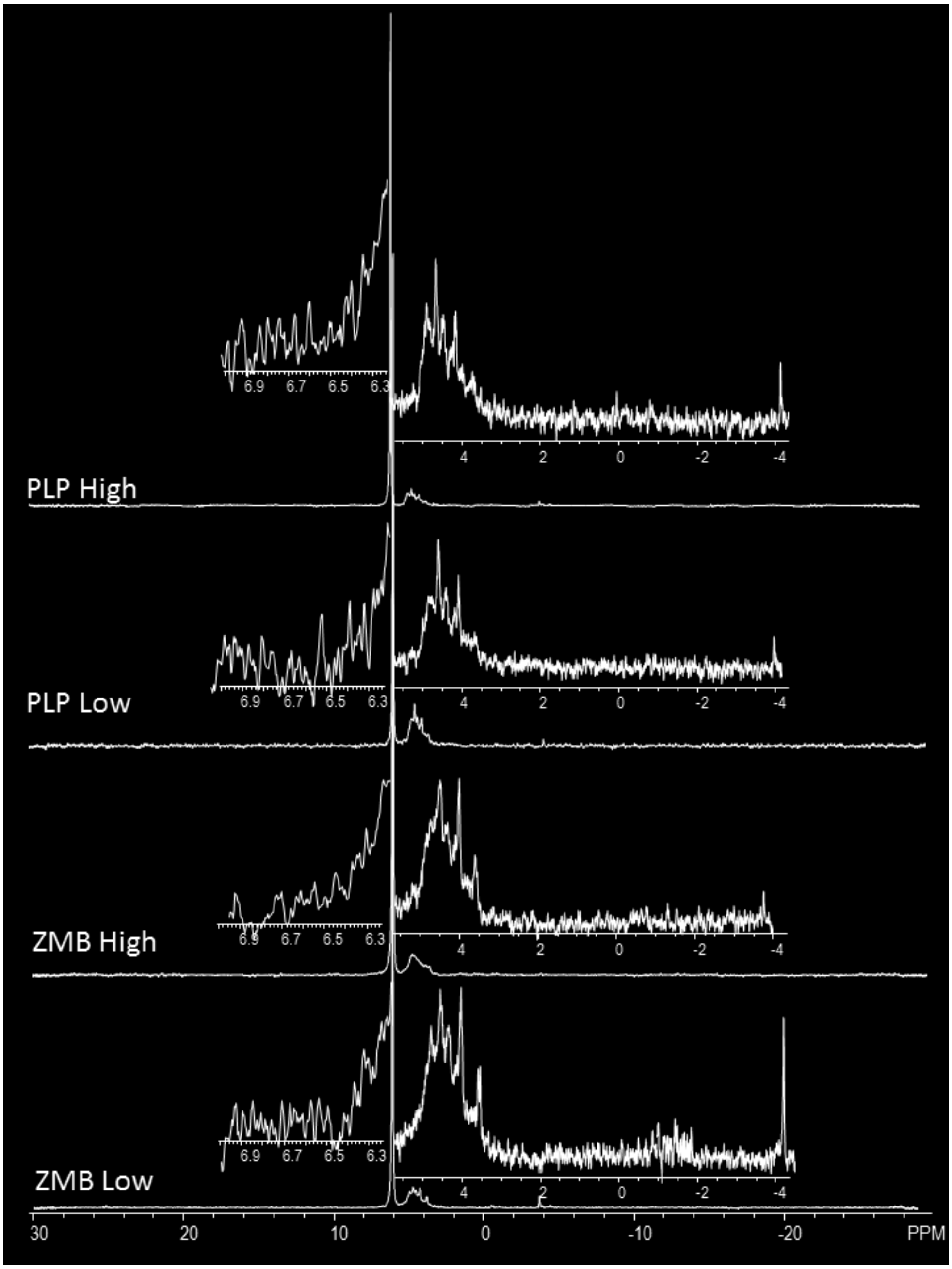


Figure 5.1. ^{31}P NMR spectra of NaOH-EDTA extract of soils PLP and ZMB for low-P and high-P samples

Peaks for P compounds ranged between 25 and –25 ppm (Table 5.2). Peaks assigned to Pi compounds were: orthophosphate at 6.00 ± 0.00ppm, pyrophosphate at –4.10 ± 0.10 ppm and polyphosphates between –4.88 ± 0.40 ppm with the poly-end-group at –3.98 ± 0.10 ppm. For OP forms, their chemical shifts ranged from 25 to 7.8 ppm. Orthophosphate monoesters were assessed at 7.7 to 6.2 and at 5.9 to 2.6 ppm; orthophosphate diesters at 2.5 to – 3.5 ppm. Chemical shifts assigned to IP_6 stereoisomers were: *myo*-IP_6 at 5.40 ± 0.05, 4.46 ± 0.04, 4.09 ± 0.05, 4.03 ± 0.05 ppm; *neo*-IP_6 at 6.33 ± 0.02, 4.25 ± 0.02 ppm; D-*chiro*-IP_6 4e/2a at 6.48 ± 0.04, 5.25 ± 0.02, 3.95 ± 0.04, and D-*chiro*-IP_6 4a/2e at 6.19 ± 0.02, 4.74 ± 0.01, 4.32 ± 0.01 ppm; and *scyllo*-IP_6at 3.68 ± 0.05 ppm (Turner et al. 2003; Turner et al. 2012; Cade-Menun 2015). Glucose 6-phosphate (5.15 ± 0.04), α-glycerophosphate (4.79 ± 0.03), ß-glycerophosphate (4.42 ± 0.03 ppm), choline phosphate (3.79 ± 0.06 ppm) and mononucleotides (4.39 ± 0.01, 4.35 ± 0.01, 4.30 ± 0.01, 4.23 ± 0.02, 4.19 ± 0.01 and 4.14 ± 0.03 ppm) were also identified through spiking experiments. Unidentified orthophosphate monoesters were divided into three regions: Monoester 1 included the unidentified peaks from 7.7 to 6.2 ppm; Monoester 2 the unidentified peaks from 5.9 to 3.7 ppm; and Monoester 3 from 3.3.5 to 2.6 ppm (Cade-Menun et al. 2010; Liu et al 2015). The chemical shifts of peaks in these regions are shown in Table 5.2. Among the peaks in the Monoester 2 region were two unidentified peaks at 4.56 ± 0.03 and 4.92 ± 0.03 ppm, identified as “Unknown 1” and Unknown 2” in Table 5.2, and “4.5” and “4.9” in Table 5.2.

Table 5.3. ^{31}P NMR forms expressed as % of extracted P in the NaOH-EDTA extracts

P status	Soil	Ort	Pyr	Poly	Phon	Myo	Scy	Neo	Chr	α-gly	β-gly	Nuc	Pcho	G6P	4.5	4.9	Mon1	Mon2	Mon3	DNA	Di1	Di2
												% TP										
H	ECO	52.8	0.3	1.2	0.7	4.7	2.3	0.8	4.6	1.6	3.1	6.2	1.6	0.8	2.3	1.6	1.6	7.7	5.4	0.1	0.5	0.1
	ZMB	63.2	0.4	1.1	0.7	2.7	2.1	0.7	4.2	0.7	1.4	4.8	0.7	0.7	2.1	1.4	2.1	5.4	2.7	0.5	1.6	0.8
	PZB	67.4	0.7	1.4	0.7	2.7	2.1	0.0	4.2	0.7	1.4	3.4	0.7	0.7	2.1	1.4	1.4	5.5	1.4	0.7	0.9	0.5
	LCV	67.9	0.4	1.0	0.7	2.8	0.7	0.7	4.2	0.7	1.4	2.8	0.7	0.7	2.1	1.4	1.4	5.5	1.4	0.3	2.7	0.5
	ADM	82.1	0.2	0.5	0.7	2.7	0.7	0.7	2.0	0.7	0.7	1.4	0.7	0.7	0.7	0.7	0.7	2.7	0.7	0.2	0.3	0.2
	PLP	64.5	0.5	1.6	0.7	4.3	1.4	0.7	2.8	0.7	1.4	3.6	0.7	0.7	2.9	1.4	0.7	7.9	2.1	0.2	1.0	0.2
	OCL	55.6	1.8	3.1	0.7	4.0	1.4	0.7	4.0	0.7	1.4	4.7	0.7	0.7	2.7	1.4	1.4	6.8	3.3	1.1	2.4	1.4
	TRB	53.9	0.2	0.5	1.5	4.3	2.1	0.7	4.2	0.7	1.4	5.7	0.7	0.7	2.8	2.1	2.1	11.4	3.5	0.5	0.6	0.4
Mean		63.4	0.6	1.3	0.8	3.5	1.6	0.6	3.8	0.8	1.5	4.1	0.8	0.7	2.2	1.4	1.4	6.6	2.6	0.5	1.3	0.5
SD		9.6	0.5	0.8	0.3	0.9	0.6	0.3	0.9	0.3	0.7	1.6	0.3	0.0	0.7	0.4	0.5	2.5	1.5	0.3	0.9	0.4
L	ECO	61.0	0.1	0.6	0.7	2.8	1.4	0.7	3.5	1.4	2.1	3.4	1.4	0.7	2.1	2.1	1.4	9.7	2.8	0.5	1.0	0.6
	ZMB	64.4	0.9	1.2	0.7	4.0	2.0	0.7	2.8	0.7	1.4	4.0	1.4	0.7	2.7	1.4	1.4	6.1	1.4	0.5	1.3	0.3
	PZB	58.0	0.5	0.9	1.4	7.2	2.1	0.7	4.2	1.4	2.9	3.6	0.7	0.7	1.4	1.4	1.4	8.7	1.4	0.2	0.9	0.3
	LCV	59.0	0.3	0.4	1.4	4.4	2.1	0.7	4.2	1.4	2.8	5.0	0.7	0.7	2.1	1.4	0.7	9.9	1.4	0.3	0.8	0.3
	ADM	62.7	0.6	0.8	2.1	4.1	1.4	0.7	3.5	1.4	2.7	4.1	0.7	0.7	1.4	1.4	0.7	8.2	1.4	0.3	0.8	0.3
	PLP	49.9	0.7	0.7	1.4	4.6	2.3	0.8	4.6	1.5	2.3	6.9	1.5	0.8	4.6	1.5	1.5	10.7	2.3	0.2	0.9	0.3
	OCL	44.2	3.3	2.9	0.7	4.1	2.0	0.7	4.0	1.4	2.0	8.1	1.4	0.7	2.0	1.4	2.0	10.2	2.7	1.9	3.1	1.2
	TRB	64.9	0.4	1.7	0.7	3.9	1.3	0.7	4.0	1.3	2.6	5.2	0.7	0.7	2.0	0.7	1.3	5.2	1.3	0.3	0.9	0.2
Mean		58.0	0.9	1.2	1.1	4.4	1.8	0.7	3.9	1.3	2.4	5.0	1.1	0.7	2.3	1.4	1.3	8.6	1.8	0.5	1.2	0.4
SD		7.3	1.0	0.8	0.5	1.3	0.4	0.0	0.6	0.3	0.5	1.7	0.4	0.0	1.0	0.4	0.4	2.0	0.6	0.6	0.8	0.3

Ort, orthophosphate; Pyr, pyrophosphate; Poly, polyphosphates; Phon, phosphonates; Myo, *myo*-IP$_6$; Scy, *scyllo*-IP$_6$; Neo, *Neo*-IP$_6$ estimated as neo1 (4e/2a) plus neo2(2e/4a) ; Chr, *D-Chiro*-IP$_6$, estimated as chiro1 (4e/2a) plus chiro2(2e/4a); MyoP1, *myo*-IP$_1$; α-gly, alpha glycerol phosphate; β-gly, beta glycerol phosphate; AMP, adenosine monophosphate; Nuc, mononucleotides; Pcho, choline phosphate; g6P, glucose 6 phosphate; Fruc, fructose; Mon (1, 2 and 3), monoesters spectral areas; DNA, Deoxyribonucleic acid; Di(1 and 2), diester spectral areas

These were present in all samples (Table 5.3), at high enough percentages (0.7-4.6 %) to show them separately from the general Monoester 2 category. This 4.56-ppm peak is similar in chemical shift to peaks for phosphoryl ethanolamine and fructose-1 phosphate, while the 4.92-ppm peak is similar to phosphatidic acid (Cade-Menun 2015). They may be lower *myo*-inositol phosphates (Turner and Richardson 2004). Further spiking experiments are required to confirm the identities for these peaks. They do not align with the sharper peaks for high-molecular weight P compounds recently identified by McLaren et al. (2015) when corrected for differences in chemical shifts of identified compounds such as α- and β-glycerophosphate. However, we should note that the areas for the Monoester 1, Monoester 2 and Monoester 3 regions were determined by integrating across each of these regions and then subtracting the areas of identified peaks. As such, the area for the broad peaks of high molecular weight P compounds (McLaren et al. 2015) would be included in the area for Monoester 2 in our study.

Peaks for DNA were confirmed in all samples at –0.75 ± 0.06 and –0.85 ± 0.08 ppm. Other orthophosphate diesters were divided into two groups: diesters-1 ranging from 2.42 ± 0.06 to –0.428 ± 0.12 ppm, and diester-2 from -1.02 ± 0.07 to -3.52 ± 0.05 (Liu et al. 2015).

Nucleotides may be derived from hydrolysis of RNA (He et al., 2011), and ß and α-glycerophosphate from phospholipids during NMR analysis (Doolette et al. 2009). This assumption was taken into account to correct the concentration of monoesters and diesters in the samples (Young et al. 2013; Liu et al. 2013; Liu et al. 2015; Schneider et al. 2016). Corrected values were calculated in two different ways: (i) α-glycerophosphate, β-glycerophosphate, mononucleotides and half of the unidentified peaks in the monoester 2 category (correction 1; Liu et al. 2013; 2015; Young et al. 2013), and (ii) α-glycerophosphate, β-glycerophosphate, and all mononucleotides (correction 2; He et al. 2011; Noack et al. 2012; Vincent et al. 2013; Schneider et al. 2016). Even with correction, the percentages of orthophosphate monoesters were always greater than diesters for all samples in this study (Table 5.4).

Table 5.4. Groups of main P forms in NaOH-EDTA extracts

P status		**extr P**	**Pi**		**Total Poly**		**OP**		**IP_6t**		**C1 Mono**		**C2 Mono**		**C1 Di**		**C2 Di**	
		mg kg^{-1}	mg kg^{-1}	% extr P	mg kg^{-1}	% extr P	mg kg^{-1}	% extr P	mg kg^{-1}	% extr P	mg kg^{-1}	% extr P	mg kg^{-1}	% extr P	mg kg^{-1}	% extr P	mg kg^{-1}	% extr P
High	ECO	141	76,6	54,3	2,1	1,5	64,4	45,7	17,5	12,4	41,7	29,6	47,1	33,4	21,8	15,5	16,4	11,6
	ZMB	347	224,5	64,7	5,2	1,5	122,5	35,3	33,7	9,7	76,7	22,1	86,1	24,8	43,4	12,5	34,0	9,8
	PZB	208	144,6	69,5	4,4	2,1	63,4	30,5	18,7	9,0	40,5	19,5	46,2	22,2	21,5	10,4	15,8	7,6
	LCV	293	203,0	69,3	4,1	1,4	90,0	30,7	24,6	8,4	55,2	18,9	63,3	21,6	32,7	11,2	24,6	8,4
	ADM	589	487,7	82,8	4,1	0,7	101,3	17,2	35,9	6,1	68,6	11,7	76,6	13,0	28,6	4,9	20,6	3,5
	PLP	218	145,2	66,6	4,6	2,1	72,8	33,4	20,1	9,2	47,2	21,7	55,8	25,6	24,1	11,1	15,5	7,1
	OCL	169	102,2	60,5	8,3	4,9	66,8	39,5	17,1	10,1	40,1	23,7	45,8	27,1	25,5	15,1	19,8	11,7
	TRB	80	43,7	54,6	0,6	0,7	36,3	45,4	9,0	11,3	23,1	28,9	27,7	34,6	12,0	15,0	7,4	9,3
Mean		**256**	**178,4**	**65,3**	**4,2**	**1,9**	**77,2**	**34,7**	**22,1**	**9,5**	**49,1**	**22,0**	**56,1**	**25,3**	**26,2**	**12,0**	**19,3**	**8,6**
SD		**159**	**138,9**	**9,3**	**2,2**	**1,3**	**26,6**	**9,3**	**9,0**	**1,9**	**17,2**	**5,7**	**18,8**	**6,9**	**9,2**	**3,5**	**7,8**	**2,7**
Low	ECO	195	120,3	61,7	1,4	0,7	74,7	38,3	16,4	8,4	46,3	23,8	55,8	28,6	27,0	13,9	17,6	17,6
	ZMB	277	184,2	66,5	5,8	2,1	92,8	33,5	26,3	9,5	59,7	21,6	68,1	24,6	31,2	11,3	22,7	22,7
	PZB	148	87,9	59,4	2,1	1,4	60,1	40,6	21	14,2	37,8	25,6	44,3	29,9	20,2	13,7	13,8	13,8
	LCV	139	83	59,7	1	0,7	56	40,3	15,8	11,4	32,5	23,4	39,3	28,3	21,6	15,6	14,7	14,7
	ADM	176	112,8	64,1	2,5	1,4	63,2	35,9	17,1	9,7	35,4	20,1	42,6	24,2	24,1	13,7	16,9	16,9
	PLP	135	69,3	51,3	1,9	1,4	65,7	48,7	16,6	12,3	40,3	29,9	47,5	35,2	23,6	17,5	16,3	16,3
	OCL	221	111,4	50,4	13,7	6,2	109,6	49,6	23,9	10,8	57,7	26,1	69	31,2	50,4	22,8	39,1	39,1
	TRB	72	48,2	67	1,5	2,1	23,8	33	7,1	9,9	13,8	19,2	15,7	21,8	9,4	13,1	7,6	7,6
Mean		**170**	**102,1**	**60,0**	**3,7**	**2,0**	**68,2**	**40,0**	**18,0**	**10,8**	**40,4**	**23,7**	**47,8**	**28,0**	**25,9**	**15,2**	**18,6**	**18,6**
SD		**62**	**41,1**	**6,3**	**4,3**	**1,8**	**25,6**	**6,3**	**5,9**	**1,8**	**14,7**	**3,5**	**17,2**	**4,3**	**11,7**	**3,6**	**9,3**	**9,3**

extr P, total phosphorus extracted by the NaOH+EDTA; Pi, inorganic phosphorus; Poly, polyphosphates; OP, organic phosphorus estimated from NMR spectra ; IP_6t, total inositol hexaquisphosphate forms; CMono, corrected monoesters spectral areas from total estimated; CDi, corrected diesters spectral areas from total estimated; Total poly= pyro+poly

Main phosphorus compounds in soils

Overall, total OP determined by ^{31}P NMR accounted on average for 35 and 40 % of extracted P in alkali extracts, in high- and low-P samples, respectively (Table 5.4). The dominant form of Pi was orthophosphate, but polyphosphates and pyrophosphates were also detected in small amounts (Tables 5.3, 5.4). The latter is usually a minor component of Pi in soils (Murphy et al. 2009). It is a short-chain polyphosphate synthesized by microorganisms as a P storage compound (Condron et al. 2005), usually related to microbial biomass in soils. Orthophosphate concentrations determined in NaOH-EDTA extracts by ^{31}P NMR spectra were linearly related to Olsen P in soil ($R^2 = 0.6$; $P < 0.001$), which reveals that an increased P availability in soil is the consequence of Pi enrichment through fertilization.

Monoesters (Mono), which dominated OP, amounted to a similar percentage of OP in low-P samples and high-P samples (48 and 56 %, respectively after C2 correction; not shown). Overall, this percentage was in the range described in other agricultural soils from other geographical areas where monoesters (e.g. around 50-60 % in Cade-Menun et al. 2015b; Liu et al. 2015). Inositol phosphates (IP_6) in four stereoisomeric configurations (*myo*, *scyllo*, *neo*, *chiro*), were the most abundant identified group of monoesters (Table 5.3), and accounteing on average for 45 % of monoesters in both types of samples, in the range described by previous studies (Liu et al. 2013, 2015; Cade-Menun et al. 2015).

The IP_6 stereoisomers *myo-, neo-, chiro- and scyllo*-IP_6 were found in all the samples (Table 5.4). *Myo*-IP_6 has been described as the main known form of OP and the inositol phosphates (Turner et al. 2012; Jørgensen et al. 2015; Cade-Menun et al. 2015). This isomer is synthetized by plants and is present in cereal straw and in seeds (Noack et al. 2012; Liu et al. 2015). *Scyllo* and *neo* stereoisomers are not present in plant tissue and they are thought to be of microbial origin, perhaps synthesized by epimerization of the *myo* isomer (Giles and Cade-Menun 2014; Jørgensen et al. 2015). *Chiro*-IP_6 may originate from plants and microbes (Liu et al. 2015). In our soils, *chr*-IP_6 was as abundant as *myo*-IP_6 (even more in high-P samples) (Table 5.4), and was more abundant than s*cyllo*-IP_6, which is often reported as the second most abundant form of IP_6 stereoisomers in soil (Giles and Cade-Menun 2014). This relative abundance of IP_6 stereoisomers contrasts with other studies (Jørgensen et al. 2015; Schneider et al. 2016). This likely reveals a different pattern of accumulation through microbial epimerization of *myo*-IP_6 (Giles et al. 2015), de novo synthesis (Liu et al. 2015), different types of plant residues, or different stabilization mechanisms in soils affecting the different IP_6 stereoisomers when compared with studies performed in soils from other regions. *Myo*- and *scyllo*-IP_6 concentrations in soil increased with increased total OP in soil determined by the combustion method (logarithmically, R^2 = 0.5 and 0.29, $P < 0.01$ and 0.05, respectively). This reveals that the concentration of these two stereoisomers may depend on the accumulation of OP in soil. On the other hand, *chiro*-IP_6 was not related to total OP, thus revealing that its accumulation is less dependent on OP accumulation in soil, suggesting it depends more on potential degradation or epimerization from other IP_6 isomers.

Orthophosphate diesters (Di) contribution to total extracted P and OP in soil extracts (Table 5.3) was in the range described by Cade-Menun et al. (2015), but lower than percentages reported by Liu et al. (2015) and Schneider et al. (2016). Overall, and for both corrections methods, the ratio Mono:Di was greater than perviously described in the literature (Liu et al. 2015; Cade-Menun et al. 2015).

Effect of soil status and soil use on organic P forms

Organic P determined by dry combustion or ^{31}P NMR was not significantly different between high- and low-P samples (Table 5.1; Table 5.4). Additionally, the concentration of orthophosphate diesters (Di), and their relative contribution to extracted P (Table 5.3) or OP (not shown) did not significantly differ between low- and high-P samples or between different agricultural uses. Crop residues can be high in Di (Noack et al. 2012), which can contribute to their accumulation in soils (Condron et al. 2005; Liu et al. 2015). Thus, it can be concluded there is not any Di accumulation in the high P samples of this study from increased plant residues due to higher nutrient availability. This can be explained, at least in part, by the traditional management of crop residues in these fields, without incorporation to soil in field crops or in tree orchards. In fact, although some more residue supply would be expected in field crops through roots and stubble than in orchards, the different agricultural use did not affect Di concentration or their relative contribution to total or OP in NaOH-EDTA extracts (data not shown). Overall, the different agricultural uses did not result in any differences in the main OP compounds (data not shown).

Although the relative contribution of monoesters to total or OP in soil extracts was not significantly different in high- and low-P samples (Table 5.3), the difference in monoester concentrations between high- and low-P samples increased for each soil with increasing differences in Olsen P between both types of samples (Fig. 5.2). This likely reveals that P accumulation as monoester forms may depend on the soil P status and it is in agreement with previous studies (Murphy et al., 2009) showing that P fertilization may result in greater monoester accumulation in soil. This result suggests that a greater P availability status results in less degradation of monoesters. Although diesters are though to be more rapidly mineralized than monoesters, these latter forms, including IP_6, are known to be also mineralized and used by plants and microorganisms (Leytem et al. 2006; Cade-Menun and Hill 2009; Doolette et al. 2010; Jørgensen et al. 2015). The degradation of monoesters is known to be enhanced under P starvation conditions (García-López et al. 2015) when plants and microorganisms are more likely to utilize less available P forms (Liu et al. 2015). This degradation may explain the increased ratio of monoesters to diester with increased Olsen P in soil (Fig. 5.3). The degradation of diesters, more easily accessible to enzymatic hydrolysis than monoesters, likely does not depend on the available P status in soil, but the degradation of monoesters may increase with a decrease available P pool.

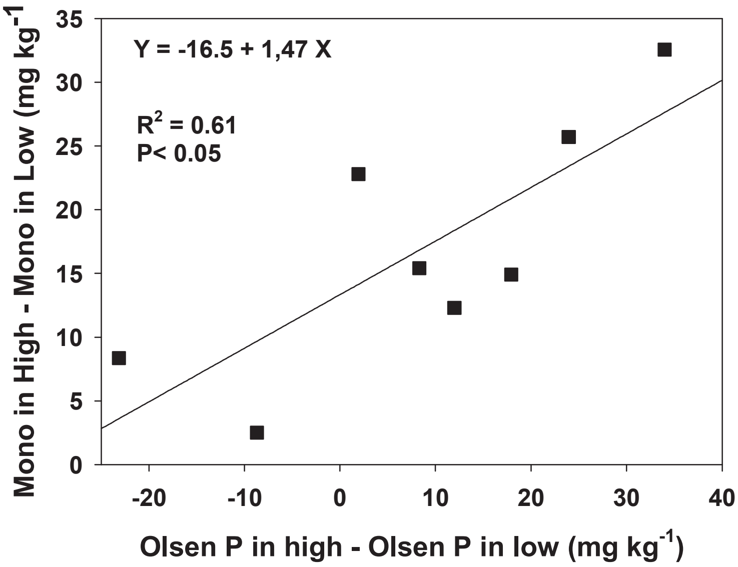


Figure 5.2. Relationship between the difference in monoester phosphates concentration between high- and low-P samples (using C2 correction for diester degradation), and the difference in Olsen P between the same samples.

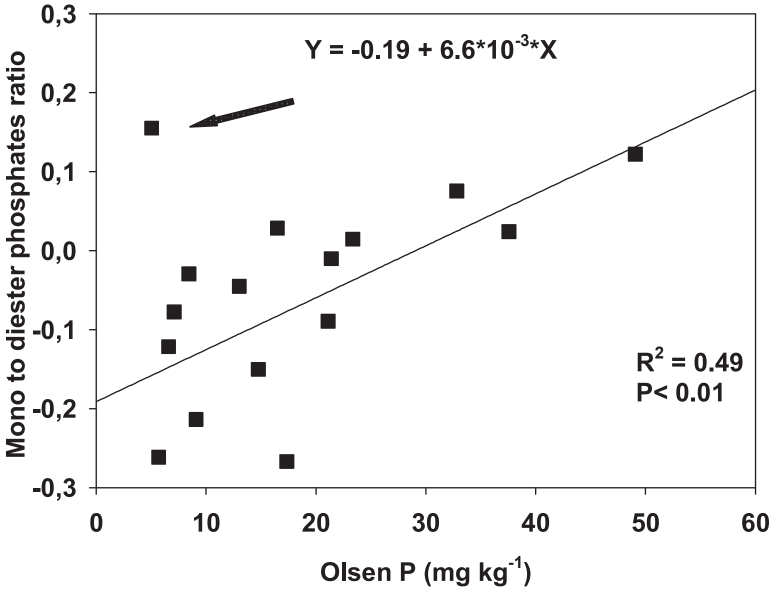


Figure 5.3. Relationship between the mono to diester phosphates ratio (C2 correction for diester degradation) and Olsen P in studied soils (all the samples, high- and low-P); data marked with an arrow was excluded from the regression.

Further evidence of monoester degradation from soil P limitation is the increased ratio of monoesters to OP (combustion method) with increased Olsen P in soil, which in our case was only evident in soils taken from vineyards or olive orchards (Fig. 5.4). This reveals that monoester mineralization is more evident when residue supply to soil is scarce, such as in tree orchards where there is not a minimum supply through decay roots and stubble as shown by its lower OP content relative to soils used for field crops. The same trend was observed for *myo-,* and *chiro*-IP_6 in soils from tree orchards (Fig.5.4), likely revealing an increased degradation of these compounds with rwhen soil available P is limited. The total amount of all IP_6 isomers, by except *scy*-IP_6, were linearly related to Olsen P ($P < 0.05$; data not shown). This may also contribute to prove an increased degradation of these organic compounds when P availability to plants in soil is low.

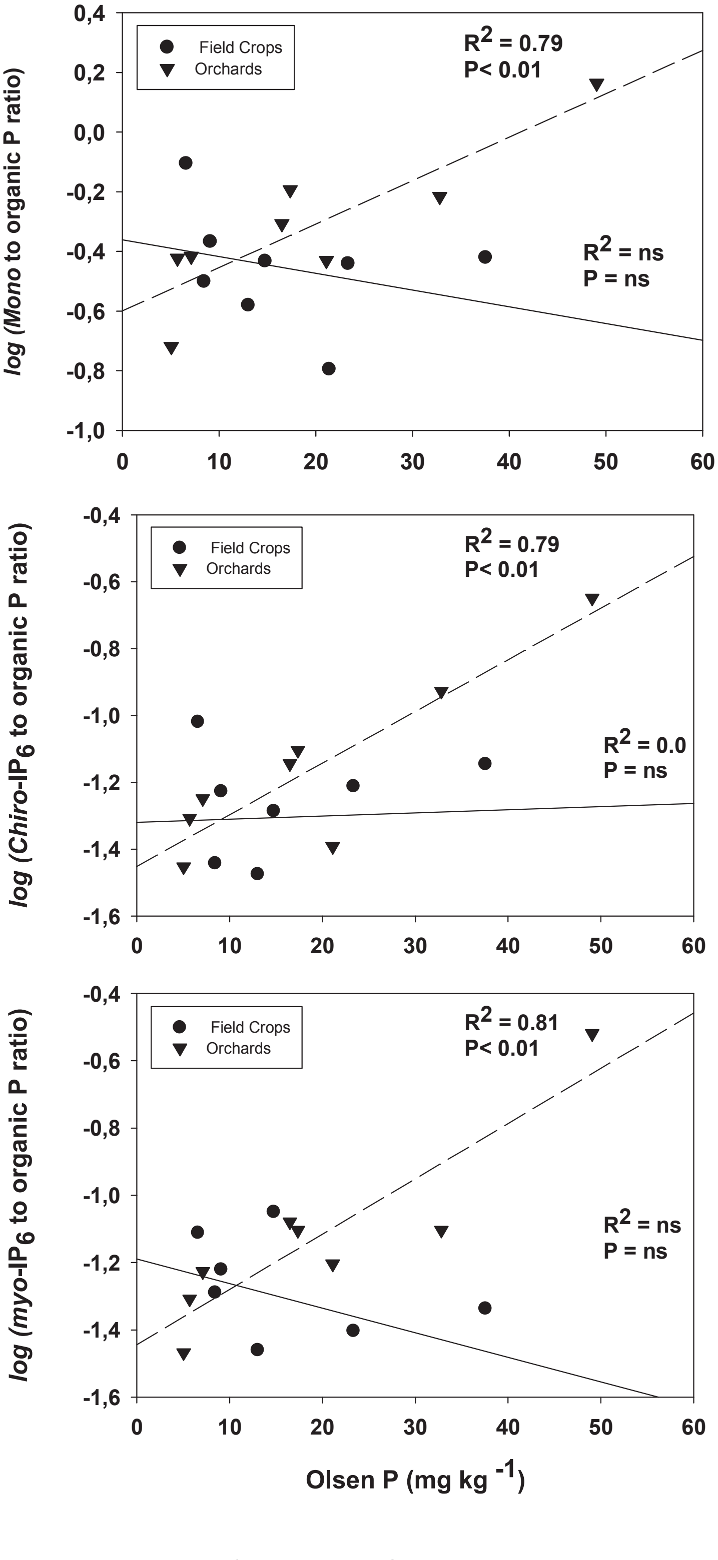


Figure 5.4. Relationship between: a) the ratio of monoester phosphates to total organic P and Olsen P; b) the ratio of chiro-IP6 to total organic P and Olsen P, and c) the ratio of myo-IP$_6$ to total organic P and Olsen P. Total organic P determined by the combustion method.

Relationship of P forms with soil properties

Overall, the relative contribution of Mono and Di to total extracted P in NaOH-EDTA extracts increased logarithmically with increasing affinity of soil for orthophosphate (increased *b* factor in the Freundlich equation; $R^2 = 0.47$, $P < 0.01$, and $R^2 = 0.77$, $P < 0.001$, for Mono and Di, respectively). Affinity of sorbent surfaces by OP and Pi is known to govern its release to the soil solution and thus its potential use by living organisms (Chacon et al. 2006). These results reveal that, in spite of an increased affinity for inorganic orthophosphate in soils, the main OP compounds, specifically IP_6 stereoisomers are preferentially retained over orthophosphate. This suggests that affinity for OP is greater than for Pi, which in agreement with Berg and Joern (2006), and explains at least in part why OP forms are less bioavailable than orthophosphate.

The concentration of *myo*-IP_6 in soils increased with increased ratios of Fe bound to oxides to clay content in soil (Fe_{ca}+Fe_d/clay) (logarithmically, $R^2 = 0.42$, $P < 0.01$), whereas for the other IP_6 isomers the relationship with the above mentioned ratio was not significant. In addition, *myo*-IP_6 was the only IP_6 isomer whose concentration in soil decreased with pH (logarithmically, R^2 0 0.29, $p < 0.05$). Clay content in soil provides an estimate of the total P sorption capacity in soil, not only by its usual correlation with Fe oxides content (in our case, $r = 0.56$, $P < 0.05$) as previously described by Giavenno et al. (2008), but because clay minerals can also absorb Pi and OP (Fuentes et al. 2014). A decreased sorption with increased pH can be ascribed to the dominance of adsorption of this OP form in soils (Celi et al. 2001; Fuentes et al. 2014). The positive relationship of *myo*-IP_6 with the ratio Fe_{ca}+Fe_d/clay, and its decreased concentration in soil with increasing pH, likely reveals a dominant role of adsorption on Fe oxides explaining its presence and stabilization in soil. In contrast, none of the other IP_6 steroisomers were significantly related to ratio of Fe in oxides to clay or to pH in soils. This perhaps reveals that processes other than adsorption on Fe oxides may explain the retention in soil of other IP_6 steroisomers, such as precipitation of Ca-phosphates or adsorption on clay minerals. A non-significant relationship of IP_6 sorption with pH in soils with high Ca saturation has been ascribed to precipitation of Ca salts (Fuentes et al. 2014). In our soils, the precipitation of Ca salts may be a relevant process contributing to IP_6 retention in soils according to the range of pH and Ca saturation (Celi et al. 2001). The lack of a significant relationship between concentration in soil and pH may likely reveal the relevance of this precipitation (Fuentes et al. 2014) which may explain the relative abundance of *chiro*- between IP_6 isomers when compared with other studies in more acidic soils (e.g. pH < 5.5 in Jørgensen et al. 2015; pH < 6.5 Giles et al. 2015). On the other hand, when compared with soils with higher pH such those studied by Schneider et al. (2016), pH ranging from 6.9 to 7.5, the ratio of *myo*- to *chiro*-IP_6 was closer to that observed by us. This supports the role of pH in IP_6 accumulation and stabilization in soils, not only through the effect on adsorption, but also likely affecting precipitation of these compounds.

Conclusion

Although, the forms of Pi and OP detected in the studied soils as by ^{31}P NMR are those usually described in literature, the relative contribution to total P was different to that reported for soils from other geographical areas. Among the IP_6 stereoisomers, *chiro*-IP_6 was as abundant as *myo*-IP_6, and the percentage of *scyllo*-IP_6 was much lower than in previous studies in other regions. The difference in monoester concentrations between high- and low-P samples increased for each soil with increasing differences in Olsen P between both types of samples. The contribution of monoesters and most of the IP_6 isomers to OP increased as increased Olsen P in soil, but only where the crop residue accumulation was scarce (vineyards and olive orchards). These results suggest that there is degradation of monoesters, including IP_6, under conditions of P limitation, indicating a potential contribution of these organic forms to P supply to plants. Adsorption on Fe oxides and precipitation of Ca phosphates appears to vary among IP_6 stereoisomers, influencing their retention and stabilization in soils.

Acknowledgement

This study was funded by the Spanish Ministry of Science and Innovation and the European Regional Development Fund of the European Union through the National Research, Development and Innovation Program (Plan Nacional I+d+i, AGL2011-29893-CO2-01) and by the Regional Government of Andalucia (Project AGR 63-85). Mr. Ramiro Recena was a PhD student with a grant from the Regional Government. The Agriculture & Agri-Food Canada Swift-Current Research and Development Centre provided facilities for the work of Mr. Ramiro Recena during his research stay under the supervision of Dr Barbara Cade-Menun. Fertiberia S.A. and OCA Lebrija collaborated in the collection of soil samples for the present study. We thank the Saskatchewan Structural Sciences Centre, and particularly Dr. Keith Brown, for assistance with the ^{31}P NMR analysis.

References

Abdi D, Cade-Menun BJ., Ziadi N, Parent LÉ (2014) Long-term impact of tillage practices and phosphorus fertilization on soil phosphorus forms as determined by P nuclear magnetic resonance spectroscopy. J Environ Qual 43:1431-1441. DOI: 10.2134/jeq2013.10.0424

Abdi D, Cade-Menun BJ, Ziadi N, Parent LÉ (2015) Compositional statistical analysis of soil 31 P-NMR forms. Geoderma 257-258:40-4. DOI: 10.1016/j.geoderma.2015.03.019

Aitchison J (1986) The Statistical Analysis of Compositional Data. 1st ed. Chapman and Hall, London, UK

Berg AS, Joern BC (2006) Sorption dynamics of organic and inorganic phosphorus compounds in soil. J Environ Qual 35:1855-1862. DOI: 10.2134/jeq2005.0420

Cade-Menun BJ, Preston CM (1996) A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. Soil Sci 161:770-785. DOI: 10.1097/00010694-199611000-00006

Cade-Menun BJ (2005) Characterizing phosphorus in environmental and agricultural samples by ^{31}P nuclear magnetic resonance spectroscopy. Talanta 66:359-371. DOI: 10.1016/j.talanta.2004.12.024

Cade-Menun BJ, Carter MR, James DC, Liu CW (2010) Phosphorus forms and chemistry in the soil profile under long-term conservation tillage: A phosphorus-31 nuclear magnetic resonance study. J Environ Qual 39:1647–1656. DOI: 10.2134/jeq2009.0491

Cade-Menun BJ, Liu CW (2014) Solution ^{31}P-NMR spectroscopy of soils from 2005-2013: A review of sample preparation and experimental parameters. Soil Sci Soc Am J 78:19-37. DOI: 10.2136/sssaj.2013.05.0187dgs

Cade-Menun BJ (2015) Improved peak identification in ^{31}P-NMR spectra of environmental samples with a standardized method and peak library. Geoderma 257-258:102-114. DOI: 10.1016/j.geoderma.2014.12.016

Cade-Menun, BJ, He Z, Zhang H, Endale DM, Schomberg HH, Liu CW (2015). Stratification of phosphorus forms from long-term conservation tillage and poultry litter application. Soil Sci Soc Am J 79(2): 504-516. DOI: 10.2136/sssaj2014.08.0310

Chacon N, Silver WL, Dubinsky EA, Cusack DF (2006) Iron reduction and soil phosphorus solubilization in humid tropical forests soils: the roles of labile carbon pools and an electron shuttle compound. Biogeochemistry 78(1): 67-84. DOI: 10.1007/s10533-005-2343-3

Condron LM, Turner BL, Cade-Menun BJ (2005) Chemistry and dynamics of soil organic phosphorus. In: Sims JT, Sharpley AN (eds) Phosphorus, agriculture and the environment. Monograph 46. Soil Sci Soc Am. Madison, WI, pp 87-121. DOI.org/ 10.1104/pp.111.175232

Celi L, Presta M, Ajmore-Marsan F, Barberis E (2001) Effects of pH and electrolytes on inositol hexaphosphate interaction with goethite. Soil Sci Soc Am J 65:753-760. DOI: 10.2136/sssaj2001.653753x

de Santiago A, Delgado A (2006) Predicting iron chlorosis of lupin in calcareous Spanish soils from iron extracts. Soil Sci Soc Am J 70:1945-1950. DOI: 10.2136/sssaj2005.0343

Delgado A, Madrid A, Kasem S, Andreu L, del Campillo MC (2002) Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. Plant Soil 245:277–286. DOI: 10.1023/A:1020445710584

Delgado A, Scalenghe R (2008) Aspects of phosphorus transfer in Europe. J Plant Nutr Soil Sci 171:552–575. DOI: 10.1002/jpln.200625052

Díaz-Espejo A, Serrano, L Toja, J (1999) Changes in sediment phosphate composition of seasonal ponds during filling. Hydrobiologia 392:21-28. DOI: 10.1023/A:1003557408640

Díaz I, del Campillo MC, Barrón V, Torrent J, Delgado A (2013) Phosphorus losses from two representative small catchments in the Mediterranean part of Spain. J Soils Sediments 13:1369-1377. DOI: 10.1007/s11368-013-0740-0

Doolette, AL, Smernik RJ, Dougherty WJ (2010) Rapid decomposition of phytate applied to a calcareous soil demonstrated by a solution ^{31}P NMR study. Eur J Soil Sci 61:563–575. DOI: 10.1111/j.1365-2389.2010.01259.x

Doolette AL, Smernik RJ, Dougherty WJ (2009) Spiking improved solution phosphorus-31 nuclear magnetic resonance identification of soil phosphorus compounds. Soil Sci Soc Am J 73:919-927. DOI: 10.2136/sssaj2008.0192

Fuentes, B, de la Luz Mora, M, Bol, R, San Martin, F, Pérez, E, and Cartes, P (2014) Sorption of inositol hexaphosphate on desert soils. Geoderma 232:573-580. DOI: 10.1016/j.geoderma.2014.06.016

Garcia-Lopez AM, Aviles M, Delgado A (2015) Plant uptake of phosphorus from sparingly available P-sources as affected by Trichoderma asperellum T34. Agr Food Sci 24:249-260

Gee GW, Bauder JW (1986) Particle-size analysis. In: Klute A (ed) Methods of soil analysis, Part 1, 2nd edn. ASA and SSSA, Madison, WI, pp. 383–411.

Giaveno C, Celi L, Cessa RMA, Prati M, Bonifacio E, Barberis E (2008) Interaction of organic phosphorus with clays extracted from oxisols. Soil Sci 173:694-706. DOI: 10.1097/SS.0b013e3181893b59

Giles CD, Cade-Menun BJ, Hill JE (2011) The inositol phosphates in soils and manures: Abundance, cycling and measurement. Can J Soil Sci 91:397-416. DOI: 10.4141/CJSS09090

Giles CD, Cade-Menun BJ (2014) Phytate in animal manure and soils: Abundance, chemistry and bioavailability. Applied manure and nutrient chemistry for sustainable agriculture. Springer, NY. DOI 10.1007/978-94-017-8807-6_9

Giles CD, Cade-Menun BJ, Liu CW, Hill JE (2015) The short-term transport and transformation of phosphorus species in a saturated soil following poultry manure amendment and leaching. Geoderma 257-258:134-141.

Gil-Sotres F, Zech W, Alt HG (1990) Characterization of phosphorus fractions in surface horizons of soils from Galicia (N.W. Spain) by ^{31}P NMR spectroscopy. Soil Biol Biochem 22:75-79. DOI:10.1016/0038-0717(90)90063-6

He ZQ, Olk DC, Cade-Menun BJ (2011) Forms and lability of phosphorus in humic acid fractions of Hord silt loam soil. Soil Sci Soc Am J 75:1712–1722. DOI: 10.2136/sssaj2010.0355

Hill JE, Cade-Menun BJ (2009) Phosphorus-31 nuclear magneticresonance spectroscopy transect study of poultry operations on the Delmarva Peninsula. J Environ Qual 37:1–9. DOI: 10.2134/jeq2007.0587

Jørgensen C, Turner BL, Reitzel K (2015) Identification of inositol hexakisphosphate binding sites in soils by selective extraction and solution 31 P NMR spectroscopy. Geoderma, 257: 22-28. DOI: 10.1016/j.geoderma.2015.03.021

Kuo S (1996) Phosphorus. In: Sparks DL (ed) Methods of soil analysis, Part 2, Chemical properties, 3rd edn. ASA, SSSA, CSSA, Madison, WI, pp 869–919.

Leytem AB, Smith DR, Applegate TJ, Thacker PA (2006) The influence of manure phytic acid on phosphorus solubility in calcareous soils. Soil Sci Soc Am J 70:1629-1638. DOI: 10.2136/sssaj2006.0003

Liu J, Yang J, Cade-Menun BJ, Liang X, Hu Y, Liu CW, Zhao Y, Li L, Shi. J (2013) Complementary phosphorus speciation in agricultural soils by sequential fractionation, solution ^{31}P NMR, and P K-edge XANES spectroscopy. J Environ Qual 42:1763-1770. DOI: 10.2134/jeq2013.04.0127

Liu, J, Hu YF, Yang JJ, Abdi D, Cade-Menun BJ (2015) Investigation of soil legacy phosphorus transformations in long-term agricultural fields using sequential fractionation, P K-edge XANES and solution P-NMR spectroscopy. Environ Sci Technol 49:168-176. DOI: 10.1021/es504420n

McDowell RW, Stewart I, Cade-Menun BJ (2006) An examination of spin-lattice relaxation times for analysis of soil and manure extracts by liquid state phosphorus-31 nuclear magnetic resonance spectroscopy. J Environ Qual 35:293-302. DOI: 10.2134/jeq2005.0285

McLaren, TI, Smernik RJ, McLaughlin, MJ, McBeath TM, Kirby JK, Simpson RJ, Guppy CN, Doolette, AL, Richardson AE (2015) Complex forms of soil organic phosphorus – a major component of soil phosphorus Environ Sci Technol 49:13238-13245. DOI: 10.1021/acs.est.5b02948

Murphy PNC, Bell A, Turner BL (2009) Phosphorus speciation in temperate basaltic grassland soils by solution 31P NMR spectroscopy. Eur J Soil Sci 60:638–651. DOI: 10.1111/j.1365-2389.2009.01148.x

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36 doi:10.1016/S0003-2670(00)88444-5. DOI:10.1016/S0003-2670(00)88444-5

Noack SR, McLaughlin MJ, Smernik RJ, McBeath TM, Armstrong RD (2012) Crop residue phosphorus: speciation and potential bio-availability. Plant Soil 359:375-385. DOI: 10.1007/s11104-012-1216-5

Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939, US Government Printing Office, Washington DC.

Recena R, Torrent J, del Campillo MC, Delgado A (2015) Accuracy of Olsen P to assess plant P uptake in relation to soil properties and P forms. Agron Sustain Dev 35:1571-1579. DOI: 10.1007/s13593-015-0332-z

Saavedra C, Delgado A (2005a) Phosphorus fractions and release patters in typical Mediterranean soils. Soil Sci Soc Am J 69:607-615. DOI: 10.2136/sssaj2004.0156

Saavedra C, Delgado A (2005b) Iron-related phosphorus in eroded sediments from agricultural soils of Mediterranean areas. Geoderma 125:1-9. DOI: 10.1016/j.geoderma.2004.06.001

Saavedra C, Velasco J, Pajuelo P, Perea F, Delgado A (2007) Effects of tillage on phosphorus release potential in a Spanish Vertisol. Soil Sci Soc Am J 71:56-63. DOI: 10.2136/sssaj2006.0162

Schneider KD, Cade-Menun BJ, Lynch DH, Voroney PR (2016) Soil phosphorus forms from organic and forage fields. Soil Sci Soc Am J. DOI: 10.2136/sssaj2015.09.0340

Soil Survey Staff (2010) Keys to soil taxonomy, 11th ed. USDA-Natural Resources Conservation Service, Washington, DC.

StatPoint. 2000. Statgraphic plus 5.1. Rockville, Maryland, USA.

Stutter M, Shand C, George T, Blackwell M, Bol R, MacKay R, Haygarth P (2012) Recovering phosphorus from soil: a root solution. Environ Sci Technol 46:1977–1978. DOI: 10.1021/es2044745

Sumner ME, Miller WP (1996) Cation exchange capacity, and exchange coefficients. In: Sparks DL (ed) Methods of soil analysis, Part 2, Chemical properties, 3rd edn. ASA, SSSA, CSSA, Madison, WI, pp 1201-1229

Trasar-Cepeda MC, Gil-Sotres F, Zech W, Alt HG (1989) Chemical and spectral analysis of organic P forms in acid high organic matter soils in Galicia (N.W. Spain). Sci Tot Environ 81/82:429-436

Turner BL, Cade-Menun BJ, Condron LM, Newman S (2005) Extraction of organic phosphorus from soil and manure. Talanta 66:294-306

Turner BL (2007) Inositol phosphates in soil: amounts, forms and significance of the phosphorylated stereoisomers. In: Turner BL, Richardson AE, Mullaney EJ (eds) Inositol phosphates: Linking agriculture and the environment. CAB International, Wallingford, UK, pp 186–206

Turner BL, Cheesman AW, Godage HY, Riley AM, Potter BVL (2012) Determination of *neo*- and D-*chiro*-inositol hexakisphosphate in soils by solution ^{31}P NMR spectroscopy. Environ Sci Technol 46:4994-5002. DOI**:** 10.1021/es204446z.

Turner BL, Mahieu N, Condron LM (2003) Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. Soil Sci Soc Am J 67:497-510

Turner BL, Richardson AE (2004) Identification of *scyllo*-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. Soil Sci Soc Am J 68:802-808

Turrión MB, Gallardo JF, Haumaier L, González MI, Zech W (2001) ^{31}P-NMR characterization of phosphorus fractions in natural and fertilized forest soils. Ann For Sci 58:89-98

Turrión MB, Lafuente F, Aroca JJ, López O, Mulas R, Ruipérez C (2010) Characterization of soil phosphorus in a fire-affected forest *Cambisol* by chemical extractions and ^{31}P-NMR spectroscopy analysis. Sci Tot Environ 408:3342-3348

Vincent, A., J. Vestergren,G. Gröbner,P. Persson,J. Schleucher, and R. Giesler. (2013) Soil organic phosphorus transformations in a boreal forest chronosequence. Plant Soil 367:149-162.

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci 63:251–263

Young, E.O., D.S. Ross, B.J. Cade-Menun, and C.W. Liu. (2013) Phosphorus speciation in riparian soils: A phosphorus-31 nuclear magnetic resonance spectroscopy and enzyme hydrolysis study. Soil Sci Soc Am J 77:1636-1647.

Zhang A, Chen Z, Zhang G, Chen L, Wu Z (2012) Soil phosphorus composition determined by 31P NMR spectroscopy and relative phosphatase activities influenced by land use. Eur J Soil Biol 52:73–77

Zohar I, Cade-Menun B, Paytan A, Shaviv A (2014) Phosphorus transformations from reclaimed wastewater to irrigated soil: A ^{31}P NMR study. Soil Sci Soc Am J 78:1884-1892

Figure Captions

Figure 5.1. ^{31}P NMR spectra of NaOH-EDTA extract of soils PLP and ZMB for low-P and high-P samples

Figure 5.2. The relationship between the difference in monoester phosphate concentrations between high- and low-P samples (using C2 correction for diester degradation), and the difference in Olsen P between the same samples.

Figure 5.3. The relationship between the mono to diester phosphates ratio (C2 correction for diester degradation) and Olsen P in studied soils (all the samples, high- and low-P); data point marked with an arrow was excluded from the regression.

Figure 5.4. The relationships between: a) the ratio of monoester phosphates to total organic P and Olsen P; b) the ratio of chiro-IP6 to total organic P and Olsen P, and c) the ratio of myo-IP$_6$ to total organic P and Olsen P. Total organic P determined by the combustion method.

Chapter VI. General Discussion

General Discussion

Accuracy of Olsen P to assess plant P uptake

To achieve an efficient management of P in agriculture which also contributes to avoid phosphorus (P) losses by runoff or drainage and consequent eutrophication, an accurate P fertilization management is required. It is difficult to develop general methods to pinpoint available P for plants (Delgado et al., 2010) due to the wide and variable nature of soils. Poor relationships are frequently observed between soil P test (SPT), and particularly the widely used Olsen P, and P uptake by plants (Kulhanek et al., 2007), even in soils with very similar physico-chemical properties (Delgado et al., 2010).

Thus, we studied which soil properties affect the accuracy of Olsen P as indicator of available P in soil by conducting a soil P depletion experiment in pots, in which the equivalent amount of soil to 1 mg of Olsen P was settled. This depletion experiment was performed with “high”, and “low” P samples for each soil, i.e., two samples per soil ranging widely in Olsen P values. Thus, with this experimental setup, in low-P pots, the ratio of theoretically non-available P to available P was higher than in high-P samples. Also, the ratio of organic P to Olsen-P was lower in "high-P" soil samples than in "low-P" soil samples. Soils, besides their different P status, ranged widely in properties, particularly in carbonates and Fe oxides, which are properties affecting P dynamics in soils. According to the ratio between the total available P to Olsen P observed by Delgado and Torrent (1997) in similar soils (1.5 times the value of Olsen-P), over 10% of applied P may remain available for crops.. P uptake by crops at both "low-P" and "high-P" soil samples were not equivalent or proportional to the corresponding Olsen-P values. Overall, P uptake values were significantly higher, 11 % on average, in high-P samples than in low-P samples. This was in agreement with previous studies (e.g. Delgado et al. (2010; Tandy et al., 2011) revealing that P uptake by plants was not well related to Olsen P values. Thus, these results confirm other previous studies showing that Olsen P, as an estimate of the "quantity" factoris not directly related to P uptake by crops (Sanchez et al., 2014). In addition, the accuracy of Olsen P seems to be also affected by the P status of soil, since P uptake was greater in high- than in low-P samples. Amounts of P extracted by crop in both high- and low-P samples decreased with increased affinity of soil particles for P. This affinity was estimated from the factor (*b*) in the Freundlich equation (P adsorbed = A $[P]^{1/b}$). This revealed the implications of factors affecting the equilibrium between solid and liquid phase on P adsorption by crops. The P release from the soil phase is thought to be partially explained by soil affinity for P (McDowell et al., 2002), and it has been described that P availability may be affected by the affinity for P (Delgado et al., 2010). The affinity of soil for P depends on the nature and concentration of the adsorbent surfaces (Colombo et al., 1994; Guzman et al., 1994). The affinity for P decreased with increased ratios of Fe in poorly crystalline to that in crystalline Fe oxides (Fe_{ca}/Fe_d) because crystalline Fe oxides has a higher affinity for P than poorly crystalline Fe oxides. On the other hand, P uptake by crops in high-P samples showed a positive correlation with the ratio Feca/Fed and the total concentration of Fe in oxides (Feca + Fed). The former correlation can be explained by the relationship between oxide type and affinity, and the second because a high content of adsorbing surfaces may imply a dominant role of P adsorption relative to P precipitation. In Mediterranean soils, adsorbed P appears to be more available than precipitated P in the form of insoluble metal phosphates (Delgado and Torrent 1997; Saavedra and Delgado 2005).

In low-P soil samples, phosphatse activity was significantly higher than in high-P soil samples. In turn, phosphatase activity were positively correlated with the total concentration of organic P in soil, but not with the absorption of P by the crops. However, when organic P concentration and phosphatase activity in the rhizosphere were included in the predictive model in low-P samples 37 % of the P uptake by crop was explained. In addition, phosphatase in rhizosphere and pH explained 50 % of P uptake by crops in low-P samples. Thus, it can be concluded that the affinity for P of soil surfaces, the P adsorption capacity (estimated form the Fe in oxides), non-readily available P forms such as organic P, and P mobilization processes such as hydrolytic activity in the rhizosphere activity were factors affecting the low accuracy of Olsen P in estimating plant available P. However, the relevance of these processes affecting accuracy of Olsen P seems to vary depending on the soil P status. It is well known that P mobilization mechanisms in the rhizosphere such as acidification, exudation of organic anions, and hydrolytic activity contribute to P supply to plants (Mollier et al., 2008; Hinsinger et al., 2011). Our results however, revealed that the relevance of these processes at the rhizosphere was greater in low-P samples where the saturation of soil by P was lower and where the ratio of non-readily available P (including organic) to available P in pots was greater than in high-P samples.

Estimation of threshold Olsen P values for fertilizer response

The involvement of organic and inorganic P compounds in P availability to plants and in the loss of P from soil are fairly well-known facts. P release from soil particles is controlled in part by the equilibrium established between the concentration of phosphate in the solid phase ("quantity" factor, *Q*) and its concentration in the solution ("intensity" factor, *I*). The Q to I ratio ("capacity" factor or P buffer capacity) controls the dynamic balance between the P present in the solid phase and the liquid phase of the soil. As the P is removed from the soil solution, for example by absorption by plants, the capacity factor is responsible of maintaining a P concentration in solution through desorption. When the P concentration in soil solution increases, due for example to fertilization, a new equilibrium is established, increasing the amount of P associated with the solid phase through the sorption process. The characteristics of the absorbent surfaces, as well as pH and the concentration of different cations and anions in the solution are relevant factors affecting the processes of sorption/desorption. Also soil organic matter can decrease P sorption in soils or, when complexed with Fe and Al, it possess the ability to retain phosphate by adsorption reactions (Gerke and Hermann, 1992).

To elucidate which soil properties affect threshold Olsen P values for fertilizer response, and to develop models to estimate accurate threshold values for a more precise use of this soil P test (SPT), a P starvation pot experiment was performed in 18 soils representative from Mediterranean areas.

As expected, the P buffer capacity (PBC) increased with increased clay content (Shirvani et al., 2005) and the affinity decreased with increased ratios of Fe in poorly crystalline to that in crystalline Fe oxides. As mentioned avove, these poorly crystalline Fe oxides have lower affinity for P than crystalline ones (Delgado et al., 2010; Colombo et al., 1994).

Threshold values for a given soil P test (SPT) can range widely among those soils for which its use is recommended (e.g. for Olsen P; Delgado et al. 2010; Sánchez-Alcalá et al., 2015). In soils with very similar properties, Delgado et al., (2010) observed a wide range in threshold values for Olsen P. In our soils, the threshold values for Olsen P ranged from 4 to 16.5 mg kg^{-1}, in the range described in the literature for many field and pot experiments (Delgado et al., 2010). Our results revealed that threshold values for P-Olsen could have been accurately predicted in our soils by multiple regressions involving different soil properties. Clay content, pH and phosphatase activity in the rhizosphere explained about 90% of the variation in threshold values for P-Olsen. In addition, clay, soil organic carbon, and P hydrolyzed by phytase in citrate-bicarbonate (CB) extracts, explained about 75% of the variation for Olsen P threshold values.

The threshold values for the usual SPT do not take into account the participation of organic P in the absorption of P by crops. However, our results reveal the importance of some factors related to organic P, such as hydrolyzable P by phytase and phosphatase activity in the rhizosphere, in the definition of threshold value for Olsen P. In addition, organic carbon seems to affect also these threshold values, which is known to affect the balance between P in solid phase and soil solution. This occurs by competing for the adsorptive surfaces and inhibiting the precipitation of insoluble metal phosphates (Delgado et al., 2002; Saavedra et al., 2007).

Threshold values for P extracted with 0.01 M $CaCl_2$ (P_{CaCl2}) were also determined. These values were influenced by the phytase hydrolyzable P in CB fraction. This also reveals the potential contribution of OP to P supply to plants. Moreover, pH, ACCE, and the Fe_{ca} to Fe_d ratio, may be taken into account in explaining P_{CaCl2} threshold values. The effect of Fe_{ca}/ on these threshold values can be explained by the difference in affinity for P between poorly crystalline and crystalline Fe oxides (Colombo et al. 1994). The relationship of pH with PBC and clay makes it difficult to show clear conclusions; the effect of pH can be ascribed not only to its influence on soil P dynamics but also by its correlation with other mineralogical properties, such as sorbent surface type, also affecting P availability to plants (Delgado and Torrent, 1997). The Fe in Fe oxides was negatively correlated with the active calcium carbonate equivalent (ACCE), which likely evidenced that mineral weathering and subsequent precipitation of oxides during soil genesis is inhibited when soil pH is buffered by carbonate.

Overall, e buffer capacity was found to be a fundamental property explaining the variability in the threshold Olsen P and P_{CaCl2} values and the effect of other properties such as clay content can be explained at least in part by their relationship with PBC.

From present results, a useful single model for estimating threshold values for Olsen P can be proposed involving clay content and pH and able to explain 80% in the variation of these threshold values. If clay content is only taken into account, about 60% of variation in threshold values can be explained. In practice, the easiest way to translate this type of model is to recommend different threshold values depending on clay content and pH, in such a way that threshold values decreased with increased clay contents and pH values in soil These models should be implemented for practical recommendations with experiments at field scale and for different crops.

Estimation of total plant available phosphorus

Soil P availability should be considered the result, not only of P accumulation, but also the consequence of complex physical, chemical, and biological interactions at the rhizosphere level in soil where P mobilization strategies by plant roots and microorganisms may crucially contribute to P uptake by plants.

Based on a P depletion experiment with 18 representative soils from Mediterranean areas, total plant available P (TAP) was estimated for each soil, considering low- and high-P samples described above as the same data set to this end. Phosphorus concentration in soil solution (i.e., in 0.01 M $CaCl_2$ extracts) at which a null P uptake in plants can be expected (threshold values for P uptake) were determined, and based on these values and on the cumulative P uptake curve (i.e.

relationship between accumulated P uptake and P in 0.01 M $CaCl_2$ extracts) TAP was estimated. P concentration for null P uptake ranged widely between soils, and depended on the ratio Fe_{ca}/Fe_d, and soil organic matter (SOM). A greater P concentration in soil solution can be expected at increased ratios of Fe in poorly crystalline to that in crystalline Fe oxides due to the nature of the sorbent surfaces. Poorly crystalline Fe oxides have less affinity for P than crystalline Fe oxides, and consequently, greater P concentration in solution can be expected with increasing values of this ratio. Besides, SOM competes for sorption sites with P and interferes in the precipitation of poorly soluble metal phosphates (Delgado et al., 2002). Therefore, for similar P enrichment in soil, and for similar amount of available P (*Q* factor), greater P concentration in soil solution can be expected in soils with higher portion of Fe in oxides ascribed to poorly crystalline ones and with a greater concentration of SOM. However, the capacity of replenish P in solution may be finished at higher P concentration in solution. On the other hand, buffer capacity for P and properties correlated with it, such as clay content negatively affected P_{CaCl2} threshold values (Sánchez-Alcalá et al., (2015); thus this probably means that the soil have the capacity of releasing P to plants from P in solid phase till low P concentration in solution

Available P portion of total P in soil depends on physical-chemical properties such as texture, pH, mineralogy and soil organic matter. Also, biological activities in rhizosphere, i.e., plants and microorganisms that produce organic acids and hydrolytic enzyme exudates (García-López et al., 2015), are also factors affecting available P from soil as mentioned above.

A single SPT is usually related to only one factor related to P availability to plants; e.g. Olsen P is an estimate of the quantity factor (*Q*), or P concentration in $CaCl_2$ extracts can be deemed an estimated of the intensity factor (*I*). However, P extractions with near-infinite sink (e.g. anion exchange resins, AER) are proved to be related not only to *Q,* but also with BC. This means that this type of extraction could provide better predictions of TAP than classical SPTs based on single chemical extractions since they involve more than one factor affecting P availability to plants. This is confirmed by our results, which revealed that more accurate estimations of TAP were achieved with AERs than with single chemical extractions such as Olsen P. Both anion exchange resins (AER) tested were able to explain at least 77% of TAP variation in TAP, much more than Olsen P which only explained 53 %. None of the other single chemical extractions such as CB or NaOH+CB improved estimations achieved with Olsen P.

Anion exchange resins in Cl^- from explained near 80 % of variation in TAP, and AER membranes in bicarbonate form explained near 90 % of this variation. The better results of AER in HCO_3^- form has than in Cl^- form (Delgado and Torrent, 1997) can be explained because in HCO_3- form they mimic better the effect of plant roots than resins in Cl- form (Sibbesen, 1978). On the other hand, when AERs in Cl^- form are used, the Cl^- accumulated in solution inhibits the exchange reaction (Myers et al., 2005) and do not simulates effects of rhizosphere on soil.

The highly significant and negative correlation between P extracted with AERs and P buffer capacity (PBC) may be explained because resins are not really near-infinite P sinks, and relatively high P concentrations in the soil solution should remain for effective P extraction. Thus, their efficiency in P extraction from soil depends on the capacity of soil to replenish P in soil solution (Delgado et al., 2010). Negative correlation between PBC and P extracted with AERs may be explained, in our case, because soils with high PBC were soils which maintained low P concentration in solution, and thus negatively affecting amounts extracted by AERs. Other properties contributing to explain equilibrium between P in soil solution and P in solid phase, such as Fe_{ca}/Fe_{cbd}, improve predictions of different SPT tested, by except HCO_3-AER. Kinetics factors in P release can be also relevant explaining TAP. The increase in the amount extracted with Cl-AER between 1 and 24 h was significantly correlated with TAP.

Overall, AER extractions performed better when compared with single chemical extractions, including Olsen P. This can be explained at least in part because AER extraction are more correlated with PBC than single extractions. Phosphorus buffer capacity (PBC) is a crucial property explaining variability in P availability indexes (Bolland et al., 1994; Ehlert et al., 2003), and the correlation of P availability indexes values with soil properties such clay or pH can be explained, at least in part, by the correlation of these soil properties with PBC.

As mentioned above, the portion of Fe in oxides ascribed to poorly crystalline oxides (Fe_{ca}/Fe_d) was relevant explaining the accuracy of extractions methods in predicting TAP. When this ratio was taken into account along with Cl-AER, 84 % of the variance in TAP was explained. It has been demonstrated that the amorphous metal oxides of Fe and Al sorb relatively greater amounts of phosphorus than crystalline Fe oxides (McLaughlin et al., 1981), but the affinity of the formers is lower than in the latters. This explain the relationship of the Fe_{ca}/Fe_d ratio with TAP.

The best results in estimating TAP obtained with HCO_3-AER can be explained not only by it being an estimation of *Q* (correlated with estimates by chemical extractions), but also by its more significant correlation with PBC than other index tested, and also with the Fe_{ca}/Fe_{cbd}, which has been shown to be relevant in explaining the effect of SPT. In addition, it was the extraction more significantly correlated with the estimate of the *I* factor (P_{CaCl2}). Thus, it seems to better reflect most of the factors affecting the equilibrium of inorganic P between solid and liquid phases in soils and consequently affecting P availability to plants.

There were some evidences of potential contribution of organic P to P supply to plants. NaOH plus CB from Ruiz et al., (1997) fractionation extracts seemed to embrace some of the potential organic P contribution to plants (Zaidi et al., 2013). In our case, when the phytase hydrolysable P in NaOH and CB extracts was taken into account, variance of TAP explained by Cl-AER increased to 86 %.

Estimation of TAP with Olsen P significantly improved with the inclusion of PBC and Fe_{ca}/Fe_{cbd} in a multiple regression. In our soils, the CCE is not related to PBC, but it is negatively correlated with TAP in agreement with Afif et al., (1993). When physicochemical soil properties related to PBC and Fe_{ca}/Fe_{cbd} were included in a multiple regression with Olsen P, i.e. clay and carbonates, 80 % of the variance in TAP was explained. This means that single models which take into account these properties can be effective in using Olsen P in assessing TAP in representative soils from Mediterranean areas.

Organic phosphorus forms as related to soil properties and fertilization

Results described above reveal that organic P seems to contribute to the availability of this element to crops. Due to the lack of knowledge on organic P forms, particularly in Mediterranean soils, their study become necessary to understand its dynamics in soils and potential contribution to P availability to plants. The scarce information is also noticed with regards to the relationships between OP forms and soil properties or soil use. Studies using ^{31}P-NMR spectra in NaOH-EDTA soil extracts during the last two decades have showed that orthophosphate forms are dominant between inorganic and organic P forms (Zhang et al., 2012). We have performed a study using this spectroscopy technique with 8 soils, selected from the collection using in the previous chapters, ranging widely in soil properties; for each soil a low-P and a high-P sample was used. Soils used include two types of use: (i) rainfed field crops (in most of the cases wheat-sunflower rotation), (ii) and rainfed trees orchards (vineyars or olive).

Orthophosphate concentrations in NaOH-EDTA extracts by ^{31}PNMR spectra were linearly related to Olsen P in soil ($R^2 = 0.6$; $P < 0.001$). Thus, an increased P availability in soil may be the consequence of Pi enrichment through fertilization. Other forms of inorganic P were also detected, such as pyrophosphates and polyphosphates. Pyrophosphates are minor components of P in soil (Murphy et al., 2009) and pyrophosphates are related to microbial biomass as a P storing form in cells (Cade-Menun et al., 2000).

Monoesters phosphate (Mono) were the dominant organic P forms. They accounted for a similar percentage of total extracted P in both "low-P" and "high-P" soil samples (28 and 25%, respectively) and of organic P (48 and 56%, respectively) in NaOH-EDTA extracts. This is in agreement with the range described in several works with the monoester correction for diester degradation (Zohar, et al., 2014; Cade-Menun et al., 2015b; Liu et al., 2015; Giles et al., 2015). Mono was always present in greater concentration than diesters in all the soil samples studied. Concentration of monoesters phosphate is thought to be affected by long-term soil management. In our soils, the difference in Monoesters phosphate concentration between "high-P" and "low-P" soil samples increased with increased differences in the Olsen-P values between "high-P" and "low-P" soil samples. This suggests that a greater P availability status in soil may result in less degradation of monoesters.

Inositol phosphate (IP_6), with its four stereoisomer´s configurations (*myo-, scyllo-, neo-, chiro-*), was found in our soils as the main form between monoesters phosphates, in agreement with many previous evidences (Liu et al., 2014). IP_6 amounted to 45% of total monoesters in both types of samples, in the range described by Cade-Menun et al., (2015). The concentration of total IP_6 showed a positive relationship with Olsen P, thus revealing that IP_6 concentration was greater in P-enriched soils.

Myo-IP_6*,* which is deemed as the dominant organic P compound in soils and the main one between inositols forms (Turner et al., 2012; Jørgensen et al., 2015; Cade-Menun et al., 2015) accounted for a similar concentration than *Chr*-IP_6, these both isomers being more abundant than Scy- IP_6. The latter is described in many studies as the usual second dominant form (Murphy et al., 2009; Turner et al., 2012). This shows that soils from the Mediterranean area may have a different pattern of stabilization of IP_6, perhaps due to microbial epimerization of *myo*-IP_6 (Giles et al., 2015), de novo synthesis (Liu et al., 2015), different types of plant residues or different stabilization mechanisms in soils affecting the different IP_6 stereoisomers.

The contribution of orthophosphates diesters (Di) either to extracted P or OP is almost equal in both types of samples and both uses of soil in the range described by Cade-Menun et al., (2015), but lower than percentages reported by Liu et al., (2015) and Schneider et al., (2016). The traditional management of crop residues, without incorporation to soil in field crops or in tree orchards, did not lead the accumulation of more Di in the high P samples relative to low-P samples due to a greater biomass production. The increased ratio of monoesters to diester with increased Olsen P in soil can be ascribed to increased monoester degradation under P limitation conditions in soils. Therefore, there are evidences that, in the long term, potential contribution of monoesters to P uptake by crops can be expected when the P availability to plants is low in soil (Ahlgren et al., 2013).

An increased ratio of monoesters to OP with increased Olsen P in soil from vineyards or olive orchards was observed. This trend was observed for *myo-,* and *chiro*-IP_6. Under this management, a lower residues supply can be expected when compared with soils used for field crops cultivation. Thus, under conditions of limited P availability to plants, and with very low crop residue accumulation, monoesters degradation, including IP_6, can be expected (Liu et al., 2015), and thus potential contribution to P uptake by plants.

Other forms of organic P were identified; glucose 6-phosphate, α-glycerolphosphate and ß-glycerolphosphate, choline phosphate and mononucleotides. Two unknown but constant peaks in their "chemicl-shift" were detected (4.56 ± 0.03 and 4.92 ± 0.03 ppm, (Cade-Menun, 2015) which are believed to be lower myo-inositol phosphates (Turner and Richardson 2004). α-glycerophosphate-, ß-glycerolphosphate and choline phosphate are phospholipids derivatives during NMR (Abdi et al., 2014).

The contribution of both, monoesters and diesters, to total P in NaOH-EDTA extracts was positively related to the Freundlich orthophosphate affinity factor (*b*) ($R^2 = 0.47$, $P < 0.01$; $R^2 = 0.77$, $P < 0.001$, respectively). In spite of an increased affinity for inorganic orthophosphate in soils, the main OP compounds are preferentially retained over orthophosphate. This suggests that affinity for OP is greater than for Pi (Berg and Joern, 2006), which contribute to explain the lower availability to plants of OP forms when compared with orthophosphate.

The concentration of *myo*-IP_6 increased with increased enrichment in Fe oxides of the clay fraction and decreased with increased pH. This reveals that in the accumulation and stabilization of this IP_6 isomer in soils, adsorption on Fe oxides play a relevant role. For the other IP_6 isomers, precipitation of Ca-phosphates and adsorption on clay minerals seems to be retention mechanisms. The lack of a significant relationship between concentration in soil and pH in IP_6 different from *myo*-IP_6 may reveal the relevance of this precipitation (Fuentes et al., 2014). One relevant difference between our results and other studies performed with soils from other geographical areas is the relative abundance of *chr*-IP_6: in our case, it is as, or even more, abundant as *myo*-IP_6. The analysis of results in literature reveals that the relative importance of *chr*-IP_6 to others IP_6 isomers seems to increase with increased pH in soil. Taking into account the pH in our soils, most above neutrality, an enhanced retention and stabilization of this isomer at high pH may explain its high relative concentration in our soils. Since at high pH precipitation can be enhanced, this process likely contributes to explain the retention and stabilization of *chr*-IP_6 in studied soils. The lack of a significant relationship between concentration in soil and pH may support the relevance of this precipitation (Fuentes et al., 2014) which may explain the relative abundance of *chiro*- between IP_6 isomers when compared with other studies (e.g. Jørgensen et al., 2015).

Chapter VII. Conclusions

Conclusions

- As concluded from different experiments in the present work, available P in soil and amounts of P adsorbed by crops was not accurately predicted by Olsen-. Thus, it cannot be considered as a precise index for P fertilizer recommendation.

- In high-P soil samples, properties affecting the equilibrium between inorganic P in soil particles and soil solution seem to explain the uptake of P by plants with the same amount of Olsen P in growing media. On the other hand, properties related to organic P dynamics are relevant at high ratios of organic P to Olsen P in growing media.

- Factors related with the soil P buffer capacity (PBC) seems the most relevant explaining the threshold values for Olsen P as SPT; also factors related to organic P dynamics can affect these threshold values.,

- Single models based clay content and pH may provide a fairly accurate estimation of Olsen P threshold values in soils from the Mediterranean area.

- Estimations for TAP performed better with P sinks (anion exchange resins, AER) than with Olsen-P or others single chemical extractors, in particular, AER membranes in HCO_3^- forms explained near 90 % of variation in TAP. Some organic P fractions seemed to contribute to TAP in soils. Correlations of P extracted with AERs and BC and F_{eca}/Fe_{cbd}, explained the performance of P sinks in assessing TAP.

- Olsen P can be suitable for estimating TAP if BC and Fe_{ca}/Fe_{cbd}, or other soil properties such as clay and carbonates related to them, are included in the models.

- The relative contribution of organic P to total P differed widely between soils, in some cases representing around 75 % of total P.

- Contribution of organic P to P uptake by plants was also concluded from the ^{31}P NMR spectra study. Results suggest that there is degradation of monoesters, including inositol-6-P(IP_6) under P starvations conditions in soil.

- The degradation of monoesters and most of the IP_6 isomers seemed to increase with decreasing Olsen P in soil, but only where the crop residue accumulation was scarce (vineyards and olive orchards).

- *Chiro*-IP_6 was as abundant as *myo*-IP_6, and the percentage of *scyllo*-IP_6 was much lower than in previous studies in other regions. Adsorption on Fe oxides and precipitation of Ca phosphates appears to vary among IP_6 stereoisomers, influencing their retention and stabilization in soils.

Conclusiones

- El P adsorbido por los cultivos y el P disponible total para el cultivo (TAP) no se predijo con exactitud por el P-Olsen. Por tanto, esta extracción no es suficientemente precisa para la recomendación de fertilizante fosfatado.
- En las muestras de suelo "high P", propiedades relacionadas con el equilibrio entre el P en las partículas y en la disolución explicaron la absorción de P por las plantas en medios con la misma cantidad de P Olsen en el medio de cultivo. Por otra parte, propiedades relacionadas con la dinámica del P orgánico fueron relevantes con altas relaciones P orgánico/P Olsen en el medio de cultivo.
- Los factores relacionados con el poder tampón de P (PBC) del suelo parecen ser los más relevantes a la hora de explicar los niveles críticos de P Olsen como índice de disponibilidad de P (SPT): factores relacionados con la dinámica de P orgánico también pueden afectar estos niveles críticos.
- Modelos simples basados en el contenido en arcilla y pH del suelo pueden proporcionar una estimación bastante precisa de niveles críticos del P Olsen en suelos de la cuenca mediterránea.
- Las estimaciones de TAP mediante sumideros de P (resinas de intercambio aniónico, AER) fueron mejores que con el P Olsen u otras extracciones químicas sencillas. En particular, el uso de AER en formas HCO_3^-, explican cerca del 90% de la variación en TAP. Los buenos resultados en la estimación del TAP con los sumideros se explican por la buena correlación de las cantidades extraídas con PBC y Fe_{ca}/Fe_{cbd}.

- El Olsen P puede ser adecuado para la estimación del TAP si la PBC y el ratio Fe_{ca}/Fe_{cbd}, u otras propiedades del suelo, tales como arcilla y carbonatos relacionados con aquellas, se incluyen en los modelos.

- La contribución del P orgánico al P total varió dependiendo del suelo, llegando a ser del 75 %.

- Los estudios con RMN del P-31 revelan que las formas de P orgánico pueden contribuir al suministro de P a las plantas.

- La degradación de los monoésteres y la mayoría de los isómeros del inositol-6-P (IP_6)aumentó a medida que disminuyó el Olsen P en el suelo, pero sólo cuando la acumulación de residuos de la cosecha fue escasa (viñedos y olivares).

- Entre los estereoisómeros IP_6, *chiro*-IP_6 era tan abundante como *myo*-IP_6, y el porcentaje de *scyllo*-IP_6 era mucho menor que en estudios anteriores en otras regiones.

- La adsorción en óxidos de Fe y la precipitación de los fosfatos de Ca parece variar entre los estereoisómeros IP_6, lo que influye en su retención y su estabilización en los suelos.

Chapter VIII. General References (Introduction and Discussion)

Abdi, D., Cade-Menun, B. J., Ziadi, N., and Parent, L. É. (2014). Long-term impact of tillage practices and phosphorus fertilization on soil phosphorus forms as determined by P nuclear magnetic resonance spectroscopy. Journal of environmental quality, 43(4), 1431-1441.

Afif E., (2005). Dinámica del Fósforo en suelos cálcicos de Areas Mediterráneas: Trabajo experimental. Universidad de Oviedo.

Ahlgren, J., Djodjic, F., Börjesson, G., Mattsson, L. (2013). Identification and quantification of organic phosphorus forms in soils from fertility experiments. Soil Use and Management, 29(s1), 24-35.

Barbanti, A., Bergamini, M. C., Frascari, F., Miserocchi, S., and Rosso, G., (1994). Critical aspects of sedimentary phosphorus chemical fractionation. Journal of Environmental Quality, 23(5), 1093-1102.

Barrow, N. J., (2015). A mechanistic model for describing the sorption and desorption of phosphate by soil. Journal of Soil Science, 34, 733–750. Commentary on the impact of Barrow (1983): by S. Staunton, B. Ludwig and J.Torrent.

Bennet, E.M., Carpenter, S.R., and Caraco, N.F., (2001). Human impact on erodable phosphorus and eutrophication: a global perspective increasing accumulation of phosphorus in soil threatens rivers, lakes, and coastal oceans with eutrophication. BioScience, 51(3), 227-234.

Berg, A. S., Joern, B. C. (2006). Sorption dynamics of organic and inorganic phosphorus compounds in soil. Journal of environmental quality, *35*(5), 1855-1862.

Bolland, M.D.A., Wilson, I.R., Allen, D.G., (1994). Effect of P buffer capacity and P retention index of soils on soil test P, soil test P calibrations and yield response curvature. Australian Journal of Soil Research, 32, 503–517.

Bowman, R.A., Cole, C.V., (1978). Transformations of organic phosphorus substrates in soils as evaluated by $NaHCO_3$ extraction. Soil Science, 125, 49–54.

Bowman, R. A., Moir, J. O., (1993). Basic EDTA as an extractant for soil organic phosphorus. Soil Science Society of America Journal, 57(6), 1516-1518.

Bray, R. H., Kurtz. L.T., (1945). Determination of total, organic and available forms of phosphorus in soils. Soil Science, 59, 39-45.

Cade-Menun, B. J., (1995). Phosphorus forms of podzolic soils of northern Vancouver Island and their use by western red cedar (Doctoral dissertation, University of British Columbia).

Cade-Menun, B. J., Preston, C. M., (1996). A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. Soil Science, 161(11), 770-785.

Cade-Menun, B. J., Liu, C. W., Nunlist, R., and McColl, J. G., (2002). Soil and Litter Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy. Journal of Environmental Quality, 31(2), 457-465.

Cade-Menun, B. J., (2005). Characterizing phosphorus in environmental and agricultural samples by ^{31} P nuclear magnetic resonance spectroscopy. Talanta, 66(2), 359-371.

Cade-Menun, B.J. Liu, C.W., (2013). Solution ^{31}P-NMR spectroscopy of soils from 2005-2013: A review of sample preparation and experimental parameters. Soil Science Society of America Journal, 78(1), 19-37.

Cade-Menun BJ (2015a) Improved peak identification in ^{31}P-NMR spectra of environmental samples with a standardized method and peak library. Geoderma, 257-258, 102-114.

Cade-Menun, BJ, He Z, Zhang H, Endale D M, Schomberg H H., and Liu CW (2015b). Stratification of phosphorus forms from long-term conservation tillage and poultry litter application. Soil Science Society of America Journal, 79(2), 504-516.

Carpenter, S. R., (2005). Eutrophication of aquatic ecosystems: bistability and soil phosphorus. Proceedings of the National Academy of Sciences of the United States of America, 102(29), 10002-10005.

Carpenter, S. R., (2008). Phosphorus control is critical to mitigating eutrophication. Proceedings of the National Academy of Sciences, 105(32), 11039-11040.

Castro, B., Torrent, J., (1998). Phosphate sorption by calcareous Vertisols and Inceptisols as evaluated from extended P sorption curves. European Journal of Soil Science, 49(4), 661-667.

Celi, L., De Luca, G., Barberis, E., (2003). Effects of interaction of organic and inorganic P with ferrihydrite and kaolinite-iron oxide systems on iron release. Soil science, 168(7), 479-488.

Celi, L., Barberis, E., Turner, B. L., Frossard, E., and Baldwin, D. S., (2005). Abiotic stabilization of organic phosphorus in the environment. Organic phosphorus in the environment, 113-132.

Celi, L.., Barberis, E., (2005). Abiotic stabilization of organic phosphorus in the environment. Organic phosphorus in the environment, 113-132.

Chang, S. C., and Jackson, M. L., (1957). Fractionation of soil phosphorus. Soil science, 84(2), 133-144.

Chen, Y. S., Butler, J. N., and Stumm, W., (1973). Kinetic study of phosphate reaction with aluminum oxide and kaolinite. Environmental Science and Technology, 7(4), 327-332.

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R., (2000). Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. Plant and Soil 220, 151–163.

Colombo, C., Barrón, V., Torrent, J., (1994). Phosphate adsorption and desorption in relation to morphology and crystal properties of synthetic hematites. Geochimica et Cosmochimica Acta, 58, 1261–1269.

Condron, L. M., Goh, K. M., Newman, R. H., (1985). Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by ^{31}P nuclear magnetic resonance analysis. Journal of Soil Science, 36(2), 199-207.

Condron, L. M., Moir, J. O., Tiessen, H., Stewart, J. W. B., (1990). Critical evaluation of methods for determining total organic phosphorus in tropical soils. Soil Science Society of America Journal, 54(5), 1261-1266.

Condron, L. M., Turner, B. L., Cade-Menun, B. J., (2005). Chemistry and dynamics of soil organic phosphorus. Agronomy, 46, 87.

Condron, L. M., Newman, S., (2011). Revisiting the fundamentals of phosphorus fractionation of sediments and soils. Journal of Soils and Sediments, 11(5), 830-840.

Cordell, D., Drangert J-O, White, S., (2009). The story of phosphorus: global food security and food for thought. Global Environmental Change 19, 293–305.

Cordell, D. and Neset, T., (2014). Phosphorus vulnerability: a qualitative framework for assessing the vulnerability of national and regional food systems to the multi-dimensional stressors of phosphorus scarcity. Global Environmental Change 24, 108–122.

Deevey Jr., E. S., (1970). Mineral cycles. Scientific American, 223(3), 149-158.

del Campillo, M. C., Van der Zee, S. E. A. T. M., and Torrent, J. (1999). Modelling long term phosphorus leaching and changes in phosphorus fertility in excessively fertilized acid sandy soils. European Journal of Soil Science, 50(3), 391-399.

Delgado, A., Torrent, J., (1997). Phosphate rich soils in the European Union: estimating total plant-available phosphorus. European Journal of Agronomy, 6, 205–214.

Delgado, A., Torrent, J., (2000). Phosphorus forms and desorption patterns in heavily fertilized calcareous and limed acid soils. Soil Science Society of America Journal, 64(6), 2031-2037.

Delgado, A., Madrid, A., Kasem, S, Andreu, L., del Campillo, M.C., (2002). Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. Plant and Soil 245, 277–286.

Delgado, A., Scalenghe, R., (2008). Aspects of phosphorus transfer from soils in Europe. Journal of Plant Nutrition and Soil Science 171, 552-575.

Delgado, A., del Campillo, M.C., Torrent, J., (2010). Limitations of the Olsen method to assess available phosphorus in reclaimed marsh soils. Soil Use and Management, 26, 133–140.

Donatello, S., Tong, D., and Cheeseman, C. R., (2010). Production of technical grade phosphoric acid from incinerator sewage sludge ash (ISSA). Waste management, 30(8), 1634-1642.

Domínguez, R., Campillo, C. D., Peña, F., Delgado, A., (2001). Effect of soil properties and reclamation practices on phosphorus dynamics in reclaimed calcareous marsh soils from the Guadalquivir Valley, SW Spain. Arid Land Research and Management, 15(3), 203-221.

Ehlert, P., Morel, C., Fotyma, M., and Destain, J. P. (2003). Potential role of phosphate buffering capacity of soils in fertilizer management strategies fitted to environmental goals. Journal of plant nutrition and soil science, 166(4), 409-415.

European Fertilizer Manufacturers Association, (2000). Phosphorus: Essential Element for Food Production. European Fertilizer Manufacturers Association (EFMA), Brussels.

Fixen, P. E., and Grove, J. H., (1990). Testing soils for phosphorus. In ‘Soil Testing and Plant Analysis’.(Ed. RL Westerman.). 141–80. Soil Science Society of America: Madison, WI.

.

Föhse, D., Claassen, N., Jungk, A., (1988). Phosphorus efficiency of plants. I. External and internal requirement and P uptake efficiency of different plant species. Plant and Soil 110, 101–109.

Frossard, E., Brossard, M., Hedley, M. J., and Metherell, A.,(1995). Reactions controlling the cycling of P in soils. Scope. 107-137

Frossard, E., Condron, L. M., Oberson, A., Sinaj, S., and Fardeau, J. C., (2000). Processes governing phosphorus availability in temperate soils. Journal of environmental quality, 29(1), 15-23.

Fuentes, B, de la Luz Mora, M, Bol, R, San Martin, F, Pérez, E, and Cartes, P (2014) Sorption of inositol hexaphosphate on desert soils. Geoderma 232, 573-580.

Garcia-Lopez, A.M., Aviles, M., Delgado, A., (2015). Plant uptake of phosphorus from sparingly available P-sources as affected by Trichoderma asperellum T34. Agricultural and Food Science, 24, 249-260

Geelhoed, J. S., Van Riemsdijk, W. H., Findenegg, G. R., (1999). Simulation of the effect of citrate exudation from roots on the plant availability of phosphate adsorbed on goethite. European Journal of Soil Science, 50(3), 379-390.

Gerke, J., and Hermann, R., (1992). Adsorption of orthophosphate to humic□Fe□complexes and to amorphous Fe-oxide. Zeitschrift für Pflanzenernährung und Bodenkunde, 155(3), 233-236.

Giaveno, C., Celi, L., Cessa, R.M.A., Prati, M., Bonifacio, E., Barberis, E., (2008). Interaction of organic phosphorus with clays extracted from oxisols. Soil Science, 173 (10), 694-706.

Gil-Sotres, F., Zech, W., Alt, H. G., (1990). Characterization of phosphorus fractions in surface horizons of soils from Galicia (NW Spain) by ^{31}P NMR spectroscopy. Soil Biology and Biochemistry, 22(1), 75-79.

Giles, C. D., Cade-Menun, B. J., Liu, C. W., and Hill, J. E. (2015). The short-term transport and transformation of phosphorus species in a saturated soil following poultry manure amendment and leaching. Geoderma, 257, 134-141.

George, T. S., Turner, B. L., Gregory, P. J., Cade-Menun, B. J., Richardson, A. E., (2006). Depletion of organic phosphorus from Oxisols in relation to phosphatase activities in the rhizosphere. European Journal of Soil Science, 57(1), 47-57.

Goldberg, S., Sposito, G., (1985). On the mechanism of specific phosphate adsorption by hydroxylated mineral surfaces: A review. Communications in Soil Science and Plant Analysis, 16(8), 801-821.

Golterman, H. L. (1976). Interaction between sediments and fresh water. In Proceedings of an international symposium. Amsterdam (Netherlands). 6-10 Sep 1976.

Golterman, H.L., Paing, J., Serrano, L., Gomez, E., (1998). Presence of and phosphate release from polyphosphates or phytate phosphate in lake sediments. Hydrobiologia, 364, 99–104.

Guzman, G., Alcántara, E., Barrón, V., Torrent, J., (1994). Phytoavailability of phosphate adsorbed on ferrihydrite, hematite, and goethite. Plant and Soil 159, 219–225.

Hayes, J. E., Richardson, A. E., Simpson, R. J., (1999). Phytase and acid phosphatase activities in extracts from roots of temperate pasture grass and legume seedlings. Functional Plant Biology, 26(8), 801-809.

Haygarth, P.M., Bardgett, R.D., Condron, L.M., (2013). Phosphorus and nitrogen cycles and their management, In: Gregory, P.J., Nortcliff, S. (Eds.), Russell's Soil Conditions and Plant Growth, 12th ed. Wiley-Blackwell, Hoboken, New Jersey, United States, 132–158.

He, Z., Honeycutt, C. W., Zhang, T., and Bertsch, P. M. (2006)., Preparation and FT–IR Characterization of Metal Phytate Compounds. Journal of environmental quality, 35(4), 1319-1328.

He, Z., Honeycutt, C. W., Xing, B., McDowell, R. W., Pellechia, P. J., and Zhang, T., (2007). Solid-state Fourier transform infrared and ^{31}P nuclear magnetic resonance spectral features of phosphate compounds. Soil science, 172(7), 501-515.

Hedley, M. J., Stewart, J. W. B., Chauhan, B., (1982). Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Science Society of America Journal, 46(5), 970-976.

Hieltjes, A. H., and Lijklema, L., (1980). Fractionation of inorganic phosphates in calcareous sediments. Journal of Environmental Quality, 9(3), 405-407.

Hill, J. E., Cade-Menun, B. J., (2009). Phosphorus-31 nuclear magnetic resonance spectroscopy transect study of poultry operations on the Delmarva Peninsula. Journal of environmental quality, 38(1), 130-138.

Hinsinger P, Brauman A, Devau N, Gérard F, Jourdan C, Laclau JP, Le Cadre E, Jaillard B, Plassard C., (2011). Acquisition of phosphorus and other poorly mobile nutrients by roots.Where do plant nutrition models fail? Plant and Soil, 348(1–2), 29–61.

Holford, I.C.R., (1980). Effects of phosphate buffer capacity on critical levels and relationships between soil tests and labile phosphate in wheat growing soils. Australian Journal of Soil Research, 18, 405–414.

Holford, I. C. R., (1997). Soil phosphorus: its measurement, and its uptake by plants. Australian Journal of Soil Research, 35(2), 227-240.

Horta, M., and Torrent, J., (2007). The Olsen P method as an agronomic and environmental test for predicting phosphate release from acid soils. Nutrient Cycling in Agroecosystems, 77(3), 283-292.

Hughes, J. M., Rakovan, J., (2002). The crystal structure of apatite, Ca5 (PO4) 3 (F, OH, Cl). Reviews in mineralogy and geochemistry, 48(1), 1-12.

Inskeep, W. P., Silvertooth, J. C., (1988). Inhibition of hydroxyapatite precipitation in the presence of fulvic, humic, and tannic acids. Soil Sci. Soc. Am. J. 52, 941–946.

Ivanoff, D.B., Reddy, K..R., Robinson, S., (1998). Chemical fractionation of organic phosphorus in selected histosols. Soil Sci 163, 36–45.

Jarlbring, M., Sandström, D. E., Antzutkin, O. N., and Forsling, W., (2006). Characterization of active phosphorus surface sites at synthetic carbonate-free fluorapatite using single-pulse 1H, ^{31}P, and ^{31}P CP MAS NMR. Langmuir, 22(10), 4787-4792.

Jordan-Meille, L., Rubæk, G. H., Ehlert, P. A. I., Genot, V., Hofman, G., Goulding, K., ... Barraclough, P., (2012). An overview of fertilizer P recommendations in Europe: soil testing, calibration and fertilizer recommendations. Soil Use and Management, 28(4), 419-435.

Jørgensen, C., Turner, B. L., and Reitzel, K. (2015) Identification of inositol hexakisphosphate binding sites in soils by selective extraction and solution 31 P NMR spectroscopy. Geoderma, 257, 22-28.

Kamprath, E.J., Watson, M.E., (1980). Conventional soil and tissue tests for assessing the phosphorus status of soils. In F.E. Khasawneh et al. (ed.) The role of phosphorus in agriculture. ASA, CSSA, and SSSA, Madison, WI.

Keyzer, M., (2010). Towards a closed phosphorus cycle. De Economist, *158*(4), 411-425.

Kulhánek, M., Balík, J., Cerny, J., Nedved, V., and Kotková, B., (2007). The influence of different intensities of phosphorus fertilizing on available phosphorus contents in soils and uptake by plants. Plant and soil environment, 53(9), 382.

Lindsay W L, Vlek P L G and Chien, S. H., (1989). Phosphate minerals. In Minerals in Soil Environments, 2nd edn. Eds. J B Dixon and S B Weed. 1089–1130. SSSA, Madison, WI.

Liu, J., Wang, H., Yang, H., Ma, Y., and Cai, O., (2009). Detection of phosphorus species in sediments of artificial landscape lakes in China by fractionation and phosphorus-31 nuclear magnetic resonance spectroscopy. Environmental Pollution, 157(1), 49-56.

Liu, J., Yang, J., Liang, X., Zhao, Y., Cade-Menun, B. J., and Hu, Y. (2014). Molecular speciation of phosphorus present in readily dispersible colloids from agricultural soils. Soil Science Society of America Journal, 78(1), 47-53.

Liu, J, Hu YF, Yang JJ, Abdi D, Cade-Menun BJ (2015) Investigation of soil legacy phosphorus transformations in long-term agricultural fields using sequential fractionation, P K-edge XANES and solution P-NMR spectroscopy. Environ Sci Technol, 49, 168-176.

Lookman, R., Freese, D., Merckx, R., Vlassak, K., Van Riemsdijk, W.H., (1995): Long-term kinetics of phosphate release from soil. Environ Sci Technol, 29, 1569–1575.

Magid, J., Tiessen H., Condron., L.M., (1996). Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. Humic Substances in Terrestrial Eco-systems. Elsevier, Oxford, UK., 429–466.

Matar, A., Torrent, J., and Ryan, J., (1992). Soil and fertilizer phosphorus and crop responses in the dryland Mediterranean zone. In Advances in Soil Science (81-146). Springer New York.

McDowell, R., Sharpley, A., Withers, P., (2002). Indicator to predict the movement of phosphorus from soil to subsurface flow. Environ Sci Technol 36, 1505–1509.

McKelvie, I.D., (2005). Separation, preconcentration and speciation techniques for organic phosphorus in environmental samples, in: B.L. Turner, E. Frossard, D. Baldwin (Eds.) Organic Phosphorus in the Environment, CAB International, Wallingford, UK, 1-20.

McLaughlin, J. R., Ryden, J. C., and Syers, J. K. (1981) Sorption of inorganic phosphate by iron- and aluminiumcontaining components: J. Soil Sci, 32, 365-377.

Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15, 1409-1416.

Mengel, K.., Kirkby, E.A., (2001). Principles of plant nutrition, 5th ed. Kluwer Academic Publishers, Dordrecht.

Mollier A, De Willigen P, Heinen M, Morel C, Schneider A, Pellerin S., (2008). A two dimensional model of phosphorus uptake including crop growth and P-response. Ecol Model 210, 453–464.

Moody, P. W., and Bolland, M. D. A.,(1999). Phosphorus. In 'Soil analysis: an interpretation manual'.(Eds KI Peverill, LA Sparrow, DJ Reuter). 187–220.

Murphy, P.N.C.,.Bell, A. and Turner, B. L. (2009). Phosphorus speciation in temperate basaltic grassland soils by solution 31P NMR spectroscopy. Eur. J. Soil Sci.60, 638–651.

Myers, R.G., A.N. Sharpley, S.J. Thien, G.M. Pierzynski., 2005. Ion-sink phosphorus extraction methods applied on 24 soils from the continental USA. Soil Sci. Soc. Am. J. 69, 511-521.

Negassa, W., Leinweber, P., (2009). How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: a review. J. Plant Nutr. Soil Sc. 172 (3), 305–325.

Negassa, W., Kruse, J., Michalik, D., Appathurai, N., Zuin, L. and Leinweber, P., (2010) Phosphorus speciation in agro-industrial byproducts: sequential fractionation, solution ^{31}P NMR, and K- and L 2,3-Edge XANES spectroscopy. Environ. Sci. Technol. 44, 2092–2097.

Newman, R. H., Tate, K. R., (1980). Soil phosphorus characterisation by ^{31}P nuclear magnetic resonance. Communications in Soil Science and Plant Analysis, 11(9), 835-842.

Oehl, F., Oberson, A., Probst, M., Fliessbach, A., Roth, H.-R., Frossard, E., (2001). Kinetics of microbial phosphorus uptake in cultivated soils. Biology and Fertility of Soils 34, 31–41.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. US Government Printing Office, Washington DC.

Peña, F. Torrent, J., (1990). Predicting phosphate sorption in soils of Mediterranean regions. Fert. Res. 23: 173-179.

Pardo, P., Lopez-Sanchez, J.F., Rauret, G., (2003). Relationships between phosphorus fractionation and major components in sediments using the SMT harmonised extraction procedure. Anal Bioanal Chem 376, 248–254.

Pierzynski, G.M., T.J. Logan, S.J. Traina., (1990a). Phosphorus chemistry and mineralogy in excessively fertilized soils: Solubility equilibria. Soil Sci. Soc. Am. J., 54, 1589–1595.

Pierzynski, G.M., T.J. Logan, S.J. Traina, J.M. Bigham., (1990b). Phosphorus chemistry andmineralogy in excessively fertilized soils: Quantitative analysis of phosphorus-rich particles. Soil Science Society of America Journal, 54,1576–1583.

Pierzynski, G. M., Logan, T. J., Traina, S. J., and Bigham, J. M., (1990c). Phosphorus chemistry and mineralogy in excessively fertilized soils: Quantitative analysis of phosphorus-rich particles. Soil Science Society of America Journal, *54*(6), 1576-1583.

Psenner, R., Pucsko, R., (1988). Phosphorus fractionation: advantages and limits of the method for the study of sediment P origins and interactions. Arch Hydrolbiol Beih Ergebn Limnol 30, 43–59.

Recena, R., Torrent, J., del Campillo, M. C., Delgado, A., (2015). Accuracy of Olsen P to assess plant P uptake in relation to soil properties and P forms. Agronomy for Sustainable Development, 35(4), 1571-1579.

Reddy, K. R., Kadlec, R. H., Flaig, E., and Gale, P. M.,(1999). Phosphorus retention in streams and wetlands: a review. Critical reviews in environmental science and technology, 29(1), 83-146.

Richardson, A.E., Lynch, J.P., Ryan, P.R., Delhaize, E., Smith, F.A., Smith, S.E., Harvey, P.R., Ryan, M.H., Veneklaas, E.J., Lambers, H., Oberson, A., Culvenor, R.A., Simpson, R.J., (2011). Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant Soil 349, 121–156.

Ruiz JM, Delgado A, Torrent J (1997) Iron-related phosphorus in overfertilized European soils. J Environ Qual, 26, 1548–1554.

Ruttenberg K.C., (1992). Development of a sequential extraction method for different forms of phosphorus in marine sediments. Limnol Oceanogr, 37, 1460–1482.

Ryan J, Hasan HM, Baasiri M, Tabbara HS (1985) Availability and transformation of applied phosphorus in calcareous Lebanese soils. Soil Sci Soc Am J 49:1215–1220

Ryan, J., (2008). A perspective on balanced fertilization in the Mediterranean region. Turkish Journal of Agriculture and Forestry, 32(2), 79-89.

Ryan, J., Ibrikci, H., Delgado, A., Torrent, J., Sommer, R., Rashid, A., (2012). Significance of phosphorus for agriculture and the environment in theWest Asia and North Africa region. Adv Agron 114, 91–153.

Ryden, J. C., Syers, J. K., Tillman, R. W., (1987). Inorganic anion sorption and interactions with phosphate sorption by hydrous ferric oxide gel. Journal of soil science, 38(2), 211-217.

Saavedra, C., Delgado, A. (2005). Iron-related phosphorus in eroded sediments from agricultural soils of Mediterranean areas. Geoderma. 125(1), 1-9.

Saavedra, C., Delgado, A., (2005). Phosphorus fractions and release patterns in typical Mediterranean soils. Soil Science Society of America Journal, 69, 607–615.

Saavedra, C., Delgado, A., (2006). Phosphorus forms in overland flow from agricultural soils representative of Mediterranean areas. Communications in soil science and plant analysis, *37*(13-14), 1833-1844.

Saavedra, C., Velasco, J., Pajuelo, P., Perea, F., Delgado, A., (2007). Effects of tillage on phosphorus release potential in a Spanish Vertisol. Soil Science Society of America Journal. 71(1), 56-63.

Sánchez-Alcalá, I., Campillo, M. C., Torrent, J., (2015). Critical Olsen P and $CaCl_2$-P levels as related to soil properties: results from micropot experiments. Soil Use and Management, 31(2), 233-240.

Sánchez-Alcalá, I., del Campillo, M.C., Barrón, V., Torrent, J., (2014). The Olsen P/solution P relationship as affected by soil properties. Soil Use Manage. 30, 454–462.

Schneider, K.D., Cade-Menun, B.J., Lynch, D.H., Voroney, P.R., (2016). Soil phosphorus forms from organic and forage fields. Soil Sci Soc Am J.

Schröder, J. J., Smit, A. L., Cordell, D., Rosemarin, A.., (2011). Improved phosphorus use efficiency in agriculture: A key requirement for its sustainable use. Chemosphere, 84(6), 822-831.

Sibbesen, E., (1978). An investigation of the anion-exchange resin method for soil phosphate extraction. Plant and Soil 50, 305-321.

Shirvani, M., Shariatmadari, H., and Kalbasi, M., (2005). Phosphorus buffering capacity indices as related to soil properties and plant uptake. Journal of plant Nutrition, 28(3), 537-550.

Spivakov, B. Y., Maryutina, T. A., and Muntau, H., (1999). Phosphorus speciation in water and sediments. Pure Applied Chemistry, 71(11), 2161–2176.

Staunton, S., Leprince, F., (1996). Effect of pH and some organic anions on the solubility of soil phosphate: implications for P bioavailability. European Journal of Soil Science, 47(2), 231-239.

Sommers, L.E., Williams, J.D., Syers, J.K., Armstrong, D.E., Harris, R.F., (1972). Fractionation of organic phosphorus in lake sediments. Soil Science Society of America Proceedings, 36, 51–54.

Tandy, S., Mundus, S., Yngvesson, J., de Bang, T. C., Lombi, E., Schjørring, J. K., and Husted, S., (2011). The use of DGT for prediction of plant available copper, zinc and phosphorus in agricultural soils. Plant and soil, 346(1-2), 167-180.

Tisdale, S.L., W.L. Nelson and J.D. Beaton., (1985). Soil and fertilizer potassium, p. 249-291. In: Soil fertility and fertilizers. (Eds.): S.I. Tisdale, W.I. Nelson and J.D Beaton. 4th ed. MacMillan publ. Co., New York.

Tiessen, H., Moir, J.O., (2008). Characterisation of available P by sequential extraction. In: Carter MR, Gregorich EG (eds) Soil sampling and methods of analysis, 2nd edn. CRC, Boca Raton, 293–306.

Torrent, J. (1997). Interactions between phosphate and iron oxide. Advances in Geoecology, 30, 321-344.

Turner, B.L., M. Papha´ zy, P.M. Haygarth, I.D. McKelvie., (2002). Inositol phosphates in the environment. Philosophical Transactions of the Royal Society B: Biological Sciences, 357, 449–469.

Turner, B.L., Mahieu, N., Condron, L.M., (2003). Quantification of myo-inositol hexakisphosphate in alkaline soil extracts by solution 31P NMR spectroscopy and spectral deconvolution. Soil Science, 168(7), 469-478.

Turner, B.L., Richardson, A.E., (2004). Identification of *scyllo*-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. Soil Science Society of America Journal, 68, 802-808

Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S., (2005). Extraction of soil organic phosphorus. Talanta, 66, 294-306.

Turner, B. L., Cheesman, A. W., Godage, H. Y., Riley, A. M., and Potter, B. V. (2012). Response to Comment on “Determination of neo-and d-chiro-Inositol Hexakisphosphate in Soils by Solution 31P NMR Spectroscopy”. Environmental science & technology, 46(20), 11480-11481.

United Nations, 2007. The 2006 Revision Population Base. http://esa.un.org/unpp/.

Vaccari, D. A., (2009). Phosphorus: A Loomıng Crisis. Scientific American, 300(6), 54.

Van Eck, G.T.M., (1982). Forms of phosphorus in particulate matter from the Holland Diep/Haringuliet, The Netherlands. Hydrobiologia 92, 665–681.

Van Vuuren, D. P., Bouwman, A. F., and Beusen, A. H. W., (2010). Phosphorus demand for the 1970–2100 period: A scenario analysis of resource depletion. Global Environ. Change 20, 428–439.

Von Wandruszka, R., (2006). Phosphorus retention in calcareous soils and the effect of organic matter on its mobility. Geochemical transactions, 7(6).

White, R. E., (1981). Retention and release of phosphate by soil and soil constituents [Prediction of phosphorus fertilizer requirements]. Soils and agriculture (USA).

Withers, P. J., Neal, C., Jarvie, H. P., and Doody, D. G., (2014). Agriculture and eutrophication: Where do we go from here?. Sustainability, 6(9), 5853-5875.

Wu, L., Willis, F., Schindler, P. W., (1991). Surface complexation of calcium minerals in aqueous solution. J. Colloid Interface Sci.147, 178–185.

Ziadi, N., Whalen, J. K., Messiga, A. J., and Morel, C., (2013). Assessment and modeling of soil available phosphorus in sustainable cropping systems. Advances in Agronomy, 122, 85-126

Zhang, Y. S., Werner, W., Scherer, H. W., and Sun, X., (1994). Effect of organic manure on organic phosphorus fractions in two paddy soils. Biology and fertility of soils, 17(1), 64-68.

Zhang, A., Z. Chen, G. Zhang, L. Chen, and Z. Wu., (2012). Soil phosphorus composition determined by ^{31}P NMR spectroscopy and relative phosphatase activities influenced by land use. European Journal of Soil Biology, 52, 73–77

Zhou, M.F., Li, Y.C., (2001). Phosphorus-sorption characteristics of calcareous soils and limestone from the southern Everglades and adjacent farmlands. Soil Science Society of America Journal , 65, 1404–1412

Zohar, I., Cade-Menun, B., Paytan, A., Shaviv, A., (2014). Phosphorus Transformations from Reclaimed Wastewater to Irrigated Soil: AP NMR Study. Soil Science Society of America Journal, 78 (6), 1884-1892.

Annexes

Main properties of studied soils, sorption curve parameters and estimated buffer capacity (BC) at different P concentration in the soil solution

	Use	**SOC**	**Clay**	**pH**	**CCE**	**ACCE**	**Fecbd**	**Feca**	**CEC**	**BC 1**	**BC 0.2**	**BC 0**	***A***	***b***	**Xmx**
		%	——————— g / kg ———————												
ECO	FC	1	492	7,9	75	31	10,1	1,3	37,9	56,1	178,3	1315,6	139,6	2,4	266,3
LC-perez	O	1,3	270	7,7	409	107	3,3	0,4	15,6	22,6	39,6	133,7	62,8	2,1	103,2
ZMB	FC	2	190	7,8	168	38	6,3	0,9	16,0	33,7	68,3	62,9	41,8	1,7	33,0
PNB	O	0,67	190	8,2	723	176	2,1	0,2	13,2	47,2	140,8	130,9	96,6	2,6	252,9
ALB	O	0,94	270	8,1	546	178	3,9	0,3	22,3	30,1	90,4	1480,5	91,3	2,6	237,7
LUE	FC	1,5	393	8,0	390	156	3,7	0,5	37,0	31,1	81,0	475,6	78,6	2,3	154,3
PZB	FC	1,5	48	7,2	0	0	2,0	0,8	9,1	6,4	9,0	9,3	16,1	1,9	---
LCV	O	0,87	412	8,1	32	0	13,2	2,4	13,6	50,5	57,6	465,0	78,0	2,3	453,4
HVR	FC	1,1	450	7,9	244	121	6,4	0,8	36,0	34,4	93,5	230,7	82,8	2,2	146,2
BLL	O	0,55	175	7,9	90	38	2,9	0,7	11,9	15,3	47,4	158,7	48,6	2,1	455,3
ADM	O	0,78	67	6,8	0	0	6,5	2,0	10,5	0,0	0,0	31,6	35,0	2,1	---
PLP	O	1,25	640	7,9	77	35	9,0	2,4	52,3	65,8	118,7	134,0	135,5	2,0	322,2
ADA	O	1	250	8,0	564	101	4,1	0,2	10,6	29,9	72,0	305,3	68,5	2,5	126
FCB	O	0,61	157	6,5	0	0	5,9	1,9	11,4	24,6	98,3	310,8	70,0	2,5	870,7
OCL	FC	1,4	360	7,9	244	86	8,3	1,6	18,6	42,4	119,9	44,3	41,7	1,5	209,4
TRB	O	0,84	400	8,1	400	156	3,6	0,8	36,3	37,5	99,8	297,5	76,9	2,1	188,4
ACL	FC	0,65	180	8,3	280	64	5,3	0,8	10,9	14,9	36,9	529,7	86,8	2,5	132,7
MCC	O	1,2	242	8,2	613	176	2,7	0,3	22,0	31,5	92,0	152,9	45,0	2,5	120,9

SOC, soil organic carbon; CCE, calcium carbonate equivalent; ACCE, active calcium carbonate equivalent,subindexca, citrate ascorbate extractable; subindex d, citrate-bicarbonate-dithionite extractable; A and b according to the Freundlich equation $Y=A\cdot X^{1/b}$, BC buffer capacity estimated from the derivate of the Freundlich equation at e=equilibrium; 0.2 mg P L^{-1} , and 1 mg P L^{-1} , Use of soil ; F Field crops, O Orchards

Main inorganic P fractions according to the fractionation scheme of Saavedra et al. (2007) according to Olsen et al. (1954) in the studied high P soils before crop experiment

Soil Sample	**Inorganic P**							
	——mg P / kg soil ——							
High	**NaOH**	**CB**	**C1**	**C2**	**CA**	**CBD**	**OAc**	**HCl**
ECO3	2,47	52,56	153,06	119,22	43,69	60,61	52,90	78,61
LC	3,08	217,81	341,93	342,47	778,23	184,68	261,82	54,95
ZMB-h	8,74	147,29	259,76	75,70	71,24	38,06	11,71	16,03
PNB-h	1,50	58,09	26,73	14,67	36,20	28,41	113,17	19,18
ALB_h	1,99	147,78	23,55	13,51	28,97	36,49	59,59	26,95
LUE-h	1,96	56,52	34,16	43,06	201,36	54,32	80,99	30,83
PZB-h	84,21	29,61	8,86	4,90	25,20	45,99	5,78	23,36
LCV-h	32,87	91,13	59,44	17,54	25,36	63,44	9,55	11,04
HVR-h	3,52	78,61	43,60	138,26	109,18	51,06	16,31	52,23
BLL-h	13,90	104,33	157,90	31,07	23,38	27,65	3,52	12,07
ADM-h	73,80	302,46	262,28	19,59	44,44	77,13	5,76	7,89
PLP-h	4,27	87,74	45,96	16,05	22,35	44,15	11,92	12,53
ADA-h	1,86	62,87	45,47	34,17	60,77	60,11	92,17	30,46
FCB-h	99,88	26,01	6,62	5,16	18,50	35,62	6,41	4,83
OCL-h	13,58	70,66	41,135	47,10	77,82	52,87	14,33	42,99
TRB279	1,30	88,52	57,97	48,01	233,67	60,90	74,15	27,25
ACL-h	6,74	74,60	130,05	105,90	56,10	40,61	9,47	69,39
MCC-h	1,85	50,67	44,103	33,38	67,29	51,65	125,58	24,95

IP inorganic P (molybdate reactive), subindexes: NaOH NaOH extractable, cb citrate-bicarbonate, c1,c2 citrate, ca citrate-ascorbate, cbd citrate-bicarbonate-dithionite, OAc acetate, HCl hydrochloric acid, H_2SO_4 + pers potassium persulfate plus sulfuric acid, NaOH 90º NaOH at 90 °C

Main inorganic P fractions according to the fractionation scheme of Saavedra et al. (2007) according to Olsen et al. (1954) in the studied low P soils before crop experiment

Soil Sample	**Inorganic P**							
	——— mg P / kg soil ———							
Low	**NaOH**	**CB**	**C1**	**C2**	**CA**	**CBD**	**OAc**	**HCl**
ECO6	4,98	36,04	146,05	70,36	56,16	59,66	20,81	26,85
Perez	11,06	50,96	120,30	216,39	133,45	52,69	34,99	32,25
ZMB-I	29,32	68,28	114,99	32,74	40,60	28,63	7,91	12,63
PNB-I	11,67	28,03	23,76	13,98	42,19	20,89	189,67	24,21
ALB_I	18,31	27,79	16,68	13,28	29,78	38,24	68,58	29,25
LUE-I	16,83	28,36	31,22	61,21	179,36	51,22	64,74	33,53
PZB-I	43,15	17,22	4,63	4,53	26,87	79,52	8,02	23,99
LCV-I	21,37	23,98	23,55	7,10	15,72	46,30	7,20	8,90
HVR-I	17,66	57,29	43,36	149,32	88,66	54,41	13,29	46,23
BLL-I	28,57	47,62	56,76	18,69	13,49	27,47	2,82	10,96
ADM-I	55,95	54,95	17,82	5,98	32,49	85,65	5,89	15,76
PLP-I	16,67	35,13	33,99	21,13	18,60	44,57	9,60	12,50
ADA-I	19,89	53,00	51,59	30,29	63,78	52,36	90,14	31,66
FCB-I	16,41	29,69	22,55	4,91	13,01	40,85	7,66	8,08
OCL-I	18,02	65,45	48,92	31,90	38,97	41,21	6,50	21,84
TRB277	11,56	51,21	35,82	32,30	135,67	30,96	15,75	22,96
ACL-I	26,52	36,62	160,98	109,23	26,94	42,11	10,92	60,07
MCC-I	13,15	29,09	31,28	24,03	49,27	48,06	103,98	26,23

IP inorganic P (molybdate reactive), subindexes: NaOH NaOH extractable, cb citrate-bicarbonate, c1,c2 citrate, ca citrate-ascorbate, cbd citrate-bicarbonate-dithionite, OAc acetate, HCl hydrochloric acid

Main organic P fractions according to the fractionation scheme of Saavedra et al. (2007) according to Olsen et al. (1954) in the studied high P soils before crop experiment

Soil Sample	**organic P**							**Recalcitrant Organic P**	
	——— mg P / kg soil ———								
High	**NaOH**	**CB**	**C1 + C2**	**CA**	**CBD**	**OAc**	**HCl**	**NaOH 90º**	**H_2SO_4 + pers**
ECO3	14,9	32,3	45,1	41,0	0,0	88,1	9,4	33,23	30,03
LC	10,2	7,2	40,5	391,8	0,0	244,4	10,3	12,85	22,18
ZMB-h	17,5	63,8	106,9	53,1	38,3	31,3	19,0	23,17	42,07
PNB-h	22,0	32,1	78,8	32,0	44,2	71,5	18,4	19,65	16,72
ALB_h	15,1	22,0	86,2	35,4	28,5	39,1	21,4	17,55	24,06
LUE-h	14,7	83,5	90,6	81,9	33,2	48,8	22,4	27,00	28,89
PZB-h	68,1	54,4	56,0	23,5	45,5	29,9	18,9	18,32	15,08
LCV-h	18,4	39,8	78,9	39,7	33,8	32,9	13,6	30,72	49,96
HVR-h	23,6	54,5	112,4	51,1	30,3	46,7	25,7	29,44	32,05
BLL-h	42,7	18,0	86,4	31,4	32,7	29,6	17,0	23,15	19,07
ADM-h	30,2	17,3	48,9	48,1	43,8	29,6	20,3	19,00	12,37
PLP-h	20,2	41,9	76,0	50,0	33,7	38,4	17,7	33,15	19,74
ADA-h	13,5	37,4	75,2	32,9	40,7	44,2	29,3	19,10	26,60
FCB-h	41,8	41,6	61,9	36,8	43,7	32,8	16,1	12,10	16,05
OCL-h	25,4	50,4	86,3	42,2	40,9	34,4	29,9	25,46	42,36
TRB279	11,6	50,3	76,7	51,9	69,7	33,5	26,2	33,69	16,80
ACL-h	16,9	48,7	107,7	32,6	32,4	35,5	33,2	12,08	17,87
MCC-h	12,9	37,5	103,2	21,1	43,9	63,0	23,5	24,02	21,91

OP organic P, subindexes: NaOH NaOH extractable, cb citrate-bicarbonate, c1,c2 citrate, ca citrate-ascorbate, cbd citrate-bicarbonate-dithionite, OAc acetate, HCl hydrochloric acid, H_2SO_4 + pers potassium persulfate plus sulfuric acid, NaOH 90º NaOH at 90 °C

Main organic P fractions according to the fractionation scheme of Saavedra et al. (2007) according to Olsen et al. (1954) in the studied low P soils before crop experiment

Soil Sample	**organic P**							**Recalcitrant Organic P**	
	mg P / kg soil								
Low	**NaOH**	**CB**	**C1 + C2**	**CA**	**CBD**	**OAc**	**HCl**	**NaOH 90º**	**H_2SO_4 + pers**
ECO6	5,0	24,3	30,0	56,0	0,0	41,8	11,3	17,19	17,42
Perez	11,1	20,7	0,0	0,7	11,6	107,0	9,1	13,67	34,32
ZMB-I	29,3	44,7	115,1	47,1	50,9	24,0	20,5	19,80	37,41
PNB-I	11,7	27,1	69,9	30,0	25,7	34,0	18,9	21,52	8,41
ALB_I	18,3	34,6	78,4	24,0	29,0	50,8	18,5	15,58	28,08
LUE-I	16,8	56,7	97,9	113,9	32,3	37,1	20,7	26,16	39,15
PZB-I	43,2	33,8	37,3	30,9	32,7	28,8	17,9	18,81	14,33
LCV-I	21,4	28,4	78,4	52,4	25,1	36,3	12,4	23,25	35,07
HVR-I	17,7	41,0	102,1	38,3	34,2	37,4	22,1	27,78	33,64
BLL-I	28,6	22,5	75,5	30,6	29,2	31,5	14,0	18,58	16,58
ADM-I	56,0	45,1	80,3	19,1	37,8	35,4	18,0	23,30	9,98
PLP-I	16,7	52,6	89,4	37,1	31,5	39,3	16,4	24,17	25,55
ADA-I	19,9	42,8	104,3	44,7	33,8	49,8	26,5	31,67	25,52
FCB-I	16,4	45,8	69,2	30,7	30,1	32,5	16,3	20,79	19,06
OCL-I	18,0	60,6	88,5	61,8	42,5	35,5	21,2	21,96	50,49
TRB277	8,9	48,9	81,5	29,5	44,1	36,6	18,9	23,08	19,66
ACL-I	26,5	76,7	131,8	83,2	32,5	34,0	30,9	33,43	38,00
MCC-I	13,2	46,4	89,2	38,9	30,1	49,6	22,0	24,41	27,20

OP organic P, subindexes: NaOH NaOH extractable, cb citrate-bicarbonate, c1,c2 citrate, ca citrate-ascorbate, cbd citrate-bicarbonate-dithionite, OAc acetate, HCl hydrochloric acid, H_2SO_4 + pers potassium persulfate plus sulfuric acid, NaOH 90º NaOH at 90 °C

NaOH + CB			
Soil	mg P / kg soil	**Soil**	mg P / kg soil
H		**L**	
ECO3	55,0	ECO6	38,2
LC	220,9	Pérez	52,7
ZMB-h	156,0	ZMB-l	76,5
PNB-h	59,6	PNB-l	29,1
ALB_h	149,8	ALB-l	28,9
LUE-h	58,5	LUE-l	29,5
PZB-h	113,8	PZB-l	90,6
LCV-h	124,0	LCV-l	35,4
HVR-h	82,1	HVR-l	60,4
BLL-h	118,2	BLL-l	53,5
ADM-h	376,3	ADM-l	112,1
PLP-h	92,0	PLP-l	36,5
ADA-h	64,7	ADA-l	54,4
FCB-h	125,9	FCB-l	42,5
OCL-h	84,3	OCL_L	105,0
TRB279	89,9	TRB277	52,5
ACL-h	81,4	ACL-l	39,3
HCC-h	52,5	HCC-l	30,2

inorganic P fractions according to the fractionation scheme of Saavedra et al. (2007) NaOH NaOH extractable plus cb citrate-bicarbonate

Olsen P (mg P / kg soil)				
High P soil samples			**Low P soil samples**	
ECO3	9,09		ECO6	6,59
LC	42,04		Pérez	10,27
ZMB-h	23,34		ZMB-l	8,43
PNB-h	21,61		PNB-l	8,73
ALB_h	18,62		ALB-l	8,18
LUE-h	18,06		LUE-l	7,16
PZB-h	37,56		PZB-l	14,75
LCV-h	32,81		LCV-l	7,1
HVR-h	18,06		HVR-l	10,17
BLL-h	18,06		BLL-l	7,16
ADM-h	49,05		ADM-l	16,49
PLP-h	21,09		PLP-l	5,68
ADA-h	21,14		ADA-l	10,81
FCB-h	22,68		FCB-l	4,77
OCL-h	21,39		OCL_L	13,03
TRB279	17,34		TRB277	5,04
ACL-h	20,13		ACL-l	6,91
MCC-h	18,6		MCC-l	6,32

Agron. Sustain. Dev. (2015) 35:1571–1579
DOI 10.1007/s13593-015-0332-z

RESEARCH ARTICLE

Accuracy of Olsen P to assess plant P uptake in relation to soil properties and P forms

Ramiro Recena [1] · José Torrent [2] · María Carmen del Campillo [2] · Antonio Delgado [1]

Accepted: 22 July 2015 / Published online: 7 September 2015

✉ Antonio Delgado
adelgado@us.es

[1] Departamento de Ciencias Agroforestales, ETSIA, Universidad de Sevilla, Ctra. Utrera km 1, 41013 Sevilla, Spain

[2] Departamento de Agronomía, Universidad de Córdoba, Edificio C4, Campus de Rabanales, 14071 Córdoba, Spain

JUNTA DE ANDALUCIA
Consejería de Innovación, Ciencia y Empresa

UNIVERSIDAD DE SEVILLA