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# A Comparison of Different Phosphorus Extraction Methods with the Phosphorus Uptake of Wheat

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Für meine Eltern

## **STATUTORY DECLARATION**

I declare that I have written my thesis independently, that I have not used any other sources than the ones declared and that I have marked all material quoted literally or by content.

Vienna, September 2013

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## ABSTRACT

Phosphorus (P) is indispensable to life and therefore essential to plant nutrition. Rock phosphate reserves are limited demanding an optimization of the usage of P fertilizers. One step in this process is to find methods to determine the plant available P in soils accurately.

The aim of this study was to identify P extraction methods best reflecting plant available P. Fifty contrasting agricultural soils were selected from Austria and Germany. Spring wheat was grown in a greenhouse pot experiment, harvested when mature and analyzed for yield parameters and P contents. The soils were extracted with the following methods/ extractants: H<sub>2</sub>O, CaCl<sub>2</sub>, LiCl, iron oxide impregnated filter papers, Olsen, calcium acetate lactate (CAL), cation and anion exchange membranes, Mehlich 3, Bray and Kurtz II, citrate-bicarbonate-dithionite, organic P, HCl, acid ammonium oxalate, total P.

Phosphorus plant uptake was in the range of the P extracted by the weakest extraction agents. In general, the P values extracted by the 14 extraction methods significantly correlated with each other. Our results showed that H<sub>2</sub>O and CaCl<sub>2</sub> extractable P correlated best with plant P uptake over one growing season. When grouping the soils according to pH, organic carbon content and clay content, weaker extraction methods showed analogous trends; also among the stronger extraction methods common tendencies were found.

Based on my results I conclude that a single method may not be sufficient to assess current and potential plant availability of P and to reliably predict the necessity of fertilizer applications in agriculture. Further investigations of additional soil types, of soils with very low P status, of experiments over more than one growing season and field trials could give supplementary information on the prediction accuracy of the examined methods.

## ZUSAMMENFASSUNG

Phosphor (P) ist für alles Leben unabdingbar und darum auch ein essentieller Nährstoff der Pflanzen. Das Vorkommen von Rohphosphat ist begrenzt, dadurch wird die Optimierung von P Düngereinsatz immer wichtiger. Ein Schritt in diese Richtung ist Methoden zu finden, die den pflanzenverfügbaren Phosphor in Böden adäquat bestimmen.

Das Ziel dieser Arbeit war es, diejenigen Bodenphosphor-Extraktionsmethoden zu ermitteln, die die pflanzenverfügbare Fraktion am besten widerspiegeln. Dazu wurden 50 unterschiedliche Ackerböden aus Österreich und Deutschland ausgewählt. Sommerweizen wurde in einem Glashaus-Topfversuch angebaut, zur Reife geerntet und auf Ernteparameter und P-Gehalte analysiert. Die Böden wurden mit folgenden Methoden/Extrakten extrahiert:  $H_2O$ ,  $CaCl_2$ ,  $LiCl$ , Eisenoxid-Filterpapier, Olsen, Calcium Acetat- Lactat (CAL), Anionen-Kationenaustauschmembranen, Mehlich 3, Bray und Kurtz II, Citrat-Bicarbonat-Dithionit, Organischer P,  $HCl$ , Ammoniumoxalat, Gesamt-P.

Die Pflanzenaufnahme lag in der Größenordnung der schwächeren Extraktionsmethoden. Generell kann man sagen, dass die Bodenphosphorgehalte, die durch die 14 Methoden extrahiert wurden, signifikant miteinander korreliert waren. Meine Ergebnisse haben gezeigt, dass P, der durch  $H_2O$  und  $CaCl_2$  extrahierbar ist, am besten mit der über eine Wachstumsperiode von Pflanzen aufgenommenen P Menge korrelierte. Bei einer Gruppierung der Böden nach pH, organischem Kohlenstoff und Tongehalt zeigten sich innerhalb der schwächeren Extraktionsmethoden ähnliche Trends. Ebenso konnten innerhalb der stärkeren Extraktionsmethoden analoge Tendenzen festgestellt werden.

Aufgrund der Ergebnisse meiner Arbeit, kam ich zu dem Schluss, dass eine einzelne Methode möglicherweise nicht ausreichend ist, um die gegenwärtige sowie die potentielle Pflanzenverfügbarkeit von P in Böden hinreichend abzuschätzen und um eine verlässliche Vorhersage über die Notwendigkeit einer Düngerapplikation in der Landwirtschaft zu treffen. Weitere Untersuchungen mit zusätzlichen Bodentypen, Böden mit sehr niedrigen P-Gehalten, Versuchen über mehr als eine Wachstumsperiode und Feldversuchen könnten ergänzende Informationen zur Schätzgenauigkeit der untersuchten Methoden liefern.

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## 1 Introduction

Phosphorus (P) is a central element to life on earth. Living organisms rely on it, as it is crucially involved in most major metabolic processes, e.g. it takes on a prime role in energy transfer as adenosine triphosphate (ATP). As phospholipids, phosphorus is part of cell membranes and as part of nucleotides, phosphorus is a major component to build up DNA and RNA. Furthermore plants are dependent on phosphorus to secure energy production in photosynthesis (SMIL, 2000, RUTTENBERG, 2009). Due to the reasons mentioned above, the fundamental significance of phosphorus in agriculture and food production is beyond question.

In nature we can identify several phosphorus pools. The largest extent of phosphorus in the lithosphere occurs in marine and freshwater sediments (about  $800\text{--}4000 \times 10^6$  Mt P), while the entire phytomass (terrestrial and marine; about 570–625 Mt P) accounts for over 90% of phosphorus stored in the totality of all living organisms (maritime and terrestrial). Phosphorus does not possess a stable gaseous compound, therefore the smallest and an only insignificant extent (0.028 Mt P) can be found in the atmosphere as dust and sea spray picked up by wind erosion (SMIL, 2000, RUTTENBERG, 2009). The flows of phosphorus between these reservoirs is slow (it may take more than 1 million years). This is due to the low solubility of phosphate in water and due to the lack of an airborne reservoir. However, despite a long retention time of phosphorus in its reservoirs and relatively constant flux quantities, human activity has increased the amount of phosphorus moving within the P cycle (LIU *et al.*, 2008).

Today, about 90% of phosphorus production is used for mineral fertilizers and fodder additives for animal husbandry, i.e. food production (EFMA, 2000, CORDELL *et al.*, 2009). The global rock phosphate mining production is continuously increasing – an annual growth of 2% is estimated by IFA (2011) as an average over the last six consecutive years. In 2011 it amounted to a total of 191,000 t (JASINSKI, 2012). According to the US Geological Survey there are over 300 billion tons of reserves of phosphate rock worldwide, nevertheless opinions are divergent on how long these reserves will last. In this context the term ‘peak phosphorus’ was introduced by CORDELL *et al.* (2009) indicating the time when the maximum rate of rock phosphate mining is approached. Beyond this point minable ores are expected to be of minor quality, harder accessible and therefore less economical to degrade, yet the demand will be on the rise. The authors concluded a peak will appear around the year 2033. How long global phosphate rock reserves will last is being discussed controversially by multiple authors. Based on differing reserve assumptions (reserve and reserve base; cf. USGS 2006) and dissimilar scenarios for the growth of earth’s population and

accordingly growing fertilizer demand, estimates for the depletion of phosphate rock reserves may vary between 50 years to 400 years (even 1000 years; cf. *EFMA, 2000*). However, a majority comes to the conclusion that the depletion will occur within the next 100 years (*CORDELL et al., 2009, CORDELL and WHITE, 2011, COHEN et al. 2011, SMIT, 2011*). Some factors considered relevant for the accelerating depletion of the reserves are the increasing world population causing rising food demand, the changing of diets (of low income economies) towards a phosphorus-intensive, meat-based diet, and the increasing production of biofuels, all of which increases the demand for fertilizers. While on the contrary increasing costs for mining (increasing oil costs), increasing costs for fertilizers and increasing consciousness of over-fertilization and of eutrophication problems may diminish demand and therefore delay a production depletion (*CORDELL et al., 2009, CORDELL and WHITE, 2011*).

Recently, *RECHBERGER et al. (2013)* have assessed the situation and phosphorus flows in Austria and presented losses, the potential to recycle and possible scenarios for regaining P from sewage sludge and meat and bone meal. In Germany, *RÖMER (2006)* assessed recycling products regarding P availability for plants and P uptake to predict their usability for fertilization.

With regard to phosphorus fertilization two major issues concerning environmental and economic problems are of interest: on the one hand contamination of fertilizers with heavy metals and radionuclides and on the other hand eutrophication. The quality of rock phosphate that is extracted is highly variable; the concentration of toxic elements depends on its origin (parent material - igneous rock or sedimentary ores), the region of mining and the manufacturing process of the fertilizers (*MORTVEDT and BEATON, 1995*). In fact, all phosphorus reserves are contaminated with heavy metals or radionuclides. In this context uranium and cadmium are of particular significance (*COHEN et al. 2011*). The degree of contamination differs considerably; higher concentrations are found in particular in phosphate rock from sedimentary origin, which accounts for about 80% of phosphate rock processed (*COHEN et al. 2011 citing STEWART, 2005*). Notable is, that a contamination of cadmium and uranium simultaneously, will multiply their toxicity. Accumulation of these toxic substances in agricultural soils and especially their runoff to groundwater can pose a potential danger to human health (*SCHNUG, 2012*).

Eutrophication plays a major role in terms of lake and fresh water pollution. The runoff of phosphorus from (agricultural) topsoil layers provides an oversupply of phosphorus in surface water, which leads to excessive plant growth and causes a major disturbance of the biologically balanced dynamics in the waters. As a consequence of abnormal algal growth – and the resultant high biological activity of decomposer

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organisms – the amount of oxygen and light in the water severely decreases, leading to fundamental change of fish species compositions and often resulting in mass mortality of fish and other oxygen dependent organisms (LIU *et al.*, 2008, SMIL, 2000).

In soils, phosphorus is mainly drawn from weathering processes of the mineral apatite (SCHLESINGER, 1997). Soil phosphate can be classified into four pools: dissolved in soil water, sorbed to surfaces of clay minerals or Fe and Al oxides, primary phosphate minerals and in organic substances and living organisms. The average total phosphorus in soils ranges from 200 mg kg<sup>-1</sup> (in older, highly weathered soils) to 800 mg kg<sup>-1</sup> (in younger soils) (ZEHETNER *et al.*, 2008), the average amount of organic P ranges between 30% and 65% of the total P (CONDRON and TIESSEN, 2005). The average concentration of phosphorus in plant tissues is variable; depending on the time of sampling, phosphorus in wheat shoots may range between 2.5 to 4.9 g kg<sup>-1</sup> (FANGERIA, 2009) and phosphorus in plant seeds can amount up to 4.0 g kg<sup>-1</sup> (TIESSEN, 2008). A shortage of P – depending on crops – causes a decrease of tillering and branching, reduced plant growth and also limited root growth. Phosphorus further improves the shoot strength and crop quality (necessary for the development of seeds, fruit and leaflets) and is a component necessary in the process of N-fixation by legumes. Typically, plants which are P deficient show dark green color of leaflets, as the reduced growth but similar amount of chlorophyll increases its proportion per leaf area; on older leaflets a purple or reddish color may be noticed (FANGERIA, 2009). Plants mainly take up phosphorus in forms of inorganic ions, either H<sub>2</sub>PO<sub>4</sub><sup>-</sup> (in acidic soils) or HPO<sub>4</sub><sup>2-</sup> (in alkaline soils) (BLUME *et al.*, 2010). The availability of phosphorus to plants is dependent on the pH of the soil solution; when the pH is low (in the acidic range) phosphate is mainly found sorbed on Al and Fe oxides while a pH close to neutral promotes a fixation as Ca phosphate (LIU *et al.*, 2008).

HOLFORD (1997) and FROSSARD *et al.* (2004) have classified phosphorus availability using the concept of intensity factor, quantity factor and capacity factor. The intensity factor gives the concentration of phosphate that is readily solved in the soil solution. The quantity factor describes the total amount of phosphorus that is in the soil and may be solved and taken up by the plant eventually. The capacity factor expresses the capacity of soils to stabilize the magnitude of the intensity factor even when the quantity factor changes. The relation of intensity and quantity factor may provide information on the ability of the soil to resupply nutrients for plant uptake.

In agriculture a balanced availability of nutrients is not only the basis to achieve satisfying yields with excellent quality but simply to produce healthy plants. Nutrient availability affects the crop and farm management as it influences the amount of fertilizers, pesticides and herbicides that have to be applied. Due to the scarcity of

phosphorus resources an efficient use of phosphorus is crucial; optimizing plant nutrition and fertilization is one step to guarantee global food production. To secure plant availability while reducing environmental risks an accurate forecast of the plant available nutrient status in agricultural soils is indispensable.

Today, there is a multitude of soil extraction methods available, and even in routine soil testing diverse extraction methods are used. While the calcium acetate lactate (CAL) extraction is used in routine soil testing in Austria and Germany, Mehlich 3 is used in the Czech Republic and in major parts of Canada and the United States of America. On the contrary, Brazil is using a more recently established extraction method on the basis of anion exchange membranes.

Based on the fact that different P extraction methods are used all over the world, which complicates the comparability of results, the aims and objectives of this work were:

- I) to compare several extraction methods in contrasting soils, and
- II) to identify extraction methods that best represent phosphorus uptake by plants through relation to plant phosphorus uptake (after one growing season)

## 2 Materials and Methods

### 2.1 Description of Investigated Soils

Soil samples were taken in the period from mid-March to mid-April 2011 in a depth of 0-30 cm from agricultural soils in Austria and Germany. The soils were chosen to be dissimilar in soil properties to cover an interesting spectrum of soil types and a wide range of phosphorus availability. In advance, the samples were not specifically fertilized with P to contain different levels of phosphorus but were solely treated according to common farm management. Hence, the farmers applied different fertilizers depending on e.g. the crop rotation and crop types. To guarantee a broad range of phosphorus levels the 50 soils were selected from a total of 83 samples according to the amount of phosphorus extracted by calcium acetate lactate (CAL) and anion exchange membranes (CAEM).

Most soils were just represented once in the experiment; however, some of the samples belonged to long-term experiments. Then, similar soils with different treatment of fertilization were compared, having otherwise the same management. All other soils were managed with diverse tillage, fertilization and crop sequences before the experiment took place.

### 2.2 Soil Analysis

In this chapter a basic overview on selected physicochemical properties and a detailed description of the applied extraction methods are given.

#### 2.2.1 Determined Basic Soil Properties

The soil data gathered for this work amounts to an extensive analysis of physicochemical parameters. Only selected soil parameters could be elaborated in this thesis, as a detailed discussion of all would go beyond the scope of this work.

A thorough analysis of the soil parameters is important as they influence the performance of the various extraction methods. For example, some of the extraction methods have a certain pH range at which they extract properly.

The pH was determined in a H<sub>2</sub>O saturation extract and also in 0.5 M KCl with a soil-to-solution ratio of 1:2.5 (volume ratio) as described in *OENORM S 2122-1 (2004)*, the electrical conductivity (EC) was measured in the water saturation extract. Total carbon contents were measured by dry combustion as described in *OENORM L 1080 (1999)* and carbonate contents (CaCO<sub>3</sub>) were determined by the Scheibler method as described in *OENORM L 1084 (2006)*. Organic carbon (OC) was calculated as the

difference of total and carbonate carbon. In order to calculate the ratios of C/N, C/P and N/P on a molar basis, additionally total soil N was determined by dry combustion according to *OENORM L 1080 (1999)* and total P contents as described in chapter XIV *Total P*. Furthermore, the KH value was calculated as described in *OENORM S 2122-1 (2004)*. This value represents the amount of water contained in the soil at saturation, which is individual for each soil.

The potential cation exchange capacity ( $CEC_{pot}$ ) was determined by extraction with a 0.4 M LiCl solution buffered at pH 8.1 with triethanolamine. To this end, 50 mL of the solution was shaken with 40 g of water-saturated soil for 2 h, centrifuged and the supernatant was transferred into a flask; the soil sample was then mixed with another 50 mL, shaken, centrifuged and the supernatant added to the flask. The solution was filtered with a membrane filter, exchangeable cations were measured and the  $CEC_{pot}$  calculated in  $cmol_c\ kg^{-1}\ DM$  (dry matter) as described in *OENORM S 2122-1 (2004)*. The base saturation and the respective cations were calculated in percent of  $CEC_{pot}$ . Oxidic iron and manganese was extracted with dithionite-citrate-bicarbonate ( $Fe_d$ ,  $Mn_d$ ) (*MEHRA and JACKSON, 1960*) and also by acid ammonium oxalate ( $Fe_o$ ,  $Mn_o$ ; extracting only the poorly crystalline forms) according to *OENORM L 1201 (2007)*; the extracted Fe and Mn was measured by atomic absorption spectroscopy (AAS). The molar ratios of  $Fe_o/Fe_d$  and  $P_o/Fe_o$  were calculated based on the measurements of Fe and P in the oxalate and dithionite extractions.

The soil particle size distribution was determined by use of wet-sieving and sedimentation in a sedigraph after adding  $H_2O_2$  to remove organic matter and dispersing with sodium metaphosphate, modified according to *OENORM L 1061-1 (2002)* and *OENORM L 1061-2 (2002)*.

### 2.2.2 Applied Soil P Extraction Methods

In advance all soil samples were dried at 50°C and sieved to smaller than 2 mm. For every chemical analysis blank and reference samples were carried along and treated similarly.

The same extracting solution was used as a background solution for the blanks, standards and for the extraction of the samples. All methods were undertaken at room temperature. If filtration was necessary the first few mL were discarded, the quantity depending on the total amount of solution output. Munktell folded paper filters (grade 14/N) were used for the filtration of all extractions.

A brief summary of the 14 applied methods is given in Table 1. The table provides an overview of the composition of the extracting solution, the soil-to-solution ratio, the extraction time and the method of measurement that was applied.

Table 1: Overview of the studied soil P extraction methods

Method	Extracting solution	Soil-to-solution ratio	Shaking time	Method of measurement
H <sub>2</sub> O	distilled H <sub>2</sub> O	1:0.3 to 1:1.2	(ca. 12 h)	ICP; Photometer
CaCl <sub>2</sub>	0.01 M CaCl <sub>2</sub>	1:10	2 h	ICP; Photometer
LiCl	0.4 M LiCl	1:1.8 to 1:4.0	(2 × 2 h)	ICP
<sup>a</sup> Fe-oxide P <sub>i</sub>	(0.01 M CaCl <sub>2</sub> )	1:40	16 h	Photometer
Olsen	0.5 M NaHCO <sub>3</sub> ; pH 8.5	1:20	30 min	ICP; Photometer
<sup>b</sup> CAL	0.3 M CH <sub>3</sub> COOH, 0.05 M C <sub>6</sub> H <sub>10</sub> CaO <sub>6</sub> × 5 H <sub>2</sub> O, 0.05 M (CH <sub>3</sub> COO) <sub>2</sub> × H <sub>2</sub> O	1:20	2 h	ICP; Photometer
<sup>c</sup> CAEM	(distilled H <sub>2</sub> O)	1:40	16 h	Photometer
Mehlich 3	0.2 M CH <sub>3</sub> COOH, 0.25 M NH <sub>4</sub> NO <sub>3</sub> , 0.015 M NH <sub>4</sub> F, 0.013 M HNO <sub>3</sub> , 0.001 EDTA	1:10	5 min	Photometer
Bray II	0.03 M NH <sub>4</sub> F, 0.1 M HCl	1:7	40 sec	Photometer
Dithionite	0.2 M NaHCO <sub>3</sub> , 0.12 M NaS <sub>2</sub> O <sub>4</sub> , 0.24 M C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> × 2 H <sub>2</sub> O	1:50	16 h	ICP
Organic P	<sup>d</sup> (1 M HCl)	1:100	16 h	Photometer
HCl	0.5 M HCl	1:10	2 h	ICP
Oxalate	0.08 M (COOH) <sub>2</sub> × 2 H <sub>2</sub> O, 0.11 M (COONH <sub>4</sub> ) <sub>2</sub> × H <sub>2</sub> O; pH 3.0	1:50	4 h	ICP
Total P	1 M HCl (after calcination)	1:100	16 h	Photometer

<sup>a</sup>Fe-oxide P<sub>i</sub> = iron oxide impregnated filter paper

<sup>b</sup>CAL = calcium acetate lactate

<sup>c</sup>CAEM = anion exchange membrane

<sup>d</sup>Organic P was determined as the difference of P extracted with 1M HCl before and after calcination

### I. Water Extraction (H<sub>2</sub>O)

In order to produce the water extraction, the steps described in *OENORM S 2122-1 (2004)* were followed. This method is based on the preparation of a water saturation extraction that is described in *OENORM L 1092 (2005)*. Accordingly, the amount of water the sample is able to take up was added until the liquid limit was reached (the quantity

is specific for each soil). About 300 g of field-moist soil sample was sieved to <5 mm and moisturized while stirring until saturation (i.e. when tilting the flask, the sample starts to flow slightly) was obtained. The solution was allowed to stand for one hour, and then the saturation level was checked. If after that hour a water film had formed on top, more soil needed to be added, if the soil suspension had stiffened after that time, more water was necessary. In case of adjustment, the suspension had to be rechecked after another hour. When the state of saturation was attained the supplied amount of water was weighed, the extraction was covered and allowed to equilibrate over night at room temperature. The next day it was centrifuged with 2500 g for 15 min and subsequently filtered by membrane filters. This extraction method was measured by ICP-OES and by photometer.

The water extraction determines the phosphorus in the soil solution, thus dissolved or readily soluble forms of phosphorus.

## **II. $\text{CaCl}_2$**

The  $\text{CaCl}_2$  method was prepared as described in *HOUBA et al. (2000)* using 5 g of soil in 50 mL of extracting solution (0.01 M  $\text{CaCl}_2$ ). The solution was shaken for 2 h and then filtered. The samples were measured by ICP-OES and colorimetrically by photometer.

This method is based on the principle of a (dilute) salt solution inducing an ion exchange but nevertheless relies mostly on the dissolution of phosphorus in the extraction solution (*VAN RAIJ, 1998*).

## **III. $\text{LiCl}$**

The  $\text{LiCl}$  extraction method was carried out according to instructions in *OENORM S 2122-1 (2004)*. In advance the field-moist soil samples were sieved to <5 mm and then a water saturation extraction was prepared as described for  $\text{H}_2\text{O}$  extraction and in *OENORM L 1092 (2005)*. Of this soil-water mixture 40 g were shaken for 2 h with 50 mL of extraction solution (0.4 M  $\text{LiCl}$ ). Thereafter it was centrifuged and the liquid phase transferred to a separate flask, while the solid phase was shaken for a second time with additional 50 mL of 0.4 M  $\text{LiCl}$  for an extra 2 h. After centrifugation the supernatant was also transferred to the flask containing the first subset. At last this suspension had to be filtered through a membrane filter. The  $\text{LiCl}$  extractions were measured by ICP-OES.

This method is based on a pH-neutral salt solution suitable for a vast range of soil properties. Due to the characteristics of Li (small ion-diameter, thick hydration shell,

weak electrostatic charge), LiCl extracts only easily exchangeable cations and soluble phosphorus forms (HUSZ, 2001).

#### **IV. Iron Oxide Impregnated Filter Paper (Fe-oxide P<sub>i</sub>)**

This method was performed on the basis of the protocol by CHARDON (2000). The preparation of the filter papers: As a first step a solution of 0.62 M FeCl<sub>3</sub> was produced in dilute HCl. Every filter paper was treated with this solution for at least 5 min; afterwards they were allowed to dry for an hour. When the papers were dry, a 2.7 M NH<sub>4</sub>OH solution was prepared and every filter paper was pulled through the solution rather quickly and constantly with (plastic) tweezers. Thereby amorphous iron-oxides formed on the filter paper; immediately following the filter papers had to be washed with distilled water to assure that any loose iron-oxide particles were removed. Next, the impregnation of the filter papers were dried and cut into quarter slices. The prepared filter papers were folded thoroughly and put into nets made of polyethylene and tied with a fishing line (likewise made of polyethylene). This protection net was put around the filter papers to keep the bigger soil particles from scratching off the iron oxides while shaking. Polyethylene was selected as material of choice because it is inert and should not affect the chemical process of dissolution and adsorption of phosphorus to the oxides on the filter paper. In Figure 1 the paper slices, folding and the nets are illustrated.

For the extraction 1 g of soil along with 1 bag of filter paper were mixed with 40 mL of 0.01 M CaCl<sub>2</sub> solution, and then this soil suspension was shaken for 16 h. After the shaking filter papers were taken out and washed properly to assure no soil particles being stuck on the filter paper. After that the filter papers were put in 40 mL of 0.1 M H<sub>2</sub>SO<sub>4</sub> and shaken for 1 h in order to elute the P. This solution was then measured by photometer.

This method is based on an infinite P sink approach. The iron oxide filter papers serve as a P sink imitating the plant root. Through sorption of P from the solution to the filter paper a further desorption/ re-supply from the soil is induced (MYERS, *et al.* 2005).



**Figure 1:** Preparation of the iron oxide impregnated filter papers: cut, folded and put into protection nets

### **V. Olsen**

This extraction method was introduced by *OLSEN et al. (1954)*, our extraction was carried out according to *SIMS (2000)* except for a minor modification; we used 2 g (instead of 1 g) of soil sample that was mixed with 40 mL (instead of 20 mL) of extracting solution, nonetheless maintaining the soil-to-solution ratio of 1:20. The extracting solution was prepared of 0.5 M  $\text{NaHCO}_3$  and adjusted to a pH of 8.5. The samples were shaken for 30 min at room temperature, filtered, and measured by ICP-OES and photometer.

In general, the Olsen method is preferably used for calcareous soils (>2%  $\text{CaCO}_3$ ) but studies have shown that also values for non-calcareous give reasonable results (*FRANK, et al., 1998*). The Olsen extraction reduces the  $\text{Ca}^{2+}$  in the solution, as  $\text{CaCO}_3$  precipitates and therefore causes a better Ca-phosphate dissolution. The phosphate adsorbed on the Ca-carbonate and Fe-oxide surfaces is extracted by this method (*SIMS, 2000, SCHOENAU and O'HALLORAN, 2007*).

### **VI. Calcium Acetate Lactate (CAL)**

The calcium acetate lactate method will be further referred to as CAL. The CAL procedure is used as the standard method in routine soil testing in Austria and Germany. This extracting method was carried out according to *OENORM L 1087 (2006)*. Although in *OENORM L 1087 (2006)* 5 g of soil is mixed with 100 mL extracting solution we used 2.5 g of soil in 50 mL extraction solution preserving the soil-to-solution ratio of 1:20. The extracting solution was prepared of 0.05 M calcium lactate ( $\text{C}_6\text{H}_{10}\text{CaO}_6 \times 5 \text{H}_2\text{O}$ ), 0.05 M calcium acetate ( $(\text{CH}_3\text{COO})_2\text{Ca} \times \text{H}_2\text{O}$ ), 0.3 M acetic acid ( $\text{CH}_3\text{COOH}$ ). The solution was shaken end-over-end for 2 h. Subsequently, the samples were filtered and then they were measured by ICP-OES and by photometer.

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According to SCHÜLLER (1969) who introduced this method, the CAL method extracts only the readily soluble, exchangeable phosphates and easily dissolved Ca-phosphates from fertilizers, neglecting the P of apatitic phosphates. However, is not well suited for soils with a pH below 6 as in acidic soils the apatitic phosphate might not be determined since it is re-adsorbed by Al- and Fe-phosphates.

### **VII. Cation and Anion Exchange Membranes (CAEM)**

The anion exchange membrane method is used in routine soil testing in Brazil. The method used in this study was performed similarly to protocols described by NUERNBERG *et al.* (1998), BISSANI *et al.* (2002) and FERNANDES and COUTINHO (1997). The cation and anion exchange membranes were obtained from Membranes International Inc. and cut to 1 cm broad and 7.5 cm long strips. The average weight of the anion strips was 0.377 g per strip with a total exchange capacity of  $1.3 \pm 0.1$  meq  $g^{-1}$  (the average weight of one cation strip was 0.443 g with a total exchange capacity of  $1.6 \pm 0.1$  meq  $g^{-1}$ ). After cutting they were put into a 0.5 M sodium bicarbonate solution ( $NaHCO_3$ ) for 62 h. The solution was renewed and after another 19 h the strips were rinsed five times with distilled water.

For the extraction procedure 1 g of soil was put into a tube of 50 mL and combined with one strip anion exchange membrane and one strip cation exchange membrane and 40 mL of distilled water. The samples were shaken end-over-end for 16 h. Afterwards the membranes were washed thoroughly until no soil particles were left. The anion exchange membranes were put into 40 mL of 0.5 M HCl solution and shaken for 90 min. After this time all the adsorbed ions should have desorbed from the membrane and should be dissolved in the solution. Subsequently the membranes were taken out of the solution and rinsed with water and as the membranes are reusable they were put into 0.5 M sodium bicarbonate for regeneration as shown in Figure 2. The amount of phosphorus in the HCl solution was detected by a photometer.

Similarly to the procedure of the iron oxide impregnated filter paper, this method is based on a sink concept. The exchange membranes simulate the root system in the soil, keeping the P solution concentration low and thus inducing re-supply from the soil solid phase (VAN RAIJ, 1998, KUO, 1996).



**Figure 2: Anion and cation exchange membranes in 0.5 M sodium bicarbonate for regeneration**

### **VIII. Mehlich 3**

The Mehlich 3 extraction is employed as a standard method for phosphorus extraction in the Czech Republic and is also widely used in routine soil testing in the USA and Canada. This method is considered to be suitable for a vast range of soils varying in their physicochemical properties (SIMS, 2000, FRANK *et al.*, 1998).

The method was introduced by MEHLICH (1978) for the extraction of the soil samples the protocol described by SIMS (2000) was used. The extracting solution was prepared of 0.2 M acetic acid ( $\text{CH}_3\text{COOH}$ ), 0.25 M ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), 0.015 M ammonium fluoride ( $\text{NH}_4\text{F}$ ), 0.013 M  $\text{HNO}_3$  and 0.001 M ethylenediaminetetraacetic acid (abbreviated as EDTA) ( $(\text{HOOCCH}_2)_2\text{NCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{COOH})_2$ ). According to the soil-to-solution ratio of 1:10, 2 g of soil were mixed with 20 mL of extracting solution and shaken for 5 min at room temperature. After filtration the samples were measured photometrically.

As this extraction is a multi-nutrient extraction method – determining P, K, Ca, Mg, Na, Cu, Zn, Mn, B, Al, Fe – many mechanisms are combined to enable the extraction of this amplitude of nutrients. For this method the approach to extract P is an acetic acid solution, promoting the dissolution of Ca-phosphates with an addition of ligand exchange by ammonium fluoride releasing the phosphate by building a new complex with  $\text{F}^-$ . The elements K, Ca, Mg and Na are extracted by ammonium nitrate and nitric acid, while Cu Zn, Mn and Fe are dissolved and complexed by ammonium nitrate and EDTA, which hereby functions as a chelating agent (SIMS, 2000, ZIADI and SEN TRAN, 2007, KUO, 1996).

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### **IX. Bray and Kurtz II (Bray II)**

This method was performed following the procedure of *BRAY and KURTZ (1945)*. 1 g of soil was weighed into a bottle and mixed with 7 mL of extraction solution. Accordingly the solution was prepared in a soil-to-solution ratio of 1:7 with an extracting solution of 0.03 M  $\text{NH}_4\text{F}$  and 0.1 M HCl. The samples were then shaken for 40 seconds. Then the solution was filtered and measured by photometer.

The Bray II extraction is considered to extract Al bound phosphates as the  $\text{F}^-$  is exchanged and forms new complexes with Al, simultaneously prohibiting a fixation by soil colloids (*KUO, 1996*), while the dilute HCl dissolves the Ca-phosphates.

### **X. Dithionite**

The dithionite extraction was introduced by *MEHRA and JACKSON (1960)* and is not considered a conventional phosphorus extraction method. Procedure was executed following the instructions of *HOLMGREN (1967)*.

In order to prepare the extracting solution 0.2 M  $\text{NaHCO}_3$ , and 0.24 M sodium citrate ( $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \times 2 \text{H}_2\text{O}$ ) were mixed. Then 2 g of soil were put into 100 mL extracting solution and 2 g of solid Na-dithionite (0.12 M  $\text{NaS}_2\text{O}_4$ ) was added. The solution was shaken for 16 h and then filtered (the first 10 mL were discarded). The samples were measured with ICP-OES.

The Na-dithionite functions as a reduction agent, the citrate as a chelating agent. With this method the total secondary free Fe with the exception of magnetite (*RAISWELL et al., 1994*) was solubilized (*MEHRA and JACKSON, 1960*).

### **XI. Organic P**

Organic P is a calculated value. It was determined from total P and inorganic P as described below.

In order to determine the inorganic phosphorus in the soils, the first step was to grind and homogenize the sample thoroughly. Subsequently, a homogeneous soil sample of 2 g was used for the extraction procedure, which was carried out (with minor modifications) according to *PARDO et al. (2003)*. The soil-to-solution ratio was 1:100; hence the 2 g were mixed with 200 mL of 1 M HCl. The extraction time was set to 16 h. The analytical determination was carried out by photometrical measurement. Organic P was then calculated by subtracting the values for inorganic P from the values gained by the total P method (s. below).

## **XII. HCl**

The HCl extraction is also not a conventional phosphorus extraction method. To carry out the extraction a 0.5 M HCl solution was prepared. The soil-to-solution ratio was 1:10 and end-over-end shaking time was 2 h. Afterwards the solution was centrifuged for 15 min at 2500 g. The supernatant was transferred to a separate flask and filtered through a membrane filter (0.20 µm). The final solution was then measured by ICP-OES.

The method is based on the dissolution of inorganic phosphorus forms. Phosphorus originating from apatite is dissolved as well as Fe oxide-sorbed phosphorus (at least partly) (KUO, 1996).

## **XIII. Oxalate**

The oxalate extraction method is not a conventional phosphorus extraction method, but derives from soil iron analysis methods. To produce this extraction the protocol of OENORM L 1201 (2007) was followed. As the solution is light-sensitive, the very first step was to ensure that all flasks (both the flasks for extracting procedure and also the ones after filtration for storage until measurement) were impermeable for light. The extraction solution consisted of 0.8 oxalic acid ((COOH)<sub>2</sub> × 2 H<sub>2</sub>O) and 0.11 M ammonium oxalate ((COONH<sub>4</sub>)<sub>2</sub> × H<sub>2</sub>O) and the pH was set to 3.0. Then 1 g of soil was mixed with 50 mL of extracting solution. The solution was shaken for 4 h and then filtered in the dark. The oxalate solutions were measured by ICP-OES.

By this method poorly crystalline Fe was extracted, which approximates the amount of ferrihydrite and Fe associated with organic matter (CAMPBELL and SCHWERTMANN, 1984).

## **XIV. Total P**

To determine the total phosphorus, 2 g of homogeneous soil sample was ignited in a muffle furnace and held at 550°C for 1 h. The residue was transferred to an extraction bottle and extracted with 1 M HCl at a soil-to-solution ratio of 1:100 for 16 h. The analytical determination was carried out photometrically.

The organic phosphorus fraction is turned to inorganic forms of phosphorus by calcination. Thereafter, the total amount of phosphorus in soil (inorganic and former organic) is extracted quantitatively by HCl (modified on the basis of PARDO *et al.*, 2003).

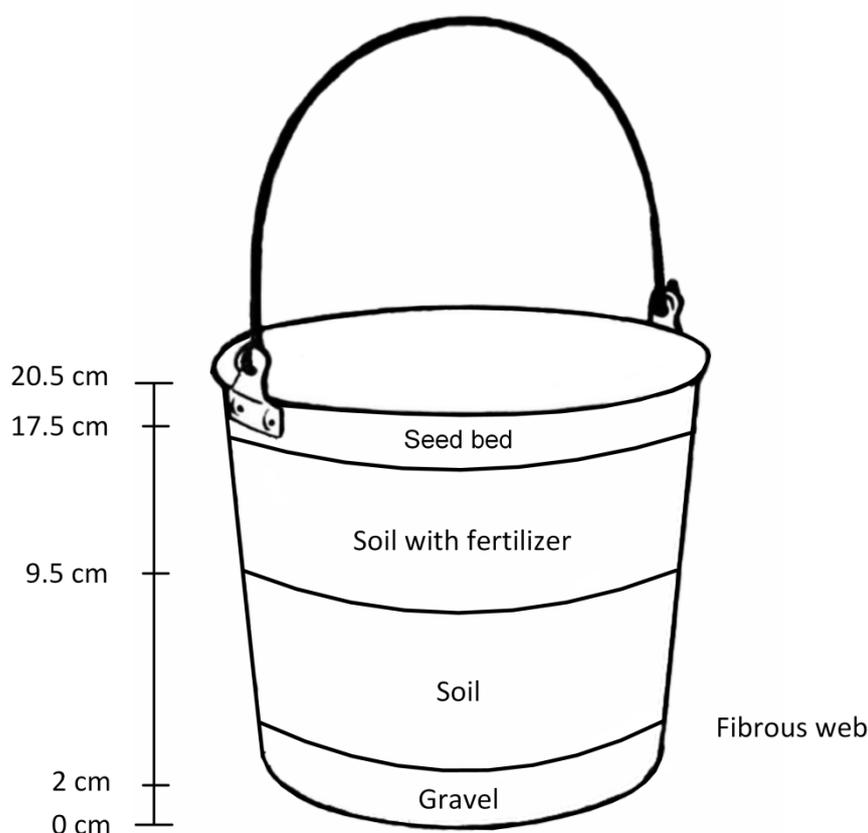
## **2.3 The Greenhouse Pot Experiment**

The pot experiment was carried out in a greenhouse with automatic climate control through ventilation but no air conditioning for cooling. As a preliminary measure the tap water in the greenhouse was tested regarding traces of phosphorus, to assure no

fertilization would take place by irrigation. The water used for irrigation was put in advance into a barrel in the greenhouse to allow it to reach “room-temperature”, avoiding a temperature shock of the plant roots.

### 2.3.1 Set-up of the Pots

The very first step was to homogenize the soil samples. The entire amount of one sample was mixed well in a container. Any big stones and other unwanted objects (wood, plastic, and so on) were removed and large aggregates were crushed. Figure 3 shows a schematic illustration of the layers of each pot.



**Figure 3:** Schematic illustration of the set-up of a pot

The pots were prepared from 12 L buckets. 13 holes – each with a diameter of 10 mm – were drilled on the bottom of each bucket to avoid stagnant water. A 2 cm thick layer of gravel was placed as drainage layer at the bottom. The quartz gravel was washed to remove any dust and to make sure that only pure gravel was left. In order to keep the soil from washing out and to provide a barrier between the soil and gravel layer, a fibrous web was placed in between. One layer of sample prepared beforehand was filled into the bucket and re-compacted a bit then another layer of soil was put thereupon. This layer was mixed well with 0.8 g KCl fertilizer (each pot) and also this

layer was again compacted a bit. The top 3 cm were sieved in order to offer an ideal seed bed later on. The total filled soil volume was 10.88 L. The sequence of the working steps is shown in Figure 4 below.



**Figure 4:** Pictured illustration of the working steps necessary to prepare the pots

### 2.3.2 The Experimental Procedure

The experiment started on May 2<sup>nd</sup>, 2011 with sowing the spring wheat and ended on July 28<sup>th</sup>, 2011 with harvesting the plant material, amounting to a total of 88 days test duration.

Pots prepared as described in chapter 2.3.1 were set up for sowing by first watering them. A master plate was used to standardize the pattern and the distance of the seeds. 19 grains were sown in each of the buckets in a depth of about 2-3 cm and afterwards watered carefully. In Figure 5 this process is illustrated in detail.



**Figure 5:** Pictured illustration of the sowing process

During the growing phase the pots were watered according to necessity depending on e.g. the weather and the growing stage of the plants. The soil water content was monitored by soil moisture sensors (four Decagon ECH2O Dielectric Aquameter sensors and one Decagon 5TE) in five representative pots highly varying in soil properties (to have references for all 50 pots included in the experiment). Soil water was kept near field capacity. On average the pots were irrigated every 1.8 days with an average of about 340 mL each time and pot. The average soil temperature measured

by the Decagon 5TE sensor amounted to 24.9°C with a normal day to night fluctuation and a minimum at 18.8°C and a maximum of 36.6°C.

A common fertilization strategy was applied for the spring wheat plants. Details on measures and the time of application are given in Table 2 below. In total, the applied fertilizers added to 221.69 kg N ha<sup>-1</sup> and 79.03 kg K ha<sup>-1</sup>.

**Table 2: Application of fertilizer over the duration of the experiment**

Date	Fertilizer application per m <sup>2</sup>
2. May	15.07 g KCl
9. May	48.97 g <sup>a</sup> NAC 27 granulate
19. May	11.09 g Ammonium nitrate
14. June	22.60 g <sup>b</sup> Urea fertilization
22. June	1.00 g <sup>b</sup> Urea leaf fertilization

<sup>a</sup> NAC 27 = NAC with 13.5% N derived from nitrate N and 13.5% N derived from ammonium N

<sup>b</sup> Urea = 46% N

The plants were constantly monitored regarding unusual changes in vegetation and diseases. When a moderate infestation of thrips occurred, measures were taken as described in detail in Table 3 below. A biologic method of pest control was implemented when spider mites infested the spring wheat plants. Only measures that would not interfere with the experimental outcome were considered. Sulphuric treatment and predatory mites were applied; in addition a high humidity was maintained to keep the infestation at a minimum. The feeding damage by the thrips infestation is shown in Figure 6 below.

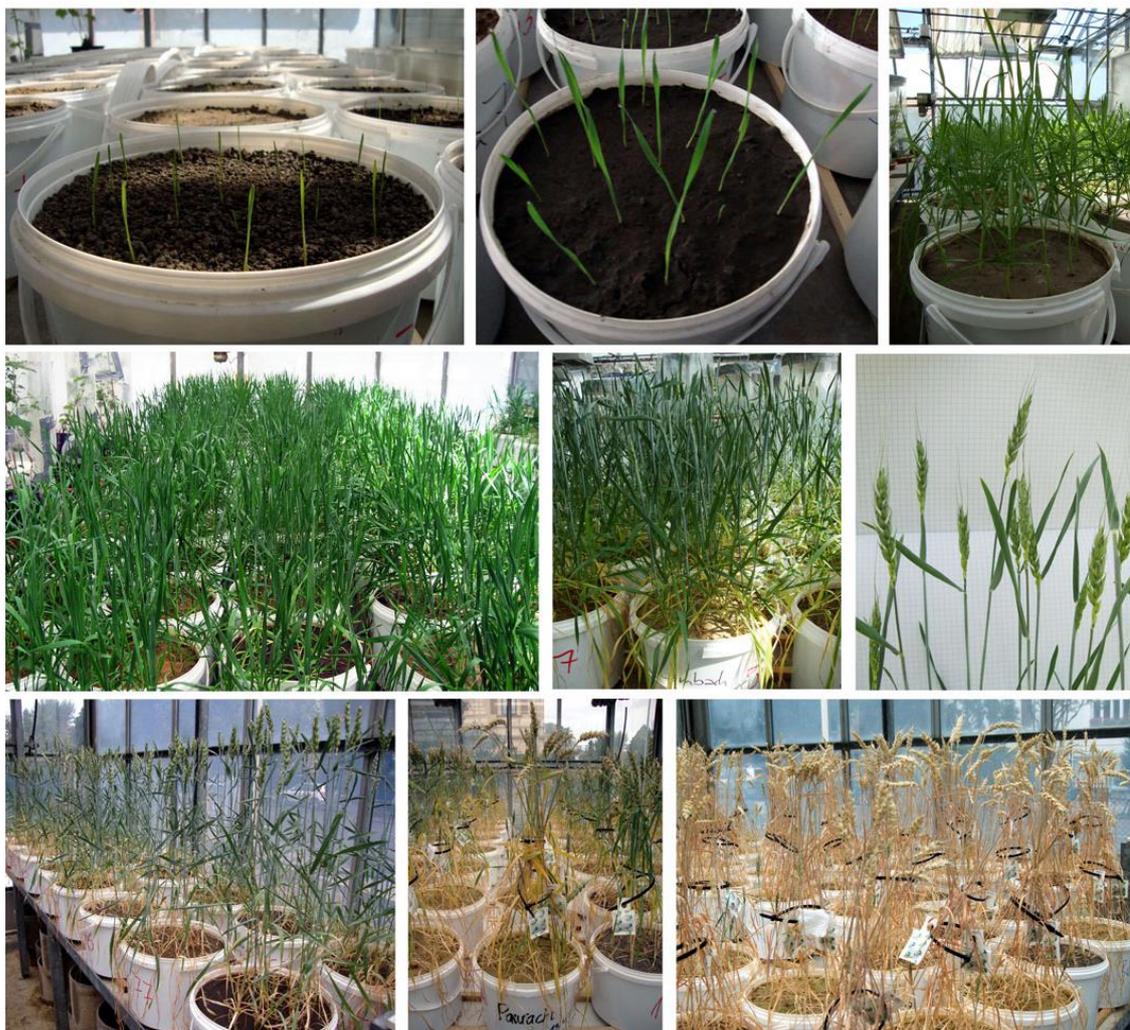


**Figure 6: Feeding damage caused by moderate thrips infestation**

**Table 3: Detailed description of pest management measures and of the time of application**

Date	Pest management application per m <sup>2</sup>
20. May	Fungicide: application of 14.05 mL H <sub>2</sub> O + 0.04 mL acetate + 0.14 mL sulfate + 0.02 mL Gladio (active agent)
21. June	Insecticide: 100 mL application of 'Campo Schädlingfrei plus' (active agent 4.59 g L <sup>-1</sup> pyrethrine)
22. June	Fungicide: application of 20 mL H <sub>2</sub> O + 0.05 mL acetate + 0.20 mL sulfate + 0.03 mL Gladio (active agent)
1. July	Insecticide: application of 20 mL H <sub>2</sub> O + 0.08 mL adhesive + 0.40 g wettable sulphur
4. July	0.5 L insecticidal soap (potassium salts of fatty acids 10.2 g L <sup>-1</sup> ) 265 predatory mites ( <i>Phytoseiulus persimilis</i> )
7. July	26 predatory mites ( <i>Phytoseiulus persimilis</i> ) 100 predatory mites ( <i>Phytoseiulus amblyseius swirskii</i> )

The progress of the experiment is illustrated graphically from the beginning of germination to the ripening of the ears in Figure 7 below.



**Figure 7: Experimental progress: development of the plants**

Before harvesting, the plants were not watered for a longer period of time in order to guarantee ripening. All plants were harvested at once, whereupon parameters were measured as described in chapter 2.4 below. Also straw was separated from the ears on-site. For analysis all the plant material was dried and milled. The grain was threshed, counted and weighed afterwards.

## 2.4 Plant Analysis

Most of the plant parameters were determined at harvest. The tillering was determined by the number of stalks (green and ripened). Additionally the number of stalks with ripe ears and without ripe ears were counted. The thousand seed weight, number of ears and number of grains per ear were recorded. The weight of the straw and the weight of the grain per m<sup>2</sup> were determined.

The straw was dried at 50°C for three days, after that it was weighed immediately. The next step was to grind each sample to improve the performance of

digestion. The grain yield was also dried and then weight and calculated back to the 14% water content that is conventionally used for the thousand seed weight. The grain was dried as it was important to have dried material for the digestion. The grain samples were also ground to assure complete digestion later on.

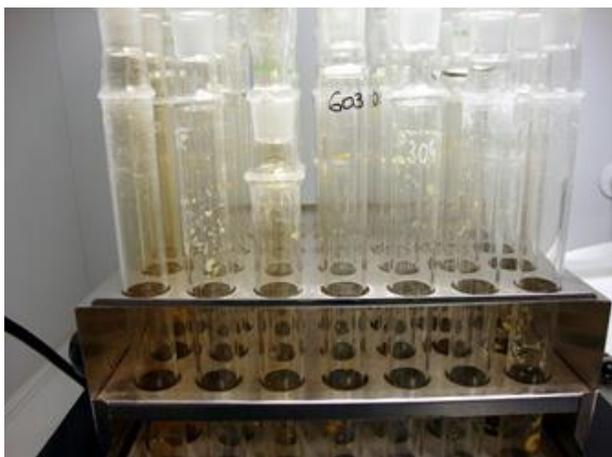
#### 2.4.1 Digestion Method

In order to analyze the plant samples the straw was dried separately from the grain. Next, each sample was ground with a hand mill to facilitate and assure a complete digestion of the samples later on. A plant material sample of 200 mg was digested with 5 mL HNO<sub>3</sub> and 1 mL of H<sub>2</sub>O<sub>2</sub>. The tubes were left in a fuming hood to react overnight. Hereby it is important to put the cooler tubes immediately onto the tubes as the reaction produces a lot of foam and the reacting material might rise within the tubes and even leak out. The next morning the digestion program was run according to program settings given in Table 4.

Table 4: Program settings for the plant digestion

Temperature	Lapse Time
[°C]	[min]
65	25
110	10
155	190

The solutions were diluted with approximately 30 mL of distilled water and vortexed. The exact weight of each sample was noted, in order to be able to calculate back on the exact dilution by the water. At last, the solutions were filter by paper filters and then measured by photometer. Note that it was necessary to acid wash all the flasks and tubes that were used for the digestion, as to avoid any contamination by residuals of previous runs. Figure 8 below displays the digestion tubes while running the program.



**Figure 8:** The tubes in the digester with plant material and  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  during the run of the digestion program

## 2.5 Instrumental Phosphorus Analysis

Two different instruments of measurement were used to determine the phosphorus in the extraction solutions. On the one hand ICP-OES and on the other hand the photometer. When using the photometer there were two further methods to color the solutions as described in chapter 2.5.2.

### 2.5.1 Inductively Coupled Plasma - Optical Emission Spectrometry (ICP - OES)

The ICP-OES measures the total amount of P in the solution, whereas the colorimetric method determines only the ortho-phosphate amount in the solution (*OENORM L 1087, 2006*). One advantage of this measuring method is that ICP-OES is able to detect more than one nutrient at once, while the colorimetric measurement is only capable to determine phosphate (*PIERZYNSKI et al., 2009*). Also, the ICP-OES can measure P in solutions where the matrix disturbs the color formation of the photometric method.

### 2.5.2 Photometer

The photometer is a device to detect phosphate colorimetrically. The method is based on the formation of a complex between phosphate and molybdate. This complex is protected from oxidation through adding ascorbic acid. The complex is stable for 4 h after a reaction time of 15 min (*OLSEN and SOMMERS, 1982*). All samples were measured by an Agilent 8453 UV-Visible spectrophotometer using either a flow cell or manual cuvettes.

#### 2.5.2.1 Molybdenum-Blue Method

This method originally published by *MURPHY and RILEY (1962)* is used as a standard method for determination of phosphorus extracted by soil testing. The detection limit is

0.3 mg P L<sup>-1</sup>. The detailed protocol for the procedure can be found in *OENORM L 1087 (2006)*.

The first step was to produce a stock solution of ammonium heptamolybdate which had to be further diluted (1:10) just before usage to gain the working solution. Additionally a 0.025 M ascorbic acid solution was prepared. For measurement 1 mL of ascorbic acid was mixed with 0.5 mL of sample and then 8 mL working solution were added (the *OENORM* instructions were modified here, the volume ratio was maintained but just half the amount of liquid was used.) For each measurement series the wavelength had to be set to the optimum. The optima for all measurements were close to 710 nm.

#### **2.5.2.2 Modified Molybdenum-Blue Method**

Due to the need for a higher sensitivity, a different method to color the extraction solutions was selected. This method allowed for a detection limit of about 25 µg P L<sup>-1</sup>.

The method of *MURPHY and RILEY (1962)* was modified accordingly: while the four solutions were prepared with the identical concentrations as in the original, the volumes mixed to prepare the working solution were changed. 10 mL of 2.5 M H<sub>2</sub>SO<sub>4</sub> was combined with 3 mL of 0.032 M ammonium molybdate, 1 mL of 0.006 M potassium antimonytartrate and 6 mL of 0.1 M ascorbic acid. The obtained solution was used for the color reaction, where 1 mL was mixed with 5 mL of sample. After a reaction time of 15 min the samples could be measured at 710 nm.

## **2.6 Statistical Methods**

For basic statistical analysis MS Excel 2007 was used while for computing the analysis of variance and multiple linear regressions SPSS 15.0 for Windows was used. SigmaPlot 11.0 was used to create all the graphical work.

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## 3 Results and Discussion

### 3.1 Basic Soil Properties

In this chapter a description of the samples with regard to basic soil characteristics will be presented. A summary is given in Table 5a and Table 5b below, the mean, median, standard deviation, minimum and maximum values and also 10% quantile and 90% quantile are presented.

The soil samples used for this experiment represented a wide range of soil characteristics. The pH (KCl) ranged from 4.3 to 7.1, the soils displayed a span from a low base saturation of 21% to a very high one of 97%. Two-thirds of the soils were carbonate free, the remaining soils had carbonate contents ranging from 1.1% to 56% with an average of about 20% CaCO<sub>3</sub>. The organic carbon was found to range between 0.93% to 4.03% and the C/N ratio of the organic matter varied from 9 to 25 and the N/P ratio ranges from 5 to 63. These ratios are usually given jointly as C/N/P ratio, in soils this is on average 186/13/1 (CLEVELAND and LIPTZIN, 2007). The texture of the soils showed a wide range. The samples were varying between a high clay content of over 50% to a very high sand content of over 80%. The potential CEC showed an average of 13 cmol<sub>c</sub> kg<sup>-1</sup>.

The Fe<sub>d</sub> (oxidic iron) ranged from 1863 mg kg<sup>-1</sup> to 42990 mg kg<sup>-1</sup>. The Fe<sub>o</sub>/Fe<sub>d</sub> ratio – giving information on the degree of weathering of the iron oxides and therefore on the age of the soil (LAIR *et al.*, 2009) – indicated that the selection of soils featured samples with mainly amorphous iron oxides (Fe<sub>o</sub>/Fe<sub>d</sub> above 0.5) to soils with primarily crystalline iron oxides (Fe<sub>o</sub>/Fe<sub>d</sub> below 0.5) The ratio P<sub>o</sub>/Fe<sub>o</sub> was ranging between 0.08 and 0.84.

Soil parameters of particular importance to P solubility, P availability and P extractability are: the prevailing pH in the soil and related to it the CaCO<sub>3</sub> content, the distribution of texture, the mineralogical composition, the total surface area, the content of organic matter and also the presence of Fe and Al (hydr-)oxides (ABDU, N. 2006). The prevalent combination of these parameters in the soil influences the solubility of phosphates. For example, ZORN and KRAUSE (1999) reported that the extraction capacity of the CAL method was affected by CaCO<sub>3</sub>. It is possible that higher CaCO<sub>3</sub> contents increase the pH in the extraction solution and may therefore diminish the effectiveness of the CAL extraction. However, samples which showed reduced extraction capacity could not be identified by their CaCO<sub>3</sub> content alone.

Table 5a: Summary of physicochemical soil properties; n=50

	pH (H <sub>2</sub> O)	pH (KCl)	<sup>a</sup> EC [mS cm <sup>-1</sup> ]	<sup>b</sup> OC [%]	CaCO <sub>3</sub> [%]	C/N (molar)	C/P (molar)	N/P (molar)	<sup>c</sup> KH mL 100g <sup>-1</sup>	Clay	Silt	Sand
												[%]
<b>Mean</b>	7.2	6.0	0.65	1.72	5.6	13.0	173	13.6	55.1	22.8	40.1	36.5
<b>Median</b>	7.3	6.0	0.61	1.47	0	11.7	129	11.4	52.3	24.4	46.2	28.1
<b>Standard Dev.</b>	0.5	0.8	0.27	0.74	11.6	3.1	103	9.1	22.1	9.9	16.6	23.8
<b>Minimum</b>	6.1	4.3	0.23	0.93	0	9.0	79	5.4	26.0	4.2	9.1	1.5
<b>10% Quantile</b>	6.5	4.9	0.37	1.02	0	10.4	90	7.8	31.7	12.1	18.2	13.1
<b>90% Quantile</b>	7.9	7.0	1.02	2.67	25.1	15.9	292	22.2	78.0	34.5	59.8	66.3
<b>Maximum</b>	8.0	7.1	1.35	4.03	56.2	25.1	564	63.0	121	53.1	63.6	86.2

<sup>a</sup> EC = electrical conductivity in saturated H<sub>2</sub>O extract<sup>b</sup> OC = organic carbon<sup>c</sup> KH = amount of water necessary to prepare the H<sub>2</sub>O saturation extract

Table 5b: Summary of physicochemical soil properties; n=50

	<sup>d</sup> CEC <sub>pot</sub> [cmol <sub>c</sub> kg <sup>-1</sup> ]		[% of CEC <sub>pot</sub> ]							[mg kg <sup>-1</sup> ]			<sup>g</sup> P <sub>o</sub> /Fe <sub>o</sub> (molar)
	<sup>e</sup> BS	Ca	Mg	K	Al	<sup>f</sup> Fe <sub>d</sub>	<sup>f</sup> Mn <sub>d</sub>	<sup>g</sup> Fe <sub>o</sub>	<sup>g</sup> Mn <sub>o</sub>	<sup>g</sup> Fe <sub>o</sub> /Fe <sub>d</sub>			
<b>Mean</b>	13.0	55.3	8.0	2.9	2.3	9770	490	4040	373	0.42	0.31		
<b>Median</b>	12.4	54.4	7.8	2.5	1.5	8370	450	3270	381	0.42	0.25		
<b>Standard Dev.</b>	4.5	19.4	3.3	1.7	2.2	6480	197	3320	195	0.14	0.20		
<b>Minimum</b>	6.2	14.7	3.0	0.5	0.2	1863	67	930	47	0.14	0.08		
<b>10% Quantile</b>	7.4	31.6	3.9	1.0	0.4	4900	275	1360	143	0.23	0.16		
<b>90% Quantile</b>	18.0	80.9	13.0	5.7	5.4	14240	704	5710	598	0.55	0.67		
<b>Maximum</b>	29.1	82.6	15.8	7.4	11.4	42990	1010	21950	821	0.82	0.84		

<sup>d</sup>CEC<sub>pot</sub> = potential cation exchange capacity (determined at pH 8.1)

<sup>e</sup>BS = base saturation

<sup>f</sup>Fe<sub>d</sub> and Mn<sub>d</sub> = dithionite-extractable Fe and Mn

<sup>g</sup>Fe<sub>o</sub>, Mn<sub>o</sub> and P<sub>o</sub> = oxalate-extractable Fe, Mn and P

## 3.2 Soil P Extractions

### 3.2.1 Comparing Applied Soil P Extraction Methods

In this chapter the data gained by the soil P extraction methods are displayed in detail. The measured values were processed and prepared statistically in Table 6. The mean, median, standard deviation, minimum and maximum values and also 10% quantile and 90% quantile are given. Average values shown in Table 6 are illustrated graphically in Figure 9. To compare the methods with each other, it was tested how well they correlate. The correlation coefficients with significance level are given in Table 7.

On average, the efficiency of extraction increased along the order of  $\text{H}_2\text{O} < \text{CaCl}_2 < \text{LiCl} < \text{Fe-oxide P}_i < \text{Olsen} < \text{CAL} < \text{CAEM} < \text{Mehlich 3} < \text{Bray II} < \text{Dithionite} < \text{Organic P} < \text{HCl} < \text{Oxalate} < \text{Total P}$  (shown in Table 6 and Figure 9). This sequence is similar to findings by other authors (*KULHÁNEK et al., 2009b, CSATHÓ et al., 2005, FERNANDES et al., 1999*).

While the  $\text{H}_2\text{O}$ ,  $\text{CaCl}_2$  and  $\text{LiCl}$  extractions extracted an average P amount below  $10 \text{ mg kg}^{-1}$ , the averages of Fe-oxide  $\text{P}_i$ , Olsen, CAL and CAEM were also in the same range of around  $100 \text{ mg kg}^{-1}$ . Mehlich 3 and Bray II obtained about the same average magnitude (below  $200 \text{ mg kg}^{-1}$ ) while Dithionite, Organic P and HCl averaged between 200 and  $300 \text{ mg kg}^{-1}$ . The mean values of Oxalate and Total P amounted to 530 and  $830 \text{ mg kg}^{-1}$ , respectively.

The widest range of measured values by the same extraction method was detected for Bray II where the highest value was of 201-fold greater magnitude than the lowest measured value that is  $3.13 \text{ mg kg}^{-1}$  vs.  $628 \text{ mg kg}^{-1}$ , respectively. The narrowest range was detected for the HCl extraction method, where the maximum extracted P ( $622 \text{ mg kg}^{-1}$ ) was less than five times higher than the lowest value ( $129 \text{ mg kg}^{-1}$ ). *SAGGAR et al. (1999)* discussed the advantage of extraction methods producing a greater range within the samples, stating that a categorization in P status groups may be more easily achieved. This is especially important in routine soil testing when results provide the basis for fertilization recommendations.

As expected, the lowest extractable amount of phosphorus from soil was found by the  $\text{H}_2\text{O}$  extraction method and the highest amount was measured by the extraction method for the total P content (c.f. Table 7 Figure 9). *KULHÁNEK et al. (2007)* stated that  $\text{CaCl}_2$  may extract lower amounts of P than the  $\text{H}_2\text{O}$  method due to formation of Ca-phosphate. The data of our experiment showed different results, probably due to the application of a saturation water extract. Also, concentrations for a  $\text{H}_2\text{O}$  extract found

by *MARSCHNER (1995)* ranged from 0.8 to 8.0 mg kg<sup>-1</sup> while our results showed lower values of 0.03 to 1.79 mg kg<sup>-1</sup> which may be for the same reason.

The total P values in our samples ranged from 298 to 1680 mg kg<sup>-1</sup>, reflecting a diverse range of soils. By comparison *CROSS and SCHLESINGER (1995)* found total P values from 64 to 908 mg kg<sup>-1</sup> in 88 different soils globally.

*ZBÍRAL and NĚMEC (2002)* measured for Mehlich 3 similar values as in our study ranging from 4 to 643 mg kg<sup>-1</sup> with an average at 107 mg kg<sup>-1</sup> over a very broad range of soils. But in general, it was found that a comparison with other studies entails some problems; soils in other studies are found to be very different regarding physicochemical parameters (e.g. tropical soils *BORTOLON and GIANELLO, 2012, MAMO et al., 1996*); other studies applied a variety of other crops (*KUMAR et al, 1994*) or tested the influence of various fertilizer applications on P extraction methods (*MENON et al., 1989a, MENON et al., 1991*).

Table 6: Comparison of the extractable P by the 14 extraction methods [mg kg<sup>-1</sup>]; n=50, Dithionite n= 48

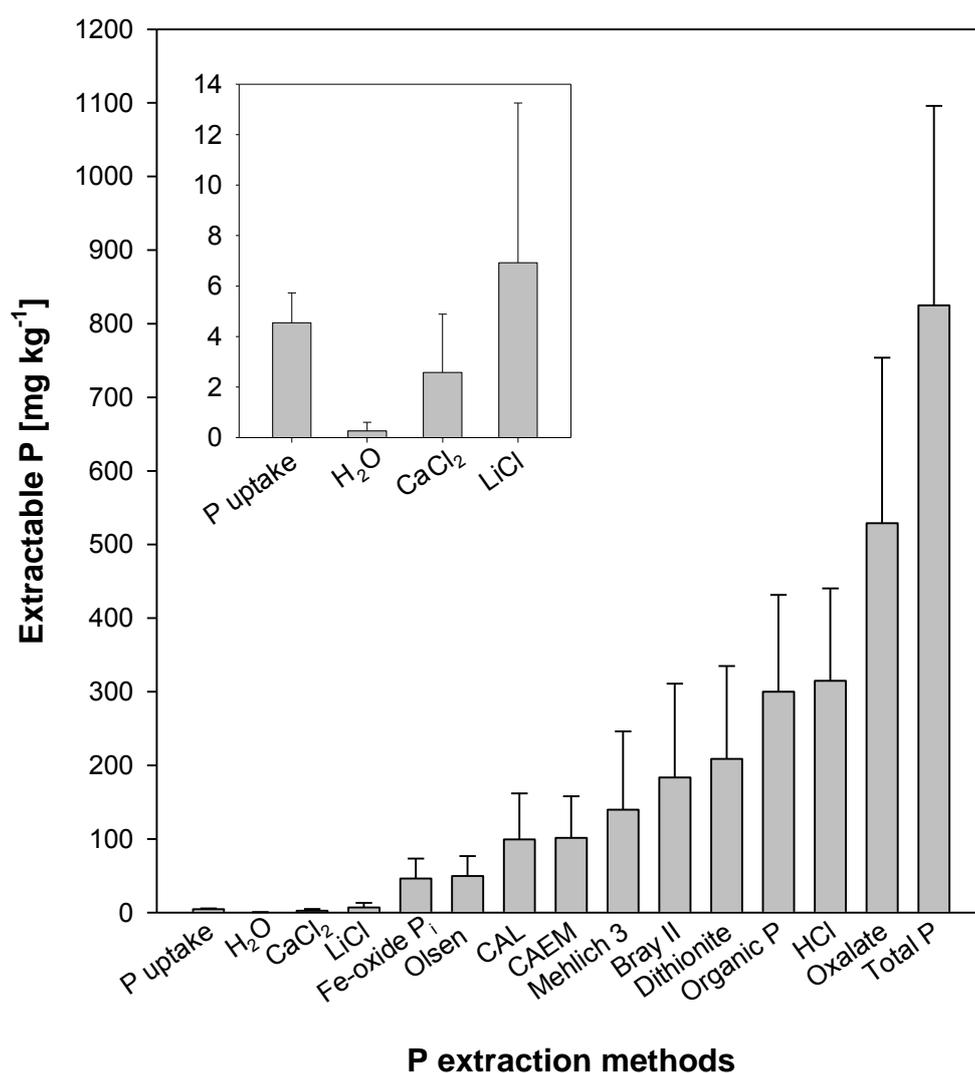
	H <sub>2</sub> O	CaCl <sub>2</sub>	LiCl	<sup>a</sup> Fe-oxide P <sub>i</sub>	Olsen	<sup>b</sup> CAL	<sup>c</sup> CAEM	Mehlich 3	Bray II	Dithionite	Organic P	HCl	Oxalate	Total P
	[mg kg <sup>-1</sup> ]													
<b>Mean</b>	0.26	2.58	6.79	46.2	49.6	99.3	102	140	183	209	300	315	529	825
<b>Median</b>	0.12	1.52	4.95	41.3	41.4	76.9	94.2	110	158	179	308	304	488	844
<b>Standard Dev.</b>	0.35	2.31	6.33	27.2	27.0	62.6	56.5	106	127	126	132	125	225	271
<b>Minimum</b>	0.03	0.10	b.d.	5.58	10.8	17.8	20.5	27.2	3.13	44.4	54.4	129	179	298
<b>10% Quantile</b>	0.04	0.74	1.64	18.3	21.7	40.3	37.5	46.5	67.0	76.1	134	158	320	449
<b>90% Quantile</b>	0.56	5.63	12.7	86.1	80.7	182	182	256	347	381	459	481	770	1150
<b>Maximum</b>	1.79	10.5	34.8	122	124	267	246	540	628	607	716	622	1300	1680

<sup>a</sup> Fe-oxide P<sub>i</sub> = iron oxide impregnated filter<sup>b</sup> CAL = calcium acetate lactate<sup>c</sup> CAEM = cation and anion exchange membrane

As mentioned in the beginning of this chapter, the magnitude of the average extracted phosphorus by the 14 methods is shown in Figure 9. Additionally, the average P uptake by the plants is given in the first column. This comparison illustrates that the weaker extraction methods (i.e. H<sub>2</sub>O, CaCl<sub>2</sub>, LiCl) were in the range of the average P uptake by plants, whereas the other extraction methods extracted much higher P amounts. It is notable that the oxalate extraction method was closest to Total P amounts in the soil samples. *MENON et al. (1989)* found that measured values of Fe-oxide P<sub>i</sub> and Olsen were about the same magnitude, which is in agreement with the results obtained in our study and displayed in Figure 9.

Plants and other organisms mainly assimilate phosphorus dissolved in the soil solution. Due to its low solubility, the concentration of dissolved phosphorus in the solution is generally low (below 5 µmol) (*CONDRON and TIESSEN, 2005*), *BLUME et al., (2010)* listed a concentration of 0.2 to 0.8 mg P L<sup>-1</sup>. Approximately that amount was extracted by our water saturation extraction. *EMSLEY (2001)* stated that the concentration in the soil solution will at any given time hold only about 1% of phosphorus essential to a healthy plant growth. In our study, the average amount of P taken up by the plants was 4.55 mg kg<sup>-1</sup>, while the H<sub>2</sub>O extraction method extracted 0.26 mg kg<sup>-1</sup> on average. Here, the P in soil solution was more than 5% of the average plant uptake.

Figure 9 displays also that the average organic P amounted to about 50% of the total P in the given soil samples which is in agreement with *CONDRON and TIESSEN (2005)* who stated a common range of 35-65%.



**Figure 9:** Comparison of applied extraction methods by the average amount of extracted P and standard deviation. Abbreviations: Fe-oxide P<sub>1</sub> = iron oxide impregnated filter paper, CAL = calcium acetate lactate, CAEM = cation and anion exchange membrane

From Table 7, it can be perceived as an overall trend that most extraction methods correlated well with each other. Two extraction methods did not follow this trend: Organic P, which only correlated with the extraction methods Dithionite, Oxalate and Total P positively and with CaCl<sub>2</sub> negatively, and Total P, which correlated (positively) with only half of the methods (Olsen, CAL, CAEM, Dithionite, Organic P, HCl, and Oxalate). Moreover, it was notable that the correlation coefficients between the weaker extraction methods, i.e. H<sub>2</sub>O, CaCl<sub>2</sub> and LiCl, and the other extraction method declined with increasing extracting intensity of the other methods. The Dithionite extraction method did not correlate with any of these weaker extraction methods, a similar picture could be noted for Oxalate.

The highest correlation coefficient was found between Bray II and Mehlich 3 (0.944), the lowest was determined for Total P and  $\text{CaCl}_2$  (being close to 0.000). The close correlation of Bray II and Mehlich 3 was most likely found due to their similar mechanism to extract the phosphorus ( $\text{F}^-$  binding on Al and releasing phosphate). Throughout the study the results by these two extraction methods exhibited similarity.

Interesting is that the  $\text{H}_2\text{O}$  method best correlated with the LiCl while the  $\text{CaCl}_2$  method was closest correlated with the Fe-oxide  $\text{P}_i$ . The close correlation by CAEM and Fe-oxide  $\text{P}_i$  was expected as these methods are based on the same mechanism. Equally, the highest correlation coefficient for Organic P and Oxalate was found with Total P as these three methods (also) extract organic bound phosphorus.

Table 7: Correlation coefficients between the extraction methods, significance in \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ 

	H <sub>2</sub> O	CaCl <sub>2</sub>	LiCl	<sup>a</sup> Fe-oxide P <sub>i</sub>	Olsen	<sup>b</sup> CAL	<sup>c</sup> CAEM	Mehlich 3	Bray II	Dithionite	Organic P	HCl	Oxalate
CaCl <sub>2</sub>	0.795 ***												
LiCl	0.851 ***	0.771 ***											
<sup>a</sup> Fe-oxide P <sub>i</sub>	0.644 ***	0.813 ***	0.686 ***										
Olsen	0.574 ***	0.657 ***	0.646 ***	0.888 ***									
<sup>b</sup> CAL	0.509 ***	0.556 ***	0.732 ***	0.663 ***	0.801 ***								
<sup>c</sup> CAEM	0.722 ***	0.721 ***	0.752 ***	0.868 ***	0.849 ***	0.661 ***							
Mehlich 3	0.437 **	0.701 ***	0.652 ***	0.865 ***	0.837 ***	0.761 ***	0.693 ***						
Bray II	0.409 **	0.643 ***	0.649 ***	0.810 ***	0.792 ***	0.826 ***	0.658 ***	0.944 ***					
Dithionite	0.194	0.193	0.173	0.528 ***	0.664 ***	0.342 *	0.607 ***	0.394 **	0.367 **				
Organic P	-0.073	-0.337 *	-0.164	-0.209	-0.047	-0.048	-0.098	-0.210	-0.152	0.378 **			
HCl	0.327 *	0.289 *	0.447 **	0.516 ***	0.727 ***	0.740 ***	0.622 ***	0.448 **	0.505 ***	0.598 ***	0.104		
Oxalate	0.293 *	0.216	0.286 *	0.538 ***	0.740 ***	0.564 ***	0.551 ***	0.497 ***	0.519 ***	0.788 ***	0.475 ***	0.695 ***	
Total P	0.165	-0.040	0.157	0.214	0.436 **	0.400 **	0.359 *	0.139	0.212	0.675 ***	0.765 ***	0.680 ***	0.813 ***

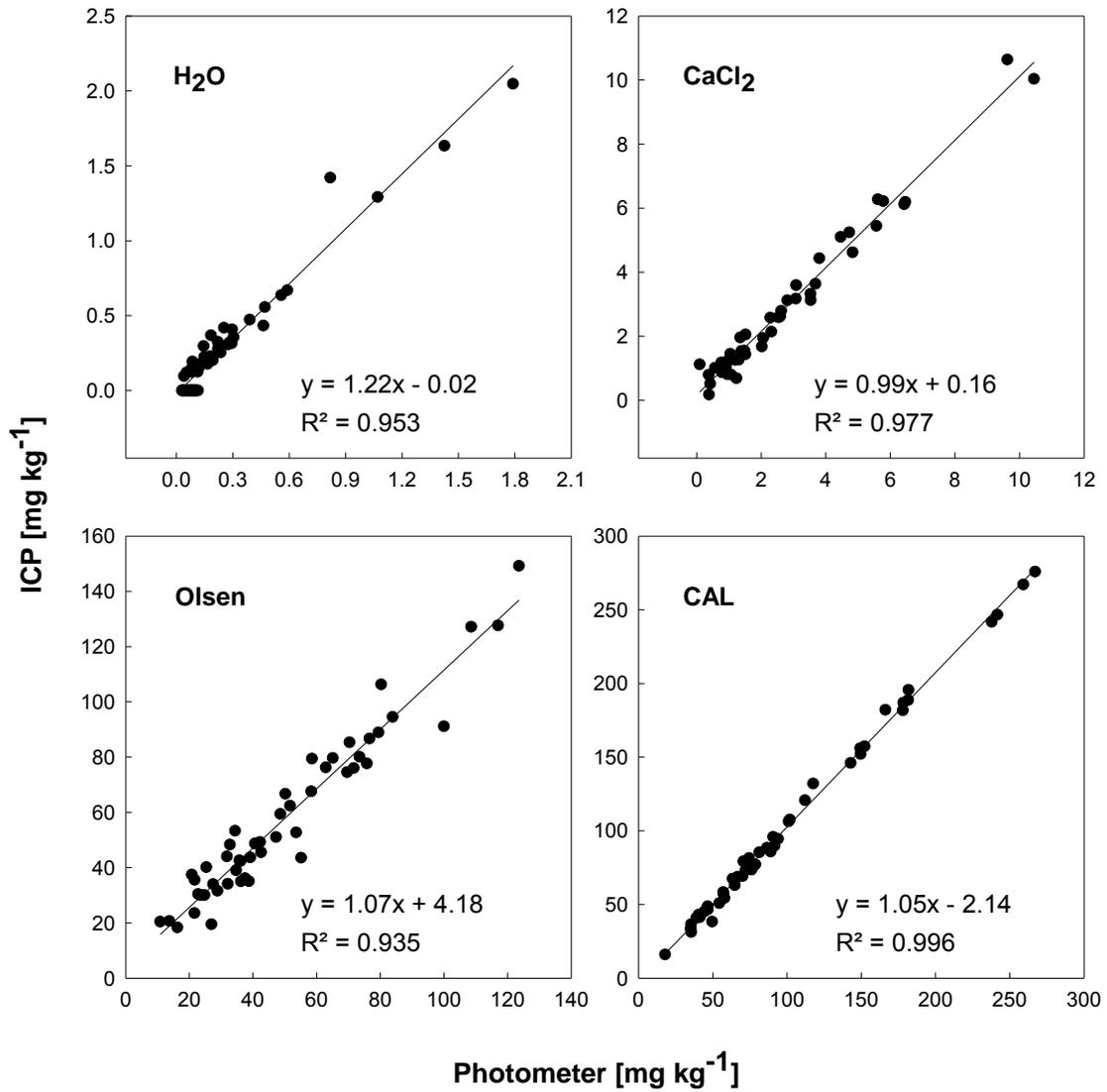
<sup>a</sup>Fe-oxide P<sub>i</sub> = iron oxide impregnated filter paper<sup>b</sup>CAL = calcium acetate lactate<sup>c</sup>CAEM = cation and anion exchange membrane

### 3.2.2 ICP versus Photometer

Figure 10 shows the difference of measurement when using ICP-OES and the colorimetric method (molybdenum blue method measured by photometer) performed as described in *OENORM L 1087 (2006)*. As mentioned in chapter 2.5.1, the photometer is only capable of detecting phosphate because it is dependent on the formation of a molybdenum blue complex. *SEELING and JUNGK (1996)* found that the  $\text{CaCl}_2$  method extracts organic P that is taken up and readily utilized by plants. However, this fraction of organic phosphorus – phytate soil phosphorus – is immeasurable by the molybdenum blue method. Even though phytate soil phosphorus is highly plant available, *STEFFENS et al. (2010)* found that routine soil tests are only limitedly able to extract and detect this fraction of soil phosphorus. . Conversely, according to *KUO (1996)*, the colorimetric method is preferable for samples containing low amounts of phosphorus or a high proportion of organic phosphorus; ICP measurement might overestimate the extractable P due to an oxidation of organic P through high plasma temperatures at measurement. *MALLARINO (2003)* found that ICP measurements determined higher P amounts than the photometer, and found a correlation coefficient of 0.84 for Mehlich 3 measured by both ICP and photometer.

In order to determine differences due to the type of measurement for the samples at hand, for four methods ( $\text{H}_2\text{O}$ ,  $\text{CaCl}_2$ , Olsen, CAL) all 50 samples were measured by both analytical techniques. The data showed similar results as reported by *PIERZYNSKI et al. (2011)*; a very close correlation between ICP and photometer was observed for all four extraction methods (see Figure 10). Furthermore, the values measured by ICP were slightly higher than the ones detected by photometer. *PIERZYNSKI et al. (2011)* found a higher variability between the two detection methods when the samples contained only low P contents (below  $60 \text{ mg kg}^{-1}$ ); this is not in agreement with the data presented in our study.

As values measured by the two analytical techniques showed only little difference, a double measurement for all methods was considered unnecessary. Coefficients of determination ranged between 0.935 for Olsen and 0.996 for CAL. For the other methods, the P contents were only determined by one of the analytical techniques, i.e. either ICP-OES or photometer (cf. Table 1).



**Figure 10:** A comparison of measurement by ICP-OES and by photometer on the basis of coefficients of determination of four soil P extraction methods. Abbreviations: CAL= calcium acetate lactate

### 3.3 Correlating Soil P Extraction Data with Soil Parameters

In this chapter, correlations for P extractions and soil parameters were calculated. Table 8a and 8b show the correlation coefficients for the soil parameters and the P extracted by the different extraction methods. In addition, correlation coefficients of soil parameters and the total P uptake by the plants are presented in these tables.

As a next step, in Table 9 the 50 soil samples were grouped according to pH (KCl), the organic carbon and clay content. The three subdivisions of pH (KCl) were selected at pH lower than 5.5, from 5.5 to 6.6 and pH higher than 6.6. The organic carbon content was subdivided to a group below 1.2%, a group from 1.2% to 2% and one above 2% organic carbon content. Also the clay content was split into three groups of clay content below 17%, ranging from 17% to 28% and one that was above 28%. The classification ranges are based on *BLUM et al. (1996)* and modified to better fit the sample data. For each P extraction method and grouping the mean values and the standard deviations were calculated. Subsequently these calculated values were compared in an ANOVA. The Levene test was used to assure homogeneity of variances. As a standard post hoc test the Duncan test disclosed significant differences of means (significance level of 0.05). If the Levene test was significant, the Games-Howell test was used instead (significance level of 0.05). A detailed summary is given below in Table 9 where mean values followed by different letters are significantly different.

As shown in Table 8a and 8b soil parameters and the P uptake by plants (in mg m<sup>-2</sup>) yielded only two significant results: CaCO<sub>3</sub> and K correlated significantly negatively. This indicated that besides the two parameters just mentioned, none of the soil properties had a major impact on the uptake of P by the plants. Generally, an increasing amount of CaCO<sub>3</sub> may decrease the amount of plant available P (*MING, 2006*). This is likely caused by sorption and co-precipitation of phosphate on calcite surfaces.

The pH was negatively correlated with CaCl<sub>2</sub>, Fe-oxide P<sub>i</sub>, Olsen, CAEM, Mehlich 3, Bray II and Dithionite. Due to the relation of pH with CaCO<sub>3</sub>, base saturation (BS) and exchangeable Ca the results were corresponding. The CAL method was found to overestimate the decrease in plant availability with increasing CaCO<sub>3</sub>, as the pH of the extraction solution may be raised by the calcium carbonate of the soil (*ZORN and KRAUSE, 1999, ZBÍRAL and NĚMEC 2002*). As an alternative *ZORN and KRAUSE (1999)* suggested the use of the Olsen extract, *ZBÍRAL and NĚMEC (2002)* proposed the use of Mehlich 3. However, results given in Table 8a and 8b show that CAL did not correlate significantly with the amount of CaCO<sub>3</sub>; however, the Olsen extraction displayed a negative correlation with this parameter, and the correlation coefficient for

Mehlich 3 indicated a similar relation. Equally, Fe-oxide  $P_i$  and CAEM and Dithionite extracted significantly ( $p < 0.001$ ) lower P when the  $CaCO_3$  amount increased. ZBÍRAL (2000) argues that acidic extraction methods for soil P are highly affected by the prevailing pH during extraction. The results gained by the ANOVA (given in Table ) revealed that the mean values for Fe-oxide  $P_i$ , CAEM and Dithionite were significantly lower for the third group of pH above 6.6. However, as the three extraction methods are not acidic extraction methods these results may not indicate a pH dependency by these three methods but propose that our samples of higher pH (and  $CaCO_3$  content) had indeed less available P for plant uptake.

ZHENG and ZHANG (2012) state that soil texture and soil particle size distribution has a very high influence on soil phosphorus fractions. The authors attribute the impact of texture to its influence on the microbial biomass and activity in soils. All fractions of P that were affected by microbial activity were equally affected by texture, and that also implied extraction by soil P extraction methods. Investigations by FERNANDES *et al.* (1999) showed that Olsen, Fe-oxide  $P_i$  and CAEM were less dependent on soil texture, while results of our experiment indicated a significant correlation ( $p < 0.01$ ) with texture, except for CAEM, indicating that CAEM was not texture dependent. A positive correlation of HCl with sand content as found by MENON *et al.* (1989b) was not revealed by the data at hand. Furthermore, only the organic P content was correlated negatively with the sand content and positively with the clay content (all other extraction methods that correlated with texture showed the opposite trend), suggesting protection of organic P pools in the clay fraction. The weaker extraction methods correlated negatively with the silt and clay content and positively with sand content; conversely, O'HALLORAN *et al.* (1987) found that low sand content correlated with higher amounts of extractable P by methods extracting the labile forms of P. Investigation of the extracted P values in an ANOVA given in Table 9 disclosed that for  $CaCl_2$ , Fe-Oxide  $P_i$  and CAEM, mean values of clay content up to 28% were higher than for clay content >28%. For Mehlich 3 and Bray II the low clay samples < 17% had a significantly higher average value and standard deviation. Organic P and Total P showed the opposite trend (cf. Table 9).

Oxalate and dithionite-extractable Mn and Fe contents correlated better with the stronger extraction methods, e.g. Dithionite, Organic P, HCl, Oxalate and Total P. Whereas with the  $Fe_o/Fe_d$  ratio all methods correlated significantly except for the weaker extraction methods (i.e.  $H_2O$ ,  $CaCl_2$  and LiCl) and Total P. Bray II only correlated significantly with parameters, when Mehlich 3 was correlated significantly with them.

The parameter of organic carbon only correlated with the methods of Organic P, Oxalate and the Total P, showing that only these extracting methods were able to extract significant amounts of P from the organic pool. Results obtained by the ANOVA in Table 9 show similar outcomes. As expected, values for Organic P and Total P revealed a positive relation with increasing OC amount. The more organic carbon, the more organic phosphorus was extracted. Additionally Fe-oxide  $P_i$  obtained significant differences, but the lowest value was found for the highest OC amounts.

It is notable that correlation coefficients of Organic P and Total P were inverse compared to the other extraction methods (e.g. for C/N, C/P, Clay, Sand,  $CEC_{pot}$ , Mg). Thus, if other extraction methods correlated significantly positively, they correlated significantly negatively. In general it was perceptible that the Total P correlation coefficients were only significant when the Organic P was also significant. One can conclude that these two extraction methods extracted a completely different pool (the organic P) in soils, not corresponding to the other methods.

*KUMAR et al. (1994)* found that not only soil properties but also the composition of fertilizers is likely to change results on which method is most suitable to determine plant available phosphorus. Due to fertilizer residues and their variable solubility, these effects may even have overlaid soil property influences. In our experiment different soil fertilizers were used beforehand, however their influence could not be considered as this would have gone beyond the scope of this work.

Table 8a: Correlation coefficients of soil P extraction methods and soil properties, significance in \* p &lt; 0.05; \*\* p &lt; 0.01; \*\*\* p &lt; 0.001

	pH (H <sub>2</sub> O)	pH (KCl)	<sup>a</sup> EC	<sup>b</sup> OC	CaCO <sub>3</sub>	C/N (molar)	C/P (molar)	N/P (molar)	<sup>c</sup> KH	Clay	Silt	Sand
H <sub>2</sub> O	-0.174	-0.111	0.154	-0.089	-0.234	0.062	0.014	-0.016	0.010	-0.011	-0.161	0.119
CaCl <sub>2</sub>	-0.435 **	-0.348 *	0.387 **	-0.223	-0.341 *	0.452 ***	0.223	0.010	-0.359 *	-0.422 **	-0.462 ***	0.500 ***
LiCl	-0.125	0.024	0.407 **	-0.067	-0.238	0.184	0.166	0.077	-0.155	-0.237	-0.291 *	0.301 *
<sup>d</sup> Fe-oxide P <sub>i</sub>	-0.471 ***	-0.423 **	0.272	-0.162	-0.478 ***	0.414 **	0.191	-0.010	-0.329 *	-0.394 **	-0.500 ***	0.515 ***
Olsen	-0.343 *	-0.319 *	0.219	0.076	-0.363 **	0.268	0.170	0.020	-0.148	-0.242	-0.463 ***	0.427 **
<sup>e</sup> CAL	-0.027	0.110	0.267	0.142	-0.143	0.129	0.262	0.171	-0.150	-0.216	-0.387 **	0.360 *
<sup>f</sup> CAEM	-0.298 *	-0.286 *	0.218	-0.104	-0.500 ***	0.127	0.182	0.141	-0.169	-0.195	-0.273	0.274
Mehlich 3	-0.390 **	-0.308 *	0.391 **	-0.081	-0.338 *	0.555 ***	0.299 *	0.030	-0.402 **	-0.549 ***	-0.574 ***	0.630 ***
Bray II	-0.290 *	-0.200	0.322 *	-0.103	-0.380 **	0.455 ***	0.214	-0.030	-0.405 **	-0.512 ***	-0.524 ***	0.579 ***
Dithionite	-0.387 **	-0.448 **	-0.188	0.043	-0.466 ***	-0.085	-0.238	-0.194	-0.108	-0.172	-0.246	0.251
Organic P	0.266	0.193	-0.355 *	0.376 **	0.201	-0.440 **	-0.582 ***	-0.420 **	0.489 ***	0.427 **	0.268	-0.364 **
HCl	0.077	0.068	0.006	0.213	-0.163	-0.134	0.072	0.081	0.027	-0.029	-0.118	0.097
Oxalate	-0.104	-0.144	-0.117	0.291 *	-0.179	-0.059	-0.167	-0.171	0.127	-0.010	-0.222	0.164
Total P	0.221	0.164	-0.267	0.392 **	0.070	-0.413 **	-0.356 *	-0.212	0.370 **	0.293 *	0.142	-0.218
P uptake	-0.131	-0.149	0.211	0.037	-0.308 *	0.000	0.069	0.018	0.093	-0.016	-0.088	0.069

<sup>a</sup> EC = electrical conductivity in saturated H<sub>2</sub>O extract<sup>b</sup> OC = organic carbon<sup>c</sup> KH = amount of water necessary to prepare the H<sub>2</sub>O saturation extract<sup>d</sup> Fe-oxide P<sub>i</sub> = iron oxide impregnated filter paper<sup>e</sup> CAL = calcium acetate lactate<sup>f</sup> CAEM = cation and anion exchange membrane

Table 8b: Correlation coefficients of soil P extraction methods and soil properties, significance in \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ 

	<sup>g</sup> CEC <sub>pot</sub>	<sup>h</sup> BS	Ca	Mg	K	Al	<sup>i</sup> Fe <sub>d</sub>	<sup>i</sup> Mn <sub>d</sub>	<sup>j</sup> Fe <sub>o</sub>	<sup>j</sup> Mn <sub>o</sub>	<sup>ij</sup> Fe <sub>o</sub> /Fe <sub>d</sub>	<sup>j</sup> P <sub>o</sub> /Fe <sub>o</sub>
H <sub>2</sub> O	-0.020	-0.129	-0.200	0.029	0.551 ***	0.031	0.010	0.241	0.051	0.294 *	0.199	-0.005
CaCl <sub>2</sub>	-0.312 *	-0.379 **	-0.411 **	-0.315 *	0.518 ***	0.173	-0.106	0.003	-0.074	0.093	0.232	0.122
LiCl	-0.205	-0.040	-0.102	0.005	0.586 ***	0.189	-0.091	0.108	-0.044	0.160	0.196	0.174
<sup>d</sup> Fe-oxide P <sub>i</sub>	-0.136	-0.519 ***	-0.529 ***	-0.370 **	0.241	0.064	0.076	0.141	0.186	0.238	0.522 ***	0.075
Olsen	0.070	-0.402 **	-0.408 **	-0.322 *	0.226	-0.077	0.146	0.099	0.307 *	0.193	0.627 ***	0.091
<sup>e</sup> CAL	-0.064	0.031	0.036	-0.173	0.274	-0.005	-0.096	0.046	0.016	0.079	0.351 *	0.316 *
<sup>f</sup> CAEM	0.004	-0.382 **	-0.414 **	-0.221	0.351 *	0.138	0.173	0.252	0.327 *	0.372 **	0.561 ***	-0.100
Mehlich 3	-0.282 *	-0.429 **	-0.423 **	-0.418 **	0.251	0.075	-0.155	-0.064	-0.054	0.041	0.427 **	0.329 *
Bray II	-0.274	-0.324 *	-0.310 *	-0.373 **	0.204	0.018	-0.147	0.062	-0.044	0.141	0.405 **	0.294 *
Dithionite	0.243	-0.556 ***	-0.573 ***	-0.201	0.012	-0.168	0.597 ***	0.337 *	0.757 ***	0.457 ***	0.573 ***	-0.362 **
Organic P	0.529 ***	0.135	0.086	0.452 ***	-0.156	-0.579 ***	0.558 ***	0.536 ***	0.370 **	0.352 *	-0.285 *	0.069
HCl	0.176	0.021	0.022	-0.030	0.065	-0.208	0.296 *	0.100	0.473 ***	0.068	0.526 ***	-0.016
Oxalate	0.353 *	-0.243	-0.264	0.018	-0.063	-0.399 **	0.455 ***	0.226	0.597 ***	0.289 *	0.486 ***	0.091
Total P	0.515 ***	0.075	0.037	0.325 *	-0.113	-0.534 ***	0.583 ***	0.433 **	0.592 ***	0.302 *	0.173	0.005
P uptake	-0.131	-0.149	0.211	0.037	-0.308 *	0.000	-0.053	-0.078	0.093	-0.016	-0.088	0.069

<sup>d</sup>Fe-oxide P<sub>i</sub> = iron oxide impregnated filter paper<sup>e</sup>CAL = calcium acetate lactate<sup>f</sup>CAEM = cation and anion exchange membrane<sup>g</sup>CEC<sub>pot</sub> = potential cation exchange capacity (determined at pH 8.1)<sup>h</sup>BS = base saturation<sup>i</sup>Fe<sub>d</sub> and Mn<sub>d</sub> = dithionite-extractable Fe and Mn<sup>j</sup>Fe<sub>o</sub>, Mn<sub>o</sub> and P<sub>o</sub> = oxalate-extractable Fe, Mn and P

Table 9: The mean values  $\pm$  standard deviation if grouped according to the pH (KCl), organic carbon and clay content, compared in an ANOVA.

	H <sub>2</sub> O	CaCl <sub>2</sub>	LiCl	<sup>a</sup> Fe-oxide P <sub>i</sub>	Olsen	<sup>b</sup> CAL	<sup>c</sup> CAEM	Mehlich 3	Bray II	Dithionite	Organic P	HCl	Oxalate	Total P
<sup>d</sup> pH < 5.5	0.26 $\pm$ 0.26	3.13 $\pm$ 2.59	5.76 $\pm$ 4.17	52.7 $\pm$ 24.0	53.2 $\pm$ 23.4	83.9 $\pm$ 42.1	108 $\pm$ 46.8	153 $\pm$ 96.0	186 $\pm$ 105	259 $\pm$ 136	296 $\pm$ 166	293 $\pm$ 121	537 $\pm$ 275	794 $\pm$ 342
pH 5.5 to 6.6	0.30 $\pm$ 0.42	2.87 $\pm$ 2.33	7.90 $\pm$ 6.12	55.0 $\pm$ 28.7	55.1 $\pm$ 30.2	103 $\pm$ 62.0	125 $\pm$ 51.5	156 $\pm$ 129	205 $\pm$ 137	230 $\pm$ 118	258 $\pm$ 107	332 $\pm$ 130	529 $\pm$ 227	788 $\pm$ 264
pH > 6.6	0.21 $\pm$ 0.36	1.49 $\pm$ 1.53	6.69 $\pm$ 8.73	26.5 $\pm$ 19.3	38.0 $\pm$ 25.4	115 $\pm$ 82.7	63.4 $\pm$ 57.8	101 $\pm$ 81.5	153 $\pm$ 144	123 $\pm$ 77.3	360 $\pm$ 88.2	321 $\pm$ 130	518 $\pm$ 155	913 $\pm$ 271
<sup>e</sup> OC < 1.2%	0.18 $\pm$ 0.15	2.59 $\pm$ 1.75	5.20 $\pm$ 3.82	48.0 $\pm$ 21.6	43.5 $\pm$ 17.6	80.6 $\pm$ 43.1	103 $\pm$ 45.5	119 $\pm$ 64.1	162 $\pm$ 72.9	201 $\pm$ 76.9	206 $\pm$ 78.5	307 $\pm$ 125	426 $\pm$ 141	688 $\pm$ 234
OC 1.2% to 2.0%	0.45 $\pm$ 0.55	3.78 $\pm$ 3.16	10.03 $\pm$ 9.60	57.7 $\pm$ 28.3	58.2 $\pm$ 28.2	104 $\pm$ 63.1	122 $\pm$ 59.7	190 $\pm$ 118	233 $\pm$ 130	239 $\pm$ 110	297 $\pm$ 121	290 $\pm$ 107	557 $\pm$ 257	783 $\pm$ 230
OC > 2.0%	0.17 $\pm$ 0.18	1.67 $\pm$ 1.46	5.55 $\pm$ 3.60	36.3 $\pm$ 27.6	47.8 $\pm$ 31.3	109 $\pm$ 73.5	85.3 $\pm$ 59.1	118 $\pm$ 114	163 $\pm$ 151	194 $\pm$ 161	373 $\pm$ 129	340 $\pm$ 140	586 $\pm$ 234	959 $\pm$ 274
<sup>f</sup> clay < 17%	0.25 $\pm$ 0.25	3.67 $\pm$ 2.52	7.92 $\pm$ 5.07	59.4 $\pm$ 28.4	58.8 $\pm$ 28.2	111 $\pm$ 68.4	115 $\pm$ 49.6	207 $\pm$ 132	248 $\pm$ 151	235 $\pm$ 132	209 $\pm$ 118	301 $\pm$ 134	527 $\pm$ 289	677 $\pm$ 277
clay 17% to 28%	0.34 $\pm$ 0.48	2.58 $\pm$ 2.28	7.68 $\pm$ 8.43	46.8 $\pm$ 22.9	49.4 $\pm$ 20.7	98.3 $\pm$ 62.2	111 $\pm$ 55.2	121 $\pm$ 66.3	180 $\pm$ 96.3	230 $\pm$ 115	340 $\pm$ 117	358 $\pm$ 104	561 $\pm$ 172	957 $\pm$ 245
clay > 28%	0.15 $\pm$ 0.21	1.06 $\pm$ 0.82	3.92 $\pm$ 2.93	27.0 $\pm$ 20.7	37.2 $\pm$ 30.4	84.9 $\pm$ 56.0	69.0 $\pm$ 58.6	74.3 $\pm$ 50.8	99.6 $\pm$ 78.4	146 $\pm$ 122	368 $\pm$ 103	272 $\pm$ 133	485 $\pm$ 201	838 $\pm$ 200

<sup>a</sup>Fe-oxide P<sub>i</sub> = iron oxide impregnated filter paper<sup>b</sup>CAL = calcium acetate lactate<sup>c</sup>CAEM = cation and anion exchange membrane<sup>d</sup>pH < 5.5 n = 18; pH 5.5 to 6.6 n = 18; pH > 6.6 n = 14<sup>e</sup>OC = organic carbon < 1.2% n = 15; organic carbon 1.2% to 2.0% n = 15; organic carbon > 2.0% n = 20<sup>f</sup>clay < 17% n = 18; clay 17% to 28% n = 19; clay > 28% n = 13

### 3.4 Plant Analysis

Table 10, given below, displays a summary of data collected at harvest or shortly after. While the average dry matter grain yield amounted to  $157.4 \text{ g m}^{-2}$ , the mean dry matter straw yield was  $219.5 \text{ g m}^{-2}$ . An average of 301 ears per  $\text{m}^2$  was counted and the number of grains per ear was varying between a minimum of 9 and a maximum of 21. An average thousand-seed weight of 35.9 g was calculated. Clearly, these yield parameters were below the average of field-grown spring wheat.

*DIEPENBROCK et al. (1999)* list an average grain yield for spring wheat at harvest of  $643 \text{ g m}^{-2}$ . Moreover, typically the thousand-seed weight of spring wheat is higher (i.e. 39.5 g), and stand density is much higher with about 526 ears per  $\text{m}^2$ ; similarly the number of grains per ear reaches a greater average of 30.8.

The minimum value for P in grain was  $2950 \text{ mg kg}^{-1}$  and the maximum value was  $5380 \text{ mg kg}^{-1}$ . The minimum content of P in straw was approximately  $340 \text{ mg kg}^{-1}$  and the maximum content was  $2820 \text{ mg kg}^{-1}$ . These values were in the range of reported average values for wheat; an average range of  $3020 \text{ mg kg}^{-1}$  to  $5580 \text{ mg kg}^{-1}$  is common for wheat grain and the range for wheat straw is  $1050 \text{ mg kg}^{-1}$  to  $1980 \text{ mg kg}^{-1}$  (*DIEPENBROCK et al., 1999*). According to *BLUME et al. (2010)* dry matter wheat grain contains approximately  $4000 \text{ mg kg}^{-1}$  and straw  $1500 \text{ mg kg}^{-1}$ . On average, the grain P content was 5 times higher than the straw content (Table 10). The total P uptake was ranging from  $460 \text{ mg kg}^{-1}$  to  $1400 \text{ mg kg}^{-1}$  thus showing a 3-fold increase from the lowest to the highest value.

Table 10: Basic parameters of the plant analysis

	<sup>a</sup> DM grain yield	<sup>a</sup> DM straw yield	Ears per m <sup>2</sup>	Grains per ear	Thousand-seed weight	P content grain	P content straw	Total P uptake
	[g m <sup>-2</sup> ]				[g]	[mg kg <sup>-1</sup> ]		[mg m <sup>-2</sup> ]
<b>Mean</b>	157.4	219.5	301	17	35.9	4190	790	842
<b>Median</b>	159.4	207.7	283	17	36.6	4210	738	855
<b>Standard Dev.</b>	22.6	45.5	35	2	3.4	528	394	193
<b>Minimum</b>	101.9	152.4	264	9	28.4	2950	338	463
<b>10% Quantile</b>	122.6	164.2	283	14	31.4	3420	450	589
<b>90% Quantile</b>	183.0	293.9	358	19	39.5	4920	1100	1030
<b>Maximum</b>	203.8	318.9	433	21	45.1	5380	2820	1400

<sup>a</sup>DM = dry matter

Figure 11 and Figure 12 show each the highest and lowest yield for straw and grain yield, respectively. Pictures were taken just before harvest.

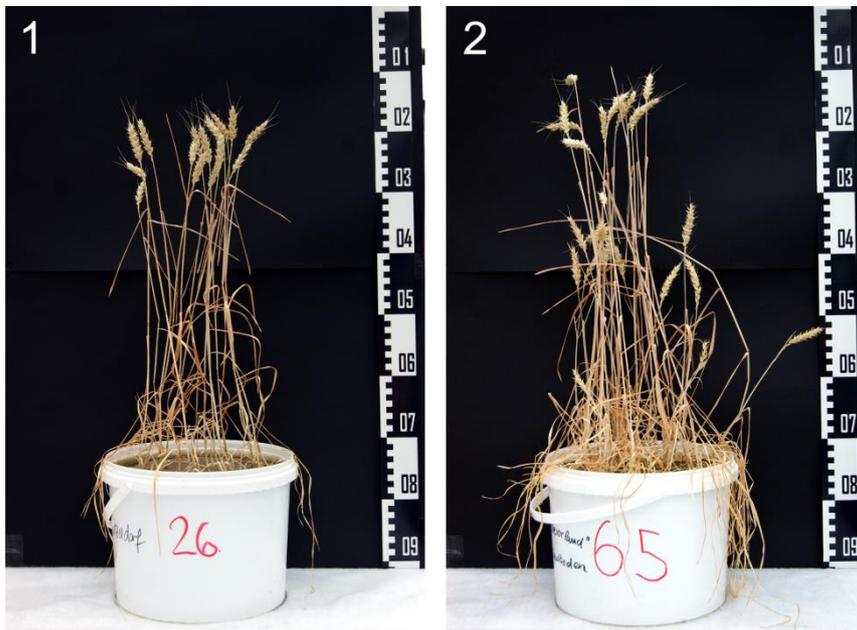


Figure 11: The pots with lowest (1) and highest (2) straw yield.

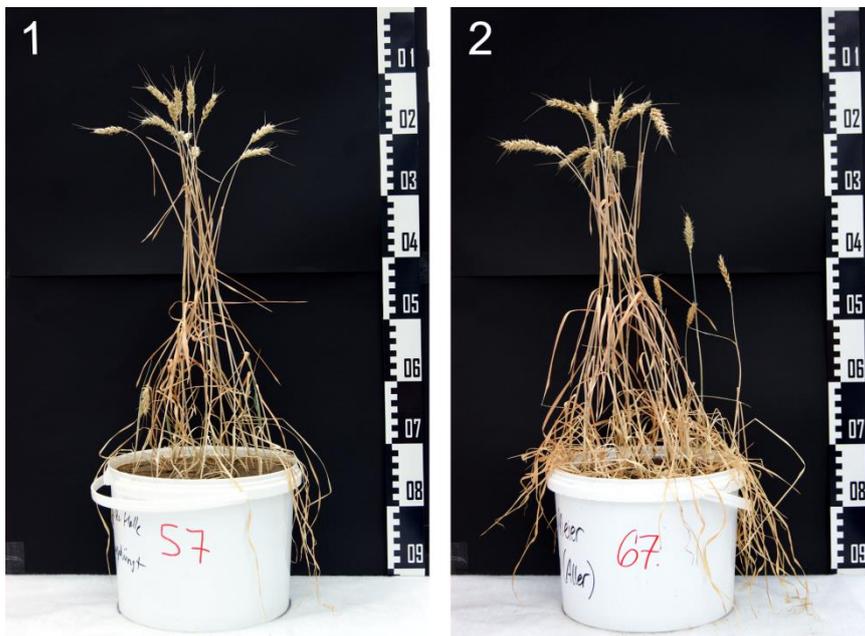
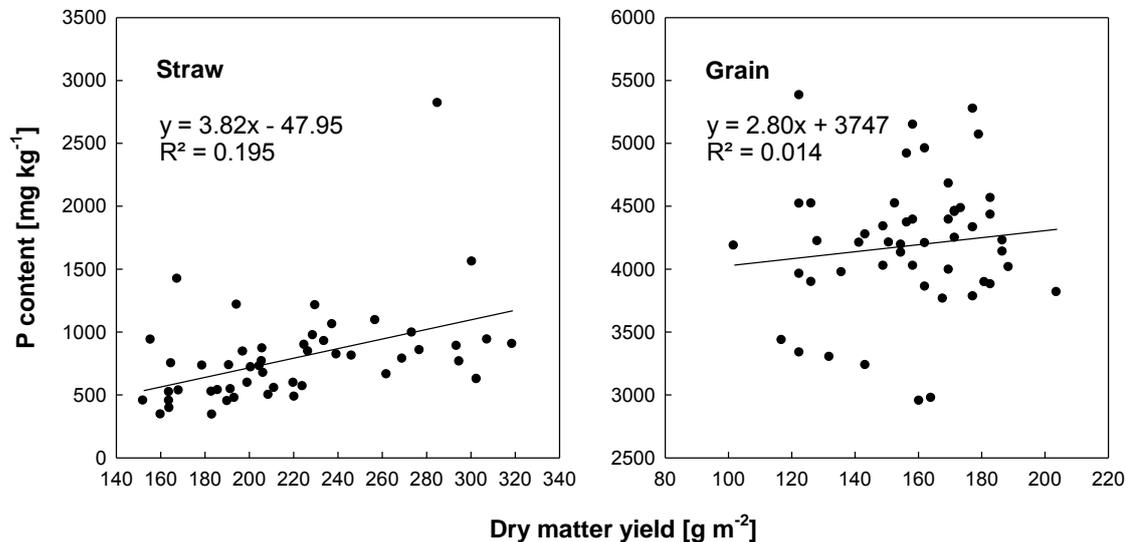


Figure 12: The pots with lowest (1) and highest (2) grain yield.

The following Figure 13 shows the relation between the phosphorus content of grain and straw, respectively and their dry matter yield. While an increasing dry matter straw yield tended to result in a higher phosphorus content of the straw, the grain yield



**Figure 13: Correlation of dry matter yield of straw and grain with the respective P content measured.**

A stepwise multiple linear regression was performed in SPSS. The Figure 14 below shows the contributing share of each of the three harvest parameters to the grain yield variation. The parameters that were taken in account were the number of grains per ear, the number of ears per m<sup>2</sup> and the thousand seed weight. All three parameters showed a similar contribution to grain yield variation. The number of ears per m<sup>2</sup> explained 34.5% of the grain yield variation, the thousand-seed weight accounted for 32.9%, and the number of grains per ear explained 29.3% of the variation. As discussed in the beginning of this chapter, the yields determined in this study were below average for common spring wheat. It may be assumed that all three parameters contributed equally to the observed low yields.

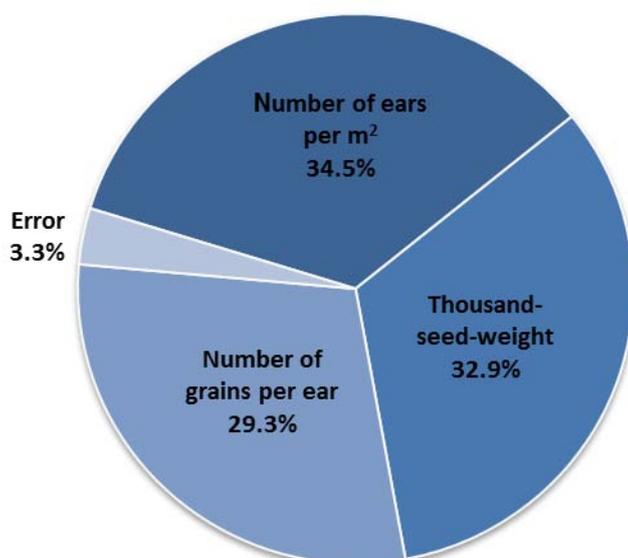


Figure 14: The proportionate contribution of three harvest parameters to grain yield variation.

### 3.5 Correlating Plant Yield and P Uptake with Extracted P

As a next step, correlation coefficients between the plant data and the P extracted with the different extraction methods were calculated. Correlation coefficients are given in Table 11 below.

In general, straw dry matter yield correlated more significantly with the extracted P by the different soil extraction methods than grain dry matter yield. This is in agreement with *BISSANI et al. (2002)*, who showed generally lower correlation coefficients of grain yield for Mehlich 1, anion exchange resin, anion exchange membrane and Fe-oxide P<sub>i</sub>. The observed results may be due to the fact that phosphorus is accumulated in grain seeds in order to provide enough P for germination and the first growing phase when roots are not yet able to take up enough phosphorus. In the plant, P is relatively mobile; therefore, P is not stored in older parts of the plant (i.e. straw) but transported back to the roots and redistributed to plant parts with P demand (*SCHACHTMAN et al., 1998*). Hence, soil-induced P limitations may be more strongly reflected in the straw compared to the grain.

It is interesting to note that the dry matter yield of grain correlated significantly with the stronger extraction methods, whereas the dry matter yield of straw showed a tendency to correlate less with the strongest extraction methods. The highest correlation coefficient of dry matter grain yield and an extraction method was obtained for the HCl extraction. Similar significance levels were reached by Olsen, CAEM, Dithionite, Oxalate and Total P. For the dry matter straw yield the best correlation coefficient was obtained with the CAEM method ( $r = 0.484$ ,  $p < 0.001$ ). Equally well

correlating were methods such as CaCl<sub>2</sub>, Fe-oxide P<sub>i</sub> and Olsen; less but still significant were others such as H<sub>2</sub>O, LiCl, CAL, Mehlich 3, Bray II, Dithionite and Oxalate.

Several extraction methods correlated with the parameter ears per m<sup>2</sup>, i.e. CaCl<sub>2</sub>, Fe-oxide P<sub>i</sub>, Olsen, CAL, CAEM, Mehlich 3 and Bray II. This shows that the P availability in our samples may have affected the tillering of the spring wheat. The parameter number of grains per ear was only correlated significantly with the dithionite extraction method, while the thousand-seed weight did not show any significant correlation with the soil extraction methods at all.

SLAFER (2007) stated that the two major yield components, number of grains per m<sup>2</sup> and average individual grain weight are negatively correlated. Thus, the observed opposite trends of thousand-seed weight and ears per m<sup>2</sup> are plausible.

**Table 11: Correlation coefficients of plant harvest data and extraction methods, significance in \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$**

	<sup>a</sup> DM Grain yield	<sup>a</sup> DM Straw yield	Ears per m <sup>2</sup>	Grains per ear	Thousand-seed weight
H <sub>2</sub> O	0.035	0.425 **	0.251	-0.093	-0.139
CaCl <sub>2</sub>	0.115	0.467 ***	0.294 *	-0.112	-0.029
LiCl	0.030	0.446 **	0.379 **	-0.203	-0.131
<sup>b</sup> Fe-oxide P <sub>i</sub>	0.224	0.456 ***	0.301 *	-0.022	0.005
Olsen	0.337 *	0.450 ***	0.336 *	0.098	-0.010
<sup>c</sup> CAL	0.204	0.351 *	0.370 **	-0.145	0.155
<sup>d</sup> CAEM	0.304 *	0.484 ***	0.375 **	0.014	0.006
Mehlich 3	0.178	0.380 **	0.309 *	-0.023	-0.024
Bray II	0.228	0.400 **	0.346 *	-0.106	0.163
Dithionite	0.304 *	0.297 *	0.212	0.290 *	-0.122
Organic P	0.139	0.145	0.111	0.160	-0.051
HCl	0.364 **	0.266	0.230	0.132	0.124
Oxalate	0.351 *	0.328 *	0.254	0.233	-0.012
Total P	0.345 *	0.249	0.228	0.220	0.039

<sup>a</sup> DM = dry matter

<sup>b</sup> Fe-oxide P<sub>i</sub> = iron oxide impregnated filter paper

<sup>c</sup> CAL = calcium acetate lactate

<sup>d</sup> CAEM = cation and anion exchange membrane

Figure 15 shows the correlation of every considered extraction method with the total plant P uptake in  $\text{mg m}^{-2}$ . The weaker extraction methods achieved a better correlation than the stronger methods. The best coefficient of determination was obtained by the  $\text{H}_2\text{O}$  extraction  $R^2= 0.337$ . followed by the  $\text{CaCl}_2$  extraction method ( $R^2=0.331$ ) and the CAEM method ( $R^2=0.24.9$ ). While  $\text{LiCl}$  and  $\text{Fe-Oxide P}_1$  revealed a tendency towards correlating, the rest of the methods failed to yield any relation to plant P uptake. Similar results were gained by *KULHÁNEK et al. (2009a)*, finding by far the best correlation with the water extract ( $R^2=0.66$ ). However, in that study  $\text{CaCl}_2$  ( $R^2=0.13$ ) was correlated less than anion exchange membrane extraction ( $R^2=0.20$ ) and Mehlich 3 ( $R^2=0.19$ ). These results were based on samples of homogeneous soil types (all loamy) with a pH close to 6.3. The authors suggested that the weak correlation by  $\text{CaCl}_2$  was a result of Ca-phosphates forming during extraction.

$\text{Fe-oxide P}_1$  and CAEM were found to be well correlated with the P uptake by plants (*KUO, 1996*) and were expected to perform better than the other extraction methods, due to their supposed independency of soil properties. This is in agreement with results shown by *SAGGAR et al. (1999)* who found that CAEM extracted higher amounts of soil (available) P than Olsen, and a plant yield prediction was superior to the one by Olsen. *MENON et al. (1988)* claimed that  $\text{Fe-oxide P}_1$  is an accurate method to determine plant available P and that it is highly independent of soil properties and fertilizer applied. However, *ZHENG and ZHANG (2012)* stated that inconsistent results were observed, deriving from the preparation of the impregnated filter paper. Depending on the type of filter papers, the  $\text{Fe-oxides}$  may distribute inhomogeneously generating irreproducible extracted P amounts.

In general, our results showed weaker coefficients of determination and lower significance than data presented in most other papers (e.g. *ZORN and KRAUSE, 1999*, *KULHÁNEK et al., 2009a*). However, this may be explained by the utilization of many different soils with a wide range of basic properties and variable P status rather than applying the method to a limited number of similar soils with altering P levels (due to fertilizer application). Experiments that are based on fewer soils have much less variability in soil parameters. However, their results are very specific for limited soil types and provide less information on a general evaluation of soil P extraction methods and how they are regionally applicable to agricultural soils in Austria and Germany.

Moreover, the plant uptake is also dependent on the plant's ability to take up phosphorus, and not only on the process of supplying by the soil. Important traits in this context are plant age, root system and root age (*HOLFORD, 1997*). Thus, the plant also influences the amount of plant available P. In this context mycorrhizae are of high agricultural interest. The symbiosis of plants with mycorrhizae increases the efficiency

of plants to take up phosphate (*RYAN and RASHID, 2006*) influencing thereby the amount of plant available P.

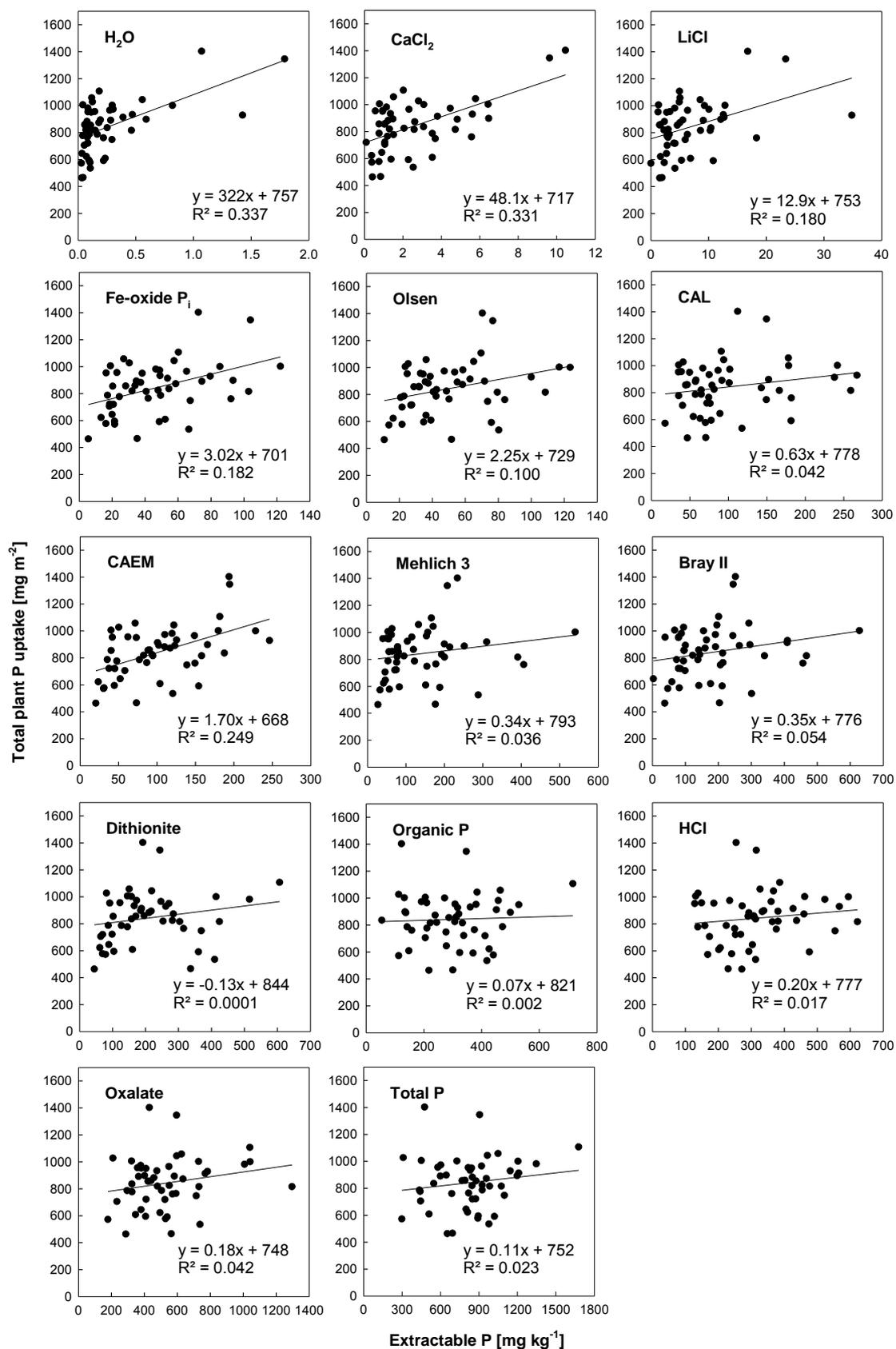


Figure 15: Regression of the plant P uptake on the extractable P of the different extraction methods. Abbreviations: Fe-oxide P<sub>i</sub> = iron oxide impregnated filter paper, CAL = calcium acetate lactate, CAEM = cation and anion exchange membrane

As mentioned in chapter 3.3 the 50 soil samples were grouped according to their pH (KCl), the organic carbon and clay content. This analysis may provide information on which methods are dependent on the soil properties. The grouping system was chosen based on the classifications by *BLUM et al. (1996)* and modified according to extents of samples, in regard of relatively equal group sizes and more profound differences between the groups per se. The groups of pH (KCl) were selected to be at pH lower than 5.5, from 5.5 to 6.6 and a pH higher than 6.6. The organic carbon content was subdivided to a group below 1.2%, between 1.2% and 2% and one above 2% organic carbon. Also the clay content was split into three groups of clay content below 17%, ranging from 17% to 28% and one that was beyond 28%. After arranging the groups, correlation coefficients between the uptake and the corresponding extractable P by each method were calculated. Results are given in Table 12; a two-tailed significance level was applied to test the significance.

In general, it could be noted that primarily the weaker extraction methods, Fe-oxide  $P_i$  and CAEM were correlated in more cases with plant P uptake than the stronger extraction methods. Mehlich 3 and Bray II obtained the similar results over all the correlation coefficients; this is likely a consequence of the similarity of their extraction mechanism. Similarly, the Dithionite, Oxalate and Total P results were corresponding. Furthermore, Fe-oxide  $P_i$  and CAEM showed some resemblance regarding significance, yet e.g. at the pH classification they correlated dissimilar with plant P uptake.

Examining the pH groups, the weaker extraction methods (i.e.  $H_2O$ ,  $CaCl_2$ ,  $LiCl$ ) were better correlated with the first two groups of pH (below 5.5 and 5.5 to 6.6). In contrast, the Fe-oxide  $P_i$  correlated significantly with middle and upper pH groups. The stronger extraction methods correlated significantly only with the last pH group above 6.6, i.e. only for calcareous soils. Our results indicate that the weaker extraction methods, being based on water-solubility and ion exchange, performed best in soils around pH 6, where P retention is relatively low, and in more acidic soils, where anion sorption becomes more important (*HOLFORD, 1997*). However, they failed to relate to plant P uptake in calcareous soils, where P is precipitated as calcium phosphate (*TUNESI et al., 1999*). Obviously, the mechanisms of water solubility and ion exchange are not of primary importance in such soils; plants likely mobilize P (and other nutrients) through acidification of the rhizosphere (*HINSINGER et al., 2003*). This mechanism, in turn, is more closely simulated by (some of) the stronger extraction methods, which explains their better performance in the high pH soils.

The organic carbon grouping resulted only in the middle (organic carbon in the range of 1.2% to 2.0%) and in the upper group (organic carbon above 2%) in significant

correlations. H<sub>2</sub>O, CaCl<sub>2</sub>, LiCl and CAEM were correlated significantly in the middle group. Others, i.e. CAEM, Fe-oxide P<sub>i</sub>, Olsen, Dithionite, Oxalate and Total P, obtained significance in the upper group.

For the grouping by clay content, the least amount of significant correlations was detected. Solely the weaker extraction methods and the methods using a sink mechanism were significant. For these extraction methods, significant correlations were found at lower and medium clay contents but not for the grouping with high clay content above 28% clay.

**Table 12: Correlation coefficients of the P uptake by plants and the extractable soil P if samples are grouped according to pH (KCl), organic carbon (OC) or clay content, significance in \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$**

	H <sub>2</sub> O	CaCl <sub>2</sub>	LiCl	<sup>a</sup> Fe-oxide P <sub>i</sub>	Olsen	<sup>b</sup> CAL	<sup>c</sup> CAEM	Mehlich 3	Bray II	Dithionite	Organic P	HCl	Oxalate	Total P
<sup>d</sup> pH < 5.5	0.641 **	0.534 *	0.560 *	0.162	0.126	0.104	0.467 *	-0.003	-0.002	-0.009	0.145	0.033	0.048	0.137
pH 5.5 to 6.6	0.744 ***	0.625 **	0.448	0.501 *	0.266	0.128	0.368	0.085	0.098	0.047	0.031	0.090	0.278	0.175
pH > 6.6	0.383	0.485	0.412	0.546 *	0.503	0.549 *	0.500	0.554 *	0.598 *	0.553 *	0.323	0.357	0.579 *	0.696 **
<sup>e</sup> OC < 1.2%	0.319	0.286	0.373	0.258	0.285	0.234	0.330	0.287	0.401	0.153	0.133	0.029	0.216	0.211
OC 1.2% to 2.0%	0.709 **	0.745 ***	0.509 *	0.434	0.226	0.206	0.551 *	0.029	0.057	-0.161	-0.052	0.054	-0.045	-0.036
OC > 2.0%	0.391	0.398	0.240	0.517 *	0.449 *	0.247	0.541 *	0.351	0.387	0.508 *	0.228	0.323	0.601 **	0.468 *
<sup>f</sup> clay < 17%	0.715 ***	0.709 ***	0.584 **	0.298	0.210	0.204	0.493 *	0.121	0.133	-0.154	-0.277	0.169	0.003	-0.052
clay 17% to 28%	0.638 **	0.649 **	0.422	0.652 **	0.431	0.185	0.577 **	0.436	0.295	0.358	0.295	-0.094	0.357	0.171
clay > 28%	0.405	0.351	0.159	0.442	0.352	0.220	0.362	0.244	0.391	0.377	0.296	0.025	0.392	0.309

<sup>a</sup>Fe-oxide P<sub>i</sub> = iron oxide impregnated filter paper

<sup>b</sup>CAL = calcium acetate lactate

<sup>c</sup>CAEM = cation and anion exchange membrane

<sup>d</sup>pH < 5.5 n = 18; pH 5.5 to 6.6 n = 18; pH > 6.6 n = 14

<sup>e</sup>OC = organic carbon < 1.2% n = 15; organic carbon 1.2% to 2.0% n = 15; organic carbon > 2.0% n = 20

<sup>f</sup>clay < 17% n = 18; clay 17% to 28% n = 19; clay > 28% n = 13

A multiple linear regression was performed in order to determine which other factors most contributed to improve the coefficient of determination for predicting plant P uptake from the amount of P extracted by H<sub>2</sub>O and CaCl<sub>2</sub>. These two methods were selected as they initially correlated best with plant uptake. Stepwise backwards multiple linear regressions were applied to exclude one by one the considered soil parameters (c.f. Table 5a and 5b).

When examining the H<sub>2</sub>O extraction method, the Fe<sub>d</sub> was the single most contributing factor and increased the R<sup>2</sup> from 0.337 to 0.381 as given in Table 13 .

Table 14 displays data of the multiple linear regression for the CaCl<sub>2</sub> extraction method, where the addition of Mn<sub>d</sub> and OC content helped to increase the R<sup>2</sup> from 0.331 to 0.481.

**Table 13: Factors most contributing to improve the coefficient of determination for predicting plant P uptake from H<sub>2</sub>O extractable P, as determined by multiple linear regression**

<b>R<sup>2</sup>=0.381</b>	<b>B</b>	<b>Standard error</b>	<b>Standardized beta</b>	<b>Statistical significance</b>
<b>Constant</b>	769	47.4		0.000
<sup>a</sup> <b>H<sub>2</sub>O-P</b> [mg kg <sup>-1</sup> ]	353	69.4	0.54	0.000
<sup>b</sup> <b>Fe<sub>d</sub></b> [mg kg <sup>-1</sup> ]	0.01	0.004	0.29	0.018

<sup>a</sup> H<sub>2</sub>O-P = H<sub>2</sub>O-extractable P

<sup>b</sup> Fe<sub>d</sub> = dithionite-extractable Fe

**Table 14: Factors most contributing to improve the coefficient of determination for predicting plant P uptake from CaCl<sub>2</sub> extractable P, as determined by multiple linear regression**

<b>R<sup>2</sup>=0.481</b>	<b>B</b>	<b>Standard error</b>	<b>Standardized beta</b>	<b>Statistical significance</b>
<b>Constant</b>	463	97.8		0.000
<sup>a</sup> <b>CaCl<sub>2</sub>-P</b> [mg kg <sup>-1</sup> ]	56.1	10.4	0.60	0.000
<sup>b</sup> <b>Mn<sub>d</sub></b> [mg kg <sup>-1</sup> ]	0.44	0.12	0.41	0.000
<sup>c</sup> <b>OC</b> [%]	72.6	32.7	0.25	0.032

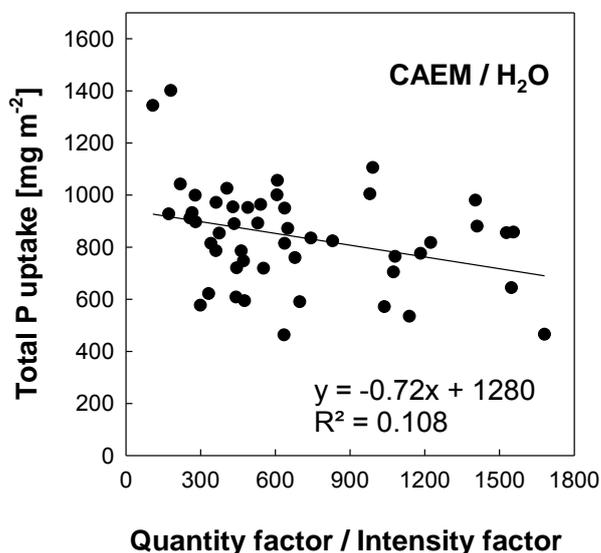
<sup>a</sup> CaCl<sub>2</sub>-P = CaCl<sub>2</sub>-extractable P

<sup>b</sup> Mn<sub>d</sub> = dithionite-extractable Mn

<sup>c</sup> OC = organic carbon

The model of categorizing phosphorus in soil in intensity, quantity and capacity factor allows to assess the soil's P buffering capacity and estimate the P supply for plants over time. The intensity factor determines the readily dissolved or weakly adsorbed phosphorus in the soil, while the quantity factor represents the amount that is not immediately available but will be desorbed and supplied with time. The weakest extraction methods, dilute salt solutions and H<sub>2</sub>O extraction, are usually applied to describe the intensity factor of soils (KULHÁNEK *et al.*, 2008). The ratio between quantity and intensity is commonly applied to define P buffering and re-supplying capacity in soils (RYAN and RASHID, 2006).

Figure 16 shows a correlation between plant P uptake on the y-axis and the ratio of quantity factor over intensity factor. Similar to literature, H<sub>2</sub>O was chosen as intensity factor while CAEM was chosen as quantity factor (selected because of best correlation with the plant uptake). A positive relation between plant P uptake and the quantity/ intensity ratio was expected. The observed negative correlation may indicate that the quantity factor did not have much impact on the total P uptake by the plants over the course of this (short-term) experiment as the intensity factor was sufficient to cover nutritional necessities by plants. However, in case of a longer test duration, the quantity factor would gain more importance as the resupply of P becomes necessary.



**Figure 16:** Regression of the plant P uptake on the ratio of quantity factor/ intensity factor. The quantity factor was approximated by CAEM-extractable P, the intensity factor by H<sub>2</sub>O extractable P. Abbreviations: CAEM = cation and anion exchange membrane

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## 4 Conclusions and Outlook

The aim of this study was to determine the amount of phosphorus taken up by wheat plants and to set this in relation to the amount of available P predicted by different soil P extracting methods. The plant P uptake was in the magnitude of P extractable by the weakest extraction agents (H<sub>2</sub>O, CaCl<sub>2</sub> and LiCl). An objective was also to identify extraction methods best representing the P uptake by plants over the course of one growing season. Results showed that weaker extracting agents, i.e. H<sub>2</sub>O and CaCl<sub>2</sub> correlated best with the uptake by spring wheat over one growing season. This indicates that these weak extraction methods may be suitable indicators of plant-available P over one growing season. However, the potential resupply by the soil may not be assessed by these methods. The observed “over-extracting” of P by the stronger extracting agents may represent the amount that will become available in subsequent growing seasons. Thus, the stronger extracting agents (e.g. CAL, Mehlich 3, etc.) may provide information on the longer-term P buffering capacity of soils.

When grouping the soils according to their pH, their organic carbon content or their clay content, weaker extraction methods correlated better for soils with lower pH (and clay contents) while stronger extraction methods behaved contrary; they correlated better for soils with high pH (calcareous soils). Furthermore, stronger extraction methods showed a tendency to correlate better for soils of high organic carbon content. In conclusion, the results of this study suggest that a single method may not be sufficient to assess both current and potential plant available P.

Further insights could be gained by focusing on soils with very low P status. This could offer better information on the suitability of the extraction methods to predict the plant available P fraction. Similarly, it would be useful to utilize the very same pots for a long-term experiment involving several cropping cycles without P fertilization. When adding up the amounts of plant-extracted P it could hint to how well the studied soil extraction methods work for long-term prediction of P application necessity. As all plants have a very specific capacity to take up P (*HOLFORD, 1997*) varying plant species would be an interesting approach for further experiments. In regard to this, the occurrence of mycorrhizae, their role in the plant-soil system and the assessment by soil P extraction methods is largely unknown (*SCHACHTMAN et al., 1998*). Certainly, further investigations on additional soil types would be interesting and as a concluding step, it would be necessary to apply the methods to field trials. Undoubtedly interesting is the exploration of new and innovative methods that may predict plant available P more accurately. *ZHANG et al. (1998)* have introduced the use of diffusive gradient in

thin films (DGT) as a new method to determine the dissolved phosphorus in waters, and *MENZIES et al. (2005)* applied the method to evaluate plant available phosphorus in soils. Recently, several experiments have proven the applicability of DGT to predict plant P uptake and response, and concluded that DGT is a very suitable method to predict fertilizer requirements (*MASON et al., 2010, TANDY et al., 2011*).

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## **6 Appendix**

All the data used for this study is given in the appendix. The Curriculum vitae can be found on the last page of the appendix.

Table 15: Raw data of soil characteristics and physicochemical properties

Sample No.	pH (H <sub>2</sub> O)	pH (KCl)	<sup>a</sup> EC	<sup>b</sup> OC	CaCO <sub>3</sub>	C/N (molar)	C/P (molar)	N/P (molar)	<sup>c</sup> KH	Clay	Silt	Sand
			[mS cm <sup>-1</sup> ]	[%]					[mL 100 g <sup>-1</sup> ]			
P3	7.5	7.0	0.64	2.03	13.0	10.2	116	11.4	52.4	26.7	25.8	46.3
P4	7.5	6.8	0.56	2.02	6.1	11.6	112	9.7	56.1	30.0	25.3	44.0
P5	7.6	6.8	0.83	1.71	1.1	11.0	139	12.6	62.7	24.9	45.1	29.1
P6	7.6	6.3	0.56	1.45	0	11.4	104	9.1	63.5	24.2	41.5	33.5
P9	7.1	6.0	0.66	1.49	0	12.4	111	8.9	56.8	24.9	45.6	29.3
P10	7.0	6.1	0.88	0.93	0	13.7	125	9.1	33.6	15.5	34.9	48.9
P15	7.8	6.8	0.73	2.36	24.9	11.2	129	11.5	117.2	37.5	47.4	14.3
P17	7.8	7.1	0.70	3.86	56.2	10.6	358	33.6	53.1	34.3	39.4	25.5
P18	7.9	6.9	0.68	4.03	27.0	11.2	273	24.4	121.2	38.9	46.8	13.7
P19	7.9	7.1	0.56	1.90	26.9	11.5	145	12.6	65.8	25.8	58.7	14.4
P20	7.9	7.1	0.63	1.92	26.8	11.3	153	13.5	68.5	25.8	58.8	14.8
P22	7.7	7.0	0.62	2.06	26.8	10.8	185	17.2	67.7	25.2	60.6	13.3
P23	7.8	7.0	0.48	2.06	18.6	12.5	120	9.6	70.4	28.1	53.6	18.3
P26	7.9	7.0	0.40	1.94	19.9	12.6	117	9.3	65.6	29.0	52.4	18.5
P28	7.9	7.1	0.69	2.02	15.5	12.8	126	9.9	59.4	30.8	47.4	20.1
P29	8.0	6.6	0.33	1.15	0.04	11.7	95	8.2	54.6	25.7	60.4	13.1
P30	7.7	6.4	0.47	1.15	0	9.7	95	9.8	49.4	25.5	60.8	13.1
P31	7.5	6.2	0.43	1.44	0	12.6	116	9.2	47.4	26.4	59.3	13.4
P32	7.5	6.1	0.37	1.15	0	11.3	141	12.5	55.7	26.7	60.0	12.9
P33	7.5	5.7	0.36	1.03	0	11.7	111	9.5	48.8	23.2	49.6	26.5
P34	7.5	5.5	0.34	1.02	0	10.9	108	9.9	45.1	23.1	48.9	27.8
P35	7.4	5.4	0.23	1.02	0	11.4	86	7.5	45.4	23.8	48.8	26.7
P36	7.3	5.4	0.32	0.97	0	9.5	90	9.5	48.5	23.2	49.4	27.0
P41	7.2	5.4	0.49	1.74	0	10.7	85	7.9	75.4	30.5	38.0	30.8
P43	7.2	5.5	0.69	1.89	0	11.7	98	8.3	77.9	23.5	54.2	21.8
P44	6.9	4.6	0.43	1.32	0	11.7	89	7.6	65.1	24.5	45.6	29.5
P49	6.7	4.5	0.46	1.37	0	15.7	84	5.4	35.2	13.2	22.9	63.8
P50	6.7	4.3	0.39	1.44	0	15.6	124	8.0	36.3	12.2	22.1	65.3
P51	6.6	6.0	0.52	1.44	0	14.1	99	7.0	38.1	14.0	23.1	62.6
P52	6.5	4.7	0.41	1.50	0	14.2	116	8.2	38.7	11.5	22.6	65.5
P57	6.3	5.1	0.45	0.97	0	14.5	222	15.3	26.6	15.9	19.1	64.3
P58	6.4	5.3	0.67	1.30	0	17.8	226	12.7	31.4	15.6	17.9	65.5
P59	6.3	4.9	1.12	1.09	0	20.2	198	9.8	29.1	15.7	18.4	65.1
P60	6.3	5.5	0.38	0.97	0	15.7	186	11.8	26.0	14.6	18.5	66.0
P61	6.1	5.1	1.32	1.11	0	15.4	208	13.5	30.1	14.5	19.6	65.2
P63	7.1	6.5	0.77	1.87	0	10.5	130	12.4	51.9	27.9	48.3	22.5
P65	7.2	6.2	1.26	1.45	0	18.0	237	13.2	32.0	4.2	9.1	86.2
P66	7.5	6.7	0.82	2.83	1.2	13.6	329	24.1	41.5	5.9	18.2	75.6
P67	7.0	5.6	1.35	2.67	0	25.1	520	20.7	39.3	5.6	12.9	80.9
P68	6.6	4.8	1.06	1.38	0	19.6	291	14.8	31.7	8.1	14.4	77.1
P69	6.9	5.8	1.01	1.32	0	13.7	301	21.9	39.8	12.5	17.6	68.8
P70	7.0	5.5	0.89	1.95	0	11.7	163	13.9	88.2	43.2	54.5	1.5
P71	7.0	5.7	0.58	0.98	0	15.4	120	7.8	37.9	12.1	48.2	39.7
P72	6.9	5.9	0.89	3.81	0	13.9	215	15.5	64.4	15.7	20.8	63.0
P73	7.1	5.8	0.64	2.67	0	9.9	253	25.6	118.6	53.2	32.2	14.7
P74	7.0	5.2	0.42	1.15	0	10.5	145	13.8	52.3	18.8	58.4	21.9
P75	6.8	5.4	0.59	2.19	0	10.4	79	7.6	59.0	25.3	49.4	25.0
P76	7.7	6.8	0.60	1.62	16.8	13.1	192	14.6	79.2	35.5	59.8	4.2
P77	7.8	6.5	0.91	1.19	0	9.0	564	63.0	43.8	15.1	56.2	28.4
P78	7.7	6.4	0.93	1.94	0	12.8	244	19.1	58.1	30.5	63.6	5.0

<sup>a</sup>EC = electrical conductivity in saturated H<sub>2</sub>O extract<sup>b</sup>OC = organic carbon<sup>c</sup>KH = amount of water necessary to prepare the H<sub>2</sub>O saturation extract

Sample No.	<sup>d</sup> CEC <sub>pot</sub>	<sup>e</sup> BS	Ca	Mg	K	Al	<sup>f</sup> Fe <sub>d</sub>	<sup>f</sup> Mn <sub>d</sub>	<sup>g</sup> Fe <sub>o</sub>	<sup>g</sup> Mn <sub>o</sub>	<sup>g</sup> Fe <sub>o</sub> /Fe <sub>d</sub>	<sup>g</sup> P <sub>o</sub> /Fe <sub>o</sub> (molar)
	[cmol <sub>c</sub> kg <sup>-1</sup> ]		[% CEC <sub>pot</sub> ]				[mg kg <sup>-1</sup> ]					
P3	13.7	94	81.7	10.1	1.2	0.6	6902	605	2280	471	0.33	0.61
P4	15.5	91	79.3	9.9	0.8	0.4	8379	681	2568	596	0.31	0.44
P5	12.0	89	67.7	13.6	6.9	3.5	8208	631	4506	576	0.55	0.31
P6	12.9	66	48.8	13.8	3.1	1.9	8329	613	3809	478	0.46	0.23
P9	11.2	60	41.6	9.9	7.4	2.0	13357	992	3901	785	0.29	0.28
P10	7.4	62	46.5	8.4	5.7	3.4	9338	495	2177	455	0.23	0.31
P15	18.0	90	75.3	8.5	5.1	0.4	9336	350	1261	87	0.14	0.72
P17	14.2	97	80.9	13.1	2.1	1.6	1863	67	930	71	0.5	0.74
P18	27.1	92	76.1	14.9	0.5	0.3	10785	446	2405	286	0.22	0.29
P19	11.7	96	81.2	11.9	2.3	1.2	8404	447	2742	53	0.33	0.27
P20	11.5	97	80.9	12.5	2.7	1.2	8320	437	2724	47	0.33	0.27
P22	12.2	95	80.2	11.9	2.6	1.3	8450	435	2771	47	0.33	0.28
P23	13.9	95	82.2	10.4	2.1	0.3	7339	470	1443	203	0.2	0.66
P26	13.8	94	82.6	9.7	1.5	0.3	7099	450	1381	181	0.19	0.65
P28	12.5	96	79.3	13.0	3.0	0.4	5476	322	1128	252	0.21	0.84
P29	10.6	85	72.0	9.6	3.1	2.2	9690	663	4876	516	0.5	0.16
P30	12.4	68	57.1	7.8	2.3	0.7	9313	661	5002	508	0.54	0.16
P31	12.1	68	58.0	7.8	2.2	0.7	9495	624	5046	509	0.53	0.16
P32	12.7	68	58.2	7.5	2.2	0.6	9673	614	5124	575	0.53	0.19
P33	11.1	63	51.0	8.1	3.2	0.7	9887	510	5629	411	0.57	0.2
P34	16.1	43	35.5	5.1	2.0	0.4	10180	499	5462	388	0.54	0.16
P35	12.9	54	45.0	6.1	2.6	0.5	10662	766	5514	418	0.52	0.18
P36	11.6	60	50.7	6.6	2.5	0.8	10827	533	5460	397	0.5	0.24
P41	17.0	60	49.2	8.5	1.7	1.1	22610	885	7132	733	0.32	0.1
P43	15.3	59	47.7	10.0	1.0	1.4	17781	583	5438	441	0.31	0.19
P44	14.1	56	44.6	6.8	3.9	1.2	17819	546	5004	468	0.28	0.21
P49	10.7	26	18.5	4.0	2.7	1.2	7731	412	3698	379	0.48	0.36
P50	11.6	21	14.7	3.0	3.2	1.0	7759	392	3451	338	0.44	0.29
P51	9.6	44	31.9	8.6	3.0	1.7	8477	415	3627	338	0.43	0.29
P52	12.6	25	18.2	3.6	2.3	1.5	8031	407	6446	623	0.8	0.36
P57	7.6	71	64.4	5.0	0.6	3.6	6791	265	2695	262	0.4	0.12
P58	13.0	42	35.7	4.8	1.3	7.1	6932	293	2621	237	0.38	0.24
P59	11.2	41	34.3	4.4	2.0	5.8	6802	293	2707	251	0.4	0.2
P60	13.0	40	33.2	3.7	2.3	3.4	7381	387	3107	312	0.42	0.23
P61	9.0	53	45.3	4.6	1.9	5.8	7342	383	3105	326	0.42	0.21
P63	12.8	70	59.9	4.1	5.8	4.4	12125	689	4745	519	0.39	0.2
P65	7.4	65	56.1	4.5	3.6	2.6	2565	234	1222	169	0.48	0.84
P66	11.8	87	79.1	3.7	4.2	3.6	7886	276	4770	244	0.6	0.28
P67	9.3	49	41.2	4.8	2.2	1.1	n.d.	n.d.	1693	150	n.d.	0.78
P68	6.2	38	25.9	4.8	6.9	2.1	3058	170	1778	153	0.58	0.44
P69	7.4	76	59.7	8.6	6.3	6.8	3178	273	1251	163	0.39	0.3
P70	20.0	64	52.7	6.4	4.0	4.8	12004	830	3550	769	0.3	0.18
P71	6.9	56	42.2	10.9	1.6	2.7	4737	566	2238	483	0.47	0.26
P72	18.4	61	44.1	15.8	0.8	2.7	42990	334	21949	265	0.51	0.08
P73	29.1	71	62.0	6.8	0.6	0.2	12565	480	10350	383	0.82	0.18
P74	10.7	53	46.7	3.9	1.7	3.3	8646	449	4097	482	0.47	0.14
P75	20.7	40	28.1	8.8	2.7	0.8	24285	1010	10856	821	0.45	0.17
P76	13.6	89	77.6	7.7	2.7	1.5	7108	292	2682	267	0.38	0.19
P77	7.3	83	72.5	6.4	3.8	11.4	4951	387	2417	356	0.49	0.24
P78	14.5	76	65.9	7.1	2.9	5.4	6304	481	1651	439	0.26	0.25

<sup>d</sup>CEC<sub>pot</sub> = potential cation exchange capacity (determined at pH 8.1)

<sup>e</sup>BS = base saturation

<sup>f</sup>Fe<sub>d</sub> and Mn<sub>d</sub> = dithionite-extractable Fe and Mn

<sup>g</sup>Fe<sub>o</sub>, Mn<sub>o</sub> and P<sub>o</sub> = oxalate-extractable Fe, Mn and P

Table 16: Raw data of the applied soil P extraction methods

Sample No.	H <sub>2</sub> O	CaCl <sub>2</sub>	LiCl	Fe-oxide P <sub>i</sub>	Olsen	CAL	AEM	Mehlich 3	Bray II	Dithionite	Organic P	HCl	Oxalate	Total P
	[mg kg <sup>-1</sup> ]													
P3	0.39	3.80	12.7	53.7	62.9	237.9	100.7	200.0	408.8	187.9	451	427	769	1210
P4	0.12	1.52	5.05	27.1	36.2	178.0	71.7	132.6	292.7	150.6	465	326	625	1049
P5	1.43	5.62	34.8	79.5	100.0	267.1	246.2	310.2	409.8	261.6	317	567	783	1144
P6	0.47	1.35	12.6	49.1	38.7	75.1	125.5	103.8	164.0	165.7	361	272	476	831
P9	1.79	9.62	23.4	103.9	76.6	149.5	194.5	207.7	245.2	243.8	347	315	596	906
P10	0.30	4.46	10.1	48.9	47.3	101.9	110.3	153.4	190.9	172.8	192	235	378	602
P15	0.10	0.76	3.21	17.2	22.8	64.8	35.4	53.6	73.4	87.0	473	224	504	926
P17	0.03	0.91	2.72	20.0	36.1	89.0	52.2	46.6	3.1	88.8	278	303	380	800
P18	0.09	0.57	1.25	16.4	24.8	35.1	41.9	40.0	38.5	91.6	381	186	382	817
P19	0.08	1.07	4.00	18.5	27.5	72.1	37.7	74.9	79.0	98.7	338	268	410	874
P20	0.09	1.39	5.31	21.5	34.7	77.6	44.6	83.1	140.8	104.2	324	293	408	894
P22	0.11	1.04	4.59	20.4	32.1	78.7	40.4	80.2	96.0	101.7	287	305	424	877
P23	0.10	0.76	2.33	16.3	21.6	70.1	31.1	57.0	81.7	69.4	441	241	529	892
P26	0.07	0.37	1.67	13.3	16.2	54.4	23.8	41.8	59.3	61.2	426	206	496	811
P28	0.08	0.10	4.19	20.8	27.0	76.2	45.1	71.1	85.3	70.0	412	252	525	849
P29	0.06	0.76	1.52	28.3	28.8	44.4	88.5	55.8	95.8	170.9	312	290	423	768
P30	0.06	1.08	1.65	34.0	31.8	46.6	90.2	64.7	140.5	195.6	312	308	440	791
P31	0.08	1.23	2.34	37.3	37.5	57.1	109.9	78.3	191.3	210.8	321	293	458	849
P32	0.27	2.32	6.37	65.2	53.6	86.6	148.7	115.4	244.2	246.9	210	360	549	920
P33	0.18	2.62	5.02	58.6	58.3	101.1	117.2	119.6	159.9	284.4	239	460	634	957
P34	0.07	1.30	2.26	32.1	40.6	64.7	82.6	77.2	122.3	253.4	244	381	480	846
P35	0.11	2.07	2.99	48.0	48.6	81.5	93.7	94.9	143.7	281.9	307	438	552	926
P36	0.29	3.68	5.96	67.4	73.5	149.3	138.9	154.5	206.4	369.1	277	554	715	1098
P41	0.11	0.95	2.75	38.3	34.4	49.5	73.1	55.1	79.1	270.9	529	129	410	843
P43	0.19	1.48	5.53	34.7	35.7	57.8	102.3	78.5	100.1	217.5	500	381	582	1198
P44	0.56	5.78	8.54	57.6	65.1	94.0	121.9	170.9	195.8	218.8	385	368	596	966
P49	0.11	2.54	4.18	66.6	80.3	117.6	120.6	288.2	300.9	409.9	419	313	737	978



Table 17: Raw data of plant analysis

Sample No.	<sup>a</sup> DM grain yield	<sup>a</sup> DM straw yield	Ears per m <sup>2</sup>	Grains per ear	Thousand-seed weight	P content grain	P content straw	Total P uptake
	[g m <sup>-2</sup> ]				[g]	[mg kg <sup>-1</sup> ]		[mg m <sup>-2</sup> ]
P3	152.8	229.0	283	14	43.9	4520	969	911
P4	156.6	307.6	433	9	45.1	4910	935	1056
P5	154.7	257.1	358	15	32.5	4190	1089	927
P6	162.3	237.7	301	17	37.9	4200	1056	932
P9	128.3	285.3	283	17	31.6	4220	2815	1343
P10	158.5	205.9	283	19	34.3	5140	763	971
P15	156.6	191.9	283	17	36.8	4360	540	786
P17	126.4	164.1	283	16	33.4	4520	448	643
P18	188.7	239.6	283	20	37.9	4010	817	951
P19	162.3	183.3	283	18	36.9	3860	520	720
P20	143.4	179.1	283	17	34.4	3230	728	593
P22	171.7	168.4	283	20	34.6	4460	530	853
P23	126.4	164.1	283	16	32.3	3890	517	576
P26	122.6	152.4	283	17	30.1	4510	450	621
P28	150.9	190.4	301	16	36.8	4210	445	718
P29	169.8	183.5	283	18	38.1	4680	338	855
P30	177.4	193.6	283	18	39.9	4330	471	857
P31	171.7	199.5	283	18	39.5	4450	591	880
P32	183.0	220.2	320	17	39.4	4560	590	963
P33	171.7	201.0	283	18	39.9	4240	715	871
P34	177.4	204.9	283	19	39.0	3780	723	817
P35	149.1	206.2	283	17	37.0	4330	864	823
P36	167.9	211.5	301	18	37.0	3760	549	746
P41	173.6	262.2	320	17	36.1	4480	658	949
P43	181.1	302.9	301	20	35.2	3890	622	892
P44	186.8	273.7	301	19	37.5	4130	990	1042
P49	132.1	186.1	283	17	32.4	3300	533	534
P50	117.0	164.2	264	17	30.2	3430	390	465
P51	154.7	224.3	283	19	32.7	4120	564	764
P52	169.8	206.6	264	21	35.5	3990	669	814
P57	101.9	155.8	283	15	28.4	4180	934	571
P58	122.6	165.0	301	13	37.2	3960	746	607
P59	141.5	226.8	320	14	37.8	4200	841	785
P60	122.6	167.8	301	14	33.7	5380	1418	896
P61	143.4	230.0	283	16	36.7	4270	1207	889
P63	164.2	208.9	283	18	36.9	2970	494	590
P65	160.4	318.9	414	14	31.5	2950	899	759
P66	183.0	220.6	301	20	36.2	3870	481	814
P67	203.8	295.0	320	18	40.3	3810	762	1000
P68	177.4	300.8	358	17	34.7	5270	1554	1400
P69	183.0	234.1	320	18	37.5	4430	922	1025
P70	158.5	293.8	301	16	37.4	4390	884	954
P71	158.5	191.2	283	18	36.6	4020	730	776
P72	169.8	277.1	301	18	35.6	4390	850	979
P73	186.8	269.3	358	17	35.4	4220	783	998
P74	162.3	225.1	283	18	37.2	4960	892	1003
P75	179.3	246.5	377	19	29.7	5060	806	1105
P76	122.6	160.3	283	16	31.0	3330	340	462
P77	149.1	194.6	264	17	38.0	4020	1212	834
P78	135.9	197.4	283	16	35.9	3970	839	704

<sup>a</sup>DM = dry matter

**Table 18: Transfer factors from different soil P pools to wheat grain (tfG) correlated with soil parameters, significance in \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$** 

	pH (H <sub>2</sub> O)	pH (KCl)	<sup>a</sup> EC	<sup>b</sup> OC	CaCO <sub>3</sub>	C/N (molar)	C/P (molar)	N/P (molar)	<sup>c</sup> KH	Clay	Silt	Sand
tfG H <sub>2</sub> O	0.161	0.119	-0.281 *	0.09	0.347 *	-0.121	-0.012	0.042	-0.015	0.118	0.245	-0.220
tfG CaCl <sub>2</sub>	0.297 *	0.296 *	-0.114	0.137	0.274	-0.101	-0.106	-0.064	0.164	0.256	0.194	-0.247
tfG LiCl	0.346 *	0.155	-0.416 **	0.116	0.252	-0.272	-0.197	-0.086	0.288 *	0.315	0.462 ***	-0.453 ***
<sup>d</sup> tfG Fe-oxide P <sub>i</sub>	0.437 **	0.449 **	-0.160	0.206	0.542 ***	-0.212	-0.036	0.044	0.404 **	0.431 **	0.444 **	-0.490 ***
tfG Olsen	0.268	0.296 *	-0.193	-0.026	0.331 *	-0.148	-0.056	0.006	0.215	0.282 *	0.362 **	-0.371 **
<sup>e</sup> tfG CAL	-0.09	-0.110	-0.172	-0.11	-0.003	-0.058	-0.074	-0.059	0.063	0.117	0.189	-0.182
<sup>f</sup> tfG AEM	0.339 *	0.390 **	-0.192	0.148	0.564 ***	-0.145	-0.069	-0.021	0.308 *	0.311 *	0.320 *	-0.353 *
tfG Mehlich 3	0.341 *	0.303 *	-0.211	0.236	0.416 **	-0.284 *	-0.039	0.059	0.403 **	0.470 ***	0.421 **	-0.491 ***
tfG Bray II	0.187	0.227	0.007	0.444 **	0.669 ***	-0.133	0.252	0.321 *	0.043	0.216	0.034	-0.115
tfG Dithionite	0.375 **	0.483 ***	0.157	0.147	0.557 ***	0.019	0.188	0.164	0.224	0.295 *	0.244	-0.300 *
tfG Organic P	-0.287 *	-0.188	0.378 **	-0.326 *	-0.234	0.295 *	0.695 ***	0.654 ***	-0.440 **	-0.422 **	-0.259	0.355 *
tfG HCl	-0.168	-0.149	0.030	-0.166	-0.005	0.076	-0.027	-0.044	0.008	0.041	0.045	-0.051
tfG Oxalate	-0.158	-0.045	0.134	-0.273	0.029	0.091	0.244	0.225	-0.205	-0.055	0.023	0.003
tfG Total P	-0.384 **	-0.266	0.238	-0.357 *	-0.177	0.323 *	0.305 *	0.181	-0.377 **	-0.290 *	-0.225	0.275

<sup>a</sup>EC = electrical conductivity in saturated H<sub>2</sub>O extract<sup>b</sup>OC = organic carbon<sup>c</sup>KH = amount of water necessary to prepare the H<sub>2</sub>O saturation extract<sup>d</sup>tfG Fe-oxide P<sub>i</sub> = transfer factor of P extractable by iron oxide impregnated filter paper<sup>e</sup>tfG CAL = transfer factor of P extractable by calcium acetate lactate<sup>f</sup>tfG AEM = transfer factor of P extractable by anion exchange membrane

	<sup>g</sup> CEC <sub>pot</sub>	<sup>h</sup> BS	Ca	Mg	K	Al	<sup>i</sup> Fe <sub>d</sub>	<sup>i</sup> Mn <sub>d</sub>	<sup>j</sup> Fe <sub>o</sub>	<sup>j</sup> Mn <sub>o</sub>	<sup>j</sup> Fe <sub>o</sub> /Fe <sub>d</sub>	<sup>j</sup> P <sub>o</sub> /Fe <sub>o</sub> (molar)
tfG H <sub>2</sub> O	-0.076	0.197	0.229	0.108	-0.347 *	-0.087	-0.131	-0.254	-0.111	-0.181	-0.094	-0.045
tfG CaCl <sub>2</sub>	0.067	0.339 *	0.334 *	0.296 *	-0.140	-0.199	-0.085	-0.153	-0.154	-0.150	-0.342 *	0.351
tfG LiCl	0.233	0.173	0.200	0.148	-0.359 *	-0.249	0.010	0.044	-0.031	0.012	-0.174	-0.123
<sup>d</sup> tfG Fe-oxide P	0.151	0.520 ***	0.540 ***	0.278	-0.190	-0.151	-0.157	-0.208	-0.265	-0.277	-0.440 **	0.106
tfG Olsen	-0.024	0.429 **	0.455 ***	0.214	-0.245	0.001	-0.194	-0.172	-0.306 *	-0.208	-0.468 ***	0.008
<sup>e</sup> tfG CAL	-0.004	0.065	0.087	0.061	-0.284 *	0.117	0.004	-0.047	-0.068	0.014	-0.230	-0.313 *
<sup>f</sup> tfG AEM	0.051	0.510 ***	0.535 ***	0.259	-0.242	-0.159	-0.210	-0.264	-0.341 *	-0.397 **	-0.528 ***	0.210
tfG Mehlich 3	0.240	0.448 **	0.464 ***	0.324 *	-0.298 *	-0.095	0.045	-0.102	-0.066	-0.132	-0.374 **	-0.074
tfG Bray II	0.074	0.246	0.234	0.256	-0.098	-0.054	-0.166	-0.325 *	-0.140	-0.244	0.035	0.293 *
tfG Dithionite	-0.050	0.584 ***	0.599 ***	0.263	-0.074	0.066	-0.414 **	-0.394 **	-0.547 ***	-0.471 ***	-0.581 ***	0.431 **
tfG Organic P	-0.462 ***	-0.123	-0.090	-0.365 **	0.167	0.738 ***	-0.356 *	-0.388 **	-0.255	-0.220	0.166	-0.081
tfG HCl	-0.084	-0.040	-0.040	-0.012	-0.041	0.266	-0.143	-0.012	-0.278	0.077	-0.361 **	-0.120
tfG Oxalate	-0.302 *	0.113	0.136	-0.085	0.011	0.503 ***	-0.345 *	-0.272	-0.392 **	-0.173	-0.273	-0.209
tfG Total P	-0.425 **	-0.123	-0.103	-0.259	0.112	0.540 ***	-0.372 **	-0.337 *	-0.356 *	-0.160	-0.092	-0.137

<sup>d</sup>tfG Fe-oxide P<sub>i</sub> = transfer factor of P extractable by iron oxide impregnated filter paper<sup>e</sup>tfG CAL = transfer factor of P extractable by calcium acetate lactate<sup>f</sup>tfG AEM = transfer factor of P extractable by anion exchange membrane<sup>g</sup>CEC<sub>pot</sub> = potential cation exchange capacity<sup>h</sup>BS = base saturation<sup>i</sup>Fe<sub>d</sub> and Mn<sub>d</sub> = dithionite-extractable Fe and Mn<sup>j</sup>Fe<sub>o</sub>, Mn<sub>o</sub> and P<sub>o</sub> = oxalate-extractable Fe, Mn and P

**Table 19: Transfer factors from different soil P pools to wheat straw (tfS) correlated with soil parameters, significance in \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$**

	pH (H <sub>2</sub> O)	pH (KCl)	<sup>a</sup> EC	<sup>b</sup> OC	CaCO <sub>3</sub>	C/N (molar)	C/P (molar)	N/P (molar)	<sup>c</sup> KH	Clay	Silt	Sand
tfS H <sub>2</sub> O	-0.046	-0.045	-0.168	-0.012	0.126	-0.043	0.051	0.073	-0.097	-0.011	0.110	-0.072
tfS CaCl <sub>2</sub>	0.216	0.236	-0.105	0.145	0.228	-0.114	-0.065	-0.018	0.180	0.255	0.161	-0.224
tfS LiCl	0.176	0.008	-0.273	0.143	0.133	-0.234	-0.097	0.002	0.303 *	0.262	0.361 *	-0.360 *
<sup>d</sup> tfS Fe-oxide P <sub>i</sub>	0.277	0.321 *	-0.016	0.166	0.383 **	-0.193	0.066	0.147	0.379 **	0.370 **	0.360 *	-0.406 **
tfS Olsen	-0.046	0.043	0.018	-0.144	0.047	-0.04	0.107	0.141	0.037	0.077	0.120	-0.117
<sup>e</sup> tfS CAL	-0.296 *	-0.250	0.011	-0.147	-0.143	0.051	0.054	0.028	-0.039	-0.013	-0.013	0.014
<sup>f</sup> tfS AEM	0.069	0.178	-0.039	0.036	0.335 *	-0.058	0.024	0.036	0.185	0.168	0.139	-0.168
tfS Mehlich 3	0.064	0.073	-0.052	0.147	0.148	-0.19	0.053	0.118	0.294 *	0.319 *	0.226	-0.292
tfS Bray II	0.158	0.204	0.022	0.455 ***	0.657 ***	-0.135	0.274	0.345 *	0.062	0.224	0.026	-0.112
tfS Dithionite	0.067	0.217	0.342 *	-0.003	0.210	0.127	0.325 *	0.272	0.060	0.090	0.034	-0.065
tfS Organic P	-0.291 *	-0.193	0.410 **	-0.300	-0.268	0.247	0.64 ***	0.632 ***	-0.377 **	-0.394 **	-0.260	0.346 *
tfS HCl	-0.350 *	-0.268	0.238	-0.229	-0.210	0.18	0.120	0.068	-0.114	-0.112	-0.133	0.138
tfS Oxalate	-0.351 *	-0.205	0.303 *	-0.313 *	-0.205	0.185	0.32 *	0.278	-0.276	-0.205	-0.175	0.205
tfS Total P	-0.464 ***	-0.322 *	0.369 **	-0.342 *	-0.299 *	0.339 *	0.35 *	0.233	-0.369 **	-0.340 *	-0.322 *	0.365 **

<sup>a</sup>EC = electrical conductivity in saturated H<sub>2</sub>O extract

<sup>b</sup>OC = organic carbon

<sup>c</sup>KH = amount of water necessary to prepare the H<sub>2</sub>O saturation extract

<sup>d</sup>tfS Fe-oxide P<sub>i</sub> = transfer factor of P extractable by iron oxide impregnated filter paper

<sup>e</sup>tfS CAL = transfer factor of P extractable by calcium acetate lactate

<sup>f</sup>tfS AEM = transfer factor of P extractable by anion exchange membrane

	<sup>g</sup> CEC <sub>pot</sub>	<sup>h</sup> BS	Ca	Mg	K	Al	<sup>i</sup> Fe <sub>d</sub>	<sup>i</sup> Mn <sub>d</sub>	<sup>j</sup> Fe <sub>o</sub>	<sup>j</sup> Mn <sub>o</sub>	<sup>j</sup> Fe <sub>o</sub> /Fe <sub>d</sub>	<sup>j</sup> P <sub>o</sub> /Fe <sub>o</sub> (molar)
tfS H <sub>2</sub> O	-0.112	0.081	0.120	0.010	-0.367 **	0.078	-0.084	-0.218	-0.072	-0.122	-0.096	-0.169
tfS CaCl <sub>2</sub>	0.100	0.323 *	0.321 *	0.296 *	-0.206	-0.132	-0.051	-0.141	-0.133	-0.118	-0.366 **	0.242
tfS LiCl	0.314 *	0.046	0.065	0.111	-0.349 *	-0.108	0.067	0.088	0.002	0.084	-0.192	-0.228
<sup>d</sup> tfS Fe-oxide P	0.150	0.444 **	0.450 **	0.272	-0.123	0.065	-0.146	-0.113	-0.290 *	-0.158	-0.508 ***	-0.039
tfS Olsen	-0.127	0.210	0.217	0.105	-0.074	0.293 *	-0.175	-0.067	-0.286 *	-0.040	-0.397 **	-0.153
<sup>e</sup> tfS CAL	-0.062	-0.060	-0.053	-0.018	-0.126	0.274	0.000	-0.023	-0.070	0.070	-0.194	-0.340 *
<sup>f</sup> tfS AEM	-0.021	0.356 *	0.371	0.182	-0.159	0.065	-0.197	-0.173	-0.341 *	-0.259	-0.516 ***	0.031
tfS Mehlich 3	0.201	0.253	0.252	0.239	-0.177	0.119	0.103	0.008	-0.019	0.018	-0.351	-0.243
tfS Bray II	0.093	0.242	0.227	0.270	-0.097	-0.021	-0.150	-0.317 *	-0.136	-0.229	0.010	0.255
tfS Dithionite	-0.146	0.333	0.322 *	0.141	0.169	0.350 *	-0.366 **	-0.199	-0.506 ***	-0.229	-0.498 ***	0.188
tfS Organic P	-0.413 **	-0.147	-0.135	-0.307 *	0.269	0.711 ***	-0.270	-0.240	-0.209	-0.106	0.124	-0.061
tfS HCl	-0.176	-0.178	-0.206	-0.081	0.248	0.425 **	-0.122	0.103	-0.234	0.202	-0.247	-0.174
tfS Oxalate	-0.326 *	-0.083	-0.088	-0.159	0.244	0.595 ***	-0.247	-0.096	-0.294 *	0.005	-0.174	-0.211
tfS Total P	-0.402 **	-0.216	-0.226	-0.253	0.322 *	0.569 ***	-0.266	-0.152	-0.268	-0.008	-0.045	-0.132

<sup>d</sup>tfS Fe-oxide P<sub>i</sub> = transferfactor of P extractable by iron oxide impregnated filter paper

<sup>e</sup>tfS CAL = transferfactor of P extractable by calcium acetate lactate

<sup>f</sup>tfS AEM = transferfactor of P extractable by anion exchange membrane

<sup>g</sup>CEC<sub>pot</sub> = potential cation exchange capacity

<sup>h</sup>BS = base saturation

<sup>i</sup>Fe<sub>d</sub> and Mn<sub>d</sub> = dithionite-extractable Fe and Mn

<sup>j</sup>Fe<sub>o</sub>, Mn<sub>o</sub> and P<sub>o</sub> = oxalate-extractable Fe, Mn and P

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## CURRICULUM VITAE

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