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PREDICTING MAIZE YIELD, NUTRIENT CONCENTRATION AND
UPTAKE IN P AND K FERTILIZED SOILS: PRESSURIZED HOT
WATER AND OTHER ALTERNATIVES TO MEHLICH I
EXTRACTION IN GUATEMALA SOILS

by

Heather M. Hunsaker-Alcântara

A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Master of Science

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Brigham Young University

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BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

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ABSTRACT

PREDICTING MAIZE YIELD, NUTRIENT CONCENTRATION AND UPTAKE IN P AND K FERTILIZED SOILS: PRESSURIZED HOT WATER AND OTHER ALTERNATIVES TO MEHLICH I EXTRACTION IN GUATEMALA SOILS

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Department of Plant and Animal Sciences

Master of Science

The inaccessibility and cost of soil testing reduce effectiveness of fertilizer use on small-scale subsistence farms, and inadequate funding promotes adoption of soil tests in developing countries with minimal validation. For example, Mehlich-I extraction of phosphorus (P) currently used extensively in Guatemala may not be suitable for its broad range of soils. At least four alternatives are available but are relatively untested [Bray 1, Mehlich III, Olsen and pressurized hot water (PHW)]. Pressurized hot water is relatively simple and inexpensive, but is not yet tested against other extraction methods under variable P or potassium (K) fertilization levels. To determine whether PHW-extracted nutrients could be used to predict maize yield, as nutrient content and uptake, soil, plant tissue and grain samples were obtained from a multiple-site field study and calibration

studies were conducted using five rates of P and three rates of K on soils incubated without plants or cropped with maize in greenhouse and field conditions. In the multiple-site field study, maize yield related significantly to PHW-extractable P ($r^2=0.36$) and to leaf P concentration ($r^2=0.23$), but Mehlich I did not. In the two soils used in the greenhouse study, maize yield, vegetative P concentration and total P uptake by maize were predicted by PHW extractable P ($R^2=0.72, 0.75$ and 0.90 , respectively). In the field experiment, grain yield was not improved by P or K application, but P content of maize leaf tissue did relate significantly with PHW-extracted P ($R^2=0.40$), but Mehlich I did not. There were no yield responses to K application in any experiment, but relationships defined between extractable K for all five K-extraction procedures and soil applied K were similarly significant. In comparing P extraction methods, PHW was as good as or better than Olsen, Bray 1 and Mehlich III for relating soil P extraction to the parameters measured in these experiments, and these four alternative extraction methods were consistently better than Mehlich I. Mehlich I extraction should be replaced by one of the four alternatives tested, and PHW is the least expensive and, thus, most viable for use in Guatemala soils.

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TABLE OF CONTENTS

Title Page.....	i
Graduate Committee Approval.....	ii
Final Reading Approval and Acceptance.....	iii
Abstract.....	iv
Acknowledgements.....	vi
Table of Contents.....	vii
List of Tables.....	x
List of Figures.....	xii
Manuscript: Predicting Maize Yield, Nutrient Concentration and Uptake in P and K Fertilized Soils: Pressurized Hot Water and Other Alternatives to Mehlich I Extraction in Guatemala Soils	
Abstract.....	1
Introduction.....	2
Materials and Methods.....	5
Soil Sampling Preparation and Analysis.....	5
Experimental Design and Treatments.....	7
Plant Management and Analysis.....	8
Statistics.....	10
Results and Discussion.....	10
Multiple Field Study.....	10

Greenhouse.....	12
Incubation.....	13
Field Experiment.....	15
Conclusions.....	17
Acknowledgements.....	18
References.....	19
Table 1.....	26
Table 2.....	27
Table 3.....	28
Table 4.....	29
Table 5.....	30
Table 6.....	31
Figure 1.....	32
Figure 2.....	33
Figure 3.....	34
Figure 4.....	35
Figure 5.....	36
Figure 6.....	37
Figure 7.....	38
Figure 8.....	39
Figure 9.....	40
Figure 10.....	41
Figure 11.....	42

Figure 12.....	43
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Appendix I: Extraction and Analytical Methods for Soil and Plant Analysis

Pressurized Hot Water Method—NO ₃ -N, P, K.....	45
Chromotropic Acid Method—NO ₃ -N, P, K.....	49
Phosphorus and Potassium: Sodium Bicarbonate.....	51
Phosphorus and Potassium: Double-Acid Procedure.....	53
Phosphorus and Potassium: Mehlich III.....	55
Phosphorus: Bray P1 Extraction.....	57
Potassium: Ammonium Acetate.....	59
Soil Texture.....	60
Cation Exchange Capacity.....	60
Organic Matter.....	60
pH: Saturated Paste.....	60
Electrical Conductivity.....	60
Calcium Carbonate Neutralization Potential.....	61
Exchangeable Aluminum.....	61
References.....	62

LIST OF TABLES

Table 1. Soil characteristics for the two soils used for the incubation and greenhouse experiments. The valley soil was obtained near Chiquimula and the mountain soil in Durazno, a district of Chiquimula. All values are the average of four measurements of the control plots.....26

Table 2. Mean and standard deviation of maize yields, soil organic matter, pH and the range of soil textures for each area in the multiple field study. Values were obtained from 26 fields among two different regions of Guatemala. The yields for the Valley locations in Chiquimula were severely affected by dry conditions.....27

Table 3. The R^2 values associated with the relationships between P extracted by five methods and whole plant yield (g pot^{-1}), leaf tissue P content (% dry matter) and total P uptake (P content x yield; g pot^{-1}) in the greenhouse experiment. Values used to establish relationships are the average of both soils for each treatment.....28

Table 4. The r^2 values associated with the relationships between rates of applied K and K extracted by each of the five methods near the time of initiation of the greenhouse and incubation experiments sampled 14 days after fertilization. Values used to establish relationships are the average of two soils for each treatment in each case.....29

Table 5. The R^2 values associated with the relationship between P extracted by five extraction methods and leaf P content (%) or P extracted by five extraction methods and the r^2 values associated with the rates of P application. Values used to establish relationships are the average of each treatment of the field experiment.....30

Table 6. The r^2 values associated with the relationships between K extracted by five methods and rates of applied K in the field experiment. Values used to establish relationships are the average of all treatments.....31

LIST OF FIGURES

Figure 1. The relationship between PHW-extractable P measured in samples obtained between the rows of maize and maize grain yield in the multiple field study. Yields were obtained at physiological maturity and represent 10 high yielding sites scattered among the 26 fields in two different regions of Guatemala.....32

Figure 2. The relationship between leaf N content at 1/3 tassel and maize corn yield in the multiple field study. Yields were obtained at physiological maturity and represent 10 high yielding sites distributed among the 26 fields in two different regions of Guatemala.....33

Figure 3. The relationship between leaf P content of maize and PHW-extractable P measured in samples obtained between rows in the multiple field study. Data represent 16 low yielding sites distributed among the 26 fields in two different regions of Guatemala.....34

Figure 4. The relationship between PHW-extractable NO₃-N measured in samples obtained next to plants and maize grain yield in the multiple field study. Yields were obtained at physiological maturity and represent 16 low yielding sites distributed among the 26 fields in two different regions of Guatemala.....35

Figure 5. The relationship between the rate of P applied and whole plant yield (g pot^{-1}), P content (% dry matter) and total P uptake (P content x yield; g pot^{-1}) in the greenhouse. Values used were the average of two soils.....36

Figure 6. The relationship between PHW-extractable P and maize yield (g pot^{-1}), whole plant P content (% dry matter) and total P uptake (P content x yield; g pot^{-1}) in the greenhouse. Values used to establish this relationship are the average of both soils for each treatment.....37

Figure 7. The relationship between P application rate and PHW-extractable P at the initiation of greenhouse and incubation experiments for soils sampled 14 days after fertilization. Values used to establish this relationship were the average of both soils for each treatment.....38

Figure 8. The relationship between K application rates and PHW-extractable K in the greenhouse and incubation soils sampled on day 14. Values used to establish this relationship were the average of both soils for each treatment.....39

Figure 9. The relationship between P application rates and PHW-extractable P at the initiation of the incubation and greenhouse experiments, sampled 14 days after fertilization and at the termination of the incubation and the greenhouse experiments sampled 56 days after fertilization. Values used to establish relationships are the average of two soils and three potassium treatments.....40

Figure 10. Relationship between leaf P concentration of maize at tassel and P extracted by PHW or between leaf P concentration and P rates applied for the field experiment.....41

Figure 11. Relationship between PHW-extractable P and rates of P applied in the field experiment. Values used to establish this relationship are the average of each treatment.....42

Figure 12. Relationship between PHW-extractable K and rates of applied K in the field experiment. Values used to establish this relationship are the average of each treatment.....43

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ABSTRACT

The inaccessibility and cost of soil testing reduce effectiveness of fertilizer use on small-scale subsistence farms, and inadequate funding promotes adoption of soil tests in developing countries with minimal validation. For example, Mehlich-I extraction of phosphorus (P) currently used extensively in Guatemala may not be suitable for its broad range of soils. At least four alternatives are available but are relatively untested [Bray 1, Mehlich III, Olsen and pressurized hot water (PHW)]. Pressurized hot water is relatively simple and inexpensive, but is not yet tested against other extraction methods under variable P or potassium (K) fertilization levels. To determine whether PHW-extracted

nutrients could be used to predict maize yield, as nutrient content and uptake, soil, plant tissue and grain samples were obtained from a multiple-site field study and calibration studies were conducted using five rates of P and three rates of K on soils incubated without plants or cropped with maize in greenhouse and field conditions. In the multiple-site field study, maize yield related significantly to PHW-extractable P ($r^2=0.36$) and to leaf P concentration ($r^2=0.23$), but Mehlich I did not. In the two soils used in the greenhouse study, maize yield, vegetative P concentration and total P uptake by maize were predicted by PHW extractable P ($R^2=0.72$, 0.75 and 0.90 , respectively). In the field experiment, grain yield was not improved by P or K application, but P content of maize leaf tissue did relate significantly with PHW-extracted P ($R^2=0.40$), but Mehlich I did not. There were no yield responses to K application in any experiment, but relationships defined between extractable K for all five K-extraction procedures and soil applied K were similarly significant. In comparing P extraction methods, PHW was as good as or better than Olsen, Bray 1 and Mehlich III for relating soil P extraction to the parameters measured in these experiments, and these four alternative extraction methods were consistently better than Mehlich I. Mehlich I extraction should be replaced by one of the four alternatives tested, and PHW is the least expensive and, thus, most viable for use in Guatemala soils.

INTRODUCTION

Fertilizer use in the developed world has declined in the last decade while concomitant use in developing countries has increased at an exponential rate to exceed in some cases levels of current use in developed countries (FAO, 2006; Vitousek et al., 1997). Due to

worldwide concern for food security and the positive impact of fertilizers on crop yields, fertilizer is made readily available to developing countries and use increases to compensate for dramatic declines in fertility over time (Arnason et al., 1981). Fertilization in tropical agriculture has the potential to dramatically increase production due to the highly weathered soils and the limited reserves of nutrients (Stewart et al., 2005), yet increased nutrient application is rarely managed by recommendations derived from soil testing and consequently this leads to misuse and associated economic (Chase et al., 1991) and environmental risks (Bundy et al., 2001; Cox and Lins, 1984).

The pressure to produce food on marginal land and the importance of fertilizers for improved production makes access to soil testing essential. Yet, there is little research to develop and calibrate soil testing regimes for the variable soils and environments found in developing countries (Raun and Barreto, 1995; Ryan et al., 2001). Instead, soil testing methods related to modern, large-scale agriculture are simply adopted for use in developing countries (Ryan et al., 2001). For example, the Mehlich I double-acid extraction for soil phosphorus (P) and potassium (K), a method developed in North Carolina for very acid soils with low cation exchange capacities and a variety of soil textures (Mehlich, 1953), is used across the entire range of soils found in Guatemala (Saín and Martínez, 2004). Common characteristics of soils in Guatemala and other Central American countries include: derivation from volcanic ash parent material, cultivation of highly sloped terrain susceptible to erosion, and a history of continuous cultivation on marginal land (Raun and Barreto, 1995). Previous research by Crane et al. (2006) on 111 soils sampled throughout Guatemala reported a range of soil characteristics as follows: pH, 4.76 to 7.43, organic matter, 2.3 to 10.8%, calcium carbonate, 0 to 10%

and textures ranging from clay to sandy loam. This broad diversity of soils suggests that Mehlich I may not be an ideal soil test for use in Guatemala, yet alternatives have not been evaluated.

The majority of agriculture in Guatemala is small-scale subsistence farming with monocultured maize or a mixed culture of maize, beans, and squash as the most common cropping systems (Saín and Martinez, 2004). For example, Saín and Martínez (2004) stated that “Approximately 60% of the basic grains produced in the country are grown on farms that are too small to satisfy the basic nutritional needs of a typical family (5-6 persons)”.

Soils of these small farms are likely in need of soil testing, but financial circumstances just as likely eliminate their use. Thus, there is an increasing need for soil testing methods that are affordable, available, and calibrated to fertilizer application in a variety of soil types used by subsistence farmers and related to yields of traditional crops. Pressurized hot water (PHW) which uses an espresso machine to extract soil nutrients, has been evaluated with several soil extraction procedures across a broad range of soils and has been shown to be a practical alternative soil nutrient extraction procedure in Guatemala (Crane et al., 2006). Pressurized hot water is economically more feasible for small-scale subsistence farmers in developing countries than other methods. The total cost of equipment and chemicals needed for analyzing soils for nitrogen (N), P, K and pH is approximately one tenth of the cost of starting up a laboratory using standard procedures (Crane et al., 2006). The procedure has also been easily and successfully taught in small laboratories in Morocco and Guatemala (Crane et al., 2006). Shiffler et al. (2005) found PHW to be effective in predicting boron (B) status of B fertilized soils

and to be related B content, uptake and yield of alfalfa. Yet studies relating pressurized hot water extraction of P and K to yield, nutrient content or uptake under controlled fertilizer application rates have not been reported. In addition, a comparison of PHW extraction to other potential methods such as Bray 1, Mehlich I, Mehlich III, and Olsen extraction methods in Guatemala on fertilized soils is needed.

The purposes of this study were to determine: (a) if PHW-extracted P and K can predict maize yield, nutrient content and/or uptake and to calibrate extraction values to known levels of applied P and K, and (b) if PHW extraction of P and K is as effective as extraction by Olsen, Mehlich I, Mehlich III and Bray 1 (for P only), and ammonium acetate (for K only) methods. To that end, intensive soil and plant sampling from multiple fields, controlled incubation, greenhouse and field experiments were completed.

MATERIALS AND METHODS

Soil Sampling Preparation and Analysis

For the multiple-site field study, an area of 15 x 15 m within each of 26 farmed fields was chosen at random and soils within this area were sampled about two weeks after planting. Ten 20-cm depth samples were obtained with a soil sampling auger during the second week of June 2004 from between maize rows (with an average of 80 cm between rows) as well as next to the maize plant where the fertilizer was placed. The ten samples of each of these two sets were mixed separately and sub-sampled, air-dried, sieved (< 2 mm), extracted and analyzed for extractable nitrate, P and K. Nitrate was extracted and analyzed using PHW (Crane et al., 2006) and water (Haby, 1989). The nitrate was quantified using the chromotropic acid (CTA) procedure (Sims and Jackson, 1971).

Phosphorus (P) was extracted by PHW (Crane et al., 2006), Olsen (Olsen et al., 1954), Mehlich I (Mehlich, 1953) and Bray 1 (Bray and Kurtz, 1945). The P was analyzed using the Murphy-Riley ascorbic acid procedure (Murphy and Riley, 1962). Potassium was extracted using the PHW and ammonium bicarbonate (Olsen) and analyzed using atomic absorption spectroscopy (AA).

In the controlled field experiment, 4 x 9 m plots were established on a sandy clay loam soil that tested below the critical level for maize of 15 ppm available P (Olsen P test) and ten samples (0-20 cm depth) were taken at random within each plot 17 June 2005 30 days after fertilization (a gentle rain of four cm occurred immediately after fertilizer application). These ten samples were mixed, subsampled and prepared for analysis in the same way as the multiple-field-study soils except that Mehlich III extraction was included in the P extractions (Mehlich, 1984). Mehlich III, Mehlich I and ammonium acetate methods were also used for extracting K (Helmke and Sparks, 1996). Both P and K were analyzed as previously described. A second composite soil sample from each plot was taken at the time of grain harvest, prepared and analyzed as just described.

In the soil incubation and maize greenhouse experiments, two soil types, a clay loam with a low pH (mountain) and a sandy clay loam with a high pH (valley; Table 1), were used. These experiments were conducted at the Centro Universitario de Oriente (CUNORI), in Chiquimula, Guatemala. After mixing soils with fertilizer treatments (described later), 500 g sub-samples of each fertilizer treatment-soil mixture were placed in plastic bottles with caps, watered to field capacity and incubated in the dark at room temperature, (27 ± 3 °C). Fourteen days later, approximately 250 g of soil were removed,

air dried, screened and analyzed in a similar fashion as the soil samples in the two field experiments. These analyses mark the fertility levels at the initiation of greenhouse and incubation experiments. After 56 days, greenhouse and incubation soil subsamples were obtained by removing the remaining soil in the incubation bottles and by taking a soil sample from each greenhouse pot at the time of the plant harvest. These samples were air-dried, screened (< 2 mm), extracted and analyzed for N, P and K as described previously. All soil analyses were performed in the Soil and Plant Analysis Laboratory, Brigham Young University, Provo, Utah.

Experimental Design and Treatments

Experimental treatments in the field, greenhouse and incubation bottles were incorporated into randomized complete block designs consisting of four blocks with five rates of P (0, 30, 60, 120 and 240 kg P ha⁻¹) and three rates of K (0, 40 and 80 kg K ha⁻¹) in a 5x3 factorial arrangement. Nitrogen was applied to each field plot [(urea, CO(NH₂)₂)] to equal 215 kg N ha⁻¹. In the field, commercial grade urea, diammonium phosphate [(NH₄)₂HPO₄], and potassium chloride (KCl) were sources of N, P and K. In the greenhouse and incubation studies, reagent grade urea, monocalcium phosphate [Ca(H₂PO₄)₂*H₂O] and potassium chloride were the nutrient sources. The field plots were broadcast fertilized by hand one week before planting. The greenhouse and incubation soils were fertilized by mixing each fertilizer rate into 26 kg of soil (mountain and valley soils mixed separately) after which four 6.0-kg subsamples were placed in greenhouse pots and four 500-g subsamples were placed in capped bottles. The two different soils (valley and mountain) received each treatment and were randomized

within each block. The greenhouse and incubation experimental units were re-randomized every two weeks to ensure uniformity over time.

Plant Management and Analysis

Control of maize cultivars used in the multiple field study was not possible, but rather there was an inherent genetic diversity in the cultivars used, about half were open pollinated varieties and the other half were hybrids developed in Guatemala. Row spacing, plant populations, and fertilizer use were equally dependent upon the individual grower. For the field experiment the maize hybrid HB-83 seed developed for tropical areas by the Instituto de Ciencia y Tecnologías Agrícolas (ICTA; López, 2002) and one of the most common hybrids used in Guatemala (Fauchère, 2000; Saín and Martínez, 2004) was planted 27 May 2005. The seed was planted into 75-cm width rows, prepared prior to fertilization with a tractor, in approximately 2.5-cm deep holes made with a traditional planting stick. The greenhouse pots were planted 19 July 2005 with four seeds per pot. Plants were thinned to 63 plants plot⁻¹ in the field and to one plant pot⁻¹, in the greenhouse. Precipitation was evenly distributed and generally adequate in the field, but irrigation water was applied 16-20 June 2005 by sprinkler to maintain optimal moisture level during one extended dry period. In the greenhouse, pots were brought to field capacity initially with tap water by weighing. Thereafter, a randomly selected subset of pots was weighed daily followed by addition of the same average amount of water to all pots. Weeds in the field were controlled with the pre-emergent herbicide, Batalla [N-(phosphonomethyl) glycine], and post-emergent herbicides, Hedonal (2,4-Dichlorophenoxyacetic acid) and Paraquat (1,1-dimethyl-4,4-bipyridylum dichloride), as

well as manual weeding on a regular basis. The insecticide Thiodan (6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-,methano-2,4,3-benzodioxathiopin-3-oxide) was used to control corn earworms, in the field, and Volaton (2-diethoxyphosphin othioyloxyimino-2-phenyl-acetonitrile), was used to control cutworms in both the field and greenhouse.

Leaf tissue samples consisting of 10 leaves (one leaf directly below and opposite the primary ear of 10 plants) were taken at the beginning of tassel for the controlled field and multiple field study. In the multiple-site field study samples were taken from 8 m of two center rows within the 15 x 15 m area of each farmed field and sampling date varied in each region because of variation in planting dates among fields. In the controlled field study, samples were taken from the two center rows of the 4 x 9 m plots 19 July 2005 (53 days after planting). All maize samples were dried in a forced-air oven for a minimum of 48 hours at 65° C, weighed, ground (< 1 mm), digested by wet ashing with nitric-perchloric acid (Johnson, and Ulrich, 1959) and analyzed for P and K using inductively coupled plasma (ICP). Nitrogen was analyzed using a total N analyzer.

Maize grain in the field was hand harvested at physiological maturity (black layer formed). Maize plants and ears were counted in 8 m of the two center rows of each 15 x 15 m area in the multiple field study consisting of 26 farmed fields and of each 4 x 9 m area in the field experiment (Rehm and Lamb, 2004). The total ear wet weight was recorded, a 10-ear subsample taken, weighed and shelled from which a grain subsample was taken, weighed and oven-dried (Pilbeam et al., 2002). The grain subsample was ground, (< 1 mm), digested by wet ashing with nitric-perchloric acid and analyzed for P and K using ICP. Nitrogen was analyzed using a total nitrogen analyzer. In the

greenhouse study, the whole plant was harvested at tassel (56 days), by clipping plants at soil level (Chien et al., 1987). Plants were oven dried for a minimum of 48 hours at 65° C, ground and analyzed as previously described for N, P and K.

Statistics

All data obtained from the soil and plant analyses were subjected to several statistical analysis procedures using Statistical Analysis Software (SAS, 2001), including analysis of variance, correlation and regression.

RESULTS AND DISCUSSION

Multiple-Site Field Study

Soil characteristics varied widely among and within the two regions and yields reflected soil and environmental variation (Table 2). Analysis of the combined data of all regions revealed few significant relationships among P, K and NO₃ extraction by various extraction methods and yield or leaf nutrient concentration (data not shown). Because of the wide variation in yield and soil characteristics, data was analyzed by grouping data into low (< 5.5 t ha⁻¹) and high yielding sites (> 5.5 t ha⁻¹). These analyses yielded several significant relationships.

Maize yields related significantly to PHW-extracted P in high-yielding sites of the multiple field study (Figure 1, $r^2=0.36$) but not in low yielding sites. Of the other four soil P extractions used, only Olsen-extractable P related positively with maize yields in this survey ($r^2=0.33$), while Mehlich I and the Bray 1-P extractions resulted in significant

negative relationships to yield in low yielding areas ($r^2=-0.69$ and $r^2=-0.55$ respectively). The latter may be due to the wide range of soil characteristics found in the areas studied, making these two extraction methods developed for acidic soils less effective (Enwezor, 1977; Westerman, 1990). Other studies also affirm that Olsen is more widely applicable to different soil types than either Mehlich I or Bray 1 (Dancer, 1984; Mallarino, 1992). In an even broader variety of soils in Guatemala, Crane et al. (2006) found that PHW-extractable P related most closely with Olsen-extracted P followed by Bray-1 and the poorest relationship was with Mehlich-I extraction. Yields in high yielding areas also related significantly to percent N in maize leaves taken at the beginning of tassel (Figure 2; $R^2=0.68$). Leaf N for 7 of 10 high yielding sites is above 2.70% N, the level established for sufficiency, and leaf N for the lowest yielding sites are below 2.70% (Mills and Jones, 1996). This confirms the importance of adequate N nutrition to avoid restriction of P response in maize.

In the 16 low yielding sites, PHW-extracted P related significantly to leaf P content of maize (Figure 3), but the relationship is not too predictable ($r^2=0.23$). Olsen P was the only other P extraction that related significantly to leaf P content ($r^2=0.31$, other data not shown). About half of the sixteen leaf P concentrations were at or above the sufficiency level of 0.25% P in the ear leaf (Mills and Jones, 1996). The relationship between yield and PHW-extracted $\text{NO}_3\text{-N}$ was also significant in the low-yielding sites (Figure 4; $r^2=0.37$) and was confirmed by similar relationship with water-CTA extraction ($r^2=0.38$; data not shown).

There were no significant relationships between extractable K with any extraction method with maize yield or leaf K concentration which suggests that K is not a limiting

factor in these soils. Only 6 of the 26 sites sampled resulted in soil K levels below the 120 ppm K critical level for maize for the Olsen method (Jones, 1980). Leaf K concentrations confirmed that K was not limiting as only eight of 26 sites were below the sufficiency level of 2.0% K (Mills and Jones, 1996).

The results of the multiple-site field study suggest that PHW and Olsen extraction methods have greater promise in extracting P than Bray I and Mehlich I, and that there is some ability to predict maize grain yields in high yielding sites despite a wide range of hybrids and native lines of maize being cultivated. Thus, further research under controlled P and K application conditions of the field and greenhouse testing PHW compared to other methods is justified.

Greenhouse

Where maize was grown on two soil types in the greenhouse for 56 days (to near the silk stage), significant increases in maize biomass yield, P concentration in the dry matter and total P uptake (P concentration x yield) were observed as rate of P fertilizer rose (Figure 5; analysis of variance, $P \leq 0.01$). More importantly, the relationships between maize yield, P concentration, total P uptake and PHW-extracted P were highly significant (Figure 6). The relationship was better for total P uptake related to PHW extracted P ($R^2=0.92$) and rate of P applied ($R^2=0.93$) than for P concentration ($R^2=0.74$ and 0.75 , respectively) or yield ($R^2=0.75$ and 0.72 , respectively). The mean P concentrations of maize at the high rates of P applied approached the commonly accepted adequate concentration of 0.30% (a concentration reported for whole plant tops at a 12-inch height, plants younger than those harvested; Figure 5; Mills and Jones, 1996). Some propose

nutrient uptake or concentration as more predictable parameters than yield to measure during methodology development in soil testing and our data support the idea (Mills and Jones, 1996; Ross et al., 2006).

The relationships between extractable P and maize yield were equally well defined for PHW, Olsen, and Bray 1 extraction methods but were less well defined for Mehlich III or Mehlich I (Table 3, Figure 6). The relationships between extractable P and P concentration as well as extractable P and total P uptake were similar with all extraction methods except with Mehlich I (Table 3, Figure 6). Thus, based on greenhouse data, Mehlich I did produce significant relationships for all three parameters but was consistently the poorest of the five P extraction methods tested, while PHW was consistently as good as or better than the other P extraction methods tested.

There was no significant biomass response to applied K (data not shown). Consequently, there were also no significant relationships between yield and extractable K by any method, nor between maize K content and any K extraction method. Whole plant K concentration was adequate with total maize K content averaging 3.5% K, at the upper end of the sufficiency range of 2.5 to 4.0% K (a concentration reported for whole plant tops, plants younger than those harvested in this experiment; Mills and Jones, 1996).

Incubation

In the soil samples taken 14 days after incubation began, which represent the beginning equilibrium levels of nutrients after treatment application at the initiation of both the incubation and greenhouse experiments, the relationships between rates of applied P and

extractable P with various methods were highly significant for PHW (Figure 7; $r^2=0.99$) and for all other P extraction procedures (r^2 between 0.96 and 0.99). The relationships between the rate of K applied and the amount extracted by each of the five extraction methods were also significant, but Olsen and Mehlich I relationships were better than those for PHW, Mehlich III and ammonium acetate (Figure 8; Table 4). In general, the K extractions with the five methods were not as strongly related to rates of K application as were the P extractions to P application rates and Olsen and Mehlich I produced the best relationships. However, PHW-K extraction related comparably to Mehlich III and ammonium acetate-extractable K, and the latter is the most commonly used K extraction method (Westerman, 1990).

Comparing PHW-extractable P levels at the initiation (14 days) and termination (56 days) of the greenhouse and incubation experiments reveals a definite impact of P uptake by maize in the greenhouse study at each of the five P levels for PHW-extractable P (Figure 9). Declines were greater between initial and final PHW-extractable P as P rate increased and this was reflected in measured leaf P concentration and total P uptake (Figure 5 and 6). There is little evidence of P fixation, except for some fixation at the 120 and 240 kg ha⁻¹ P rates. These relationships for uptake and fixation were similar in the mountain and valley soils (Figure 9 is the average of two soils). This is surprising since the mountain soils in this region are reported to exhibit high P fixation capacity (Bueso-Campos, M.L., personal communication, 2005; Ruan and Barreto, 1995). Both soils are high in clay, while the mountain soil contains some exchangeable aluminum and the valley soil has excess CaCO₃ at relatively low levels (Table 1). Each of these factors

enhance P fixation (Havlin et al., 2005). The other four P extraction methods showed P uptake and P fixation relationships similar to PHW-extraction (data not shown).

Evidence for K uptake by maize or K fixation was not as strong for K as for P (data not shown). Although some treatments showed a decline in K over time, the decline was inconsistent and unpredictable. This unpredictability was apparent with all methods of K extraction (data not shown).

Field Experiment

Even though yields in the field experiment ranged from 2.2 to 5.2 t ha⁻¹, no significant relationships between yield and soil extractable P or K could be established with any soil extraction method (data not presented). This is surprising since the same application rates to this soil in the greenhouse produced responses to P application (Figure 5). Early vegetative growth in the field appeared to be related to P rates, leaf P increased with P rate (Figure 10), and other leaf nutrient concentrations were at or above reported critical levels (Mills and Jones, 1996; Ramirez, 1981). In addition, there was early and in-season weed control and adequate N application. Consistent rainfall distribution and irrigation during the one extended dry-period should have minimized impacts of environment on yield. Yield responses can be more common in greenhouse than field conditions because of greater root–fertilizer contact (Eghball and Sander, 1989; Ussiri et al., 1998). Also, the greenhouse study was harvested near the end of the vegetative stage rather than at the end of the reproductive stage, a period in the field when P fertilizer application appeared to impact growth, but no data were collected. The middle to upper range of yields observed in this study (5.7 t ha⁻¹) is in the high range of maximum yields reported for this

hybrid (Fauchère, 2000). Thus, although widely cultivated, perhaps the maize hybrid HB-83 lacks the genetic potential to respond to high rates of P for grain production. Genetics play a major role in developing crop cultivars responsive to fertilizer application and is a factor needing attention in developing countries (Havlin et al., 2004). However, external conditions known to complicate yield responses to fertilizer (Locke and Hanson, 1991; Viets et. al, 1954) cannot be completely ruled out.

There were significant predictable improvements in leaf P content with increasing rates of P application, but all of the concentrations were in the sufficiency range (> 0.25%) for maize (Mills and Jones, 1996). The increased leaf P as P rate increased was reflected in PHW-extractable P (Figure 10) and extractable P of three of the other four P extraction methods (Table 5). Only Mehlich I failed to extract P in relation to the concentration of P in the plant. Since yields usually relate to leaf P content or P uptake (Tyner, 1946; Viets et al., 1954), this lack of relationship by Mehlich I is another evidence that Mehlich I is not the appropriate P soil test for Guatemala soils (Perez et al., 2003).

The relationship between the rate of P applied in the field and PHW-extractable P was good (Figure 11) and similar strong relationships were observed with other extraction methods except Mehlich I (Table 5). These relationships are not as strong as observed in the greenhouse and incubation studies (r^2 between 0.96 and 0.99), and all P extraction methods produced significant relationships. By nature field experiments are more variable than greenhouse or incubation experiments in which relatively small quantities of soil are mixed thoroughly improving fertilizer distribution and increasing root-soil contact. The relatively poor relationship between Mehlich I-extractable P and

rate of P in the field ($r^2=0.35$) compared to other extraction methods ($r^2=0.79$ to 0.89) further identifies the weaknesses of Mehlich I on variable soils of Guatemala.

Relationships between extractable K and yield were not established due to lack of K response by maize in the field (data not shown). All other relationships i.e. PHW-extractable K and rates of K application or PHW-extractable K and leaf K were relatively poor. However, the amount of PHW-extracted K related significantly to rates of K applied (Figure 12), but as with the incubation and greenhouse studies, this relationship was poor compared to those for extractable P (Figure 11; Table 6). The worst relationship between rates of K applied and extractable K was with ammonium acetate (Table 6), the most common method for K extraction. None of the K extraction methods produced strong relationships with applied K (r^2 from 0.22 to 0.48; Figure 12; Table 6), but relationships were better defined with data from the greenhouse/incubation studies (r^2 from 0.62 to 0.85; Figure 8; Table 4). Extractable K is the one area in which Mehlich I is as good as or better than other extraction methods but Olsen is consistently better than others. Thus, PHW is almost as effective as other K extraction methods, but none of the extraction methods are as effective in predicting K as they were for predicting P relationships.

CONCLUSIONS

Our data from these studies confirm that the PHW-P extraction procedure successfully reflects levels of P applied to soils and illustrate that PHW can effectively be used to predict maize yield, P concentration and uptake under varying soil P status. Pressurized

hot water was as effective as Olsen, Bray 1 and Mehlich III extraction methods and more effective than the widely used Mehlich I extraction methods in predicting yield, P uptake and P content of maize. These data confirm earlier results on a broader set of Guatemalan soils (Crane et al., 2006) that the Mehlich-I P extraction method that is widely used in Guatemala needs to be replaced with another extraction method more adapted to the diverse soils inherent to the region. Although results were not as strong for K, PHW-K extraction was similarly significant compared to the other four K extraction methods used. Additional field work is recommended to improve this K data and to further define maize yield and P-extraction relationships in a broader group of soils of Guatemala and to develop and refine fertilizer recommendations for PHW, Olsen, Bray 1, or Mehlich III—all potential improvements over Mehlich I extraction methods (Heckman et al., 2006). Pressurized hot water extraction is a viable option for replacing Mehlich I for recommending P application in Guatemala and regions with similar soils.

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Table 1. Soil characteristics for the two soils used for the incubation and greenhouse experiments. The valley soil was obtained from near Chiquimula and the mountain soil from Durazno, a district of Chiquimula. All values are the average of four measurements of the control plots.

Characteristic	Valley Soil	Mountain Soil
Texture	Sandy Clay Loam	Clay Loam
Sand, %	49	31
Silt, %	25	33
Clay, %	26	36
CEC, meq 100 g ⁻¹	31	22
NO ₃ -N, mg kg ⁻¹	88	48
Organic matter, %	6.3	2.3
pH	7.3	5.5
Electrical Conductivity, dS M ⁻¹	1.3	0.5
CaCO ₃ , %	2.1	0.7
Exchangeable Al, meq 100 g ⁻¹	0.2	0.8

Table 2. Mean and standard deviation of maize yields, soil organic matter, pH and the range of soil textures for each area in the multiple field study. Values were obtained from 26 fields among two different regions of Guatemala. The yields for the Valley locations in Chiquimula were severely affected by dry conditions.

AREA	Yield, t ha ⁻¹	Organic Matter, %.	Texture Range	pH
-----Coban -----				
Tanchí	7.1 ± 0.9	6.2 ± 0.6	Loam - Clay	5.9 ± 0.9
Nueva Viñaroz	5.0 ± 0.6	4.9 ± 0.9	Clay Loam - Clay	5.7 ± 0.6
-----Chiquimula-----				
San Juan Ermita	7.1 ± 0.5	4.0 ± 0.5	Sandy Clay Loam -Clay	5.6 ± 0.7
Valley	2.5 ± 0.2	9.9 ± 0.1	Sandy Loam	7.1 ± 0.2

Table 3. The R^2 values associated with and equations defining relationships between P extracted by five methods and whole plant yield (g pot^{-1}), leaf tissue P content (% dry matter) and total P uptake (P content \times yield; g pot^{-1}) in the greenhouse experiment. Values used to establish relationships are the average of both soils for each treatment.

P Extraction Method	R^2	Equation
----- Yield -----		
Pressurized Hot Water	0.72	$Y=13.8 + 3.37X - 0.101X^2$
Olsen	0.70	$Y=10.7 + 1.21X - 0.0119X^2$
Mehlich III	0.66	$Y=13.4 + 0.859X - 0.00657X^2$
Mehlich I	0.62	$Y=11.1 + 1.86X - 0.0285X^2$
Bray 1	0.71	$Y=9.91 + 1.39X - 0.0152X^2$
----- Plant P -----		
Pressurized Hot Water	0.75	$Y=0.155 + 0.011X - 0.00025X^2$
Olsen	0.73	$Y=0.143 + 0.00407X - 3.17X^2$
Mehlich III	0.73	$Y=0.148 + 0.00314X - 1.96 e^{-5}X^2$
Mehlich I	0.68	$Y=0.123 + 0.00876X - 0.00013X^2$
Bray 1	0.76	$Y=0.144 + 0.00429X - 3.41 e^{-5}X^2$
----- P Uptake -----		
Pressurized Hot Water	0.93	$Y=0.0101 + 0.0105X - 0.00027X^2$
Olsen	0.91	$Y=-0.00043 + 0.0038X - 3.27 e^{-5}X^2$
Mehlich III	0.89	$Y=0.00593 + 0.00284X - 1.92 e^{-5}X^2$
Mehlich I	0.84	$Y=-0.0084 + 0.00694X - 0.000101X^2$
Bray 1	0.91	$Y=0.00013 + 0.00409X - 3.77 e^{-5}X^2$

Table 4. The r^2 values associated with and equations defining relationships between rates of applied K and K extracted by each of the five methods near the time of initiation of the greenhouse and incubation experiments sampled 14 days after fertilization. Values used to establish relationships are the average of two soils for each treatment in each case.

Extraction Method	K Applied	Equation
	r^2	
Pressurized Hot Water	0.65	$Y=127.7 + 0.225X$
Olsen	0.85	$Y=254.6 + 0.251X$
Mehlich III	0.62	$Y=405.8 + 0.354X$
Mehlich I	0.77	$Y=166.4 + 0.29X$
Ammonium Acetate	0.67	$Y=277.7 + 0.397X$

Table 5. The R^2 values associated with and equations defining relationship between P extracted by five extraction methods and leaf P content (%) or P extracted by five extraction methods and the r^2 values associated with and equations defining rates of P application. Values used to establish relationships are the average of each treatment of the field experiment.

Extraction Method	Parameter Regressed	Equation
-----Leaf P Content-----		
	R^2	
Pressurized Hot Water	0.40	$Y=0.277 + 0.0036X - 5.52 e^{-5}X^2$
Olsen	0.36	$Y=0.260 + 0.0025X - 2.15 e^{-5}X^2$
Mehlich III	0.38	$Y=0.281 + 0.0028X - 2.58 e^{-5}X^2$
Mehlich I	NS	-----
Bray 1	0.38	$Y=0.275 + 0.00082X - 3.57 e^{-6}X^2$
-----Rate of P Application-----		
	r^2	
Pressurized Hot Water	0.79	$Y=5.73 + 0.0601X$
Olsen	0.89	$Y=16.1 + 0.118X$
Mehlich III	0.85	$Y=6.18 + 0.0576X$
Mehlich I	0.35	$Y=14.4 + 0.0275X$
Bray 1	0.88	$Y=24.9 + 0.111X$

Table 6. The r^2 values associated with and equations defining relationships between K extracted by five methods and rates of applied K in the field experiment. Values used to establish relationships are the average of all treatments.

Extraction Method	Rate of K Applied	Equation
	r^2	
Pressurized Hot Water	0.31	$Y=105.7 + 0.145X$
Olsen	0.48	$Y=238.5 + 0.35X$
Mehlich III	0.32	$Y=269.9 + 0.26X$
Mehlich I	0.48	$Y=589.6 + 1.24X$
Ammonium Acetate	0.22	$Y=291.4 + 0.31X$

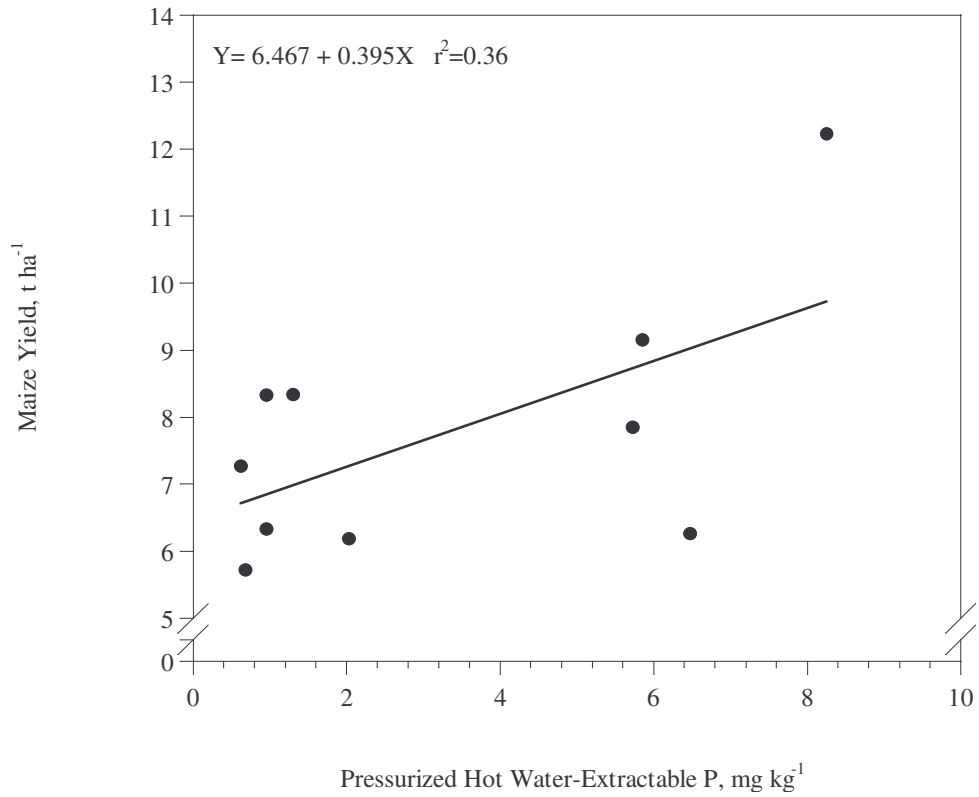


Figure 1. The relationship between PHW-extractable P measured in samples obtained between the rows of maize and maize grain yield in the multiple field study. Yields were obtained at physiological maturity and represent 10 high yielding sites scattered among the 26 fields in two different regions of Guatemala.

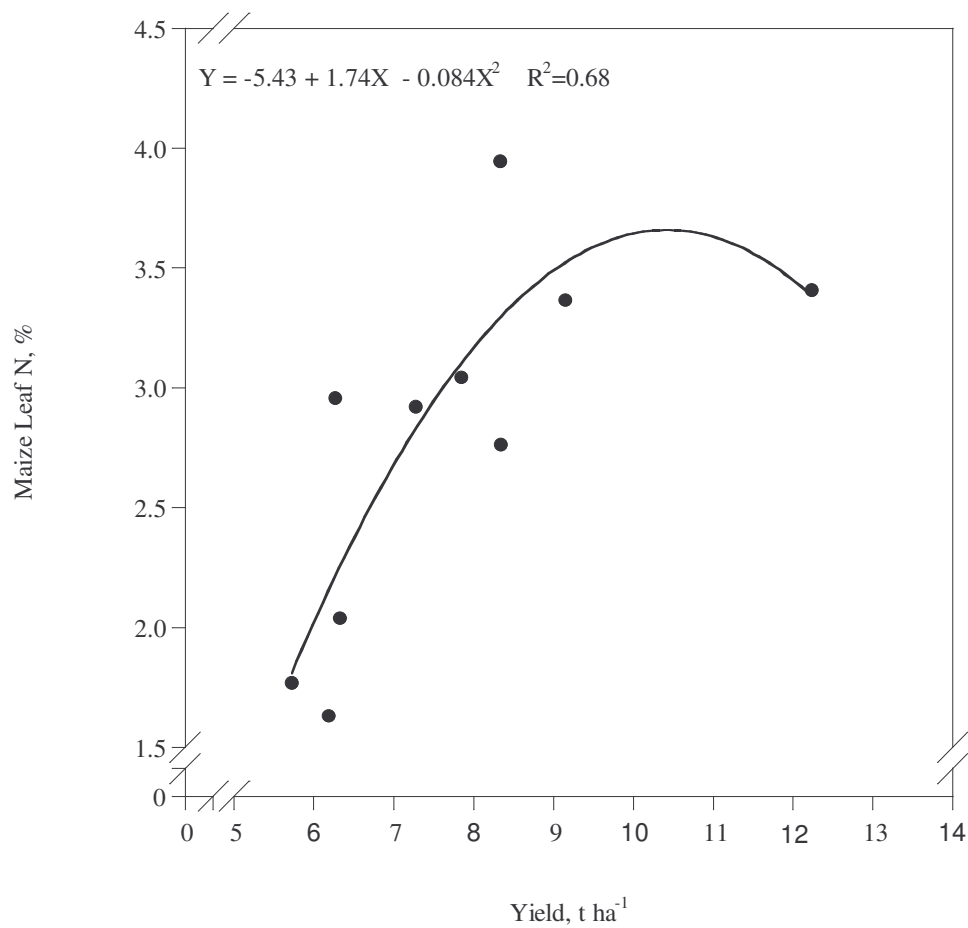


Figure 2. The relationship between leaf N content at tassel and maize corn yield in the multiple field study. Yields were obtained at physiological maturity and represent 10 high yielding sites distributed among the 26 fields in two different regions of Guatemala.

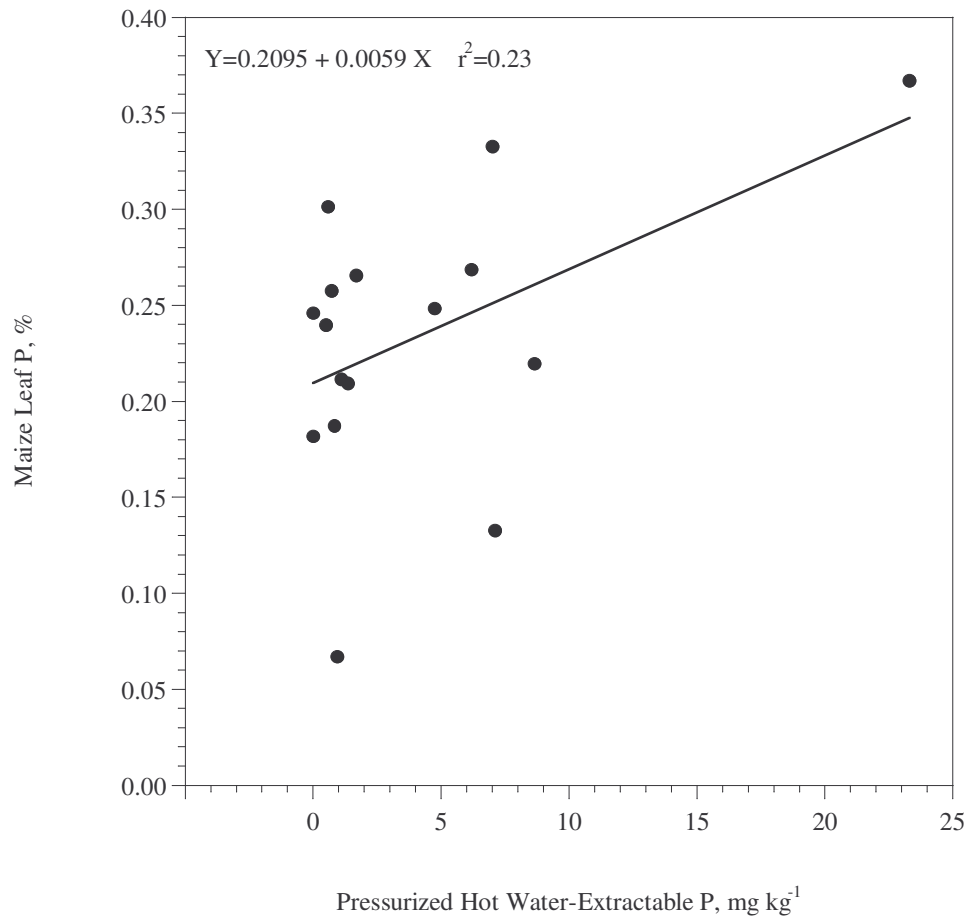


Figure 3. The relationship between leaf P content of maize and PHW-extractable P measured in samples obtained between rows in the multiple field study. Data represent 16 low yielding sites distributed among the 26 fields in two different regions of Guatemala.

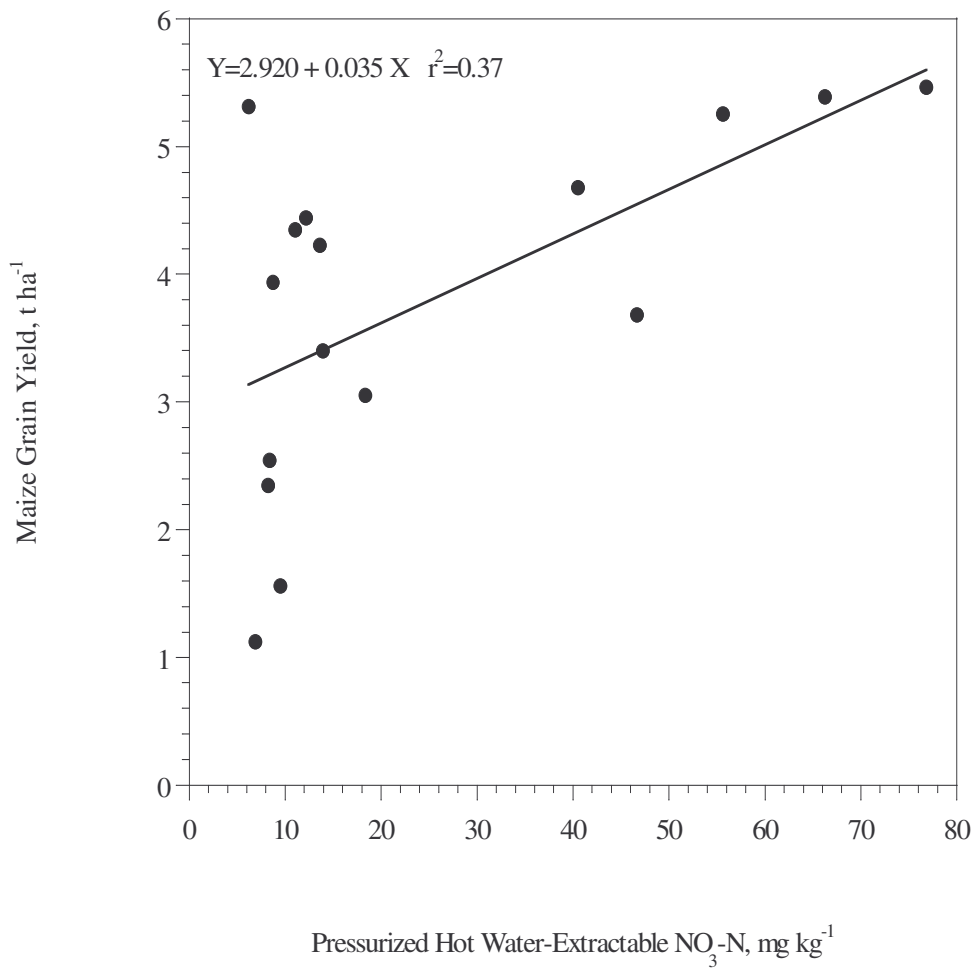


Figure 4. The relationship between PHW-extractable NO₃-N measured in samples obtained next to plants and maize grain yield in the multiple field study. Yields were obtained at physiological maturity and represent 16 low yielding sites distributed among the 26 fields in two different regions of Guatemala.

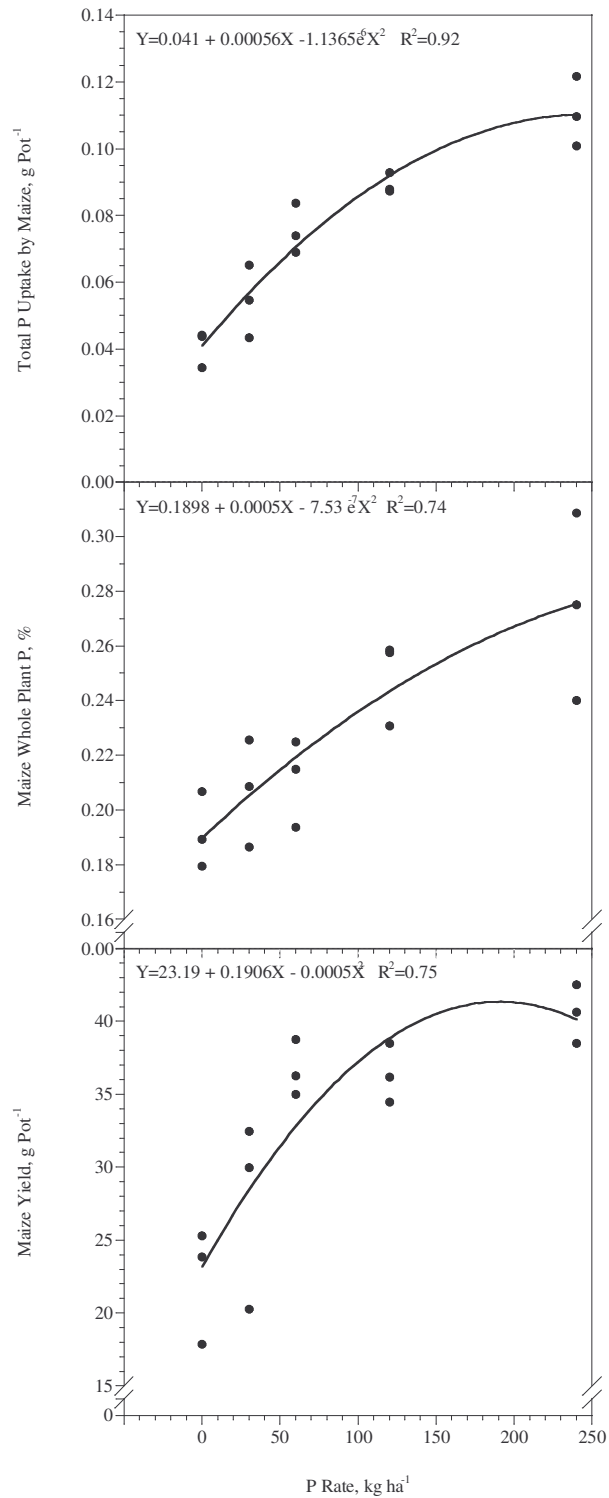


Figure 5. The relationship between the rate of P applied and whole plant yield (g pot⁻¹), P content (% dry matter) and total P uptake (P content x yield; g pot⁻¹) in the greenhouse. Values used were the average of two soils.

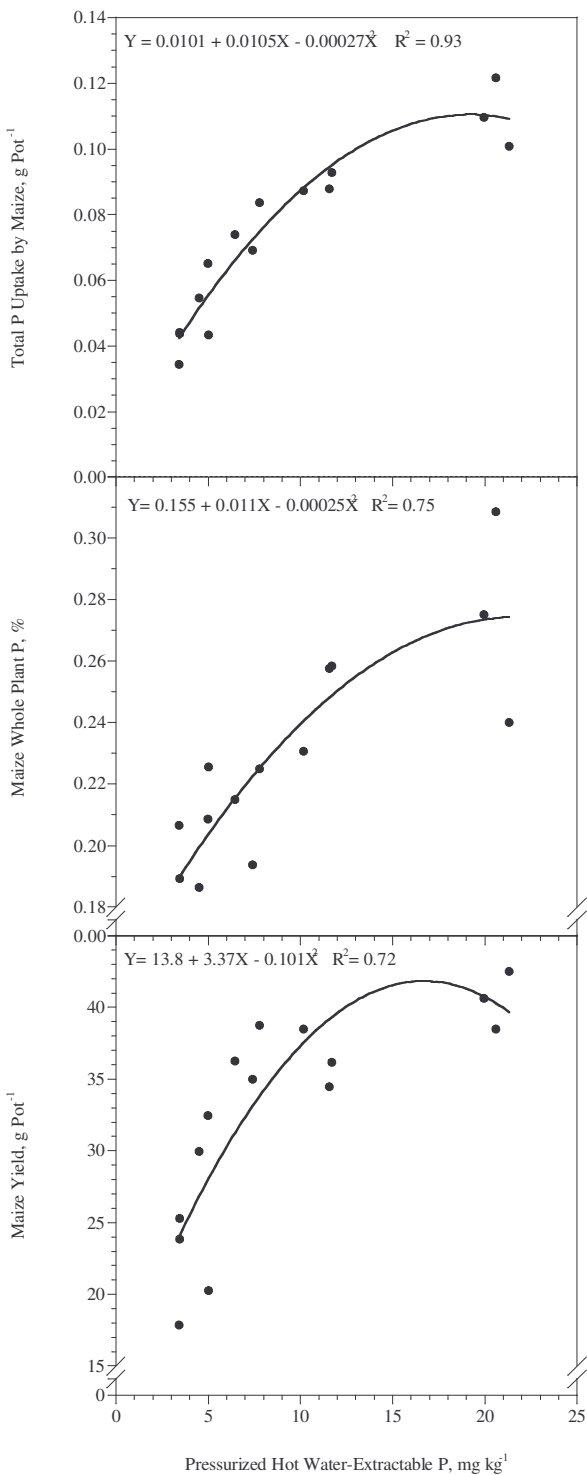


Figure 6. The relationship between PHW-extractable P and maize yield (g pot⁻¹), whole plant P content (% dry matter) and total P uptake (P content x yield; g pot⁻¹) in the greenhouse. Values used to establish this relationship are the average of both soils for each treatment.

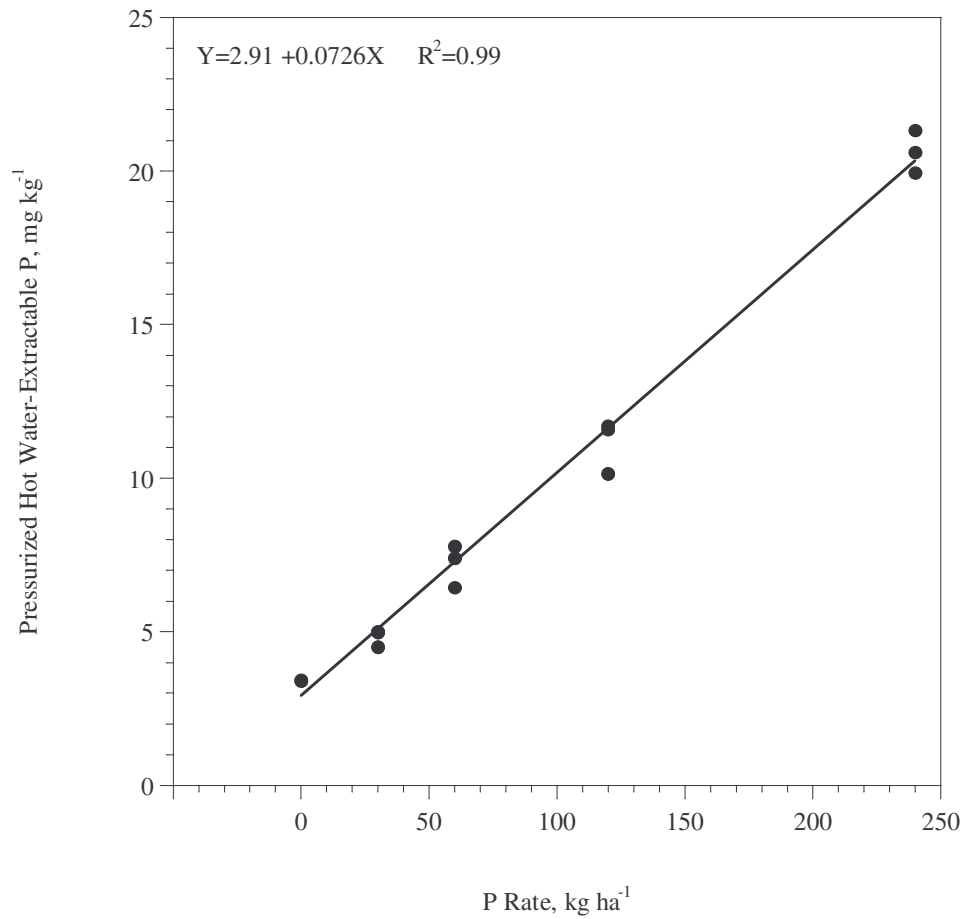


Figure 7. The relationship between P application rate and PHW-extractable P at the initiation of greenhouse and incubation experiments for soils sampled 14 days after fertilization. Values used to establish this relationship were the average of both soils for each treatment.

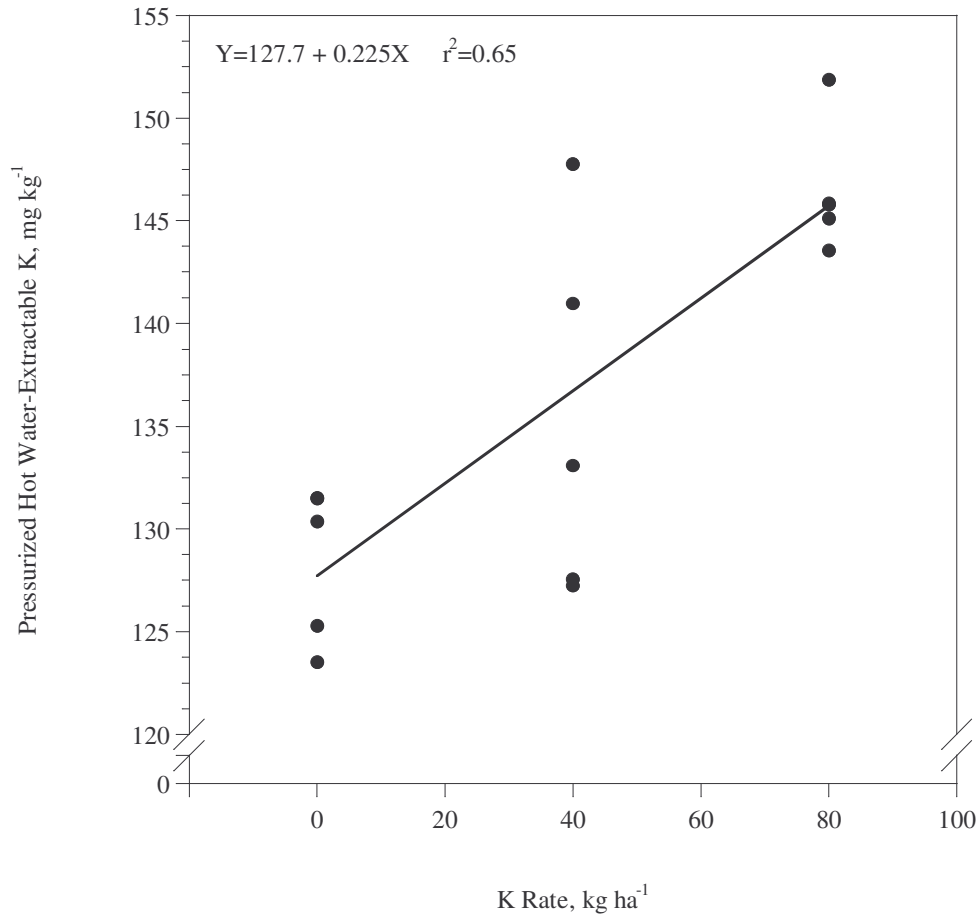


Figure 8. The relationship between K application rates and PHW-extractable K in the greenhouse and incubation soils sampled on day 14. Values used to establish this relationship were the average of both soils for each treatment.

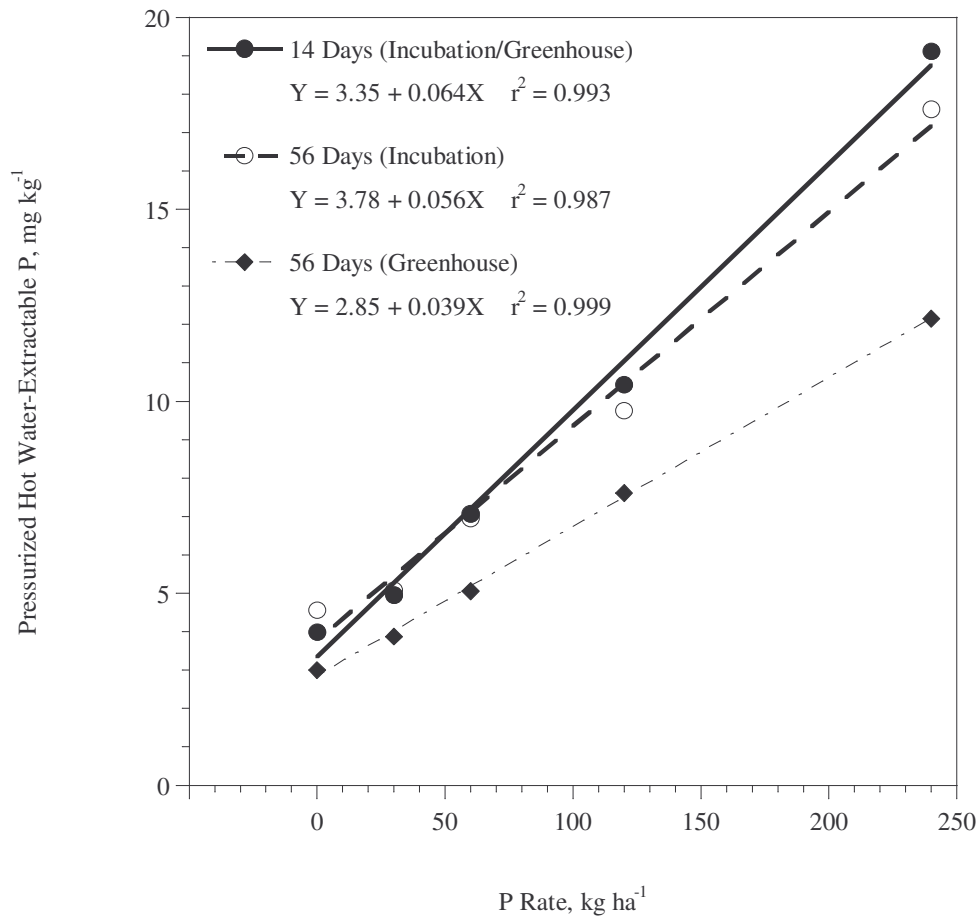


Figure 9. The relationship between P application rates and PHW-extractable P at the initiation of the incubation and greenhouse experiments, sampled 14 days after fertilization and at the termination of the incubation and the greenhouse experiments sampled 56 days after fertilization. Values used to establish relationships are the average of two soils and three potassium treatments.

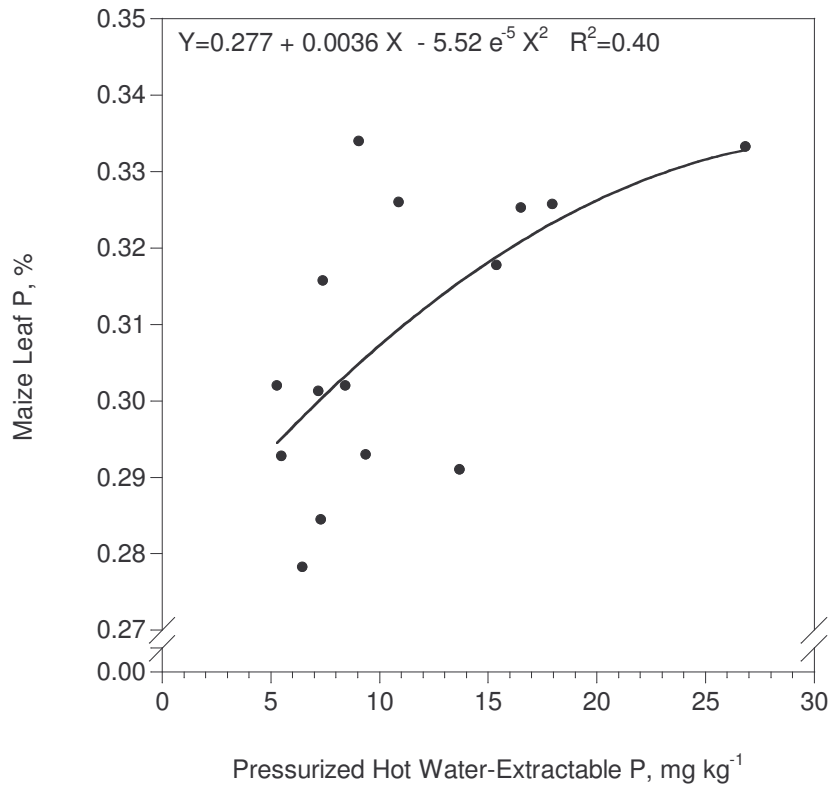
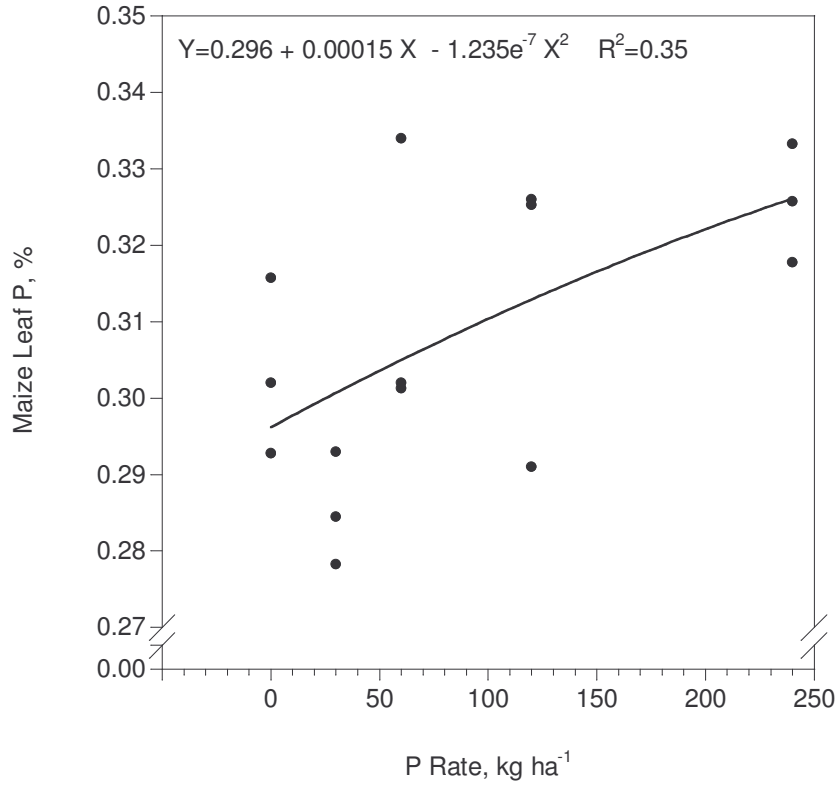


Figure 10. Relationship between leaf P concentration of maize at tassel and P extracted by PHW or between leaf P concentration and P rates applied for the field experiment.

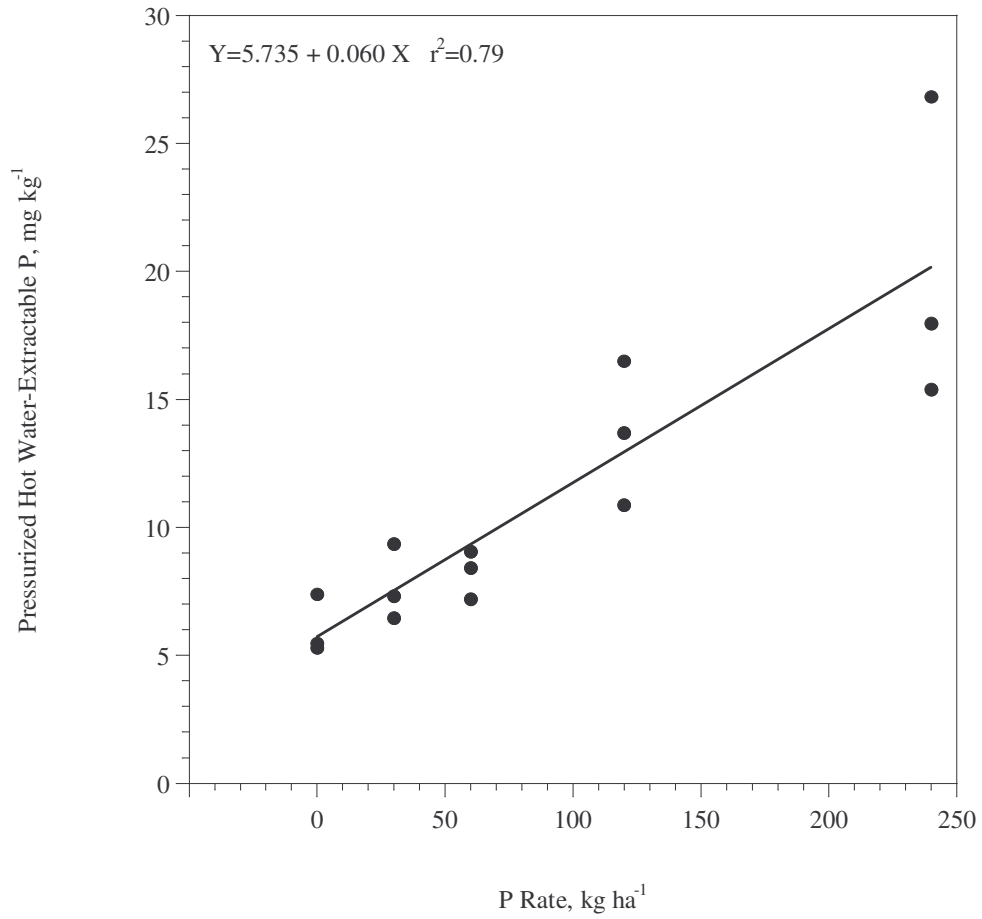


Figure 11. Relationship between PHW-extractable P and rates of P applied in the field experiment. Values used to establish this relationship are the average of each treatment.

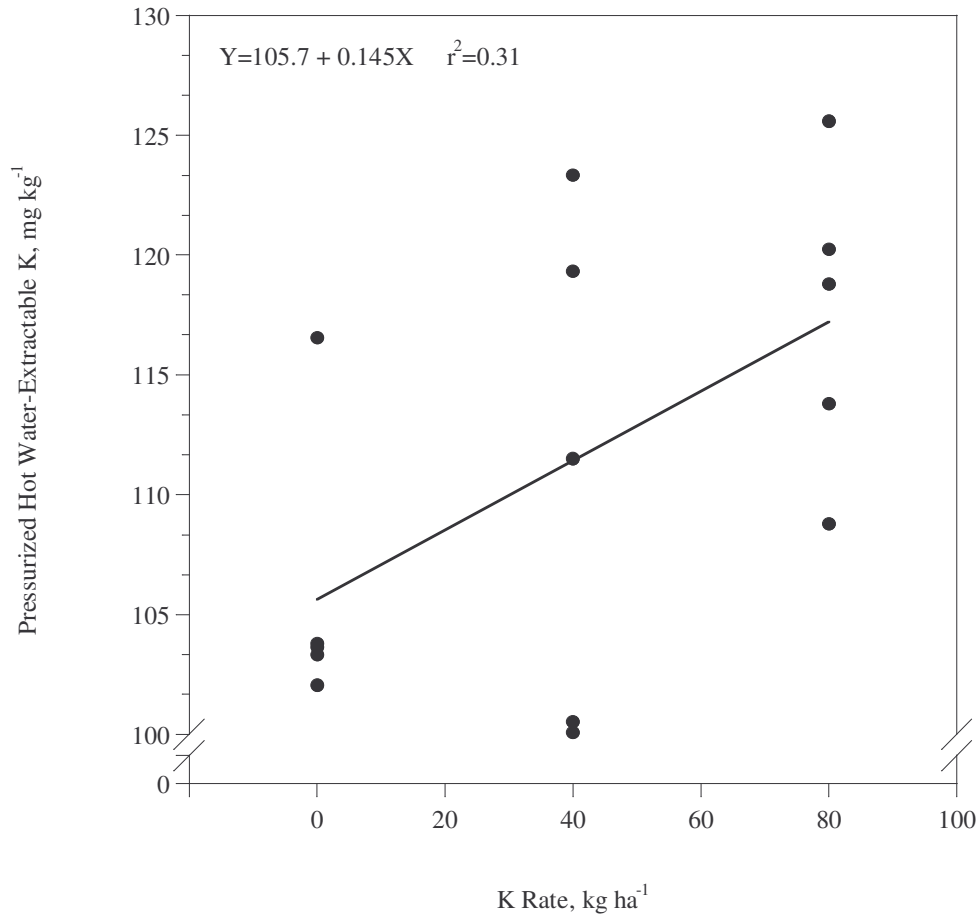


Figure 12. Relationship between PHW-extractable K and rates of applied K in the field experiment. Values used to establish this relationship are the average of each treatment.

APPENDIX I

Extraction and Analytical Methods for Soil and Plant Analysis

SOIL ANALYSIS

Pressurized Hot Water Method—NO₃-N, P and, K (Crane, 2004)

Apparatus

- Espresso Machine (Melitta® MEX1B)
- 5 cm medium filter paper
- Plastic cup

Reagents

Nitrate Nitrogen: Chromotropic Acid (CTA) (Sims and Jackson, 1971).

- 0.1% solution of chromotropic acid disodium salt (CTA) (4,5-dihydroxy-2,7-Naphthalenedisulfonic acid): Dissolve 0.184 g of CTA in a 100 ml volumetric flask by adding 100 ml of reagent grade H₂SO₄. (Do not heat the solution to dissolve.)
- Sulfite-urea solution: Dissolve five g analytical grade urea and five grams of reagent grade anhydrous sodium sulfite in distilled water and dilute to 100 ml.
- Antimony sulfate solution: Dissolve 0.5 g of antimony (Sb) metal in 80 ml of concentrated H₂SO₄ at room temperature. Heat the solution until it is clear to dissolve the antimony metal. After the antimony is dissolved, let the solution cool and then bring it to volume with 20.0 ml of distilled water (in a volumetric).
- NO₃-N standard solutions: To develop the standard curve, use 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 µg ml⁻¹ NO₃-N in 100 ml distilled water standard solutions and complete the same procedure as found in the chromotropic acid

analysis. Plot the log transmittance of each standard sample on the y-axis against the concentration on the x-axis.

Phosphorus: Molybdic Acid (Murphy and Riley 1962)

- Reagent A: Dissolve 12.0 g of ammonium molybdate in 250 ml of distilled water and in a separate volumetric flask, dissolve 0.2908 g of antimony potassium tartrate in 100 ml of distilled water. In a third flask add 148 ml of concentrated H₂SO₄ to distilled water and bring to volume of one liter to make 5 N H₂SO₄. Mix these three solutions together in a 2000 ml volumetric flask and bring the solution to a volume of 2000 ml with distilled water. Store in the dark at about 25° C.
- Reagent B: Dissolve 0.528 grams of ascorbic acid in 100 ml of Reagent A. Reagent B should be made fresh every day.

Pressurized Hot Water Extraction

Warm up the machine by running one to two runs of distilled water only. Weigh 5.0 g of air-dry (2 mm) soil into a weigh boat. Place filter paper into the filter basket of the espresso machine, place 5.0 g of soil into the basket, and lock it into the machine. Add 100.0 ml of distilled water into the water reservoir at the top of the machine and tighten the lid. Label the 70 ml plastic cup (SOLO no. P71), place it under the filter basket and turn the machine on. Collect all of the water dripping from the basket and then release any steam from the machine. Throw the soil sample away, rinse the basket with distilled water and then repeat with the next sample.

Preparation of PHW extract for Nitrate Analysis

After obtaining a soil extract as above, perform the tests for potassium and phosphorus. These tests do not require clarification of the extract and should be done before nitrate analysis. To perform the nitrate procedure with pressurized hot water, add 0.15 g Ca(OH)_2 to the remaining extract and swirl gently in the plastic cup until the mixture is cloudy. Filter the extract/ Ca(OH)_2 mixture through medium filter paper set on the opening of a 125 ml wide mouth high density polyethylene Nalgene bottle. Collect at least 6 ml of filtrate and discard the filter paper. The filtrate should be clear with an opalescent sheen on the water. If the sample is still colored, repeat the filtering process. After the filtrate is clear, follow the procedure for nitrate-nitrogen. The rest of the filtrate can then be discarded.

Nitrate Nitrogen Analysis Curve: Chromotropic Acid (CTA)

The pressurized hot water-method utilizes the pressurized hot water extraction and the chromotropic acid colorimetric analysis. Nitrate ($\text{NO}_3\text{-N}$) working standards are made from 1000 $\mu\text{g ml}^{-1}$ stock solution of $\text{NO}_3\text{-N}$. Working standards of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 $\mu\text{g ml}^{-1}$ $\text{NO}_3\text{-N}$ in 100 ml distilled water are made for the chromotropic acid procedure.

To make these working standards place a 2.5 ml aliquot of filtrate into 50 ml Erlenmeyer flask, along with one drop of sulfite-urea solution and 2.0 ml of antimony-sulfate solution. Mix the reagents by swirling the flask for a couple of seconds and then add 1.0 ml of CTA solution and 4.5 ml of concentrated H_2SO_4 to the mixture. Allow the

flask and its contents to cool in a water bath and then read the transmittance at 410 nm on the spectrophotometer.

Phosphorus Analysis: Molybdc Acid (Wantanabe, 1965)

The pressurized hot-water method uses the pressurized hot-water extraction and an ascorbic acid colorimetric analysis procedure. Phosphate working standards are made from 1000 $\mu\text{g ml}^{-1}$ stock solution of $(\text{PO}_4)^{3-}$. Working standards of 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 5.0, 6.0, and 7.0 $\mu\text{g ml}^{-1}$ $(\text{PO}_4)^{3-}$ in 100 ml of distilled water were made for the molybdc acid procedure.

In a 95 ml plastic test tube, combine a 5.0 ml aliquot of the soil extract with 10.0 ml of distilled water. Add 5.0 ml of Reagent B to the diluted extract and swirl the test tube vigorously to allow for CO_2 evolution. Allow the solution to stand for 15 minutes and then read the transmittance on the spectrophotometer at 880 nm.

Potassium Analysis: Atomic Absorption Spectroscopy (AA)

In a 95 ml plastic test tube, combine 1.0 ml of the soil extract and 15.0 ml of distilled water (1:16 dilution). Read the absorbance of the dilute solution on the A.A. using 0.0, 2.0, 5.0, 10.0 $\mu\text{g ml}^{-1}$ solutions of K in ammonium acetate to calibrate the machine.

Chromotropic Acid Method—NO₃-N (Haby, 1989)

Reagents

- 0.1% solution of chromotropic acid disodium salt (CTA) (4,5-dihydroxy-2,7-Naphthalenedisulfonic acid): Dissolve 0.184 g of CTA in 100 ml reagent grade H₂SO₄. (Do not heat to dissolve)
- Sulfite-urea solution: Dissolve five grams analytical grade urea and five grams of reagent grade anhydrous sodium sulfite in distilled water and dilute to 100 ml.
- Antimony sulfate solution: Dissolve 0.5 grams of antimony (Sb) metal in 80 ml of concentrated H₂SO₄ at room temperature. Heat the solution until it is clear to dissolve the antimony metal. After the antimony is dissolved, let the solution cool and then bring it to volume with 20 ml of distilled water.
- NO₃-N standard solutions: To develop the standard curve, use 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 µg ml⁻¹ NO₃-N in 100 ml distilled water standard solutions and complete the same procedure as found in the chromotropic acid analysis. Plot the log transmittance of each standard sample on the y-axis against the concentration on the x-axis.

Nitrate Water Extraction

Weigh 10.0 g of soil into a 250 ml Erlenmeyer flask and add 0.25 g Ca(OH)₂ using a 0.4 cm³ scoop. Then add 50.0 ml of distilled water and shake the solution for 15 minutes on a reciprocating shaker at 180 oscillations per minute. Filter the soil solution through a 15 cm medium filter paper and collect the extract in a 70 ml plastic cup.

Nitrate Analysis Procedure

In a 50 ml Erlenmeyer flask, place a 2.5 ml aliquot of filtrate, one drop of sulfite-urea solution and 2.0 ml of antimony-sulfate solution. Mix the reagents by swirling the flask and then add 1.0 ml of CTA solution and 4.5 ml of concentrated H_2SO_4 to the mixture. Allow the flask and its contents to cool in a water bath and then read the transmittance at 410 nm on the spectrophotometer. Treat the standards just like the soil samples, but without soils.

Phosphorus and Potassium: Sodium Bicarbonate (Wantanabe, 1965)

Reagents

- 0.5 M sodium bicarbonate extracting solution: In a 100 ml volumetric flask, dissolve 42 g of NaHCO_3 in distilled water, bring to volume and let the solution stand overnight to equilibrate. The solution should be stored in a plastic container and the pH adjusted to 8.2 before each use with 6.0 N NaOH and 6 N HCl.
- Reagent A: Dissolve 12.0 g of ammonium molybdate in 250 ml of distilled water and in a separate volumetric flask, dissolve 0.2908 g of antimony potassium tartrate in 100 ml of distilled water. In a third flask add 148 ml of concentrated H_2SO_4 to distilled water and bring to volume at one liter to make 5 N H_2SO_4 . Mix these three solutions together in a 2000 ml volumetric flask and bring the solution to a volume of 2000 ml with distilled water. Store in the dark at about 25° C.
- Reagent B: Dissolve 0.528 grams of ascorbic acid in 100 ml of Reagent A.
Reagent B should be made fresh every day.
- Phosphorus standard solutions: To make curve, use 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 5.0, 6.0, and 7.0 $\mu\text{g ml}^{-1}$ P in 100 ml bicarbonate standard solutions and complete the same procedure as found in the ascorbic acid analysis. To find the equation for the standard curve, plot the log transmittance of each standard sample on the y-axis against the concentration on the x-axis.

Phosphorus and Potassium Extraction

Weigh 5.0 g of soil into a 250.0 ml Erlenmeyer flask, add 50 ml of sodium bicarbonate (pH 8.2). Shake the solution for 30 minutes on a reciprocating shaker at 180 oscillations per minute and filter the soil solution into 15 cm medium fast filter paper. Collect the extract in a 70 ml plastic cup.

Potassium Analysis Procedure

In a 95 ml plastic test tube, combine 1.0 ml of the soil extract and 15 ml of distilled water (1:16 dilution). Read the absorbance of the dilute solution on the A.A. using 0.0, 2.0, 5.0, 10.0 $\mu\text{g ml}^{-1}$ solutions of K with the same amount of NaHCO_3 as the dilute sample to calibrate the instrument.

Phosphorus Analysis Procedure

In a 95 ml plastic test tube, combine a 5.0 ml aliquot of the soil extract with 10.0 ml of distilled water. Add 5.0 ml of Reagent B to the diluted extract and swirl the test tube vigorously to mix. Allow the solution to stand for 15 minutes and then read the transmittance on the spectrophotometer at 880 nm.

Phosphorus and Potassium: Double-Acid Procedure (Mehlich, 1953)

Reagents

- Extracting reagent: In a 1,000 ml volumetric flask, add 4.0 ml concentrated HCl and 0.7 ml of concentrated H₂SO₄. Bring to volume with distilled water.
- Reagent A: Dissolve 12.0 g of ammonium molybdate in 250 ml of distilled water and in a separate volumetric flask, dissolve 0.2908 g of antimony potassium tartrate in 100 ml of distilled water. In a third flask add 148 ml of concentrated H₂SO₄ to distilled water and bring to volume at one liter to make 5 N H₂SO₄. Mix these three solutions together in a 2000 ml volumetric flask and bring the solution to a volume of 2000 ml with distilled water. Store in the dark at about 25° Celsius.
- Reagent B: Dissolve 0.528 grams of ascorbic acid in 100 ml of Reagent A. Reagent B should be made fresh every day.
- Phosphorus standard solutions: To make curve, use 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 5.0, 6.0, and 7.0 µg ml⁻¹ P in 100 ml of extracting solutions and complete the same procedure as found in the ascorbic acid analysis. To find the equation for the standard curve, plot the log transmittance of each standard sample on the y-axis against the concentration on the x-axis.

Phosphorus and Potassium Extraction

Weigh 5.0 g of soil and place in a 250 ml Erlenmeyer flask. Add 25.0 ml of the extracting agent and shake the mixture for five minutes on a reciprocating shaker at 180

oscillations per minute. Filter the soil solution through a 15 cm medium filter paper into a 70 ml plastic cup.

Potassium Analysis Procedure

In a 95 ml plastic test tube, combine 1.0 ml of the soil extract and 15.0 ml of distilled water (1:16 dilution). Read the absorbance of the dilute solution on the A.A. using 0.0, 2.0, 5.0, 10.0 $\mu\text{g ml}^{-1}$ solutions of K with the same amount of Mehlich I extractant as the diluted sample to calibrate the instrument.

Phosphorus Analysis Procedure

In a 95 ml plastic test tube, combine a 5.0 ml aliquot of the soil extract with 10.0 ml of distilled water. Add 5.0 ml of Reagent B to the diluted extract and swirl the test tube vigorously to mix. Allow the solution to stand for 15 minutes and then read the transmittance on the spectrophotometer at 880 nm.

Phosphorus and Potassium: Mehlich III (Mehlich, 1984)

Reagents

- Extracting reagent: Add 11.49 ml of concentrated CH_3COOH (17.4 M), 20.0 g of NH_4NO_3 , 0.56 g NH_4F , 0.84 ml of concentrated HNO_3 (15.5 M), and 0.29 g EDTA to about 800 ml of deionized water in a 1000 ml volumetric flask mix and dilute to 1000 ml with deionized water.
- Reagent A: Dissolve 12.0 g of ammonium molybdate in 250 ml of distilled water and in a separate volumetric flask, dissolve 0.2908 g of antimony potassium tartrate in 100 ml of distilled water. In a third flask add 148 ml of concentrated H_2SO_4 to distilled water and bring to volume at one liter to make 5 N H_2SO_4 . Mix these three solutions together in a 2000 ml volumetric flask and bring the solution to a volume of 2000 ml with distilled water. Store in the dark at about 25° C.
- Reagent B: Dissolve 0.528 g of ascorbic acid in 100 ml of Reagent A. Reagent B should be made fresh every day.
- Phosphorus standard solutions: To make curve, use 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 5.0, 6.0, and 7.0 $\mu\text{g ml}^{-1}$ P in 100 ml of extracting solutions and complete the same procedure as found in the ascorbic acid analysis. To find the equation for the standard curve, plot the log transmittance of each standard sample on the y-axis against the concentration on the x-axis.

Phosphorus and Potassium Extraction

Weigh 2.5 g of soil and place it in a 250 ml Erlenmeyer flask. Add 25.0 ml of the extracting agent and shake the mixture for five minutes on a reciprocating shaker at 180 oscillations per minute. Filter the soil solution in 15 cm filter paper and collect the extract in a 70 ml plastic cup.

Potassium Analysis Procedure

In a 95 ml plastic test tube, combine 1.0 ml of the soil extract and 15.0 ml of distilled water (1:16 dilution). Read the absorbance of the dilute solution on the A.A. using 0.0, 2.0, 5.0, 10.0 $\mu\text{g ml}^{-1}$ solutions of K (same as other standards above).

Phosphorus Analysis Procedure

In a 95 ml plastic test tube, combine a 1.0 ml aliquot of the soil extract with 15.0 ml of distilled water (1:16 dilution). Add 5.0 ml of Reagent B to the diluted extract and swirl the test tube vigorously to mix. Allow the solution to stand for 15 minutes and then read the transmittance on the spectrophotometer at 880 nm.

Phosphorus: Bray P1 Extraction (Bray, 1945)

Reagents

- 1.0 N NH_4F : In a 1,000 ml volumetric flask, dissolve 37.0 g of ammonium fluoride in distilled water then fill the flask to volume. The solution should be stored in a polyethylene container to avoid prolonged solution contact with glass.
- 0.5 N HCl: In a 500 ml volumetric flask, dilute 20.4 ml of 12.0 N HCl to volume with distilled water.
- Extracting Solution: In a 1000 ml volumetric flask, mix 30 ml of 1.0 N NH_4F and 50 ml of 0.5 N HCl. Dilute the mixture to volume using distilled water and store it in a polyethylene bottle.
- Reagent A: Dissolve 12.0 g of ammonium molybdate in 250 ml of distilled water and in a separate volumetric flask, dissolve 0.2908 g of antimony potassium tartrate in 100 ml of distilled water. In a third flask add 148 ml of concentrated H_2SO_4 to distilled water and bring to volume at one liter to make 5 N H_2SO_4 . Mix these three solutions together in a 2000 ml volumetric flask and bring the solution to a volume of 2000 ml with distilled water. Store the Reagent A in a dark cupboard at about 25° C.
- Reagent B: Dissolve 0.528 g of ascorbic acid in 100 ml of Reagent A. Reagent B should be made fresh every day.
- Phosphorus standard solutions: To develop the standard curve, use 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 5.0, 6.0, and 7.0 $\mu\text{g ml}^{-1}$ P in 100 ml of extracting solutions and complete the same procedure as found in the sample analysis. To find the equation for the standard

curve, plot the log transmittance of each standard sample on the y-axis against the concentration on the x-axis.

Phosphorus Extraction

Weigh 2.5 g of soil and place it in a 250 ml Erlenmeyer flask. Add 25.0 ml of the extracting agent and shake the mixture for five minutes on a reciprocating shaker at 180 oscillations per minute. Filter the soil solution in 15 cm medium fast filter paper and collect the extract in a 70 ml plastic cup.

Phosphorus Analysis Procedure

In a 95 ml plastic test tube, combine a 5.0 ml aliquot of the soil extract with 10.0 ml of distilled water. Add 5.0 ml of Reagent B to the diluted extract and swirl the test tube vigorously to mix. Allow the solution to stand for 15 minutes and then read the transmittance on the spectrophotometer at 880 nm.

Potassium: Ammonium Acetate (Helmke and Sparks, 1996)

Reagents

- Ammonium acetate: Add 114.0 ml glacial acetic acid to a 2,000 ml volumetric flask and bring to about 1,000 ml with distilled water. Add 138.0 ml concentrated ammonium hydroxide and bring to about 1950 ml. Adjust the pH to 7.0 with acetic acid or ammonium acetate and bring to volume of 2,000 ml with distilled water.
- K working standards: Make working standards of 0.0, 2.0, 5.0 and 10.0 ppm K in 100.0 ml volumetric flasks with 6.25 ml ammonium acetate and filled to volume with distilled water.

Potassium Extraction

Weigh 5.0 grams of soil and place it in a 250 ml Erlenmeyer flask. Add 25.0 ml ammonium acetate (pH 7.0) and shake for 15 minutes on a reciprocating shaker at 180 oscillations per minute. Filter the soil solution into 15 cm medium fast filter paper. Collect the extract in a 70 ml plastic cup.

Potassium Analysis Procedure

In a 95 ml plastic test tube, combine 1.0 ml of the soil extract and 15.0 ml of distilled water (1:16 dilution). Read the absorbance of the dilute solution on the A.A. using the above working standards to calibrate the instrument.

Soil Texture (Day, 1965)

The hydrometer method of determining soil texture was carried out according to the procedure cited by Day in Methods of Soil Analysis Part 1 published in 1965.

Cation Exchange Capacity (Chapman, 1965)

Analysis for cation exchange capacity was completed in accordance with the procedure cited by Chapman in Methods of Soil Analysis Part 2 published in 1965.

Organic Matter (Walkley and Black, 1934)

The Walkley-Black dichromate oxidation was used in the determination of soil organic matter.

pH: Saturated Paste (Rhodes, 1982)

The pH was determined by the saturated paste method described by Rhodes, 1982.

Electrical Conductivity (Rhoades, 1996)

Analysis for electrical conductivity for the determination of soil salinity was completed in accordance with the procedure cited by Rhoades in Methods of Soil Analysis Part 3 published in 1996.

Calcium Carbonate Neutralization Potential (Allison and Moode, 1965)

Analysis for the acid neutralization potential for the determination of soil carbonates was completed in accordance with the procedure cited by Allison and Moode in Methods of Soil Analysis Part 2 published in 1965.

Exchangeable Aluminum (Bertsch and Thomas, 1996)

Analysis for the determination of exchangeable aluminum was completed in accordance with the procedure cited by Bertsch and Thomas in Methods of Soil Analysis Part 3 published in 1996.

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