



GOCE DELCHEV
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VALIDATION OF A PHOSPHOVANADOMOLYBDATE UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF PLANT-AVAILABLE PHOSPHORUS IN AGRICULTURAL SOILS AND EVALUATION OF DISSOLVED SILICON INTERFERENCE

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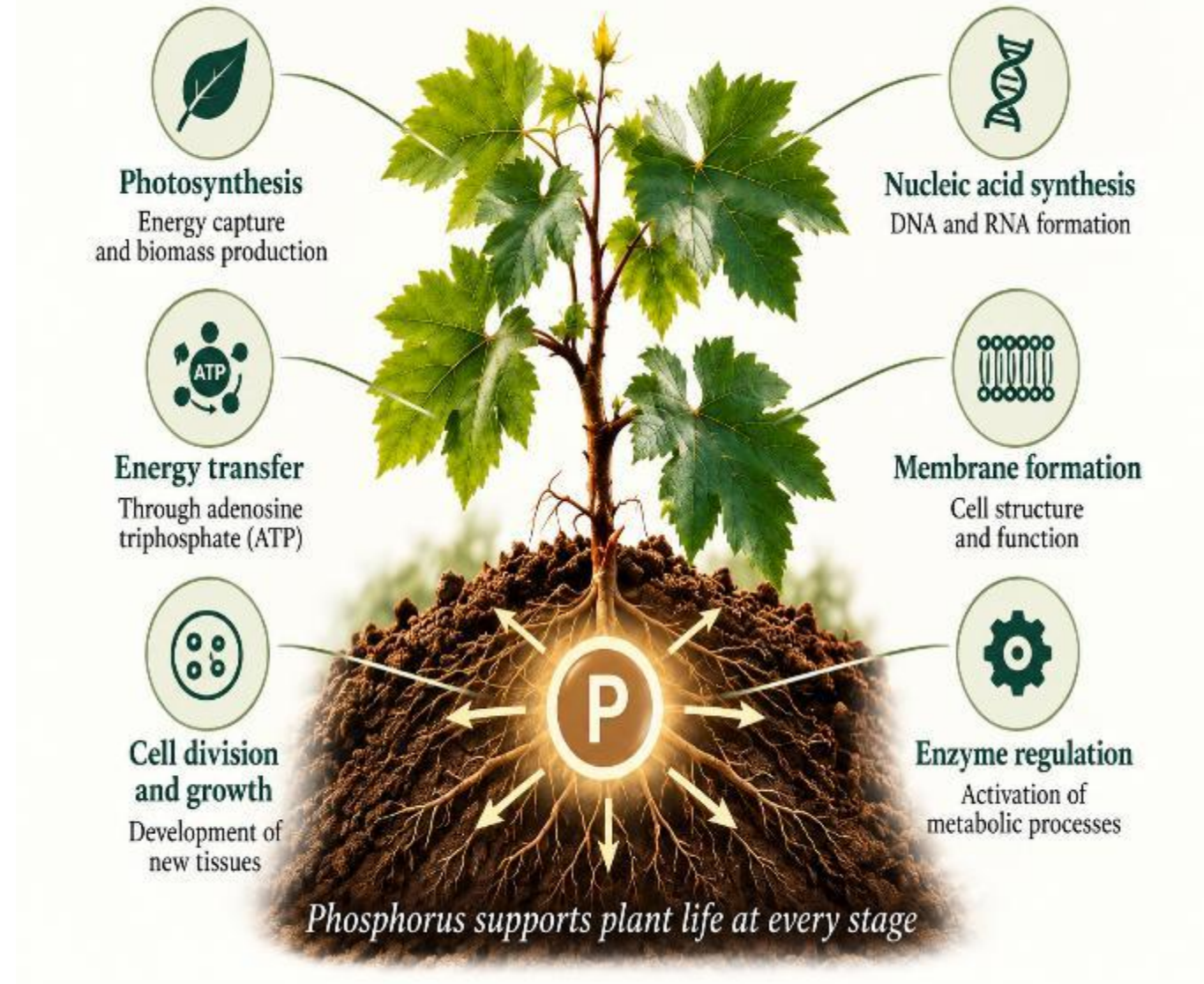


INTRODUCTION

PHOSPHORUS IN PLANTS AND AGRICULTURE

An essential element for growth, productivity, and sustainable agricultural systems

- Phosphorus (P) is one of the essential macronutrients required for plant growth and development and plays a fundamental role in agricultural productivity, food security and ecosystem sustainability.
- It participates in numerous physiological and biochemical processes, including photosynthesis, respiration, energy transfer through adenosine triphosphate (ATP), nucleic acid synthesis, membrane formation, cell division and enzyme regulation.
- Furthermore, phosphorus is an indispensable structural component of DNA, RNA, phospholipids and phosphorylated proteins, making it essential for cellular metabolism, plant growth and reproductive development.



INTRODUCTION

Behavior of phosphorus in different soils

➤ Acidic soils (low pH)



Fe/Al oxides

Phosphorus is predominantly retained by adsorption onto iron and aluminum oxides, reducing its bioavailability.

Fe/Al oxides

➤ Neutral and calcareous soils (higher pH, CaCO_3)

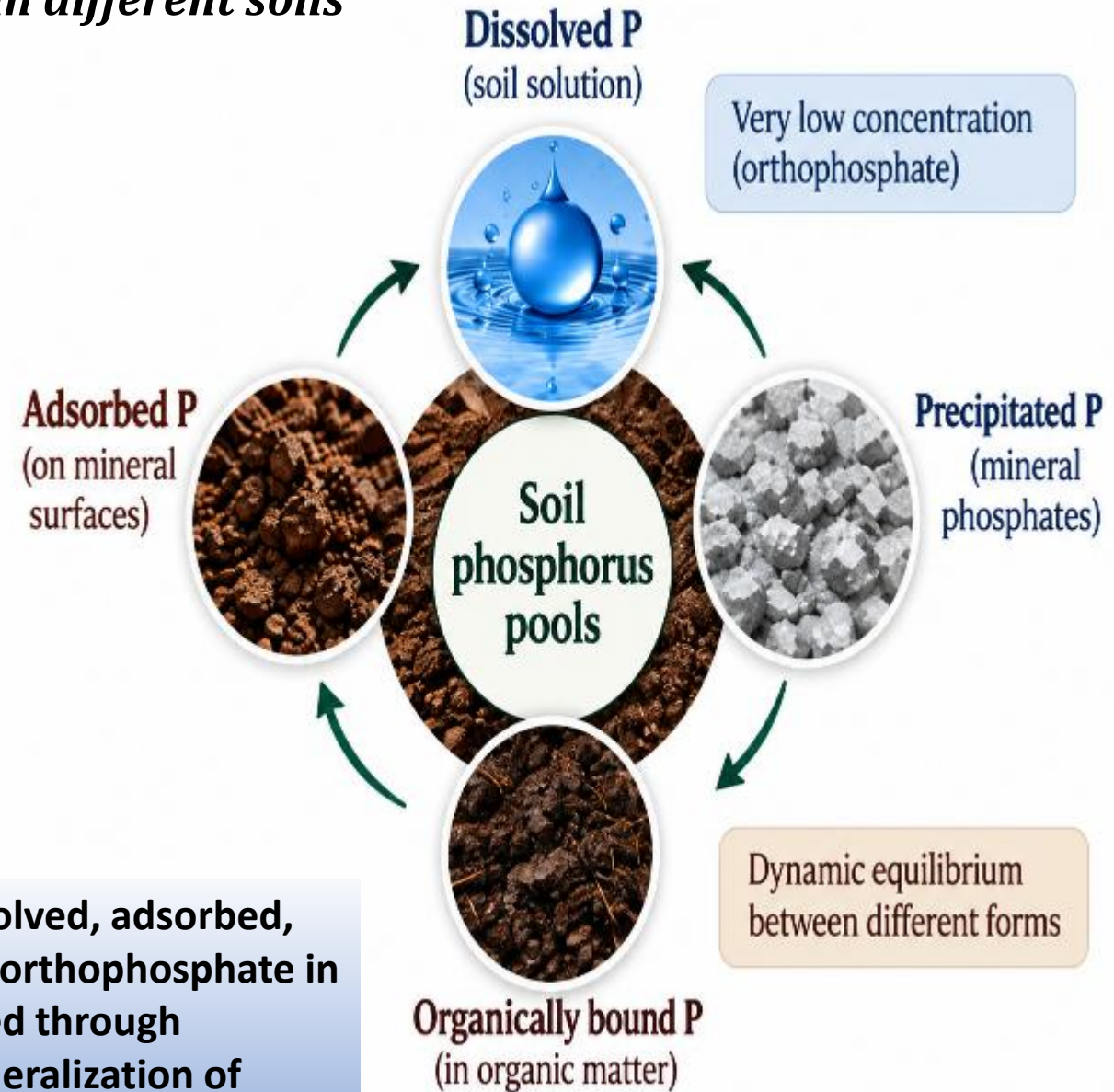


Ca phosphates

Phosphorus is mainly immobilized through precipitation with calcium phosphates, substantially reducing its bioavailability.

Ca phosphates

In soils, phosphorus exists in a dynamic equilibrium among dissolved, adsorbed, precipitated and organically bound forms. The concentration of orthophosphate in the soil solution is extremely low and is continuously replenished through desorption, dissolution of P-bearing minerals and biological mineralization of organic matter.



INTRODUCTION

Determination of Plant-Available Phosphorus



➤ Why determine plant-available phosphorus?

- Reliable determination of plant-available phosphorus is essential for soil fertility assessment, fertilizer recommendation and sustainable nutrient management.
- Insufficient phosphorus supply restricts root development, delays crop maturation and significantly reduces crop yield
- Therefore, accurate determination of plant-available phosphorus is important not only in agriculture but also in environmental monitoring and ecosystem protection.

Analytical Techniques for Phosphorus Determination

UV-Visible Spectrophotometry



Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)



Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

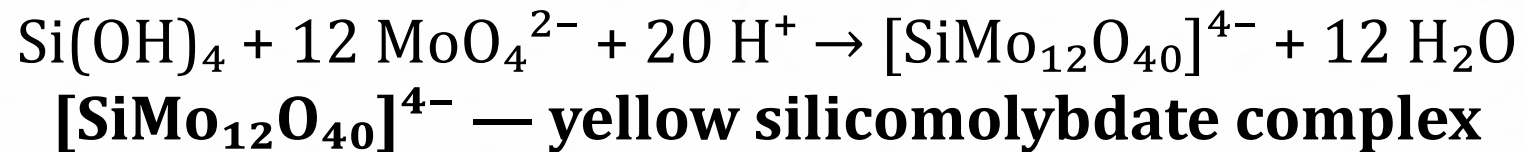


Ion Chromatography (IC)



AIM OF THE STUDY

- To validate the phosphovanadomolybdate UV–Vis spectrophotometric method for determining plant-available phosphorus in ammonium lactate (AL) soil extracts.
- To evaluate the potential interference of dissolved silicon **in AL soil extracts**, as silicon can also react with molybdate under acidic conditions to form a yellow silicomolybdate complex, potentially affecting phosphorus determination.



MATERIALS AND METHODS

Phosphovanadomolybdate (Molybdenum Yellow) Method

- Orthophosphate reacts with ammonium molybdate and ammonium metavanadate in acidic medium, forming a stable **yellow phosphovanadomolybdate complex** suitable for visible spectrophotometric determination.
- Stock standard: **100 mg P L⁻¹ (KH₂PO₄)**
- Working standards: **1, 2, 5, 10 and 20 mg P L⁻¹**

Phosphovanadomolybdate reagent

- 25.0 g (NH₄)₆Mo₇O₂₄·4H₂O → ~500 mL H₂O
- 1.25 g NH₄VO₃ → ~300 mL hot H₂O
- Add 125 mL concentrated HNO₃
- Mix solutions and dilute to 1000 mL

1. AL extraction

Weigh 5.00 g of soil



Add 100 mL AL extraction solution



Shake for 3 h at room temperature (rotary shaker)

Filter through P-free quantitative filter paper



AL soil extract

2. Colour development

10.0 mL soil extract (or P calibration standard) + 10.0 mL phosphovanadomolybdate reagent

Dilute to 50 mL with deionized water

Mix and allow to stand for 10 min



0 1 2 5 10 20
P concentration (mg L⁻¹)

Measure absorbance at 420 nm

MATERIALS AND METHODS

Investigation of Silicon Interference in Phosphorus Determination

To evaluate the influence of dissolved silicon on analytical performance, three matrix-matched calibration series were prepared.

- 1. Series 1:** Phosphorus standards prepared only in the AL extraction solution (without added silicon).
- 2. Series 2:** Phosphorus standards with the addition of 2.0 mL of 100 mg Si L⁻¹ standard solution.
- 3. Series 3:** Phosphorus standards with the addition of 5.0 mL of 100 mg Si L⁻¹ standard solution.

Calibration curves were constructed by plotting absorbance against phosphorus concentration.

The corresponding regression parameters were compared to evaluate the effect of dissolved silicon on analytical sensitivity.



MATERIALS AND METHODS

Method Validation and Statistical Analysis

1. Linearity and Working Range

- Matrix-matched calibration: 1–20 mg P L⁻¹
- Linear regression: slope, intercept and R²

2. Selectivity and Matrix Effect

- Calibration curves with and without dissolved silicon.
- Evaluation of analytical sensitivity.

3. Accuracy

- Standard addition using BIPEA reference soil and agricultural soils.
- Recovery (%).

4. Precision

- Repeatability: 10 determinations (intra-day)
- Intermediate precision: 3 days × 3 replicates
- Expressed as RSD (%)

5. Trueness

- BIPEA certified reference soil material
- Comparison with assigned value and z-score:

6. Detection and Quantification Limits

- Based on calibration slope (S) and response SD (σ)
- LOD = $3.3\sigma/S$
- LOQ = $10\sigma/S$



RESULTS AND DISCUSSION

Linearity of the Proposed Method

Table 1. Calibration data for phosphorus determination by the phosphovanadomolybdate UV-Vis spectrophotometric method (n = 3).

Phosphorus concentration mg P L ⁻¹	Absorbance nm (Mean ± SD)
1	0.011 ± 0.001
2	0.022 ± 0.001
5	0.053 ± 0.002
10	0.106 ± 0.001
20	0.222 ± 0.003

Table 2. Analytical characteristics of the calibration curve.

Parameter	Value
Working range mgPL ⁻¹	1-20
Regression equation	y = 0.0111x – 0.0014
Slope	0.0111
Intercept	- 0.0014
Coefficient of determination	0.9994
LOD (mgPL ⁻¹)	0.30
LOQ (mgPL ⁻¹)	0.90

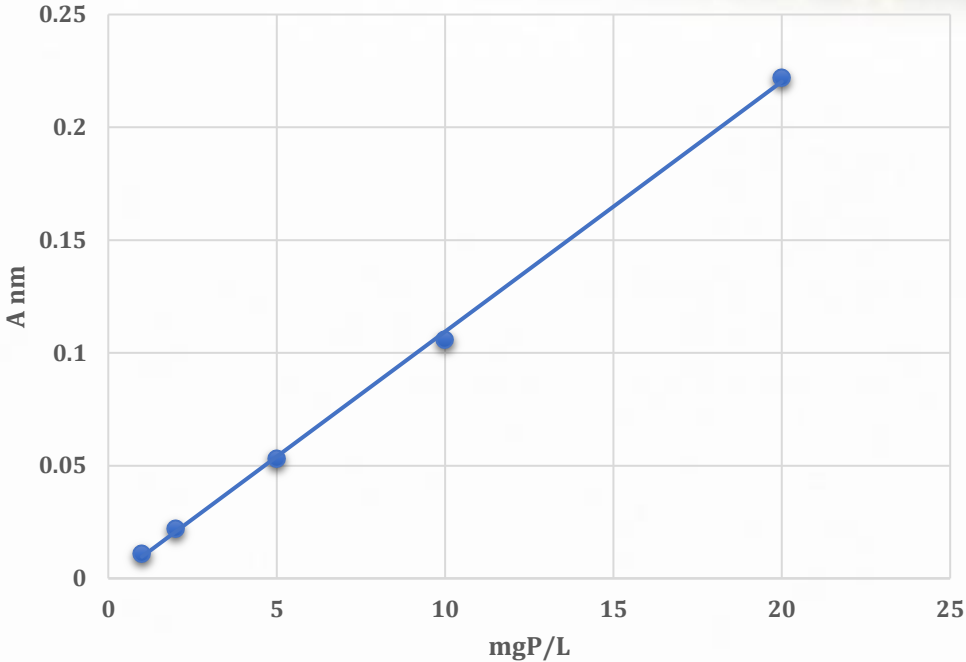


Figure 1. Calibration curve for phosphorus determination by the phosphovanadomolybdate UV-Vis spectrophotometric method.

RESULTS AND DISCUSSION

Effect of Dissolved Silicon on Calibration Characteristics

Table 3. Calibration data obtained in the absence and presence of dissolved silicon.

P (mgL ⁻¹)	A (nm), without Si	A (nm), +4mgSiL ⁻¹	A (nm), +10mgSiL ⁻¹
1	0.011	0.012	0.013
2	0.022	0.024	0.022
5	0.053	0.055	0.057
10	0.106	0.110	0.113
20	0.222	0.226	0.224

Table 4. Regression parameters of the calibration curves obtained in the absence and presence of dissolved silicon.

Silicon concentration	Slope	Change of slope, (%)	R ²
0mgSiL ⁻¹	0.0111	-	0.9994
4mgSiL ⁻¹	0.0112	+0.90	0.9996
10mgSiL ⁻¹	0.0112	+0.90	0.9999

The calibration slope increased from 0.0111 to 0.0112, corresponding to an increase in analytical sensitivity of approximately 0.9%. Considering the excellent linearity of all calibration curves and the minimal variation in slope, this difference can be regarded as analytically insignificant and indicates the absence of a measurable matrix effect caused by dissolved silicon.

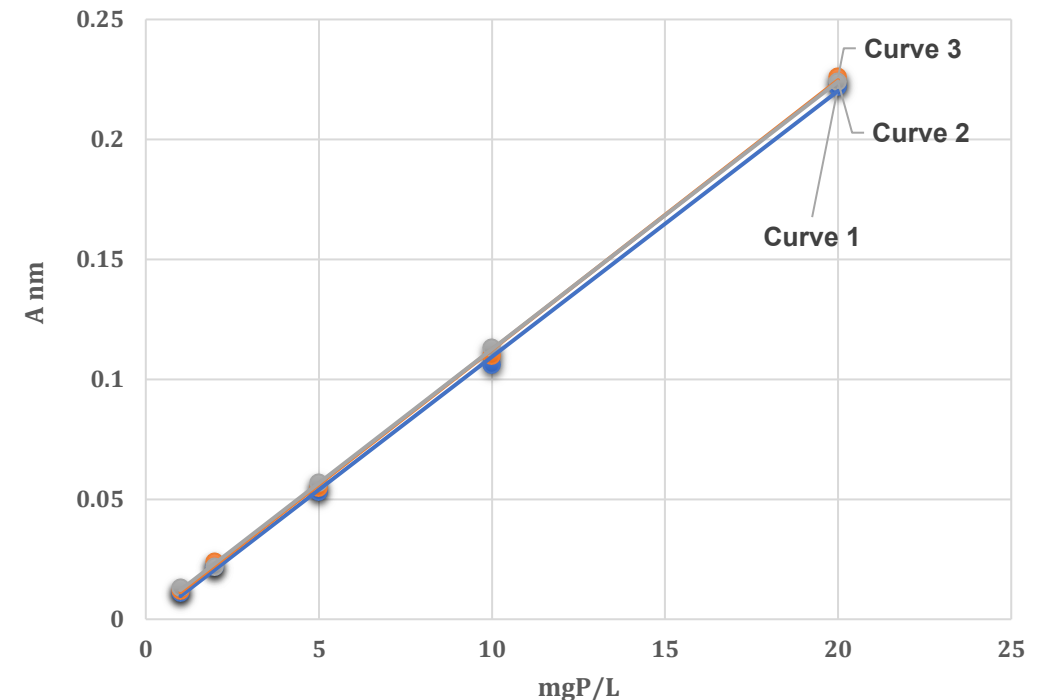


Figure 2. Matrix-matched calibration curves prepared with different silicon concentrations.

RESULTS AND DISCUSSION

Trueness of the Proposed Method

Evaluation Using BIPEA Reference Soil Material

- Trueness assessed using BIPEA certified reference soil material.
- 10 independent determinations ($n = 10$).
- Phosphorus quantified using external calibration and expressed as $\text{mg P}_2\text{O}_5 \text{ } 100 \text{ g}^{-1}$ soil.

Table 5. Trueness assessment using the BIPEA certified reference soil material ($n = 10$).

Parameter	Value
Certified value ($\text{mgP}_2\text{O}_5 100 \text{ g}^{-1}$)	12.97
Certified acceptance range ($\text{mgP}_2\text{O}_5 100 \text{ g}^{-1}$)	9.17 – 16.77
Determined value $\pm$ SD ($\text{mgP}_2\text{O}_5 100 \text{ g}^{-1}$).	16.36 ± 0.36
Relative standard deviation (RSD %)	2.18
z – score	1.78
Evaluation	Satisfactory

- The calculated z-score was 1.79, which satisfies the commonly accepted criterion of $|z| \leq 2$ for satisfactory performance.

RESULTS AND DISCUSSION

Accuracy of the Proposed Method

- Evaluated using the standard addition method.
- Two matrices: BIPEA certified reference soil and agricultural soil.
- Phosphorus additions: 5 and 10 mg P₂O₅ 100 g⁻¹.
- Triplicate determinations (n = 3).
- Accuracy expressed as phosphorus recovery (%).

Table 6. Accuracy assessment of the BIPEA certified reference soil material using the standard addition method (n = 3).

Sample	Original Value, (mg P ₂ O ₅ 100 g ⁻¹)	Added, (mg P ₂ O ₅ 100 g ⁻¹)	Found, (mg P ₂ O ₅ 100 g ⁻¹)	Recovery, (%)
BIPEA (mgP ₂ O ₅ 100 g ⁻¹)	16.36 ± 0.36	-	-	-
BIPEA +5 (mgP ₂ O ₅ 100 g ⁻¹)	16.36	5	20.99 ± 0.48	92.6
BIPEA +10 (mgP ₂ O ₅ 100 g ⁻¹)	16.36	10	26.57 ± 0.41	100.1

Table 7. Accuracy assessment of the agricultural soil sample using the standard addition method (n = 3).

Sample	Original Value, (mgP ₂ O ₅ 100 g ⁻¹)	Added, (mgP ₂ O ₅ 100 g ⁻¹)	Found, (mgP ₂ O ₅ 100 g ⁻¹)	Recovery, (%)
Soil sample (mgP ₂ O ₅ 100 g ⁻¹)	38.35 ± 0.24	-	-	-
Soil sample +5 (mgP ₂ O ₅ 100 g ⁻¹)	38.35	5	42.76 ± 0.41	95.6
Soil sample +10 (mgP ₂ O ₅ 100 g ⁻¹)	38.35	10	46.75 ± 0.24	96.3



RESULTS AND DISCUSSION

Precision of the Proposed Method

Repeatability (Intra-day Precision)

- 10 consecutive determinations (n = 10) per sample.
- Measurements performed under identical analytical conditions within a single day.
- Evaluated using BIPEA and agricultural soil samples.

Intermediate Precision (Inter-day Precision)

- BIPEA certified reference soil material.
- 3 consecutive days × 3 replicate determinations per day.
- Evaluation of analytical variability between days.

Precision parameter	Experimental design	Mean ± SD (mgP ₂ O ₅ 100g ⁻¹)	RSD (%)
Repeatability (intra-day)	10 replicate determination (n=10)	16.36 ± 0.36	2.18
Intermediate precision (Inter-day)	3 days x 3 replicates (n=9)	16.33 ± 0.02	0.13

Table 8. Precision of the proposed phosphovanadomolybdate UV-Vis spectrophotometric method determined using the BIPEA certified reference soil material.



Soil sample	Mean ± SD (mg P ₂ O ₅ 100 g ⁻¹)	Repeatability (RSD, %, n = 10)
Soil 1	35.43 ± 0.06	0.17
Soil 2	38.45 ± 0.05	0.12
Soil 3	28.55 ± 0.04	0.13
Soil 4	19.88 ± 0.06	0.33
Soil 5	24.21 ± 0.08	0.33
Soil 6	29.26 ± 0.04	0.14
Soil 7	47.23 ± 0.04	0.09
Soil 8	9.90 ± 0.07	0.67
Soil 9	13.51 ± 0.03	0.25
Soil 10	23.51 ± 0.04	0.20

Table 9. Repeatability of the proposed phosphovanadomolybdate UV-Vis spectrophotometric method determined using ten representative agricultural soil samples.

CONCLUSION

- The phosphovanadomolybdate UV-Visible method was successfully validated for determining plant-available phosphorus in agricultural soils following Egner-Riehm ammonium lactate extraction.
- Excellent linearity was achieved over 1–20 mg P L⁻¹ ($R^2 > 0.999$), with adequate sensitivity for routine analysis.
- Silicon showed no measurable interference under the tested analytical conditions.
- Reference material analysis and recovery studies demonstrated satisfactory trueness and accuracy.
- Excellent repeatability and intermediate precision were obtained, with RSD values of 0.09–0.67% for agricultural soil samples.
- The method provides a simple, reliable and cost-effective tool for routine soil phosphorus analysis and soil fertility assessment.

Thank you for your attention!

Questions are welcome.

