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Assessment of available phosphorus in planted mangrove sediments in Thailand by using the Olsen extraction method

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Abstract Phosphorus (P) is an essential macronutrient for plant growth, carbon sequestration, and nutrient cycling within the dynamics of mangrove ecosystems, where its bioavailability is often a limiting factor. One important factor is available P, which represents the P fraction weakly bound to minerals or organic matter that is easily consumed by plants and microbes. This available P concentrations in two sediment cores from a planted mangrove forest located in southern Thailand was investigated. The pH levels of sediment across the sites ranged from 7.13 to 7.90, indicating neutral to slightly alkaline condition, which is found to be suitable for application of the Olsen method to extract P in the sediments. Results showed that available P in sediment samples commonly decreased with increasing depth, which varied from 19.38 ± 0.06 to 47.50 ± 0.06 mg/kg P, with an average value of 28.29 ± 3.80 mg/kg P. To ensure reliability of the data, the analytical performance of the lsen extraction method ($NaHCO_3$, pH 8.5) iscoupled with UV-Vis spectrophotometry was validated. The analytical performance of the method showed excellent linearity ($R^2 = 0.9980$) and robust precision were %RSD 1.23–3.45% for within-day and %RSD 1.24–4.35% for between-day. Accuracy, evaluated through spike recovery ranged from 82% to 99%, indicating that the method is highly reliable. Collectively, these validation metrics indicated the effectiveness of the Olsen method as a quantitative analytical tool to determine the available P in mangrove sediment samples. This research is provided critical information on the P status in restored mangrove areas, playing a significant role of enhancing the understanding of nutrient dynamics within these ecosystems and promoting the critical potential of mangroves for supporting plant growth and blue carbon sequestration in coastal environments.

Keywords: Blue carbon, Nutrient, Environment, Restoration of mangrove forest, Spectrophotometry

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Introduction

Phosphorus (P) is a macronutrient that is important for plant development, microbial activity, and soil richness. It is generally found as a phosphate compound and plays a vital role in soil biogeochemical cycle. P supports plant growth, a wide range of physiological functions, photosynthesis, energy metabolism (via ATP and ADP synthesis), enzyme activation, and reproduction (Khan *et al.,* 2023). While P is abundant in the lithosphere, its biologically available forms are often limited, making it a crucial nutrient for both terrestrial and water environments. In soil and sediment systems, P accessibility is a main limiting factor for productivity, especially in many mangrove ecosystems, where nutrient limitation can occur depending on sediment composition and geochemical conditions (Mack *et al*., 2024). Therefore, understanding P dynamics is important for preserving ecosystem health and improving nutrient management, especially in sensitive coastal areas.

The sources of P in the environment are from both natural and anthropogenic input (Yuan *et al*., 2011). Under natural conditions, it is introduced to ecosystems through the weathering of P-bearing minerals and the decomposition of organic matter (Zhang *et al*., 2020). In contrast, human activities such as agronomy, aquaculture, industrial effluents, and urban runoff have contributed to the excessive P in environmental systems. Discharge of this excess P into marine and estuarine systems can cause eutrophication, which leads to algal blooms, oxygen depletion, biodiversity loss, and reduced ecosystem functions (Akinnawo, 2023). Furthermore, increasing nutrients concentrations in coastal areas can accelerate the digestion of seagrasses leaves, leading to increase carbon dioxide (CO_2) into atmosphere. This altered activity reduces the ability of seagrasses ecosystems to function efficiently as blue carbon sinks for climate change mitigation (Liu *et al.,* 2023).

P in tropical sediment is found in inorganic and organic forms. Inorganic P is generally bound to metal oxides and inorganic substances, particularly iron (Fe), aluminum (Al), and calcium (Ca). These interactions create compounds, the readiness of which depend on pH and redox conditions (Olego *et al*., 2022). In contrast, organic P is obtained from plant residues, soil microbial mass, and animal waste. This form of P is thought to be within complex organic compounds and must undergo microbial mineralization to become available for biological uptake (Elhaissoufi *et al.,* 2022). Together, these various P forms make unique and significant contributions to the entire nutrients availability, playing distinct and crucial roles in the ecosystem nutrient flow and long-term preservation of P within the sediment column (Moushmi *et al*., 2022).

P readiness is a significant limitation for mangrove ecosystems, which are defined by their high ecological complication and key productivity.

P availability is critical for supporting the growth of main mangrove species such as *Rhizophora* and *Avicennia*, which act as coastal shields, carbon sequestration, and important resources for local communities (Sunkur *et al.*, 2023). The P level in mangrove sediments is affected by multiple factors, including tidal fluxes, organic matter degradation, redox potential, and microbial activity. To study P content in sediments, researchers typically employ two main analytical approaches, i.e., determination of total P (TP) and available P analyses. The TP analysis elucidates all P species of both labile and non-labile fractions, which is advantageous for understanding long-term storage and biogeochemical cycling. This method requires strong acid digestion methods such as perchloric acid ($HClO_4$) and sulfuric acid (H_2SO_4). In contrast, the available P analysis focuses on the fraction of P that is easily consumed by plants and microbes. This form is especially relevant for agricultural and ecological studies on assessing short-term nutrient accessibility (Mogashane *et al.*, 2025). Among the various analysis methods, the Olsen method is widely used for the determination the available P content in sediments (FAO, 2021). The Olsen method employs sodium bicarbonate ($NaHCO_3$) as the extracting solution and is particularly effective for neutral to moderately alkaline soils where the pH exceeds 7. In this study, the Olsen method was selected because of the slightly basic condition of the sediment samples. This method also offers a dependable of available P in plant uptake, making it suitable for comparing soil fertility across different land-use types, such as natural mangroves, reforested plantations, or different ages of forests.

This research is focused to on assesse the available P in sediments via the Olsen method from two sites (8 years and 10 years old) across planted mangrove forest in Tha Sala, Nakhon Si Thammarat, Thailand. The purposes were to study the impacts of available P in sediments in affecting plant growth and the composition of the ecosystem and to study the impact of planted mangrove trees on the levels of available P in the sediment.

Materials and methods

Study area and sediment sampling

The sampling locations are plotted in Figure 1 as red dots. Sediment samples were collected from two sites, namely, PK1 and PK2, within mangrove forests located in the Tha Sala district, Nakhon Si Thammarat province, Thailand. These sites represent distinct environmental and ecological conditions within the Gulf of Thailand's coastal mangrove ecosystems. The undisturbed sediment cores were collected using a cylindrical PVC core with 100 cm in length and 69 mm in inner diameter. The cores were extracted out of the PVC corer to preserve the natural stratification of the sediments. Each core was then transported to the laboratory in an upright position, sliced into 2 cm intervals, and the sediment slices were kept in zip lock plastic bag in 4°C refrigerator prior to analysis. Subsamples were taken at defined intervals for the determination of pH and available P content. All samples were dried using a hot air oven (Model: UNB500, Memmert, Germany) at 60°C.

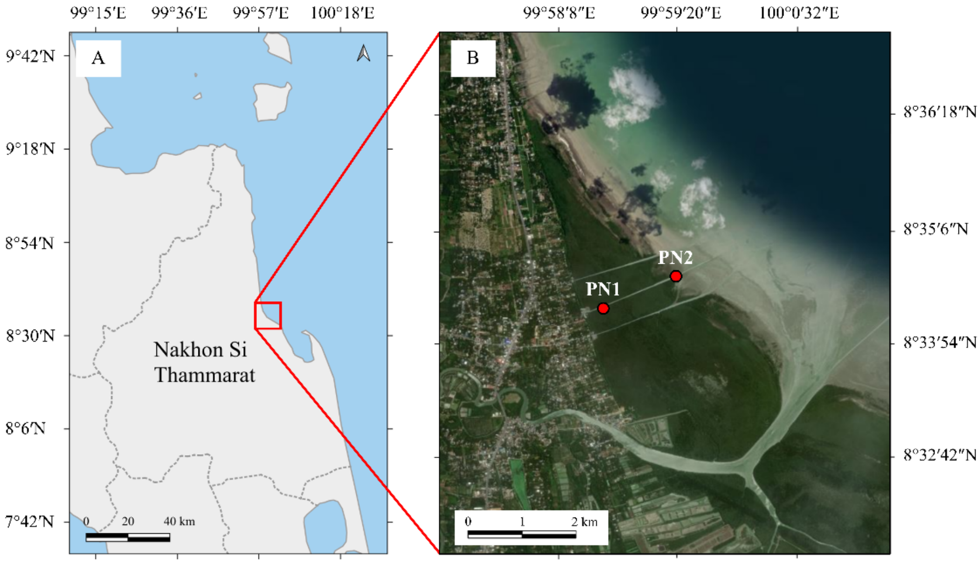

Figure 1. Sampling locations

Chemicals and reagents

Chemical grades analytical reagents were used in this study, including sodium bicarbonate ($NaHCO_3$; 99.0%, KEMAUS, Australia), sodium hydroxide (NaOH; LOBA CHEMIE PVT. LTD., India), potassium dihydrogen phosphate (KH_2PO_4; 99.5%, LOBA CHEMIE PVT. LTD., India), sulfuric acid (H_2SO_4; 98%, QReC, New Zeeland), ammonium molybdate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$; 99.3%, LOBA CHEMIE PVT.LTD., India), potassium antimony tartrate ($K_2Sb_2(C_4H_2O_6)_2$; 99.0% LOBA CHEMIE PVT. LTD., India), and L-ascorbic acid ($C_6H_8O_6$; 99.5% LOBA CHEMIE PVT. LTD., India). All chemicals were purchased from local suppliers.

Determination of sediment pH

The pH of sediment samples was measured using a 1:2.5 ratio of air-dried sediment to distilled water. The mixture was thoroughly mixed and allowed to settle under room temperature. Then, the pH of the supernatant was determined using a calibrated pH meter (Model: ID1000, Index, USA). All measurements were achieved in triplicate, and the mean pH level was reported (Pazi *et al*., 2021).

Determination of available Phosphorus in sediments using the Olsen method

The available P content in sediment samples was determined on the Olsen extraction method. Precisely 5 g of air-dried sediment was mixed with 0.5 g of activated charcoal and 100 mL of sodium bicarbonate solution (pH 8.5, 0.5 M) in a 250 mL Erlenmeyer flask. The mixture was shaken at 200 rpm for 30 min using a shaker (Model: Promax 2020, Heidolph, Germany), following agitation, the suspension was filtered with a filter paper (Whatman No. 42, Cytiva, China), and the resultant extract was stored at 4 °C until further analysis.

For spectrophotometric analysis, 3 mL of the prepared sample was pipetted into a test tube, then mixed with 3 mL of mixed reagent. The mixture was homogenized with a vortex mixer (Model: Genie 2, Scientific Industries, USA) and maintained under room temperature for 1 h to enable the development of a blue color indicating of completion of chemical reaction. The absorbance of the developed color was determined at 882 nm wavelength with a UV-Vis spectrophotometer (Model: SP-8001, Metertech, Taiwan) (FAO, 2021).

Method Validation for available phosphorus determination

The linearity and range for the determination of available P in sediment samples were based on the absorbance values to a standard calibration curve prepared from known concentrations of standard P solutions. The amount of P in each sample was computed by applying linear regression formula of $y = mx + c$ (where x represents P content and y represents absorbance). The calibration curve was required to have a coefficient of determination (R^2) of no less than 0.995 (AOAC, 2016).

The limit of detection (LOD) and the limit of quantification (LOQ) were determined from the blank's standard deviation and the slope of the calibration curve. The LOD and LOQ can be determined using these formulas: $\text{LOD} = 3.3 \times (\sigma / S)$ and $\text{LOQ} = 10 \times (\sigma / S)$ (here σ is the standard deviation ($n = 5$), and S is the slope of the calibration curve) (AOAC, 2016).

The precision of the analysis was assessed through replicate analyses of standard P solutions at low, medium, and high dosages under the same analytical conditions. The intra-day precision was assessed by analyzing five replicates within a single day, while the inter-day precision was established by repeating the analyses over five continuous days. The precision was represented as the relative standard deviation (RSD), reflecting the method’s repeatability and reproducibility (AOAC, 2016).

The accuracy of the analysis was confirmed through a recovery test. Known concentrations of standard P solution were spiked into sediment extracts, and the spiked samples were processed and analyzed using the same method as the unspiked samples. The percentage recovery was calculated using the following equation: Recovery (%) = (Measured concentration / Added concentration) × 100. The recovery percentage ranges from 90% to 110% indicate acceptable accuracy in quantification (AOAC, 2016).

Statistical analysis

The data on pH value and available P from two different sites were evaluated using statistical analysis ANOWA (t-Test: two-sample) to determine significant variance at a 95% confidence level.

Results

pH of sediment samples

The pH values of sediment samples were measured across two different sites PN1 and PN2. Results across their respective depth profiles are presented in Figure 2A. At site PN1, the pH values ranged from 7.13 to 7.80. At site PN2, pH levels ranged from 7.70 to 7.80. Overall, the sediment pH at these locations showed conditions that varied from neutral to mildly alkaline across the sampled depths. The two sites showed a statistically significant difference at a 95% confidence level (p-value = 0.0044).

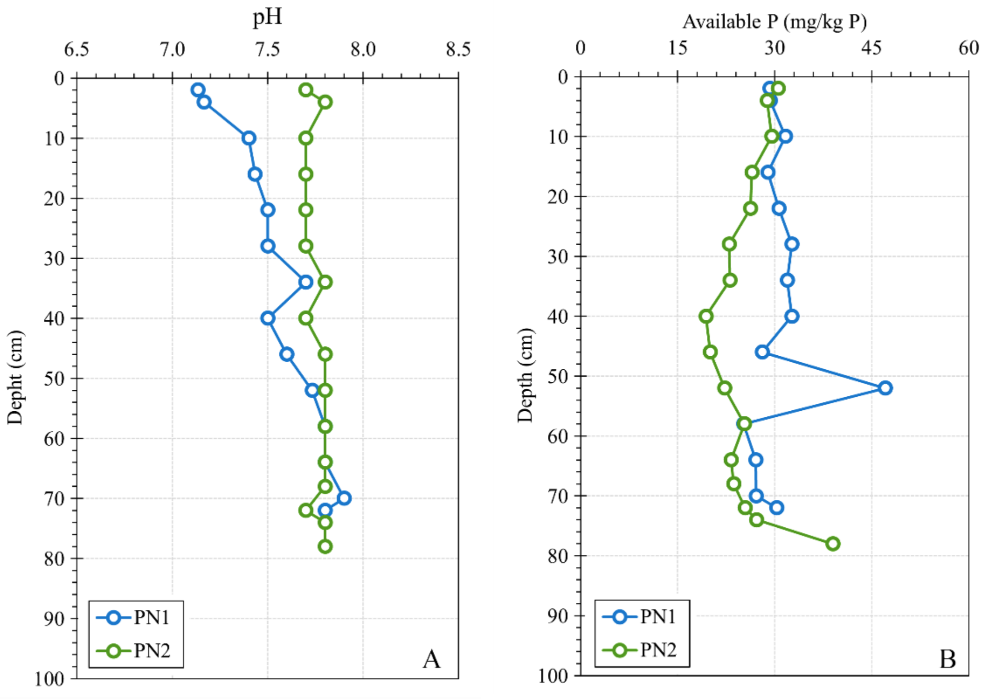

Figure 2. Depth profiles of geochemical variables in sediment samples from two locations: (A) the pH levels and (B) the concentrations of available P

Evaluation of analytical performance of the Olsen method

The analytical performance of the Olsen method was thoroughly assessed to confirm its suitability for the exact and precise measurement of available P in sediment samples. This calculation included linearity, working range, method detection and quantification limits, precision, and accuracy.

The analytical performance of the Olsen method was assessed regarding its linearity and working range. A linear calibration curve was created for the concentration range of 0.40 to 2.00 mg/L, yielding the regression formula $y = mx + c$ with a strong correlation coefficient (R^2) of 0.9980. The detection limit (LOD) was 0.27 mg/L and the limit of quantification (LOQ) was 0.40 mg/L, as listed in Table 1.

Table 1. Analytical parameters of the Olsen method employed for assessing available phosphorus in sediment samples

Parameter	Olsen Method
Linearity	$y = 0.3140x - 0.0029$
Range	0.40 – 2.00 mg/L
R^2	0.9980
LOD	0.27 mg/L
LOQ	0.40 mg/L

The precision of the Olsen method was thoroughly evaluated with internal quality control (IQC) samples at three different levels of low (0.50 mg/L), medium (1.00 mg/L), and high (1.50 mg/L). Both intraday (n = 5 replicates within a day) and interday (n = 5 replicates with different days) analyses were performed to assess the method's repeatability and intermediate precision under standard laboratory conditions. The method verified satisfactory precision across all tested concentrations as summarized in Table 3. The intraday relative standard deviations (%RSDs) were 3.13%, 1.59%, and 1.23% for low, medium, and high concentrations, respectively. For the interday measurements, the %RSDs were 4.21%, 1.62%, and 1.63%, respectively. All recorded %RSD values were found to be the normally accepted criteria for method validation, indicating the high reproducibility and consistency of the Olsen method.

Table 2. Precision of the Olsen method for determining available P

Concentration P (mg/L)	Intra-day (N =5)		Inter-day (N=5)	
	Analysis value (mg/L)	%RSD	Analysis value (mg/L)	%RSD
0.50 (Low)	0.49 ± 0.0154	3.13	0.52 ± 0.0219	4.21
1.00 (Medium)	1.00 ± 0.0159	1.59	1.01 ± 0.0165	1.62
1.50 (High)	1.50 ± 0.0185	1.23	1.53 ± 0.0249	1.63

The accuracy of Olsen method was evaluated by spiked known standard P solution with sediment samples. The percentage recovery (%Recovery) was calculated to determine whether the method produce reliable and accurate results. Sediment samples (N = 2) were at three concentrations at 0.50, 1.00, and 1.50 mg/L. The recoveries were 84.60% and 87.19% for the 0.50 mg/L, 92.58% and 93.99% for 1.00 mg/L, and 97.37% and 99.67% for 1.50 mg/L, respectively. The high recovery results indicated that the method demonstrates good accuracy across the tested concentration range and is found to be suitable for quantifying available P in sediment within the acceptable range of 80–120% as recommended by AOAC (2016) as shown in Table 3.

Table 3. Accuracy assessment of the Olsen method for determining available P

Concentrations P (mg/L)		**%Recovery ± S.D.**	**%RSD**
Target value	**Amount found**		
0.5	0.423 ± 0.0019	84.60 ± 0.3738	0.44
	0.436 ± 0.0081	87.19 ± 1.6294	1.87
1.0	0.926 ± 0.0032	92.58 ± 0.3237	0.35
	0.940 ± 0.0019	93.99 ± 0.1869	0.20
1.5	1.461 ± 0.0037	97.37 ± 0.2492	0.26
	1.495 ± 0.0208	99.67 ± 1.3875	1.39

Available P in sediment samples

The available P in sediment samples was determined using the Olsen method, which is mainly appropriate for basic to neutral soils and sediments with pH values above 7.00. The pH of all samples exceeded this threshold, hence the Olsen extraction method was selected as an appropriate technique for evaluating P availability. The results revealed spatial variation in available P concentrations across the two planted mangrove forest sites. Specifically, concentrations ranged from 25.22 to 47.05 mg/kg P at site PN1 and 19.38 to 38.99 mg/kg P at site PN2 (Figure 2B). According to the results, the available phosphorus across two sites were significantly different at a 95% confidence level (p-value=0.0095). Moreover, when averaged available P value from all results was 28.29 ± 3.80 mg/kg P (Figure 3). This distribution is highlighted the heterogeneity of nutrient availability within the sediments, which may reflect differences in sedimentation patterns, organic matter input, and vegetation coverage among sites.

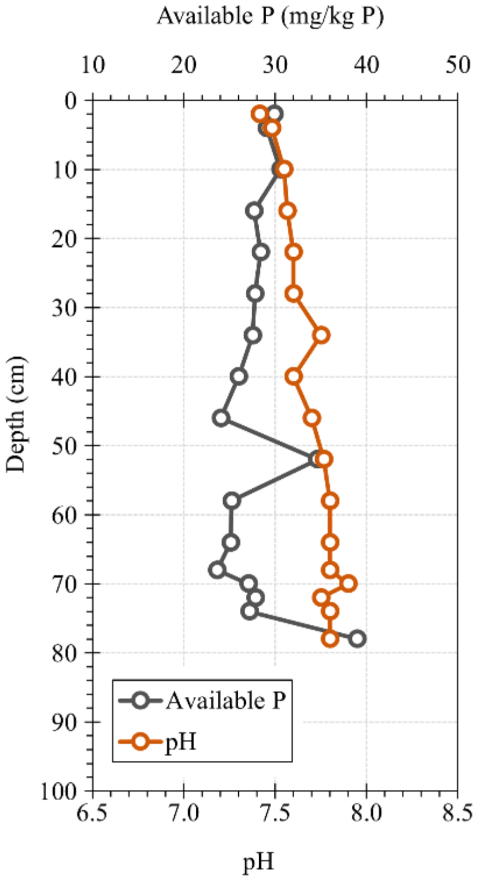

Figure 3. The relation between average pH and available P in planted mangrove forests

Discussion

The pH values of the sediment samples across the study sites primarily indicated a range from neutral to slightly alkaline conditions. While visual inspection of depth profiles showed some variability in pH with increasing depth for individual sites, a statistically significant have a difference in pH values among the two locate planted mangrove forest sites at confident 95%. The average concentrations of available P in sediment samples across the two sites ranged from 23.67 to 38.99 mg/kg P. The available P levels in the layers ranged from 0 to 22 cm, higher than in the deeper layers. The release of P may be due to decomposition of biomass at the sediment surface and microbial activity. However, the depth-related distribution of available P was complex and site-specific. Contrary to the pH trend, the available P concentrations generally decreased with increasing sediment depth (Zhou *et al.*, 2021). This inverse relationship between pH and available P, where increased in pH values corresponded to decrease in available P that could be due to the reaction of dissolved phosphorus with cations such as calcium

(Ca^{2+}) to form calcium phosphate ($Ca_3(PO_4)_2$). This reaction is removed free phosphate from solution as insoluble precipitates, resulting in lower P availability (Gandaseca *et al*., 2016; Wang *et al*., 2023; Cerozi *et al.,* 2016). The available P is varied across different locations. The mangrove area demonstrated greater variability. The differences in available P between areas may be related to factors such as pH, plant, TOC, TN, and microbial activity (Wang *et al*., 2023; Wu *et al*., 2020). These values generally fall within a range reported to support mangrove productivity and are found to be crucial for enhancing nutrient retention and plant establishment in reforested coastal areas.

Table 4. Comparative available P in various locations

Environment	**Area**	**Available P**	**Ref.**
		mg/Kg	
Environment	Global distribution	48.56	Wang *et al*., 2023
Mangrove	China	11.08	You *et al*., 2022
Mangrove	Vietnam	1.134	Toan *et al*., 2023
Mangrove	Thailand	28.29	The present study

Analytical performance of the Olsen method showed LOQ at 0.4 mg/L, and the results of precision from %RSD values demonstrated at low, medium, and high levels (0.50, 1.00, and 1.50 mg/L) which were all below 15%, indicating acceptable precision. The Olsen method demonstrated a %Recovery range of 84.60 to 99.67, indicating its effectiveness for P measurement in sediment samples according to standard of AOAC (2016).

In conclusion, this research successfully studied the available P content in sediments from a planted 2-site using the Olsen analysis method. Mangrove ecosystems play a crucial role in capturing carbon, achieved via the accumulation of biomass and stabilization of sediments. As a result, the increasing availability of P results in enhanced plant productivity and promotes the sustained storage of blue carbon. The approach used to assess available P in this study shows strong consistency, verifying its dependability for P assessment in sediment samples. This study is reinforced the importance of accessible P in promoting plant growth and maintaining the P cycle in mangrove forests. Moreover, the results are emphasized the approach's appropriateness for use in environmental monitoring and geochemical evaluations. However, this study was limited by insufficient supporting data. In the future investigation should include microorganisms, TOC, TN, and TP to provide more comprehensive support for the data.

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Conflicts of interest

The authors declare no conflict of interest.

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