Validating Reflectoquant™ Method for Phosphorus Quantification in Uruguayan Waters

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Abstract

This study evaluates the Reflectoquant™ (REFL) method for quantifying soluble reactive phosphorus (SRP) in surface waters, using UV-Vis spectrophotometry (SPEC) as a reference. Statistical analyses—including Spearman correlation, linear regression (simple, logarithmic, square-root, polynomial), and Bland-Altman tests—were conducted to assess method agreement. Spearman correlations were strong (ρ = 0.979 for standards; ρ = 0.890 for river samples). Simple linear regression showed excellent fits (adjusted R^2 = 0.972, RMSE = 0.010 mg PO_4-P L^{-1} for standards; adjusted R^2 = 0.995, RMSE = 0.018 mg L^{-1} for rivers), outperforming transformed models (e.g., log adjusted $R^2 \leq 0.856$). Residuals from river samples deviated from normality and homoscedasticity, except in the polynomial model for standards. Bland-Altman analysis revealed a small overall bias (+0.009 mg L^{-1}), with REFL overestimating SRP at high concentrations (≥ 0.100 mg L^{-1}, mean difference = +0.015 mg L^{-1}) and underestimating at low concentrations (< 0.025 mg L^{-1}, mean difference = −0.022 mg L^{-1}). Despite limitations at trace levels, Reflectoquant's portability and low cost make it a valuable field tool. In Uruguay, SRP typically represents ~60% of total phosphorus (TP), a key driver of eutrophication. Since TP thresholds for eutrophication are commonly set at 0.025 mg L^{-1}, SRP values above this level may indicate high eutrophication risk. Reflectoquant enables rapid field screening of potential tipping points in freshwater ecosystems, supporting timely interventions.

Keywords: soluble reactive phosphorus, eutrophication, fresh waters, Bland-Altman test.

Validación del Método Reflectoquant™ para Cuantificar Fósforo en Águas Uruguayas

Resumen

Este estudio evalúa el método Reflectoquant™ (REFL) para cuantificar fósforo reactivo soluble (PRS) en aguas superficiales, utilizando la espectrofotometría UV-Vis (SPEC) como referencia. Se realizaron análisis estadísticos, incluyendo correlación de Spearman, regresión lineal (simple, logarítmica, raíz cuadrada, polinómica) y pruebas de Bland-Altman para evaluar la concordancia entre métodos. Las correlaciones de Spearman fueron fuertes ($\rho = 0.979$ para estándares; $\rho = 0.890$ para muestras de río). La regresión lineal simple mostró ajustes excelentes (R^2 ajustado = 0.972, RMSE = 0.010 mg PO_4-P L^{-1} para estándares; R^2 ajustado = 0.995, RMSE = 0.018 mg L^{-1} para ríos), superando a los modelos transformados (por ejemplo, log R^2 ajustado ≤ 0.856). Los residuos de las muestras de río se desviaron de la normalidad y homocedasticidad, excepto en el modelo polinómico para estándares. El análisis de Bland-Altman reveló un sesgo general pequeño (+0.009 mg L^{-1}), con REFL sobreestimando el PRS en concentraciones altas (≥ 0.100 mg L^{-1}, diferencia media = +0.015 mg L^{-1}) y subestimando en concentraciones bajas (< 0.025 mg L^{-1}, diferencia media = −0.022 mg L^{-1}). A pesar de sus limitaciones en niveles traza, la portabilidad y bajo costo del Reflectoquant lo convierten en una herramienta útil en campo. En Uruguay, el PRS representa aproximadamente el 60 % del fósforo total (PT), principal impulsor de la eutrofización. Dado que el umbral de PT para eutrofización se establece comúnmente en 0.025 mg L^{-1}, valores de PRS superiores pueden indicar alto riesgo. Reflectoquant permite una evaluación rápida en campo de puntos críticos en ecosistemas acuáticos, facilitando intervenciones oportunas.

Palabras clave: fósforo reactivo soluble, eutrofización, agua dulce, prueba de Bland-Altman.

Validação do Método Reflectoquant™ para Quantificar Fósforo em Águas Uruguaias

Resumo

Este estudo avalia o método Reflectoquant™ (REFL) para quantificar fósforo reativo solúvel (PRS) em águas superficiais, utilizando espectrofotometria UV-Vis (SPEC) como referência. Foram realizadas análises estatísticas, incluindo correlação de Spearman, regressão linear (simples, logarítmica, raiz quadrada, polinomial) e testes de Bland-Altman para avaliar a concordncia entre os métodos. As correlações de Spearman foram fortes (ρ = 0.979 para padrões; ρ = 0.890 para amostras de rio). A regressão linear simples apresentou excelentes ajustes (R^2 ajustado = 0.972, RMSE = 0.010 mg PO_4-P L^{-1} para padrões; R^2 ajustado = 0.995, RMSE = 0.018 mg L^{-1} para rios), superando os modelos transformados (por exemplo, log R^2 ajustado ≤ 0.856). Os resíduos das amostras de rio desviaram-se da normalidade e homocedasticidade, exceto no modelo polinomial para padrões. A análise de Bland-Altman revelou um pequeno viés geral (+0.009 mg L^{-1}), com REFL superestimando o PRS em concentrações altas (≥ 0.100 mg L^{-1}, diferença média = +0.015 mg L^{-1}) e subestimando em concentrações baixas (< 0.025 mg L^{-1}, diferença média = −0.022 mg L^{-1}). Apesar das limitações em níveis traços, a portabilidade e o baixo custo do Reflectoquant tornam-no uma ferramenta útil para uso em campo. No Uruguai, o PRS representa cerca de 60 % do fósforo total (PT), principal responsável pela eutrofização. Como o limite de PT para eutrofização é geralmente definido em 0.025 mg L^{-1}, valores elevados de PRS podem indicar alto risco. O Reflectoquant permite triagens rápidas em campo de pontos críticos em ecossistemas aquáticos, apoiando intervenções oportunas.

Palavras-chave: fósforo reativo solúvel, eutrofização, águas doces, teste de Bland-Altman.

1. Introduction

The monitoring of environmental contaminants, such as phosphorus in surface waters, relies on laboratory methods like UV-Vis spectrophotometry (SPEC) for their accuracy, precision, and reproducibility. However, these methods require costly equipment (e.g., >$10,000 for spectrophotometers), sample transport risking degradation over hours to days, and a chain of custody, limiting responsiveness to dynamic events like post-rainfall nutrient spikes. Portable systems like Reflectoquant™ (REFL) address these challenges by enabling rapid, field-based screening, reducing the need for extensive laboratory analyses while prioritizing samples for confirmation(1,2).

Phosphorus in surface waters is categorized into total phosphorus (TP), measured after digestion and hydrolysis; soluble reactive phosphorus (SRP), primarily orthophosphate, quantified after filtration with 0.45 µm membranes; and a non-reactive dissolved fraction (TP minus SRP). SRP is measured via the molybdenum blue method, where samples react in an acidic medium with ammonium molybdate and potassium antimony tartrate to form phosphomolybdic acid. Reduced with ascorbic acid, this produces a blue color proportional to SRP concentration, quantified by SPEC (3,4).

Portable analyzers like Reflectoquant® are part of a growing trend in high-frequency, in situ nutrient monitoring (1). Reflectoquant provides a promising alternative for SRP monitoring using color-reactive strips evaluated by reflectometry. Capable of quantifying multiple analytes (e.g., PO_4^{3-}, NO_3^-, As), its accuracy and precision, particularly at trace levels (< 0.025 mg PO_4-P L^{-1}), remain underexplored (5–7). Eutrophication is associated with TP concentrations > 0.025 mg L^{-1} in oligotrophic systems, with SRP often > 0.010 mg L^{-1} indicating mesotrophic conditions (Carpenter, 2008; Sharpley and Wang, 2014). A pollution process tha sometimes is related to watershed disturbance of increasing land use intensity that can result in increased prduction and eutrophication, with potential for seasonal development of algal blooms (8–10). In a overview of the freshwater studies of phosphorus in Uruguay and neighborn countires, the values of PT and the ratio PRS/PT are in the range of 0.040 - 0.900 and 17 - 88%, respectively (Table 1 and 2). Therefore, with PRS values higher than 0.025 mg PO_4-P L^{-1} could be a high probability that TP is over the eutrophication limits. To evaluate REFL as a complementary technology to SPEC for monitoring eutrophic waters, this study tests two null hypotheses: (1) no significant relationship exists between REFL and SPEC across all SRP concentrations, limiting REFL's ability to detect varying trophic states; and (2) systematic bias exceeds the 95% limits of agreement (approximately ±0.036 mg L^{-1}), preventing equivalence in detecting eutrophic conditions (SRP > 0.010 mg L^{-1}).

Rejection of these hypotheses would yield a mathematical model linking REFL to SPEC with statistically significant coefficients ($p < 0.05$), high adjusted R^2, and low RMSE/MAE, though residual normality and homoscedasticity may vary by sample type. Bland-Altman analysis (11,12) should confirm acceptable bias and limits of agreement for moderate-to-high SRP detection, with potential calibration needs for trace levels. This study analyzed synthetic samples (0.020–0.250 mg PO_4-P L^{-1}) and Uruguayan river samples (n=300; 0.010–2.400 mg L^{-1}), measuring SRP simultaneously with REFL and SPEC. Statistical analyses included Spearman correlation, linear regression simple and some transformations (logarithmic, square-root, polynomial) according to that simple regression perhaps is not always is the best

145 option (13–15), and Bland-Altman tests to validate REFL as a field tool for
146 eutrophication survey, and monitoring.

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148 Table 1 – Phosphorus concentration in water expressed in (10^{-3} mg PO_4–P L-1) for some case of Uruguay (Post-1980).

Water Body	PT	PRS	PRS/PT (%)	Department	Year(s)	References
Río Santa Lucía rivers	0.050 - 0.180	0.020 - 0.080	~76%	Canelones, Montevideo	2004-2016	(16)
Santa Lucía river	0.300 - 0.900	0.680 (median)	~88%	Canelones	2008-2009	(17)
Río Negro and Río Uruguay rivers	0.050 - 0.150 means: 0.086 (Río Negro) and 0.099 (Río Uruguay)	0.020 - 0.080	~40 - 60% (estimated)	Río Negro, Soriano	2009-2018	(18)
Queguay River	Rivers: 0.044 – 0.051; Streams: 0.051 – 0.097; Floodplain lakes: 0.074 - 0.109	n.d.	-	Paysandú	2019-2021	(19)
Laguna del Sauce	0.050-0.150	~0.050 - 0.080	~50-80% (estimated)	Maldonado	2015-2017	
Salto Grande Reservoir (Argentine - Uruguay)	0.030 - 0.090	0.010 - 0.065	~23-39%	Salto (Uruguay), Entre Ríos	2000-2001	(20)

Water Body	PT	PRS	PRS/PT (%)	Department	Year(s)	References
				(Argentine)		
Paso Severino Reservoir (Santa Lucía river)	0.380 (annual mean)	n.d.	-	Florida	2013-2015	(21)
Canelón Grande and Canelón Chico streams	> 0.100	n.d.	-	Canelones	2004-2016	(16)
Coastal Lagoons	0.060 - 0.300	0.010 - 0.100	~17-33%	Maldonado, Rocha	2007-2011	(22)
Artificial ponds	0.200 - 0.250	n.d.	-	Montevideo	2006-2010	
Río Negro river	0.158	~0.010–0.050	~20–40%	Río Negro	2009–2010	(23)
Laguna de Rocha lagoon	0.046 - 0.167	0.011–0.065	~23–39%1	Rocha	2017	(24)
Streams – Laguna Merín Basin	0.046 - 0.150	1.100 - 1.300	>100%	Cerro Largo, Treinta y Tres	2015–2019	(25)

Water Body	PT	PRS	PRS/PT (%)	Department	Year(s)	References
99 Nationwide (various rivers)	0.005–0.110 (Autumn)	0.002–0.030 (Autumn)	~20–30%	15 departments	2014	(26)

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Table 2 – Phosphorus concentration in water expressed in (10^{-3} mg PO_4–P L-1) for some case of Argentina, Brazil, Paraguay (Post-1980).

Water Body	PT	PRS	Year(s)	Reference
Ypacaraí Lake (Paraguay)	0.124 - 0.256	n.d.	1988; 2012–17	(27)
Paraná lakes (Argentina)	0.005 to 0.004	0.015 to 0.124	1992–1997	(28)
Salado River (Argentina)	0.200–1.320	n.d.	2006–2007	(29)
Pampulha Reservoir (Brazil)	0.100 to 0.300	n.d.	1990s–2000s	(30)
Orós Reservoir (Brazil)	0.030 to 0.140	n.d.	2008–2012	(31)

2. Materials and Methods

2.1 Quantification Methods

2.1.1 Spectrophotometry (SPEC)

The molybdenum blue reaction is the basis for most manual determinations of SRP (9), with several accepted reductants—ascorbic acid among them—modifying the original Murphy and Riley (1962) protocol. Soluble reactive phosphorus (SRP) was measured using spectrophotometry (SPEC) with the molybdenum blue method (3,9). A 50 mL sample, filtered through 0.45 µm glass microfiber filters (Ahlstrom-Munksjö, formerly Munktell), made of high-purity borosilicate glass microfiber, binder-free, biologically inert, and thermally stable up to 500°C. The filtered was combined with 5 mL of reagent solution containing 10 mL ammonium molybdate (24.28 mM), 25 mL sulfuric acid (5 N), 10 mL ascorbic acid (0.31 M), and 5 mL potassium antimony tartrate (2.2 mM). The mixture was stirred and allowed to react for 30 minutes at room temperature. The resulting blue color was measured at 885 nm with a single-beam UV-visible spectrophotometer (Milton Roy Spectronic 401, Spectronic Instruments, USA). SRP concentrations were calculated by interpolating values from a calibration curve prepared with KH_2PO_4 solutions ranging from 0.010 to 0.250 mg PO_4-P L^{-1}. Samples with concentrations exceeding 0.250 mg PO_4-P L^{-1} were diluted with distilled water before analysis.

2.1.2 Reflectometry (REFL)

Reflectometric methods such as Reflectoquant® have been used in environmental and food matrices, offering portability and rapid analysis (5,6). Reflectometric determination (REFL) utilized the Reflectoquant® kit for orthophosphate (PO_4^{3-}, Cat. No. 1.17942.0001, Merck, USA), with a detection range of 0.1–5 mg PO_4 L^{-1} (0.033–1.63 mg PO_4-P L^{-1}, where 1 mg PO_4 L^{-1} equals 0.326 mg PO_4-P L^{-1}). For samples below 0.033 mg PO_4-P L^{-1}, a 4:1 sample-to-fortification solution (0.480 mg PO_4-P L^{-1}) was used to adjust concentrations within the kit's range, followed by back-calculation to the original concentrations. Measurements were conducted with a portable RQflex® plus 10 reflectometer (Cat. No. 1.16955.0001, Serial No. 2000332/480, Merck KGaA, Germany) using a cuvette adapter, in accordance with the instructions for the Reflectoquant® plus Phosphate Test kit.

2.2 Water Samples

Filtration through 0.45 µm membranes was used to operationally define the dissolved phase (9). Samples were stored at 4 °C or frozen, in line with best practices for phosphorus species preservation. The sampling design across 99 watersheds try to catch whole potential disturbance similar to that used by Zampella (1994) to assess land use impacts on water quality.

2.2.1 Orthophosphate Solutions

A stock solution of orthophosphate (250 mg PO_4 L^{-1}, equivalent to 81.5 mg PO_4-P L^{-1}) was diluted with Milli-Q™ water to 2.5 mg PO_4 L^{-1} (0.815 mg PO_4-P L^{-1}). From this, 13 dilutions (0.010, 0.020, 0.030, 0.040, 0.050, 0.060, 0.070, 0.080, 0.090, 0.100, 0.150, 0.200, 0.250 mg PO_4-P L^{-1}) were prepared, assigned as solutions of known concentrations, and analyzed in triplicate. Data are available in the “ss” file on GitHub (https://github.com/lcarrascol/reflectoquant_prs).

2.2.2 Surface Water Samples

A total of 300 surface water samples from 99 Uruguayan watersheds were collected in November 2014 using a Kemmerer bottle as part of the INIA national watershed study [2]. Samples were filtered in the field using 0.45 µm glass microfiber discs (grade MGC, 25 mm diameter, Munktell & Filtrak GmbH, Germany), pre-calcined at 500 °C.

Samples were filtered in the field using 0.45 µm glass microfiber filters (Ahlstrom-Munksjö, formerly Munktell), made of high-purity borosilicate glass microfiber, binder-free, with a nominal particle retention of 0.45 µm. These filters are chemically resistant (except to hydrofluoric acid and strong alkali), hydrophilic, biologically inert, and thermally stable up to 500°C. These glass microfiber discs (grade MGC, 25 mm diameter, Munktell & Filtrak GmbH, Germany), pre-calcined at 500 °C for their use in field. They are suitable for environmental analysis, including the operational definition of the dissolved phase in water quality studies, and are available in standard laboratory disc diameters.The filtrates were stored at 4 °C in plastic bottles and transported to Dr. Gabriela Eguren’s laboratory (Faculty of Sciences, University of the Republic) within 8 hours. Samples not analyzed on the same day were frozen at −20 °C and analyzed within one month to reduce SRP degradation. Data are available in the “Rivers” file on GitLab (https://gitlab.com/lcarrascol/reflectoquant_prs/-/releases/v1.0).

2.3 Data Analysis

Correlation coefficients (e.g., Pearson's r) are sensitive only to random error and are not sufficient for method comparison (32). Therefore, we used Spearman's ρ, regression models, and Bland–Altman analysis to assess agreement. Moreover, the quality of analytical input data often has more influence on regression reliability than the model itself (33), justifying our use of residual diagnostics and heteroscedasticity tests. According to these criteria the statistical analysis was conducted in four stages:

Correlation between Methods: Monotonic relationships between Reflectoquant (REFL) and spectrophotometry (SPEC) were evaluated using Spearman's correlation coefficient (ρ), without assuming linearity. A strong relationship was defined as $\rho > 0.8$ with $p < 0.05$, with potential variations at low concentrations (< 0.025 mg PO_4-P L^{-1}) due to sensitivity limitations.

Prediction Models: Models to predict SPEC from REFL included simple linear regression, logarithmic, square-root, and polynomial (degree 2) regressions.

Model Efficiency at Low Concentrations: Model performance was assessed for concentrations < 0.025 mg PO_4-P L^{-1} to evaluate utility near oligotrophic-mesotrophic thresholds.

Bias and Agreement Evaluation: The Bland-Altman method analyzed bias (mean difference, SPEC − REFL) and 95% limits of agreement (mean ± 1.96 × SD). Homogeneity of differences was assessed graphically and via Breusch-Pagan and Levene's tests for heteroscedasticity. A bias within approximately ±0.036 mg PO_4-P L^{-1} was considered acceptable for method interchangeability.

Analyses were performed in R (version 4.5.1, 2025) using packages readr, dplyr, tidyr, stats, lmtest, BlandAltmanLeh, MethComp, blandr, car, broom, Metrics, ggplot2, and gridExtra.

2.5 Model Evaluation Criteria

Models were evaluated using root mean squared error (RMSE), mean absolute error (MAE), adjusted coefficient of determination (R^2), and residual diagnostics for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). Evaluations assessed predictive accuracy (low RMSE and MAE), explanatory power (high adjusted R^2), and

residual assumptions, though deviations from normality and homoscedasticity were anticipated for complex river matrices.

3. Results

3.1 Spearman's correlation

Spearman's correlation analysis of soluble reactive phosphorus (SRP) concentrations measured by spectrophotometry (SPEC) and Reflectoquant™ (REFL) in standard solutions (ss, n = 39, Table 3) showed a strong positive monotonic relationship (ρ = 0.979, S = 95.8, p < 2.2 × 10^(-16)). For river samples (rivers, n = 300, similarly filtered, Table 3), the correlation was also strong (ρ = 0.890, S = 476,621, p < 2.2 × 10^(-16)).

Table 3: Descriptive statistics of SRP concentrations (mean ± SD, range, mg L^{-1}) in standard solutions and river samples, stratified by low (< 0.025 mg L^{-1}) and high (≥ 0.100 mg L^{-1}) levels.

Dataset	Subset	n	SPEC Mean ± SD (Range)	REFL Mean ± SD (Range)
Standards	All	39	0.098 ± 0.068 (0.010–0.250)	0.080 ± 0.058 (0.033–0.228)
Rivers	All	300	0.135 ± 0.259 (0.010–2.320)	0.126 ± 0.248 (0.010–2.480)
Rivers	Low (< 0.025 mg L^{-1})	29	0.017 ± 0.005 (0.010–0.020)	0.040 ± 0.000 (0.040–0.040)
Rivers	High (≥ 0.100 mg L^{-1})	136	0.281 ± 0.367 (0.100–2.320)	0.266 ± 0.355 (0.100–2.480)

3.2 Simple Linear Regression

Simple linear regression for standard solutions (ss) showed a strong fit for predicting REFL from SPEC (adjusted R^2 = 0.972, RMSE = 0.010 mg L^{-1}, MAE = 0.009 mg L^{-1}; equation: predicted REFL = −0.001 + 0.939 × SPEC). The slope was significant (t = 31.744, p < 2.2 × 10^(-16)), but the intercept was not (t = −0.376, p = 0.710). Residuals were marginally normal (Shapiro-Wilk: W = 0.930, p = 0.050) and homoscedastic (Breusch-Pagan: BP = 2.632, p = 0.105; Levene's: F = 0.929, p = 0.441). For river samples, the model (predicted RFL_rivers = −0.012 + 1.015 × SPC_rivers) had a stronger fit (adjusted R^2 = 0.995, RMSE = 0.018 mg L^{-1}, MAE = 0.013 mg L^{-1}), with both coefficients significant (p < 2.2 × 10^(-16)). However, residuals were non-normal (Shapiro-Wilk: W = 0.869, p = 3.8 × 10^(-15)) and marginally heteroscedastic (Breusch-Pagan: BP = 60.888, p = 6.0 × 10^(-15); Levene's: F = 2.445, p = 0.064). At concentrations < 0.025 mg L^{-1}, REFL values were often constant (~0.040 mg L^{-1}), reducing precision due to fortification adjustments.

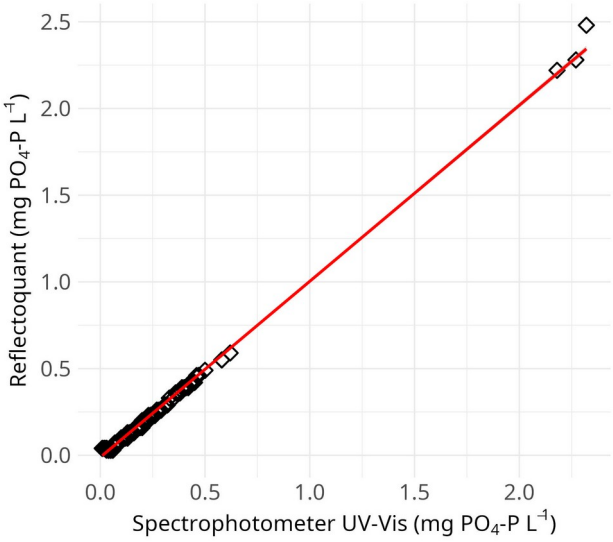

Figure 1: Scatter plots of REFL vs. SPEC for standard solutions and river samples, with simple linear regression lines.

3.3 Linear Regression with Logarithmic Transformation

For standard solutions, logarithmic regression showed a moderate fit (adjusted R^2 = 0.856, RMSE = 2.634 mg L^{-1}, MAE = 2.583 mg L^{-1}; equation: log(REFL) = −3.444 + 8.817 × SPEC), with significant coefficients ($p < 2.2 \times 10^{-16}$). Residuals deviated from normality (Shapiro-Wilk: W = 0.871, p = 0.002) but were homoscedastic (Breusch-Pagan: BP = 2.748, p = 0.097; Levene's: F = 1.934, p = 0.149). For river samples, the fit was weaker (adjusted R^2 = 0.549, RMSE = 2.631 mg L^{-1}, MAE = 2.616 mg L^{-1}; equation: log(RFL_rivers) = −2.898 + 2.880 × SPC_rivers), with significant coefficients ($p < 2.2 \times 10^{-16}$) but non-normal (Shapiro-Wilk: W = 0.874, $p = 7.3 \times 10^{-15}$) and heteroscedastic residuals (Breusch-Pagan: BP = 231.94, $p < 2.2 \times 10^{-16}$; Levene's: F = 4.312, p = 0.005).

3.4 Linear Regression with Square Root Transformation

Square-root regression for standard solutions yielded an intermediate fit (adjusted R^2 = 0.937, RMSE = 0.205 mg L^{-1}, MAE = 0.202 mg L^{-1}; equation: sqrt(REFL) = 0.151 + 1.399 × SPEC), with significant coefficients ($p < 2.2 \times 10^{-16}$). Residuals were non-normal (Shapiro-Wilk: W = 0.883, p = 0.003) but homoscedastic (Breusch-Pagan: BP = 0.595, p = 0.441; Levene's: F = 1.907, p = 0.153). For river samples, the fit was similar (adjusted R^2 = 0.855, RMSE = 0.199 mg L^{-1}, MAE = 0.194 mg L^{-1}; equation: sqrt(RFL_rivers) = 0.219 + 0.727 × SPC_rivers), with significant coefficients ($p < 2.2 \times 10^{-16}$) but non-normal (Shapiro-Wilk: W = 0.908, $p = 1.7 \times 10^{-12}$) and heteroscedastic residuals (Breusch-Pagan: BP = 249.51, $p < 2.2 \times 10^{-16}$; Levene's: F = 2.287, p = 0.079).

3.5 Polynomial Regression of Degree 2

Polynomial regression for standard solutions showed a fit comparable to simple linear regression (adjusted R^2 = 0.973, RMSE = 0.010 mg L^{-1}, MAE = 0.008 mg L^{-1}; equation: REFL = 0.080 + 0.414 × poly(SPEC,1) − 0.019 × poly(SPEC,2)). The linear term was significant (t = 28.43, $p < 2.2 \times 10^{-16}$), but the quadratic term was not (t = −1.25, p = 0.222). Residuals were normal (Shapiro-Wilk: W = 0.942, p = 0.101) and homoscedastic (Breusch-Pagan: BP = 7.707, p = 0.021; Levene's: F = 0.186, p = 0.905). Polynomial regression for river samples was not feasible due to data constraints.

316

317 3.6 Regression in River Samples with SRP < 0.025 mg PO_4-P L^{-1}

318 3.6.1 Simple Linear Regression

319 For river samples with SRP < 0.025 mg L^{-1} (rivers_low, n = 29), simple linear
320 regression showed a moderate fit (adjusted R^2 = 0.455, F = 24.38, df = 1 and 27, p = 3.6
321 × 10^(-5); equation: predicted RFL_rivers = −0.001 + 2.095 × SPC_rivers). The
322 intercept was significant (p < 2.2 × 10^(-16)), but the slope was not (p = 0.582).
323 Constant REFL values (~0.040 mg L^{-1}) limited model reliability, with residuals showing
324 non-normality (Shapiro-Wilk: W = 0.246, p = 3.9 × 10^(-11)) and homoscedasticity not
325 assessable due to low variability (Breusch-Pagan: BP = 0.363, p = 0.547).

326

327 3.6.2 Logarithmic Transformation

328 Logarithmic regression yielded a similar fit (adjusted R^2 = 0.455, F = 24.38, p = 3.6 ×
329 10^(-5); equation: log(RFL_rivers) = −3.184 + 2.095 × SPC_rivers). The intercept was
330 significant (p < 2.2 × 10^(-16)), but the slope was not (p = 0.582). Residuals were non-
331 normal (Shapiro-Wilk: W = 0.246, p = 3.9 × 10^(-11)), and homoscedasticity was not
332 assessable (Breusch-Pagan: BP = 0.363, p = 0.547).

333 3.6.3 Square Root Transformation

334 quare-root regression slightly improved the fit (adjusted R^2 = 0.492, F = 28.16, p = 1.3 ×
335 10^(-5); equation: sqrt(RFL_rivers) = 0.200 + 2.718 × 10^(-15) × SPC_rivers). The
336 intercept was significant (p < 2.2 × 10^(-16)), but the slope was not (p = 0.582).
337 Residuals remained non-normal (Shapiro-Wilk: W = 0.246, p = 3.9 × 10^(-11)), and
338 homoscedasticity was not assessable (Breusch-Pagan: BP = 0.363, p = 0.547).

339 3.6.4 Polynomial Regression of Degree 2

340 Polynomial regression was not feasible for rivers_low due to insufficient unique values
341 in SPC_rivers, preventing coefficient estimation and diagnostics.

342

343 3.7 Bland-Altman Analysis

344 Bland-Altman analysis for river samples (n = 296 after excluding 4 NA cases, Table 4)
345 showed a mean bias of +0.009 mg L^{-1} (95% limits of agreement: −0.027 to 0.045 mg

L^{-1}, t-test p = 5.5 × 10^(-16)), indicating REFL slightly underestimated SPEC overall. For low concentrations (< 0.025 mg L^{-1}, n = 29), REFL overestimated SPEC (mean bias = −0.022 mg L^{-1}, 95% limits: −0.031 to −0.014 mg L^{-1}, t-test p < 2.2 × 10^(-16)). For high concentrations (≥ 0.100 mg L^{-1}, n = 136), REFL underestimated SPEC (mean bias = +0.015 mg L^{-1}, 95% limits: −0.021 to 0.052 mg L^{-1}, t-test p < 2.2 × 10^(-16)). No specific outliers were identified, but scatter in low-concentration data suggested potential analytical variability.

Table 4: Bland-Altman metrics (bias, 95% limits of agreement, SD) for river samples and subsets.

Subset	n	Mean Bias (mg L^{-1})	95% Limits of Agreement (mg L^{-1})	SD of Differences (mg L^{-1})
All	296	+0.009	−0.027 to 0.045	0.018
Low (< 0.025 mg L^{-1})	29	−0.022	−0.031 to −0.014	0.004
High (≥ 0.100 mg L^{-1})	136	+0.015	−0.021 to 0.052	0.019

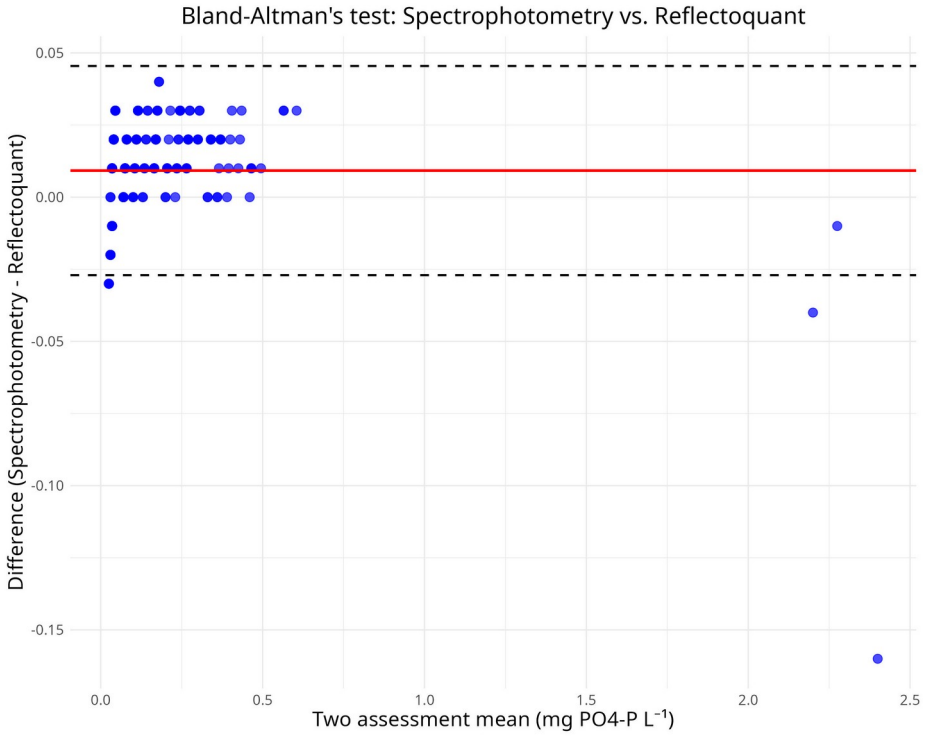

Figure 2: Comparison of two methods of phosphorus reactive soluble determination throught spectrometry (reference method) and Reflectoquant.

4. Discussion

This investigation assessed the Reflectoquant™ (REFL) technique as a field-deployable substitute for UV-Vis spectrophotometry (SPEC) in quantifying soluble reactive phosphorus (SRP) within surface waters, uncovering substantial concordance between the two approaches, especially for standard preparations and river samples exhibiting moderate to elevated SRP levels (5,6). These outcomes refute the initial null hypothesis of no meaningful association between REFL and SPEC measurements, as demonstrated by robust Spearman correlations and well-fitted linear regressions (13–15). Notably, the straightforward linear model achieved adjusted R^2 values above 0.97, underscoring REFL's viability as a supplementary method to SPEC for on-site assessments (32,33). This aligns with the demand for swift, economical solutions to identify nutrient excesses and mitigate eutrophication risks (8,9). That said, violations of regression prerequisites—such as non-normal residuals and heteroscedasticity in river data—highlight how natural water complexities, including organic interferents or fluctuating ionic compositions, can complicate analyses beyond the uniformity of lab standards (9).

Alternative regression forms, including logarithmic and square-root transformations, produced subpar performance relative to the basic linear approach, implying that nonlinear refinements offer limited gains in SRP forecasting accuracy (13). While the quadratic polynomial marginally enhanced fits for standards, it proved inapplicable to river data owing to distributional irregularities. Collectively, these results affirm the practicality of simple linear modeling in real-world scenarios, where interpretability trumps elaborate formulations (14). Still, the recurring fixed REFL readings (approximately 0.040 mg L^{-1}) at trace SRP levels (< 0.025 mg L^{-1}) expose an inherent detection threshold, potentially amplified by the sample fortification step, leading to inflated SRP estimates in low-nutrient, oligotrophic settings (6).

Complementing this, the Bland-Altman evaluation dismisses the second null hypothesis, revealing biases confined to the 95% limits of agreement (±0.036 mg L^{-1}) and thus endorsing methodological comparability across typical ranges . Yet, the observed bias variability—overestimation in low-SRP regimes and underestimation in high ones—flags challenges in precise low-level detection, which is essential for flagging incipient eutrophication (e.g., SRP surpassing 0.010 mg L^{-1} as a mesotrophic signal) (16–18). Such trends could arise from reflectometry's dependence on chromatic reflectance, prone to saturation at concentration extremes, or unaddressed matrix effects like residual turbidity (3,4). In contrast to earlier evaluations of handheld phosphorus assays, which document analogous field-based discrepancies (5,6), REFL holds its own; however, high-end underestimations (≥ 0.100 mg L^{-1}) risk downplaying contamination in severely eutrophic systems, warranting SPEC verification (19–22).

From an applied standpoint, REFL's mobility and affordability (roughly $5 per assay versus over $50 for SPEC, factoring in instrument amortization) facilitate immediate evaluations in isolated Uruguayan catchments, streamlining workflows by flagging elevated-SRP sites for detailed lab scrutiny (1). This capability proves especially pertinent for real-time surveillance amid episodic events, such as runoff surges after storms or peak farming seasons, as echoed in local investigations (17,18). Key drawbacks encompass possible SRP instability from prolonged cryogenic preservation (up to one month at −20 °C), which might skew results (9), alongside the research's regional scope on Uruguayan fluvial systems, curbing broader applicability to atypical waters (e.g., brackish or sediment-laden) (27–31). Ultimately, although REFL cannot supplant SPEC amid low-concentration hurdles, it excels as a frontline screener for phosphorus surveillance, bolstering eco-friendly water governance in under-resourced locales (8,10).

5. Conclusions

The evaluation of Reflectoquant™ (REFL) as a field-based method for quantifying soluble reactive phosphorus (SRP) in surface waters, compared to UV-Vis spectrophotometry (SPEC), demonstrates its potential as a practical tool for water quality monitoring, particularly in resource-limited settings. The study rejects both null hypotheses, confirming a strong monotonic relationship between REFL and SPEC (Spearman's $\rho \geq 0.890$, $p < 2.2 \times 10^{(-16)}$) and acceptable bias within 95% limits of agreement (± 0.036 mg L^{-1}) across a wide concentration range (0.010–2.400 mg PO_4-P L^{-1}). Simple linear regression models provided the best predictive performance (adjusted $R^2 \geq 0.972$, RMSE ≤ 0.018 mg L^{-1}), outperforming logarithmic, square-root, and polynomial transformations, which suggests that REFL can reliably estimate SRP in most environmental conditions without complex adjustments.

However, REFL's limitations at low concentrations (< 0.025 mg L^{-1}), where constant values (~0.040 mg L^{-1}) and poor model fits (adjusted $R^2 \leq 0.492$) indicate reduced sensitivity, restrict its use for detecting early eutrophication in oligotrophic waters. Concentration-dependent biases—overestimation at low levels (-0.022 mg L^{-1}) and underestimation at high levels (≥ 0.100 mg L^{-1}, $+0.015$ mg L^{-1})—further highlight the need for range-specific calibration.

REFL's portability and low cost make it a valuable screening tool for identifying high-phosphorus areas in Uruguayan watersheds, enabling efficient prioritization of samples for laboratory confirmation. This supports dynamic monitoring during events like post-rainfall nutrient spikes. Future work should refine REFL calibration for trace levels, validate its performance in diverse water matrices, and explore optimized filtration protocols to enhance accuracy. Overall, REFL complements SPEC, advancing sustainable phosphorus monitoring in environmental management programs.

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Transparency of data

The author has to indicate one of the following options:

- Data not available: The data set that supports the results of this study is not publicly available.
- Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

For each author of your manuscript, please indicate the types of contributions the author has made with the CRediT form (https://credit.niso.org/contributor-roles-defined/). For example:

LCL: Conceptualization; Investigation; Writing – original draft

GE: Conceptualization; Methodology

AH: Methodology

AB: Methodology; Writing – review & editing

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