Supplementary Material

A green analytical assay for the quantitation of bioavailable phosphorus in soils: *in situ* extraction and smartphone-based measurement

Stella Sales Macedo*, Edilene Cristina Ferreira*, Arnaldo Alves Cardoso*[a]

*São Paulo State University (UNESP), Rua Francisco Degni 55, Araraquara, SP, 14800-970, Brazil

Table S1. (a) Particle size fractions and (b) chemical properties of the certified soil samples used in this study.

(a)

Sample	Clay	Silt	Sand		
			Total	Coarse	Fine
		g/kg			
549	387	95	516	342	174
555	395	255	345	87	251
560	388	96	516	342	176
564	271	91	638	351	285
574	487	226	284	196	88
583	390	93	516	343	176
586	416	245	339	136	200

(b)

	P resin	OM	pH CaCl2	K	Ca	Mg	H+Al	Al	S phosphate	B	Cu	Fe	Zn	Na	Si
Sample	mg/dm³	g/dm³		mmol/dm³					mg/dm³					mmol/dm³	mg/dm³
549	100	24	5.1	5	33	11	34	0	5	0.26	2.6	42	3.6	0.1	7
555	78	23	4.9	6.7	41	22	41	1	12	0.57	3.5	143	6.6	0.1	17
560	119	23	5.1	5.3	3.4	11	35	0	5	0.27	2.7	41	4	0.1	7
564	48	29	4.7	1.1	25	7	40	1	5	0.26	3.5	122	6.4	0.1	1
574	160	52	5.2	9.4	65	34	37	0	8	0.37	4.2	236	13.3	0.2	16
583	115	24	5.1	5.3	36	12	34	0	7	0.28	2.9	41	3.9	0.2	7
586	130	44	5.9	6.4	83	37	22	0	7	0.4	5.1	57	7.7	0.2	20

OM= organic matter determined by the Walkley–Black method; pH $CaCl_2$: soil pH measured in 0.01 mol L^{-1} $CaCl_2$; P-resin: available phosphorus extracted by the ion-exchange resin method; Ca, Mg and K: exchangeable calcium, magnesium and potassium extracted by ion-

exchange resin; H+Al: potential acidity determined by SMP buffer or calcium acetate extraction; Al: exchangeable aluminum extracted with KCl; S-SO_4^{2-}: sulfate extracted as calcium phosphate; B: boron extracted with hot water; Cu, Fe, Mn and Zn: available micronutrients extracted with DTPA. All analyses were performed by the Agronomic Institute of Campinas (IAC), Campinas, Brazil, following the standard methods adopted in its Soil Proficiency Testing Program.

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Figure S1. Photographs of the ultrasonic baths used in the sample preparation experiments: (1) ultrasonic bath 1 (USC-1400, UNIQUE); (2) ultrasonic bath 2 (YT-80 ultrasonic cleaner).

Table S2. Chemical properties of certified soil sample A555 employed in the optimization of extraction using ultrasonic bath 2, performed according to the 2^3 factorial design.

Sample	pH CaCl2	P resin mg/dm^3	Ca mmol/dm^3	H+Al mmol/dm^3	Fe mg/dm^3
555	4.9	78	41	41	143

(a)	**(b)**

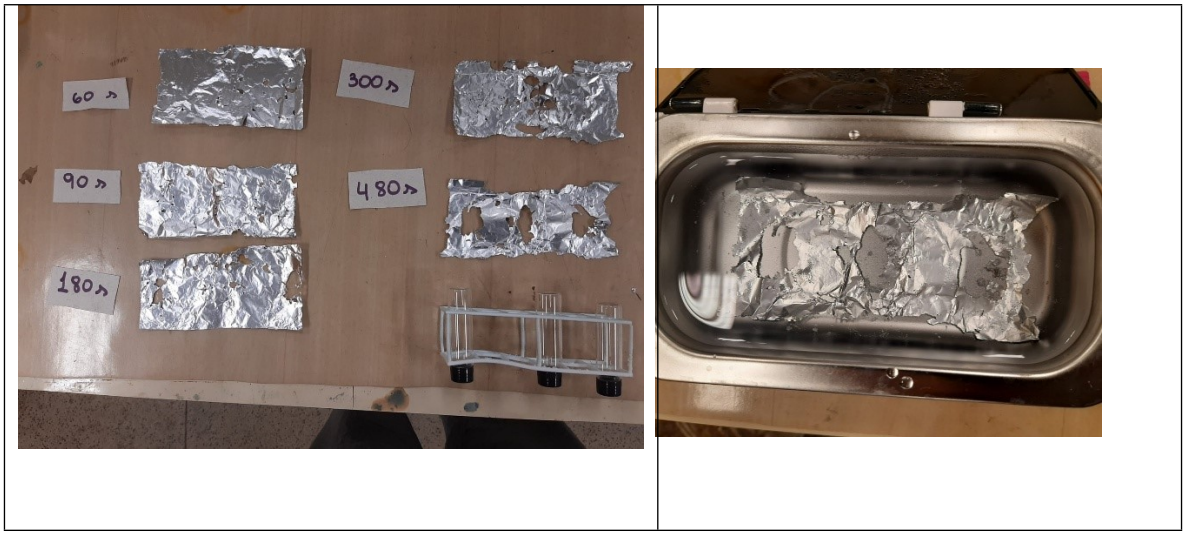


Figure S2. (a) Aluminum foils removed after different sonication times in tests performed with the portable ultrasonic bath to evaluate the distribution and intensity of cavitation within the tank. (b) Image of the aluminum foil after 480 s of sonication, showing perforations and eroded areas that indicate regions of higher cavitational activity in the ultrasonic bath.

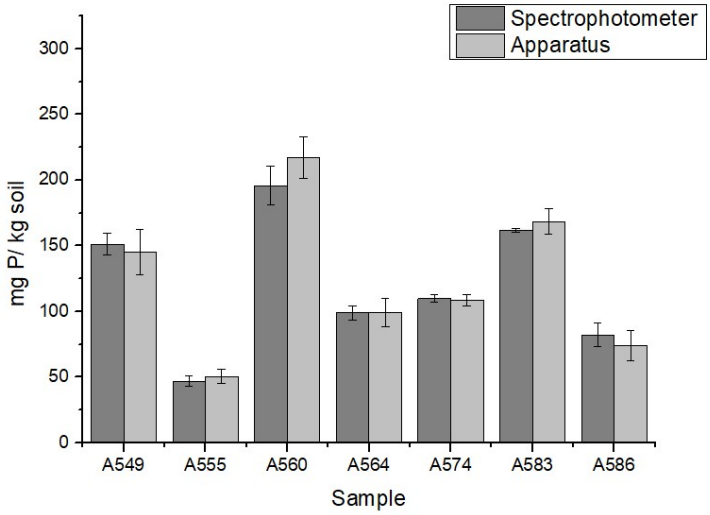
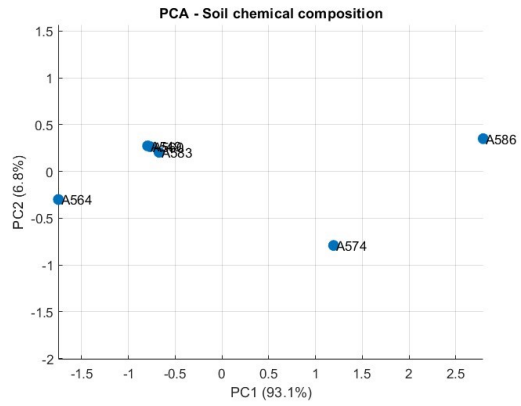


Figure S3. Column graph comparing the available phosphorus values obtained by the spectrophotometric and DIP methods, using the analytical signal: $S = -\log(R/R_0)$.

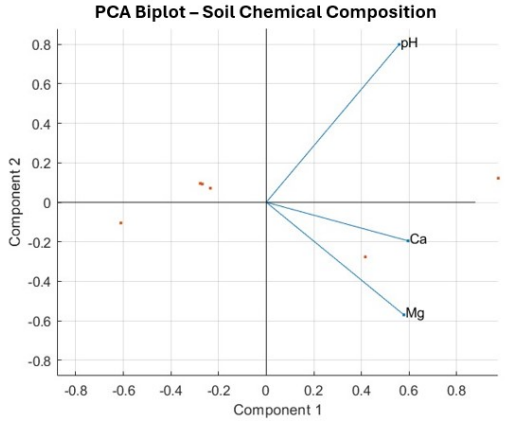
Table S3. Phosphorus concentrations (mg P kg^{-1} soil, mean ± standard deviation) determined by the reference spectrophotometric method and by the proposed Digital Image Processing (DIP) method using the portable apparatus. Measurements were performed in triplicate (n = 3). The data were used for statistical comparison by analysis of variance (ANOVA).

	Spectrophotometry		Apparatus	
Sample	mg P kg^{-1} soil (mean ± SD)			
549	151.07	±8.18	145.18	±17.3
555	46.84	±3.997	50.5	±5.34
560	195.64	±14.82	271.4	±15.88
564	98.803	±5.53	98.793	±10.809
574	109.837	±2.637	108.42	±4.133
583	161.85	±1.356	168.38	±9.63
586	82.11	±8.9	74.01	±11.308

(a)



(b)



(c)

(d)

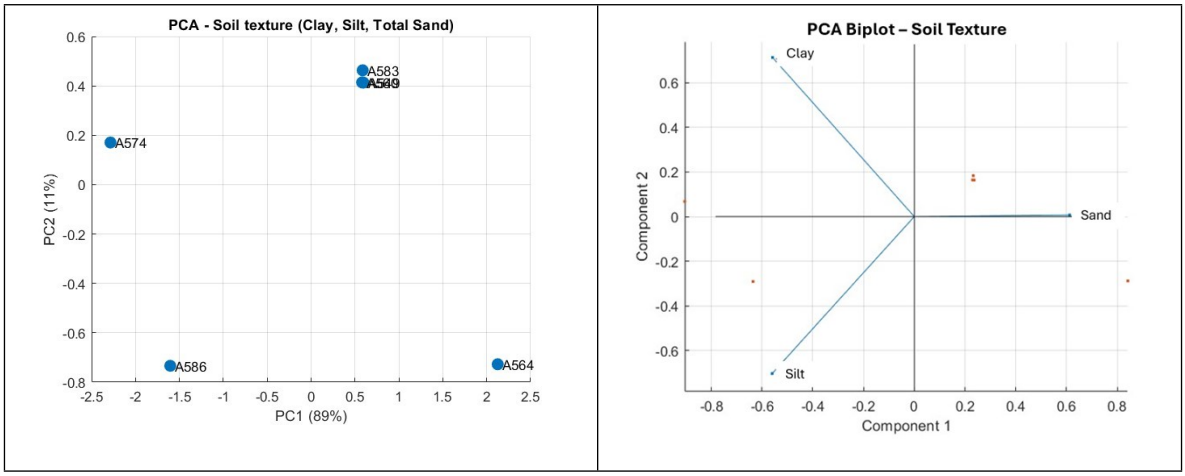


Figure S4. Principal component analysis (PCA) applied to the soil samples, based on ((a) and (b)) chemical composition (Ca, Mg, and pH), and ((c) and (d)) texture (clay, silt, and total sand). Plots (a) and (c) show the distribution of the samples in the space defined by the first two principal components (PC1 and PC2), which explained most of the data variance (93.1% and 6.8% for chemical composition; 89% and 11% for texture). Biplots (b) and (d) display the correlations between the original variables and the main components, highlighting the contribution of each parameter to sample differentiation.

Table S4. Absolute Red (R) channel values obtained for a 1.00 mg P L^{-1} phosphate standard solution under different environmental and lighting conditions, with and without the use of the photographic box. Results are expressed as mean ± standard deviation (n = 3) under intra-day repeatability conditions. The region of interest (ROI) area was fixed at 4421 px^2 for all measurements.

Absolute R values – 1,00 mg P L^{-1}

Location	Using the photography box	Without using the photography box	ROI Area (px^2)
Inside the laboratory	102,3 ± 7	64 ± 27	4421
Ouside the laboratory – Sunny day	109,3 ± 7	43,1 ± 14	4421
Outside the laboratory - cloudy	109,2 ± 7	16 ± 4	4421

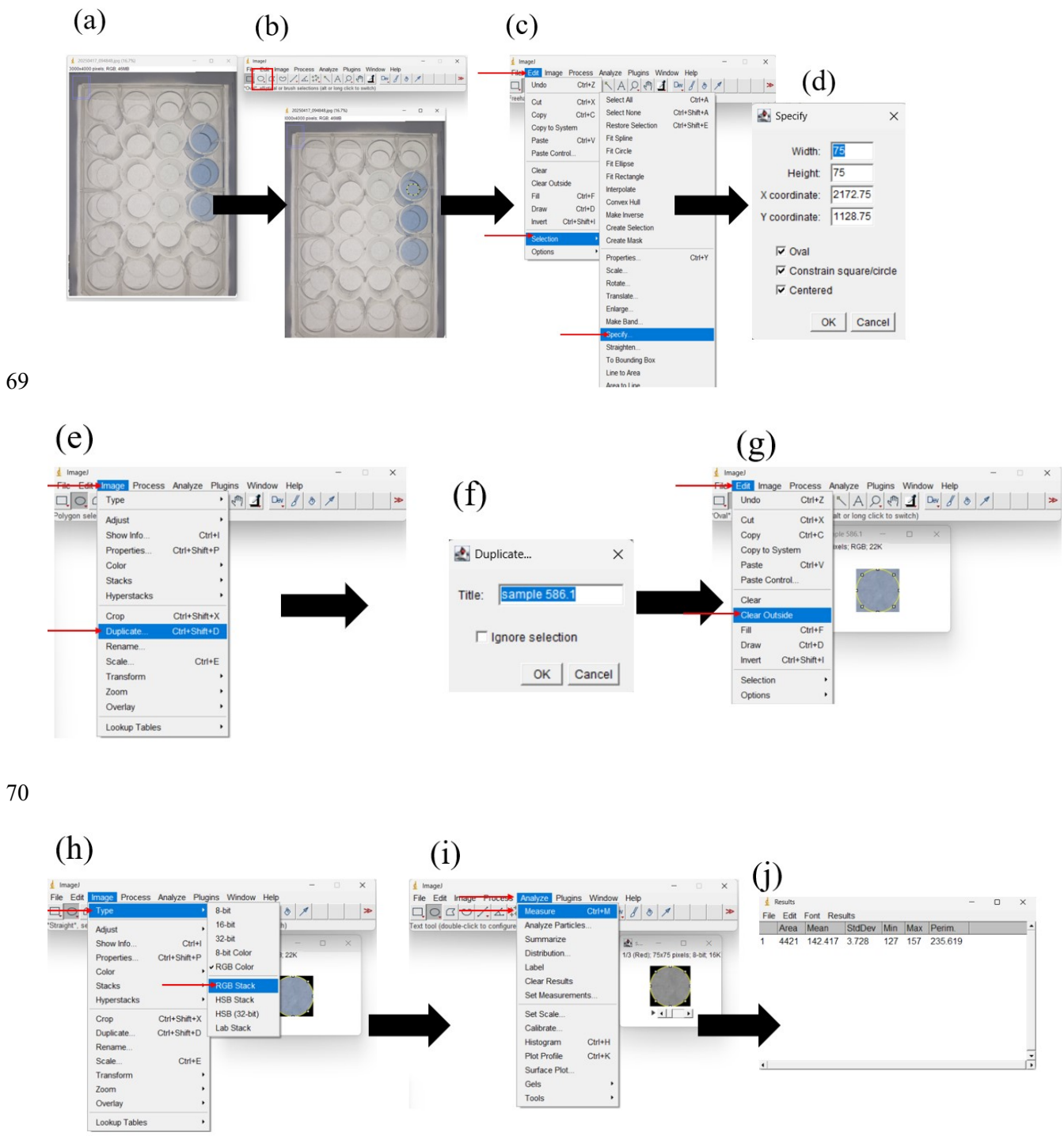


Figure S5. Step-by-step guideline of the procedure performed using ImageJ. (a) The sample image was opened in the software. (b) The oval selection tool was used to define the region of interest (ROI) on the original image. (c) The selected area was standardized by accessing *Edit* > *Selection* > *Specify*. (d) Predefined width and height values were entered to ensure consistent ROI dimensions. (e) The selected region was duplicated via *Image* > *Duplicate*.

(f) The duplicated image was renamed and confirmed by clicking “OK”. (g) The area outside the circular ROI was removed using *Edit > Clear Outside*. (h) The duplicated image was separated into RGB channels by selecting *Image > Type > RGB Stack*, generating three sequential stacks corresponding to the Red, Green, and Blue channels. (i) The intensity of the Red channel was determined using *Analyze > Measure*. (j) The software generated a results window containing the area, mean R value, standard deviation, minimum and maximum pixel values, and perimeter. The results were exported and saved as a *.csv* file for subsequent analysis.