Supplementary material

Low-Cost Precision Agriculture for sustainable farming using paper based analytical devices

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Calcium

When using RGB color space to quantify calcium, concentrations of the nutrient above 4 mmol L^{-1} did not display significant color intensity changes (Figure S1A). As stated in the main text, for calcium detection, the color changed from pink to orange. The more orange the image became, the less the green channel changed, which would be expected since green (the absorbed color) is complementary to pink (the reflected color). However, the intensity of the blue channel changed seamlessly, probably because the RGB model is based on the human vision perception, so it is more sensitive to green than blue. This can also explain why hue color space displayed good performance, but in a limited range as well (Figure S1C). On the other hand, the grayscale (Figure S1D) preserves the same luminance (standard model of human vision) of the original images, which can be why it was the best quantifying method. The parameter “lightness” from the CIELab color space was also a good candidate (Figure S1B), but for calcium concentrations higher than 4 mmol L^{-1} the signal saturated.

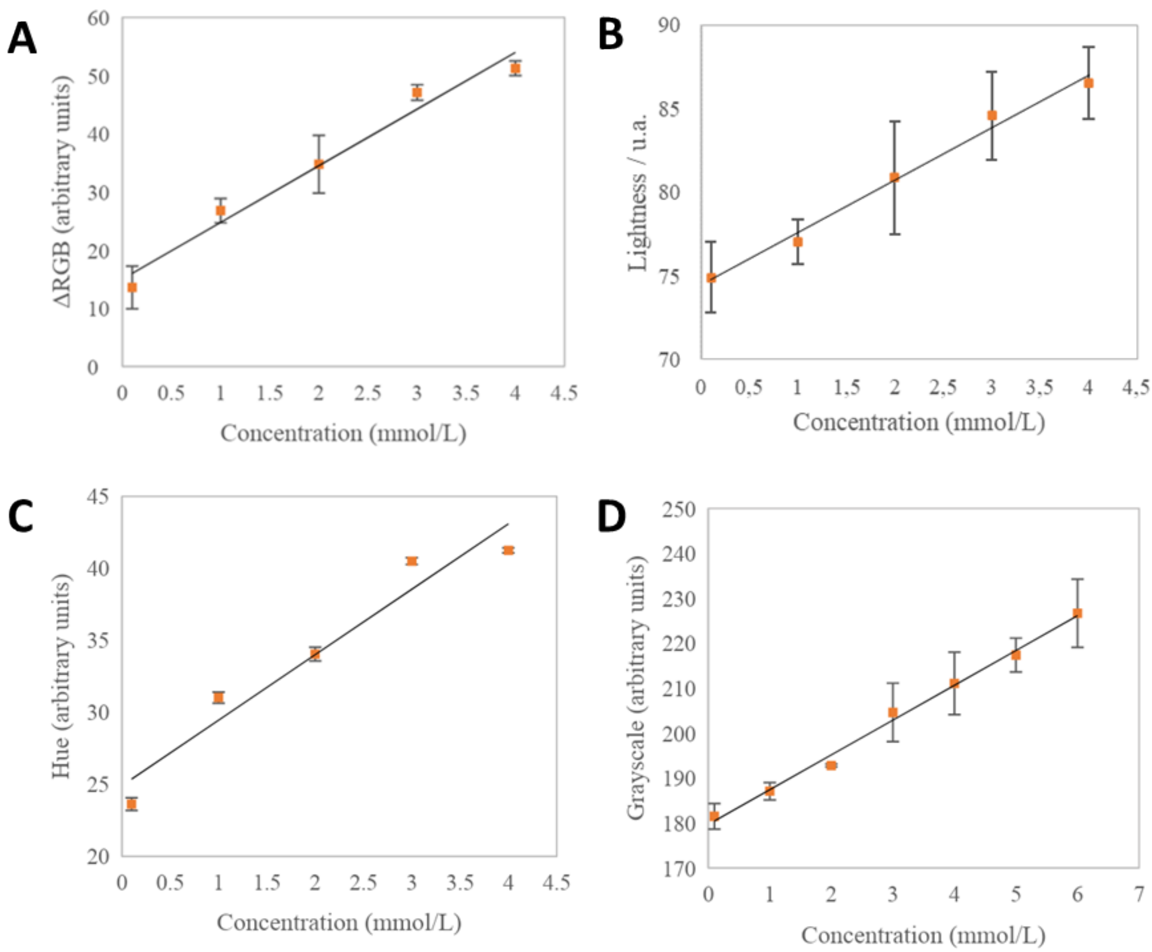

Figure S1 – Calibration curves for calcium using a) ΔRGB; b) lightness (L* component of L*a*b* color space); c) hue (H component of HSV color space) and d) grayscale.

Table S1. Comparison of the correlation coefficient and sensitivity for the detection of calcium in paper-based devices for different color spaces.

Quantification method	R^2	Sensitivity (u.a. L/mol)
ΔRGB	0.973	9.74
HSV	0.989	3.14
CIELab	0.940	4.54
Grayscale	0.993	7.74

Table S2. Comparison of analytical parameters for the detection of calcium in paper-based devices using ΔRGB and gray scale.

Quantification method	LOD (mmol L^{-1})	LOQ (mmol L^{-1})	Intermediate precision (n=5)	Repeatability (n=10)
ΔRGB	0.728	2.428	3.5 %	17 %
Grayscale	0.595	1.984	3 %	4 %

Table S3. Recovery test for ions commonly present in soil. The tests were performed with a fixed concentration of calcium (3.0 mmol L^{-1}), while each interfering agent was added in typical concentrations found in soil solutions. Each ion was individually tested.

Interferent	Interferent concentration (mmol L^{-1})	Relative error
Nitrate	3.0	-1 %
Potassium	2.0	-5 %
Magnesium	3.0	-4%
Ammonium	1.0	-10%
Nitrite	0.1	-9%
Dihydrogen phosphate	0.025	-7%

Table S4. Recovery test for mixes of ions commonly present in soil. The tests were performed with a fixed concentration of calcium (3.0 mmol L^{-1}), while each interfering agent was added in typical concentrations found in soil solutions.

Interferent	Relative error
Magnesium (3.0 mmol L^{-1}) + nitrate (3.0 mmol L^{-1})	1%
Ammonium (1.0 mmol L^{-1})+ magnesium (3.0 mmol L^{-1})	0%
Ammonium (1.0 mmol L^{-1})+ nitrate (3.0 mmol L^{-1})	1%

Magnesium

Hue displayed the best performance to determine magnesium concentrations (Figure S2C). Figure S2A and D shows that RGB and grayscale present no significant correlation with analyte concentration. This might be due to the fact that in these color spaces the intensity was saturated. Indeed, values for all three RGB channels were around 200, which might be the maximum that the camera is capable of detecting under the studied conditions. The parameter b (axis blue-yellow) from the CIELab color space presented a linear correlation with magnesium concentration (Figure S2B). The increase in analyte concentrations led to less negative values of b, representing the blue color. In this case, however, the standard deviation of the measurements was high and the slope was smaller than for the curve obtained using hue (Figure S2C). We believe that hue was able to detect the small changes in color (from blue to purple) with increasing amount of magnesium in solution because it is ideal to indicate color gradients.

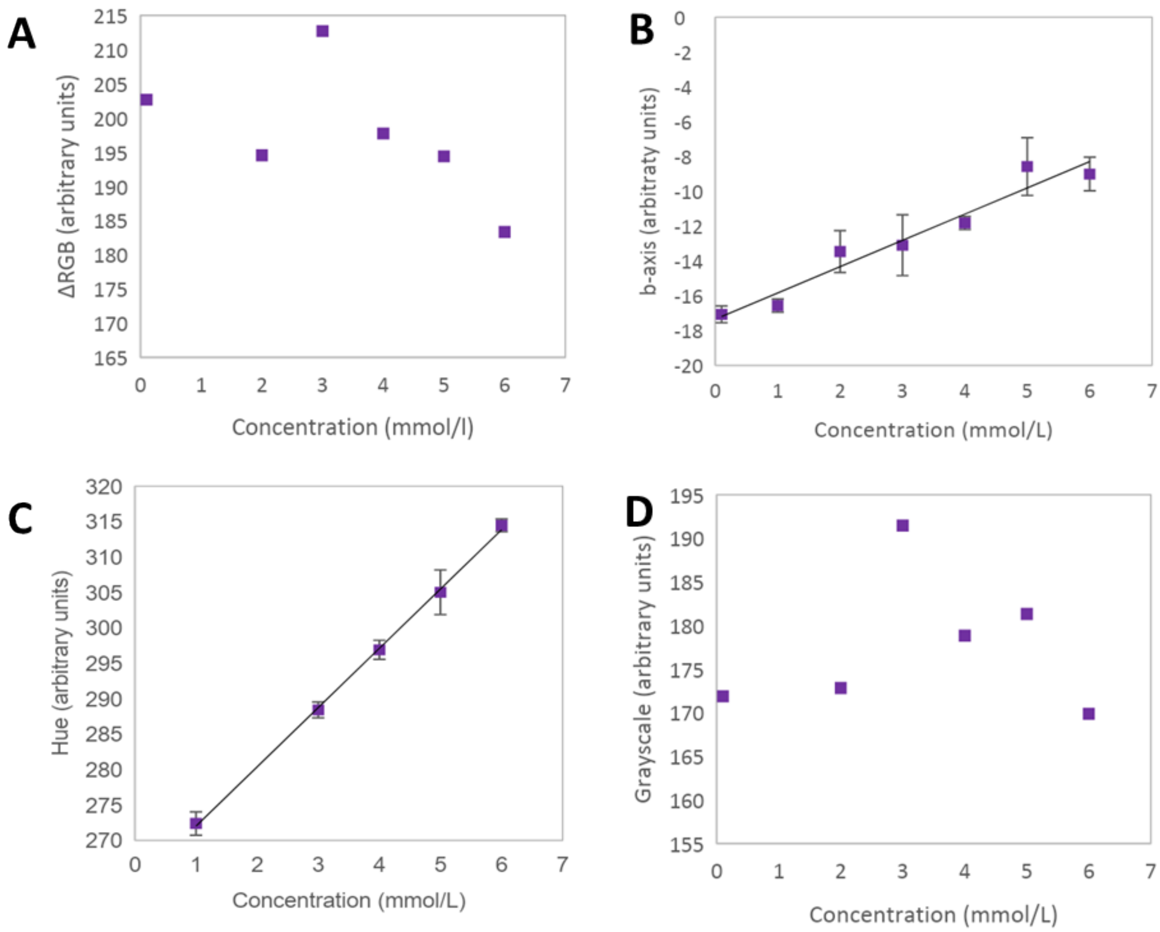

Figure S2 – Calibration curve for magnesium obtained using a) ΔRGB; b) b* parameter from L*a*b* color space; c) hue (H of the HSV color space) and d) grayscale.

Table S5. Summary of analytical parameters for the detection of magnesium in paper-based devices using the HSV color space.

Analytical parameter	**Calculated value**
R^2	0.999
Sensitivity	8.37 a.u. L/mmol
LOD	0.144 mmol L^{-1}
LOQ	0.481 mmol L^{-1}
Intermediate precision (n=5)	0.2%
Repeatability (n=10)	1.0%

Table S6. Recovery test for ions commonly present in soil. The tests were performed with a fixed concentration of magnesium (3.0 mmol L^{-1}), while each interfering agent was added in typical concentrations found in soil solutions. Each ion was individually tested.

Interferent	**Interferent concentration (mmol L^{-1})**	**Relative error**
Calcium	3.0	-0.4%
Potassium	2.0	0.3%
Ammonium	1.0	-0.4%
Nitrite	0.1	0.5%
Nitrate	3.0	-0.6%
Dihydrogen phosphate	0.025	0.7%

Table S7. Recovery test for mixes of ions commonly present in soil. The tests were performed with a fixed concentration of magnesium (3.0 mmol L^{-1}), while each interfering agent was added in typical concentrations found in soil solutions.

Interferents	**Relative error**
Calcium (3.0 mmol L^{-1}) + nitrate (3.0 mmol L^{-1})	-1.1%
Ammonium (1.0 mmol L^{-1}) + calcium (3.0 mmol L^{-1})	1.6%
Ammonium (1.0 mmol L^{-1}) + nitrate (3.0 mmol L^{-1})	0.1%

Ammonium

For ammonium, the parameter which promoted the best analytical performance was hue (Figure S3C). When using RGB (Figure S3A) it was impossible to distinguish different analyte concentrations. Lightness (Figure S3B) has shown a linear response for different ammonium concentrations, but the reproducibility of the measurements was poor probably due to the reflection of light in wet spots. Hue was sensitive enough to detect color changes as a response to the increasing amount of ammonium in the solution. Although the product of the reaction is blue, the values correspond to green because of the excess of reagent sodium nitroprusside, which is yellow.

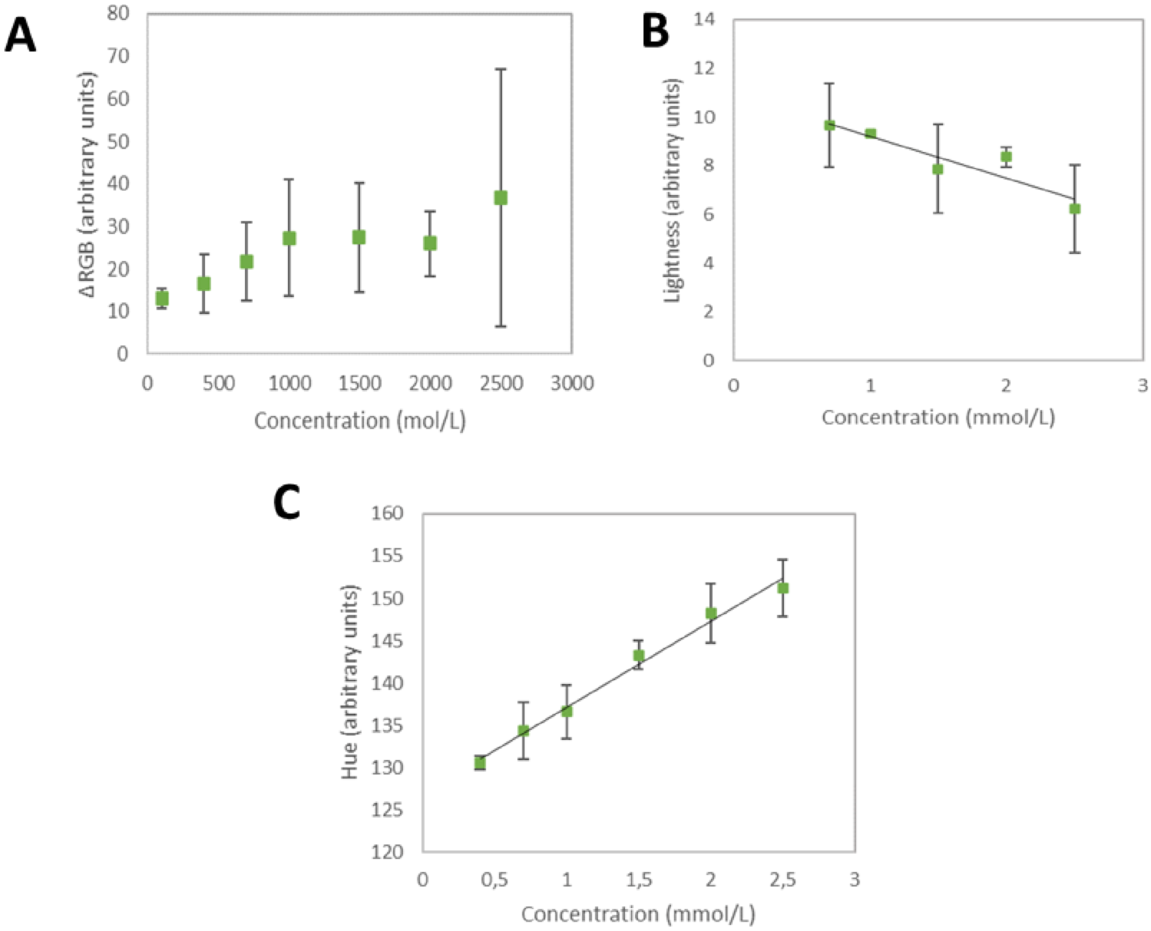

Figure S3 – Calibration curve for ammonium obtained using a) ΔRGB; b) Lightness (L* parameter from L*a*b* color space); c) hue (H of the HSV color space).

Table S8. Summary of analytical parameters for the detection of ammonium in paper-based devices using the HSV color space.

Analytical parameter	Calculated value
R^2	0.988
Sensitivity	10.15 a.u. L/mmol
LOD	0.181 mmol L^{-1}

LOQ	0.602 mmol L^{-1}
Intermediate precision (n=5)	0.8 %
Repeatability (n=10)	0.4 %

Table S9. Recovery test for ions commonly present in soil. The tests were performed with a fixed concentration of ammonium (1.0 mmol L^{-1}), while each interfering agent was added in typical concentrations found in soil solutions. Each ion was individually tested.

Interferent	**Interferent concentration (mmol L^{-1})**	**Relative error**
Calcium	3.0	3%
Potassium	2.0	2%
Magnesium	3.0	2%
Nitrate	3.0	3%
Dihydrogen phosphate	0.025	1%

Table S10. Recovery test for mixes of ions commonly present in soil. The tests were performed with a fixed concentration of ammonium (1.0 mmol L^{-1}), while each interfering agent was added in typical concentrations found in soil solutions.

Interferent	**Relative error**
Calcium (3.0 mmol L^{-1}) + magnesium (3.0 mmol L^{-1})	-5%
Calcium (3.0 mmol L^{-1}) + nitrate (3.0 mmol L^{-1})	-4%
Magnesium (3.0 mmol L^{-1}) + nitrate (3.0 mmol L^{-1})	-4%

Nitrate

Among the channels of the RGB color space, the green channel presented the best linearity with increasing concentration (R^2 = 0.972) of nitrate (Figure S4B). This is because the method chosen for determining the intensity of the colors on the paper is based on the light reflected from the surface of the detection zone. The azo dye formed by the Griess method absorbs in the green region and the observed color is the reflected one - in this case, purple. It was observed that the higher the intensity of the purple color in the detection zone, the lower the intensity in the green channel. When subtracting the intensity of the green channel from the white value (Figure S4C), an increasing difference in intensity is observed, which is linear with the increase in the nitrate concentration in the sample - possibly explaining the better correlation of this channel when compared to blue and red. Table S11 summarizes the parameters for each of the obtained curves.

The L component of the CIELab color space was the one that resulted in the best linearity with the concentration of the analyte (Figure S4D). The L axis refers to brightness and ranges from 0 (black) to 100 (white). When increasing the concentration of nitrate, a decrease in luminosity values was observed. Subtracting the white value from the luminosity intensities, there is an increase in the difference, linear with the increase in the nutrient concentration. However, the sensitivity of the quantification method was one of the lowest among the evaluated methods.

The grayscale can assume values between 0 (black) and 255 (white), referring to 8-bit scanning. In the ColorScan software, gray intensity is given in terms of the saturation of the image. Therefore, with increasing concentration of nitrate in the sample, the region of the detection zone became darker, reaching lower values in the grayscale. Subtracting from the blank value, the intensities increased linearly with the analyte concentration (R^2 = 0.997), with intermediate sensitivity among the evaluated methods (slope = 16.59). Considering the linearity of the method, sensitivity and detection and quantification limits (Table S5), the ΔRGB quantification method was chosen for subsequent studies.

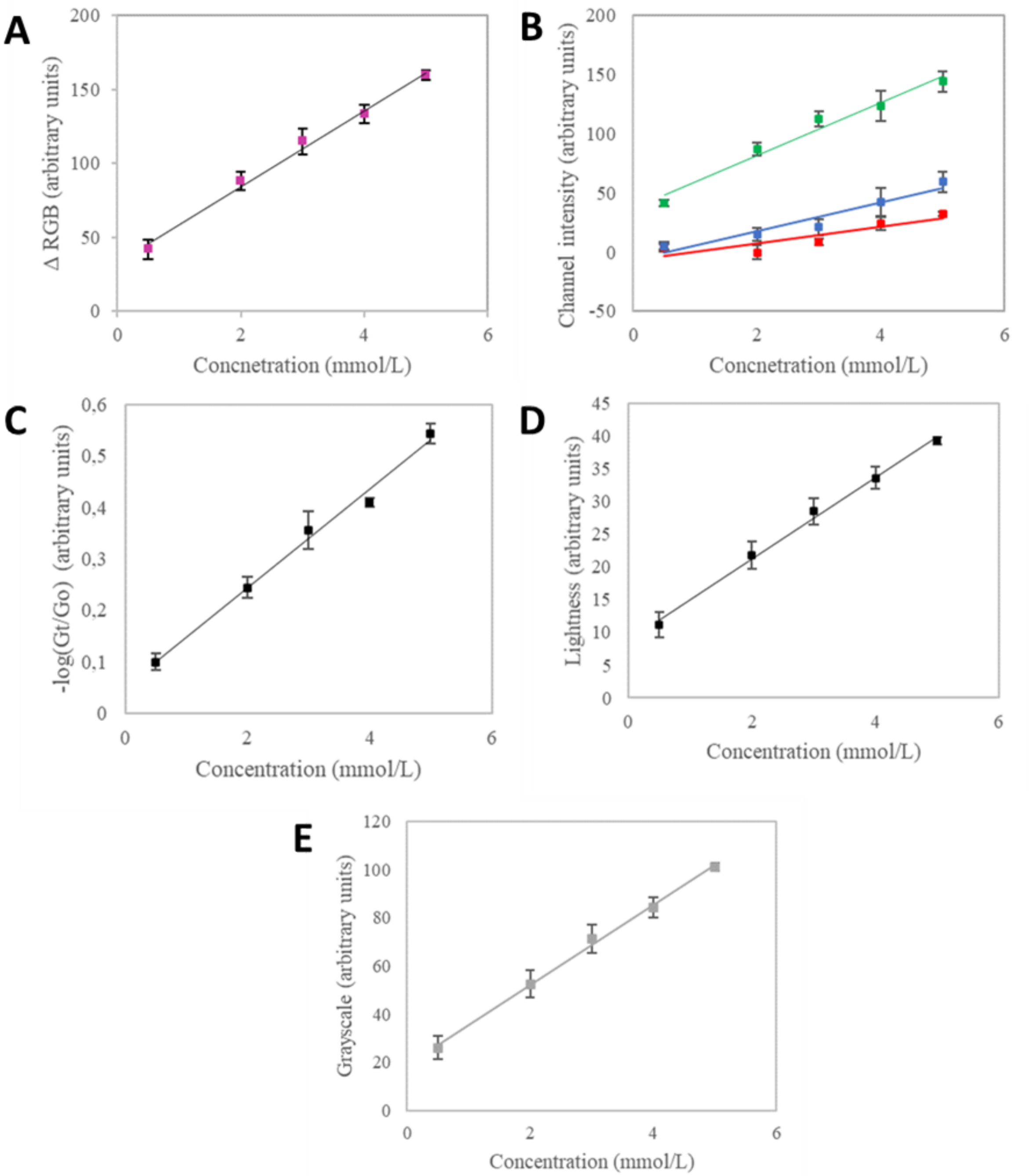

Figure S4 - Calibration curve for nitrate obtained using a) ΔRGB b) R, G, and B channels from RGB; c) -log(Gt/Go), being Gt = intensity of green channel at the concentration t; Go = intensity of green channel for the blank; d) Luminosity (L* component of the L*a*b* color space); e) grayscale.

Table S11. Comparison of the correlation coefficient and sensitivity for the detection of nitrate in paper-based devices for different color spaces.

Quantification method	R^2	Sensitivity
ΔRGB	0.992	25.6
B (RGB)	0.972	22.3
Lambert-Beer (G - RGB)	0.991	0.096
L (Lab)	0.995	6.22
Greyscale	0.997	16.6

Table S12. Comparison of analytical parameters for the detection of nitrate in paper-based devices using ΔRGB and grey scale.

Quantification method	LOD (mmol L^{-1})	LOQ (mmol L^{-1})	Intermediate precision (n=5)	Repeatability (n=10)
ΔRGB	0.229	0.996	8%	4%
Greyscale	0.365	1.220	2%	3%

Table S13. Recovery test for ions commonly present in soil. The tests were performed with a fixed concentration of nitrate (3.0 mmol L^{-1}), while each interfering agent was added in typical concentrations found in soil solutions. Each ion was individually tested.

Interferent	Interferent concentration (mmol L^{-1})	Relative error
Calcium	3.0	-7%
Potassium	2.0	2%
Magnesium	3.0	-4%
Ammonium	1.0	-1%
Nitrite	0.1	-1%
Dihydrogen phosphate	0.025	-4%

Table S14. Recovery test for mixes of ions commonly present in soil. The tests were performed with a fixed concentration of nitrate (3.0 mmol L^{-1}), while each interfering agent was added in typical concentrations found in soil solutions.

Interferent	Relative error
Ammonium (1.0 mmol L^{-1}) + calcium (3.0 mmol L^{-1})	1%
Ammonium (1.0 mmol L^{-1}) + magnesium (3.0 mmol L^{-1})	2%
Magnesium (3.0 mmol L^{-1}) + calcium (3.0 mmol L^{-1})	-22%