5. Detection Methods

5.2 Photometry

5.2.4 Malachite green in lowest concentration range, in soil science

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Principle

Similar to the molybdenum blue method (chapter 5.2.3) a green colour complex is formed under acidic conditions by molybdate, malachite green and phosphate. The extinction of the blue green colour complex is measured at a wavelength of 623 nm and used for determination of P concentrations. The method of Altmann et al. (1971) is used in the working group Soil Science to measure P concentrations in $NaHCO_3$- and NaOH-extracts of soil samples. The calibration line in table 5.2.4-1 was created in a water matrix to test the linear range of the method.

Concentration range

The linear range of the method is between 20 µg or 0.6 µmol and 250 µg or 8 µmol l^{-1}. For this range, the limit of quantification was with 39 µg P l^{-1} (1.3 µmol l^{-1}) very high, even if the reagent blank was subtracted from all blanks. Here, the limit of quantification should be calculated according to the calibration line method (chapter 6.2).

Procedure

Preparation:

- Fill graduated 25 ml test tubes or other volumetric flaks with ultra-pure water (RW) and leave to stand overnight for emaciation.
- If necessary, dry KH_2PO_4 for 2 hours at 40 °C and cool down in desiccator, alternatively use commercial P standards.

Calibration line:

- Pipette from P working solution in table 5.2.4-1 specified ml in 25 ml graduated test tubes or volumetric flasks for standards.
- Fill to 25 ml with RW.
- Calibrants contain up to 250 g P l^{-1} or 8,1 µmol l^{-1}.

- For a linear calibration line, it is recommended to set standards between 20 and maximal 250 µg P l^{-1} (Tab. 5.2.4-1).

Table 5.2.4-1 Calibrant number, volume of working solution, absolute P amounts and P concentrations for calibration line for testing the linear range after filling to 25 ml in a water matrix

Cal. No.	0	1	2	3	4	5	6	7	8
Working solution ml	0	1	2.5	3.5	5	6	7.5	10	12.5
µg P absolute in 25 ml	0	0.5	1.25	1.75	2.5	3	3.75	5	6.25
µg P l^{-1}	0	20	50	70	100	120	150	200	250
µmol P l^{-1}	0	0.65	1.61	2.26	3.23	3.87	4.84	6.46	8.07

Setting of samples and measurement:

- Pipette in the standard vessels the same amounts of calibrants as samples are given into the sample vessels (e.g. 10 ml $NaHCO_3$-extract).
- Calibrants should be dissolved in the same matrix like the samples.
- Add, for example, 10 ml sample or blank into the vessels for samples and blanks.
- Fill all vessels (standards, samples and blanks) to ca. 12 ml with RW.
- Add 1.5 ml of 24 % H_2SO_4 in all vessels and wait 10 min for silicate elimination.
- Add 2.5 ml malachite-PV-solution and swing for mixing (do not shake too strong, to avoid creation of foam). Wait around 5 min.
- Add 2.5 ml molybdate solution, fill vessels with RW to 25 ml and swing for mixing. Wait 1 hour for colour development.
- Fill aliquots into cuvettes and measure extinction at 623 nm.
- Dilute samples with extinctions > 1, set them again in 25 ml test tubes or volumetric flasks with all reagents and measure again.

A very long calibration line in a water matrix shows the restricted linear range (Fig. 5.2.4-1). A concentration higher than 250 µg P l^{-1} (extinction 0.62 in 1 cm cuvettes) saturation effects are visible. At a concentration of 1000 µg P l^{-1} the malachite green flocculates.

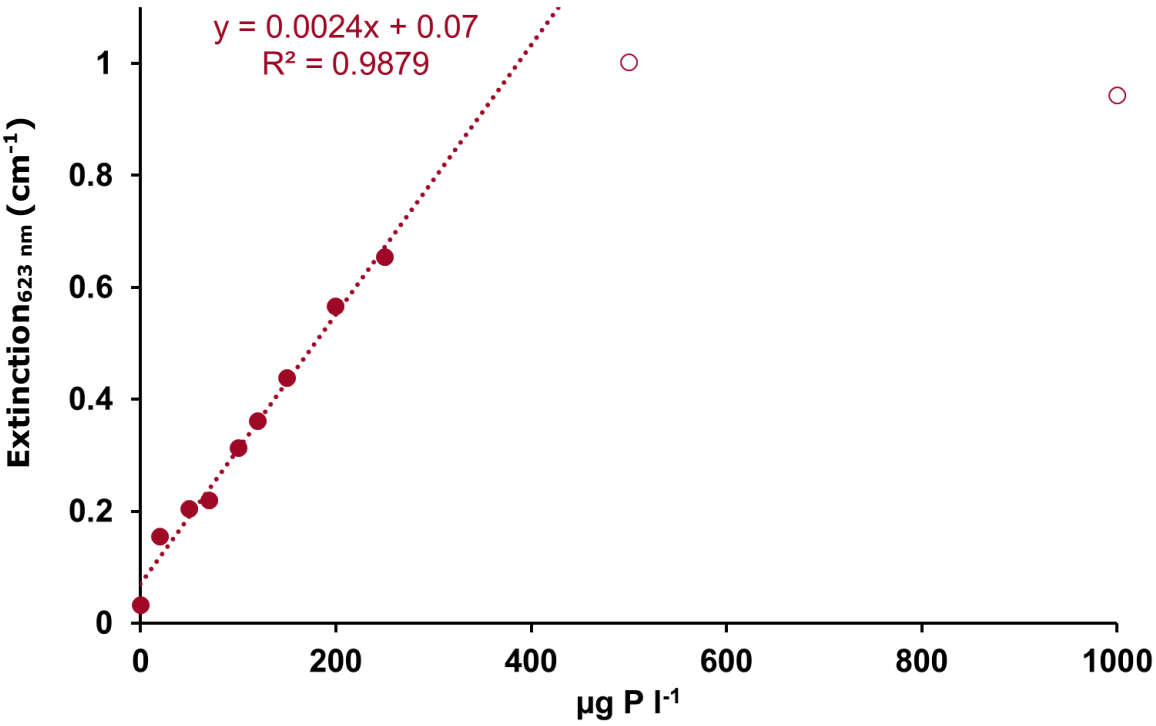

Figure 5.2.4-1 Example of a calibration line for malachite green in a water matrix. The factor for conversion into µg P l^{-1} is here for a 1 cm cuvette 410. Open circles are higher concentrations outside the linear measurement range.

Quality management

Reagents of the malachite green method have a weak green-yellow inherent colour. That means that the extinction of the reagent blank has to be subtracted from all values (samples, blanks and calibrants).

Sometimes it is recommended, and often the software offers it as well, to set this value in the photometer or set it as reference in the photometer. This is not recommended, because simple operating errors can have serious consequences. Errors can be for example: at reference measurement the cuvettes were not completely clean, the blank could be turbid by lint or something similar. Additionally, a drift or a suddenly higher blank is not visible and cannot be corrected later.

Calculation

According to the inherent colour of the reagents, the subtraction of the reagent blank is urgently required. Another question is the stability of the reagent blank. For the calibration line itself, it is not important for calculation of the conversion factor. The increase is shifted parallelly along the y-axis. In each case, either the reagent blank of the sample or the reagent blank of the calibration line has to be subtracted.

$PO_4^{3-} = F \cdot (E_{sample} - E_{RBW} - E_{FBW})$

PO_4^{3-}	Phosphate concentration (mg P l^{-1})
F	Factor of calibration line[1]
E	Extinction[2]
RBW	Reagent blank
FBW	Absorption of the filtrates

Chemicals

- Ultra-pure water (RW) is deionized silicate free water: molybdate reacts also with silicate, which can be released from certain glasses or ion exchangers in larger amounts. Measurement of natural (low) silicate concentrations is suppressed by the measurement conditions. Anyhow, deionat from silicate free ion exchangers has to be used, e.g. from Rostocker Kraftwerk or high-quality ultra-pure water systems have to be used, e.g. Milli-Q.
- **24 % H_2SO_4:** give around 50 ml RW in a 100 ml volumetric flask, set the flasks in a cold-water bath and add slowly 25 ml concentrated H_2SO_4 (96 %, 18 M). Attention, the solution gets very hot! Fill with RW a bit below the calibration mark, cool down until the next day and fill to 100 ml.
- **PVA-solution:** dissolve 5 g polyvinyl alcohol by cooking (e.g. above Bunsen burner) in a 500 ml RW in a beaker (use a glass rod for stirring). Add PVA in small amounts (hardly soluble) and filter subsequently by folded filters. The PVA is not dissolved completely, some of it remains in the filter!
- **Malachite-polyviol-solution:** give 250 ml of PVA solution into a beaker or Erlenmeyer flask, add 92.5 mg malachite green, add a magnetic stirrer. Stir for around 3 hours on the stirrer. Transfer into a 500 ml volumetric flask, rinse with RW, fill to 500 ml. The solution can be stored in darkness for 1 month.
- **0.015 M Molybdate solution:** dissolve 1.854 g hexaammonium heptamolybdate $(NH_4)_6Mo_7O_{24}$ x 4 H_2O in around 50 to 70 ml RW in a beaker with magnetic stirrer on the stirrer. Transfer into a 100 ml volumetric flask and fill to 100 ml with RW.

[1] Increase of calibration line, if extinction is on the x-axis and concentration on the y-axis.
[2] Always take the same cuvette length!

- **Standards**
  - P stock solution: weigh in 0.2197 g potassium dihydrogen phosphate (KH_2PO_4) (dried at 40 °C for 2 h), transfer into a 1 litre volumetric flask with RW. The solution contains 50 mg P l^{-1} and can be stored for 1 week in the refrigerator.
  - P working solution: give 10 ml of P stock solution into a 1 litre volumetric flask and fill with RW. The solution contains 500 µg P l^{-1}.

References

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For citation: Zimmer D, Schumann R *(year of download)* Chapter 5.2.4 Malachite green in lowest concentration range, in soil science (Version 1.1) in Zimmer D, Baumann K, Berthold M, Schumann R: Handbook on the Selection of Methods for Digestion and Determination of Total Phosphorus in Environmental Samples. DOI: 10.12754/misc-2020-0001