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Cover paint: Wenceslaus, "August", from the "Cycle of the Months" frescoes, painted around 1400 in the Eagle Tower of the Castle of Buonconsiglio, Trento, Italy.
MONITORING NITRATES IN DRINKING WATER WITH A PORTABLE REFLECTOMETER

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Introduction
The maximum admissible concentration (MAC) of nitrate in drinking water is 50 mg/l (EEC, 1991). This limit was established because of the toxicity of nitrate/nitrite to human health, though the discussion about the acceptable daily intake of nitrate persists (Boink and Speijers, 2001).

Bragança is a rural district of the NE Portugal with a significant part of its population dispersed among little villages with 50 to 500 inhabitants. Despite practically the whole territory having a public supply of piped potable water many people prefer to collect the water for drinking from old fountains of local and shallow captation of water, perhaps to avoid the taste of chlorine. Due to the great number and dispersion of those fountains the sanitary authorities have not the means to monitor their water quality. Taking into account that this is a farm region, it is logical to suppose that nitrate could be an important contaminant of water.

In this work results of the nitrate concentration in water, determined with a portable reflectometer, of more than 40 fountains and some rivers, streams and shallow lakes of the region were presented. The objective of the work was also to evaluate the potentialities of the portable tool to determine on site the concentration of nitrate in water.

Material and methods
The concentration of nitrate in water was determined several times during the period June, 2001-March, 2002. The analyses of nitrate in water were performed on site using Reflectoquant® test strips and the portable reflectometer RQflex® (MERCK). When a test strip is immersed into a solution that contains nitrate a red-violet azo dye is formed, the concentration of which is determined reflectometrically. The RQflex measures the amount of light reflected and converts the reflectance into nitrate in solution. After every one of the RQflex-nitrate readings the temperature of the water was also recorded.

The accuracy of the RQflex reflectometer was checked in the laboratory using standard calcium nitrate solutions within the measuring range of the test strips. The sensivity of the test strips to water temperature was also calibrated in the range of 2 to 30 °C.

Results and discussion
The calibration of the RQflex results with standard solutions of Ca(NO₃)₂ showed that nitrate concentrations were underestimated by the RQflex (figure 1a). The statistical test of hypothesis $H₀: β₁ = 1$ showed that the slope of the straight line was significantly lower than 1 ($β₁ = 0.98$), it being necessary to correct on site measurements. On the other hand, the coefficient of determination associated to the linear model was very high ($r² = 0.998$). Calibration of RQflex results was also needed depending on the temperature of the water. The reactivity of test strips had a linear decrease for temperatures of water lower than 20 °C (figure 1b).

The results of the six fountains most polluted with nitrates are presented in figure 2. In the autumn, after the first rainfalls, the concentration of nitrate in water increased slightly, maybe due to the leaching of nitrates from soils which are available after the mineralization of summer crop residues and N-fertilization of winter crops. However, among the 40 fountains monitored, only in one of them did the values of nitrate in water exceed MAC (50 mg NO₃/l). The result could be justified by the fact that the points of captation of water were in the upper and sloped lands and therefore in the areas with less intensive agriculture.
The nitrate concentration in water of rivers, streams and shallow lakes was always lower than 5 mg/l NO₃⁻ (data not shown). The large volumes of water involved (dilution factor), the fact that the agriculture in general is not intensive in the use of fertilizers and the large areas of sloped and uncultivated soils involved could combine to explain the results.

![Graph](image)

Figure 1 – (a) relation between nitrate concentration in standard Ca(NO₃)₂ solutions and RQflex readings; and (b) reactivity of test strips to water temperature.

![Graph](image)

Figure 2 – Water nitrate concentration of the six most contaminated fountains.

Conclusions
The results showed (i) an environmentally-sound region where the water is not yet contaminated with nitrates, and (ii) the usefulness of that portable tool in monitoring the nitrate concentration in water, although previous calibration of its results seems to be necessary.

References
USE OF CHLOROPHYLL-SPAD METER ON POTATO AND SORGHUM

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Introduction
The SPAD-502 chlorophyll meter measures the transmittance of leaves at 430 nm, which is a peak of maximum absorbance by chl, providing an indication of its chl content. Chlorophyll content of leaves or SPAD readings can be used as a nitrogen nutrition index of plants since most leaf N is contained in chl molecules. In order to avoid the interference of factors besides N fertility on the interpretation of results, Peterson et al. (1993) suggest the use of a N sufficiency index calculated as follows:

\[ \text{Sufficiency index} = \frac{\text{Average bulk readings}}{\text{Average reference strip readings}} \times 100\% \]

The reference strips must be well supplied with N. A sufficiency index lower than 95% indicates a shortage of N. In this work we study the relation between SPAD readings and laboratory measurements of chl pigments on potato and sorghum as a function of N applied. We inquire into some aspects of the use of the N sufficiency index related to the reference strips.

Material and methods
In the experimental farm of the Polytechnical Institute of Bragança two N-fertilizer experiments with potato and sorghum were imposed on a loamy textured soil. During the growing season the chlorophyll content of leaves was measured in the field with the SPAD meter and determined in the laboratory. The laboratory method uses 1 cm² of green tissue and methanol as solvent and the extracts are read spectrophotometrically in 3 wavelengths: 470, 651 and 664 nm. Lab chl analysis and SPAD readings were made on 15 randomly chosen plants per plot at mid-length for sorghum and at the distal leaflet for potato on the uppermost fully expanded leaves. The leaf's midrib was avoided in both crops.

Results and discussion
The quadratic relations found between laboratory and SPAD readings of chlorophyll indicated that the SPAD meter underestimated the chl content of leaves for most fertilized plants, with higher chl content (fig 1), relatively to the lab method. Changes in the relation between the two chl pigments as a result of N fertilization (fig 2) with probable changes in peaks of absorbance and the fact that the laboratory method reads in two wavelengths (651 nm for chl a and 654 for chl b), whereas the SPAD meter only reads one wavelength (430 nm), can justify the result. The chlorophyll content of leaves (lab method) as well as the SPAD readings increased with moderate N rates and decreased with high N rates (fig 3 and 4). The initial increase is justified by the synthesis of more chl associated with more available N. The decrease is attributed to the lesser thickness of the leaves, with less optical density, caused by higher N rates and higher mutual shading of plants. With SPAD readings that effect is more evident and the reasons for this are the same as those presented to justify the results of fig 1. The effect was also more evident for the potato crop because of the higher extinction coefficient of its canopy.

Conclusion
The SPAD meter seems to have limitations in detecting minor changes in chlorophyll pigments that could occur, motivated by crop management and ecological factors, in so far as the portable tool only reads one wavelength.

The results suggest that there must be caution in the use of N sufficiency index on crops like the potato, where the effect of high N rates on chlorophyll-SPAD readings could be confounded with the effect of insufficient N. In this crop the reference strips would never be supplied with excessive N.
Potato, 38 days after emergence

Potato, 65 days after emergence

Sorghum, 4 leaf stage

$R^2 = 0.30$

$R^2 = 0.47$

$R^2 = 0.68$

Figure 1 – Relation between leaf chlorophyll (lab. method) and SPAD readings.

Chlorophyll $a$ (µg ml$^{-1}$)

Chlorophyll $b$ (µg ml$^{-1}$)

Figure 2 – Relation between chlorophyll $a$ and chlorophyll $b$ for all N-fertilizer treatments.

= Confidence limits of means ($\alpha < 0.05$)

Figure 3 – Relation between N applied and total ($a+b$) leaf chlorophyll.

SPAD readings

Figure 4 – Relation between N applied and SPAD readings.

References