

Development of a quick on-farm test to determine nitrate levels in soil

Urs Schmidhalter^{1*}

¹ Chair of Plant Nutrition, Technical University of Munich, Am Hochanger 8, D-85354 Freising, Germany

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Summary—Zusammenfassung

Crop management can be optimized and nitrogen (N) losses can be reduced with a better knowledge of soil-nitrogen availability, especially if this information becomes directly available on-site in a fast and cost-effective way. In this paper, simple on-farm methods to determine nitrate-N in field-moist soil samples immediately after sampling are described. The procedures include volumetric soil sampling, extraction based on manual shaking with tap water as universally available extractant, filtering soil/extractant mixtures on-site, on-site determination of the soil water content, and reflectometric nitrate measurements based on test strips. Using correction factors can compensate the impact of the temperature during the final nitrate measurement. An excellent agreement was found between the developed quick-test procedures and the standard laboratory procedure. The proposed quick-test has great potential to enable economical savings for farmers as well as benefiting the environment.

Key words: nitrate / nitrogen availability / on-farm test / reflectometer / soil / soil water content

1 Introduction

A better knowledge of soil-N availability is important for optimizing crop management and for reducing N contamination of groundwater and other N losses. Taking into account soil mineral-N content (*i.e.*, extractable nitrate- + ammonium-N) in fertilization decisions during the growing season has a long tradition in some countries. In Germany, where such strategies have long been advised, they are mostly applied to vegetables. However, in agricultural crop rotations less than one percent of all fields are sampled for available soil N each year. Even less information on soil N status is available for farmers in most developing countries.

Extension services and farmers most often carry out the soil sampling, while the analysis for soil mineral N is done *ex-situ* in a laboratory. Unwanted mineralization is a particular problem if soil samples are not adequately cooled after sampling and during shipping/storage and may lead to erroneous values. If samples are frozen, thawing may substantially increase the nitrate content (Esala, 1994). Furthermore, the necessity of sending the soil samples to a laboratory means that it may take several days to weeks until the farmer

Entwicklung eines Schnelltestes zur Bestimmung des Nitratgehaltes in feldfeuchten Böden vor Ort

Eine bessere Kenntnis der Stickstoff (N)-Verfügbarkeit des Bodens trägt zu Verbesserungen in der Bewirtschaftung und zu reduzierten Stickstoffverlusten bei, insbesondere wenn diese Information unmittelbar vor Ort rasch und billig zur Verfügung steht. Einfache Schnellmethoden zur direkten Bestimmung von Nitrat in feldfeuchten Bodenproben unmittelbar nach der Entnahme vor Ort werden beschrieben. Die vereinfachte Prozedur ermöglicht es, volumenbezogene Bodenproben zu entnehmen und die Lagerungsdichte des Bodens verlässlich zu bestimmen. Als universal erhältliches Extraktionsmittel kann Leitungswasser eingesetzt werden. Minimale Schüttelzeiten für die Extraktion wurden bestimmt. Die Nitrat-Messungen werden danach im klaren Überstand der Lösung oder nach einer stark vereinfachten Filterprozedur reflektometrisch mit nitratsensitiven Teststäbchen durchgeführt. Der Bodenwassergehalt kann unmittelbar vor Ort vereinfacht bestimmt werden. Die Temperaturabhängigkeit der Nitrat-Bestimmung kann durch die Nutzung von Korrekturfaktoren kompensiert werden. Die vorgeschlagenen Schnelltestmethoden zeigten eine sehr gute Übereinstimmung mit Standardbestimmungen im Labor. Die Methoden sind anwenderfreundlich und können zu ökonomischen Einsparungen bei den Landwirten führen sowie einen Beitrag zur Entlastung der Umwelt leisten.

receives the results. Finally, it has to be considered that for most farmers in developing countries, costs for this analytical service are unaffordable.

In recent years, substantial efforts have been directed towards improving this situation with a focus on simplifying the analytical part of the soil-mineral-N test. Quick-test methods such as nitrate-sensitive colorimetric test strips (Schaefer, 1986; Jemison and Fox, 1988; Roth et al., 1991) for immediate nitrate analysis are frequently used for soil, plant, or water analyses nowadays. However, previous nitrate-sensitive colorimetric test strips of reagent-impregnated paper (Hunt et al., 1979), reflectometric quick-tests (*e.g.*, Schaefer, 1986), or nitrate-selective electrodes (Hartz et al., 1993) have to be characterized as semi-quantitative. Moreover, detection limits of 5 mg NO₃⁻-N (kg dry soil)⁻¹ for these tests meant that they were appropriate only for detecting relatively high soil nitrate contents. Nevertheless, it has also been shown previously that after careful calibration, accurate results can be obtained (Schlaghecken, 1984; Nitsch, 1985). Recent technical improvements have resulted in significantly enhanced nitrate sensitivity of the test strips and an improved reflectometer using modern bar-code technology requiring no extra calibration (Schmidhalter, 1994).

Comparatively little effort has been directed towards simplifying the steps preceding the nitrate analysis. Once soil sam-

* Correspondence: Prof. Dr. U. Schmidhalter;
e-mail: schmidhalter@wzw.tum.de

ples have been obtained, they need to be transported elsewhere for nitrate extraction and filtering for the subsequent analysis. Furthermore, the determination of soil water content requires drying of the soil samples, which further delays the availability of the information. These steps have been identified as main limiting factors for using quick-tests (Scharpf and Wehrmann, 1986). An adequate assessment of soil bulk density also influences the accuracy of soil-nitrate analyses (Schmidhalter et al., 1991). Gravimetric soil sampling is used nearly exclusively to obtain samples for soil-nitrate analysis, and the rather imprecise soil-bulk-density estimates may entail further deviations from the correct result.

To overcome the above-mentioned limitations, all steps of the procedure need to be simplified so that they can be carried out on-site. This paper describes such simplified on-farm soil-nitrate analysis procedures.

2 Materials and methods

2.1 Soils

The soils used for the development of the procedure cover a wide range of soil textures, ranging from sandy to clayey soils with soil nitrate contents varying from close to 0 up to 100 kg NO₃-N ha⁻¹ in a 30 cm soil layer. Most soils originated from temperate regions (Switzerland, Germany), although the method was also tested with soils from arid (Niger) and tropical zones (Thailand, Brazil).

The development of a quick-test to determine on-site the gravimetric soil water content included 66 soil samples varying in gravimetric soil water contents from 0.0 to 0.50 g g⁻¹. For the determination of shaking times to extract nitrate, four soils representing different soil textural classes (clay, clay loam, silt loam, and sandy loam, respectively) were chosen. These soils were spiked with nitrate to obtain three different nitrate levels for each soil.

2.2 Soil sampling

Soils were either sampled with a stainless-steel auger (inner diameter 6 cm) down to a soil depth of 90 cm (3 layers: 0–30 cm, 30–60 cm, and 60–90 cm) or using a 5 cm (soil layer 0–30 cm) and a 3.5 cm (soil layer 30–60 cm) diameter stainless-steel soil-sampling tube, respectively. This hand-held sampler consists of a 30 cm long stainless-steel outer tube with an inner tube holding a PVC casing in which the undisturbed soil sample is collected resulting in a volumetric sampling (Max Hug, Luzern, Switzerland). The 3.5 cm tube with a longer handle was inserted in the previously sampled 5 cm hole and then driven to a soil depth of 60 cm.

Based on previous investigations, 10–15 soil cores were pooled to obtain a representative sample (Schmidhalter et al., 1991, 1992). The soil sample was thoroughly blended by hand, and passed through a 10 mm sieve for adequate homogenization.

2.3 Soil bulk density

Soil bulk density was determined with a classical method using cylinders 100 mm wide and 60 mm in height according to Grossmann and Reinsch (2002) and compared to the hand-held volumetric soil sampling-tube method. Thirty-six soil cores, with 12 for each soil depth from 0–30, 30–60, and 60–90 cm, were used for this comparison. The weights of the volumetric soil cores were determined with an inexpensive solar-driven balance. The gravimetric water content of bulked soil samples was determined immediately thereafter with the quick-test procedure described below. Soil bulk density was calculated as dry mass per unit volume of soil.

2.4 Extraction

As universal applicability was the main objective when developing the procedure, tap water was thought to be the extractant being available everywhere. Therefore, 22 soils were selected, and nitrate content was determined using tap water as well as 0.01 M CaCl₂, which is one of the standard extractants in commercial labs and was also used for the other investigations in this study. Minimal shaking times for the extraction of nitrate were determined. Soil/extractant mixtures were manually shaken for 1, 3, or 5 min, and compared to 1 h shaking using a laboratory head-over-head shaker as a reference.

2.4.1 Standard extraction procedure (quick-test method A)

A subsample of about 100 g field-moist soil, weighed to a precision of ±1g, was filled into a 325 mL plastic beaker, and 150 mL of extractant was added. The beaker was then capped and the soil/extractant mixtures were manually or mechanically shaken. Generally this was below 10 min and compared to 1 h shaking by a laboratory head-over-head shaker as a reference. The soil water content of a separate subsample was determined by weighing before and after drying.

2.4.2 Cylinder method with simultaneous determination of soil water content (quick-test method B)

A graduated 500 mL plastic cylinder (50 mm diameter) was filled with 250 mL extractant, and about 100 mL field-moist soil were added. The weight of the added moist soil was determined to a precision of ±1g. Then the cylinder was capped with a stopper and thoroughly shaken for a recommended time to obtain adequate recovery of nitrate. Vigorously shaking the soil/extractant mixture removes trapped air so that the exact sample volume could be determined.

The particle densities of most mineral soils vary between 2.60 and 2.75 g cm⁻³ (e.g., Flint and Flint, 2002). Using a value of 2.65 g cm⁻³ as an average for the particle density of the investigated soils, gravimetric soil water contents can be calculated according to the following formula:

$$\theta_g = (W_i - W_d) / W_d$$

and

$$W_d = \rho_p \cdot (W_i - W_w) / (\rho_p - \rho_w),$$

where θ_g is the gravimetric soil water content [g g^{-1}], W_i is the initial weight of the soil sample [g], W_d is the dry weight of the soil sample [g], W_w is the weight of water contained in a volume corresponding to the soil-sample volume [g], ρ_p is the particle density of the soil sample [g cm^{-3}], and ρ_w is the density of water [g cm^{-3}].

Gravimetric soil water contents of 145 field-moist as well as partly dried and completely dried soil samples used for the development of the cylinder method were analyzed with this procedure and compared with the standard procedure, *i.e.*, drying in an oven until constant weight is obtained.

2.5 Nitrate-measurement procedure

A filter paper is placed in a funnel-like fashion in the muddy soil solution immediately after shaking. Nitrate is measured in the clear solution collected inside the funnel-shaped filter paper after 1–2 min. Nitrate concentration was determined reflectometrically using nitrate-test strips (Reflectoquant® Nitrate Test, Cat. No. 1.16995.0001, E. Merck, Darmstadt, Germany) and a reflectometer (Reflectometer RQflex, Cat. No. 1.16970.0001, E. Merck, Darmstadt, Germany). The reactive zone of the nitrate-test strip is wetted with the extraction solution for approximately 2 s. Excess liquid is then shaken off. Nitrate ions are reduced to nitrite with Griess reagent. In the presence of an acidic buffer, the nitrite ions react with an aromatic amine to form a diazonium salt that in turn reacts with N-(1-naphthyl)-ethylene-diamine to form a red-violet azo dye, the intensity of which is determined reflectometrically. About 10 s before the end of the 60 s reaction time, the test strip is inserted into the test-strip reader. The nitrate concentration is indicated on the display of the reflectometer. Quick-test measurements were compared with a standard laboratory nitrate-determination procedure using ion chromatography (LC 75, Perkin Elmer, Norwalk, CT, USA).

If the extractant itself contains nitrate (*e.g.*, nitrate-containing tap water), the formula to calculate the nitrate content of the soil sample is as follows:

$$\text{Nitrate content [mg NO}_3\text{-N (kg soil)}^{-1}\text{]} = (M_s \cdot 0.226 \cdot (V_e + V_{sw}) - (M_e \cdot 0.226 \cdot V_e)) \cdot (1 + \theta_g) / W_i,$$

where M_s = measured nitrate value of soil sample/extractant mixture [mg L^{-1}], 0.226 = factor to convert NO_3^- into $\text{NO}_3\text{-N}$, V_e = volume of extractant [L], V_{sw} = volume of water contained in the soil sample [L], M_e = measured nitrate value of nitrate-containing extractant [mg L^{-1}], W_i = initial weight of the sample [kg], and θ_g = gravimetric soil water content [g g^{-1}]. V_{sw} is obtained from the weight of water contained in the soil sample (W_{sw}) by assuming a density for water of 1 kg L^{-1} , and where $W_{sw} = W_i - W_d$, with W_d = dry weight of the soil sample [kg]. To calculate the quantity of nitrate per ha, the apparent bulk density (which might vary between 1000 and 1600 kg m^{-3}) and the soil depth from which the sample has been sampled has to be taken into consideration as well.

The temperature dependence of the nitrate-test strips was investigated in the range from 6°C to 30°C , with the test strips, reflectometer, and solutions used being stored at the

respective temperatures for at least 2 h. Triplicate measurements were conducted at six nitrate concentrations from 5 to $90 \text{ mg NO}_3^- \text{ L}^{-1}$, and correction factors for the respective temperatures were calculated.

2.6 Statistical analysis

Analyses of variance, least significant differences (LSD), and regression analyses were carried out using SPSS, version 12.0.1 (SPSS, 2003).

3 Results

3.1 Soil bulk density

Undisturbed soil samples were obtained using hand-held volumetric soil samplers down to a soil depth of 90 cm. Soil bulk density determined by the classical technique and the volumetric soil sampler were statistically not different. Mean values obtained for the two methods were 1.52 and 1.49 ($0\text{--}30 \text{ cm}$), 1.62 and 1.63 ($30\text{--}60 \text{ cm}$), and 1.70 and 1.69 g cm^{-3} ($60\text{--}90 \text{ cm}$).

3.2 Efficiency of nitrate extraction using tap water

Tap water (nitrate-free or nitrate-containing) can be used successfully as extractant. For the 22 soils tested in this series, the relationship between tap water and 0.01 M CaCl_2 is highly significant ($R^2 = 0.966$), and the slope of the regression line is close to 1 (Fig. 1). Nitrate-free or nitrate-containing bottled mineral waters can be used for soil nitrate analysis as well (data not shown).

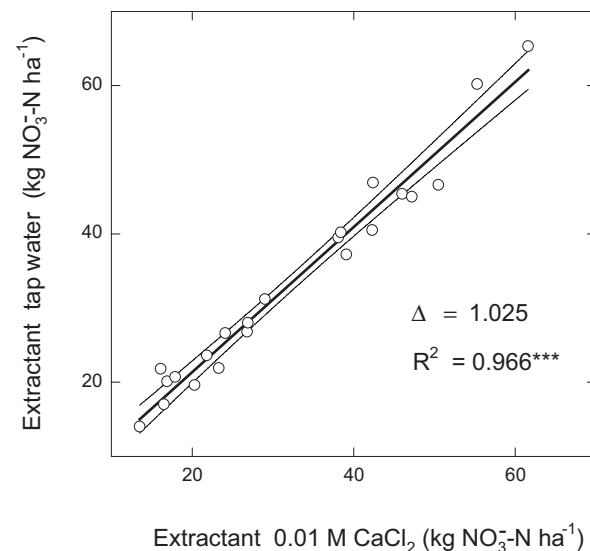


Figure 1: Comparison of soil nitrate-N contents in 22 soils using 0.01 M CaCl_2 or tap water as extractant. Regression coefficient (slope Δ), coefficient of determination, and confidence intervals for prediction are indicated.

Abbildung 1: Nitrat-N-Gehalte in 22 Böden bei Verwendung von $0,01 \text{ M CaCl}_2$ oder Leitungswasser als Extraktionsmittel. Regressionskoeffizient und Bestimmtheitsmaß sowie Konfidenzintervalle der Vorhersage sind angegeben.

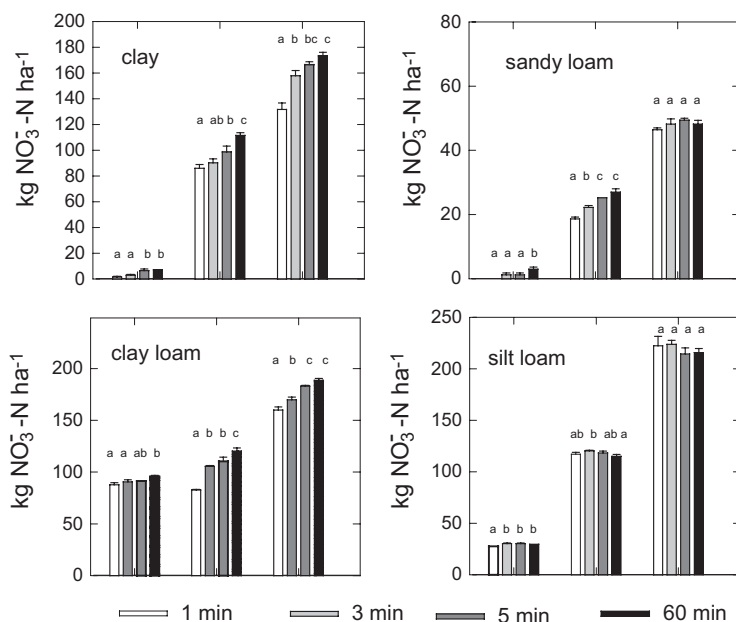


Figure 2: Impact of extraction time (1, 3, or 5 min manual shaking, 60 min mechanical head-over-head shaking) on nitrate-N content in four soils, each with three different nitrate-N levels. Error bars indicate standard errors ($n = 3$). Columns labeled with the same letter within each nitrate-N level and soil are not statistically different at $p < 0.05$.

Abbildung 2: Einfluss der Schüttelzeit auf die Extraktion von Nitrat-N aus vier verschiedenen Böden bei jeweils drei unterschiedlichen Nitrat-N-Gehalten. Die Bestimmung von Nitrat-N im Boden erfolgte nach 1, 3 oder 5 min manuellem Schütteln im Vergleich zu 60 min mechanischem Schütteln. Standardfehler sind angegeben ($n = 3$). Statistische Unterschiede zwischen den Schüttelzeiten sind getrennt für die drei Nitrat-Level und vier Böden durch unterschiedliche Buchstaben gekennzeichnet.

3.3 Extraction time

As expected, different nitrate levels were achieved by spiking four samples with different soil texture with nitrate. Overall, adequate extraction of nitrate could be obtained by vigorously manual shaking of the extractant/soil mixtures for 3–5 min (Fig. 2). Whereas this was satisfactory for the sandy loam, shaking for more than 5 min has to be recommended for clayey soils to completely disintegrate aggregates. No significant differences in performance were observed between 3 min and longer shaking times for the silt loam. This was also the case for 5 min of manual shaking *versus* 60 min of mechanical shaking for the sandy loam (not considering the nonspiked soil). For the clay loam and clay, nitrate values were about 4% and 9% lower, respectively, with 5 min manual shaking as compared to 60 min of mechanical shaking. Still, results indicate that manual shaking as compared to mechanical laboratory head-over-head shaking for 1 h adequately extracted nitrate after a few minutes.

3.4 Determination of soil water content

The quick-test procedure described to instantaneously obtain gravimetric soil water contents without any drying of the soil sample is accurate and was largely comparable to the standard method, *i.e.*, drying in an oven until constant weight is obtained. Using appropriate values for the particle density (*i.e.*, 2.65 g cm^{-3} in this study), the gravimetric soil water content of moist field soil samples could be sufficiently precisely determined with the cylinder method (Fig. 3). Separately determined values of the soil particle density revealed slightly higher values and did not lead to improved determinations. Slight under- or overestimation of the soil water content, can be ascribed to a number of factors such as the assumption of the soil particle density, overestimation of the soil volume because of trapped air, or deviations during the volume recording.

3.5 Nitrate content as determined by the standard and the cylinder method

The agreement between both quick-test methods and the standard laboratory method that includes the drying and weighing of the soil samples was excellent as indicated by the regression coefficients and the coefficients of determination (Fig. 4). Whereas the standard quick-test method (A) slightly underestimated the nitrate values by about 4% compared to the laboratory method, the agreement with the cylinder method (quick test method [B]) was nearly perfect.

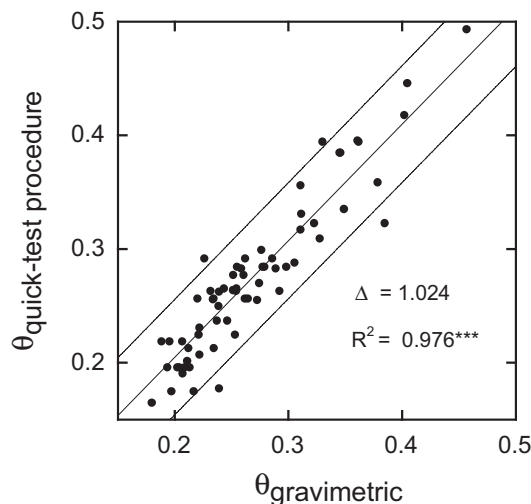


Figure 3: Gravimetric soil water content determination by oven drying ($\theta_{\text{gravimetric}}$) as compared to a quick-test procedure ($\theta_{\text{quick-test procedure}}$). Regression coefficient (slope Δ), coefficient of determination, and confidence intervals for the prediction are indicated.

Abbildung 3: Bestimmung gravimetrischer Bodenwassergehalte durch eine Schnelltest-Methode im Vergleich zur gravimetrischen Bestimmung nach 24-stündiger thermischer Trocknung. Regressionskoeffizient und Bestimmtheitsmaß sowie Konfidenzintervalle der Vorhersage sind angegeben.

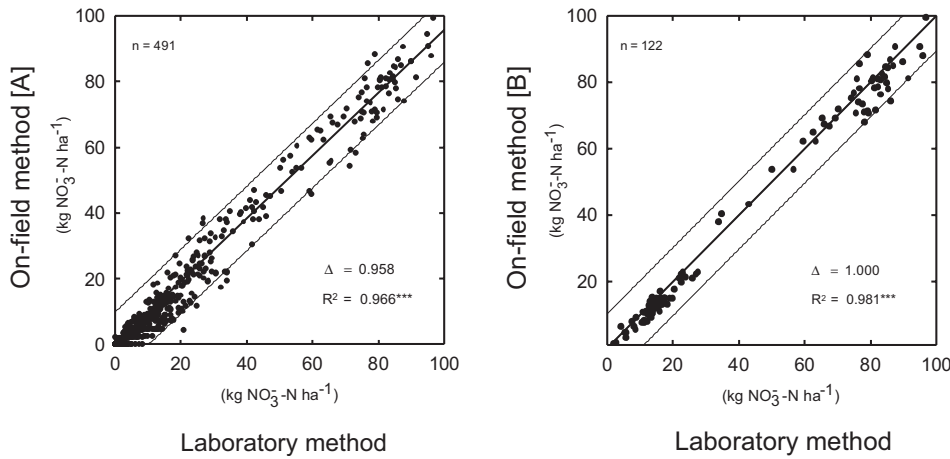


Figure 4: Comparison of soil nitrate-N contents measured by a standard laboratory procedure and two on-field methods using plastic beakers (quick-test method [A]) or graduated cylinders (quick-test method [B]), respectively. Regression coefficient (slope Δ), coefficient of determination, and confidence intervals for prediction are indicated.

Abbildung 4: Vergleich der Standardlabormethode zur Bestimmung von Nitrat-N-Gehalten in Böden mit zwei Schnelltest-Methoden (Extraktion in Plastikdosen [A] oder in Messzylindern [B]). Regressionskoeffizient und Bestimmtheitsmaß sowie die Konfidenzintervalle der Vorhersage sind angegeben.

3.6 Temperature dependence

The temperature dependency of nitrate analysis based on test strips in the range from 6°C to 30°C is shown in Fig. 5. At a temperature around 20°C, a good relationship between the nitrate concentrations measured using test strips and the reference values can be obtained over the whole range from 5 to 90 mg NO₃⁻ kg⁻¹. Nitrate values obtained from test-strip readings at temperatures noticeable below or above 20°C have to be divided by appropriate correction factors as indicated in Fig. 5. This is especially important at higher nitrate concentrations.

4 Discussion

Conventional gravimetric soil sampling for soil-nitrate analysis is relying on an assumption of soil bulk density, which probably represents one of the largest sources of error in the procedure (Schmidhalter et al., 1991; Scholefield and Titchen, 1995). The simple hand-held-sampling-tube tech-

nique described to obtain volumetric soil samples is much more effective than using the classical cylinder technique to determine soil bulk density. Data obtained with both procedures show a very good correspondence, giving evidence that the volumetric soil sampling can be used for the determination of soil bulk density.

In previous quick-test procedures, the use of fairly small amounts of soil of about 20–25 g soil was recommended (e.g., Schaefer, 1986; Jemison and Fox, 1988; Roth et al., 1991; Hartz, 1994). However, in view of the high heterogeneity of soil nitrate in soils, subsampling may lead to large errors (Scholefield and Titchen, 1995). The use of larger soil samples, as recommended in both quick-test procedures described, is expected to improve the accuracy of soil-nitrate determinations.

Extraction solutions containing flocculating agents such as Ca ions allow sufficiently clear supernatants to be obtained in a short time and without filtering. Low concentrated CaCl₂

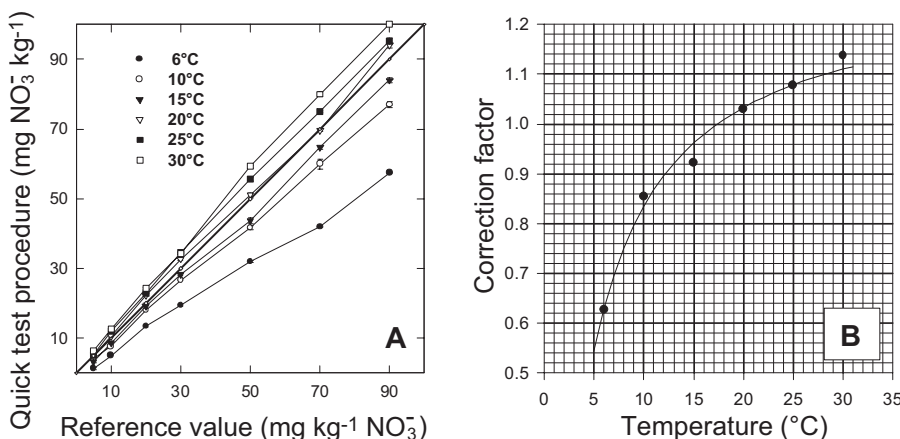


Figure 5: Temperature dependency of nitrate-test-strip measurements [A] and correction factors [B] for the respective temperatures. Error bars indicate standard deviations of the temperature dependency and are not indicated if smaller than the symbol size.

Abbildung 5: Temperaturabhängigkeit der reflektometrischen Nitrat-Bestimmungen mit Teststäbchen (A) und Angabe von Korrekturfaktoren (B) in Abhängigkeit von der Temperatur. Standardabweichungen der Temperaturabhängigkeit sind angegeben, sofern sie größer sind als die Symbole.

(e.g., 0.005 or 0.01 M CaCl₂) is preferred as an extractant, because it flocculates suspended particles and obtains a clear supernatant after a short settling time. Higher concentrations should not be used, because Cl ions interfere with the test-strip measurements. However, the use of tap water (or alternatively bottled mineral water) as an universally available extraction solution requires no preparation and simplifies the extraction procedure markedly: neither deionized water (Nitsch, 1985; Jemison and Fox, 1988) nor chemicals (Schaefer, 1986; Roth et al., 1991; Hartz, 1994) are required.

A simplified filtering procedure (i.e., placing filter paper in a funnel-like fashion in the muddy solution) allows the collection of clear solution to moisten the test strip after a few minutes, which decreases the measuring time dramatically. Nitrate can also be measured without any filtering in the supernatant after 10–20 min settling time. A slight horizontal bending of the vertically inserted test strip allows wetting of the test-strip pad within a thin layer of supernatant.

Intensive manual shaking obtained adequate recovery of nitrate within a few minutes. For most soils, only 3–5 min of shaking was sufficient, whereas more than 5 min are necessary for clayey and clay loam soils. Shaking times of about 15–20 min (Jemison and Fox, 1988) and settling times of up to 6 h (Jemison and Fox, 1988; Hartz, 1994) are obviously not necessary. On the other hand, extremely short shaking times of 1–2 min as recommended in previous quick-test procedures for dry soils (Jemison and Fox, 1988; Roth et al., 1991) are too short and therefore inadequate at least for moist soil samples.

Previous studies suggested drying of soil samples either for the nitrate analysis itself (Jemison and Fox, 1988; Roth et al., 1991) or the separate determination of the soil water content (Scharpf and Wehrmann, 1986; Liebig et al., 1996). The results from our investigation show that there is no need for soil drying in quick-test procedures. This can dramatically decrease the time requirement for the analysis. The quick-test procedure based on the cylinder method to instantaneously obtain soil water contents without any drying of the soil sample has been shown to be accurate. The soil water contents can be determined with this technique to a precision of $\pm(2\%–3\%)$ of the actual gravimetric soil water content.

Little attention has been paid to the potential influence of temperature on nitrate-test-strip readings. Such an influence has not been noticed before, because most investigations were carried out at room temperature. Wetselaar et al. (1998) recommended that samples should be compared with standard solutions analyzed at the same temperature. In our investigation, optimal temperatures for reflectometric readings were found to be around 20°C, with the readings being sufficiently precise between 15°C and 25°C. Under field conditions (e.g., under cool-spring conditions in temperate regions or hot conditions in tropical countries), appropriate correction factors should be applied. If thermometers are not available, temperature readings can be obtained with inexpensive temperature-sensitive test strips.

5 Conclusions

The excellent agreement between the developed quick-test procedures and the standard laboratory procedure allows the replacement of the latter one without losses in precision. These on-farm procedures not only allow more frequent determinations, but they are also cheaper and deliver the result in a much shorter time, because of their reliance on universally available extractants and simplified extraction/filtering procedures. Transportation and storage, which have to be seen as critical handling steps concerning unwanted mineralization, are no longer required. The possibility to immediately determine on-site gravimetric soil water contents represents a significant improvement. Consideration of the temperature can increase the precision further. The volumetric-soil-sampling method can markedly improve determination of soil nitrate contents in soil because subjective estimates of soil bulk density are eliminated. The procedure developed revealed to be simple and reliable in use that was repeatedly tested in training courses with students. Further simplifications can be obtained by developing nomograms that require no further calculation steps. The dimensions of the extraction equipment for any of the methods can be adapted to the locally available resources. The nitrate analysis itself is user-friendly and does not require a high degree of technical expertise, nevertheless being best placed in the hands of farm advisors or trained farmers. The proposed quick-test procedures have great potential and will contribute to economical savings for farmers as well as benefiting the environment.

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