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Sample Temperature Affects Measurement of Nitrate with a Rapid Analysis Ion Selective Electrode System Used for N Management of Vegetable Crops

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Abstract: The practical value of portable hand-held ion selective electrode sensors (ISE) for on-farm $[\text{NO}_3^-]$ measurement to assist with crop N management of vegetable crops has been demonstrated in numerous previous studies. They provide rapid, in-situ measurement of the nitrate concentration ($[\text{NO}_3^-]$) in nutrient and soil solutions, and in petiole sap. Sample temperatures, for on-farm measurements, vary appreciably. This study evaluated the effects of sample temperature on $[\text{NO}_3^-]$ measurement using two different models of a commonly used, commercially available, portable ISE meter. The temperatures (5, 10, 15, 20, and 25 °C) examined were in the range likely to be encountered in practical on-farm work. Aqueous solutions of 6, 12, and 18 mmol $\text{NO}_3^- \text{L}^{-1}$ were prepared from KNO_3 , $\text{Ca}(\text{NO}_3)_2$ and NaNO_3 . $[\text{NO}_3^-]$ was measured in three replicate samples of each of the three concentrations, made from each NO_3^- compound, at each temperature. The results consistently and clearly demonstrated a strong negative linear relationship between temperature-induced errors and sample temperatures. The temperature-induced error was considerable for cooled samples, being +50% at 5 °C and +31% at 10 °C. At sample temperatures of 17–20 °C, the temperature effects were minimal. Above this range, the temperature effect caused underestimation. At 25 °C, the temperature-induced error was –24%. These results show that care must be taken to ensure that sample temperatures do not erroneously affect the measurement of $[\text{NO}_3^-]$ with ISE meters. Particular care needs to be taken with both refrigerated and warmer samples.

Keywords: quick tests; ISE; LAQUAtwin; Horiba; N monitoring; nutrient solution; soil solution; petiole sap; fertigation

1. Introduction

Intensive vegetable production characteristically uses substantial inputs of N fertiliser and is commonly associated with appreciable N contamination of nearby water bodies [1–3]. Greenhouse vegetable production, in general, has been recognised as a “hot spot” of N loss to water bodies, and of the greenhouse gas nitrous oxide (N_2O) releases to the atmosphere [4]. Internationally, there are an estimated 5.6 million ha of greenhouses used for vegetable production [4], with 4.7 million ha in China [4], and more than 200,000 ha in the Mediterranean Basin [5,6]. Most greenhouse vegetable production takes place in soil [6,7]. Production in substrates with recirculation is mostly restricted to a relatively small area in northern Europe [6].

Nitrate (NO_3^-) leaching loss from intensive vegetable production is a major source of N contamination of underlying aquifers and adjacent water bodies [1–3]. Conventional crop management of intensive vegetable crops is based on accumulated local experience of what ensures profitable production [8,9], which can be associated with increased risk of NO_3^- leaching loss [3,8]. Reducing NO_3^- leaching loss from intensive vegetable production requires both improved N and irrigation management [3,7,10]. Increasingly, combined fertigation and drip irrigation systems are being used for vegetable production; these systems are particularly common in greenhouses [11,12].

Crops grown with combined fertigation and drip irrigation systems are well suited to the use of frequent monitoring, which enables rapid subsequent correction to ensure optimal irrigation and nutrient management [12,13]. Greenhouse grown crops are particularly suited to monitoring approaches because of their relatively small size and the intensity of management [12,14]. Practical farmer-friendly tools for the frequent measurement of soil water status are widely available to optimise irrigation management of vegetable crops [6,14–17]. For N management, relatively simple monitoring approaches are particularly useful for optimising crop N status [9,12,13]. Two established approaches are measurement of the NO_3^- concentration ($[\text{NO}_3^-]$) in petiole sap to evaluate crop N status [18–25], and the $[\text{NO}_3^-]$ in the root zone soil solution to inform of immediately available root-zone N [13,18,19,26].

Timely and accurate analysis of the $[\text{NO}_3^-]$ in petiole sap and soil solutions is fundamental for effective implementation of these two monitoring approaches. The time delays and logistical issues associated with commercial laboratory analysis generally make such laboratory analyses impractical for use with N monitoring approaches [27–29]. Numerous studies have overcome this issue with the use of rapid analysis systems that provide on-farm analysis in petiole sap [18,19,30–35] and in soil solution [18,19,35]. In addition to the measurement of $[\text{NO}_3^-]$ in petiole sap and soil solution, measurement of $[\text{NO}_3^-]$ in nutrient solutions, applied by fertigation, will enhance crop N management by ensuring application of the desired concentration and amounts of N [3,35].

Portable colorimetric and refractometric analysis systems can provide rapid and accurate measurement of $[\text{NO}_3^-]$ on the farm [29,36,37]. Ion selective electrode systems (ISE) have numerous practical advantages for rapid on-farm measurement because there is no requirement to add reagents or to wait for colour development as with colorimetric and refractometric approaches [13,29]. A rapid analysis ISE system provided accurate measurement of $[\text{NO}_3^-]$ in a comprehensive assessment examining nutrient and soil solution, and petiole sap from diverse vegetable crops [35]. This study suggested that portable rapid analysis ISE systems can routinely provide accurate measurement of the $[\text{NO}_3^-]$ in petiole sap, and soil and nutrient solutions, subject to adequate equipment handling, sample preparation, and handling [35]. Concerns have been raised about the effects of chemical interferences from other ions affecting ISE measurement $[\text{NO}_3^-]$ in agricultural solutions [38].

Ion selective electrode measurement can be affected by temperature [39,40]. However, there is very little published information on temperature effects on rapid analysis ISE systems used for on-farm $[\text{NO}_3^-]$ measurement of agricultural solutions. For on-farm analysis, sample temperatures vary appreciably. Measurement may take place immediately following storage in a refrigerator, on samples obtained from plants and fertigation systems at very different ambient temperatures, or from soils subject to appreciable temperature variation. Given the strong and on-going interest in using ISE systems for on-farm measurement of $[\text{NO}_3^-]$, e.g., [13,18,19,22,23,29,33,41,42], there is a need to assess how sample temperature affects the accuracy of $[\text{NO}_3^-]$ measurement.

In this study, the effects of sample temperature on two similar models of a commercially available ISE were examined. Aqueous solutions of three different $[\text{NO}_3^-]$ were examined; the concentration range covered those likely to be measured for the management of fertigated vegetable crops. Similarly, the temperature range covered those likely to be encountered for samples that were either refrigerated or collected immediately prior

to measurement. The aqueous solutions were prepared using salts that enabled possible interactions from commonly occurring cations to be examined. The evaluation of different factors that may affect accurate measurement of these ISE systems will contribute to the development of guidelines to optimise their accuracy for on-farm measurement.

2. Materials and Methods

The NO_3^- concentration ($[\text{NO}_3^-]$) of aqueous samples, at different temperatures, was measured with two rapid ion selective electrode (ISE) systems. The concentration values measured at 5, 10, 15, 20, and 25 °C with the ISE systems were then compared with those of known $[\text{NO}_3^-]$ and verified using laboratory analytical equipment at room temperature. Aqueous solutions with $[\text{NO}_3^-]$ of 6, 12 and 18 mmol L^{-1} were prepared using reagent grade potassium nitrate (KNO_3), calcium nitrate ($\text{Ca}(\text{NO}_3)_2$) and sodium nitrate (NaNO_3).

All measurements with both the rapid analysis systems and laboratory analytical equipment were conducted in a laboratory at the University of Almeria. All results are reported as $\text{mmol NO}_3^- \text{L}^{-1}$.

2.1. Rapid Analysis Ion Selective Electrode (ISE) System for NO_3^- Measurement

Two LAQUAtwin (Horiba, Kyoto, Japan) ISE pocket meters were used. These were model NO3-11 and model B343; both models are physically very similar and have the same mode of operation. Both measure $164 \times 29 \times 20$ mm, and weigh 55 g. Model NO3-11 has a S040 sensor. Model B343 was initially sold with a No. 0243 sensor; however, a S040 sensor was used for the current work.

Liquid samples (1 mL) were placed directly in the small measurement well of the ISE systems using a Pasteur pipette. No prior sample preparation or addition of reagents is necessary with this equipment. Each individual measurement was made in approximately one minute. The operating ranges for $[\text{NO}_3^-]$ measurement reported by the manufacturer, are 1–100 mmol L^{-1} for model B343 and 0.1–157 mmol L^{-1} for model NO3-11 [43]. The manufacturer suggests that accuracy is $\pm 10\%$ [43]. Before commencing measurement, and at frequent intervals (described subsequently), a two-point calibration was conducted on each ISE system, using 150 and 2000 $\text{mg NO}_3^- \text{L}^{-1}$ (2.4 and 32 mmol L^{-1}) standards prepared by the manufacturer.

2.2. Laboratory System for NO_3^- Measurement

The measurements of $[\text{NO}_3^-]$ using laboratory equipment were conducted with a SKALAR SAN++ segmented flow analyser (Breda, The Netherlands). Nitrate was determined as nitrite (NO_2^-) using the Griess–Illosvay method following reduction of NO_3^- to NO_2^- using a cadmium column. Measurements were made on three replicate samples of each of the 6, 12 and 18 $\text{mmol NO}_3^- \text{L}^{-1}$ solutions, prepared from each of KNO_3 , $\text{Ca}(\text{NO}_3)_2$ and NaNO_3 .

2.3. Effect of Temperature on $[\text{NO}_3^-]$ Measurement with Rapid Test Systems

The aqueous solutions with $[\text{NO}_3^-]$ of 6, 12 and 18 mmol L^{-1} prepared from KNO_3 , $\text{Ca}(\text{NO}_3)_2$ and NaNO_3 were kept refrigerated at 4 °C. The following experimental procedure was repeated three times, once for each of the solutions prepared using KNO_3 , $\text{Ca}(\text{NO}_3)_2$ or NaNO_3 . The studies with the solutions with each nitrate salt were conducted on different days.

Forty mL solution samples of each concentration were placed in straight-sided 50 mL polypropylene containers (urine bottles), with a diameter of 45 mm. Three replicate containers were prepared for each concentration for subsequent measurement at different temperatures with each of the ISE meters. Two identical 50 mL containers with 40 mL of deionised water were prepared for temperature measurement. Immediately after preparation, the nine containers with the aqueous solutions of NO_3^- and the two with water were transferred to a refrigerator and were stored overnight at 4 °C. Additionally, 40 mL of each

concentration was placed in three separate 50 mL containers which were refrigerated for subsequent determination of the actual $[\text{NO}_3^-]$ using the laboratory analytical system.

The next morning, the nine samples for measurement with the ISE meters and the two water samples for temperature measurement were sequentially placed in water baths formed using 7 L polypropylene trays, in which the water temperature was 5, 10, 15, 20 and 25 °C. Within each water bath, the water level outside each container was above that of the solution/water inside the sample containers. A grid formed from a flexible and firm rubber-like material, 3 cm high, ensured that each container was stable within the water bath. Temperature in the two containers with only deionised water was measured using an electric thermometer (model Checktemp1, Hanna Instruments, Woonsocket, RI, USA) with a stainless-steel temperature probe inserted into the solutions. When required, warmer or cooler water was added to the water bath to ensure the desired temperature in the samples within the containers.

When the desired temperature was reached in the water samples, 1 mL of the aqueous NO_3^- solution, in each container, was taken and immediately measured with each ISE meter. Each reported measurement with each ISE meter, on each replicate sample, was the mean of three sequential measurements. After each measurement was completed on each replicate sample, the ISE meters were rinsed with deionised water and dried with soft paper. For measurements at 5 and 10 °C, the laboratory air temperature was reduced to 14 °C to reduce the warming of the samples during measurement. Later the laboratory air temperature was increased to 15 °C and then to 20 °C. Before commencing measurement at each sample temperature, a two-point calibration was conducted, as described previously.

In total, there were six combinations of ISE meter (LAQUAtwin models NO3-11 and B343) and of nitrate salt (KNO_3 , $\text{Ca}(\text{NO}_3)_2$ and NaNO_3). For each combination, there were three replicate samples of $[\text{NO}_3^-]$ of 6, 12 and 18 mmol L^{-1} , which were each measured at 5, 10, 15, 20 and 25 °C.

The effect of temperature was evaluated, for each nitrate salt, by (1) calculating the percentage difference of the $[\text{NO}_3^-]$ measured with the ISE meter from the known $[\text{NO}_3^-]$, for each ISE measurement (which was the mean of three separate measurements), and (2) by performing linear regression analyses. The linear regressions examined the relationship, for each combination of nitrate salt and ISE meter to determine the relationship between (a) the percentage difference the $[\text{NO}_3^-]$ measured with the ISE meter and the known value, and (b) sample temperature. Individual replicate measurements, rather than mean values, were used for the regression analysis.

3. Results

3.1. Laboratory Analysis of the Prepared Aqueous Solutions

The laboratory analysis confirmed that the $[\text{NO}_3^-]$ of the prepared solutions were 6, 12 and 18 $\pm 0.1 \text{ mmol L}^{-1}$. Consequently, comparisons of $[\text{NO}_3^-]$ measured with the ISE meters were made with the values of the prepared $[\text{NO}_3^-]$ of 6, 12 and 18 mmol L^{-1} .

3.2. Absolute $[\text{NO}_3^-]$ Measured with ISE Meters at Different Temperatures

For each of the six combinations of ISE meter (LAQUAtwin models NO3-11 and B343) and source of NO_3^- (KNO_3 , $\text{Ca}(\text{NO}_3)_2$ and NaNO_3) there were very clear and consistent effects of temperature on the measurement of $[\text{NO}_3^-]$ (Figure 1a–f). For the three known $[\text{NO}_3^-]$ of 6, 12 and 18 mmol L^{-1} , there was an increasing positive error with declining temperature, negligible error between 15 and 20 °C, and an increasing negative error at higher sample temperatures (Figure 1a–f). The average coefficient of variation for all measurements using both ISE meters was 1.8%.

3.3. Relation of Relative Error, in Measurements of $[\text{NO}_3^-]$ with ISE Meter, with Temperature

For each of the six combinations of ISE meter (LAQUAtwin models NO3-11 and B343) and source of NO_3^- (KNO_3 , $\text{Ca}(\text{NO}_3)_2$ and NaNO_3), there were strong linear relationships between the relative error of measurements of $[\text{NO}_3^-]$ with the ISE meter and temperature

(Figure 2a–f; Table 1). The relationships were generally similar. For the linear regressions of each of the six different combinations of ISE meter and NO_3^- source, the range of slopes was -3.23 to -4.22 , the range of intercepts was 54.1 to 85.1, and the range of coefficient of determination (R^2) values was 0.87 to 0.94 (Table 1). The temperature values for when the relative error was zero were 17.0 to 20.2 °C (Figure 2a–f and Table 1).

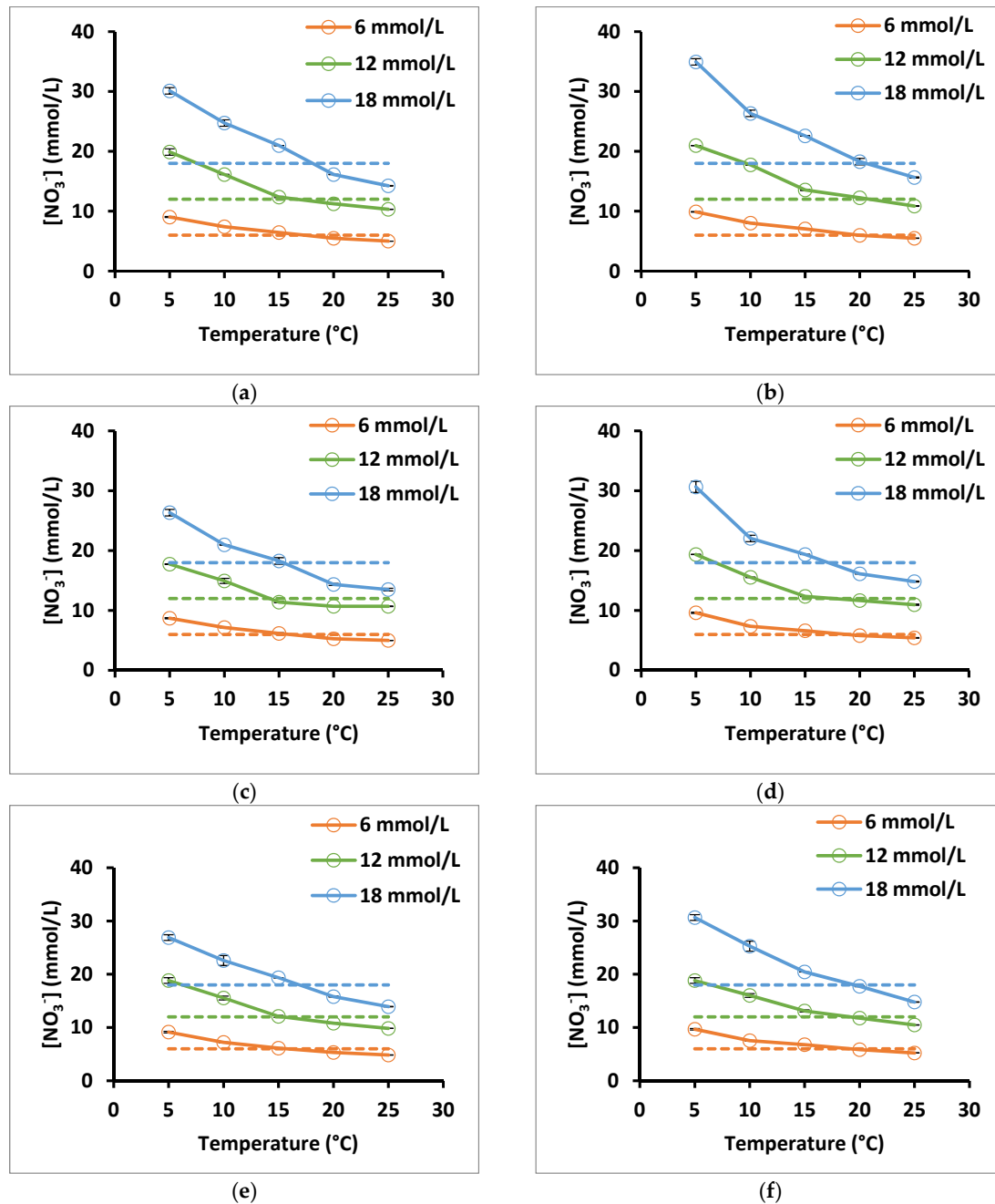


Figure 1. Values of $[\text{NO}_3^-]$ concentration measured with two different ion selective electrode (ISE) meters (LAQUAtwin models NO3-11 and B343) in aqueous NO_3^- solutions of known $[\text{NO}_3^-]$ of 6, 12 and 18 mmol $\text{NO}_3^- \text{L}^{-1}$, when measured at sample temperature of 5, 10, 15, 20 and 25 °C. The aqueous NO_3^- solutions were prepared using three different salts. There were six different combinations of ISE meter and source of NO_3^- : (a) LAQUAtwin NO3-11 and KNO_3 ; (b) LAQUAtwin B343 and KNO_3 ; (c) LAQUAtwin NO3-11 and $\text{Ca}(\text{NO}_3)_2$; (d) LAQUAtwin B343 and $\text{Ca}(\text{NO}_3)_2$; (e) LAQUAtwin NO3-11 and NaNO_3 ; and (f) LAQUAtwin B343 and NaNO_3 . The unbroken lines connect the values measured at different sample temperatures for a given known $[\text{NO}_3^-]$ and the broken horizontal lines represent the known $[\text{NO}_3^-]$. The error bars are mean values \pm standard error.

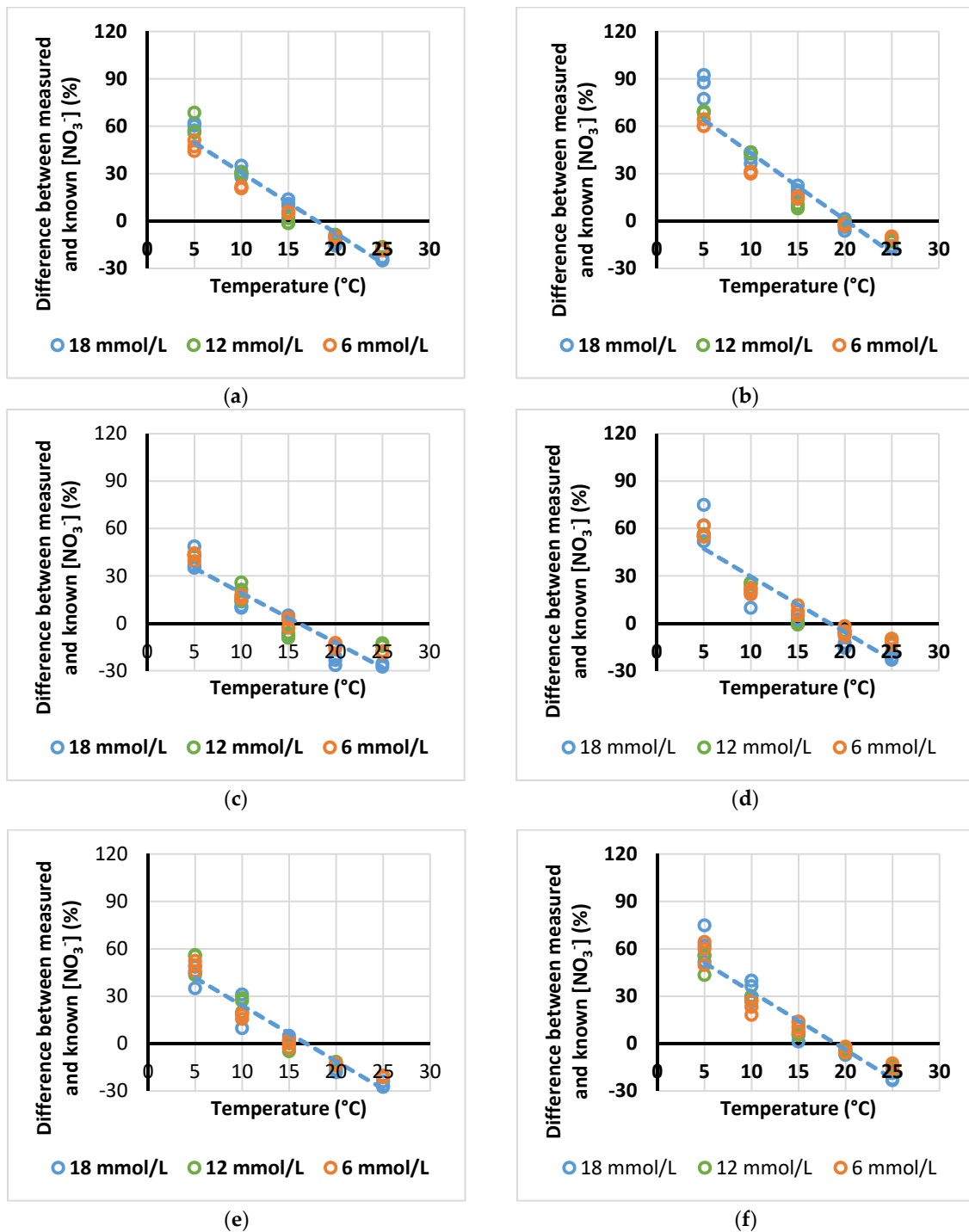


Figure 2. The relationship between the relative difference (expressed as percentage) between $[\text{NO}_3^-]$ measured with an ion selective electrode (ISE) meter and the known $[\text{NO}_3^-]$, and the temperature of the sample. Measurements were made with the two different ISE meters (LAQUAtwin models NO3-11 and B343) in aqueous solutions prepared with three NO_3^- different salts, with concentrations of 6, 12 and 18 mmol $\text{NO}_3^- \text{ L}^{-1}$. There were six different combinations of ISE meter and source of NO_3^- : (a) LAQUAtwin NO3-11 and KNO_3 ; (b) LAQUAtwin B343 and KNO_3 ; (c) LAQUAtwin NO3-11 and $\text{Ca}(\text{NO}_3)_2$; (d) LAQUAtwin B343 and $\text{Ca}(\text{NO}_3)_2$; (e) LAQUAtwin NO3-11 and NaNO_3 ; and (f) LAQUAtwin B343 and NaNO_3 . ISE measurements were conducted at sample temperatures of 5, 10, 15, 20 and 25 °C. The lines of the linear regression equations describing the relationships are presented in each panel for 5–25 °C (blue, dotted line). The equations, R^2 values, and the values of the intercept with the x-axis are presented for 5–25 °C, for each combination of ISE meter and NO_3^- source, in Table 1.

Table 1. Summary of linear regression equations, R^2 values, and the value of the intercept with the x-axis for the relationship of the relative difference between the $[\text{NO}_3^-]$ measured with an ISE meter and the known $[\text{NO}_3^-]$, and sample temperature. Data are presented for each combination of ISE meter (LAQUAtwin NO3-11 and LAQUAtwin B343) and source of NO_3^- , in the sample temperature range of 5–25 °C. The known $[\text{NO}_3^-]$ were 6, 12 and 18 mmol L^{-1} . In the regression equations, y is the percentage value of the deviation from the known value, i.e., $((\text{measured } [\text{NO}_3^-] \text{ with ISE meter minus (known } [\text{NO}_3^-]))/\text{known } [\text{NO}_3^-])$ and x is sample temperature in °C.

LaquaTWIN Meter	Source NO_3^-	Equation	R^2	Intersection x Axis (°C)
NO3-11	KNO_3	$y = -3.81x + 68.6$	0.93	18.0
NO3-11	$\text{Ca}(\text{NO}_3)_2$	$y = -3.23x + 54.8$	0.90	17.0
NO3-11	NaNO_3	$y = -3.54x + 60.2$	0.93	17.0
B343	KNO_3	$y = -4.22x + 85.1$	0.92	20.2
B343	$\text{Ca}(\text{NO}_3)_2$	$y = -3.62x + 69.4$	0.87	19.2
B343	NaNO_3	$y = -3.69x + 70.4$	0.94	19.1
All data		$y = -3.68x + 68.1$	0.88	18.5

For the pooled data set, that combined all data for each of the six combinations of ISE meter and NO_3^- source, the linear regression $y = -3.68x + 68.1$, with $R^2 = 0.88$, described the data (Table 1). The intercept value with the x-axis, signifying zero relative error, was 18.5 °C for the pooled data (Table 1). Applying this linear regression to the sample temperatures used in the study, indicated that the average temperature-induced errors in this study were +49.7, +31.3, +12.9, −5.5, and −23.9% for sample temperatures of 5, 10, 15, 20 and 25 °C, respectively (Table 2).

Table 2. Estimated relative error of $[\text{NO}_3^-]$ measurement at 5, 10, 15, 20 and 25 °C when using the ISE meters. Error values were derived the equation $y = -3.68x + 68.1$ for combined data set of all measurements (both ISE meters, all NO_3^- sources, all $[\text{NO}_3^-]$) for the temperature range 5–25 °C.

Temperature (°C)	Estimated Relative Error in Measurement of $[\text{NO}_3^-]$ (%)
5	49.7
10	31.3
15	12.9
20	−5.5
25	−23.9

3.4. Effect of NO_3^- Source on the Temperature-Induced Error in ISE $[\text{NO}_3^-]$ Measurement

Combining the data sets for the two ISE meters for each NO_3^- source, suggested a difference in the linear regression for KNO_3 compared to those for $\text{Ca}(\text{NO}_3)_2$ and NaNO_3 which were very similar to one another (Table 3). The linear regression for KNO_3 had a somewhat higher slope and intercept value, which resulted in a slightly higher value for zero relative temperature effect of 19.1 °C compared to 17.3 and 17.9 °C for the other two NO_3^- sources (Table 3). The regression equations and the value for zero relative temperature effect for each NO_3^- source, are similar to the regression equation and value for zero relative temperature effect obtained for the pooled data set (Table 1).

Table 3. Summary of linear regression equations, R^2 values, and the value of the intercept with the x-axis for the relationship of the relative difference between $[\text{NO}_3^-]$ measured with a ISE meter and the known $[\text{NO}_3^-]$, and sample temperature for each source of NO_3^- , in the sample temperature range of 5–25 °C. The measured $[\text{NO}_3^-]$ were the combined data sets from the two different ISE meters (LAQUAtwin models NO3-11 and B343). The known $[\text{NO}_3^-]$ were 6, 12 and 18 mmol L^{-1} . In the regression equations, y is the percentage value of the deviation from the known value, i.e., ((measured $[\text{NO}_3^-]$ with ISE meter) minus (known $[\text{NO}_3^-]$))/known $[\text{NO}_3^-]$ and x is sample temperature in °C.

Source NO_3^-	Equation	R^2	Intersection x Axis (°C)
KNO_3	$y = -4.01x + 76.9$	0.93	19.1
$\text{Ca}(\text{NO}_3)_2$	$y = -3.33x + 57.7$	0.88	17.3
NaNO_3	$y = -3.59x + 64.4$	0.93	17.9

3.5. Comparison of Two Different ISE Meters for Measurement of NO_3^- in Aqueous Solution

The two different ISE meters (LAQUAtwin models NO3-11 and B343) were each used to measure $[\text{NO}_3^-]$ of each sample in the study. There was a consistent tendency for the model B343 to measure slightly higher than the model NO3-11 (Figure 3). The slope of the linear regression relating the measurements of the two ISE models to one another indicated that the model measured approximately 12% higher than model NO3-11 (Figure 3).

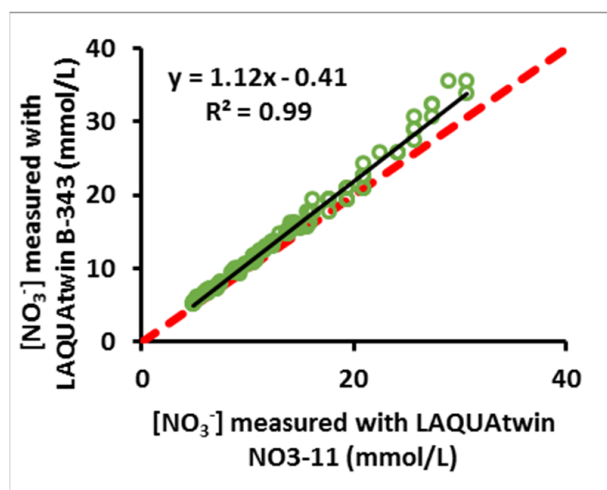


Figure 3. Relationship between values of $[\text{NO}_3^-]$ measured with two different ISE meters (LAQUAtwin models NO3-11 and B343) in aqueous NO_3^- solutions of known $[\text{NO}_3^-]$ of 6, 12 and 18 $\text{mmol NO}_3^- \text{L}^{-1}$, when measured at sample temperature of 5, 10, 15, 20 and 25 °C. The aqueous NO_3^- solutions were prepared using three different salts, KNO_3 , $\text{Ca}(\text{NO}_3)_2$, and NaNO_3 . $[\text{NO}_3^-]$ was measured with both ISE meters for at each combination of known $[\text{NO}_3^-]$, sample temperature, and NO_3^- source. There were three replicate measurements made for each of these combinations. The green circles are the data points. The black line is the fitted equation. The red broken line is the 1:1 line.

4. Discussion

The results obtained clearly demonstrated that sample temperature strongly affects measurement of $[\text{NO}_3^-]$ with the type of ISE meters used in the present work. At sample temperatures of 17–20 °C, the temperature effects were negligible. The temperature effects were large for cooled samples, the average temperature-induced error being +50% at 5 °C and +31% at 10 °C. These results show that considerable care should be taken with refrigerated samples when using these ISE meters. Additionally, notable underestimation occurred at higher temperatures, with the average temperature-induced error being −24% at 25 °C. These results indicate that there is a requirement to ensure, prior to measurement, that sample temperatures are at, or close, to 17–20 °C. With sample temperatures close to

this range, the temperature-induced error is similar to the measurements error of the ISE meter [35,43]. Sample temperatures outside of this range can cause erroneous results.

These results are of considerable practical importance given the many research e.g., [18,20,21,31,34] and Extension publications e.g., [19,22] that have used these ISE meters or their predecessor (Cardy nitrate meter) for measurement of $[\text{NO}_3^-]$ to assist in N management of vegetable crops. In numerous studies, the combined use of these ISE meters and petiole sap analysis were presented as a crop N management package e.g., [30,32,33,41,42]. While a number of studies have evaluated the accuracy of these or similar ISE systems with a particular type of sample (petiole sap or soil solution) [18,19,31,35,44–46], none of these considered how temperature affected the accuracy of measurement. The large number of studies that have used these or similar ISE meters highlights the importance of the results of the present study for the effective use of these ISE meters in crop N management.

For practical, rapid, on-farm measurement, the requirement to ensure adequate sample temperature adds a further recommendation for the use of these ISE systems, to those proposed by [22,35]. Ensuring adequate temperature, for on-farm measurement, is relatively simple. Probe-type thermometers, as used in this study, are a simple and effective way to measure sample temperature and are relatively cheap. Nutrient and soil solution samples that have been cooled, and those collected under hot conditions can be left at room temperature for sufficient time for sample temperature to equilibrate with room temperature. Also, as in the present study, water baths can be used. Care must be taken with refrigerated samples of petiole sap, because of changes that may occur in the sap if it is left standing on a bench [22]. The temperature effects observed in this work are consistent with the general temperature sensitivity of ISE technology [39,40]. All ISE systems intended for on-farm analysis of NO_3^- should be assessed for temperature effects affecting measurement accuracy. It is recommended that IES systems intended for on-farm measurement include a sensor for sample temperature measurement, and that the systems provide users with the option of automatic temperature correction.

Comparison of the linear regressions for the three different aqueous solutions suggested that the curve for KNO_3 was slightly different to those for $\text{Ca}(\text{NO}_3)_2$ and NaNO_3 which were very similar to one another. The linear regression between results from each of the two models of ISE meter indicated a systematic difference of approximately 12% (Figure 3). The effects of NO_3^- source and ISE model were within the measurement error of these ISE meters [35]. For practical purposes, the two models can be considered as having provided very similar results, and the source of NO_3^- did not affect the observations on temperature effects.

The results of the current study taken together with the results of [35] suggest that the ISE meters examined here can provide accurate measurement of $[\text{NO}_3^-]$ in samples of nutrient and soil solution, and of petiole sap, given that appreciable care is taken. The accuracy of measurement depends on following a number of procedures, such as regular calibration of the ISE meter, cleaning the measurement cell, checking accuracy with samples of known $[\text{NO}_3^-]$, ensuring that the electrode is viable, dilution of highly concentrated samples such as petiole sap, and ensuring that the sample temperature is as close as possible to 17–20 °C.

5. Conclusions

Sample temperature affects $[\text{NO}_3^-]$ measurement with portable hand-held ISE meters intended for on-farm use. The temperature effects were appreciably large for refrigerated samples, the temperature-induced error being +50% at 5 °C and +31% at 10 °C. Notable underestimation occurred at higher temperatures; the average temperature-induced error at 25 °C was –24%. At sample temperatures of 17–20 °C, the temperature effects were minimised. These results show that considerable care should be taken when using ISE meters. Sample temperatures should be at, or close to, 17–20 °C. Sample temperatures noticeably outside of this range are likely to have temperature effects causing inaccurate results.

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