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Use of a Portable Rapid Analysis System to Measure Nitrate Concentration of Nutrient and Soil Solution, and Plant Sap in Greenhouse Vegetable Production

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Abstract: A rapid analysis ion-selective electrode (ISE) system for measurement of [NO_3^-] in nutrient solution (NS), soil solution (SS) and petiole sap (PS) was evaluated. For each material, there were 797–2010 samples from 5 to 6 different crops, and from 2 to 4 different species. Accuracy was evaluated by linear regression (LR) with laboratory analysis (automated colorimetry, Cd reduction), and by relative error (RE), the average percentage deviation from laboratory analysis. For NS, the LR was y = 0.982x + 0.76, R^2 = 0.962 (n = 2010), for combined data from 5 crops (3 pepper, 2 cucumber). For SS, the LR was y = 0.975x + 1.13, R^2 = 0.965 (n = 797), for combined data from 5 crops (3 pepper, 2 cucumber). For undiluted PS, the LR relationship was y = 0.742x + 168.02, R^2 = 0.892 (n = 1425), for combined data from 6 crops (3 pepper, 2 cucumber, 1 melon). The underestimation was most pronounced at [NO_3^-] of >1500 mg NO_3^-–N L^{-1}. For diluted petiole sap (dilution by 10 for pepper and melon, 5 for other species), the LR relationship was y = 1.010x + 99.26, R^2 = 0.927 (n = 1182), for combined data from 6 crops (2 pepper, 2 cucumber, 1 melon, 1 tomato). RE values for all measurements in composite datasets were 14%, 22%, 24% and 25% for NS, SS, undiluted PS and diluted PS respectively, and they were lower in concentrations most likely to be measured in practical on-farm work. The ISE system measured [NO_3^-] in NS, SS and diluted PS with sufficient accuracy to effectively guide on-farm decision making.

Keywords: ion-selective electrode; petiole sap analysis; N fertilizer management; quick test; on-farm; greenhouse

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1. Introduction

In recent decades, there has been a large rapid growth in the surface area of greenhouses and large plastic tunnels in regions such as the Mediterranean Basin and east Asia [1,2]. In the Mediterranean Basin, there are an estimated 200,000 ha of greenhouses [1], of which there are approximately 100,000 ha in southern Europe [3], 50,000 ha in Turkey [4] and 24,000 ha in Morocco [5]. There are 4 million ha in China [6]. The vast majority of these greenhouses crop in soil [2,3,6]. A low and variable percentage have free-draining substrate systems, and generally <1% use recirculation with substrate [2,3].

Substantial nitrate (NO_3^-) leaching loss occurs from greenhouse vegetable production in soil [7–9]. Larger NO_3^- leaching loss, per unit area, occurs from crops grown in free-draining substrate production [8,10]. The NO_3^- leaching losses from soil-grown greenhouse vegetable crops are generally substantially larger than from those from open-field vegetable crops [9]. In addition to NO_3^- leaching loss, substantial nitrous oxide (N_2O)

emissions occur from soil-grown greenhouse vegetable crops [6,11]. The NO_3^- leaching loss and N_2O emissions, from soil-grown greenhouse crops, are so large that greenhouse vegetable production is considered to be a global hot spot for both types of N losses [6].

The NO_3^- leached from greenhouse vegetable production is commonly associated with appreciable NO_3^- contamination of aquifers and eutrophication of surface water bodies [12,13]. The increasing pressure on vegetable growers to reduce nitrogen (N) losses, associated with contamination of water bodies (e.g., Reference [14]), requires the adoption of improved crop N management practices [13,15,16].

Many European greenhouse vegetable growers, that crop in soil, have combined fertigation and drip irrigation systems [17,18]. In other regions, while there is variable adoption, there is generally considerable interest in these technologies [19]. Combined fertigation and drip irrigation systems provide vegetable growers with the technical capacity for a high degree of control over crop N management; however, management tools are required to effectively exploit this technical capacity [18,20]. Fertigation systems are commonly used to apply nutrient solutions in which N is applied on the basis of NO_3^- concentration ($[NO_3^-]$) [18,20]. However, with conventional fertigation systems, the applied $[NO_3^-]$ is uncertain because it is generally based on calculation and on measurement of nutrient solution electrical conductivity (EC) with poorly maintained, industrial quality EC sensors [18]. Having the means to rapidly and accurately measure nutrient solution $[NO_3^-]$ on the farm would ensure that growers apply the intended concentrations and amounts of N.

Regular crop and/or soil monitoring are very useful approaches to ensure optimal N management throughout a vegetable crop [20,21]. They are particularly well-suited to fertigated and drip-irrigated crops because N management can be rapidly adjusted to overcome detected problems [17,18,20]. Monitoring the soil solution $[NO_3^-]$, sampled with ceramic cup suction samplers, assesses the supply of readily available N in the root zone [22–24]. Monitoring petiole sap $[NO_3^-]$ informs of crop N status [21,23,25–27]. However, to be fully effective, both monitoring approaches require rapid and accurate NO_3^- analysis.

Small, portable analytical systems suitable for on-farm use are commercially available and may be suitable for on-farm measurement of $[NO_3^-]$ in nutrient solution, soil solution and petiole sap. There are two major classes of these systems: (1) ion-selective electrode (ISE) systems, and (2) refractometer systems [21,28]. For measurement of $[NO_3^-]$, the much larger reported measurement range of the ISE systems provides a considerable practical advantage [21]. The reported measurement range of the most commonly used ISE system covers the expected ranges of $[NO_3^-]$ in nutrient and soil solution, and in petiole sap [29].

Nutrient solution, soil solution and petiole sap are complex media with numerous other chemical species present. Interference from other chemical species can affect the measurement of $[NO_3^-]$ using ISE systems [28,30]. This may be particularly problematic with petiole sap in which there are high concentrations of various chemical species, such as potassium (K^+) and calcium (Ca^{2+}) ions that may interfere with ISE measurement of $[NO_3^-]$ [29]. A thorough evaluation is required to assess the adequacy of rapid analysis ISE systems, to measure $[NO_3^-]$ in the various media in which such measurements can assist with N management of vegetable crops. Such an evaluation requires large numbers of samples of nutrient and soil solution, and petiole sap, from different crops and different species.

There are no reported evaluations of rapid analysis ISE systems with nutrient solutions. There have been a small number of studies that used ISE systems with soil solution [31–33] or petiole sap [34,35]. Generally, these studies used individual crops or single species. Given that the high concentrations of various ions and compounds in petiole sap may interfere with ISE measurement of $[NO_3^-]$ [28,30], dilution of petiole sap may enhance ISE measurement of $[NO_3^-]$. However, there are no reports of the effect of dilution of petiole sap on the accuracy of ISE measurement.

The objectives of this study were to assess the accuracy of a rapid analysis ISE system to measure $[NO_3^-]$ in nutrient solution, soil solution, undiluted petiole sap and diluted

petiole sap. For each type of sample, there were several hundred samples taken from several different vegetable species and crops.

2. Materials and Methods

Samples of nutrient solution, soil solution and petioles sap were collected throughout various vegetable crops grown in soil, in greenhouses similar to those used for commercial vegetable production in southeast (SE) Spain, during the period 2010–2020. The samples were analyzed for [NO_3^-] using both a small portable rapid analysis ISE system and laboratory analysis. All crops were grown with above-ground drip irrigation, and nutrients were applied using fertigation. The nutrient solutions supplied all nutrients, apart from N, in concentrations to ensure that they were not limiting.

2.1. Cropping Sites

2.1.1. Greenhouse 1

Six of the seven crops were grown in a greenhouse at the Experimental Station of the University of Almería, in southeastern (SE) Spain (36°51′ N, 2°16′ W and 92 m elevation) (Table 1). This greenhouse is representative of medium technology greenhouses used for commercial vegetable production in SE Spain [36]. It has a multi-span design, with polycarbonate walls and a low-density polyethylene roof with passive ventilation through roof windows and lateral panels. The cropped area was 1300 m^2. The crops were grown in an artificial layered soil, known locally as "enarenado", which is the most common cropping medium in this production system [37]. The greenhouse soil consisted of a 30 cm layer of loam soil, imported from a quarry, placed over the naturally occurring sandy loam soil, with 10 cm of fine gravel mulch covering the imported soil. A detailed description of the greenhouse and soil is given in [38].

Irrigation and mineral fertilizer were supplied through above-ground drip irrigation, arranged in paired lines with a 0.8 m spacing within the paired lines and a 1.2 m spacing between adjacent paired lines. There was a 50 cm spacing between drip emitters in each drip line, and the dripper discharge rate was 3 L h^{-1}. Individual plants were positioned 6 cm adjacent to individual drip emitters, giving a planting density of 2 plants m^{-2}. For the tomato and 3 pepper crops (Table 1), the greenhouse was organized into 24 replicate plots measuring 6 by 6 m, plus border areas. Each plot had 3 paired lines with 12 emitters per line. For these four crops, the plots were organized into six different irrigation sectors, each consisting of four plots in a randomized block design. Four to five of the sectors were used in these four crops. For the Cucumber 18 and Melon 20 crops (Table 1), the greenhouse was organized into 12 replicate plots measuring 6 m long by 12 m wide, with 12 paired lines per plot. For these two crops, there were three irrigation sectors, each with four plots in a randomized block design. The three sectors were used in each crop.

The water, to which nutrients were added, was desalinated sea water. The electrical conductivity (EC) of this water, prior to nutrient addition, was 0.4–0.6 dS m^{-1}. Greenhouse 1 had its own dedicated fertigation system, which prepared complete nutrient solutions for each treatment by adding concentrated fertilizer solutions to the irrigation water using displacement pumps and a mixing tank.

Table 1. Information of the crops from which samples were obtained. Presented are the crop species, crop identifier, crop type, cultivar, the dates of the crops, the N treatments, the days after transplanting (DAT) when the N treatments commenced and published references (where available), where detailed descriptions of the crops are available.

Crop Identifier (Species, Year of Planting as YY)	Crop Type	Cultivar/s	Greenhouse (GH)	Crop Start Date	Crop End Date	N Treatments (mmol L^{-1})	Start N Treatment (DAT)	Reference Describing Crop and N Treatments
Tomato 10	Cluster	Razimo	GH1	5 August 2010	25 January 2011	0.8, 4.2, 12.4, 18.4	26	[38]
Pepper 14	Sweet, Bell	Melchor	GH1	12 August 2014	29 January 2015	2.4, 6.2, 12.6, 16.1, 20.0	1	[39]
Pepper 16	Sweet, Bell	Melchor	GH1	19 July 2016	24 March 2017	2.0, 5.3, 9.7, 13.5, 17.7	9	[39]
Cucumber 17	Dutch	Strategos, Pradera, Mitre	GH2	30 March 2017	22 June 2017	4.8, 9.7, 19.0	9	M.T. Peña-Fleitas, unpublished data
Pepper 17	Sweet, Bell	Melchor	GH1	21 July 2017	20 February 2018	2.0, 5.7, 9.7, 13.1, 16.7	10	[39]
Cucumber 18	Dutch	Strategos, Pradera, Mitre	GH1	24 April 2018	3 July 2018	2.4, 8.5, 14.8	9	[40]
Melon 20	Cantaloupe	Tezac, Magiar, Jacobo	GH1	27 February 2020	11 June 2020	2.7, 8.3, 14.0	1	M.T. Peña-Fleitas, unpublished data

2.1.2. Greenhouse 2

One crop (Cucumber 17) was grown in another greenhouse (greenhouse 2) located at the Experimental Station of the University of Almeria. This greenhouse was very similar to greenhouse 1, in terms of design and construction. It had a simplified "enarenado" soil, in which 10 cm of fine gravel mulch was placed directly over the naturally occurring sandy-loam soil. The major differences between greenhouses 1 and 2 were the soil type and that they had different fertigation systems. Greenhouse 2 used the shared fertigation system of the research station, which provided fertigation to 15 greenhouses (total area of 2.2 ha). Nutrients were added to the irrigation water by displacement pumps and the use of a mixing tank. The water, to which nutrients were added, was desalinated sea water, the EC of this water, prior to nutrient addition, was 0.4–0.6 dS m^{-1}.

The total cropping area of greenhouse 2 was 1800 m^2, of which 1300 m^2 was used for treatments in this study. The area used was organized into twelve replicate plots (18 m long × 18 m wide) with a randomized block design, and the plots were grouped into three irrigation/fertigation sectors, each with four plots. An additional irrigation/fertigation sector, of the same size, was used to discharge the high N treatments from the irrigation pipes to enable re-establishment of the low N treatment. Irrigation and mineral fertilizer were supplied by above-ground drip irrigation. The drip lines were organized in paired lines with 0.8 m spacing between lines and 1.3 m between adjacent paired lines, and there was 0.5 cm spacing between adjacent emitters in drip lines. Emitter flow rates were 3 L h^{-1}. Plants were positioned 6 cm adjacent to individual drip emitters.

2.2. Crops and Treatments

The seven crops were: (i) an indeterminate spring tomato crop grown from March to June 2010 (Tomato 10), (ii) a sweet pepper crop grown from August 2014 to January 2015 (Pepper 14), (iii) a sweet pepper crop grown from July 2016 to March 2017 (Pepper 16), (iv) a cucumber crop grown from March to June 2017 (Cucumber 17), (v) a sweet pepper crop grown from July 2017 to February 2018 (Pepper 17), (vi) a cucumber crop grown from April to July 2018 (Cucumber 18) and (vii) a melon crop grown from February to June 2020 (Melon 20) (Table 1). Essential details of the crops, including crop type, cultivar/s, dates of the crops and a description of the N treatments are provided in Table 1. Crop management, except for N fertilization, followed local grower practice.

The N treatments consisted of maintaining different N concentrations in the nutrient solution throughout most of each crop. In each crop, one of the N treatments provided an adequate N supply. In the tomato and 3 pepper crops, 4–5 different N concentrations were applied, which ranged from very deficient to very excessive N supply (Table 1). In the two cucumber crops and the melon crop, three different N concentrations were applied to three different cultivars, and the three concentrations ranged from very deficient to a slightly excessive N supply (Table 1). The different treatments commenced 1–26 days after transplanting (DAT); in six of seven crops, the different N treatments commenced within ten DAT (Table 1). Prior to starting the N treatments, either water or moderate N concentrations of approximately 4 mmol L^{-1} were applied. Once the N treatments commenced, they were part of complete nutrient solutions applied in each irrigation throughout the crop. Irrigation was every 1–4 days depending on climate conditions and crop growth.

2.3. Collection and Handling of Samples

2.3.1. Nutrient Solution

Samples of nutrient solution (NS) were collected from five of the seven crops used in this study (Pepper 14, Pepper 16, Cucumber 17, Pepper 17 and Cucumber 18 crops, Table 2). Two to four times each week, samples of NS were collected for each N treatment of each crop. Two replicate samples were collected, for each treatment, from separate emitters, each from a different replicate plot. Collected samples were refrigerated at 4–5 °C until analysis within 1–4 days of sample collection. Detailed information on which crops were

sampled, and on the number of samples from and the sampling frequency of each crop, are presented in Table 2. In the Cucumber 17 crop, the numbers of samplings of nutrient and soil solution were reduced as a consequence of a malfunction of the fertigation equipment, which had little effect on the numbers of petiole sap samplings.

2.3.2. Soil Solution

Samples of soil solution (SS) were collected from five of the seven crops (Pepper 14, Pepper 16, Cucumber 17, Pepper 17 and Cucumber 18 crops, Table 2). Every 1–2 weeks, SS samples were collected from each N treatment in each sampled crop, using ceramic cup suction samplers (Model SPS200 3.1 cm diameter, 35 cm length, SDEC, Reignac Sur Indre, France). One suction sampler was installed in each replicate plot of the three pepper crops, and two samplers were installed in each replicate plot of the two cucumber crops and the melon crop. In the two cucumber crops, soil solution was only sampled in the variety Strategos, and in the Melon 20 crop, in the variety Tezac (Table 1). Details of the crops sampled, and on the number of samples collected and sampling frequency of each crop, are presented in Table 2.

The ceramic cup suction samplers were installed at 12 cm depth and at 8 cm from the plant and 5 cm from the emitter line. Samples were collected after applying a vacuum (−70 kPa) for 24 h before collection. No fertigation was made during the 24 h prior to the application of the vacuum. Sample storage was as described for NS samples in Section 2.3.1.

2.3.3. Petiole Sap

Samples of petiole sap were obtained from all seven crops (Table 2). Sap samples were obtained every week from the Pepper 14, Cucumber 17, Cucumber 18 and Melon 20 crops, and every two weeks from the Tomato 10, Pepper 16 and Pepper 17 crops. Details of the crops sampled, the number of sap samples collected and the sampling frequency of each crop are presented in Table 2.

On each sampling date, the most recently expanded leaves were removed from 6, 8 and 16 plants per replicate plot in the tomato, cucumber and melon, and pepper crops, respectively. Leaves were sampled at 07:00–09:00 h. Immediately after sampling, each sampled leaf was placed in a sealed plastic bag, from which air was pressed, which was placed in a chilled cooler box. Immediately following completion of leaf sampling, petioles were separated from leaf blades in a nearby laboratory at the research farm. Petioles were then immediately placed in sealed plastic bags, from which air was pressed, which were then placed in a chilled cooler box in which they were promptly transported (within 20 min) to a laboratory at the University of Almeria (UAL).

In the laboratory at UAL, petioles were stored at 5 °C, for up to one hour, prior to being cut into 1 cm long sections that were immediately pressed with a domestic garlic press. A portion of each extracted sap sample was diluted with demineralized water for analysis with the laboratory analytical equipment. Dilution was conducted using pipettes and volumetric flasks. The dilution factors were 1:10 for pepper and melon sap samples, and 1:5 for tomato and cucumber sap samples. Dilution was conducted immediately after sap extraction. The diluted sap samples were centrifuged at 4500 rpm for 15 min, at a temperature of 4 °C, and stored at 5 °C prior to analysis. Centrifugation substantially reduced the particulate material that otherwise left a visible residue throughout the laboratory analytical equipment. In all crops, apart from Tomato 10, a portion of undiluted sap was analyzed immediately after sap extraction, with the rapid analysis system. In the Pepper 14 crop, no diluted sap samples were analyzed.

The [NO_3^-–N] values for diluted samples, measured with the rapid analysis system, are presented as equivalent values for sap prior to dilution, as the product of the directly measured [NO_3^-–N] in the diluted sap multiplied by the dilution factor.

Table 2. Samples of nutrient solution, soil solution and petiole sap analyzed using the rapid analysis ISE system and by laboratory analysis. Number of samples and sampling frequency are given for each crop. Where petiole sap was diluted, the number of diluted samples and the dilution factor are presented. Samples were collected from each treatment. At each sampling date, two samples of nutrient solution were collected from each treatment, and one sample of soil solution and one sample of petiole sap were collected from each replicate plot of each treatment. Samples of nutrient solution, soil solution and petiole sap were collected throughout the crops, following the imposition of the N treatments, unless otherwise indicated.

Crop Identifier (as in Table 1)	Nutrient Solution—No. of Samples	Nutrient Solution—Sampling Frequency	Soil Solution—No. of Samples	Soil Solution—Sampling Frequency	Undiluted Petiole Sap—No. of Samples	Diluted Petiole Sap—No. of Samples	Diluted Petiole Sap—Dilution Factor	Petiole Sap—Sampling Frequency
Tomato 10	n.a.	n.a.	n.a.	n.a.	n.a.	56 [1]	1:5	Two weekly [1]
Pepper 14	661	3 times/week	144	Weekly	319	n.a.	n.a.	Weekly
Pepper 16	360 [2]	3–4 times/week [2]	262	Weekly [2]	160 [2]	160 [2]	1:10	Two weekly [2]
Cucumber 17	46 [3]	2 times/week	27 [3]	Two weekly	214	214	1:5	Two weekly
Pepper 17	773	3 times/week	194	Two weekly	180	200	1:10	Two weekly
Cucumber 18	170	2 times per week	170	Weekly	252	252	1:5	Weekly
Melon 20	n.a.	n.a.	n.a.	n.a.	300	300	1:10	Weekly
Total numbers of samples	2010	n.a.	797	n.a.	1425	1182	n.a.	n.a.

n.a.: not applicable. [1]: during the period 27 October to 16 December 2010. [2]: data are for 11 November 2016 onwards; prior to that date, the rapid analysis IES system gave erroneous values because the electrode had deteriorated. [3]: The reason for the relatively low number of nutrient and soil samples in the Cucumber 17 is provided in Section 2.3.1.

2.4. Analysis of Nitrate Concentration in Samples

The NO_3^- concentration ($[NO_3^-]$) of samples was measured with a rapid analysis ion-selective electrode (ISE) system, and with laboratory analytical equipment that is described subsequently. All samples of nutrient and soil solution were analyzed with both systems. Diluted sap samples were analyzed with both systems. Undiluted sap samples were analyzed only with the rapid analysis system, and the determined values were compared with the values from the corresponding diluted sample analyzed with the laboratory analytical equipment.

All measurements with both the rapid analysis system and laboratory analytical equipment were conducted in a laboratory at the University of Almeria. Previously refrigerated samples were analyzed when they reached room temperature of 20 °C. Results for NS and SS are reported as mmol NO_3^- L^{-1}, and petiole sap as mg NO_3^-–N L^{-1}.

2.4.1. Rapid Analysis System

Two LAQUAtwin (Horiba, Kyoto, Japan) ion-selective electrode (ISE) pocket meters were used. Model B343 was used for all samples from the Tomato 10, Pepper 14, Pepper 16 and Cucumber 17 crops. Model NO3-11 was used for all samples from crops Pepper 17, Cucumber 18 and Melon 20. Both models are physically very similar and have the same mode of operation. Model NO3-11 measures 164 × 29 × 20 mm and weighs 55 g. Model NO3-11 has a S040 sensor. Model B343 was initially sold with a No. 0243 sensor, which was used for the Tomato 10 crop; thereafter, the S040 sensor was used for the remaining crops.

Liquid samples (0.3–2.0 mL) were placed in the small measurement well of the ISE system by use of a Pasteur pipette. No reagent addition or sample preparation is required with this system. Measurement takes approximately 1 min per sample. The operating ranges, reported by the manufacturer, are 14–1400 mg NO_3^-–N L^{-1} (1–100 mmol L^{-1}) for model B343 and 1.4 to 2200 mg NO_3^-–N L^{-1} (0.1–157 mmol L^{-1}) for model NO3-11 (Horiba Scientific, 2017). Before each measurement session, a two-point calibration was conducted using the 150 and 2000 mg NO_3^- L^{-1} standards prepared by the manufacturer. For all crops (Tables 1 and 2), re-calibration was conducted every 12–24 samples. The manufacturer suggests that accuracy is ±10%.

2.4.2. Laboratory Analysis System

The $[NO_3^-]$ in nutrient and soil solution samples and in diluted sap samples was analyzed with an automatic continuous segmented flow analyzer (model SAN++, Skalar Analytical B.V., Breda, The Netherlands). Nitrate was determined as nitrite (NO_2^-) using the Griess–Illosvay method [41] following reduction of NO_3^- to NO_2^- using a cadmium column [41]. Analytical accuracy was verified throughout all laboratory analyses, by the inclusion of four different independent standards of $[NO_3^-]$ or $[NO_2^-]$ after every 12 samples.

2.5. Statistical Analyses

The accuracy of the measurements with the rapid analysis system was assessed by linear regression against laboratory analysis. These regression analyses were conducted for all samples from each crop, and for the combined dataset of all samples for each sample type. On one occasion, a power regression was also calculated.

The average relative error (RE) was calculated for each crop and for all samples combined, for each sample type, as the integer of (value from rapid analysis – value from laboratory analysis)/value from laboratory analysis × 100. To avoid the distorting effect of large REs associated with very low values, $[NO_3^-]$ of <0.5 mmol L^{-1}, as measured with the SKALAR laboratory system, were not considered for RE estimation for nutrient and soil solution, and $[NO_3^-$–N] of <50 mg L^{-1}, as measured with the SKALAR laboratory system, were not considered for RE estimation for petiole sap.

2.6. Examination of Effect of Sensor Deterioration

In 108 samples of nutrient solution samples from the Pepper 16 crop, [NO_3^-] was measured with a model B343 LAQUAtwin meter, with a deteriorated electrode, and then subsequently with a new electrode. Sensor life is estimated to be approximately 1500 measurements by the manufacturer.

3. Results

3.1. Nutrient Solution

For the total of 2010 individual samples of nutrient solution, collected throughout five different crops, the overall relationship between determination of [NO_3^-] with the rapid analysis ISE system and the laboratory analytical system was described by the linear relationship y = 0.982x + 0.76, with a coefficient of determination (R^2) value of 0.962 (Figure 1; Table 3). For the five individual crops, the ranges of slopes and intercepts of the linear regression equations were 0.946–0.998 and +0.35 to +0.95, respectively (Table 3). The R^2 values for the individual crops ranged from 0.949 to 0.995. Apart from the Pepper 14 crop (R^2 = 0.949), the R^2 values for the four other crops were 0.967–0.995 (Table 3).

The relative error for all measurements of nutrient solution with the rapid analysis ISE system in all crops was 14% (Table 3). It was 16% in the Pepper 14 crop; for the other four crops, it was 8–13%. For values > 5 mmol L^{-1}, as determined with the laboratory system, the relative error for all measurements in all crops was 10% (Table 3). For corresponding values in the Pepper 14 crop, it was 11%, and for the other four crops, it was 7–10%.

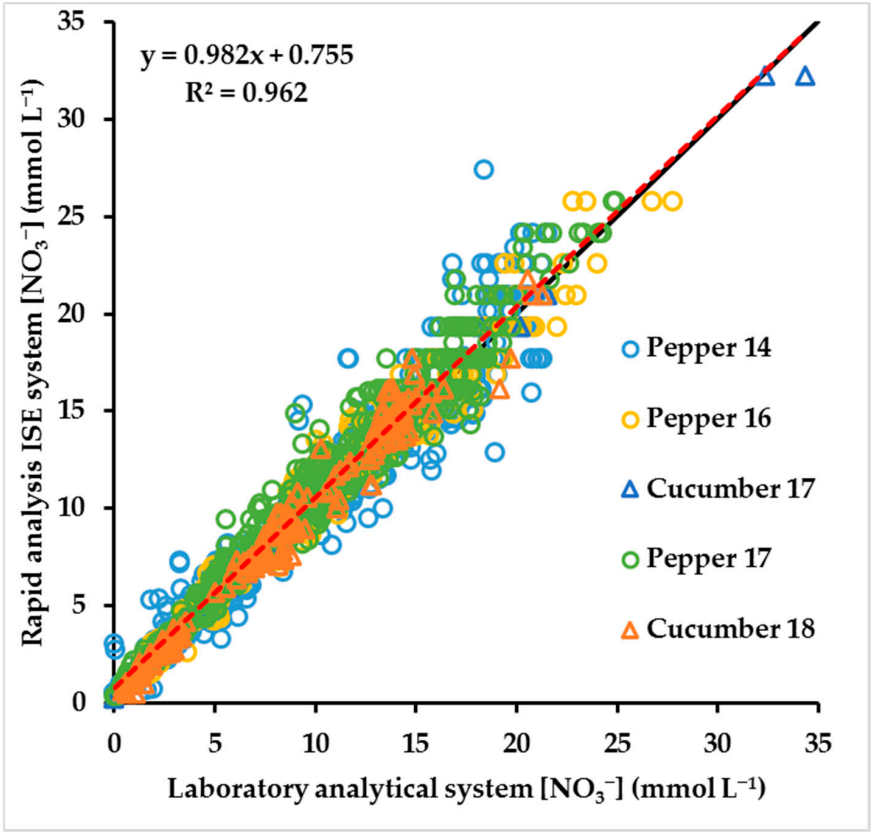

Figure 1. Nutrient solution. The relationship between [NO_3^-] measured with the rapid analysis ISE system and with a laboratory analytical system in 2010 different samples of nutrient solution obtained regularly throughout five different vegetable crops. The five crops were Pepper 14, Pepper 16, Cucumber 17, Pepper 17 and Cucumber 18. The dashed red line corresponds to the linear regression. The solid black line corresponds to the 1:1 linear relationship. The crops, their management and the two analytical systems are described in the Materials and Methods Section.

Table 3. Nutrient solution. Summary of the numbers of samples, the linear regression equations, R^2 values and the relative error for the relationship between the [NO_3^-] measured with the rapid analysis ISE system and with a laboratory analytical system. Relative error is the percentage difference between measurements with the two analytical systems, in relation to the value measured with the laboratory system. The samples were from five different vegetable crops during a four-year period. The crops, their management and the two analytical systems are described in the Materials and Methods Section.

Crop	Number of Samples	Equation	R^2 Value	[1] Relative Error All Values (%)	Relative Error, Values > 5 mmol L^{-1} (%)
Pepper 14	661	y = 0.977x + 0.826	0.949	16	11
Pepper 16	360	y = 0.956x + 0.889	0.967	12	9
Cucumber 17	46	y = 0.946x + 0.947	0.995	10	7
Pepper 17	773	y = 0.998x + 0.695	0.968	13	10
Cucumber 18	170	y = 1.004x + 0.352	0.979	8	8
All samples	2010	y = 0.982x + 0.755	0.962	14	10

[1] Relative error for all concentrations > 0.5 mmol L^{-1}, as measured with the SKALAR laboratory system. At lower concentrations, the relative error values were sometimes appreciably amplified by small denominator values.

3.2. Soil Solution

For the 797 samples of soil solution, collected throughout five different crops, the overall relationship between measurement of [NO_3^-] with the rapid analysis ISE system and the laboratory system was described by the linear relationship y = 0.975x + 1.13, with a R^2 value of 0.965 (Figure 2; Table 4). For each of the five individual crops, the ranges of slopes and intercepts of the linear regression equations were 0.935 to 1.010 and +0.35 to +1.76, respectively (Table 4). The R^2 values' range for the individual crops was 0.810–0.979; apart from the Cucumber 17 crop, for which the range was 0.960–0.979 (Table 4).

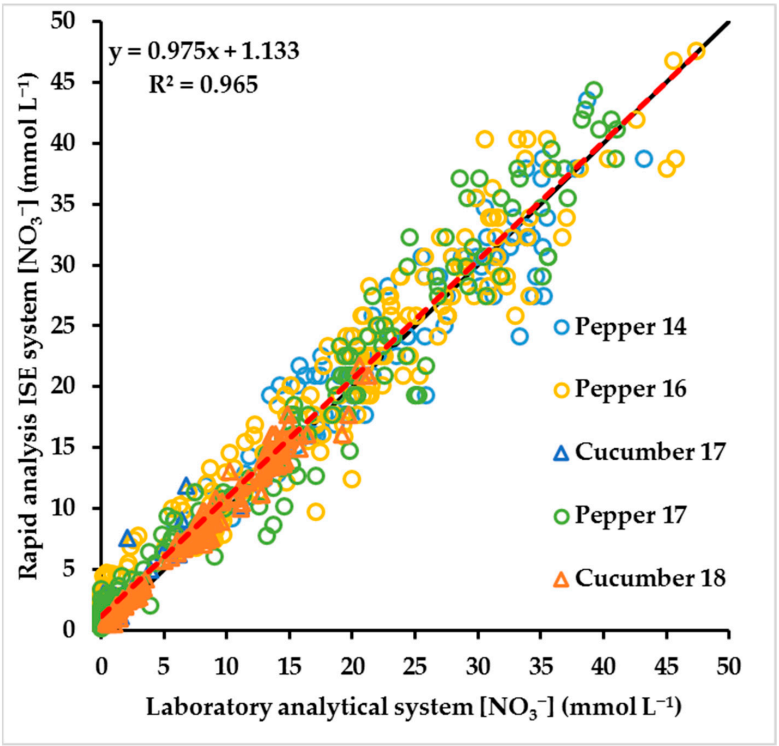

Figure 2. Soil solution. The relationship between [NO_3^-] measured with the rapid analysis ISE system and with a laboratory analytical system in 797 different samples of soil solution obtained regularly throughout five different vegetable crops. The five crops were Pepper 14, Pepper 16, Cucumber 17, Pepper 17 and Cucumber 18. The red dashed line is the linear regression equation between the two methods, and the solid black line is the 1:1 linear relationship. The crops, their management and the two analytical systems are described in the Materials and Methods Section.

Table 4. Soil solution. Summary of the numbers of samples, the linear regression equations, R^2 values and the relative error for the relationship between [NO_3^-] measured with the rapid analysis ISE system and with a laboratory analytical system. Relative error is the percentage difference between measurements with the two analytical systems, in relation to the concentration measured with the laboratory system. The samples were from five different vegetable crops during a four-year period. The crops, their management and the two analytical systems are described in the Materials and Methods Section.

Crop	Number of Samples	Equation	R^2 Value	[1] Relative Error (%)	[1] Relative Error, Values >2 mmol L^{-1} (%)	[1] Relative Error, Values >5 mmol L^{-1} (%)
Pepper 14	144	y = 0.935x + 1.761	0.964	11	11	11
Pepper 16	160	y = 0.973x + 1.463	0.960	28	19	14
Cucumber 17	27	y = 1.010x + 1.217	0.810	14	10	9
Pepper 17	194	y = 0.992x + 0.970	0.967	37	16	14
Cucumber 18	170	y = 1.004x + 0.352	0.979	12	9	8
All samples	797	y = 0.975x + 1.133	0.965	22	14	12

[1] Relative error for all concentrations > 0.5 mmol L^{-1}, as measured with the SKALAR laboratory system. At lower concentrations, the relative error values were sometimes appreciably amplified by small denominator values.

The average relative error for all measurements of soil solution with the rapid analysis ISE system was 22% and ranged from 12% to 37% for individual crops (Table 4). In three of the five crops, the relative error was 11–14%. For values > 2 mmol L^{-1} (the range commonly observed in vegetable crops), the average relative error for all measurements was 14%, and was 9–11% in three of the five crops (Table 4). For values > 5 mmol L^{-1}, the average relative error for all measurements in all crops was 12% (Table 4).

3.3. Petiole Sap

3.3.1. Undiluted Sap

The overall relationship between [NO_3^-–N] measured with the rapid analysis ISE system in undiluted petiole sap compared to analysis with the laboratory analytical system, for 1425 samples collected throughout six different crops, was described by the relationship y = 0.742x + 168.02, with an R^2 of 0.892 (Figure 3a, Table 5). The slope of the linear regression equation was appreciably less than one. The use of two separate linear regression equations for each of the ranges of 0 to 1500 mg NO_3^-–N L^{-1} and 1500 to 2650 mg NO_3^-–N L^{-1} demonstrated improved agreement between the two methods up to 1500 mg NO_3^-–N L^{-1}, with a slope closer to one (y = 0.834x + 115.99, R^2 = 0.856), and an appreciable and progressively larger underestimation at higher [NO_3^-–N] (y = 0.650x + 312.65, R^2 = 0.389) (Figure 3b).

Amongst the six individual crops, there was variation in the degree of underestimation by the rapid analysis ISE system, for measurement of [NO_3^-–N] in undiluted sap, as indicated by differences in the slopes of the linear regression equations (Table 5). The three pepper crops had the lowest slope values, and these pepper crops had higher percentages of values that were >1500 mg NO_3^-–N L^{-1} (Table 5) compared to the crops of the other species. The relative error for all samples was 24%, and for samples > 200 mg NO_3^-–N L^{-1}, it was 18% (Table 5).

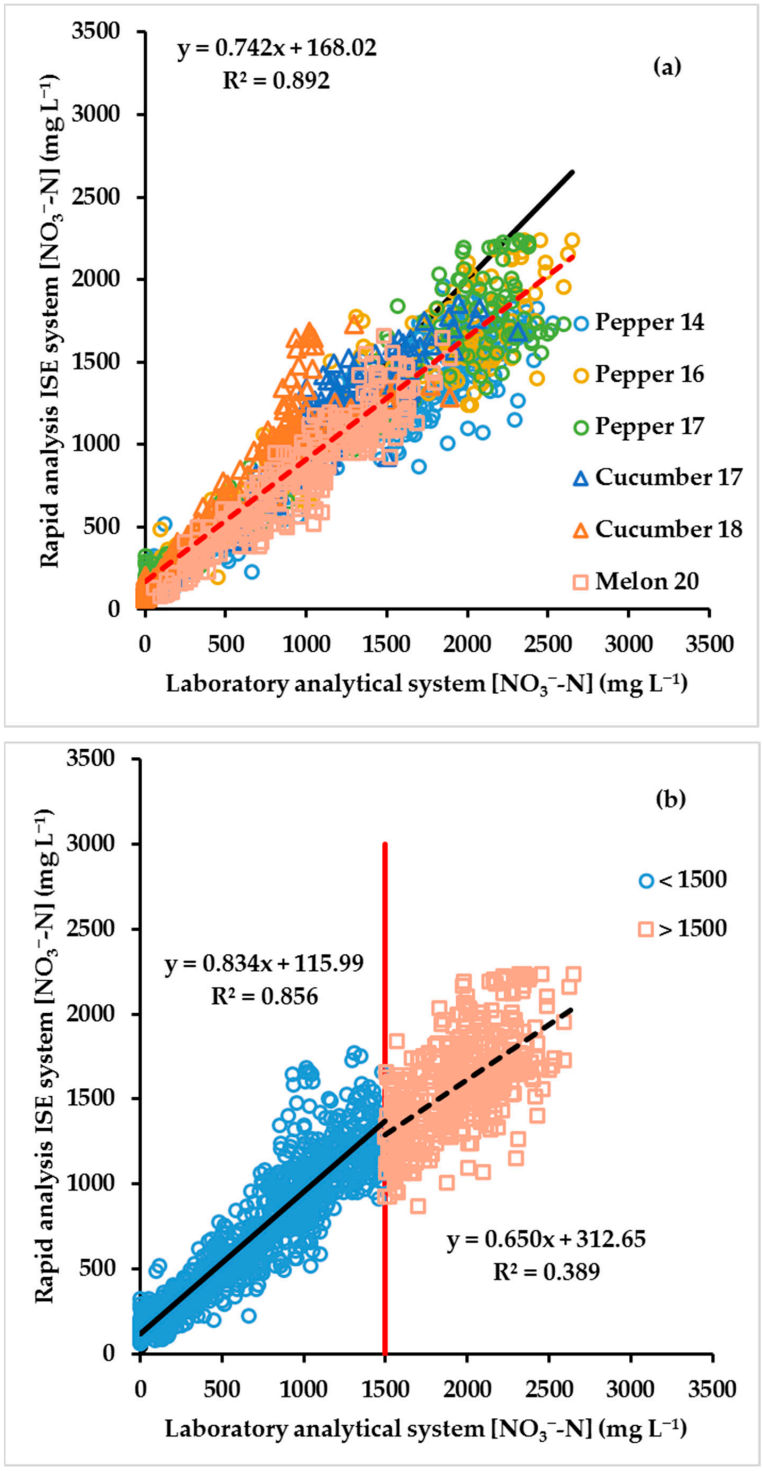

Figure 3. Undiluted petiole sap. The relationship between [NO_3^-–N] measured with the rapid analysis ISE system and with a laboratory analytical system in 1425 samples of petiole sap obtained regularly throughout six different vegetable crops. (**a**) A single linear regression equation was derived for all data points for the full range of concentrations measured with both systems. The dashed red line is the linear regression, and the solid black line corresponds to the 1:1 linear relationship. (**b**) Two linear regression equations were derived, one for [NO_3^-–N] of <1500 mg L^{-1} and the other for [NO_3^-–N] of > 1500 mg L^{-1}, as measured with the laboratory analytical system. The six crops were Pepper 14, Pepper 16, Pepper 17, Cucumber 17, Cucumber 18 and Melon 20. The laboratory system measured the [NO_3^-–N] in diluted sap. The crops, their management and the two analytical systems are described in the Materials and Methods Section.

Table 5. Undiluted petiole sap. Summary of the numbers of samples, the linear regression equations, R^2 values and the relative error for the relationship between the [NO_3^-–N] measured with the rapid analysis ISE system and with a laboratory analytical system. Relative error is the percentage difference between measurements with the two analytical systems, in relation to the concentration measured with the laboratory system. The samples were from five different vegetable crops during a five-year period. The crops, their management and the two analytical systems are described in the Materials and Methods Section.

Crop	Number of Samples	Percentage of Samples with >1500 mg NO_3^-–N L^{-1} (%)	Equation	R^2 Value	[1] Relative Error (%)	[1] Relative Error, Values >200 mg NO_3^-–N L^{-1} (%)
Pepper 14	319	55	y = 0.707x + 117.78	0.925	29	21
Pepper 16	160	53	y = 0.725x + 228.90	0.865	24	18
Cucumber 17	214	15	y = 0.866x + 128.80	0.885	12	11
Pepper 17	180	57	y = 0.756x + 184.21	0.923	27	17
Cucumber 18	252	18	y = 0.946x + 146.61	0.879	36	27
Melon 20	300	10	y = 0.777x + 65.74	0.886	18	17
All samples	1425	33	y = 0.742x + 168.02	0.892	24	18

[1] Values of 50 mg NO_3^-–N L^{-1}, as measured with the laboratory system, were excluded because very low denominator values would have distorted the results.

3.3.2. Diluted Sap

The overall relationship between the [NO_3^-–N] measured with the rapid analysis ISE system in diluted petiole sap compared to analysis with the laboratory analytical system, for 1182 samples collected throughout six crops, was described by the relationship y = 1.010x + 99.26, with a R^2 of 0.927 (Figure 4, Table 6). In contrast to undiluted sap, for diluted sap, there was good agreement between the rapid analysis ISE and laboratory systems for the full range of [NO_3^-–N] (Figure 4). The ranges of slope and intercept values for the individual crops were 0.912 to 1.041 and −33.53 to +308.16 respectively, and the range of R^2 values was 0.898 to 0.959 (Table 6). The relative error for all samples was 25%, and for samples > 200 mg NO_3^-–N L^{-1}, it was 17% (Table 6).

3.3.3. Direct Comparison of Results of Undiluted and Diluted Petiole Sap Both Measured with the Rapid Analysis System

There was a curvilinear relationship between [NO_3^-–N] of undiluted sap and diluted sap, when both were measured with the rapid analysis ISE system, described by the power equation of $y = 1.625x^{0.9064}$, R^2 = 0.918 (Figure 5a). The best fit linear regression equation, for the full range, was y = 0.734x + 120.13, with a R^2 of 0.887 (Figure 5a). A linear equation with a slope value relatively close to one (y = 0.898x + 5.880, R^2 = 0.868) described the relationship between undiluted sap and diluted sap [NO_3^-–N] up to 1500 mg NO_3^-–N L^{-1} (as measured in diluted sap) when both media were measured with the rapid analysis system (Figure 5b). For [NO_3^-–N] of >1500 mg NO_3^-–N L^{-1}, there was a substantial reduction in the slope of the linear relationship, the equation being y = 0.496x + 583.88, R^2 = 0.448 (Figure 5b).

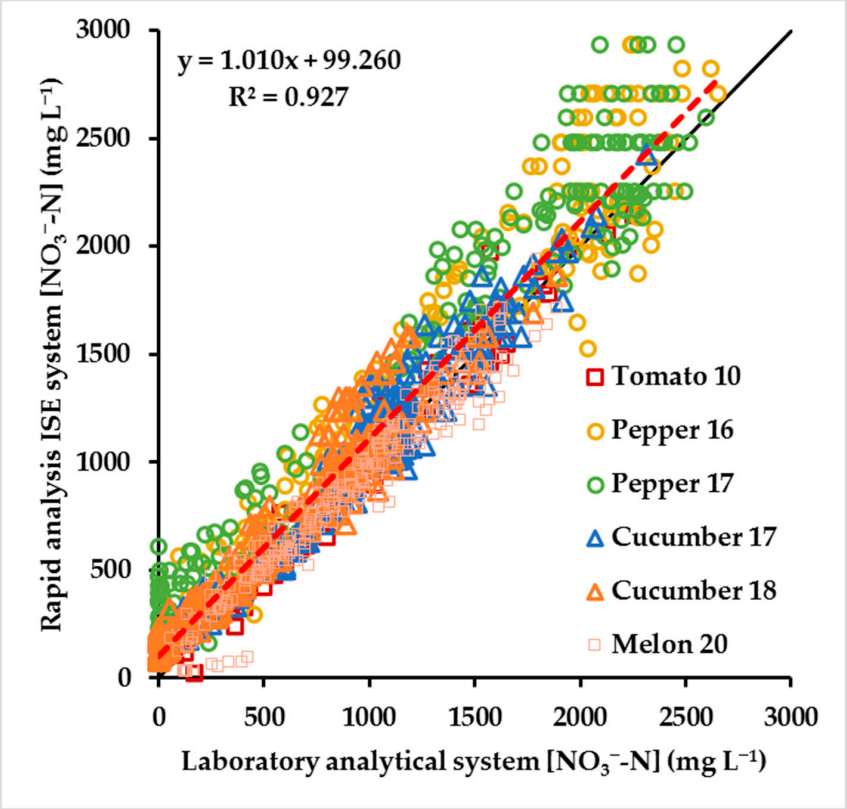

Figure 4. Diluted petiole sap. The relationship between [NO_3^-–N] measured with the rapid analysis ISE system and with a laboratory analytical system in 1182 samples of petiole sap obtained regularly throughout six different vegetable crops. The six crops were Tomato 10, Pepper 16, Pepper 17, Cucumber 17, Cucumber 18 and Melon 20. The dilution factors were 1:10 for the tomato and cucumber, and 1:5 for the pepper and melon crops. A single linear regression equation was derived for all data points for the full range of concentrations measured with both systems. The dashed red line corresponds to the linear regression, and the solid black line corresponds to the 1:1 linear relationship. The [NO_3^-–N] values for diluted samples, measured with the rapid analysis system, are presented as equivalent values for sap prior to dilution, as the product of the directly measured [NO_3^-–N] in the diluted sap multiplied by the dilution factor. The laboratory system measured the [NO_3^-–N] in diluted sap. The crops, their management and the two analytical systems are described in the Materials and Methods Section.

Table 6. Diluted petiole sap. Summary of the numbers of samples, linear regression equations, R^2 values and the relative error for the relationship between [NO_3^-–N] measured with the rapid analysis ISE system and with a laboratory analytical system. Relative error is the percentage difference between the two measurements in relation to the concentration measured with the laboratory analytical system. The samples were from six different vegetable crops. The crops, their management and the two analytical systems are described in the Materials and Methods Section.

Crop	Number of Samples	Equation	R^2 Value	[1] Relative Error (%)	[1] Relative Error, Values > 200 mg NO_3^-–N L^{-1} (%)
Tomato 10	56	y = 1.041x − 33.53	0.959	13	10
Pepper 16	160	y = 0.981x + 265.79	0.898	32	25
Cucumber 17	214	y = 1.007x + 60.44	0.940	13	11
Pepper 17	200	y = 0.965x + 308.16	0.946	51	28
Cucumber 18	252	y = 0.978x + 128.86	0.944	31	20
Melon 20	300	y = 0.912x + 75.11	0.937	14	12
All samples	1182	y = 1.010x + 99.26	0.927	25	17

[1] Values of < 50 mg NO_3^-–N L^{-1}, as measured with the laboratory analytical system, were excluded because very low denominator values would have distorted the results.

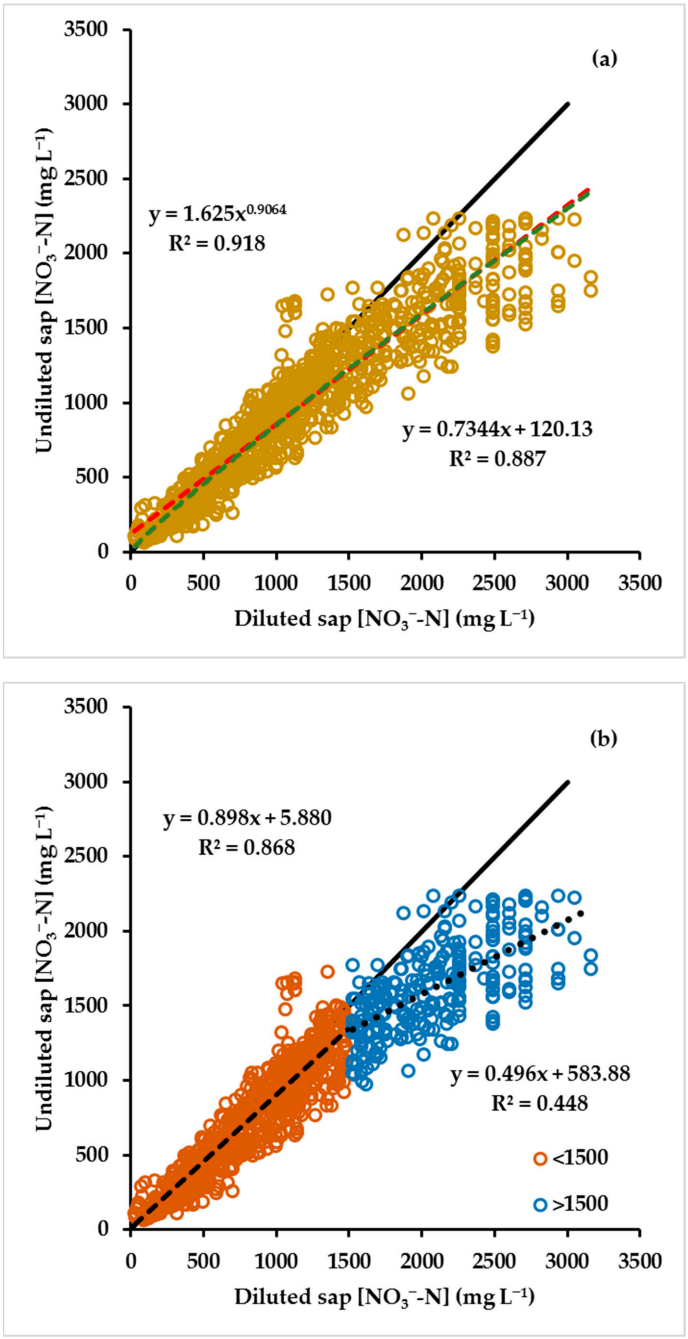

Figure 5. Undiluted and diluted petiole sap. The relationship between $[NO_3^-–N]$ in undiluted and diluted petiole sap, measured with the rapid analysis system. There were 1106 equivalent samples of both undiluted and diluted sap, taken throughout five different crops. The five crops were Pepper 16, Pepper 17, Cucumber 17, Cucumber 18 and Melon 20. In panel (**a**), both a power and a linear regression equation were derived for the full range of concentrations measured in equivalent samples of undiluted and diluted petiole sap. The green dashed line represents the power equation and the dashed red the linear regression equation. In panel (**b**), linear regression equations were derived for each of the ranges 0–1500 and >1500 mg L^{-1} (as measured in diluted sap). The dashed black line corresponds to the linear regression for the range 0–1500 mg L^{-1}, and the dotted black line corresponds to the linear regression for the range >1500 mg L^{-1}. In both panels, the black unbroken line is the 1 to 1 line. The $[NO_3^-–N]$ values for diluted samples, measured with the rapid analysis system, are presented as equivalent values for sap prior to dilution, as the product of the directly measured $[NO_3^-–N]$ in the diluted sap multiplied by the dilution factor. The crops, their management and the rapid analysis system are described in the Materials and Methods Section.

3.4. Comparison of Results with LAQUAtwin Models B343 and NO3-11

For nutrient solution and soil solution samples measured with the LAQUAtwin model B343 (Pepper 14, Pepper 16 and Cucumber 17 crops), and with the LAQUAtwin model NO3-11 (Pepper 17 and Cucumber 18 crops), the results were generally very similar in terms of slope, intercept, R^2 values and RE values (Table 7). For undiluted and diluted petiole sap samples, measured with the LAQUAtwin model B343 (Tomato 10, Pepper 16 and Cucumber 17 crops), and with the LAQUAtwin model NO3-11 (Pepper 17, Cucumber 18 and Melon 20 crops), the results were generally very similar in terms of slope, intercept, R^2 values and RE values (Table 8). Considering the results with nutrient and soil solution, and with undiluted and diluted sap, both the model B343 and model NO3-11 behaved similarly compared to laboratory analysis (Tables 7 and 8).

Table 7. Nutrient and soil solution. For each of the two models of rapid analysis ISE system used, the numbers of samples, linear regression equations, R^2 values and the relative error for the relationship between [NO_3^-] measured with each ISE system and with the laboratory system. Relative error is the percentage difference between measurements with the two analytical systems, in relation to the concentration measured with the laboratory system. The relevant data for individual crops are presented in Tables 3 and 4, and which ISE system was used with individual crops is described in Section 2.4.

Material	Model ISE	No. Samples	Equation	R^2 Value	[1] Relative Error (%)	Relative Error, Values > 5 mmol L^{-1} (%)
Nutrient solution	B-343	1067	y = 0.968x + 0.85	0.957	14	10
Nutrient solution	NO3-11	943	y = 1.002x + 0.61	0.970	13	9
Soil solution	B-343	433	y = 0.960x +1.54	0.962	21	13
Soil solution	NO3-11	364	y = 0.998x + 0.66	0.969	23	11

[1] Relative error for all concentrations > 0.5 mmol L^{-1}, as measured with the SKALAR laboratory system. At lower concentrations, the relative error values were sometimes appreciably amplified by small denominator values.

Table 8. Undiluted and diluted petiole sap. For each of the two models of rapid analysis ISE system used, the numbers of samples, linear regression equations, R^2 values and the relative error for the relationship between [NO_3^-] measured with each ISE system and with the laboratory system. Relative error is the percentage difference between measurements with the two analytical systems, in relation to the concentration measured with the laboratory system. The relevant data for individual crops are presented in Tables 5 and 6, and which ISE system was used with individual crops is described in Section 2.4.

Material	Model ISE	Number of Samples	Equation	R^2 Value	[1] Relative Error (%)	Relative Error, Values > 200 mg NO_3^-–N L^{-1} (%)
Undiluted sap	B-343	693	y = 0.720x + 187.13	0.880	18	17
Undiluted sap	NO3-11	732	y = 0.770x + 150.60	0.892	25	20
Diluted sap	B-343	430	y = 1.045 + 70.46	0.916	20	16
Diluted sap	NO3-11	752	y = 0.989 + 110.44	0.927	29	18

[1] Values of 50 mg NO_3^-–N L^{-1}, as measured with the laboratory system, were excluded because very low denominator values would have distorted the results.

3.5. Effect of Sensor Deterioration

Measurement of [NO_3^-] in nutrient solution with a deteriorated sensor, through normal ongoing wear, resulted in appreciable overestimation and increased relative error compared to laboratory analysis (Figure 6). The relevant linear regression equation was $y = 1.401x - 0.17$, $R^2 = 0.921$, which indicated an overestimation of approximately 40%. The relative error with the deteriorated sensor was 30% (data not presented). For the same model LAQUAtwin rapid analysis system with a new sensor, the linear regression of the relationship with laboratory analysis was $y = 0.945x + 0.73$, with an $R^2 = 0.980$ (Figure 6), and a relative error of only 12% (data not presented).

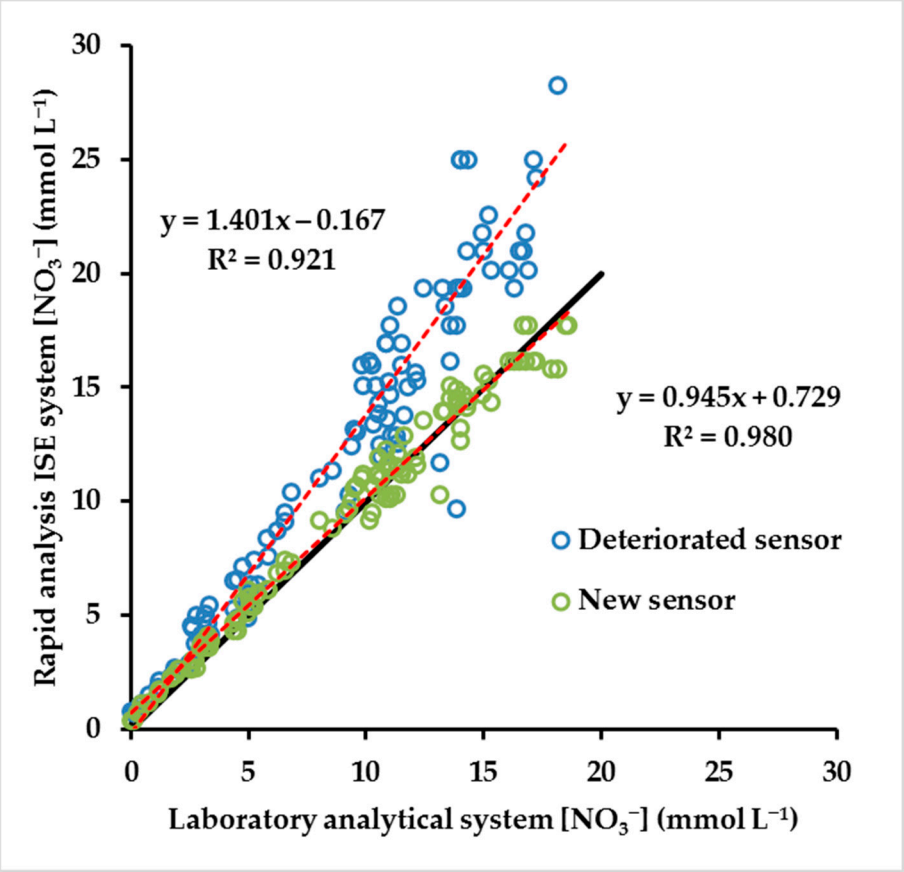

Figure 6. The relationships of [NO_3^-] measured with a deteriorated sensor and with a new sensor, both in a model B343 LAQUAtwin meter, compared to laboratory analysis, for 108 samples of nutrient solution from the Pepper 16 crop. The 108 samples were measured with the same LAQUAtwin meter with both sensors. The dashed red line corresponds to the new sensor linear regression. The dotted red line corresponds to the deteriorated sensor linear regression. The solid black line corresponds to the 1:1.

4. Discussion

The results of the linear regression and relative error (RE) analyses indicated that the rapid analysis ISE system provided measurement of the [NO_3^-] of nutrient solution, soil solution and diluted petiole sap, with sufficient accuracy to effectively guide on-farm decision making. The results obtained indicated that this rapid analysis system cannot be recommended for the measurement of the [NO_3^-–N] of undiluted petiole sap of vegetable crops. A notable feature of the results was that, for each media evaluated, the results were very similar between species and between different cropping cycles (autumn–winter versus spring–summer).

The overall RE values were 14% and 22% for nutrient and soil solution, respectively. The overall RE value for soil solution was inflated by relatively high RE values for two of the five crops. These RE results suggest that with optimal handling and measurement, this rapid analysis system can measure [NO_3^-] in nutrient and soil solution samples with an RE of approximately 14%. For the likely practical ranges of soil and nutrient solution of >5 mmol NO_3^- L^{-1} and >2 mmol NO_3^- L^{-1} respectively, the RE values were approximately 10%. The RE values were generally higher and more variable for petiole sap, both undiluted and diluted. For the common practical range of >200 mg NO_3^-–N L^{-1}, the RE for petiole sap was approximately 18%. The relative complexity of petiole sap extraction and instability of sap likely contributed to the higher and more variable RE values. The RE values in the present work are somewhat higher than the error value of 10% reported by the manufacturer.

Accurate measurement of [NO_3^-] in soil solution was consistent with two studies using similar rapid analysis ISE systems. Both [32,33] reported linear regressions close to the one to one line with R^2 values of 0.96 and 0.91 for 161 and 100 samples, respectively. The results of these and the current study demonstrate that these rapid analysis ISE systems can provide accurate measurement of the [NO_3^-] of soil solution extracted with ceramic cup suction samplers.

Of the few studies directly comparing measurements with rapid analysis ISE systems to laboratory analysis of [NO_3^-] in plant sap, two [35,42] reported appreciable underestimation, as in the present study, and one [34] reported good agreement. In general, the available results suggest that ISE systems underestimate the [NO_3^-] of undiluted plant sap. There are no published studies examining the accuracy of rapid analysis ISE systems with nutrient solution or comparing performance in diluted and undiluted sap.

Interference effects on the measurements of [NO_3^-] with ISE rapid analysis systems from chloride (Cl^-), sulphate (SO_4^{2-}), potassium (K^+), calcium (Ca^{2+}) and organic acids have been reported [28,30]. High ionic strength can contribute to underestimation of [NO_3^-] by ISE measurement [30]. The effects of interfering ions are most pronounced at higher concentrations of [NO_3^-] and interfering ions [30]. The effects of higher ionic strength and of interference from others ions, at relatively high [NO_3^-], may explain the underestimation observed in undiluted sap in the current study and in [35,42]. However, the results of the current work indicate that chemical interferences and ionic strength effects did not affect the accuracy of [NO_3^-] measurement in nutrient and soil solution, and in diluted petiole sap.

Sensor deterioration, as reported here, can induce appreciable measurement error. The user manual of the rapid analysis ISE systems used suggests that an electrode should provide approximately 1500 measurements [29]. The same manual suggested changing electrodes before commencing an annual field campaign. To minimize the possible effects of chemical interferences and sensor deterioration, it is recommended that users periodically check their results by having some samples analyzed by a reputable analytical laboratory. Also, users should consider analyzing samples and/or aqueous standards of known [NO_3^-] when using rapid analysis ISE systems. An additional important consideration is that sample temperature can notably affect measurement (M.T. Peña-Fleitas, unpublished data). All samples and calibration standards should be at room temperature (approximately 20 °C) when measurements are made. To further enhance accuracy, it is also recommended that, after developing a clearly defined protocol, that one user makes all measurements, particularly for petiole sap.

Because of their accuracy and analytical range, rapid analysis ISE systems are preferable to rapid analysis refractometers systems, which require dilution of nutrient and soil solution, and considerable dilution of sap [43,44]. Optimal measurement with a rapid analysis ISE system requires that recommended practices be very strictly followed. These recommendations include frequent calibration, use of standards appropriate for the range being analyzed, rinsing and drying of sample cells before each measurement, measurement of samples of known concentration different to the calibration standards and attention to sample temperature [21,28,30]. The results of the current study, together with available published results, suggest that rapid analysis ISE systems can provide sufficiently accurate measurement of [NO_3^-] of nutrient and soil solutions, and of diluted petiole sap to guide farm management. They should not be regarded as a replacement for laboratory analysis, particularly for certification purposes. Their accuracy should be periodically checked with laboratory analysis.

5. Conclusions

The rapid analysis ISE system measured the [NO_3^-] of nutrient solution, soil solution and diluted petiole sap, with sufficient accuracy to effectively guide on-farm decision making. With undiluted petiole sap, there was appreciable underestimation. The overall measured relative error (RE) values were 14% and 22% for nutrient and soil solution,

respectively. For the likely practical ranges of soil and nutrient solution of >5 mmol NO_3^- L^{-1} and >2 mmol NO_3^- L^{-1} respectively, the RE values were approximately 10%. For petiole sap, both undiluted and diluted, the overall RE was approximately 25% and the RE for the common practical range of >200 mg NO_3^- L^{-1} was approximately 18%. The higher error with sap measurement appears to be related to how sap extraction and handling procedures interact with the instability of petiole sap. Sensor deterioration can result in appreciable error with this rapid analysis IES system. The rapid analysis ISE can assist in on-farm management of N, but considerable care is required with its preparation and use, and with sample handling.

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