

Measurement of ion levels of spinach grown in different fertilizer regimes using ion chromatography

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Several samples of spinach grown under different ion supplies in both soil and irrigation water have been analysed by determining their contents of anions and cations using ion chromatography. The ionic contents in the spinach may be correlated with the ionic intakes during growth, and especially so in the case of the nitrate ion.

INTRODUCTION

Spinach (*Spinacea oleracea* L.), an annual cool-season plant, is popular as a green, leafy vegetable. It can be grown in both spring and autumn, giving high yields in a short time (Peavy & Greig, 1972).

Data collected during the past several years show that spinach yields in excess of 1.3 quintals (130 kg)/ha can be achieved in Spain (Ministerio de Agricultura, Pesca y Alimentación, 1991).

Vegetables provide a major portion of our dietary intake of nitrate (Olday *et al.*, 1976), and there are certain health implications involved in consuming nitrate in our diet, which have been reviewed in the authors' previous paper (Vera *et al.*, in press). Because of this, the concentration of nitrate in spinach is an important consideration (Phillips, 1971), and especially so in the preparation of baby foods (Cantliffe, 1972c).

Therefore, particular attention has been focused on the use and effect of fertilizer on those plants employed for human consumption (Cantliff, 1972b) because nitrate accumulates in the edible tissue (Bathurst & Mitchell, 1958; Eichenberger *et al.*, 1971; Cantliffe, 1972a; Maga *et al.*, 1976; Ministère de l'Environnement, 1982).

In the case of leafy vegetables, such as spinach, it is common practice to apply additional N as sidedressing several weeks before harvest (Maga *et al.*, 1976).

Recently, increased attention has been devoted to the utilization of organic fertilizers as substitutes for the commonly used mineral fertilizers (Peavy & Greig, 1972), because ammoniacal fertilizers may lead to lower nitrate accumulations in plants than nitrate-fertilizers used as sidedressing to a growing crop (Schuphan, 1974). Split application of mineral fertilizers would

hardly maintain low nitrate contents in leafy vegetables, and this shows that the absorption rate of nitrogen by the plant still remains higher than its assimilation rate. Despite the fact that the processing of organic nitrogen sources by micro-organisms in the soil is not now under control, numerous data suggest that application of a proper organic fertilization, excluding readily mineralizable nitrogen sources alone, would maintain low nitrate levels in leafy vegetables (Lairon *et al.*, 1984).

However, if the fertilizers are spread at planting, the advantage of the ammoniacal fertilizers is lost, due to nitrification (Peavy & Greig, 1972).

There are other factors that can be the cause of changes in nitrate levels; these are, for example, nitrate reductase activity in plants, especially in leaves, which is induced by the presence of nitrate in the medium (Bathurst & Mitchell, 1958; Burrige *et al.*, 1964; Cantliffe, 1972; Seith *et al.*, 1991). An inverse relationship between nitrate accumulation and nitrate reductase activity has been shown (Hageman and Flesher, 1960; Cantliffe, 1972a). Certain herbicides can lead to the accumulation of nitrate in the plant (Stahler & Whitehead, 1950).

Other factors such as soil (aeration, nitrate level, moisture, microbe activity) (Cantliffe, 1972c; Maynard *et al.*, 1976; Aworh *et al.*, 1980) and environmental effects (light intensity (Hoff & Wilcox, 1970; Cantliffe, 1972b), photoperiod (Cantliffe, 1972c) can also lead directly to nitrate build-up in vegetables.

Several methods have been proposed for the determination of nitrate in waters and in soils (Sawicki, 1978; Dick & Tabatabai, 1979; Thompson & Blankley, 1984) and of sulphates (Beaton *et al.*, 1968; Dick & Tabatabai, 1979), but the availability of the ion chromatograph

(IC) offers the opportunity of determining all ions (cations and anions) simultaneously and more rapidly than is possible by other procedures.

This study was undertaken to investigate the possible involvement of fertilizer source and ion levels in the irrigation water on ion accumulation in spinach using a simple and automatic method — the IC — that requires little pretreatment of samples with stable reagent solutions and which has an analysis rate of three samples per hour. For the water, no sample pretreatment is required (Thompson & Blankley, 1984).

MATERIALS AND METHODS

Samples

Spinach samples (*Spinacea oleracea*, L.) of F₁ Correta variety (tardy cycle hybrid), were obtained from Industrias Prieto, S. A. (Murcia, Spain). Samples for series 1, 2 and 3 were grown on 18 October 1992 (series 1 and 2 in the Algaida field) and 19 October 1992 (series 3 in the Alhama narrow valley). Seeds were planted at levels from the range 50–70 kg/ha. Natural rainfall was supplemented by sprinkler irrigation three times. The organic fertilizer (manure) was spread at series 3 planting. Fifty kg N/ha was applied for this series as organic N. The inorganic fertilizer was sidedressed on all three occasions with the irrigation water. These series were grown with 50 kg/ha NPK for series 1 and 100 kg/ha NPK for series 2 and 3, using cycloate(N-ethyl-cyclohexyl thiolcarbamate 5-ethyl) as herbicide in the first irrigation.

The plants were harvested by cutting them 2 cm above the soil level 85 days after seeding, and they were immediately sent by truck to the laboratory plant where they were passed through shakers to remove any debris.

Soils used were taken from the Algaida field and the Alhama narrow valley, both near Murcia town. Soil cores (25 cm diameter, 20 cm deep) were taken from five subplots of equal area in each paddock immediately before and during growth of the spinach. Subplots were mixed and used to provide single samples for the assessment of the precision of the techniques used for measurements. The soil samples were stored at 5°C prior to analysis (Bristow & Jarvis, 1990). Before analysis, the soils were passed through a 3 mm mesh sieve. Subsamples of each soil were dried at 105°C for 36 h (Peech, 1965).

Samples of the river water used for the several irrigations were stored at –18°C prior to analysis.

Apparatus

The IC used was a Dionex Model 2000 i/sp (Dionex corp., Palo Alto, CA, USA), consisting of an anion separator column (Dionex AS4A column (P/N 37041)), a guard column containing the same resin AG4A (P/N 37042) and a cation separator column (Dionex HPIC-

CG1 column (P/N 030831)) with a guard column HPIC-CG1 (P/N 030830); and an anion fiber suppressor (AFS) Dionex, with autosampling Model 035274 Dionex. The detector used, was a conductivity cell Model CDM. A Spectra-Physics 4270 as integrator.

The elemental analyser CHNS was a Carlo Erba 1108 model with a porapak PQS (25 cm) GC column and a Thermo Conductivity Detector, with autosample AS200, coupled to an Epson EL3S VGA automatic computer with 3C87-SX16 MHz EAGER 200 software.

Reagents and standards

All chemicals were of chromatographic grade. The eluents used were 0.65 mM NaHCO₃ + 1.5 mM Na₂CO₃, with 0.01 N H₂SO₄ regenerant for anions; and 0.3 mM HCl, with 0.4 M KOH regenerant for cations. Pump pressure was 900 psi.

For the IC, identification was made according to retention times for each anion which is determined by injecting a standard solution containing only the anion of interest.

Calibration curves for the ions were linear in the low concentration ranges up to 25–30 ppm. The correlation coefficients for Cl⁻, NO₃⁻, PO₄³⁻, SO₄²⁻, Ox²⁻, Na⁺, NH₄⁺ and K⁺ are 0.997, 0.988, 0.998, 0.997, 0.998, 0.992, 0.994 and 0.995, respectively. Minimum detectable levels are defined as the concentrations of ion necessary to give a peak height of 2% of full scale for the conductivity detector (Cantliffe, 1972) set at its maximum sensitivity (about 30 μs/cm full scale). These were Cl⁻ (0.1), NO₃⁻ (0.2), PO₄³⁻ (0.1), SO₄²⁻ (0.2) mg/litre.

For the elemental analysis; standard sample materials (sulphanilamide for calibrated detector) were placed in a tared standard tin capsule for calibration and performance testing using He gas as carrier at 140 ml/min and 1020°C in the range from 100 ppm to 100% and with a standard deviation of 10 ppm.

Sample preparation

Samples were freeze-dried in a Virtis Quickseal Valves freeze-dryer (The Virtis Company, New York) and the moisture content was then determined (Murcia & Rincón, 1991). Subsequently, freeze-dried spinach was ground to a fine powder with a 980 Moulinex mill. Determination of corrected moisture was performed by the Karl-Fischer method modified for food as described in Moibroek and Shahwecker (1984).

Ions were extracted following the AOAC methods (1980) for dried vegetables and flours, using 0.5 g spinach flour and 1:10, 1:25, 1:100 and 1:150 dilutions, except Cl⁻ ion which was extracted according to Hertz and Baltensperger (1984).

Elemental analysis was made according to Belomonte *et al.* (1987) from a 2 mg sample with an analysis cycle of 740 s in the following conditions: reduction tube temperature 1020°C, chromatographic oven temperature 75°C and filament temperature 190°C.

Analysis for soil pH was done following Peech

(1965) and that for soil ions was performed as reported by Dick and Tabatabai (1979); the soil extracts were obtained by shaking 10 g of sample with 50 ml of extractant in a stoppered 125 ml bottle for 1 h, and filtering the resulting suspension (Whatman no. 42 filter paper). The following reagents were used as soil extractant: H₂O, 10 mM LiCl, 10 mM KCl and 30 mM NaHCO₃ + 1.8 mM Na₂CO₃; since significant differences were not found in the results, the authors proceeded to use water in agreement with Dick and Tabatabai (1979). The soil extracts thus obtained were analysed by IC.

Urease activity was made following the methods of McGarity and Myers (1967) from dried soil (20 g) with toluene and potassium citrate-citric until a brown colour was seen in the filtered extract. This was read at 630 nm with phenolate solution and sodium hypochlorite solution.

RESULTS AND DISCUSSION

The high precision of the IC method used for analysing the ion levels gave good results for the replicate analysis of standards, with standard deviations ranging from 0.09 to 0.12. Further evidence that the IC method is satisfactory for the determination of ions in sample extracts was shown by the 98–102% recovery of ions. These recovery tests were performed by analysing samples before and after treatment using concentrations of 5, 10 or 50 mg/g of these ions to make up the samples.

The reproducibility of sample injections was demonstrated with a single extract of sample prepared as normal, and a reproducibility with a coefficient of variation of 1.6–3.2%. Figure 1 shows a representative chromatogram of spinach (a), soil (b) and water (c) samples. As can be seen, the anions studied can be resolved easily under the described conditions and show excellent baseline resolutions. This method was used for the anions studied, except Cl⁻, because the

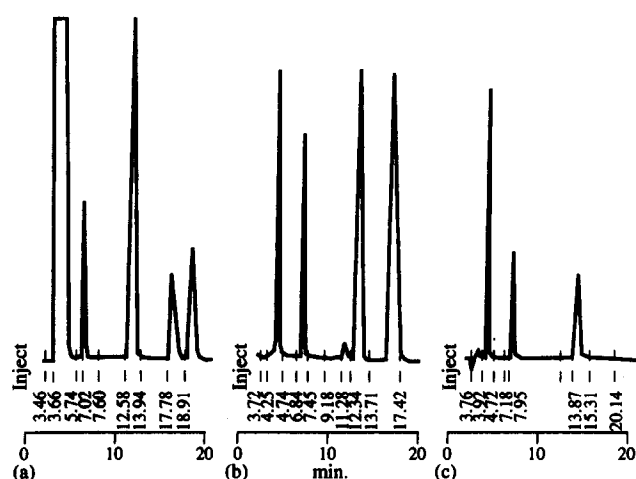


Fig. 1. Anion chromatograms of spinach (a), water (b) and soil (c) samples. Samples containing Cl⁻ (retention time, 4.74 min), NO₃⁻ (retention time, 7.45 min), PO₄³⁻ (retention time, 13.71 min), SO₄²⁻ (retention time, 17.42 min) and Ox²⁻ (retention time, 18.91 min).

Table 1. Anion levels in spinach at different growth conditions

Spinach	Cl ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻	Ox ²⁻
1	2768	1800	2720	843	2739
2	4660	3384	6647	921	2592
3	4190	4813	7302	476	2603

Data presented are means (ppm) of five determinations that differed by no more than 10%.

extraction procedure of the AOAC method, uses CdCl₂ and CaCl₂ as principal solvent components. Hence, the authors used a different extraction method for Cl⁻ (see Materials and Methods, subsection 'Sample preparation'), which was subsequently analysed in a separate chromatogram, not shown). Similar resolutions were obtained for cation chromatograms of the same samples, when detecting Na⁺, NH₄⁺ and K⁺ cations.

Content of ions in spinach in different fertilizer regimes

Tables 1 and 2 show the results obtained by ionic exchange chromatography for the different anions and cations studied in spinach samples at different growth conditions. Analysis of the nitrate values shows significant differences between the samples. Table 1 shows how the concentration of nitrates increases significantly from samples 1 to 2 to 3, reaching values of 1800, 3384 and 4813 ppm, respectively. Generally speaking, the levels of nitrate and other ions in spinach are closely related both to the fertilizing regimes and, as pointed out in the references, to the nitrogen content and other ions available in the soil and water.

Samples 1 and 2 were subjected to the same mineral dressing although in different proportions (50 and 100 kg/ha % NPK, respectively), from which it can be deduced that the increases in nitrate values are due to increased quantities of fertilizer. These results are in accordance with those obtained by other authors in studies of nitrate induction in vegetables by mineral fertilizers (Barker *et al.*, 1971, 1974; Eichenberger *et al.*, 1981).

However, these levels are higher in sample 3, to which an organic fertilizer had been previously added before the 100 kg/ha of the mineral one, a common agricultural practice which gives higher crop yields (Peavy & Greig, 1972; Maga *et al.*, 1976).

As regards the other ions analysed and whose values are shown in Tables 1 and 2, the clear increases in concen-

Table 2. Cations in spinach at different growth conditions

Spinach	Na ⁺	NH ₄ ⁺	K ⁺
1	2623	509	24300
2	2989	743	27100
3	8084	760	17555

Data presented are means (ppm) of five determinations that differed by no more than 10%.

Table 3. Anion levels in irrigation water in different farming zones

Water	Cl ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻	Ox ²⁻
1 and 2	345	7.2	1158	27.6	ND
3	892	147	ND	ND	ND

Data presented are means (ppm) of five determinations that differed by no more than 10%.
ND, None detected.

trations of the chloride and phosphate ions are due to the increased doses of mineral fertilizer. However, only small differences are seen in sample 3 to which organic fertilizer had been added.

With respect to the sulphate ion, similar levels are observed in samples 1 and 2 with a decrease of 52% in sample 3. This may be explained by the difference in levels of the sulphate ion in the irrigation waters used.

Very small variations are seen in the levels of the oxalate ion which, although appearing to be quite high, should not, according to Shaller (1961), pose any problems in a normal diet since calcium deficiency in both children and adults is rare as long as this element appears in the diet (Ackermann, 1958; Schuphan & Weinmann, 1958).

Similar results are obtained for the levels of sodium, ammonium and potassium ions (Table 2).

Analytical parameters evaluated in soil during the spinach cultivation

Studies of the soils began with the determination of urease activity as an indicator of the microbial biomass (McGarity & Myers, 1967), which through successive oxidation of the ammonium to nitrite and nitrate (Wolfe *et al.*, 1988) might seriously influence the levels of these ions in the soil and consequently in spinach.

However, the results for urease activity, expressed as mg of ammonium ion per 100 g of soil, show no significant differences in the experiments realized during the approximate two months of spinach cultivation and growth, varying between 30.5 and 40 mg of NH₄⁺/100 g of sample in soils 1 and 2 and between 19 and 22.5 in soil 3.

Parallel to microbial activity the pH of the soils was also evaluated to confirm that there were no great changes in this activity and to ensure that the irrigation waters had not deposited significant quantities of minerals or substances which might alter the mean composition of the soils during the cultivation period (Bristow & Jarvis, 1991).

The results show pH values of between 8.01 and 8.33 for suspensions of soils 1 and 2 in distilled water and between 7.21 and 7.51 for suspensions of soils 1 and 2 in a potassium chloride solution. For soil 3, the results were 7.04–7.92 and 7.0–7.4 for similar suspensions.

As can be seen, the values obtained with KCl are slightly below those obtained in the aqueous solutions,

Table 4. Cation levels in irrigation water in different farming zones

Water	Na ⁺	NH ₄ ⁺	K ⁺
1 and 2	344	133	9.6
3	1077	12.6	35.6

Data presented are means (ppm) of five determinations that differed by no more than 10%.

probably due to the clay acting as an ion-exchange resin, retaining a charge so that the pH with KCl is more acidic.

The ionic levels of the Cl⁻, NO₃⁻, PO₄³⁻, SO₄²⁻ and oxalate²⁻ Ox²⁻ for soils 1, 2 and 3 were also studied. Soil 2 is similar to soil 1 (data not shown). Serious changes in sulphate ion and nitrate ion are to be noted, a consequence of the fertilization undergone, and probably of the ionic levels of the irrigation water.

In spite of this proportionality and of the increased nitrification, this situation is not very pronounced, as the authors have shown with the urease activity and with the levels of carbon and nitrogen found in the soil, indicative of biomass changes (Bristow & Jarvis, 1991). Values for carbon ranging over 7.2–7.5 and N values ranging 0.09–0.12 were obtained for soil 1; values of 7.3–7.4 and 0.13–0.14 were obtained for soil 2; and values of 7.3–7.6 and 0.2–0.3 were obtained for soil 3; this is in agreement with Nandi *et al.* (1983). The increase in biomass shown by soil 3 could be explained by the addition of organic fertilizer received by it.

Irrigation waters

Tables 3 and 4 show the anion and cation levels detected in the irrigation waters used in the different cultivation areas. The waters used in soils 1 and 2 came from the river Segura, while the water used for soil 3 was a subterranean water, which would explain the pronounced differences between the levels of these ions. The different waters used for irrigation had different contents in nitrate and this may have contributed something to the observed different contents in nitrate of the different samples of spinach. However, the variations seen in the spinach are much more marked than those of the different waters. Therefore, the variations on nitrate of the spinach due to the water should be much less important than those due to the fertilizer.

In conclusion, in order to show the influence of the initial soil nitrate levels, i.e. after N-fertilizer had been added and the nitrate contributed by the irrigation waters on the final nitrate composition of the spinach, the histograms of Fig. 2 were elaborated. As can be seen, the spinach accumulated nitrate in proportion to the original nitrate level of the soil (compare the results of samples 1 and 2).

The greatest accumulation of nitrate is seen in sample 3. In this case both the original nitrate levels in the soil and those in the water were high.

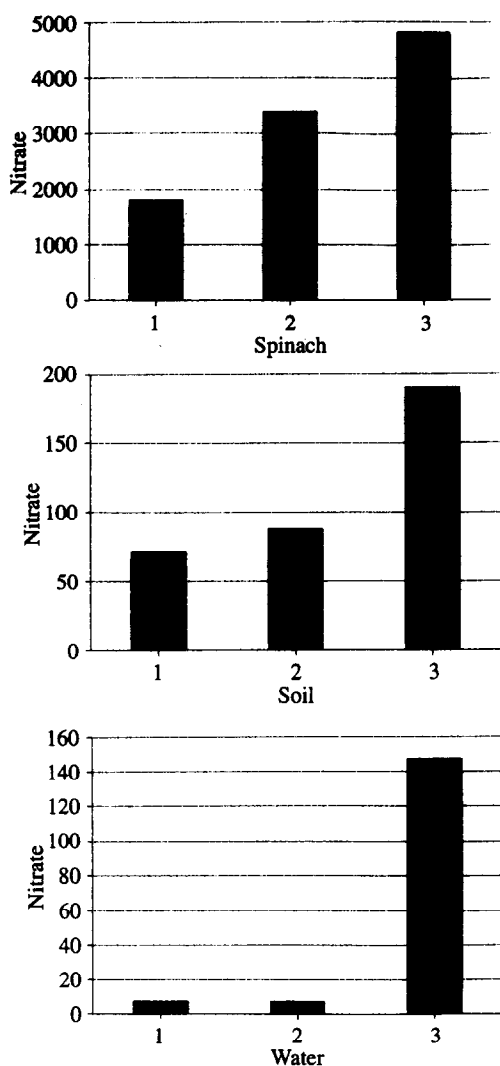


Fig. 2. Histograms with the nitrate levels in spinach, soil and irrigation waters.

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