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Original article

Evaluation of nitrate residual in lettuce irrigated with different concentrations of the anion

Evaluación de la residualidad de nitratos en lechuga (*Lactuca sativa* L.) regadas con distintas concentraciones del anión

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ABSTRACT: In Corrientes province, Argentina, lettuce cultivation represents 54 % of the total national crop, but also presents the problem of high nitrate concentrations in water used for irrigation and consumption. The aim of this study was to determine the nitrate residual in lettuce leaves (*Lactuca sativa* L.) var Brisa and growth parameters of plants irrigated with water containing different concentrations of nitrate. The trial was carried out in a greenhouse, in an Udipsament-argic soil, in 3 L pots. Treatments, with five replicates, were: T0 (control): pots were irrigated with water containing 0 mg NO_3^- L^{-1}, T1: pots irrigated with water containing 25 mg NO_3^- L^{-1}, T2: irrigated with 50 mg NO_3^- L^{-1}, T3: irrigated with 75 mg NO_3^- L^{-1}, T4: irrigated with water containing 100 mg NO_3^- L^{-1}. Lettuces, produced in winter period and that received nitrate through irrigation, presented higher foliar nitrate concentration compared to the control. In addition, irrigation with 100 ppm nitrate was associated with leaf nitrate concentration, whole plant dry mass, and plant aerial part dry mass and leaf length. While, the control and treatment with 25 ppm nitrate were associated with the number of leaves. Lettuces produced with irrigation with 75 and 100 ppm nitrate had leaf nitrate values that considerably exceeded the standards, making them unsuitable for human consumption.

Key words: nitrogen fertilizers, growth, leafy vegetables, public health.

RESUMEN: En la provincia de Corrientes, Argentina, el cultivo de lechuga representa el 54 % del total entregado a nivel nacional, pero también presenta la problemática de altas concentraciones de nitratos en agua utilizada para riego y consumo. El objetivo del presente trabajo fue determinar la residualidad de nitratos en hoja de lechuga (*Lactuca sativa* L.) var Brisa y parámetros de crecimiento de plantas regadas con agua conteniendo distintas concentraciones de nitrato. El ensayo se realizó en invernadero, en un suelo Udipsament árgico, en macetas de 3 L. Los tratamientos, con cinco repeticiones, fueron: T0 (testigo): las macetas fueron regadas con agua que contenía 0 mg de NO_3^- L^{-1}, T1: macetas regadas con agua que contenía 25 mg de NO_3^- L^{-1}, T2: regadas con 50 mg de NO_3^- L^{-1}, T3: regadas con 75 mg de NO_3^- L^{-1}, T4: regadas con agua conteniendo 100 mg de NO_3^- L^{-1}. Las lechugas, producidas en periodo invernal y que recibieron nitrato mediante el riego, presentaron mayor concentración de nitrato foliar respecto del testigo. Además, el riego con 100 ppm de nitratos se asoció con la concentración de nitrato foliar, masa seca de la planta entera, masa seca parte aérea de la planta y con la longitud de hojas. Mientras que, el testigo y tratamiento con aporte de 25 ppm de nitrato se asociaron con el número de hojas. Las lechugas producidas con riego con 75 y 100 ppm de nitratos tuvieron valores de nitrato foliar que superan considerablemente los estándares, por lo que no son aptas para consumo humano.

Palabras clave: abonos nitrogenados, crecimiento, hortalizas de hoja, salud pública.

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INTRODUCTION

Worldwide, lettuce (*Lactuca sativa* L.) is the most economically important crop among leafy vegetables, due to the possibility of being grown all year round, under different production systems (1). This vegetable is generally grown in irrigated soils, mainly in furrows, and it is associated with high water consumption. The hygienic-sanitary characteristics of water and food are compromised by the presence of excessive amounts of nitrates (NO_3^-) (2), a problem generated by the excessive use of nitrogen fertilizers.

The consumption of high nitrate content in the human diet is dangerous because this ion contributes to diseases such as methemoglobinemia, which causes a decrease in the oxygen transport capacity of red blood cells, causing a decrease of oxygen in organs and tissues throughout the body, with damage to them and even death. It also produces gastric problems, renal damage, neurological disorders and even cancer, effects that can be noticed in a medium to long term period and cause greater affectation in children under six months of age (3,4).

Vegetables, particularly leafy vegetables, accumulate higher nitrate contents than other types of food (1,5,6). Because of this problem, NO_3^- concentration limits are established in vegetable leaves, above which their consumption would not be advisable (7,8). In several European countries, a limit of 2500-4500 mg NO_3^- kg^{-1} of fresh vegetable mass has been set for consumption (9). Lettuce, consumed in salads, it is one of the species with the greatest tendency to reach high NO_3^- concentrations in leaves and ribs (10), which varies among genotypes (11) and the growing season (12,13).

In Argentina, specifically Corrientes province, has an important participation in the commercialization of lettuce, representing 54 % of the total delivered at national level; while Buenos Aires has 20 % and Santa Fe 15 %. The average volume throughout the year is 980 tons, the highest production volume is obtained between October and February. It should be noted that the participation of Corrientes is relatively constant throughout the year; however, Buenos Aires enters with its production between November and March (14).

In studies carried out by researchers from the Faculty of Agricultural Sciences of the National University of the Northeast (UNNE) with horticultural producers in Corrientes, they found NO_3^- concentrations of between 10.65 and 240.65 mg L^{-1} in water samples used for irrigation and consumption (15). Due to the fact that the producer uses water from the supply source of his farm (borehole) without, in many cases, a previous analysis of it, the aim of this work was to determine the residual nitrate in lettuce leaves (*Lactuca sativa* L.) var Brisa, irrigated with water containing different nitrate concentrations.

MATERIALS AND METHODS

The present study was carried out in the greenhouse and the Analytical Chemistry laboratory of the Faculty of Agrarian Sciences, National University of the Northeast (UNNE) Corrientes, Argentina. The soil used in this trial corresponds to an arganic Udipsament, belonging to the Ensenada Grande series (16), from the Experimental Field of the Faculty of Agricultural Sciences, UNNE; (27°28'23.36 "S and 58°47'3.36 "W), with average, average maximum and average minimum temperatures of 25.7; 31.3 and 20.9 °C, in the summer period and 19, 23.9 and 14.9 °C, in the winter period.

The chemical characteristics of the soil are shown in Table 1.

Quantitative determination of soil OM was carried out by the Walkey and Black method, P by the Bray Kurtz I method, K by flame photometry, Ca and Mg by EDTA complexometry. The pH was measured potentiometrically in a mixture of soil and water in a 1:2.5 ratio.

The physicochemical analysis of the water to be used in the test was carried out at the Analytical Chemistry laboratory (UNNE) and results are shown in Table 2.

For water analysis, the following methods were used

pH: by Potentiometry, using a peachimeter equipped with a combined glass electrode, determination *"in situ"*; electrical conductivity (Ce): by Conductimetry, using a standardized conductivity meter at 25 ºC; Calcium and Magnesium: by Complex Formation Volumetry (17). Total Hardness: by Complex Formation Volumetry (17); Chlorides: by Precipitation Volumetry, Mohr Method (17); Sulfate: by Turbidimetry (ASTM D 516-90 Method) (18); Sodium and Potassium: by atomic absorption spectrometry (17); Phosphorus: by Molecular Absorption Spectrophotometry, using the molybdenum blue method (17); Nitrate: by Molecular Absorption Spectrophotometry, by the sodium salicylate method (19).

Culture management

First, the soil was disinfected by solarization (20) for three months. Subsequently, lettuce (*Lactuca sativa* L. var Brisa) was planted in 3 L pots at a depth of 0.3 cm (seed length), considering that the planting depth should be the minimum possible. The following treatments with five replications were used:

Table 1. Chemical characteristics of the soil from the Experimental Field of the Faculty of Agricultural Sciences, UNNE Corrientes, Argentina

OM (g kg^{-1})	N (g kg^{-1})	P (ppm)	K (cmol kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	pH (H_2O)	Textural type
0,66	0,03	6,40	0,23	2,94	0,80	6,76	Sandy

T0 (control): pots were irrigated with water containing 0 mg NO_3^- L^{-1}.

T1: pots irrigated with water containing 25 mg of NO_3^- L^{-1}

T2: watered with 50 mg NO_3^- L^{-1}

T3: irrigated with 75 mg NO_3^- L^{-1}

T4: irrigated with water containing 100 mg NO_3^- L^{-1}

The solutions used were prepared with KNO_3^-, dissolving in tap water the amount corresponding to the dose of each treatment.

Plants were kept in the greenhouse of the Faculty of Agricultural Sciences (UNNE), with average temperatures of 26 ºC and, knowing the field capacity and the mass of the pot, the soil moisture was maintained by gravimetry at 80 % of the field capacity by means of irrigation with a test tube, restoring it every time the total mass of the pot decreased.

The trial began with the sowing of the seeds in April, placing four seeds per pot. The first irrigation was carried out with tap water, without the addition of NO_3^-, in order to ensure the germination of seedlings, due to the increase in osmotic potential that the anion can generate.

After 13 days, seedlings were thinned and repotted into the pots that presented failures, so that each pot had one plant, and for each treatment there were 5 plants in individual pots. During this period, irrigation was carried out with tap water, without the addition of anion.

Three days after thinning and subsequent repotting, all pots with established plants were observed and irrigation was started with the solution corresponding to each treatment.

Parameters evaluated

Leaf analysis of lettuce: lettuce was harvested in a staggered manner, according to the development of the plants. The first harvest was carried out 60 days after sowing and 12 days later another harvest was carried out, finishing with the total number of plants, identifying each one. The fresh mass was measured and then the material was dried in an oven at a temperature no higher than 60 ºC. Once dry, the material was ground in a mortar until a suitable particle size was obtained.

The determination of nitrates in lettuce leaves, using the colorimetric method by nitration with salicylic acid (21), whose rationale is as follows: the complex formed by nitration of salicylic acid under strongly acidic conditions presents maximum absorption at a wavelength of 410 nm in basic solutions (pH>12). The absorbance of the chromophore is directly proportional to the amount of $N\text{-}NO_3^-$.

It was worked in tubes with sample and counter-sample, to eliminate the effect of absorption caused by plant pigments and thus quantify only the absorbance of the complex formed. The procedure followed is detailed below.

- Extraction of $N\text{-}NO_3^-$: by boiling 0.5 g of sample in 50 mL of distilled water for 30 minutes.

Table 2. Physical and chemical characteristics of the water used to prepare the irrigation solutions for treatments

pH	5,97
Conductivity (µS cm^{-1})	286
Alkalinity (mg L^{-1} $CaCO_3$)	46
Nitrates (mg L^{-1})	N/A
Calcium (mg L^{-1})	24
Magnesium (mg L^{-1})	12
Hardness (mg L^{-1})	110,10
Sulfate (mg L^{-1})	2,87
Sodium (mg L^{-1})	0,06
Potassium (mg L^{-1})	2,93
Phosphate (mg L^{-1})	N/A

n/a- not available

- Filtration and quantitative transfer to a 50 mL flask.
- Pipetting of aliquots of 0.2 mL of extract in duplicate in 25 mL glass tubes (control tube and test tube).
- Addition of 0.8 mL of 5 % (w/v) salicylic acid solution in concentrated sulfuric acid to the test tube. Addition of 0.8 mL of concentrated sulfuric acid to the control tube.
- Slow addition, after 20 minutes, of 19 mL of NaOH 2N solution to each tube.
- Color development during 24 hours (stable color up to 72 hours).
- Preparation of nitrate standard solutions with 10, 20, 30, 40, 50, 75 and 100 mg g^{-1} from a stock solution of 1000 mg NO_3K g^{-1}.
- Pipetting of 0.2 mL aliquots of the standard solutions. Addition to each tube of the same reagents as for the test tube.
- Reading of the absorbance of the standard solutions in a double beam spectrophotometer at wavelength = 410 nm. With the obtained values a regression line is fitted and the angular coefficient (m) is calculated to establish the concentration of $N\text{-}NO_3$ in the test samples.
- Absorbance reading of each sample with its blank.
- Growth variables were also evaluated: plant height, dry mass of the whole plant and dry mass of the aerial part, number of leaves and leaf length.

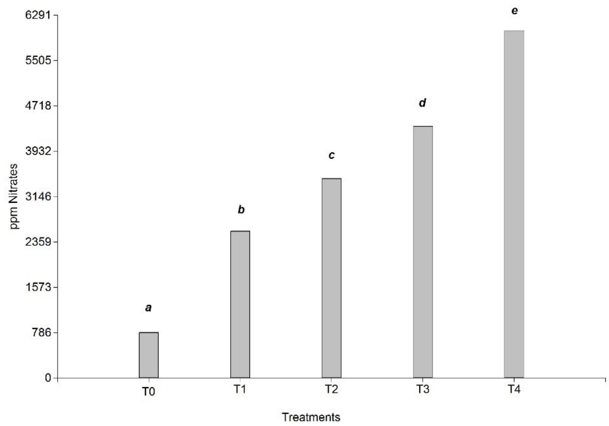
Statistical design

A randomized complete block experimental design with five replications was used. The data obtained were subjected to normality tests using the goodness-of-fit test with the modified Shapiro-Wilks statistic (α=0.05) and were analyzed statistically by ANOVA and Duncan's test ($p \leq 0.05$), using Infostat software (22). To analyze the interdependence of variables, a principal component analysis (PCA) was performed and the data were standardized to perform the analysis on the correlation matrix of variables.

RESULTS AND DISCUSSION

The analysis of chemical characteristics of the soil used in the trial showed very low organic matter values (<0.7 %), according to the type of soil texture, so it would not be contributing nitrates to the soil/crop (Table 1), since the organic matter value is used as an indicator of soil fertility (23). Low levels of nutrients in the soil cause plants to acquire less growth; even so, this should not influence the final objective of the work.

Regarding the water used, the main parameters that define the risk of using a certain type of water are the saline content and the electrical conductivity. When the salt content is higher than 2 g L^{-1} or the electrical conductivity is higher than 3,105 µS cm^{-1}, salinity problems can be very serious (24). According to the analysis of the water used for the test, it shows that it does not entail any risk of salinity, since the values obtained are below the reference parameters (8), and it is also noted that the water does not contain nitrates in solution, so it would not be modifying what is provided in each treatment.



T0: control; T1: 25 ppm nitrate solution; T2: 50 ppm nitrate solution; T3: 75 ppm nitrate solution; T4: 100 ppm nitrate solution]. Values represent the mean of five replicates. Different letters indicate significant differences ($p \leq 0.05$) according to Duncan's test. Standard Error (S.E.): T0: 6.04; T1: 2.95; T2: 2.53; T3: 1.92; T4: 0.97

Figure 1. Nitrate concentration in lettuce leaves irrigated with different nitrate concentrations

Nitrate concentrations in lettuce leaves

The analysis of lettuce leaves showed significant differences among all treatments (Figure 1), with a proportional increase in leaf nitrate content as a function of the dose of nitrate in the different treatments. The control treatment (without nitrate supply) showed significantly lower values, differing from treatments 1, 2 and 3, with intermediate values, and from treatment 4, which showed significantly higher values. The concentration of nitrate in lettuce irrigated with different concentrations of nitrate was higher than that obtained in previous research (25), where 1 079 mg kg^{-1} of leaf nitrate was recorded in lettuce var. *Acephala*, cv *Levistro*, in lettuce var. Capitata cv. Española, grown with rainbow trout (*Oncorhynchus mykiss*) residues.

The results indicate values well above 4 000 mg kg^{-1} of foliar NO_3^- in treatments with 75 and 100 ppm of nitrate in the irrigation water, considerably exceeding the maximum values of nitrate in lettuce (Table 3), so that its use in human food could cause health problems. Nitrate concentration in the plant is a dynamic state and represents the difference between absorption and assimilation. When nitrate uptake exceeds assimilation, it can accumulate in the vacuoles of plant cells. Nitrate uptake by irrigation was reflected in results. Therefore, water quality, in terms of nitrate concentration, will influence its accumulation in lettuce plants. Thus, the higher the nitrate concentration in the irrigation water, the greater the possibility of accumulation in the crop. It should be noted that nitrate contamination of groundwater has also been reported in the region (26). Thus, excess nitrates can not only accumulate on crop leaves, but can also become a risk of environmental contamination.

Principal Component Analysis (PCA) and Biplot of the growth variables analyzed and nitrate concentration in leaves explained 80.1 % of the association between the variables in two components. Principal components 1 and 2 (PC 1, PC 2) accounted for 51.7 and 30.4 %, respectively, of the total variation (Figure 2).

The treatment with the highest nitrate supply (100 ppm nitrate (T4)), presented a greater association with foliar nitrate concentration, favoring high nitrate accumulation in tissues and growth measured through the parameters leaf length, whole plant dry mass and plant dry mass without root.

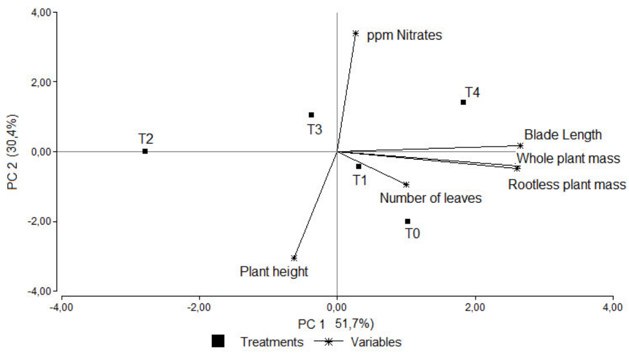
It is important to mention that this treatment considered an excessive nitrate input for the crop, which in practice is extremely detrimental to its cultivation and the environment, therefore, a nitrate input that achieves a balance in terms of yield, quality, safety and environmental care should be determined.

Table 3. Maximum levels of nitrate in lettuce regulated by the European Commission

Crop	Harvest period	mg of NO_3^- kg^{-1}
Lettuce (*Lactuca sativa* L)	Harvested between October 1 and March 31:	
	Greenhouse grown	4500
	Grown outdoors	4000
	Harvested between April 1 and September 30:	
	Greenhouse grown	3500
	Grown outdoors	2500

Similar results were recorded in lettuce plants (27),(28), where growth and leaf nitrate content increased with a higher nitrate supply.

The application of nitrate at concentrations of 50 and 75 ppm was not associated with any plant growth parameter; however, the control (T0) and the treatment with 25 ppm nitrate (T1) were associated with the number of leaves.



[T0: control; T1: 25 ppm nitrate solution; T2: 50 ppm nitrate solution; T3: 75 ppm nitrate solution; T4: 100 ppm nitrate solution]

Figure 2. Biplot resulting from the Principal Component Analysis (PCA) of the growth variables and foliar nitrate concentration in the five treatments tested

CONCLUSIONS

- The supply of different concentrations of nitrate by irrigation in lettuce plants resulted in a higher foliar nitrate residual compared to the control, without nitrate supply.
- Irrigation with 100 ppm nitrate was associated with leaf nitrate concentration and growth parameters, whole plant dry mass and plant dry mass without root and leaf length.
- The nitrate content of lettuce leaves irrigated with water containing 75 and 100 ppm nitrate exceeded the maximum leaf nitrate content required in the European Community member countries, which would indicate that they are not suitable for human consumption.

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