



Nitrate and sodium contents on lettuce and drained water as function of fertilizing and irrigation water quality in Brazil

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Abstract

Contamination of plants and waters with nitrate and sodium is an important environmental problem that can affect humans. An experiment was carried out aiming to evaluate the effect of fertilization and irrigation water quality on NO₃⁻ and Na⁺ contents of drained water and lettuce (*Lactuca sativa* L.) plants in Rio Claro, São Paulo State, Brazil. Treatments were distributed in a factorial arrangement 5 x 2, referring to fertilization and irrigation water quality (treated and polluted water), respectively, with three repetitions. The following fertilizers were used: Biofertilizer (BIO), bovine manure (BM), poultry manure (PM) and mineral fertilizer (MF). Additionally a non fertilized (NF) treatment also was studied. The following variables were registered: a) Drained water: Nitrate and sodium contents and presence of fecal and total coliforms; b) Lettuce leaves: nitrate content. The average nitrate and sodium contents of drained water indicated that contamination slowly advances on soil profile in areas of disposal of organic and inorganic residues. The fertilizer used on lettuce cultivation affected sodium and nitrate contents of leaves and drained water, and, in contrast irrigation water quality had no effect.

Keywords: Environmental problem; Water reuse; Nitrate; Sodium.

Introduction

In last years, mineral fertilizers have been used increasingly aiming to reach greater yield. However, 50% of the nitrogen applied as fertilizer is not removed by crops or stored in soil, being expected leaching as nitrate (Tisdale et al., 1985) the main reason for losses that leads to water and plant contamination, specially in intensive crops as lettuce (*Lactuca sativa*) in Brazil.

Concerns about high nitrate levels in vegetables have led some governmental organizations as European Union to introduce limits on nitrate contents in some salad crops including lettuce, aiming to decrease nitrate intake by consumers. Escobar-Gutierrez et al.

(2002) explain that nitrate limits are very important in regions where leafy vegetables can accumulate high foliar contents due to low light levels or supplemental mineral fertilizing.

Monitoring nitrate content is important, mainly in lettuce, because this salad has high ability to accumulate nitrate on leaves (Roorda van Eysinga, 1984), and, particularly, this organ is consumed by humans. Studies in the scientific literature found that foliar nitrate content depends on some factors as temperature (Richardson and Hardgrave, 1992) and nitrogen fertilizers (Urrestarazu et al., 1998), but these results were not observed in the present study.

Nevertheless, to minimize the nitrate contamination of foods and water some researchers have indicated the use of alternative organic fertilizers, as municipal garbage (Al-Redhaiman et al., 2003; Mantovani et al., 2005) and biofertilizers (Lag Reid et al., 1999). Additionally, water contamination as a non renewable resource is an environmental problem that affects many countries and tends to be a world problem that has led many studies about water reuse, as evidenced Brandão et al. (2003).

The water reuse is not common for Brazilian society due to the abundance of hydro resources that induces a temporary solution for pollution of superficial water resources with domestic sewage and industrial residues.

Crop cultivation using organic fertilizers has contributed for deposition of residues, improving physical and chemical properties of soil that is important for biological development. However, these fertilizers can contain fecal coliforms (Lag Reid et al., 1999), and, nitrogen can be transformed in nitrate, nevertheless contaminating water and, in consequence, foods.

Hence the present study aimed to evaluate the effect of organic fertilization and irrigation water quality on NO_3^- and Na^+ contents of drained water and lettuce plants.

Material and Methods

Plant materials and growth conditions

Lettuce (*Lactuca sativa* L.) plants of “Verônica” cultivar (the most commercially cultivated in Brazil), were used in this study. The experiments were carried out in a greenhouse of São Paulo State University (Brazil), evaluating three cultivation cycles of lettuce: (a) First: from 05/21/04 to 06/26/04 (37 days); (b) second: from 08/09/04 to 09/16/04 (38 days), and (c) third: from 11/27/04 to 01/08/05 (43 days). Seedlings with similar height and diameter were transplanted 15 days after sowing and spaced 25cm between lines and 20cm between plants.

Treatments were distributed in a factorial arrangement 5×2 , referring to fertilization and irrigation water quality (treated and polluted water), respectively, with three repetitions. The following fertilizers were used: Biofertilizer (BIO), bovine manure (BM), poultry manure (PM) and mineral fertilizer (MF). Additionally a non fertilized (NF) treatment also was studied. The organic fertilizers used in these studies were from Faculty of Agrarian and Veterinarian Sciences of University of São Paulo State, Brazil.

The soil used (see physical and chemical characteristics in Tables 1 and 2, respectively) was filled into thirty asbestos boxes of 0.5 m^3 and a superficial area of 1 m^2 , seated on a cement base. The bulk density of the soil used was 1.1 g cm^{-3} .

Table 1. Physical characteristics of the soil used in the experiment. Jaboticabal, Brazil, 2007.

Sand*	Silte* g kg ⁻¹	Clay *	Textural Class average – clay ¹
80	40	280	

*Densimeter method.¹According to Prevedello (1996).

Table 2. Chemical characteristics of the soil used in the experiment. Jaboticabal, Brazil, 2007.

pH	O.M. g kg ⁻¹	P mg kg ⁻¹	S-SO ₄ ⁻² mg kg ⁻¹	K	Ca	Mg	Al mg kg ⁻¹	H + Al	SB	CEC	V%	M%
4.3	13	1	35.4	24	160	24	27	243	208	451	46	22

pH in CaCl₂; O.M. = organic matter (g kg⁻¹); P and S-SO₄⁻² (mg kg⁻¹); K, Ca, Mg, Al, H+Al, SB (sum of bases) and CEC = cationic exchangeable capacity (mg kg⁻¹); V% = saturation of bases; %M = saturation of aluminium.

One layer (5 cm thickness) of triturated stones was placed in the deep of each box to avoid soil loses, and obstruction of the orifice used for water excess exit at 60cm depth. The soil used was previously sieved and the particles bigger than 1 cm³ were removed.

Lettuce plants were irrigated with water from “Ribeirão Claro” river, that receives the domestic sewage from Rio Claro County (São Paulo State, Brazil) and good quality water from a water treatment station. The physical, chemical and microbiological characteristics of irrigation waters are presented in Table 3.

Table 3. Characteristics of waters used in the experiments. Jaboticabal, Brazil, 2007.

Characteristics	Treated Water	Polluted Water
pH	7.02	6.6 – 8.8
Turbidity (UNT)	2.17	66.9 - 89
Electrical conductivity (µS cm ⁻¹)	123	96 - 350
Salinity (%)	0.1	0.2 – 0.4
Dissolved Total Solids (mg L ⁻¹)	58	45 - 166
Nitrate (mg L ⁻¹)	0.05 – 0.09	0.3 – 3.50
Ammonia (mg L ⁻¹)	0.04 – 0.11	0.13 – 3.55
Sodium (mg L ⁻¹)	1 – 16	27
TC/100 ⁻¹ ml (MPN)*		> 2419.2 100 ⁻¹ mL
FC/100 ⁻¹ ml (MPN)*		2419.2 100 ⁻¹ mL

* MPN = more probable number of quantified fecal (FC) and total coliforms (TC).

The water supply was done manually based on a daily evaporation of 5mm and corrected according to the lettuce culture coefficient (Kc) reported by Pereira and Allen (1997), direct on soil; leaves were not washed. Plants were daily irrigated (once a day) during the experiment.

The incorporation of organic fertilizers was conducted according to Lopes and Guidolin (1989) and Raij et al. (1996), thus applying 8 Kg m⁻² of bovine manure, 2 Kg m⁻² of poultry manure and 32 L m⁻² of biofertilizer, and, in the treatments with mineral fertilization were used 250 g m⁻² of simple superphosphate, 30 g m⁻² of potassium chloride and 10 g m⁻² of ammonium nitrate. As soil acidity corrective, 300 g m⁻² of rock calcareous was applied. All the organic and inorganic fertilizers were applied at 20 cm depth. In each cycle nitrogen fertilizer was applied once, applying 10 Kg ha⁻¹ of ammonium nitrate.

Water collectors were building using polystyrene tubes of 2.5cm in diameter, with longitudinal cut, thus forming a gutter. Each sample depth (15, 30 and 60cm) had a central collector with six lateral collectors, three in each side).

Variables analyzed

For nitrate analysis [Cataldo et al. (1975) methodology] six plants per parcel were collected in the end of each cultivation cycle, conducted to the laboratory, dried under 60°C (for registration of plant dry mass), triturated in a mill (Wiley, USA) and sieved (2mm of mesh).

The drained water was collected at each 15 days of each cultivation cycle (at 15, 30 and 60cm depths) for quantification of NO_3^- content, filtered in a glass fiber filter paper of 1,2 μm (Whatman GF/C) according to cadmium reduction method. Sodium analysis were performed with a selective electrode (ISE, Ion Specific Eelectrode "Sodium Eelectrode Model 44520 HACH") connected. The presence of coliforms in the water was determined by "Colilert" method proposed by Clescerl et al. (1999).

Statistical analysis

Statistical analysis included analysis of variance (ANOVA), mean separation on cultivation cycles, fertilizers and depths of sample data using Tukey test (Ferreira, 2000) and correlation analysis between nitrate contents of PM, BM and BIO and the respective nitrate contents promoted on drained water. SAS software was used and terms were considered significant at $P \leq 0.01$.

Results and discussion

In the present study, no significant interactions between fertilizing and irrigation water quality were registered.

Foliar nitrate content

As previously expected, non fertilized plants presented significant lower foliar nitrate.

Visible toxicity symptoms were observed in plants fertilized with PM of second and third cultivation cycles as function of high nitrate content of this fertilizer (Table 4). From results it can be inferred that the toxicity symptoms are also related to the synergy of other factors contents, beyond foliar NO_3^- content (Marshner, 2002), because plants fertilized with BIO presented foliar nitrate content higher than PM (Table 5), but not visible toxicity symptoms. Additionally, foliar NO_3^- content of others treatments [except NF (Table 5)], even high, not showed visual toxicity symptoms, as burning and wilting, but presented turgidity and intense green leaves.

Richardson and Hardgrave (1992) concluded plant nitrate content was unaffected as function of nitrogen fertilization, so in disagreement with present results.

Table 4. Chemical characteristics of organic fertilizers [poultry manure (PM), bovine manure (BM) and biofertilizer (BIO)] used in the experiments. Jaboticabal, Brazil, 2007. Total N, P, K, Ca, Mg and S are in g kh⁻¹ and Cu, Fe, Mn, Zn, Cd, Cr, Ni and Pb are in mg kg⁻¹.

O.F.	Total-N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	Cd	Cr	Ni	Pb
PM	32.9	9.3	15.1	24.5	3.5	4.1	411	565	255	256	0	0	1	0
BM	13.1	3.9	19.9	11.2	4.9	2.8	102	3740	430	77.5	0	0.5	5.5	0
BIO	20.3	5.6	7.3	22.1	3.7	4.3	67.5	2555	279	235	0.5	28	9	7

O.F. = organic fertilizer.

Table 5. Average foliar nitrate content of lettuce as function of fertilizer, cultivation cycle and irrigation water quality (IWQ). Jaboticabal, Brazil, 2007.

Fertilizers	Nitrate (mg kg ⁻¹)	IWQ	Nitrate (mg kg ⁻¹)	Cultivation Cycle	Nitrate (mg kg ⁻¹)
NF	1.65 b	Treated	4.26 a	1	2.69 b
PM	8.98 a	Polluted	7.04 a	2	7.00 b
BIO	10.83 a			3	13.78 a
MF	8.88 a				
BM	8.77 a				
Smd	9.70		3.68		4.36

Followed averages of same letter, in the column, do not differ between itself for the test of Tukey (5%); smd = significant minimum difference

As can be seen in Figure 1 and Table 5, from the first to the last lettuce cycle, leaf NO₃⁻ content remarkably increased (nearly 80%) independently of fertilizer that is directly related to the higher nitrogen availability. Higher plants absorb nitrogen from different forms: N₂ (gaseous, as leguminous and other species), amino acids (RCHNH₂COOH), urea [CO(NH₂)₂], NH₄⁺ and, predominantly, under natural and aerobic conditions, as NO₃⁻. However, Chaillou and Lamaze (1995), varying external nitrate content concluded that the separation of nitrate uptake system in kinetic phases, in relation to external nitrate content has not been supported recently. Nevertheless, the increase on foliar nitrate content from the first to the third cycle could be caused by longer time exposure to nitrate or urea fertilization. Tischner (2000) explains that exposing plants to nitrate, after several hours nitrate uptake rate reaches a maximum, and this uptake rate increases continuously with time.

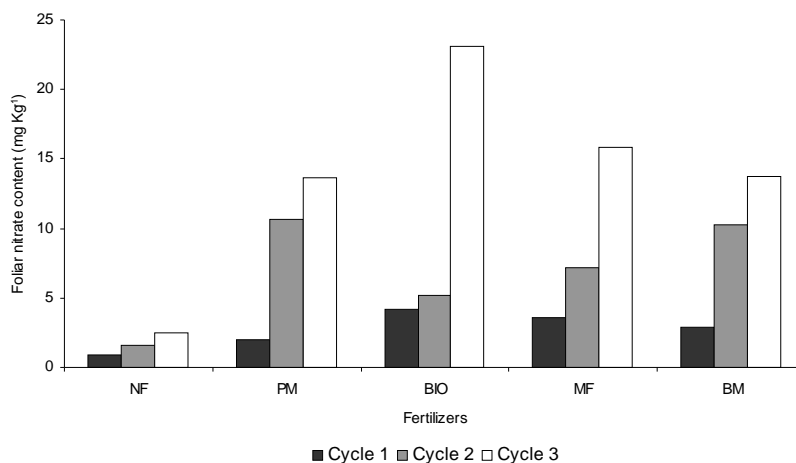


Figure 1. Foliar nitrate content (mg.kg⁻¹) of lettuce on three cultivation cycles studied as function of fertilizer abioticabal, Brazil, 2007.

The average foliar nitrate content (9.7 mg Kg⁻¹) presented in Table 5 is lower than 15 mg Kg⁻¹ reported by Beninni et al. (2002) and Mantovani et al. (2005). All foliar nitrate contents registered in this study are above the maximum value allowed in Europe indicated by Escóin-Peña et al. (1998).

Foliar NO₃⁻ results of lettuce indicate a direct relation between its absorption and availability on water (Table 3) and fertilizer (Table 4) nevertheless in agreement with Rodrigues (1990) and Katayama (1990). However, NO₃⁻ uptake probably not presents a direct correlation with lettuce yield, as supposed Stopes et al. (1989) that registered low NO₃⁻ leaf content in high yield plants. In addition, Tischner (2000) reported that most of the data available on nitrate were gained by measuring nitrate net uptake, i.e., the depletion of the surrounding medium for nitrate, thus, from these experiments it is difficult to draw conclusions on directional fluxes as influx and efflux on plants and the effects of it on higher plants, as lettuce used in the present study.

It's important to register that NH₄⁺ uptake increases acidity due to H⁺ exit from H₂CO₃ respiratory dissociation, and, on the other way, NO₃⁻ uptake reduces acidity due to OH⁻ addition from the same nitrate reduction, as proposed by Malavolta et al. (1997). Anonymous (1978) and Anonymous (1987) observed that domestic sewers contain various and significant amounts of carbonated and nitrogen compounds and, except for nitrogen, other possible pollutants contents are irrelevant.

Nitrate content of drained water

Nitrate contents of drained water depended on soil depth of sample (Table 6). The highest NO₃⁻ contents were registered at 60 cm depth that evidences leaching, with accumulation below root activity zone of lettuce, i.e., 50cm depth, according to Filgueira

(2000). These results are in agreement with Flores et al. (2001) that reported intense NO_3^- leaching associated with root activity.

There were no statistical significant differences among fertilizers and irrigation water quality in relation to nitrate contents of drained water (Table 6). Whether compared the N-total content of the organic fertilizers studied (PM, BIO and BM) with NO_3^- of drained water, it's observed that fertilizers with more N-total (Table 4) promoted higher nitrate content on drained water (Table 6), with an significant ($P \leq 0.01$) correlation coefficient (r) of 0.99. Thus, PM promoted the highest nitrate content of drained water.

Table 6. Nitrate average content of drained water from lettuce cultivation as function of fertilizer, irrigation water quality (IWQ) and depth sample. Jaboticabal, Brazil, 2007.

Fertilizers	Nitrate (mg L^{-1})	IWQ	Nitrate (mg L^{-1})	Depth sample (cm)	Nitrate (mg L^{-1})
NF	2.95 b	Treated	20.60 a	15	3.10 c
PM	32.15 a	Polluted	21.60 a	30	25.80 a
BIO	23.85 a			60	38.30 b
MF	23.70 a				
BM	22.85 a				
smd	11.18		16.09		11.05

Followed averages of same letter, in the column, do not differ between itself for the test of Tukey (5%); smd = significant minimum difference.

Results of NO_3^- contents on drained water (Table 6) evidence that leaching of compounds from mineralization and solubilization of fertilizers depends on chemical and physical characteristics of the fertilizer as structure, hydraulic conductivity and water volume under percolation on soil profile, either from irrigation or precipitation (Alves and Klar, 1997).

NO_3^- ion is a harmful pollutant of soil and water, but agricultural practices, in most situations, not preserve water and soil quality. So, the rational use of fertilizers must be done to maintain the qualitative parameters of water resources and prevent environmental degradation (Anonymous, 1986).

In the present study was expected that polluted water promoted higher nitrate contents on drained water, independently of fertilizer applied, although, it was not registered, perhaps due to leaching or root activity.

Sodium content of water drained

In spite of irrigation water quality had no statistical influence on NO_3^- and Na^+ contents, independent of cycle and fertilizer applied (Tables 6 and 7), polluted water presented quantitative averages above treated water. This tendency was previously expected due to the higher sodium content of polluted water observed in Table 3, as predicts studies of Qadir and Oster (2004) and Cavalcante and Cavalcante (2006), concluding that soil irrigated with high sodium content water had this ion content increased at the end of the experiment. This result could be caused by indicates leaching of Na^+ on soil profile, as can be seen in Table 7 that shows significant effect of fertilizers and sample depths on Na^+ contents of drained water.

Na⁺ content data demonstrate high solubility and leaching on soil profile. Sodium contents of drained water depend on fertilizer (Table 7). Among fertilizers, PM promoted the higher sodium content on drained water, 51% more than NF treatment (the lowest). In general, under no fertilizing (NF) conditions, Na⁺ contents of drained water also were high.

Table 7 indicates more Na⁺ leaching, observing significant differences between sample depths with superiority of 60cm, that shows deposition of Na⁺ in a layer below root zone, or, on another way, Na⁺ uptake, that is also deleterious for plants in higher amounts, as also reported by Cavalcante et al. (2005) and Parida and Das (2005).

Table 7. Sodium average content of drained water from lettuce cultivation as function of fertilizer, irrigation water quality (IWQ) and depth sample. Jaboticabal, Brazil, 2007.

Fertilizers	Sodium (mg L ⁻¹)	IWQ	Sodium (mg L ⁻¹)	Sample depth (cm)	Sodium (mg L ⁻¹)
NF	60.0 c	Treated	87.80 a	15	22.0 a
PM	124.0 a	Polluted	90.20 a	30	27.0 a
BIO	81.0 bc			60	190.0 b
MF	100.5 ab				
BM	79.5 bc				
smd	30.21		36.36		25.20

Averages followed by the same letter in columns not differ by the Tukey test at 0.01 probability error; smd = significant minimum difference.

Fecal coliforms (CF) were not verified in the drained water at 60 cm depth, while total coliforms (CT) were observed in all water samples of all lettuce cycles. In addition, the purification capacity verified along soil profile depends on some factors as amount of organic material incorporated, period of irrigation with polluted water and time of exposition to irrigation.

Conclusion

Thus, our studies demonstrate that: (i) the fertilizer used on lettuce cultivation not statistically affects nitrate contents of leaves and drained water; (ii) the sodium content of drained water depends on fertilizer used; (iii) fertilizers with higher nitrate contents also promote high values on drained water; (iv) irrigation water quality not statistically affects nitrate and sodium contents of drained water, foliar nitrate content and not promotes fecal coliforms presence at 60cm depth of lettuce cultivation.

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