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**NITRATE CONTENT IN LETTUCE (*Lactuca sativa* L.) AFTER FERTILIZATION WITH SEWAGE SLUDGE AND TREATED WASTEWATER IRRIGATION**

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3 1 **NITRATE CONTENT IN LETTUCE (*Lactuca sativa* L.) AFTER**  
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5 2 **FERTILIZATION WITH SEWAGE SLUDGE AND TREATED WASTEWATER**  
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8 3 **IRRIGATION**  
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24 10 **Abstract**  
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26  
27 11 A romaine type lettuce (*Lactuca sativa* L.) was cultivated during three crop seasons  
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29 12 (spring 2005, spring 2006 and autumn-winter 2006) in the same soil in Alcázar de San  
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31 13 Juan, Spain. Six plots of 36 m<sup>2</sup> each, were set up in agricultural land. A drip irrigation  
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33 14 system was used to water all plots: five plots with drinking water and one plot with  
34  
35 15 wastewater from Wastewater Treatment Plant (WWTP) from an Activated Sludge  
36  
37 16 system. One drinking water irrigated plot was not fertilized and was considered as a  
38  
39 17 control. Five different treatments were applied to the soil: Three organic mixtures  
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41 18 (sewage sludge, sewage sludge mixed with pine bark and municipal solid waste with  
42  
43 19 composted sludge) and a conventional fertilizer were applied to the soil in each of four  
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45 20 plots irrigated with drinking water. The last plot was irrigated with treated wastewater.  
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47 21 These treatments were tested for their effects on plant growth and nitrate concentration  
48  
49 22 in vegetable tissue. An increase in fresh weight in the lettuce was linked to the dosage  
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51 23 of sewage sludge. The highest nitrate level was observed in the sewage sludge treatment  
52  
53 24 in all crops and seasons, although in general, all values were below the maximum limits  
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55 25 established by the European Commission for nitrate content in fresh romaine lettuce. In  
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1 the third crop season, a significant increase in nitrate content was observed in lettuce  
2 from organic treatments. Nitrate concentration in lettuce from irrigated treated  
3 wastewater was higher than control plants, although significant differences were not  
4 found.

5 **Key words:** Nitrate, Lettuce, Sewage sludge, Wastewater

## 6 **Introduction**

7 Nitrogen is one of the principal nutrients that determine crop production. Nitrogen  
8 deficiency lowers production, but an excess can be harmful to people and the  
9 environment. This is due to the fact that nitrate is one of the most important nitrogen  
10 compounds in the soil for plant nutrition, it is very soluble and can be transported in  
11 water to the aquifers. Generally, high quantities of nitrogen are applied to horticultural  
12 crops using both inorganic and organic fertilisers. In addition, there is a high potential  
13 of groundwater nitrate pollution due to the low efficiency of nitrogen utilization by this  
14 type of crop. Excessive fertilization can lead to cumulative levels of nitrate exceeding  
15 the maximum levels recommended for human consumption (Ramos 2001).

16  
17 Nitrate in vegetables, as well as in water and generally other type of foods is relatively  
18 non-toxic, but approximately 5% of all ingested nitrate in saliva and the gastrointestinal  
19 tract is converted into nitrite, which is more toxic (Santamaria 2006). Nitrite interacts  
20 with haemoglobin, causing blood to be less efficient in transporting oxygen and  
21 resulting in a condition known as methaemoblobinaemia (Merino 2006). Nitrite may  
22 also combine with secondary or tertiary amines to form N-nitroso compounds. These  
23 compounds have been shown to produce cancer in a wide range of laboratory animals  
24 (Zeegers et al. 2006). An acceptable daily intake (ADI) for 0-3.7 mg kg<sup>-1</sup> body weight

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1 for nitrate and of 0-0.07 mg kg<sup>-1</sup> body weight for nitrite was established by the  
2 European Commission Scientific Committee on Food (SCF 1995; Speijers 1996).

3  
4 It has been estimated that vegetables constitute a major source of human exposure to  
5 nitrates contributing approximately 80-92% of the average daily intake (Prasad 2008).  
6 The regulatory limits for tissue nitrate concentrations in fresh romaine lettuce are as  
7 follows (in each case values are presented for crops grown either under cover or in the  
8 open air, on a fresh weight basis): 4500 and 4000 mg NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> for lettuces harvested  
9 from 1st October to 31<sup>st</sup> March; 3500 and 2500 mg NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> for lettuces harvested  
10 from 1<sup>st</sup> April to 30<sup>st</sup> September (European Commission 2005).

11  
12 Accumulation of nitrates in vegetables, especially lettuce, has been shown to be affected  
13 by several factors (genetic, environmental and agricultural). Studies have identified  
14 nitrogen fertilization and light intensity as the major factors that influence nitrate  
15 content in lettuce (Maynard et al. 1976, Tamme 2006). Photosynthetic activity depends  
16 on the intensity of light and if this intensity is reduced, the transformation of the  
17 absorbed nitrates into amino acids and proteins will decrease. In conditions of low  
18 luminosity the accumulation of nitrate will be higher and there will be a higher  
19 concentration of nitrates in the plants. Due to this, the maximum limits allowed are  
20 higher in crops grown in autumn - winter or in a greenhouse (Gunes et al. 1995).

21  
22 On the other hand, among the possibilities of water resource availability, the re-  
23 utilization of water is considered a non-conventional resource that can increase the  
24 utility of water. Reu-se for agricultural irrigation is the most common application in  
25 practically the whole world (Qadir & Oster 2004). However, water re-use should be

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3 1 planned for increasing water flow levels available in a specific area, substitute flows,  
4  
5 2 reduce the negative impact of waste and improve the integrated management of water  
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8 3 (Iglesias & Ortega 2008). Within the water treatment process, it is impossible to exclude  
9  
10 4 the generation of a solid waste, called sludge, for which an efficient application must  
11  
12 5 also be found and described following the specific norms for this waste product. This  
13  
14 6 material can be an important source of nutrients for the soil (Mena et al. 2003). Using  
15  
16 7 this material as a fertilizer can benefit the environment by turning wastes into valuable  
17  
18 8 resources; otherwise, the sludge would have to be disposed of by landfilling, storage in  
19  
20 9 lagoons, incineration, or ocean dumping. On the other hand, heavy metals found in  
21  
22 10 sewage sludge may sometimes present environmental problems. Several practices,  
23  
24 11 similar to those used with other organic fertilizers, will maximize the benefits of using  
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26 12 sewage sludge while minimizing the risks (Dai et al. 2006).  
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34 14 Nitrogen in sludge is in the form of ammonium or organic nitrogen, with only a small  
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36 15 fraction in the nitrate form. All of the nitrogen in the ammonium and nitrate forms is  
37  
38 16 immediately available to the plant. Organic nitrogen in sludge must be mineralized  
39  
40 17 before it can be assimilated by crops. Research shows that 10 to 50 percent of organic  
41  
42 18 nitrogen will become available the first year after application, depending on the type of  
43  
44 19 treatment used in producing the sludge (Pascual 2003).  
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51 21 In this study the nitrate concentration was compared in lettuce leaves treated with  
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53 22 different fertilizers including organic and inorganic fertilizers and irrigated with  
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55 23 different types of water, including treated wastewater. This allowed us to examine the  
56  
57 24 cumulative effect of these treatments on nitrate content and the influence of the season  
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59 25 in which crops were cultivated. In addition, the residual effect of sewage sludge on  
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1 nitrate concentration in vegetable tissue was studied. Fertilizers were applied at doses  
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1 nitrate concentration in vegetable tissue was studied. Fertilizers were applied at doses  
2 greater than the nutritional needs of the crop except for inorganic fertiliser, at a dosage  
3 equal to nutritional requirements.

#### 4 **Materials and methods**

##### 5 *Location and design of experiments*

6 The experiments were carried out during three successive crop seasons: Late spring (the  
7 first, 10th May – 29th June 2005 and the second, 22nd April – 8th July 2006) and  
8 autumn (the third, 1st October 2006 – 19th January 2007), in an area near the  
9 Wastewater Treatment Plant (WWTP) of Alcázar de San Juan (29,000 inhabitants) in  
10 central Spain (39° 24' N; 3° 12' W, altitude 644m). This treatment plant purifies  
11 wastewater using the biological system of Activated Sludge. This is a sewage treatment  
12 process in which air or oxygen is forced into sewage liquid to develop a biological floc  
13 which reduces the organic content of the sewage. The origin of wastewater was mixed:  
14 70% of domestic origin and 30% industrial from two industrial zones. Most of these  
15 industries are related to cheese and wine manufacturing. Treated wastewater is reused  
16 for the conservation of the Natural Reservation of Alcázar de San Juan, composed of  
17 two steppe lagoons. To prepare for this use, at the end of the wastewater treatment  
18 process nitrogen and phosphorus are eliminated from the treated water. The solid waste  
19 from water purification is re-used by some farmers in the area as organic amendment for  
20 cereals and grapevines.

21 For the experiments, treated wastewater and sludge from the WWPT of Alcázar de San  
22 Juan were air-dried, and drinking water from urban underground supply were used.

1 Six plots of 36 m<sup>2</sup> each, were set up in agricultural land. Two types of water treatment  
2 were considered: drinking water in five plots and treated wastewater in the last plot.

3  
4 One drinking water irrigated plot was not fertilized (neither organic nor inorganic  
5 fertiliser) and was considered the control (DW). The irrigated, treated wastewater plot  
6 did not receive fertilizer treatment (WW). In the other three plots, high doses of sewage  
7 sludge were used: 40 t ha<sup>-1</sup> of air-dried sewage sludge (SS), 40 t ha<sup>-1</sup> of air-dried sewage  
8 sludge mixed with pine bark (SSPB) in a 3:1 ratio (three parts sludge and one part pine  
9 bark) and 40 t ha<sup>-1</sup> municipal solid waste with composted sludge (MSWC) in each. In  
10 the last plot a conventional fertilizer dose adapted to crop needs (1000 kg ha<sup>-1</sup>) was  
11 used. The treatments were carried out a week before the transplant and no other type of  
12 treatment was performed during the crop growth period.

13  
14 The plant selected was *Lactuca sativa* L. var. "Líbano" (lettuce) and planting was  
15 carried out every season with a density of 60,000 plants ha<sup>-1</sup> due the fact that this crop is  
16 considered one of the highest accumulators of nitrates in the leaves (Merino 2006).  
17 Dates of transplanting and harvesting, crop duration and climatic characteristics in each  
18 crop season are described in Table 1.

19  
20 Drip irrigation was used regularly to avoid drought stress in each plot. To calculate the  
21 water needs for this crop we followed the methodology proposed by the FAO  
22 (Doorenbos & Pruitt 1984), which calculates the Evapotranspiration of Reference (ET<sub>o</sub>)  
23 using the Penman-Monteith method and applies the crop coefficient (K<sub>c</sub>). Since the ET<sub>o</sub>  
24 and K<sub>c</sub> values are known, Evapo-transpiration, and therefore water needs, could be  
25 calculated.

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During the second and third crop seasons, SS and SSPB treatment plots were divided into two subplots. In one half the same dose of treatment as in the previous crop season was applied to study the cumulative effect. In the other half, no treatment was applied to evaluate the residual effect.

### 6 *Sampling*

7 Treated wastewater and drinking water were analysed once a month while the crop was  
8 in the field. Significant differences between both types of water (drinking and treated  
9 wastewater) were recorded (Table 2). The composition of the two types of irrigation  
10 water did not vary significantly over the study period, so the mean values  $\pm$  standard  
11 deviation for the chemical properties are presented. Electrical conductivity (E.C.),  
12 carbonate, bicarbonate, total phosphorus, nitrogen, nitrite, sulphate, chloride, inorganic  
13 carbon (IC), organic total carbon (TOC), total carbon (TC), sodium, potassium,  
14 magnesium, copper, iron and manganese have significantly higher values in treated  
15 wastewater than in drinking water. However, nitrate content was significantly lower in  
16 treated wastewater.

17

18 Dried sewage sludge, sewage sludge mixed with pine bark and municipal solid waste  
19 with composted sludge were analysed once a crop season before incorporating them into  
20 the soil. Chemical characteristics of the organic mixtures are given in Table 3.

21

22 Before transplanting in the first crop season and after harvest in the third crop season  
23 soil was sampled for characterization from ten samples at random inside each plot, at a  
24 depth of 15 cm, with three repetitions (S.A.F., 2005).

25

1 In each crop season, when plants were estimated to have reached their highest  
2 commercial size they were harvested by cutting the stems at the soil level. Ten plants  
3 from each plot were harvested randomly for nitrate determination.

#### 4 *Analytical methods*

5 Immediately after harvest the fresh weight of lettuces was measured. The leaves were  
6 washed off with doubly-distilled water and blotted dry with absorbing paper. To carry  
7 out nitrate extraction, samples of 300 g of fresh lettuce were taken and dried in a forced  
8 air oven (Memmert, model 800) to 70 °C for 48 h. The dried leaves were then ground in  
9 a mill. A quantity of 100 mg of milled sample was added 20 ml of doubly-distilled  
10 warm water (80 °C) and the mix was shaken for 30 min, using a mechanical agitator  
11 (Heidolph Unimax, mod. 2010). The resulting solution was filtered through borosilicate  
12 membrane filters of 0.45 µm porosity. Nitrate content in the solution was determined  
13 by ionic chromatography using a module of chemical suppression and conductivity  
14 detection using the ISO method (UNE-EN 10304-1 1995).

15  
16 Soil and sewage sludge samples (0.5 g dry weight) were prepared for analysis with acid  
17 digestion in 4 ml of HNO<sub>3</sub>, 0.25 ml of H<sub>2</sub>O<sub>2</sub> and 2 ml of HF by applying the  
18 temperature program according to Milestones Cookbook of microwave application  
19 notes for MDR technology. Samples were analysed with the following techniques: pH  
20 with a previously calibrated CRISON GLP22 pH meter and 1:2 w v<sup>-1</sup> suspension of soil  
21 in water (Peech 1965); organic matter using the Walkley Black method (Allison 1965);  
22 electrical conductivity (Bower & Wilcox 1965); total N by the Kjeldahl procedure  
23 (Bremner 1965); soil and sewage sludge available P using the Olsen method (Olsen &  
24 Dean 1965); Na and K using the atomic emission spectroscopy method (MAPA, 1994),

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1 Ca and Mg, using the atomic absorption spectroscopy method (MAPA, 1994), Fe, Zn  
2 and Mn using the DTPA extraction method (Lindsay & Norvell 1978).

3  
4 Wastewater samples were prepared for analysis with a previous acid digestion applying  
5 the temperature program according to Milestones Cookbook of microwave application  
6 notes for MDR technology. Wastewater and drinking water samples were analysed with  
7 atomic emission method to determinate Na (limit of detection (LOD): 0.05 mg l<sup>-1</sup>) and  
8 K (LOD: 0.01 mg l<sup>-1</sup>); atomic absorption spectroscopy method to determine Fe (LOD: 1  
9 µg l<sup>-1</sup>), Al (LOD: 3 µg l<sup>-1</sup>), Cu (LOD: 2.5 µg l<sup>-1</sup>) and Mn content (LOD: 0.15 µg l<sup>-1</sup>)  
10 according APHA-AWWA-WPCF (1998); and ionic chromatography method to  
11 determine chloride (LOD: 2 mg l<sup>-1</sup>), sulphate (LOD: 1 mg l<sup>-1</sup>), nitrate content (LOD: 1  
12 mg l<sup>-1</sup>). Other chemical parameters such as N-Kjeldahl, total P, BOD<sub>5</sub>, COD, Electrical  
13 conductivity, Carbonate and Bicarbonate were determined according APHA-AWWA-  
14 WPCF (1998).

#### 15 16 *Statistical design*

17 Data were subjected to ANOVA treatments and the method used to discriminate among  
18 the means was Fisher's least significant difference (LSD) for p<0.05. To ensure that  
19 data came from a normal distribution, standardized skewness and standardized kurtosis  
20 values were checked. Percentage values considered were transformed by arcsine.

## 21 22 **Results and discussion**

### 23 *Soil*

1 No significant changes were recorded for pH and MgO in the soil throughout the study  
2 in any of the plots that had received any type of treatment (Table 4). However,  
3 significant increases in organic matter, phosphorus and copper in organic treatment  
4 plots (SSPB, SS and MSWC) were obtained. A significant increase in nitrogen in these  
5 plots was observed. The highest content of this nutrient at the end of the study was in  
6 the SS treatment plot, mainly due to the sewage sludge dose applied and to the sewage  
7 sludge mineralization during the study. These results have been reported in other studies  
8 (De las Heras et al. 2005). Few changes in DW and WW plots were recorded during the  
9 study. Only K<sub>2</sub>O content increased in the plot irrigated with treated wastewater after  
10 harvesting in the third crop season, and in both plots there was a decrease in the C:N  
11 ratio for the same time period. Micronutrient content (Fe, Mn, Cu and Zn in organic  
12 treatment plot and DW and WW plots) was relatively constant throughout the study.

### 13 *Lettuce plant growth*

14 In the first crop season and after harvesting, the average weight of fresh lettuce from the  
15 SS plot (846 g) was significantly higher than in all treatments (Figure 1). Significant  
16 differences among the fresh weight of plants from FER, SSPB and MSWC treatments  
17 plots were not observed. Lettuce fresh weight from DW and WW treatment plots was  
18 very low (below 100 g), indicating that growth was very deficient due to the lack of  
19 important nutritional elements (Maynard & Hochmuth 1997).

20  
21 In the second crop season solar radiation was higher, although temperatures were  
22 similar to those in the previous crop season. Also, in the second crop season, an increase  
23 in weight was produced in all treatments except in plants from DW plot. As in the first  
24 crop season, lettuce growth from the SS treatment plot was significantly higher. During

1 the third crop season (autumn-winter), characterized by the lowest temperatures and the  
2 least daily sunlight, reduced plant growth was observed in all treatments. An exception  
3 to this was for lettuce from the SSPB treatment plot, where significant differences in  
4 fresh weight were not observed between the three crop seasons.

5  
6 It is possible to affirm that an increase in fresh weight in the lettuce is linked to the dose  
7 of sewage sludge. These results have been reported in other studies (Mañas 2006) in  
8 which increasing the dose of sludge leads to an increase in the weight of fresh matter. In  
9 this study the quantity contributed in the SS treatment was  $10 \text{ t ha}^{-1}$  higher than in SSPB  
10 treatments.

#### 11 *Nitrate content in lettuce*

12 A total of ten lettuces from each treatment were analysed in each crop season. The  
13 maximum average value found was  $1832 \text{ mg kg}^{-1}$  in the SS treatment plot in the third  
14 crop season, which is below the maximum limits for levels of nitrate in lettuce  
15 established by European Commission (2005).

16  
17 In the first cultivation period the median nitrate content in lettuces leaves from the SS  
18 treatment plot ( $940 \text{ mg kg}^{-1}$ ) was significantly higher than the concentration observed in  
19 lettuces from the FER ( $387 \text{ mg kg}^{-1}$ ) and SSPB ( $439 \text{ mg kg}^{-1}$ ) treatment plots, where  
20 significant differences were not observed (Figure 2).

21  
22 In the second crop season an increase in nitrate concentrations was observed in all  
23 treatments but it was only significant in SSPB treatment. The plants from the SS and  
24 SSPB treatment plots presented significantly higher nitrate content ( $1262 \text{ mg kg}^{-1}$  and

1 1220 mg kg<sup>-1</sup>) (Figure 3). Significant differences between lettuces from DW, WW, FER  
2 and MSWC treatment plots were not observed. European regulatory limits were not  
3 exceeded for any treatment. However, Figure 3 shows that the nitrate content in a  
4 sample from the SSBB treatment reached the limit without exceeding it (2477 mg kg<sup>-1</sup>).  
5 It is important to emphasize the low nitrate concentration in lettuces from DW and WW  
6 treatments, as these plants had deficient growth.

7  
8 In the last crop season, nitrate concentration in lettuces from the SS treatment was  
9 significantly higher (1832 mg kg<sup>-1</sup>). Pavlou et al. (2007) reported similar results in  
10 lettuce treated with organic fertilizer during the fall crop season. Significant differences  
11 between the plants treated with SSPB and MSWC were not observed. Nitrate content in  
12 lettuces from the FER treatment was lower than nitrate levels in plants treated with  
13 organic fertilizer (Figure 4).

14  
15 When crop seasons are compared, an increase in nitrate content in lettuce leaves was  
16 observed from one crop season to another. This was consistent for all treatments,  
17 although significant differences among DW, WW and FER treatments were not  
18 observed. These results show that the continued application of SS, SSPB and MSWC  
19 treatments for the three cultivation periods increased the nitrate concentration in  
20 vegetable tissue in each crop season, although in the last crop season the reduction of  
21 hours of sunlight could influence the increase in nitrate content in lettuce. Byrne et al.  
22 (2004) demonstrated that winter-sown crops generally have higher tissue nitrate  
23 concentration levels than summer crops within the same environment. It has been  
24 demonstrated that Northern European crops have higher tissue nitrate concentration than  
25 corresponding Southern European crops (European Commission 2005). This did not

1 occur in treatments DW, FER and WW in which an increase was produced, but it was  
2 not significant. For these three treatments and specifically for the FER treatment, an  
3 adjusted dose based on the requirements of the crop was applied for the three crop  
4 seasons. No significant differences in nitrate concentration from the FER treatment were  
5 observed between crop seasons, despite the reduction in hours of sunlight in the third  
6 crop season. It can therefore be stated, as in other studies (Pavlou et al. 2007), that the  
7 nitrate concentration of romaine lettuces leaves depends more on the fertilizer type,  
8 dose, and repeated applications (accumulative effect) than on the time of the year in  
9 which the crop is grown. Brand & Molgaard (2001) showed that the production method  
10 (organic or conventional farming) has great importance in obtaining low nitrate levels in  
11 vegetables.

12  
13 The residual effect of the SS and SSPB treatments (Figure 5) was that plants treated  
14 with SS in the first crop season showed a significant decrease in nitrates during the  
15 second and third crop seasons. Plants treated with SSPB showed an increase in nitrate  
16 levels during the second and third crop seasons, even when no extra treatments were  
17 applied during these second and third crop seasons. The nitrate content in lettuce  
18 samples harvested from the third crop season was  $833 \text{ mg kg}^{-1}$ , higher than was  
19 observed in lettuces treated with FER treatment during the third crop season.

## 20 **Conclusions**

21 In this study, nitrate content in romaine lettuce leaves was shown to depend on fertilizer  
22 type and dose rather than on crop season. The hours of sunlight in winter are lower than  
23 in spring, so crop development in autumn-winter requires a longer time in the field than  
24 spring crops. In this study, the total daily hours of sunshine in both seasons was

1 approximately equal, meaning that lettuce cultivation in different seasons does not  
2 affect the nitrate concentration in vegetable tissue. Irrigation with treated wastewater  
3 from the WWPT of Alcázar de San Juan did not produce high nitrate levels in lettuce  
4 leaves, mainly due to the elimination of a high quantity of nitrogen from the water,  
5 which avoids eutrophication when it is re-used.

6  
7 On the other hand, high application rates of sewage sludge produces an increase in N, P  
8 and organic matter reserves in the soil, which will be converted into chemical forms  
9 available to crops over time. This does not occur when treated wastewater is used.

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18 English text.

## 19 20 **References**

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	First crop season	Second crop season	Third crop season
Lettuce transplanting	May 10 <sup>th</sup> 2005	April 22 <sup>nd</sup> 2006	October 1 <sup>st</sup> 2006
Lettuce harvesting	June 29 <sup>th</sup> 2005	July 8 <sup>th</sup> 2006	January 19 <sup>th</sup> 2007
Crop duration (days)	52	75	109
Sunshine duration (h)	662	991	839
Sun radiation (MJ m <sup>-2</sup> )	1317	2055	1005
Average max Temperature (°C)	29.5	29.3	15.4
Average min Temperature (°C)	12.3	12.3	4.6

Table 1: Cultivation practices and climatic conditions during the crop seasons.

	DW	WW
COD (mg l <sup>-1</sup> )	< 10	60.2 ± 65.8
BOD <sub>5</sub> (mg l <sup>-1</sup> )	Undetected	8 ± 5.5
pH	8.1 ± 0.3a	7.8 ± 0.6a
E.C. (microscm <sup>-1</sup> )	624 ± 30.2a	2085 ± 602b
Carbonate (mg l <sup>-1</sup> )	Undetected	43.8 ± 28.5
Bicarbonate (mg l <sup>-1</sup> )	180 ± 15.6a	396 ± 98.9b
T. Phosphorus (mg l <sup>-1</sup> )	0.3 ± 0.05a	3.3 ± 2.1b
N-Kjeldahl (mg l <sup>-1</sup> )	Undetected	20.8 ± 15.5
N-Ammoniacal (mg l <sup>-1</sup> )	Undetected	9.4 ± 9.4
Nitrite (mg l <sup>-1</sup> )	< 0.01	2.2 ± 1.1
Nitrate (mg l <sup>-1</sup> )	27.9 ± 0.9b	6.9 ± 6.9a
Sulphate (mg l <sup>-1</sup> )	73.6 ± 3.1a	301 ± 101b
Chloride (mg l <sup>-1</sup> )	53.5 ± 5.9a	227 ± 28.7b
IC (mg l <sup>-1</sup> )	46.2 ± 2.6a	135 ± 24.8b
TOC (mg l <sup>-1</sup> )	0.3 ± 0.5a	33.9 ± 38.5b
Na (mg l <sup>-1</sup> )	20.9 ± 1.2a	318 ± 103b
K (mg l <sup>-1</sup> )	1.9 ± 0.1a	106 ± 34.3b
Ca (mg l <sup>-1</sup> )	85.7 ± 12a	138 ± 74.8a
Mg (mg l <sup>-1</sup> )	32.3 ± 13.3a	54.7 ± 21.8b
Zn (mg l <sup>-1</sup> )	<0.2	0.4 ± 0.2
Cu (ug l <sup>-1</sup> )	2.4 ± 2.7a	144 ± 112b
Mn (ug l <sup>-1</sup> )	0.4 ± 1.2a	102 ± 128b
Fe (ug l <sup>-1</sup> )	8.2 ± 4.7a	2314 ± 2427b

Table 2: Chemical characteristics of drinking water and wastewater. Different letters mean significant differences among columns (p<0.05)

	FER	SS	SSPB	MSWC
pH		6.8	6.9	7.4
E.C. (mmhos cm <sup>-1</sup> )		9.3	6.6	11.5
Organic matter (%)		29.1	73	7.9
C:N		4	38	5
N (%)	10	4	1.1	0.9
P <sub>2</sub> O <sub>5</sub> (mg kg <sup>-1</sup> )	120000	5289	4566	989
K <sub>2</sub> O (mg kg <sup>-1</sup> )	170000	3622	1485	6526
Na (mg kg <sup>-1</sup> )		3622	989	3199
CaO (%)		1.1	0.4	1.4
MgO (%)	2	0.2	0.2	0.3
Zn (mg kg <sup>-1</sup> )		259	108	300
Cu (mg kg <sup>-1</sup> )		109	23	210
Fe (mg kg <sup>-1</sup> )	2000	5421	4007	9301
Mn (mg kg <sup>-1</sup> )		8.6	45	178

Table 3: Chemical characteristics of sewage sludge (SS), sewage sludge + pine bark (SSPB), municipal solid waste compost (MSWC) and fertilizer (FER).

1		DW	WW	FER	SSPB	SS	MSWC
pH	B	7.8 ± 0.5a	7.9 ± 0.1a	7.7 ± 0.1a	7.7 ± 0.2a	7.8 ± 0.8a	7.9 ± 1a
	A	9.2 ± 1.2a	9.6 ± 1.2a	8.7 ± 1.1a	8.5 ± 1.1a	8.5 ± 0.4a	8.6 ± 1.1a
Organic matter (%)	B	0.8 ± 0.05a	0.9 ± 0.2a	0.6 ± 0.1a	1.2 ± 0.2a	2 ± 0.05a	1.8 ± 0.2a
	A	0.6 ± 0.1a	0.8 ± 0.1a	0.6 ± 0.1a	2.5 ± 0.3b	3.1 ± 0.4b	3.2 ± 0.4b
N (%)	B	0.04 ± 0.01a	0.1 ± 0.02a	0.1 ± 0.01a	0.1 ± 0.01a	0.05 ± 0.01a	0.1 ± 0.01a
	A	0.1 ± 0.01b	0.1 ± 0.01b	0.1 ± 0.01a	0.2 ± 0.03b	0.3 ± 0.04b	0.3 ± 0.03b
P <sub>2</sub> O <sub>5</sub> (mg kg <sup>-1</sup> )	B	27.5 ± 1.8a	18.3 ± 1.3a	103 ± 5.7a	148 ± 14.9a	114 ± 4.a	135 ± 17.4a
	A	27.5 ± 3.5a	14.4 ± 5.3a	141 ± 18.3b	822 ± 106b	1259 ± 162b	446 ± 57.5b
K <sub>2</sub> O (mg kg <sup>-1</sup> )	B	769 ± 50.3a	830 ± 12.6a	699 ± 10.2a	506 ± 22.5a	647 ± 15.8a	1506 ± 194b
	A	628 ± 81a	1388 ± 179b	605 ± 78a	398 ± 51.4a	414 ± 53.4b	853 ± 110a
CaO (%)	B	0.7 ± 0.05a	0.8 ± 0.02	2.8 ± 0.2b	2.5 ± 0.2a	2.7 ± 0.1b	3.3 ± 0.1b
	A	0.8 ± 0.10a	0.7 ± 0.1a	1.9 ± 0.3a	2.2 ± 0.28a	1.7 ± 0.22a	1.5 ± 0.2a
MgO (%)	B	0.1 ± 0.01a					
	A	0.1 ± 0.01a	0.1 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.01a	0.1 ± 0.01a	0.1 ± 0.01a
Fe (mg kg <sup>-1</sup> )	B	25136 ± 1643a	25592 ± 56a	24883 ± 3492a	33252 ± 1003b	24494 ± 1655a	21339 ± 2752a
	A	24295 ± 3133a	22279 ± 2873a	21013 ± 2710a	19114 ± 2465a	24329 ± 3137a	25986 ± 3351a
Mn (mg kg <sup>-1</sup> )	B	212 ± 21.3a	196 ± 5.6a	195 ± 25.6a	205 ± 13.5b	220 ± 22.1b	153 ± 19.8a
	A	180 ± 23.3a	178 ± 24a	172 ± 22.1a	167 ± 21.6a	145 ± 18.7a	180 ± 23.2a
Zn (mg kg <sup>-1</sup> )	B	37.5 ± 3.8a	38.5 ± 5.1a	40.5 ± 5.6b	56.5 ± 2a	47 ± 4.7a	41 ± 5.3b
	A	31.8 ± 4.1a	30.1 ± 3.4a	30.2 ± 3.9a	49 ± 6.3a	54.1 ± 6.9a	10 ± 1.3a
Cu (mg kg <sup>-1</sup> )	B	11 ± 1.1a	11 ± 2a	11.5 ± 2.3a	11 ± 0.3a	11.5 ± 1.2a	12.7 ± 1.6a
	A	13 ± 1.7a	13 ± 1.7a	13.5 ± 1.7a	22.5 ± 2.9b	21.7 ± 2.8b	53.8 ± 6.9b
C:N	B	12 ± 0.8b	7 ± 1b	5 ± 0.6a	9 ± 1a	10 ± 0.6b	9 ± 1.2b
	A	4 ± 0.5a	4 ± 0.5a	4 ± 0.5a	7 ± 0.9a	6 ± 0.8a	7 ± 0.9a

2

3 Table 4: Chemical characteristics of the soil before transplanting in the first crop season

4 (B) and after harvesting in the third crop season (A). For the same parameter, averages

5 followed by different letters denote significant differences according to Fisher's

6 multiple range test (LSD;  $p < 0.05$ ).

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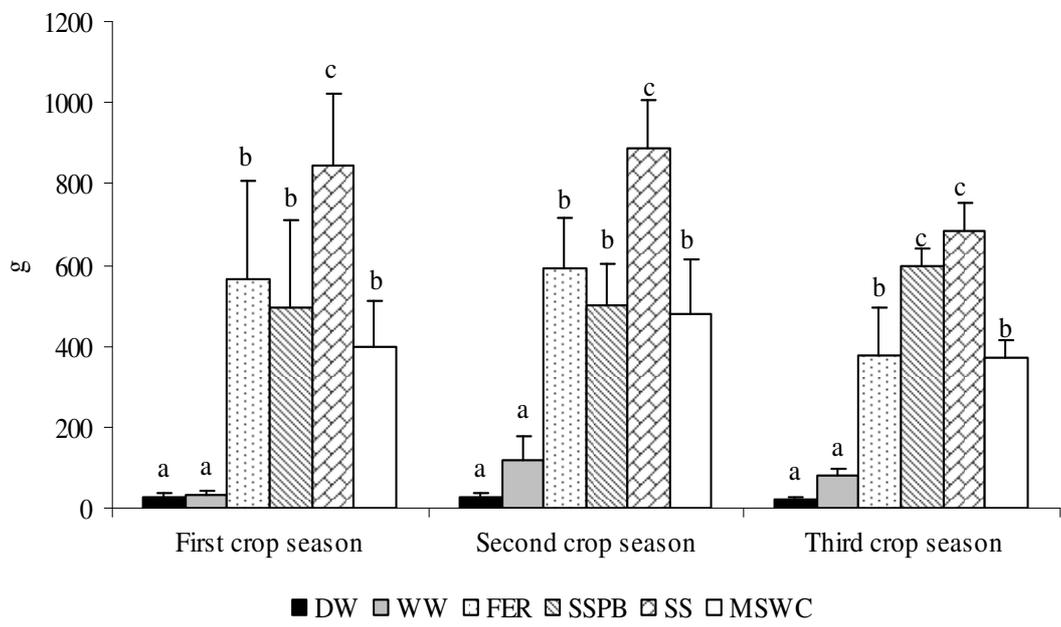


Figure 1

Review Only

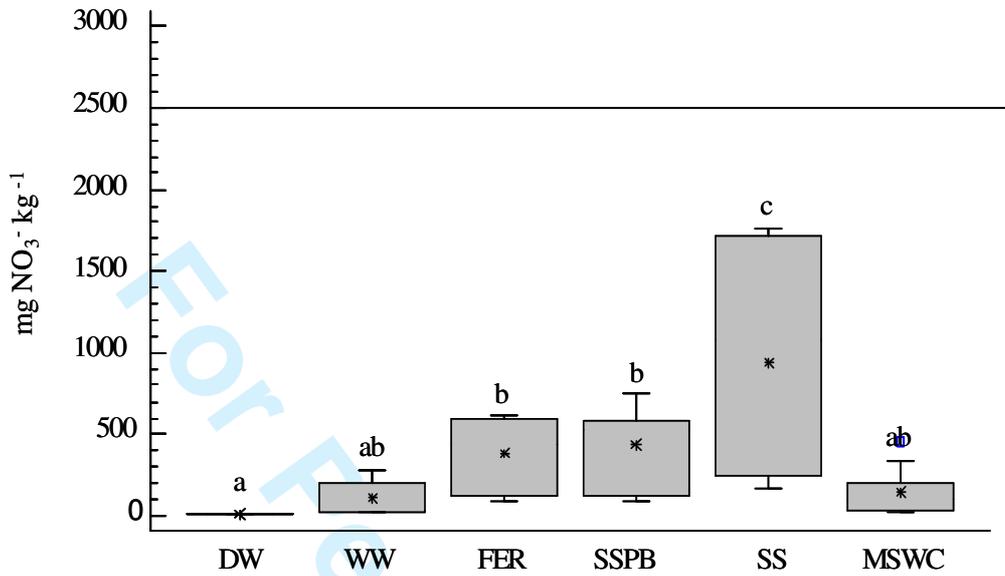


Figure 2

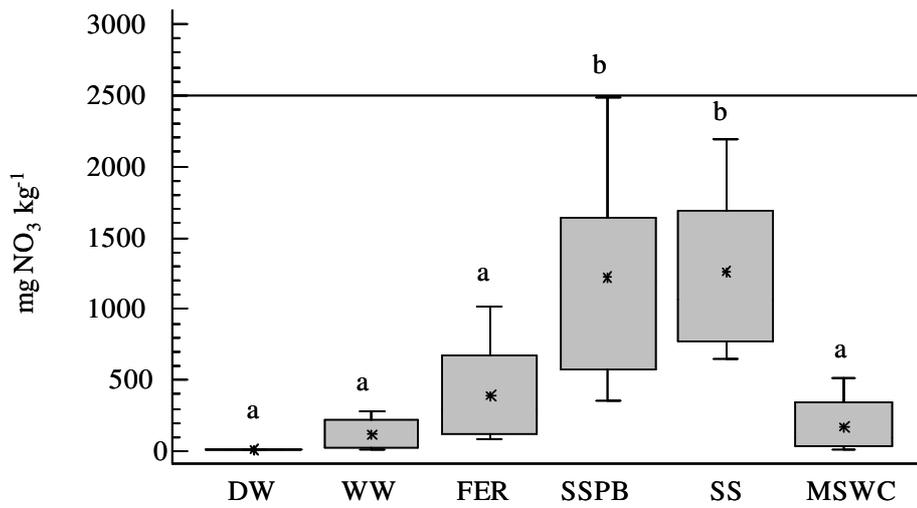


Figure 3

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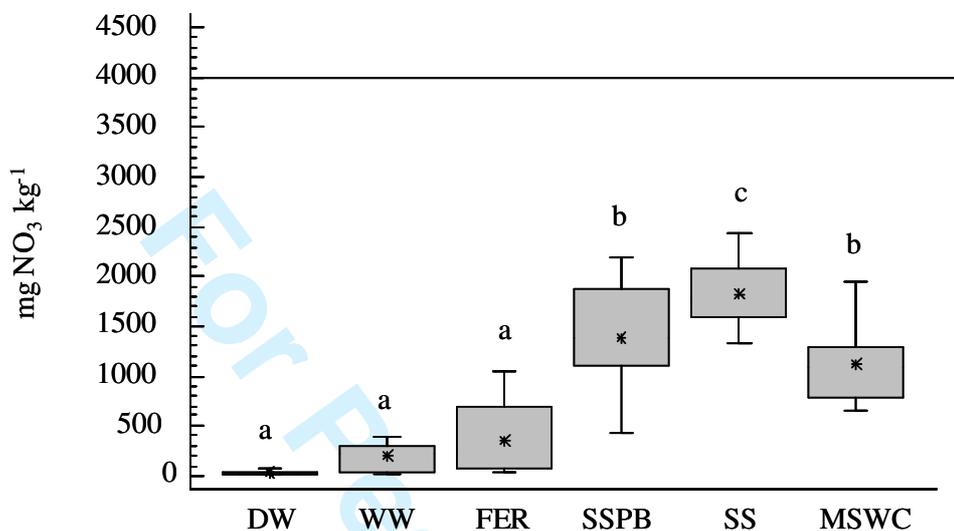


Figure 4

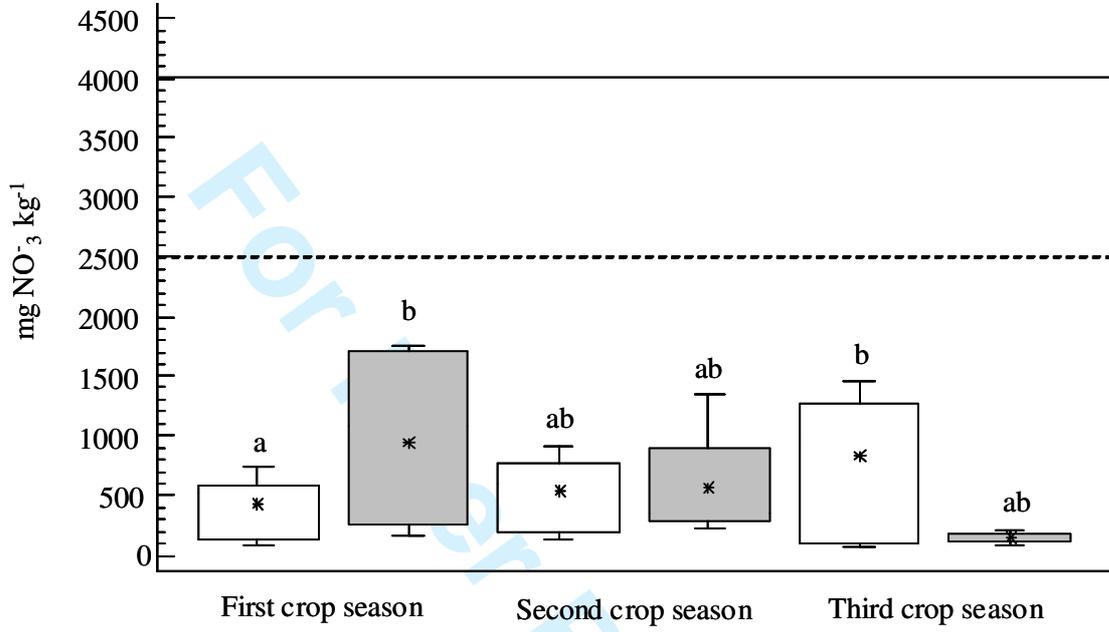


Figure 5

1 Figure 1: Average fresh weight of ten lettuce plants. In every crop season, letters denote  
2 significant differences according to Fisher's multiple range test (LSD) ( $p < 0.05$ ).

3  
4 Figure 2: Nitrate content of fresh lettuce in the first crop season (spring 2005) for all  
5 treatments and maximum level (ML summer = 2500 mg kg<sup>-1</sup>). Different letters denote  
6 significant differences according to Fisher's multiple range test (LSD) ( $p < 0.05$ ). The dot  
7 represents the mean value.

8  
9 Figure 3: Nitrate content of fresh lettuce in the second crop season (spring 2006) for all  
10 treatments and maximum level (ML summer = 2500 mg kg<sup>-1</sup>). Different letters denote  
11 significant differences according to Fisher's multiple range test (LSD) ( $p < 0.05$ ). The dot  
12 represents the mean value.

13  
14 Figure 4: Nitrate content of fresh lettuce in the third crop season (autumn 2007) for all  
15 treatments and maximum level (ML winter = 4000 mg kg<sup>-1</sup>). Different letters denote  
16 significant differences according to Fisher's multiple range test (LSD) ( $p < 0.05$ ). The dot  
17 represents the mean value.

18  
19 Figure 5: Residual nitrate content of fresh lettuce in second and third crop season SS  
20 and SSPB treatment and maximum levels (ML summer = 2500 mg kg<sup>-1</sup>, ML summer =  
21 4000 mg/kg<sup>-1</sup>). Different letters for the same treatment denote significant differences  
22 according to Fisher's multiple range test (LSD) ( $p < 0.05$ ). The dot represents the mean  
23 value.

24