



## Sorghum recovery and immobilization of $^{15}\text{N}$ labeled urea-N as affected by sewage sludge co-application

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### ABSTRACT

A pot experiment was conducted to assess the fate and recovery of urea-N applied to sorghum (*Sorghum sudanense*) both in the presence and in the absence of sewage sludge. For a better understanding of the interaction between urea N and N originating from sewage sludge,  $^{15}\text{N}$  isotope technique was used.  $^{15}\text{N}$ -labeled urea was added to soil at 0, 60, 100 kg N.ha<sup>-1</sup>, and unlabeled sewage sludge was added at 0 and 45 kg N.ha<sup>-1</sup>. In the absence of sewage sludge,  $^{15}\text{N}$  recovery ( $^{15}\text{NR}$ ) was 34% of the  $^{15}\text{N}$ - Urea applied at 60 kg N.ha<sup>-1</sup>. It increased to 55% as the urea N rate increased to 100 kg N.ha<sup>-1</sup>. Co-application of sewage sludge with the highest dose of urea led to a decrease of urea  $^{15}\text{NR}$  by 11% as compared to that in the absence of sewage sludge. Application of sewage sludge significantly improved the immobilization of urea-derived N in soil, from 13 to 42 % and from 24 to 31% of 60 and 100 kg N urea applied respectively. Thus, sewage sludge ensured prolonged and continued availability of fertilizer N to plants thereby leading to reduce N loss and to higher fertilizer use efficiency.

### Keywords

$^{15}\text{N}$ -urea recovery;  $^{15}\text{N}$ -urea immobilization;  $^{15}\text{N}$ -urea loss; sorghum sudanense; sewage sludge co-application.

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## INTRODUCTION

Nitrogen is the nutrient element that most often limits the crop productivity since most cultivated soils do not provide sufficient N. Therefore, nitrogen fertilizer application is becoming increasingly important for crop production in dryland agriculture to avoid the occurrence of N deficiency. Urea is widely used due to its high N content, solubility and nonpolarity and could be co-applied with organic amendment in crop production systems (Eghball and Power, 1999; Choi et al., 2001). In the soil, urea is quickly hydrolyzed by urease to  $\text{NH}_3$ , which can be volatilized, immobilized and nitrified once  $\text{NH}_3$  is converted to  $\text{NH}_4^+$ . In the last case, the nitrate formed from the nitrification process may be leached and denitrified (Reddy and Sharma, 2000; Han et al., 2004).

Many studies showed that land application of organic amendment is expected to increase microbial immobilization of co-applied chemical fertilizer N by stimulating the activities of heterotrophs that are responsible for immobilization of  $\text{NH}_4^+$  derived from co-applied chemical N fertilizers (Hadas et al., 1996; Siva et al., 1999; Han et al., 2004). Microbial immobilization of  $\text{NH}_4^+$  could reduce the rate of nitrification which produces  $\text{NO}_3^-$  susceptible to loss via leaching and denitrification, because heterotrophs carrying out N immobilization compete for the substrate ( $\text{NH}_4^+$ ) with the nitrifiers (Castells et al., 2004; Choi and Chang, 2005).

On the other hand, co-addition of organic amendment such as sewage sludge can be used as a practical method of improving soil physical and chemical properties (Webber et al., 1996; Mohammad and Athamneh, 2004; Camberato et al., 2006; Bipfusa et al., 2008). Sludge provides a short-term input of plant available nutrients, particularly N (Kchaou et al. 2010; 2011; 2013), and stimulation of microbial activity and it contributes to long-term maintenance of nutrient and organic matter pools. The fertilizer N value of sewage sludge can be significant, but varies considerably depending on origin and processing to application (Peterson, 2003).

It is the interest of a technique such as isotope labeling to monitor the nitrogen cycling and to calculate the balance sheet of the different N sources. The direct measurement of the recovery of fertilizer N in the soil, and the subsequent calculation of the N that is lost from the crop/soil system can only be made using  $^{15}\text{N}$ -labeled fertilizer (Limaux et al., 1998).

Therefore, the objectives of this study, using  $^{15}\text{N}$  isotope technique, were:

- to quantify the recovery of  $^{15}\text{N}$  labeled urea by sudangrass, the N remaining in soil and lost from the soil plant system in the absence and in the presence of sewage sludge.
- to investigate the impact of sewage sludge co-application on the urea N balance.

## MATERIAL AND METHODS

For this study, surface layer (0-20 cm) samples of loam clayey soil was collected of an experimental station Cherfech (Tunis, Tunisia). Soil testing results showed a pH at 8.1; carbon content at 1.23 % and Nitrogen content at 0.13%.

Sewage sludge was obtained from a wastewater treatment plant in Korba, Tunisia and it was collected from sludge drying beds. The main chemical characteristics of the sludge used in this study are given in the Table 1.

**Table 1. Composition of applied sewage sludge compared to Tunisian standards (NT 106.20) (2002).**

Parameters	Sewage sludge	NT 106.20
H <sub>2</sub> O	7.8	
pH	8.08	
		% MS
<b>C Total</b>	16.06	
<b>N Total</b>	1.5	
<b>P Total</b>	1.73	
<b>K</b>	0.4	
<b>Ca</b>	8.7	
<b>Mg</b>	0.58	
		Mg.Kg-1 MS
<b>Cd</b>	0.89	20
<b>Co</b>	9.28	-
<b>Cr</b>	97.5	500
<b>Cu</b>	180.4	1000
<b>Mn</b>	155	-
<b>Ni</b>	28.6	200
<b>Pb</b>	79.5	800
<b>Zn</b>	490.2	2000
<b>Fe ‰</b>	12.5	-



The sewage sludge was relatively rich in nutrients especially in C, and Ca. The heavy metals concentrations in the sludge were generally low to moderately low. None of the heavy metals exceeds the maximum limits allowed by Tunisian standards regulations (Table), indicating the possibility of using sewage sludge as fertilizer with no immediate threat of soil or plant contamination (Dumitru et al., 2000; Lavado, 2006).

Soil samples were air-dried, sieved, filled in plastics pots (8 kg soil per pot) and treated as follows:

Control: Soil only (control).

SS: Soil + 217 mg N.pot<sup>-1</sup> as unlabeled sewage sludge.

U1\*: Soil + 160 mg N as <sup>15</sup>N labeled Urea (equivalent to 60 kg N.ha<sup>-1</sup>).

U1\*+SS: Soil + 160 mg N as <sup>15</sup>N labeled Urea + 217 mg N.pot<sup>-1</sup> as unlabelled Sewage Sludge (equivalent to 45 kg N.ha<sup>-1</sup>).

U2\*: Soil + 270 mg N as <sup>15</sup>N-urea (equivalent to 100 kg N.ha<sup>-1</sup>).

U2\*+ SS: Soil + 270 mg N as <sup>15</sup>N-urea+217 mg N.pot<sup>-1</sup> as unlabeled sewage sludge.

(\*): labeled <sup>15</sup>N-Urea with <sup>15</sup>N excess of 10 % atom.

All treatments were replicated for times.

Sudangrass (*Sorghum sudanense*) was sown at the rate of 4 seeds per pot. Sewage sludge was applied before two weeks before sowing. <sup>15</sup>N-labelled urea (46% N, 10% atom excess) was dissolved in water and applied in two equal fractions, with half being applied at emergence and the remainder being applied at tillering stage. The pots were kept in the greenhouse. Irrigation water rates were applied to maintain moisture level of the soil at 70% of the field capacity to prevent the loss of N applied by lixiviation.

The plants were harvested at the beginning of the flowering stage. Both roots and shoot portions were collected and dried to a constant weight at 80°C. After harvesting the plants, soil samples were collected from each pot. The plant and soil samples were then analyzed for total N and <sup>15</sup>N using a Dumas analyzer coupled with a mass spectrometer (Europa Scientifica, UK).

The abundance obtained by mass spectrometry was transformed into atom % <sup>15</sup>N excess by subtracting the natural abundance (0.3663 atom % <sup>15</sup>N) from the % N abundance of plant and soil samples. To reduce the likelihood of cross-contamination, all samples were ground and analyzed from least <sup>15</sup>N concentration to greatest <sup>15</sup>N concentration. The data set was then statically analyzed using STAT-ITCF (Ver. V). Analysis of variance was conducted using the Fisher test at the 0.05 level of probability. Differences among means were then evaluated using the New man and Kewls test. All data shown represent means ± standar eleviations of quadruplicate measurements.

Labeled urea <sup>15</sup>N recovered (<sup>15</sup>NR) in plants was calculated as a percentage of the urea-N-applied.

$$^{15}\text{NR (\% of input)} = 100 (\text{Npl} * \text{Epl} / \text{Nu} * \text{Eu})$$

Where Npl is the quantity of nitrogen taken up by the plant, E<sub>pl</sub> is the atom percent excess measured in the plant, Nu is the quantity of urea N applied and Eu is the atom percent excess of applied urea.

Labeled urea <sup>15</sup>N recovered (immobilized) in the soil (<sup>15</sup>Nr) at the end of experiment was calculated by:

$$^{15}\text{Nr \%} = 100 * (\text{Ns} * \text{Es} / \text{Nu} * \text{Eu})$$

Where Ns is the quantity of nitrogen remained in soil (organic or NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup>)

Es is the atom percent excess measured for the soil in fertilized pots, Nu is the quantity of urea N applied and Eu is the atom percent excess of applied urea.

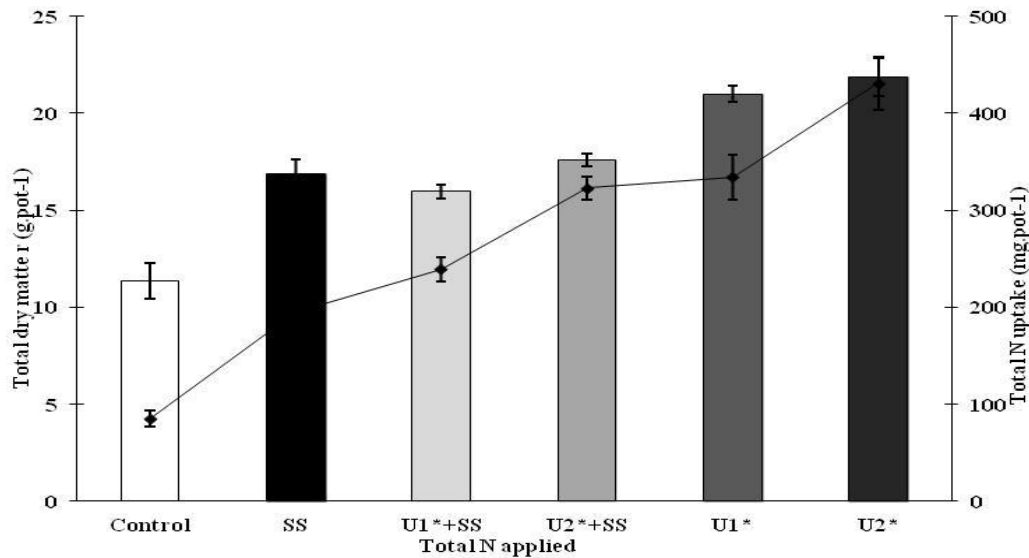
## RESULTS AND DISCUSSION

### 1. Plant production and nitrogen uptake

Independently to the N fertilization source, addition of nitrogen led to an increase in dry matter production and N uptake by sudangrass when compared to the unfertilized control (Figure 1). The highest dry matter and N uptake were recorded with urea N applied alone. No significant yield effect due to the application rate of urea was observed (average of 21 g.pot<sup>-1</sup>),



while plant N uptake was enhanced from 334 to 431 mg.pot<sup>-1</sup> as the rate of N-urea increased from 160 (U1) to 270 mg N (U2).



**Figure 1. Sorghum dry matter and N uptake of the whole plant for the different treatments applied.**

Application of sewage sludge alone proved a significant effect in increasing dry matter production and total N uptake as compared with the control (Figure 1). Also, the combination of urea and sewage sludge resulted significant in increasing the sorghum yield and N uptake, which raised from 11.4 g.pot<sup>-1</sup> and 85 mg.pot<sup>-1</sup> of the control to 17.6 g.pot<sup>-1</sup> and 323 mg.pot<sup>-1</sup> with the highest level of urea N (270 mg N). In comparison to the application of urea alone, sewage sludge co-application decreased the dry matter production and N uptake by 23 and 25% in U1\* + SS and by 20 and 28% in U2\* + SS respectively compared to the application of urea alone.

Benefits of urea N in term of increased crop biomass are universally reported. In comparison to urea N, the effect of organic amendments, is variable depending upon their chemical composition especially N concentration. Benefits of organic amendments, including application of sewage sludge, are derived mainly from a net release of nitrogen from decomposing organic matter with high N concentration and narrow C/N ratio. Thus sewage sludge amendments are reported to have a positive effect on crop yields (Kchaou et al. 2011, 2013; Akdeniz et al., 2006). However, the benefits are derived more from overall positive effects rather than from N supply alone (Ladd et al., 1983; Azam, 1990).

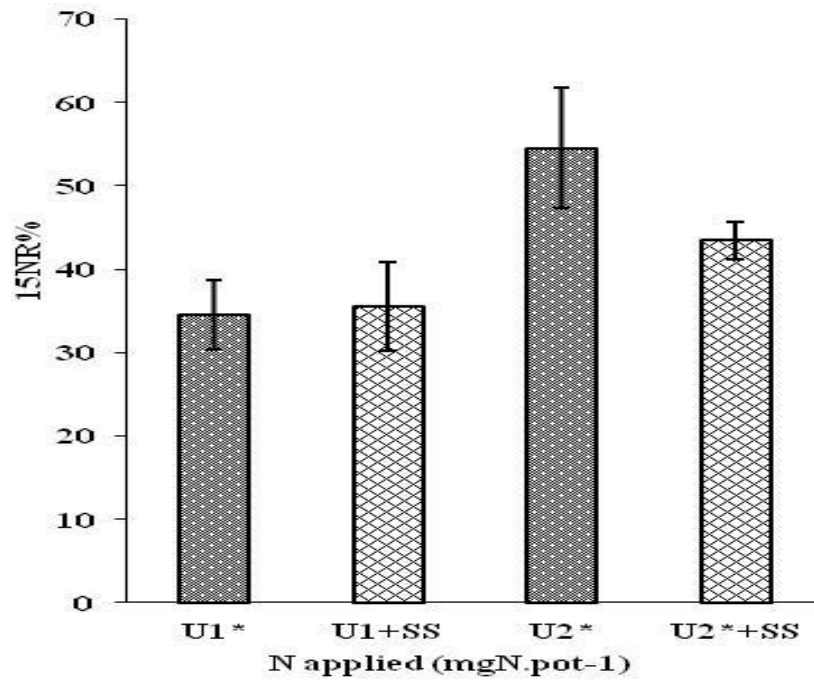
Sewage sludge used in the present study had a relatively wider C/N ratio (10.7) and low N concentration (1.5%). Therefore, the positive effect observed may not be solely due to the additional N supply by sewage sludge, but could have resulted from additional benefits in terms of increased microbial activity. Organic amendment such as sewage sludge, have been reported to increase the crop yields as a results of improvement in physical-chemical and biological characteristics of the soil and availability of plant nutrients

especially N (Hani et al., 1996; Lerch et al., 1992; McGrath et al., 1994). Hence, any increase in sorghum yield will be partially attributable to improvement in physical-chemical and biological characteristics of the soil amended with sewage sludge.

The trends in N uptake were in general similar to those observed for dry matter. This clearly demonstrated the dependence of dry matter yield on N content of the plant and thus the availability of the later. Thus, sewage sludge appeared to exert a negative effect on dry matter yield through its contribution to N urea availability, probably through N urea immobilization in the soil. These results are in agreement with those reported by Azam et al. (1999) and Azam and Lodhi (2001) who found that sewage sludge serve as an additional source of N as well as to conserve N fertilizer. According to these same authors, this may mean that sludge conserved fertilizer N, by providing additional C for microbial immobilization, and the plants were able to make use of it over an extended period of time. Also, the studies of Lerch et al. (1992) showed that, in view of being a labile source of carbon but not that of N, sewage sludge could expected to cause an immobilization of fertilizer N, but with restricted release of N over short term.

## 2. Plant and soil recoveries of <sup>15</sup>N labeled urea applied

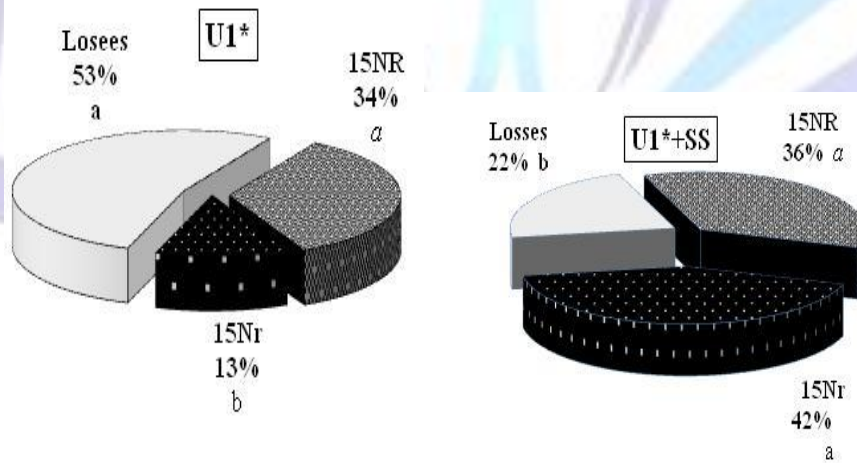
Sorghum <sup>15</sup>N-urea recovery (15NR) was significantly affected by the rate of N-urea application. It increased from 34 to 55% as the urea N rate increased from 160 to 270 mg N pot<sup>-1</sup>.

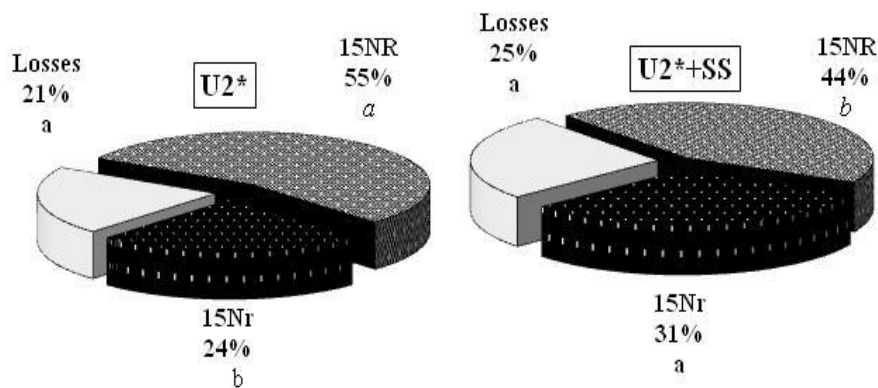


**Figure 2. <sup>15</sup>N Recovery (<sup>15</sup>NR ) in the whole plant of sorghum as a % of total nitrogen urea**

Co-application of sewage sludge with the lowest dose of urea (160 mg N pot<sup>-1</sup>) had no negative effect on the recovery of the <sup>15</sup>N labeled urea; nearly 35% of the labeled urea was taken up by the sudangrass (Figure 2).

However, co-application of sewage sludge with the highest dose of urea (270 mg Npot-1) led to a decrease of urea <sup>15</sup>NR by 11% as compared to that in the absence of sewage sludge.





**Figure 3. The nitrogen balance sheet**

(a,b,c), (a,b,c) and (a,b,c) indicate significant differences in urea <sup>15</sup>N Recovery in the whole plant (<sup>15</sup>NR), in residual <sup>15</sup>N remaining in the soil (<sup>15</sup>Nr) and in losses respectively, as affected by sewage sludge co-application .

Average values followed by the same letter are not significantly different at p>0.05.

In the absence of sewage sludge, about 13% of the applied 15N urea was found in soil at the lowest rate of urea application. The proportion of total 15N remaining in soil increased from 13 to 24% as the 15N labeled urea rate increased from 160 to 270 mg N pot<sup>-1</sup> (Figure 3).

Independently to the N urea dose applied, soil 15N urea recovery (15Nr) was significantly higher in the presence of sewage sludge than in the absence of sewage sludge.

Soil 15N recoveries in these treatments were in the decreasing order U1 + SS > U2 + SS > U2 > U1.

The greatest 15Nr was attained with the lowest N urea application rate co-applied with sewage sludge (42%). In this treatment, addition of sewage sludge increasing the urea immobilization by about 29% as compared to the application of urea alone (up to three times more).

By causing immobilization of fertilizer N, sewage sludge ensured its prolonged and continued availability to plants thereby leading to higher fertilizer use efficiency. Thus sewage sludge had a stabilizing and conserving effect on fertilizer N (Azam et al., 1985). In the other hand, it contributed to improve soil properties (Ayuso et al., 1996; Hernandez et al., 1991) especially soils having low natural fertility.

### 3. <sup>15</sup>N urea unaccounted for

The portion of the applied urea N unaccounted for was the difference between the amount of N applied and the amount of applied N recovered in the soil and crop.

We assumed that losses of applied 15N urea due to leaching were excluded and we estimated that 15N losses were essentially attributed to denitrification and volatilization.

The percentage of applied urea N unaccounted for (assumed to be lost) dropped from 53 to 22% when 160 mg 15N-urea was co-applied with sewage sludge. However, it was unaffected by the presence of sewage sludge with the highest N-urea application rate (average 23%).

In summary, the nitrogen balance sheet showed that there were significant benefits from application of sewage sludge. Less than 25% of the 15N urea applied was lost when urea was co-applied with sewage sludge. More urea-N was immobilized in the soil. This finding is consistent with the results of several other studies which showed that microbial and physico-chemical immobilization of fertilizer N mediated by organic matter reduced N loss in agricultural and forest soils (Chang and Peterson, 2000; Choi et al., 2001; Choi et al., 2005) and contributed to improve properties of soil and enriched the fertility status of soil. Siva et al. (1999); Devevre and Horwath (2001) and Castells et al. (2004) showed that co-application of organic amendment can enhance the binding of NH<sub>4</sub><sup>+</sup> onto negatively charged sites of organic matter. Such abiotic N fixation can also contribute to retention of co-applied N, since it reduces the NH<sub>4</sub><sup>+</sup> concentration in soil solution which is susceptible to NH<sub>3</sub> volatilization loss (Siva et al., 1999). This may be viewed as a positive interaction of the co-





application of sewage sludge and urea in term of N loss and may be verified in long term experiments with or without crop cultivation. Similar behaviors was reported with Motta et Maggiore (2013) who concluded that plant N uptake, along with rapid microbial immobilization and nitrification was likely responsible for the N leaching. The losses of nitrogen as nitrate from the soil were minimal for the plots treated with the maximum rate of sewage sludge, probably due to the characteristic stability of this organic compound.

The results of this study indicate that sewage sludge applications might be an attractive option to conserve fertilizer N by its immobilization in soil and simultaneously minimize its losses. Sewage sludge can be used as a soil conditioner and inexpensive source of nutrient within agriculture.

Sewage sludge application will get considerable attention in socio-economic conditions in Tunisia due to sudden increase in fertilizer prices and increased depletion of soil quality.

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