Original Research

Isotopic Evaluations of Dynamic and Plant Uptake of N in Soil Amended with ^{15}N-Labelled Sewage Sludge

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Abstract

Field experiments were conducted to evaluate the use of a novel ^{15}N isotope technique for comparing the dynamics of N derived from sewage sludge applied to sorghum to the dynamics of N derived from the commercial fertilizer, urea. The treatments included a control, sludge applied at three rates (3, 6 and 9 t/ha, or 113, 226 and 338 kg N/ha) and N-urea applied at three rates (150, 250 and 350 kg N/ha). Recovery of ^{15}N-labelled sludge was similar for the different nitrogen rates applied, with a mean value of 27%. However, the recovery of ^{15}N-urea decreased as the rate of N application increased (from 38% to 27%). Approximately 22% and 19% of the ^{15}N from sludge and urea, respectively, remained in the 0-60 cm layer of soil, most of which was present in the 0-20 cm layer. Furthermore, losses of ^{15}N-labelled fertilizer were not affected by the N fertilization source, and the greatest losses, which were measured in response to the highest N application rate, were 59%.

Keywords: ^{15}N, ^{15}N balance, sewage sludge, urea, *Sorghum sudanense*

Introduction

In Tunisia, agricultural soils have a low organic matter content that seldom exceeds 2%. To improve soil fertility, organic wastes such as sewage sludge are widely used as soil conditioner and an inexpensive source of nutrients [1]. However, the possibility of accumulation of heavy metals in soils that received sewage sludge is notorious and must be taken into account, especially when the addition is done in a sequential way for long periods of time [2]. Sewage sludge is also considered to be beneficial for crop production, particularly as a natural source of N; therefore, it may be useful as an alternative to mineral fertilizers. However, the application rate of sewage sludge to land must be determined based on the crop N requirement to avoid potential hazards associated with excessive NO_3^- in soil [3].

Many studies have reported the beneficial effects of sewage sludge [4-6]. In addition, [7] noted increases in the fresh and dry matter yield of ryegrass of 60 to 144% following the application of 4 and 8 t/ha of sewage sludge, respectively, and these increases corresponded with an increase in the total crop N content and uptake. Sims et al. [8] noted that the application of sewage sludge consistently resulted in corn yields and plant tissue N levels that were greater than or similar to the yields obtained when ammonium nitrate or urea was applied.

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Table 1. Main physical and chemical properties of the experimental soil.

Sand %	Argil %	Loam %	pH	C %	N %
80	13	7	7.7	1.99	0.055

In most cases, the availability of N was examined indirectly through crop yields or based on the total N content of succeeding crops. For example, N uptake by a crop is measured in the presence and absence of added residues, and the difference is attributed to mineralization of N present in the applied residues. However, there are serious limitations associated with this approach. N release in practical situations is often rather small compared with total crop N uptake, so measurement precision is poor [9]. As a result, techniques such as isotope labelling are often used to distinguish the origins of N removed and to calculate the balance sheet of different N sources. Indeed, direct measurement of the recovery of fertilizer in the soil and the subsequent calculation of N that is lost from the crop/soil system can only be conducted using ^{15}N-labelled fertilizer [10].

The availability of N to plants from applied inorganic fertilizers has been evaluated using ^{15}N isotope in many studies [11-13]. In addition, this method has been utilized to evaluate the turnover and plant availability of N applied as plant residue [11, 14, 15]. However, this technique has not been widely used to evaluate the effects of sewage sludge on the balance sheet of soil/plant systems.

Therefore, in this study, ^{15}N-labelled sewage sludge and ^{15}N-labelled urea applied as fertilizer were used for the following:

(i) to quantify the recovery of ^{15}N-labelled fertilizer (sewage sludge or urea) by sudangrass, a forage crop grown on approximately 8,000 ha in Tunisia (DGPA-Ministry of agriculture, 1999, cited by [16]),

(ii) to quantify the N remaining in soil and lost from the soil-plant system, and

(iii) to discuss determination of the fertilizer N balance using the isotope dilution method.

Materials and Methods

Experimental Design

This study was conducted on a sandy loam soil at the experimental field of the Rural Water and Forest Research Institute, INRGREF, of Tunisia in 2004 and 2005. Some physical and chemical properties of the experimental soil are presented in Table 1.

An experiment using microplots (1m²) was conducted utilizing a completely randomized block design with four replicates. The microplots were limited with galvanized metal barriers that penetrated to a depth of 20 cm and stood 10 cm above the soil surface.

The ^{15}N-labelled nitrogen was applied at three rates: as ^{15}N-labelled sewage sludge (3, 6 and 9t DM/ha, providing a total of 113, 226 and 338 kgN/ha), or as ^{15}N-labeled urea (46% N, 150, 250 and 350 kgN/ha). A control treatment (0N) was also included. Sludge was collected from sludge-drying beds. The main chemical characteristics of the sludge utilized in this study are given in Table 2. The sewage sludge was relatively rich in nutrients (C, N and Ca). The heavy metal concentrations in the sludge were generally low to moderately low. None of the heavy metals exceeded the maximum value in the sludge. They were below the maximum limits allowed by Tunisian standards regulations (Table 2), indicating the possibility of using sewage sludge as fertilizer with no immediate threat of soil or plant contamination [17-19].

Table 2. Average chemical composition of applied sewage sludge compared to Tunisian standards (NT 106.20) (2002).

Parameter	Sewage sludge	NT 106.20
H_2O	7.8	
pH	8.08	
	%DM	
Total C	19.49	
Total N	3.76	
NH_4^+-N	0.89	
Total P	1.73	
K	0.4	
Na	0.24	
Ca	8.7	
Mg	0.58	
	mg/Kg DM	
Cd	0.89	20
Co	9.28	-
Cr	97.5	500
Cu	180.4	1000
Fe ‰	12.5	-
Mn	155	-
Ni	28.6	200
Pb	79.5	800
Zn	490.2	2000

To label the sewage sludge, ^{15}N urea (approximately 10% atom excess) diluted with distiller water was mixed with the sewage sludge. The mixture was then covered with impermeable paper to minimize the evaporation of water and the loss of N by volatization and denitrification. Incubation was then conducted under laboratory conditions for 20 days. The sludge nitrogen had a ^{15}N atom excess of 2.3%.

Analysis of 5 different samples gave similar results (± 5% difference between results), which indicates that the labelling was homogeneous.

Sudangrass (*Sorghum sudanense*) was planted on 31 May 2004. The microplots were arranged to include four 15 cm rows of sorghum. Each row contained seven plants and there was a distance of 30 cm between rows. ^{15}N-labelled sewage sludge was applied to the soil surface 10 days before sowing. Conversely, ^{15}N-labelled urea (10% atom excess) was dissolved in water and applied using a watering can. The ^{15}N-labelled urea was applied in two equal fractions, with half being applied at emergence and the remainder being applied after the first harvest. Irrigation water was applied to compensate for evapotranspiration, which was 6-7 mm/day during the experimental period (165.3 mm/June, 236.4 mm/July and 200.2 mm/August). Overall, approximately 40 mm of water per week were added to each microplot with a watering can, at a frequency of two irrigations per week.

Harvesting and Analysis of Samples

Two harvests were conducted when the sudangrass was at the beginning of the flowering stage, after which the yield was measured. Ten central sorghum plants were used to estimate the fertilizer N recovery. In the first harvest, only the above-ground portions of the plants were collected, while in the final harvest, the whole plants were collected. All plant samples were dried at 80°C, weighed and then ground. In addition, after the second harvest, soil samples were collected from three depths at three locations in each microplot using an auger (0-20, 20-40 and 40-60 cm) and then composited. The plant and soil samples were then analyzed for total N and ^{15}N using a Dumas analyzer coupled with a mass spectrometer (Europa Scientific, UK).

Method of Calculations

The % ^{15}N abundance determined by mass spectrometry was transformed into the atom % ^{15}N excess by subtracting the natural abundance (0.3663 atom % ^{15}N) from the % N abundance of the plant and soil samples. The data set was then statistically 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 Newman and Keuls test. All data shown represent means ± standard deviations of quadruplicate measurements.

The percentage nitrogen derived from fertilizer (NDFF%) and soil (NDFS%) were calculated as follows:

$$\text{NDFF \%} = (E_{pl}/E_f)\cdot 100$$

...where E_{pl} is the atom percentage excess in the plant, and E_f is the atom percentage excess in fertilizer.

$$\text{NDFS \%} = 100 - \text{NDFF\%}$$

The ^{15}N recovery ($^{15}NR\%$) fraction for plants in fertilized plots was calculated by:

$$^{15}NR\% = (N_{pl}\cdot E_{pl}/N_f\cdot E_f)\cdot 100$$

...where N_{pl} is the amount of nitrogen taken up by the plant, N_f is the amounts of fertilizer nitrogen applied, E_{pl} is the atom percent excess in the plant, and E_f is the atom percent excess in fertilizer.

The residual ^{15}N that remained in the soil was calculated as follows:

$$^{15}N_r\% = (N_s\cdot E_s/N_f\cdot E_f)\cdot 100$$

...where N_s is the amount of nitrogen derived from the soil, N_f is the amount of fertilizer nitrogen applied, E_s is the atom percent excess in soil and E_f is the atom percent excess in fertilizer.

Results and Discussion

Dry Matter Production and Nitrogen Uptake by Sudangrass

Both the urea and sewage sludge application led to an increase in dry matter production and nitrogen uptake by sudangrass when compared to the controls (Table 3). In addition, the maximum dry matter and N uptake was attained when the lowest rate of N was applied as urea (150 kg N/ha) or sewage sludge (113 kg N/ha). Urea or sewage sludge application rates above these levels did not lead to additional increases in dry matter production (average 14,000 kg/ha) of nitrogen uptake (average 260 kg N/ha) by sudangrass (Table 3). In agreement with the results of a study conducted by [20], the results of the present study suggest that application of nitrogen as urea at a rate of 150 kg N/ha or as sewage sludge at a rate of 113 kg/ha appears to be sufficient for covering sudangrass N needs, leading to maximum yields. Furthermore, sludge-N leads to similar crop yield and nitrogen uptake than N-urea (Table 3) suggesting a higher efficiency of sewage sludge as N fertilizer. Similar behaviour was reported by [21], who demonstrated that the effects of the application of sewage sludge as a N source on ratoom cane yield and on some technological characteristics (brix, pol, reducing sugars and purity index in sugar juice and pol) were similar to the effects of the application of N via a mineral source (urea). However, [22] reported that nitrogen from urea produced higher wheat yield than N from sewage sludge when both were applied at an equal rate.

Table 3. Effect of different nitrogen rates applied as sewage sludge or urea on the above-ground dry matter and N uptake by sudangrass.

N sources	^{15}N applied	Total dry matter (DM)	Total N uptake	NDFF	NDFS	^{15}NR
	Kg/ha			%		
Control	0	11645 b	182 b			
Sewage sludge	113	15032 a	272 a	11 d	89 a	27 b
	226	13956 ab	243 a	23 bc	77 b	25 b
	338	13309 ab	250 a	31 a	69 c	23 b
urea	150	14148 ab	243 a	20 c	80 b	32 a
	250	13139 ab	259 a	28 b	72 c	29 b
	350	14769 ab	301 a	29 b	71 c	25 b

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

Moreover, our results showed that no significant difference in crop production and total N uptake with respect to the N application timing (splitting the urea application, single sewage sludge application). It appears that the crop's ability to meet the yield level depends on the level of N available to the crop throughout the growing season prior to the timing and chemical form of fertilizer application and requires the synchronisation of fertilizer-N availability and crop-N demand.

Nitrogen Derived from Fertilizer (Urea or Sewage Sludge) and from Soil in Sudangrass

Total N recovery (^{15}NR), nitrogen derived from urea, or from sewage sludge (NDFF %) and from soil (NDFS %) are presented in Table 3. The major fraction of nitrogen absorbed by sudangrass from both sources came from soil. Furthermore, the fraction of nitrogen absorbed from the soil was from two to eightfold higher than the fraction of nitrogen absorbed from the urea and sewage sludge, which suggests an extensive turnover of soil N and applied N through immobilization and mineralization. Moreover, the NDFS% decreased significantly as the N rate increased. This reduction was somewhat compensated for by an increase in nitrogen derived from both urea and sewage sludge (NDFF%). In fact, the proportion of plant N derived from fertilizer (NDFF%) increased from 11 and 20% for low application of rates of sludge and urea, respectively, to 31 and 29% for high N application rates of sewage sludge and urea, respectively (Table 3). These findings are consistent with the results of other studies conducted on sudangrass that demonstrated that a decrease in N derived from soil did not affect the total N uptake by the plant because it was compensated for by an increase in N fertilizer uptake [23].

Recovery of ^{15}N-Labelled Fertilizer (^{15}NR)

Independently of the N fertilization source, the greatest ^{15}NR, measured in the above-ground crop at final harvest, was attained when the lowest N application rate was used (between 110 and 150 kg/ha) (Table 3). Recovery of ^{15}N-labelled sludge from the above ground portion of sudangrass was similar at the different applied nitrogen rates, with a mean value of 25%. These levels of sewage sludge N recovered by sudangrass were similar to those reported by [24] for Fescue grass in soils amended with municipal sludge, who found that the total recovery of applied N in the harvested grass was 24% of the municipal-sludge N for the first five harvests.

The recovery of ^{15}N-urea decreased significantly from 32 to 25% as the nitrogen level increased from 150 to 350 kg N/ha. Similar results were reported by [13], who found that the ^{15}N recovery from urea dropped as the fertilizer application rate increased. It is likely that a significant amount of the applied N moved below the root zone when there was a high application rate, and that this resulted in much of the N not being available to crops. These findings indicate that the application rate needs to be matched to the needs of the crops to minimize the risk of pollution.

Residual ^{15}N Remaining in the soil ($^{15}N_r$)

Approximately 22 and 19% of the ^{15}N labelled fertilizer remained in the 0-60 cm layer of soil following the final harvest after fertilization with either sewage sludge or urea (Fig. 1). The distribution of residual ^{15}N at different depths in the soil indicated that, in the case of sewage sludge treatment, all of the ^{15}N was found in the top 20 cm of soil, regardless of the application rate. This indicates that leaching of nitrates beyond 20 cm can be discounted, denitrification and/or volatilization processes being the greatest factor influencing N-sewage sludge losses.

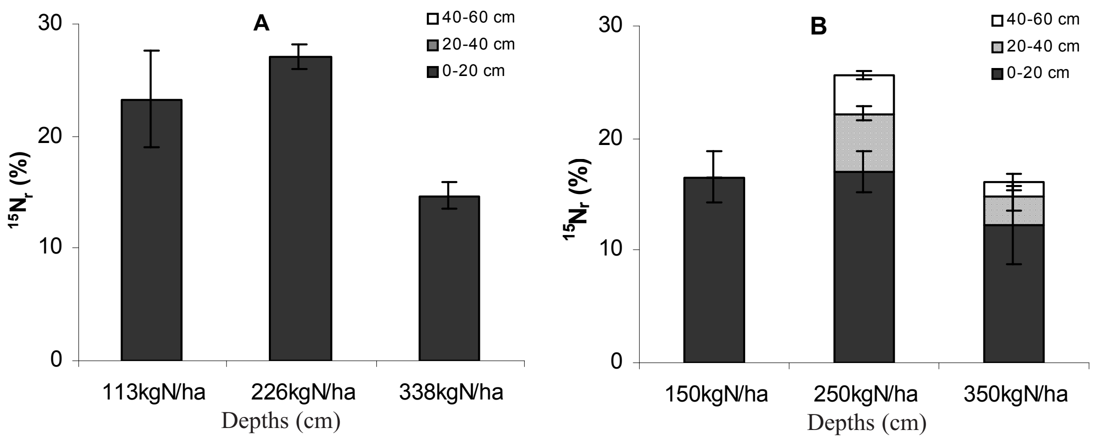

Fig. 1. Percent of residual ^{15}N remaining in the 0-60 cm layer of soil as a function of sewage sludge (A) or urea (B) application rates and different depths.

In agreement with the findings of [13], when the lowest application rate of N-urea (150 kg/ha) was used, all of residual ^{15}N remaining in the soil was recovered in the surface 0-20 cm layer. However, increasing N rate application resulted in an increased proportion of residual ^{15}N being found beyond 0-20 cm. Although the level of ^{5}N found beyond the 0-20 cm range was small (Fig. 1), these findings indicate that the applied nitrogen was subject to losses via leaching.

Nitrogen Losses

The losses of the applied N can be estimated by the difference between the amount of ^{15}N recovered from soil and crop, and the known amount of ^{15}N applied. High losses of N from both sewage sludge and urea application were observed, and these losses increased as the N rate increased (Fig. 2). Indeed, the proportion of ^{15}N-labelled fertilizer that was unaccounted for and unaffected by the N fertilization source ranged from 45% to 61%, with the lowest and highest values corresponding to the lowest and highest N application rates (Fig. 2).

Losses can affect the nitrogen fertilizer efficiency; therefore, it is important to know how most of the losses of fertilizer nitrogen occur. The most likely mechanism responsible for the loss of urea is ammonia volatilization, which accounts for 45 to 57% of the nitrogen loss associated with urea. In the present study, the ^{15}N-labeled urea in the experiment was applied as a solution of unincorporated urea and the daily air temperature during the study period

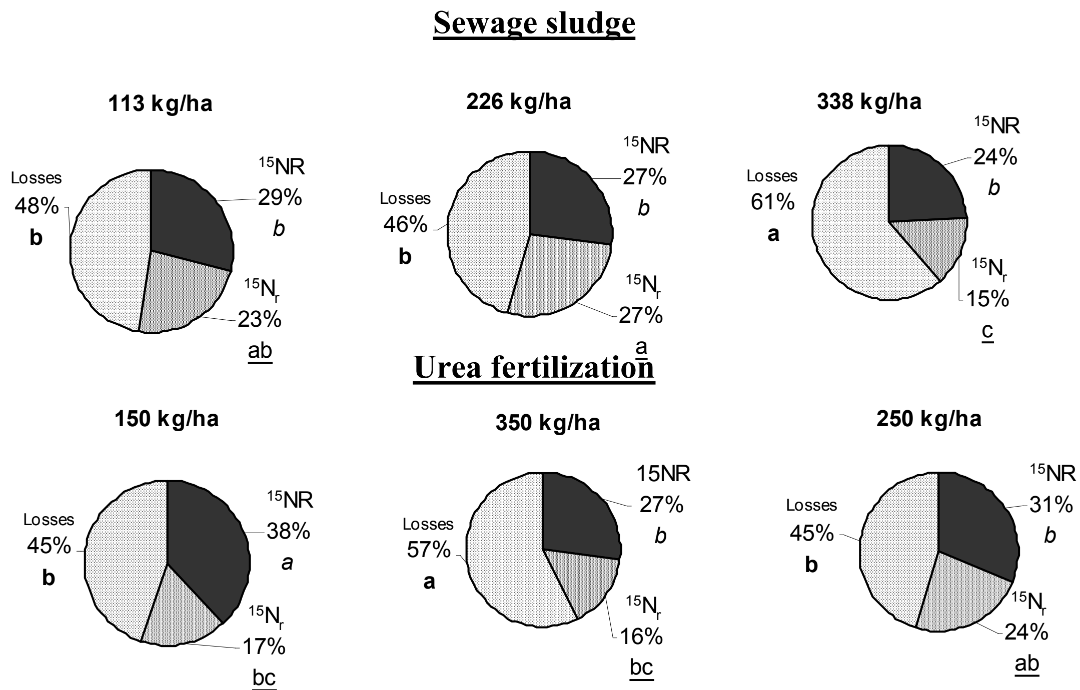

Fig. 2. Balance sheet of applied ^{15}N (%).
(*a*, *b*, *c*), (a, b, c) and (**a**, **b**, **c**) indicate significance differences in fertilizer N recovery in the whole plant (^{15}NR), in residual ^{15}N remaining in the soil ($^{15}N_r$) and in losses respectively, as related to the rate of applied ^{15}N.
Average values followed by the same letter are not significantly different at $p > 0.05$.

ranged from 25 to 38°C; therefore, conditions were favourable for N-loss via ammonia volatilization. Similar behaviour was reported by [25], who found that nitrogen losses from surface-applied unincorporated urea could be as high as 48%. However, as mentioned above, the leaching of nitrates below the sampling depth during this experiment cannot be excluded.

Losses of nitrogen derived from sewage sludge likely occurred as gaseous losses via denitrification [26-29]. Loro et al. [30] also found that denitrification and N_2O production were enhanced following manure application when compared with fertilizer application, but that this enhancement did not occur in the subsurface soil. They also found that denitrification did not occur at depths of 40 cm or more. Many other studies have reported low or negligible denitrification rates with depth [31, 32]. Moreover, in the present study, all of the observed N sewage sludge losses occurred in the top 20 cm of soil, which is similar to the results of previous studies. The loss of approximately 46% to 59% of the applied nitrogen provides an explanation for the failure of N recoveries in the above ground crop to exceed 30%, regardless of the method by which nitrogen is lost.

Nitrogen Balance

The data described above were used to create a nitrogen balance sheet (Fig. 2). The nitrogen balance for this experiment shows that the total recovery of ^{15}N labelled urea in whole sorghum plants decreased significantly from 38 to 27% as the rate of N application increased from 150 to 350 kg N/ha. Approximately 27% of the ^{15}N labelled sewage sludge was recovered for all rates of N applied at final harvest (Fig. 2). The total residual ^{15}N fertilizer remaining in the soil after the second harvest ranged from 15 to 23% and from 16 to 24% for sewage sludge and urea fertilization, respectively. The maximum N immobilization occurred at the intermediate N rate in response to fertilization with both materials. At the lowest N rate, the proportion of ^{15}N labelled sewage sludge remaining in the soil was higher than that of the urea (23 vs. 17%), which explains the relatively low ^{15}N recovery that was observed (29 vs. 38 %) in response to the application of sewage sludge fertilization at this N rate (Fig. 2). Contrary to total recovery of ^{15}N fertilizer, labelled N that was unaccounted for increased as the N application rate increased. This finding is consistent with the results of other studies conducted on sorghum [33]. However, the N fertilization source did not affect the proportion of ^{15}N-labelled fertilizer that was unaccounted for.

As mentioned above, great losses of nitrogen occurred during the growth of sudangrass, and these losses occurred via N volatilization or denitrification. The greatest part of N fertilizer lost after plant harvest (45% to 61%) was associated with a higher concentration of ^{15}N in the upper soil layer. This phenomenon was likely due to the presence of unincorporated surface applied urea or sewage sludge [34]. These high losses were responsible for the observed decrease in N-fertilizer efficiency [27, 35] and led to much lower ^{15}N recovery values than reported in previous studies conducted on sudangrass [33, 36]. Based on these findings, we suggest that injecting sewage sludge and urea into the soil could be a more efficient method of attaining higher N recoveries. Similar results were reported by [37-39], who concluded that applying nitrogen as a surface band or injecting it into the soil enhanced nitrogen-use efficiency and increased yields.

Other management practices may be adopted to improve N use efficiency in plants, increase nitrogen retention in soils and minimizing N losses. These include the use of nitrification inhibitors to reduce leaching and denitrification losses of fertilizer N from the root zone but the use of these materials requires the synchronization of soil-N availability and crop-N uptake to prevent N deficiencies [40, 41]. In addition, nitrogen management techniques should also include uses of other nutrients to enhance effectiveness of soil-applied nitrogen. Two types of mechanisms have been shown to reduce ammonia volatilization: hydrogen ion donors [42], and added cations [25]; a third mechanism works indirectly by preventing urea hydrolysis [43]. Moreover, according to [44-49] the selection of optimum fertilization (rate, type, nitrogen application timing, specific soil placement of N fertilizer) as a function of the crop needs, soil characteristics and climate, along with effective management practices, should attempt to draw up on all the possible options of increasing N-use efficiency, which untimely can lead to more profitable crop production and a safer environment.

Conclusion

The data obtained from this study highlights the suitability of the use of the isotope labelling technique for direct measurement of the real recovery of sewage sludge nitrogen and subsequent calculation of the N that is lost from the crop/soil system. In addition, the results of this study indicate that the behaviour of sewage sludge is similar to that of urea fertilizer as regards N supply to plant. Thus, sewage sludge could be used as an alternative to this synthetic fertilizer. Results also indicate that greater care regarding fertilization, such as careful evaluation of the method of application, is necessary to optimize the effects of sewage sludge application on soil fertility and crop yields.

In addition to several advantages that accrue from the use of sewage sludge for crop nutrition, certain limitations should be taken into consideration: these sludges contain varied amounts of heavy metals that have implications for soil and quality of crop. Even if the use of this sludge, with low metal content, may have no immediate threat, long-term sewage sludge application may result in the accumulation of some heavy metals in the soil and their entry into plants in quantities above the maximum permitted concentrations. There is still much to be learned from this study and this investigation needs to continue to determine whether the agricultural and ecological objectives are satisfied over the long term. Thus, the intended scope of this research is to evaluate the cumulative and residual effects of repeated application of sewage sludge, on plant production, soil sorption for nutrients and trace metals, over years with and without sewage sludge application during a long period.

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