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Indirect ^{15}N Isotope Techniques for Estimating N Dynamics and N Uptake by Rice from Poultry Manure and Sewage Sludge

[1,2]Adel Mohamed Ghoneim, [3]Hideto Ueno, [3]Naomi Asagi and [4]Takeshi Watanabe
[1]Agricultural Research Center, Rice Research and Training Center, Sakha, 33717, Kafr El-Sheikh, Egypt
[2]Department of Soil Science, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh, 11451, Saudi Arabia
[3]Ehime University Farm, Faculty of Agriculture, Ehine University, 498 Ko, Hattanji, Ehime, 799-2424, Japan
[4]Japan International Research Center for Agricultural Sciences, 1-1 Ohwashi, Tsukuba, Ibaraki, 305-8686, Japan

Corresponding Author: Adel Mohamed Ghoneim, Agricultural Research Center, Rice Research and Training Center, Sakha, 33717, Kafr El-Sheikh, Egypt

ABSTRACT
Sole use of chemical fertilizers as a nutrient source for intensive paddy rice production has likely caused a decline in soil fertility and rice yields. This study examined the effects of poultry manure and sewage sludge application on nitrogen (N) uptake, dynamics and efficiency of usage. A ^{15}N isotope dilution method was used to estimate the amount of N in rice plants derived from the poultry manure and sewage sludge. The results showed that the percentage of ^{15}N recovered from sewage sludge and poultry manure was 19 and 36%, respectively. Most of the N uptake by rice was from the soil and varied from 54-64%. The relative efficiency of poultry manure and sewage sludge was 80 and 85%, respectively. No significant difference was observed in the N loss rate and the N residual into soil post harvesting between the poultry manure and sewage sludge.

Key words: Nitrogen^{15} dilution method, nitrogen uptake, organic matter

INTRODUCTION
One of the measures being adopted for relieving environmental problems arising from agricultural production is to recycle poultry manure, sewage sludge and other organic products as fertilizers and soil amendments. Nitrogen (N) is the most limiting soil nutrient in rice production. The efficiency of applied N usage in rice is very low, ranging from 15-35% due to dentrification, volatilization and leaching. Furthermore, continuous use of chemical fertilizers over time may accelerate the depletion of soil organic matter. Crop N recovery from organic inputs such as poultry manure and sewage sludge or manures is often less than 20% (Nishida *et al*., 2004). However, it has been widely accepted that organic inputs play a significant role in the long-term build up of soil organic matter. To maximize the potential N benefit of organic inputs it is necessary to be able to predict the amount of N supplied to the rice from organic inputs. The amount of N supplied to the rice from an organic input is dependent on the mineralization of organic forms to plant available inorganic nitrogen (Ghoneim *et al*., 2008). The use of poultry manure and domestic sewage sludge in agriculture is being considered as one of the methods for recycling of these wastes in an environmentally beneficial manner. For soil management, it’s well known that organic matter

application plays an important role in supplying nutrients (Takahashi *et al.*, 2003), stabilizing pH, EC and CEC, controlling the soil moisture and enhancing microbial activity and circulation of nutrients (Ghoneim *et al.*, 2006). These processes are necessary for sustainable agricultural systems. As a source of organic matter, poultry manure and sewage sludge are the most abundant organic materials used in farming systems (Mubarak *et al.*, 2003). Thus, evolution of poultry manure and sewage sludge from the viewpoint of N is very important for integrated soil management for sustainable food production and conservation of agricultural land (Ebid *et al.*, 2008). The utilization of sewage sludge in agricultural fields is gaining popularity as a means of waste disposal. This organic material can enhance soil productivity as a consequence of its high organic matter and plant nutrient content. There is currently much interest in agricultural use of sewage sludge to reap its benefits as a fertilizer and as an aid in moisture retention. Information regarding the effect of poultry manure and domestic sewage sludge on yield and N uptake by rice is scarce. In practical farming, although the relative efficiency of organic materials (relative uptake of organic material N to chemical fertilizer N) has been used as an index of organic material efficiency (Nishida *et al.*, 2004), this index has not been estimated using ^{15}N labeled poultry manure and domestic sewage sludge. In a previous study (Ghoneim, 2007), it was reported that application of poultry manure and sewage sludge increased grain yields by 34.8 and 38.3%, respectively over the control. Therefore, the objective of this study was to investigate the fate of N in poultry manure and sewage sludge in terms of N distribution and N uptake by rice.

MATERIALS AND METHODS

Study area and experimental design: The greenhouse experiment was carried at the Experimental Farm, Ehime University, Matsuyama city, Japan, (33°57'N, 132°47'E) with an elevation of 20 m above sea level. The soil was a low fertility Fluvisol and key chemical characteristics are presented in Table 1. The experiment was set up as a Completely Randomized Block Design (CRBD) with five replications and the following treatments: control without fertilization or amendment, chemical fertilization, sewage sludge and poultry manure. Chemical fertilizer ($^{15}NH_4Cl$, 10.5 atom %) was applied at rate of 8.0 g m^{-2} in three splits (basal, tillering and panicle initiation). Sewage sludge was obtained from Nishida Industry Inc., Matsuyama, Ehime, Japan with the flowing chemical properties: pH (H_2O): 6.1, total N: 7.3%, P_2O_5: 2.5%, K_2O: 5%, CaO: 0.9%, Zn: 49 mg kg^{-1}, Cu: 720 mg kg^{-1}, Hg: 0.43 mg kg^{-1}, As: 5.6 mg kg^{-1}, Cd: 0.4 mg kg^{-1},

Table 1: Chemical properties of the upper 20 cm of soil at the study site (n = 5)

Characteristic	Mean
pH	7.50
EC (dS m^{-1})	0.37
Total soil C (%)	1.46
Total soil N (%)	0.17
C:N ratio	8.58
Available P (g kg^{-1})	1.90
Exchangeable K (g kg^{-1})	5.70
Exchangeable Ca (g kg^{-1})	1.45
Clay (%)	13.40
Sand (%)	58.50
Silt (%)	28.10

pH and EC was measured in the soil suspension (1:2.5 and 1:5, w/v), respectively

C/N ratio: 5.7 and moisture content: 10%. While the poultry manure was obtained from Asakawa Farm Inc., Matsuyama, Ehime, Japan with chemical analysis: pH (H_2O): 8.1, total N: 2.7%, P_2O_5: 6.9%, K_2O: 3.9%, CaO: 2.0%, C/N ratio: 8.3 and 12% moisture content. The application rates of sewage sludge and poultry manure were 160 and 200 g Fresh Weight (FW) m^{-2}, respectively added in one application just before transplanting. The application rate was based on the fresh weight because these materials are applied on a fresh weight basis in practical farming. In sewage sludge and poultry manure pots was labeled with a solution containing 0.30 g N m^{-2} as $^{15}NH_4Cl$ (1.0 atom %) injected carefully into the soil as ^{15}N tracer one week after transplanting. Phosphorus as P_2O_5 and K as KCl were applied as a basal dose to all pots at the rate of 8 g m^{-2} one dose just before transplanting. Wagner pots (0.025 m^2) were filled with 3.50 kg air-dried soil mixed with an equivalent volume of sewage sludge and poultry manure. Three 25-day-old seedlings of rice cultivar Sakha 103 were transplanted into the center of each pot with five replicates on 22 June. The pots, which were maintained under flooding conditions until harvesting, were drained thereafter.

Plant sampling and measurements: Plant height, number of tillers and leaf chlorophyll content were measured at different growth stages. Chlorophyll content was measured with a chlorophyll meter (SPAD-502; Minolta Co. Ltd., Japan). The rice plants were harvested at maturity and then separated into straw and grain and oven dried at 70°C to a constant weight. The dried samples were weighed and ground into a fine powder using a vibrating mill (TI-100, C.M.T. Co. Ltd., Saitama, Japan). Total N and ^{15}N were determined using a stable isotope mass spectrometer (ANCA-SL; PDZ Europa Ltd., Cheshire, UK). The loss of N resulted from sewage sludge and poultry manure was calculated using the following equation:

$$L = 1-(P+I)$$

where, L denotes loss, P denotes plant uptake and I is the amount of N remaining in the soil (assimilation, immobilization and residual N).

Statistical analysis: The obtained data were analyzed statistically and the differences among the mean was analyzed by the Tukey-Kramer test using the software KyPlot (KyensLab Inc., Tokyo, Japan).

A-value approach as indirect ^{15}N isotope method: The poultry manure and sewage sludge derived N in rice was estimated by the A-value method as one of the indirect ^{15}N approach. It is assumed that when as sources of N are present in the soil, the rice will absorb from each of these sources in proportion to the respective quantities available (Stevenson *et al.*, 1998). The A-value is a time-integrated estimate of the plant available nitrogen. The main advantage of the A-value (Fried and Dean, 1952) is that it allows comparisons of treatment with different rates of N applied. The assumption is that percentage of N derived from a source is proportional to the N available (A). The A-value is a measure of the soil N in fertilizer equivalent.

In present study, the chemical fertilizer pots, ^{15}N-labelled N fertilizer was applied, while in poultry manure and/or sewage sludge pots, ^{15}N tracer and unlabeled poultry manure and sewage were added.

An A-value of the soil, i.e., As in the chemical fertilizer can be estimated from Eq. 1:

$$As = \frac{100 - N_{dfcf}\ \%}{N_{dfcf}\ \%} \times Acf \qquad (1)$$

where, Acf is the A-value of the chemical fertilizer which represents the amount of ^{15}N labeled chemical fertilizer applied to chemical fertilizer pots. N_{dfcf}% is the parentage of plant N uptake from chemical fertilizer applied in chemical fertilizer treatment and can be calculated as follows:

$$N_{dfcf}\ (\%) = \frac{\text{Atom \% }^{15}\text{N excess of the plant}}{\text{Atom \% }^{15}\text{N excess in chemical fertilizer}} \times 100$$

The A-value of the chemical fertilizer and poultry manure and/or sewage sludge in the poultry manure or sewage sludge pots can be calculated as:

$$As + OF = \frac{100 - N_{dft}\ \%}{N_{dft}\ \%} \times At \qquad (2)$$

where, $As_{+}OF$ is the A-value of the chemical fertilizer plus poultry manure and/or sewage sludge and N_{dft}% is the percentage of rice N uptake from the amount of ^{15}N tracer applied in poultry manure and/or sewage sludge pots and can be estimated from the following equation:

$$N_{dft}\ (\%) = \frac{\text{Atom \% }^{15}\text{N excess of the plant}}{\text{Atom \% }^{15}\text{N excess in }^{15}\text{N tracer}} \times 100$$

The A-value of ^{15}N tracer (At) is the amount of ^{15}N tracer applied in poultry manure and/or sewage sludge treatment.

Since the A-value of the soil is constant regardless the amount of poultry manure and/or sewage sludge added to the soil, therefore, the A-value of the poultry manure and/or sewage sludge (A_{OF}) can be calculated by subtracting A_s from As+OF:

$$A_{OF} = As + OF - As$$

The percentage of rice N uptake from the poultry manure and/or sewage sludge applied (N_{dfOF}%) can be estimated as follows:

$$N_{dfOF}\ (\%) = \frac{N_{dfOF}\ \%}{At} \times A_{OF} \qquad (3)$$

RESULTS AND DISCUSSION

Total N uptake, N uptake originating from poultry manure or sewage sludge and dry weight are presented in Table 2. The total N uptake in the chemical fertilizer treatment was significantly higher than that in the poultry manure or sewage sludge treatments. It was also observed that the total N in poultry manure tended to be higher compared with sewage sludge. Since the application

Table 2: Dry matter and total nitrogen uptake by rice grown in unamended soil and soil amended with either chemical fertilizer, sewage sludge, or poultry manure

	Dry weight (g pot^{-1})			Total N (mg pot^{-1})		
Treatment	Grain	Straw	Total	$^{a}N_{dff}$	$^{b}N_{dfs}$	Total
Control	14.0	19.0	33.0^{c}	-	98.1^{d} (100)	98.1^{d}
Chemical fertilizer	17.3	27.4	44.7^{b}	117.4^{a} (46)	135.1^{b} (54)	252.5^{a}
Sewage sludge	19.4	25.7	45.1^{b}	32.8^{c} (19)	140.8^{a} (81)	173.6^{c}
Poultry manure	25.1	32.1	57.2^{a}	71.6^{b} (36)	125.1^{c} (64)	196.7^{b}

^{a}N derived from chemical fertilizer or organic materials, Values in parentheses are N uptake% derived from chemical fertilizer, sewage sludge, poultry manure and soil, ^{b}N derived from soil, different letters in each column reflect significant differences within treatment (Tukey-Kramer test, $p<0.05$, n = 5)

rate of the poultry manure and sewage sludge was based on fresh weight, the amount of applied N affected the total N uptake. The high total N uptake in poultry manure was due to the high application rate of N as well as high uptake rate. Poultry manure resulted in the highest total dry weight compared with other treatments. The higher dry matter yield of rice in soils amended with poultry manure may be due to better nutrient balance and relatively lower levels of toxic factors in the material (Matsuyama *et al*., 2003).

The data showed that most of the N uptake by rice was from the soil and ranged from 54 to 64%. Compared with chemical fertilizer, the percentage of ^{15}N recovered from sewage sludge and poultry manure was 19 and 36%, respectively. The lower N uptake from organic materials could be attributed mainly to the rapid immobilization of N due to microbial activity, leading to a significantly lower amount of available N compared with chemical fertilizer (Zaman *et al*., 2004; Ghoneim *et al*., 2008). Nitrogen from many organic fertilizers often shows little effect on crop growth in the year of application, because of the slow-release characteristics of organically bound N. Furthermore, N immobilization can occur after application, leading to an enrichment of the soil N pool. However, this process finally increases the long-term efficiency of organic nitrogen (Stevenson *et al*., 1998). It is difficult to directly measure the N uptake from the poultry manure and sewage sludge using the indirect ^{15}N isotope technique because immobilization and mineralization occur simultaneously. Since poultry manure and sewage sludge are frequently added consecutively in practical rice farming, it is essential to predict the effect of accumulated residual N in soil using the ^{15}N labeled poultry manure and sewage sludge. In addition, the relationship between the poultry manure, sewage sludge application rate and N efficiency would be required.

Table 3 shows N use efficiency, relative efficiency and A-value estimated indirectly by the ^{15}N method. The A-values were ranked as poultry manure>sewage sludge>chemical fertilizer. The A-values obtained for sewage sludge and poultry manure were higher than the A-value of the soil by 6.5 and 7.6 fold, respectively. It was hypothesized that in the current experiment the immobilization capacity of soil with poultry manure and sewage sludge applied was different to that of the no residue control. In the control treatment there was less immobilization of inorganic N than in the poultry manure and sewage sludge treatments, resulting in a large labeled N pool available for mixing with the unlabelled N from basal mineralization. This in turn lead to a higher ^{15}N enriched pool in this treatment than in residue treatments. In the poultry manure and sewage sludge treatments it is hypothesized that there was a greater degree of N immobilization, leaving

Table 3: Fertilizer N use efficiency (FNUE) determined by ^{15}N dilution (FUE–^{15}N) and A-values for the final harvest of rice as affected by sewage sludge and poultry manure application

	FNUE*		A-value (g pot^{-1})		
Treatment	%	Relative efficiency (%)	Fertilizer	Soil	Total
Chemical fertilizer	41	100	0.275	0.095	0.370
Sewage sludge	33	80	0.618	0.095	0.713
Poultry manure	35	85	0.720	0.095	0.815

*Fertilizer N use efficiency (relative efficiency) is defined as the uptake rate of sewage sludge or poultry manure N/uptake rate of chemical fertilizer N

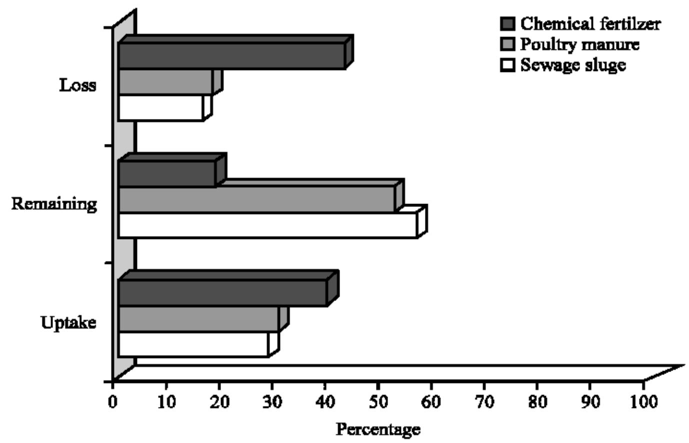

Fig. 1: Changes in N distribution of applied chemical fertilizer, sewage sludge and poultry manure

less available labeled N for mixing with a similar quantity of N from mineralization, thus resulting in a lower ^{15}N abundance in the final inorganic N pool (Hood *et al.*, 1999).

Interactions between added fertilizer N from poultry manure, sewage sludge and native soil N that change the N content in a given pool are called added N interactions (Jenkinson *et al.*, 1985). These interactions may result in different estimates for Fertilizer Use Efficiency (FUE) as shown in Table 3. The relative efficiency of the poultry manure and sewage sludge can be defined as the uptake rate of poultry manure, sewage sludge/uptake rate of chemical fertilizer. The relative efficiencies of sewage sludge and poultry manure were 80 and 85%, respectively (Table 3). The FUE–^{15}N value estimated in this study is comparable to those estimated by direct method (Nishida *et al.*, 2004).

Figure 1 shows the changes in the distribution rate of applied sewage sludge, poultry manure and chemical fertilizer. There were no significant differences observed in the N loss rate and the N remaining into soil after harvesting among treatments. However, some trends may be related to the properties of the poultry manure and sewage sludge such as higher residual rate of sewage sludge and poultry manure compared with chemical fertilizer. Further studies should be conducted to confirm these properties. By monitoring the behavior of chemical fertilizer added to the soil it was concluded that most of chemical fertilizer loss was due to dentrification (Nishida *et al.*, 2004). Although the role of soil microorganisms was not studied, soil microbes are considered to be closely associated with the uptake of organic matter and one of the important factors that control the efficiency of organic matter applied. For instance arbuscular mycorrhizal symbiosis can enhance the decomposition and increase N capture from complex organic materials in soil (Chantigny *et al.*, 2001). However, to evaluate the effect of organic matter application in soil, further studies with various organic materials labeled with stable isotopes should be carried out in a range of soils, plants and environmental conditions.

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