SHORT COMMUNICATION

Comparison of Total Nitrogen estimation by Kjeldahl Method and CHNS Analyzer in Dry Tropical Grassland

Vijay P. Gautam[1*], Swati Mishra[2] and Haseen Ahmed[3]

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Abstract

The Dumas method (CHNS analyzer) is replacing the traditional Kjeldahl method as the method of choice for N analysis due to advancements in dry combustion nitrogen (N) analyzer technology and the high cost of disposing of hazardous laboratory waste chemicals. As a result, a comparison of the Dumas method (CHNS analyzer) with the Kjeldahl method is critical. Typically, such comparisons were conducted on a small number of distinct samples. The goal of this study was to compare the performance of instruments that use the automated Dumas method (CHNS analyzer) and the Kjeldahl method for N analysis of agricultural materials in a high-throughput laboratory setting. Using both instruments, N concentrations in manure, sewage sludge, plant tissue, plant seeds, and feedstuff fibres were determined. We collected samples for the analysis of organic matter from the horticulture area at the Banaras Hindu University campus in Varanasi, and total Nitrogen (N) was estimated using the Kjeldahl (wet oxidation) and Perkin-Elmer 2.400 Series II-CHN Mode techniques (dry oxidation or combustion). The positive correlation (r = 0.94**) of the Kjeldahl method with N- determination from a CHNS analyzer demonstrates that the method is practical for routinely determining the total nitrogen content of soils.

Keywords: Total Nitrogen, CHNS Analyzer, Kjeldahl method, Tropical grassland, Carbon, Hydrogen, Sulphur, fertilizers, combustion

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Introduction

The CHNS(O) Analyzer find utility in determining the percentages of Carbon, Hydrogen, Nitrogen, Sulphur and Oxygen of organic compounds, based on the principle of "Dumas method" which involves the complete and instantaneous oxidation of the sample by "flash combustion".

Nitrogen determination using the Kjeldahl method. One of the five major elements found in organic materials such as protein is nitrogen. The Kjeldahl nitrogen analysis method is the global standard for calculating protein content in a wide range of materials including human and animal food, fertiliser, waste water, and fossil fuels. Nitrogen (N) is a nutrient that is required by all natural and managed ecosystems. As a result, the import and loss of N will affect vegetation growth and, as a result, carbon cycling and storage in the ecosystem. To feed the world's growing population, an increasing amount of reactive nitrogen is required, which must be converted from inert atmospheric molecular nitrogen (N_2) via fixation (Galloway *et al.,* 2004).

Soil includes a wide range of chemical species, from low molar mass molecules to complex decomposition-resistant chemicals, despite the fact that organic nitrogen (N) predominates (Cantarela and Trivelin 2001). Because it converts organic nitrogen found in plant cells into simple inorganic forms, organic matter mineralization is a crucial stage in the nitrogen cycle (Franzluebbers, Hons, and Zuberer 1994). N is measured using distillation or colorimetric reactions when it is present in soil as ions like NH_4^+, NO_3^-, or NO_2^-. N_2O and N_2 are difficult to differentiate, whereas NO and NO2 are the most reactive and simplest reactive gases (Tedesco *et al.,* 1995). The Kjeldahl method (wet oxidation) and the combustion method are the two techniques that are most often employed to quantify total N in soils, also known as the EuroEA 3000 series CHNS analyzer. The Kjeldahl technique is most widely used for regular soil and plant N analysis because to its efficacy and simplicity, use of standard equipment, and low cost of tests. On the other hand, semi-automatic dry combustion equipment is still widely accessible. While requiring more human labour and supplies, the Kjeldahl technique of soil N measurement is routinely employed in laboratories lacking a dry combustion automated analyzer. However, for N concentrations in organic soils or soils with a high organic matter content, the Kjeldahl method is less accurate than dry combustion (Bremner& Shaw, 1958). In mineralized soils or those with little organic content, these two techniques produced results that were comparable (Bremner& Shaw, 1958). In this study, the total N in soil samples from a horticulture field on the BHU campus in Varanasi were determined using the EuroEA 3000 series CHNS (dry oxidation or combustion) and Kjeldahl (wet oxidation) methods.

[1]Department of Botany Brahmanand PG College Rath, Hamirpur.

[2]Department of Botany Government Degree College, Haripur, Nihastha, Raebareli.

[3]Department of Botany, Shaheed Mangal Pandey Rajkiya Mahila Mahavidyalaya, Ballia.(Affiliated to Jananayak Chandrashekhar University (JNCU), Ballia.

***Corresponding author:** Vijay P. Gautam, Department of Botany Brahmanand PG College Rath, Hamirpur, Email: vijayp.gautam17@bhu.ac.in

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Materials And Methods

Site Description

We have conducted our research on a horticulture area in the campus of Banaras Hindu University in Varanasi, India,

located between 24°18'N and 83°30'E. All four seasons were present at the research site: summer (April to June), the mild rainy season (July to September), and winter. A dry tropical monsoon day prevailed during November to February. Rainy and winter seasons, in addition to the period between winter and summer, are symbolized by the months of March and October, respectively. The maximum monthly temperatures during the experiment ranged from 25.6-35.6°C to 7.3-25.4°C, and the annual precipitation averaged 932mm (Singh H. and Singh K.P. 1994). Type III nature of the soil found in the campus of Banaras Hindu University (Piper C.S. 1944). The soil is silty, light brown in color, and has a non-reactive reaction. Most of the soil is alluvial, has good drainage, is pretty fertile, and has access to phosphorus and potassium but little nitrogen (Sagar R. and Verma P. 2010). The nature of the soil pH varies from neutral to alkaline(Sagar et al. 2008).

Plot Design

24 experimental plots measuring 1×1 m^2 each were set up on the horticulture field at BHU in Varanasi. These plots were arranged in six parallel rows (4 plots in each row). Six N treatments, each with four replicates, were chosen at random to create this plot. No N treatments were used on the four control plots. The next four plots received treatments of 30 kg N/ha/yr, 60 kg N/ha/yr, 90 kg N/ha/yr, and 120 kg N/ha/yr, and the final four plots received treatments of 150 kg N/ha/yr. A relatively high nitrogen dose was used in order to guarantee a measurable response. Due to the low temperature in the nights, which decreases the activation energy of the urease enzyme and lowers N loss by volatilization, treatments in the form of urea were administered at intervals of one month (Makoi J.H.J.R. and Ndakidemi P.A. 2008). Urea contains 46% nitrogen by weight and has the chemical formula NH_2CONH_2. In order to achieve the desired results, 6.520g, 13.042g, 19.56g, 26.08g, and 32.604g of urea were spread over a 1 m^2 plot each year. These amounts correspond to 30 kg, 60 kg, 90 kg, 120 kg, and 150 kg of nitrogen per ha, respectively. Urea was used as a dry nitrogen source because it was simple to handle, had a high nitrogen content, and was inexpensive.

Soil sampling and analysis

Using the EuroEA 3000 series CHNS and Kjeldahl equations, total nitrogen was calculated (Bremner 1996). (Combustion or dry oxidation). In order to create NH_4^+ from organic N, it is necessary to digest it using H_2SO_4 and catalysts (such as and selenium (Se) copper (Cu) that speed up the conversion and help withstand high temperatures throughout the digestion process. After distillation, the digestion extract is treated with a potent NaOH solution to produce a vapour that separates the NH_4^+ ions. The ammonia that is produced is transported by water vapour, and it is then gathered with the detection substance in a solution of boric acid. H_2SO_4 standardization during a retro titration of borates. The concentration of N- NH_4^+ in the sample determines how much acid is used in the titration. Using a soil sample analyzer from the EuroEA 3000 series and a dry combustion method, total nitrogen was determined (0.5-1.5 mg). Acetanilide served as a guide in this instance. The device and function of the EuroEA 3000 series CHNS are covered in great detail by Jimenez and Ladho (1993).

Statistical Analysis

Miller and Miller (1993) compared the total nitrogen content derived from the two procedures using linear regression ($Y = b_0 + b_1X$) as the statistical approach. Both the null hypothesis intercept (b_0) and declivity (b_1) were identical. Confidence intervals at a 95% level for both coefficients were calculated to test these hypotheses. The statistical study was carried out using the data analysis feature in Microsoft Excel 97.

Results And Discussion

Table 1 shown the total N values that were determined using both techniques, with only minor differences between treatments. The Kjeldahl method had a N value of 1.61 g/kg, the highest N value, and a N value of 0.80 g/kg, the lowest. While the CHNS EuroEA 3000 series analyzer's values for total nitrogen varying from 0.92 g/kg to 1.98 g/kg, with the 90 kg/ha/yr N treatment providing the maximum value.

When the sample contained significant nitrate-nitrogen, researchers (Jimenez *et al.,* 1983; McGeehan *et al.,* 1988; Schmitter *et al.,* 1989; Smith 1991) found that the Kjeldahl method did not measure as high a N concentration as the Dumas method (CHNS Analyzer). To evaluate the performance of the two instruments on samples with high nitrate-nitrogen concentrations, plant tissue samples and soil samples with nitrate-nitrogen concentrations equal to or greater than 0.40% were removed from the plant tissue population.

Table 1: Values of soil total nitrogen calculated using the Kjeldahl and EuroEA 3000 series CHNS methods at the horticulture field on the BHU campus in Varanasi.

S. No.	*N Treatment (kg/ha/yr)*	*N Kjeldahl (g/kg)*	*N EuroEA 3000 series CHNS (g/kg)*
1	0	0.8054025	0.92275
2	30	1.0855425	1.16225
3	60	1.26063	1.3425
4	90	1.610805	1.9775
5	120	1.330665	1.6775
6	150	1.26063	1.4825

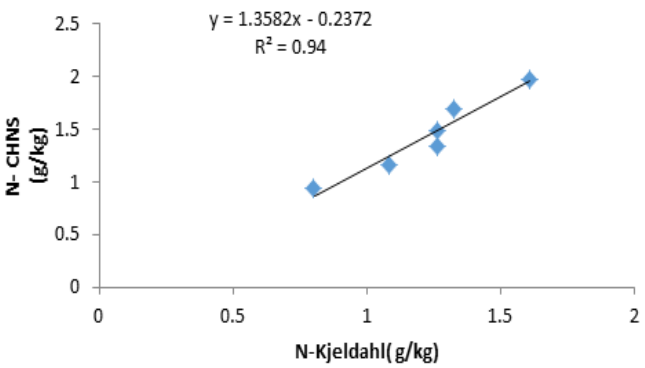

Fig.1: Correlation between total N values determined by the EuroEA 3000 series CHNS and Kjeldahl techniques of soil from horticulture field, BHU campus Varanasi

Table 2: For calculating total N from the two methods, Miller and Miller (1993) proposed a linear regression intercept and slope ($Y = b_0 + b_1 X$).

Coefficients	*Minimum*	*Intermediate*	*Maximum*
Linear	-0.81	-0.24 (b_0)	0.34
Angular	0.90	1.36 (b_1)	1.82

Determination of total N by using these two approaches very strongly related to each other (R^2= 0.94). (Fig. 1). The correlation between the total N values determined using the EuroEA 3000 series CHNS method and the kjeldahl method are described by the equation: EuroEA 3000 series CHNS = 1.3582 *(Total N Kjeldahl) - 0.2372. Results obtained from the Kjeldahl technique and the dry oxidation methods were statistically equivalent (CHNS method). As a result, in standard total N analyses, thefirst option may be preferred. Simple linear correlation analysis and the test of significance of the coefficients were performed on nitrogen data for data comparison purposes in accordance with the statistical procedures described by Steel and Torrie (1960). For each analysis comparison, the simple linear correlation coefficient (r) and regression equation of the Macro-N data on the KjelFoss data are provided. Table 2 coefficient values show that regression analysis is used to determine whether the different approaches to calculating total N are equivalent.

The linear coefficient for the intercept average value (b_0) ranged from -0.81 to 0.34, while the angular coefficient (b_1) remained within the range of 0.90 to 1.82. Both methods are equivalent because these differences were not statistically significant.

Conclusion

The results of the evaluated methods were statistically compatible, and because of its effectiveness and simplicity, the kjeldahl technique may be selected for regular total N measurement even in soils with high amounts of organic matter.

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Author's Contribution

Manuscript written and experimental design by Vijay Pratap Gautam ; Data analysis by Swati Mishra and Experimental design and plagiarism check by Dr. Haseen Ahmed.

Conflict Of Interest

None

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