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Determination of NH_3 Evolved from Foliar Parts of Crop Plants

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Abstract
A substantial portion of fertilizer N is lost from foliar parts of plants. However, the data on this aspect of gaseous N loss is sketchy due to the limitations existing with the methodologies. A simple and economical method was devised and standardized for studying such type of N losses. The results demonstrated the suitability of the method for the quantification of oxidized and reduced N gases evolved/absorbed from foliar parts of the plants especially the plants with low leaf area.

Keywords: Gaseous N loss, Ammonia, crop plants, Semi-open perspex chamber

Introduction

A significant portion of the fertilizer nitrogen (N) applied to crops is lost from soil-plant system through NH_3 volatilization, denitrification and NO leaching etc. (Allison, 1966, Azam *et al*., 1992, Azam *et al*., 1993, Daigger *et al*., 1976). In addition to these loss mechanisms, substantial quantities of N are also lost from plant foliage both during active growth as well as senescence (Harper *et al*. 1987, Harper *et al*., 1996, Parton *et al*., 1988, Schjorring *et al*., 1989, Wetselaar and Farquhar, 1980). The extent of gaseous N loss assessed either by collecting the N gases evolved from plant leaves enclosed in a polyethylene bag (Stutte and Weiland, 1978, Weiland and Omholt, 1985) or a perspex cylinder (Da Silva and Stutte, 1981) and enclosing the whole plants into sealed chambers (O'Deen and Porter, 1986, Parton *et al*., 1988) as well as by micrometeorological methods (Harper *et al*., 1987, Schjorring *et al*., 1989). However, reliable data on the extent of foliar N loss from plant tops is inadequate due to the limitations existing with the methodologies.

The methods used to detect microquantities of NH_3 evolved by plant foliage or in the atmosphere include: flow injection, ion chromatographic, differential pulse polarography and chemiluminescent or coulometric method. However, all these methods are tedious and shphisticated. Of these the one colorimeteric method used by Kempers (1974) for the determination of NH_4^+-N in soil extracts is relatively simple and inexpensive. One drawback of the method, however, is that it is sensitive to changes in pH. Therefore, it can not be applied for determination of NH trapped in acidic solutions. The objectives of our studies were i) to devise and standardize a simple economical method for determination of NH_3 and other N gases evolved from foliar parts of the plants, and, ii) to modify a colorimetric method, to suite the determination of micro-quantities of NH evolved from plant tops, especially the plants with low leaf area.

Materials and Methods

a) Reagents

I) Phenol-nitroprusside

Dissolve 7 g phenol and 0.034 sodium-nitroprusside (di-sodium-penta-cyano nitrosylferrate) in 80 ml of deionized water in a volumetric flask. Mix well and dilute the contents to 100 ml. Store at refrigerated temperature in an amber colour bottle.

2) Hypochlorite solution

Dissolve 1.48 g NaOH in about 70 ml of deionized water. Add 4.98 g of $NaHPO_4$ and 10 mL commercial hypochlorite. The hypochlorite must be of almost 10% chlorine content, otherwise adjust the volume according to the chlorine content. Adjust the pH of the reagent between 9.9-12.1 by increasing or decreasing the quantity of NaOH. Mix well and dilute to 100 ml. This solution should be prepared just before use.

3) Standard solution of $(NH_4)_2SO_4$ (5 ug NH_4^+-N ml^{-1})
4) 1*M* NaOH
5) 0.1*M* and 0.5M H_2SO_4

b) Modification and standardization of NH_3 determination method

Kempers (1974) described a colorimetric method for determination of microquantities of NH_4^+-N in the soil samples. In that method an aliquot of soil extract or digested soil sample was distilled into phenol nitroprusside reagent followed by addition of alkaline hypochlorite. The extinction of the color complex developed by the reaction of NH_3 and phenol nitroprusside reagent was determined on spectrophotometer. This method have the limitations of being sensitive to pH and interference by divalent and trivalent cations. As the method works at pH range 9.9 to 12.1 and NH_3 evolved from plant foliage was trapped in acids, the method was modified to prevent the drop in pH of the reagents when treated with these acidic solutions.

A standard solution of $(NH_4)_2SO_4$(5 ug NH_4^+-N ml^{-1}) was prepared either in 0.1M or in 0.5M H_2SO_4. Five ml of the solution from either case was treated with 2 ml of Phenol nitroprusside reagent and 4 ml of buffered hypochlorite reagent and the volume was adjusted to 25 ml with deionized water. After one hour colour stability period the absorption of the colour complex was taken at 636 nm using Shimadzu UV-120-02 spectrophotometer and 10 mm optical cell.

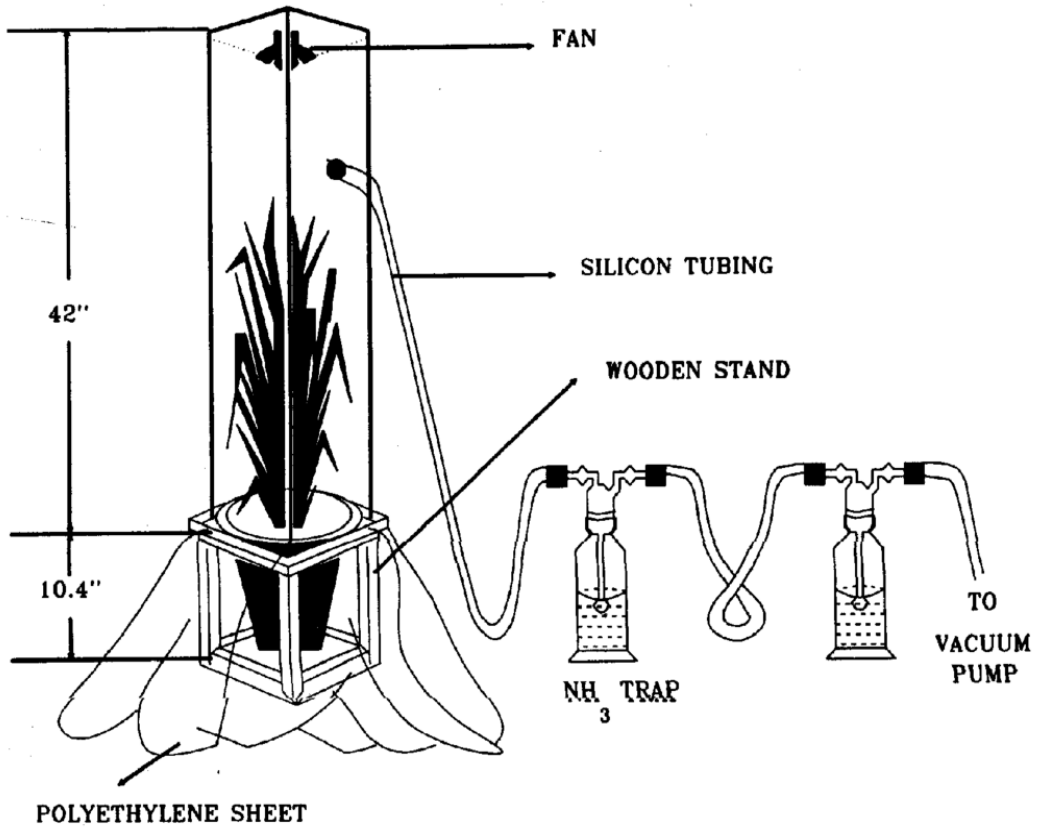

Figure 1. Setup ofsemi-open perspex chamber system for gaseous N exchange studies from foliar parts of plants

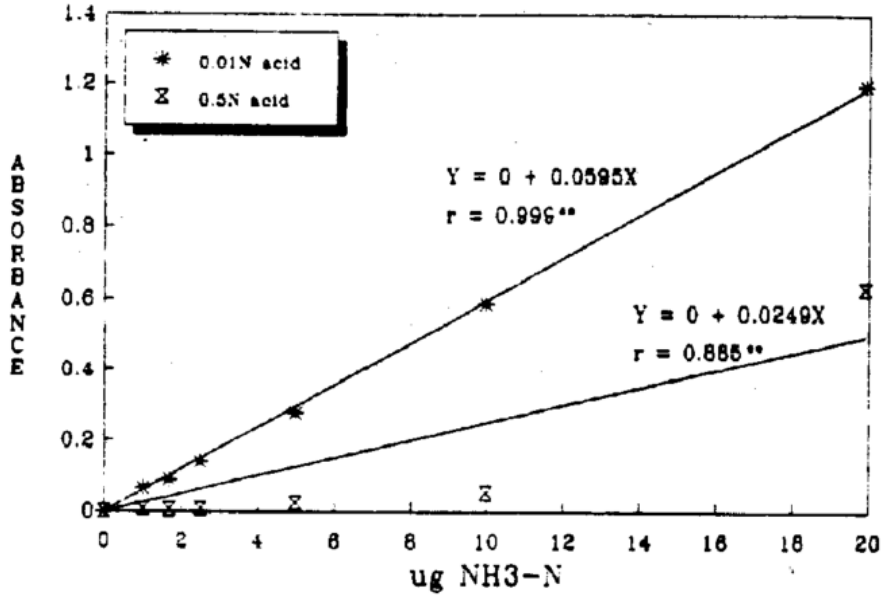

Fig. 2: A comparison of two standard curves generated for determination of NH_3-N using a standard solution of $(NH_4)_2SO_4$ in 0.01 and 0.5 N H_2SO_4

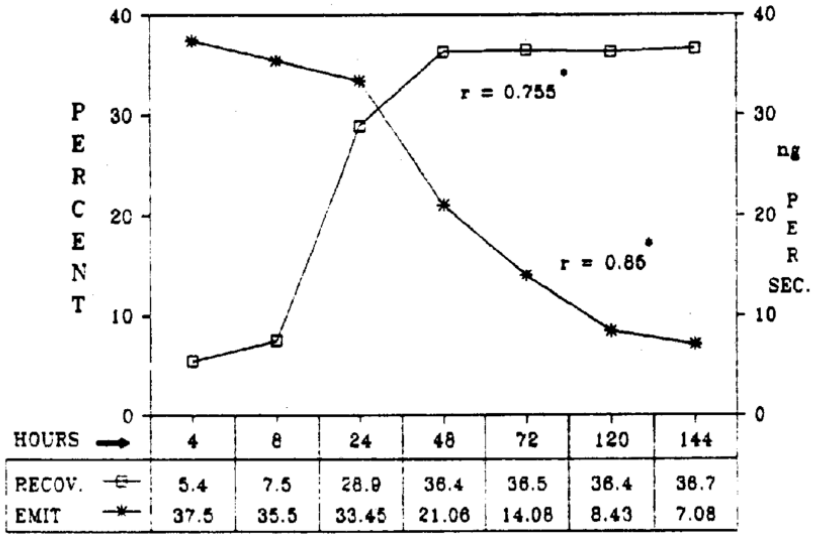

Fig. 3: Percent recovery and per unit time NH_3-N emission from semi-open perspex chamber system

c) Fabrication and standardization of plant canopies for gaseous N exchange studies

Leak proof and air tight perspex cuvettes (Fig. 1) of height x length x width of 42 x 9 x 9 inch (55.7 litre capacity) were fabricated in the workshop of the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. One end of these rectangular Perspex chambers was open and these were provided with two nozzles in one of the side walls. The top of the cuvette was provided with a fan for mixing the inside air. The temperature and humidity of the chamber was determined by placing a thermometer and a hygrometer (Zeal, UK) at the base of this perspex chamber.

At commencement of the experiment one nozzle was closed while the other was attached via silicon tubing (164 cm) to a sampling trail comprising ammonia traps. These ammonia traps consisting of two gas wash bottles (250 ml capacity), containing 100 ml of 0.5M H SO were separated by an empty gas wash bottle to avoid the overflow of the solution. The ammonia sampling trail was ultimately connected to a vacuum pump through an air flow meter for measuring volume of the air passing through the traps.

For standardization purposes the cuvettes in triplicate were placed on the floor in an inverted position i.e. the open end directing upward. The open end of the chamber was sealed with a piece of polyethylene sheet. Before sealing, 100 ml of 1M NaOH was taken into these chambers. Ten ml of a standard solution of $(NH_4)_2SO_4$(1 mg NH_4^+-N ml^{-1}) was then injected for generation of NH_3 in cuvettes. To ensure the completion of NH_3 generating reaction the cuvettes were kept over night at room temperature. On next day alongwith a blank the cuvettes were fixed onto cuboid wooden stands (26.5 cm^2, Fig. 1) by extending the sealing polyethylene sheet two to three feet on the floor and the surrounding area. One of the two nozzles of the chambers was attached to an ammonia sampling trail through silicon tubing as described above. After positioning on the stands and start of the suction of enclosed air at the rate of 4.5 litre/min. these chambers were unsealed to get a semi-open perspex chamber system. The trapping solution was sampled at different time intervals and the ammonia content of samples and residual solution was determined by a modified method as described above.

Results and Discussion

The standard curve generated by modified method of Kempers (1974) showed a correlation of 0.999 and 0.885 between NH concentration and absorbance at 0.01M and 0.5M acid, respectively (Fig. 2). Although both the correlations were highly significant (P = 0.01), the standard curve drawn for 0.01M showed linear curve, while for that of 0.5M it was curvilinear (Fig. 2).

The original method described by Kempers (1974) for the determination of sub-microquantities NH_4^+-N in soils relied on the distillation of soil extracts into nitroprusside reagent and reacting the resulting solution with a buffered hypochlorite solution. The optimal colour development in that case was obtained in a pH range of 9.5-12.1. Below this range the method was insensitive and gave erroneous results. Nevertheless, the distillation of soil extracts into one of the reagents did not cause a decline in pH, therefore the method worked well. However, in gaseous N exchange studies the NH_3 entrapment was made into 0.5M H_2SO_4, considered to be better than other NH_3 trapping agents (O'Halloran, 1992). In the present studies when this trapping solution was treated with the prescribed reagents, a drop in pH occurred which gave erroneous results for NH_4^+-N determination. The dilution of this acid to 0.01*M* however, maintained the desired pH range, therefore, this modified method worked

well for determination of 0.005 ppm to 20 ppm of NH_3 in the atmosphere. A good correlation between different concentrations of a standard solution of $(NH_4)_2SO_4$ and the obsorption spectra as well as linear curve confirmed the suitability of the method.

Fig. 3 presents the date on the recovery and per unit time emission of the NH_3^+-N generated in the canopy. Percent NH_3^+-N recovery and per unit time emission showed a correlation of 0.755 and 0.85 (P = 0.05), with the sampling intervals, respectively. The results also exhibited the maximum per unit time NH_3 emission from the cuvettes during first four hours and a steady decline onward until the termination of the experiment.

The percent N recovery of the NH_3-N generated in the canopy during first 8 hours was very low but after that period it increased and maximized at 48 hours of sampling period. After 48 hours the percent recovery attained a steady state which prevailed until the termination of the experiment. The cumulative data showed the system was 36.5% efficient for gaseous N exchange studies. Efficiency was comparable to that reported by other workers (Parton *et al*. 1988) using more sophisticated and costly setups for gaseous N exchange studies.

As the N metabolic activities of younger and older leaves very from each other, the N metabolism of the entire plant may be a heterogenous mixture of N assimilating and dissimilating activities. Therefore, the results of the methods based on the entrapment of gaseous N evolved from single or multiple leaves encased in a polyethylene bag (Stutte and Weiland, 1978, Weiland and Omholt, 1985) or a cylindrical perspex chamber (Da Silva and Stutte, 1981) could not be a representative of the whole plants. Others (O'Deen and Porter, 1986, Parton *et al*., 1988) following complete enclosure of plants in sealed growth chambers and sampling air into different traps before passing over the plants are also expected to extrapolate the results. The entrapment of native N gases of air before passing over the plants affects the concentration these N gases in the atmosphere which may result in lowering of compensation points of the N gases (i.e., the concentration of N gases in the atmosphere at which no net exchange of these N gases between plants and the atmosphere takes place). Moreover, costly equipment requirements and the limitations to grow plants for longer periods in these sealed chambers make such methods relatively impracticable. Some meteorological methods (Harper *et al*., 1996, Schjorring *et al*., 1989) are considered good for assessment of gaseous N exchange. However, these also have the demerits of including a wide range of background atmosphere of heterogenous origin. The N gases evolved from the plants can not be differentiated from the N gases evolved from soil. The semi-open perspex chamber system described here is low cost, feasible, easy to handle and efficient enough for studies on gaseous N exchange of whole plants.

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