SOME FACTORS INFLUENCING STUDIES ON NITROGEN FLUCTUATIONS IN SOIL PLOTS.

By A. SREENIVASAN, *M.A., D.Sc., A.I.C. and* V. SUBRAHMANYAN, *D.Sc., F.I.C., Department of Biochemistry, Indian Institute of Science, Bangalore.*

(*Read at Symposium, August 29-30, 1936.*)

Fluctuations in the total nitrogen, ammonia, and nitrate contents of arable land have been reported by a number of workers (Leather, 1911 ; Russell, 1914 ; Russell and Appleyard, 1917 ; Waynick, 1918 ; Waynick and Sharp, 1918 ; Lemmermann and Wichers, 1920 ; Thornton and associates, 1927, 1930 ; Clarke, 1922 ; Annett *et al.*, 1928 ; Smith, 1928; Prescott and Piper, 1930 ; Batham and Nigam, 1930 ; Penman and Rountree, 1932; Bartholomew, 1932 ; Sreenivasan and Subrahmanyan, 1934 ; Wilshaw, 1934 and others). A few workers (Prescott and Piper, 1914, 1930 ; Annett *et al., loc. cit.* ; Batham and Nigam, *loc. cit.* ; Sahasrabuddhe and Co-workers, 1927, 1931, 1932, 1936 ; Wilshaw, *loc. cit.* and others) have noted that such variations depend on season, temperature, rainfall, moisture content of soil and other factors. Definite recuperation of nitrogen in the soils has also been shown to proceed under favourable conditions of moisture, temperature, aeration, and manuring (Wilsdon and Ali, 1922 ; Annett *et al., loc. cit.* ; Sahasrabuddhe and Co-workers, *loc. cit.* ; Desai, 1933 ; Garlach, 1934 ; Vandecaveye and Villanueva, 1934, Wilshaw, *loc. cit.* ; and others).

The magnitude of experimental error attaching to investigations of this kind is a function of two probable errors, namely, (1) the laboratory error, i.e. the error of analytical determination, and (2) the field error, which is the error due to the normal variation in the composition of the soil from point to point in the field. The former may also include the error of sampling from the laboratory sample. In the present communication it is proposed to examine the significance of these two factors in regard to the nature and extent of the changes in soil nitrogen.

THE ERROR IN ANALYTICAL METHODS OF NITROGEN DETERMINATION.

Total nitrogen in soils is usually determined by the Kjeldhal method. The application of this method to soils has generally been considered to be reliable until Bal (1925) showed that certain types of soils—such as those of the black cotton areas of the Central Provinces in India—offer considerable resistance to acid digestion and yield invariably low and inconsistent estimates of total nitrogen. He showed that this was due to inadequate penetration of the soil by concentrated sulphuric acid and recommended pre-treatment with water to overcome this difficulty. Higher and more consistent estimates of nitrogen were thus obtained. The significance of this important observation was not first realised, due probably to the impression that it related only to a particular type of soil. The later observations of Sreenivasan (1932) showed, however, that it applied to other types of soils as well. 'Dry' digestion generally yielded low estimates of nitrogen, whereas pre-treatment with water or, preferably, dilute acid led to smoother digestion and to higher and more consistent values being obtained. Thus, in Table I are recorded the values for total nitrogen obtained by the 'Dry' and 'Wet' methods for a number of Indian soils (Sreenivasan and Subrahmanyan, 1933).

TABLE I

Nitrogen contents of some tropical soils as determined by 'Wet' and 'Dry' methods.

Soil from	Description	Nitrogen as p.p.m. (averages)		Difference ('Wet'-'Dry') per cent. 'Wet'=100
		'Dry'	'Wet'	
Kottayam ..	Red gravel-surface ..	978	972	−(0·7)
Cuttack ..	Low land-surface ..	754	768	+1·8
Kandy-Ceylon ..	Laterite-tea soil ..	1,437	1,471	+2·3
Jaffna-Ceylon ..	Sandy-surface ..	313	322	+2·8
Dacca ..	High land-surface ..	820	843	+2·8
Kottayam ..	Sandy-wet cultivation ..	433	442	+2·0
Travancore ..	Sandyloam-surface ..	140	144	+3·1
Tellicherry ..	Red Sandyloam-surface ..	679	692	+1·9
Bangalore ..	Lateritic-surface ..	211	220	+4·1
Do. ..	Red loam-surface ..	536	566	+5·3
Punjab ..	Rice land-surface ..	944	988	+4·5
Nasik ..	Dry land-surface ..	856	908	+5·7
Bangalore ..	Light-surface ..	901	934	+3·5
S. Bihar ..	Alluvial-surface ..	440	470	+6·6
N. Bihar ..	Calcareous ..	389	419	+7·4
Tindivanam ..	Loam-alkaline-surface ..	175	191	+8·2
Tellicherry ..	Heavy clay-surface ..	967	1,049	+7·8
Sholapur ..	Light clay-surface ..	297	339	+12·4
Mandalay ..	Rice land-surface ..	472	544	+13·2
Nandyal ..	Red clay-subsoil ..	273	313	+12·8
Do. ..	Do. surface ..	248	304	+18·3
Nagpur ..	Heavy black-surface ..	648	746	+13·1
Sholapur ..	Medium black-subsoil ..	326	395	+17·5
Do. ..	Heavy black-subsoil ..	310	411	+24.4
Indore ..	Black cotton-surface ..	395	533	+25·9

While invariably low and discordant values for nitrogen are obtained by the official 'dry' method, the inaccuracy of the estimate obtained as above is sometimes as high as 26 per cent.

The advantages of pre-treatment with water are being recognised by other workers as well (Martin and Griffith, 1935; Walkley, 1935; Ashton, 1936). Walkley (1935) has also recorded instances of certain English (heavy alkaline) soils which give highly inaccurate results by the usual 'dry' method.

Similar errors are introduced in following the course of nitrogen transformations in the soil as may be seen from the following experimental results. Soil (100 g. lots) was treated with dried blood (corresponding to 50 mg. N). In one set of experiments, the specimens were moistened with water and maintained at 60 per cent saturation, while the others were treated with enough water to maintain the mixtures in a water-logged condition. At stated intervals, samples were removed and the total nitrogen determined both by the official 'dry' method and after pre-treatment with water (Table II).

TABLE II

Transformations of nitrogen in soils.

Treatment	Method of digestion	Total nitrogen in parts per million (averages).							
		Time of days							Standard error
		0	8	15	22	29	57	78	
Maintained at 60 per cent saturation	'Dry'	711	689	669	655	645	617	595	±0·2
	'Wet'	730	724	700	689	677	659	626	Small
Water-logged	'Dry'	711	675	636	589	541	464	422	±1·2
	'Wet'	730	714	677	626	589	523	489	Small

The difference between the values obtained by the two methods becomes increasingly prominent with time. This is strikingly so in the case of the water-logged specimens in which the loss of nitrogen on the 78th day is apparently 16 per cent more according to the 'dry' method than by the 'wet' one. The results thus show that the present official methods are not reliable for the accurate study of nitrogen transformations in soils.

It has been observed by the present authors that even in finely ground and apparently homogeneous specimens of soils, such as are used for analytical work in the laboratory, the distribution of nitrogen is not uniform, so that it is not possible to obtain concordant estimates unless the entire specimen is mixed up repeatedly and carefully resampled from time to time. It is therefore to be recognised that the variability of the total nitrogen content of soils will not be great unless the method of determining total nitrogen is accurate and reliable. The present official 'dry' method is not sufficiently accurate to cause appreciable variability among a huge number of samples taken from a small area, and, as emphasised by Bal (1927), 'the important factor of the method of nitrogen determination has to be taken into consideration while formulating any conclusions regarding the work on nitrogen fixation in soils in general and heavy soils in particular'.

THE ERROR IN FIELD SAMPLING.

The total nitrogen contents of different plots (11′×11′)—apparently uniform—and manured with farmyard manure at 22 lbs. per plot were examined by the authors, from time to time, for a period of over 5 months during the life of a crop of ragi (*Eleucine coracana*) raised on the plots. A number of samples were collected from each plot to a depth of 6″ and the different samples thus collected from each plot, at one time, were mixed together and treated as a whole specimen. They were then examined for their total nitrogen contents according to the method of Sreenivasan (1932) (Table III).

TABLE III.

Nitrogen distribution in soil plots.

Plot	Date of sampling						Mean and standard deviation
	18th Sep.	3rd Oct.	1st Nov.	1st Dec.	5th Jan.	4th Feb.	
	Nitrogen as p.p.m. (averages).						
A	604	553	610	613	607	585	595 ± 21
B	692	515	572	553	567	547	574 ± 56
C	552	590	560	558	570	620	575 ± 24
D	605	610	534	673	656	525	600 ± 56
E	488	483	350	542	674	645	530 ± 108
F	539	539	612	617	548	525	563 ± 37
G	582	550	..	645	609	647	606 ± 37
H	504	656	658	528	564	558	578 ± 59
Mean and Std. deviation ..	571	562	557	591	599	581	
±	61	51	92	50	43	47	

The most striking observation to be noticed from the above table is the great variation in the nitrogen content existing between the various samples within each plot from time to time, and also in the different plots at the same time. Thus, taking the samples collected on 1st November, the coefficient of variability is sixteen, which means that over five-sixths of the total determinations may be expected to lie within the range on either side of the mean. In other words, the range within which five-sixth of the determinations may be expected to fall is 464 to 649 p.p.m. in this case. The extreme range would of course be much greater. In a like manner, the variations between the nitrogen figures of the same plot at different intervals of time also appears to be considerable. Thus with plot E, the mean is 530 and the standard deviation 108, *i.e.*, the coefficient of variability (C.V.) is about a fifth part.

Similar results were obtained by the authors in regard to ammonia, nitrate and organic matter contents of manured as well as unmanured plots.

From a consideration of the analytical error involved in the study of nitrogen balance in the soil, Pfeiffer and Blanck (1913) have concluded that the non-homogeneous character of the soil is itself a serious factor which would cause large differences between duplicate determinations or even successive samples. Robinson and Lloyd (1915), Waynick (1918), Prince (1923), Bear and McClure (1920) and others have also drawn attention to the existence of normal variations in the composition of the soil from point to point in the field.

It would appear, therefore, that random specimens from a field or even a uniform plot of land would not give concordant results and that a high sampling error may be expected. The values obtained for either independent specimens from the same area or for mixtures representing collections from different spots would exhibit a variation which might easily be mistaken for periodic fluctuations in the land itself.

Furthermore, the major part of the nitrogen of the soil is in organic form, being mostly derived from either added manures or plant residues decomposing in it. Since it is not possible in ordinary field practice to distribute the organic matter so evenly as to facilitate accurate sampling for analytical work, it would follow that although the total amounts of nitrogen spread over large areas may be the same, the quantities present in small samples collected as representative specimens may vary considerably. Additional error is also introduced by cattle grazing or sheep-folding during off-seasons, thus resulting in the droppings being unevenly distributed over the surface of the soil. In assessing the influence of season on processes like nitrogen fixation the effects of different external factors on the movement of different soil constituents would also require careful consideration. Thus, it is well known that during periods of fallow, a large part of the nitrogen, particularly the soluble forms, moves into the sub-soil and does not become available until the planting of the subsequent crop. During the rainy season, the finer fractions containing the highest percentage of fertilising ingredients would be washed from the surface of a sloping or uneven soil and carried to the lower regions exposing only the less rich, coarse fractions at the surface. Even on the same plot of land considerable variations in the distribution of different soil constituents can be brought about by washing or silting as the case may be.

It is generally believed that among the nitrogenous constituents of the soil, only the nitrates are capable of periodic movement from one stratum to another. In the ordinary soil, nitrates are present only in small quantities so that their movements may not lead to any considerable change in the total nitrogen content. There is evidence to show, however, that certain forms of organic nitrogen are also capable of movement through the soil. Soil albuminoids are generally insoluble in water, but they are partly peptised by dilute salt solutions. All soils contain soluble salts (though to varying extents), so that when rain or irrigation water soaks in, it dissolves the salts which, in turn, peptise a portion of the albuminoids. This action is no doubt helped by the reaction of the soil. In soils of pH 7·5 or more, the peptising action will be more pronounced than in those of pH 7. Acidity of the soil (pH 6·5 and below) may also help to facilitate the peptisation of certain forms of nitrogen. In this manner, there may be steady movement of organic nitrogen from one stratum to another in the soil.

The quantity of organic nitrogen peptised at any one time may be comparatively small ; but repeated action, as may be naturally expected during the months of heavy and continuous rainfall, may carry down a considerable portion of the organic nitrogen to the lower strata of the soil and, if the soil conditions permit, even shift it from one locality to another. When the dry weather comes round, the saline extract, together with the albuminoids, will be carried up to the surface layers through capillary action. This would suggest that during the post-monsoon period, and especially the Spring months, the surface layers of the soil will show an appreciable gain in nitrogen. In a like manner, the nitrogen content of the surface layers of a heavily irrigated field will show an appreciable increase as soon as the watering is stopped. This is actually what happens when the crop approaches the ripening stage and the reported increase in nitrogen at the time of and shortly after harvest (and which is attributed by some workers to a possible flow-back of nitrogenous constituents from the plant) may be accounted for by this phenomenon.

The foregoing explanation may account for the apparent discrepancy between laboratory observations on the one hand and field results on the other. The laboratory experiments are generally carried out in glass or Chinaware so that the peptised nitrogen, if any, has no chance to move out of the system. There is, in consequence, no appreciable change in the total nitrogen content of the laboratory sample. On the other hand, the field samples permit of movement of nitrogen so that on taking the samples from the usual surface (0-3′) layers, one may find apparent loss of nitrogen in certain seasons and gain in others.

Further work on this aspect of the problem is already in progress and it is hoped that the results will throw some fresh light on the mechanism of periodic fluctuations in the nitrogen contents of field samples.

Summary

Studies on periodic changes in nitrogen contents of soils are complicated by two factors, namely the error due to analytical methods and the error due to soil heterogeneity. The significance of these two factors as affecting the interpretation of results obtained from field data are fully considered. The former can be overcome by adopting one of the improved methods of estimating total nitrogen. The error due to field experimentation can be overcome by repeating the experiment over long periods, by multiplying the number of plots in a given field, by examination of a sufficiently large number of samples and by statistically inquiring into the mathematical error involved in such determinations.

Evidence has been adduced to show that in addition to nitrates (which are present in comparatively small quantities) organic nitrogen present in field samples may move from one stratum to another and, under favourable conditions, even laterally from one area to another. This may at least partly account for the periodic fluctuations observed in the total nitrogen contents of field samples.

REFERENCES.

Annett, H. E., Iyer, A. R. P. and Kayasth, R. N., *Mem. Dept. Agric. India*, Chem. Ser., Vol. **9**, p. 155, (1928).

Ashton, F. L., *Jour. Agric. Sci.*, Vol. **26**, p. 239, (1936).

Bal, D. V., *Jour. Agric. Sci.*, Vol. **15**, p. 454, (1925).

Bal, D. V., *Trans. First Internat. Cong. Soil Sci.*, Vol. III, Comm. III, p. 190, (1927).

Bartholomew, R. P., *Univ. Arkansas Agric. Expt. Sta. Bull.*, No. **270**, (1932).

Batham, H. N. and Nigam, L. S., *Soil Sci.*, Vol. **29**, p. 181, (1930).

Bear, F. E. and McClure, G. M., *Soil Sci.*, Vol. **9**, p. 65, (1920).

Clarke, G., *Agric. Jour. India*, Vol. **17**, p. 463, (1922).

Desai, S. V., *Ind. Jour. Agric. Sci.*, Vol. **3**, p. 301, (1933).

Garlach, M., *Landw. Jahrb.*, Vol. **80**, p. 73, (1934).

Leather, J. W., *Mem. Dept. Agric. India*, Chem. Ser., Vol. **2**, p. 101, (1911).

Lemmermann, O. and Wichers, L., *Centrabl. Bakt.* (II), Vol. **50**, p. 33, (1920)

Martin, W. S. and Griffith, G., *Jour. Soc. Chem. Ind.*, Vol. **54**, p. 234T, (1935).

Prescott, J. A. and Piper, G. R., *Jour. Agric. Sci.*, Vol. **20**, p. 517, (1930).

Penman, F. and Rountree, P., *Jour. Agric. Victoria*, Vol. **30**, p. 496, (1932).

Pfeiffer, Th. and Blanck, E., *Landw. Versuchs-Stat.*, Vol. **78**, p. 367, (1913).

Prince, A. L., *Soil Sci.*, Vol. **15**, p. 395, (1923).

Robinson, G. W. and Lloyd, W. E., *Jour. Agric. Sci.*, Vol. **7**, p. 144, (1915).

Russell, E. J., *Jour. Agric. Sci.*, Vol. **6**, p. 18, (1914).

Russell, E. J. and Appleyard, A., *Jour. Agric. Sci.*, Vol. **8**, p. 385, (1917).

Sahasrabuddhe, D. L. and Daji, J. A., *Mem. Dept. Agric. India*, Chem. Ser., Vol. **8**, p. 53, (1927).

Sahasrabuddhe, D. L. and Ghatikar, B. H., *Ind. Jour. Agric. Sci.*, Vol. **1**, p. 631, (1931).

Sahasrabuddhe, D. L. and Kanitkar, N. V., *Ibid.*, Vol. **2**, p. 455, (1932).

Sahasrabuddhe, D. L. and Abhyankar, V. S., *Ibid.*, Vol. **6**, p. 268, (1936).

Smith, J. B., *Soil Sci.*, Vol. **26**, p. 347, (1928).

Sreenivasan, A., *Ind. Jour. Agric. Sci.*, Vol. **2**, p. 525, (1932).

Sreenivasan, A. and Subrahmanyan, V., *Ibid.*, Vol. **3**, p. 646, (1933).

Sreenivasan, A. and Subrahmanyan, V., *Jour. Ind. Inst. Sci.*, Vol. **17A**, p. 113, (1934).

Thornton, H. G. and Fisher, R. A., *Soil Sci.*, Vol. **23**, p. 253, (1927).

Thornton, H. G. and Gray, P. H. H., *Proc. Roy. Soc.*, Sec. B, Vol. **106**, p. 399, (1930).

Vandecaveye, S. C. and Villaneuva, B. R., *Soil Sci.*, Vol. **38**, p. 204, (1934).

Walkley, A., *Jour. Agric. Sci.*, Vol. **25**, p. 598, (1935).

Waynick, D. D., *Univ. Calif. Publns. Agric. Sci.*, Vol. **3**, p. 243, (1918).

Waynick, D. D. and Sharp, L. T., *Ibid.*, Vol. **4**, p. 121, (1919).

Wilsdon, B. H. and Ali, B., *Soil Sci.*, Vol. **14**, p. 127, (1922).

Wilshaw, R. G. H., *Malayan Agric. Jour.*, Vol. **28**, No. 1, (1934).