AN IMPROVED TECHNIQUE FOR ESTIMATING THE NITRIFYING CAPACITY OF SOILS

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It is now well known that the nitrate-content of the soil fluctuates from time to time depending upon the macro- and micro-climatic conditions. This has been confirmed by various workers like Russell,[9] Olsen,[7] Braun-Blanquet[3] and others. Therefore, though the nitrate-content of the soil does no doubt influence the plants that grow upon it that they are called " Nitrophilous " plants,[2] yet it is evident that we cannot directly correlate the fluctuations in the nitrate-content of the soil with those of the cell sap of the plants growing upon it due to various reasons. These are:

(i) the quick leaching of the nitrates from the soil;

(ii) the bacterial activities of the soil;

(iii) the utilization of nitrates by plants and bacteria; and

(iv) the ærobic and anærobic conditions which might result in nitrification or denitrification of the soil.

Therefore, to measure the influence of the soil nitrates upon the plants, it is best to study the nitrification of the soil and not its nitrate-content. The nitrification process is carried out by the agency of the microorganisms and it can be measured either by their activities or by the products of their activities.

According to Waksman,[11] Olsen[7] and Russell[9], the nitrifying capa y of a soil is studied by allowing it to nitrify in Omeliansky's medium[8] for a certain fixed number of days namely 25 and then measuring quantitatively the product of its nitrification *i.e.*, nitrates formed within that period.

We, therefore, studied the nitrifying capacity of the soils taken from t e root regions of various plants by the above method but instead of estimating only the nitrates as the above workers, we measured all the three products formed namely, ammonia, nitrites and nitrates at short intervals of 2 to 3 days till all the nitrites disappeared from the medium. This was followed according to the suggestion of Basu and Rosario[1] who stated that it is risky

to fix an arbitrary period of incubation because the nitrification in soils varies with the soil types and with the nature of the materials used for the study of nitrification. Our investigations (Table I) have supported this view.

However, Omeliansky's medium was modified by using calcium carbonate instead of magnesium carbonate as a base because according to Warington[12] and our own experiments, in the presence of the former as a base, the soils showed faster nitrification than with the latter.

The actual technique consisted of filling each 250 c.c. Erlenmyer flask with 20 c.c. Omeliansky's medium with 0·4 gram of calcium carbonate. After sterilization, each flask was supplied with 1 c.c. of 5 per cent. ammonium sulphate solution, (= 0·53 mgs. or 530 p.p.m. of nitrogen as ammonia was supplied to each c.c. of the medium) and 0·4 gram of air-dried sieved soil. A set of about 20 flasks containing the same type of soil was kept in a dark chamber.

After every alternate day two flasks containing the same type of nitrified soil were analysed for ammonia, nitrites and nitrates and the average of the two were taken as final. By this method, we not only estimated the different products of nitrification but also measured the rapidity with which the amount of nitrogen given in the form of ammonium sulphate gets transformed to nitrite and nitrate-nitrogen in different soils.

Ammonia was estimated by the qualitative spot test method with Nessler's reagent as recommended by Fiegel[5] and the nitrites were measured quantitatively by Griess-Llosway's colorimetric method.[4] The nitrates were detected by colorimetric phenol-disulphonic acid method as recommended by Harper.[6]

To test our modified method we chose three types of soils of contrasting characters: (i) soil from the roots of *Amarantus spinous* L. which, as shown in our previous paper (2), is the most dominant species of the nitrophilous association of Bombay and which shows also the highest amount of nitrates in its tissues as compared to other plants. The soil from the roots of this plant, therefore, was presumed to show the highest nitrification; (ii) the water-logged soil dominated by *Astercantha longifolia* Nees. which shows no nitrates in its tissues (*ibid*) and presumably it would show the lowest nitrification; (iii) the dry fallow soil covered by *Eleusine indica* Gaertn. which was neither nitrophilous nor water-logged and as such would presumably show nitrification which is intermediate between the above two types.

The following Table shows nitrification results of the three soils:—

TABLE I

Date of Inoculation of Soils: 5-8-1950

Incubation period in days	*Amaranthus spinosus* soil (Fertile)			*Eleusine indica* soil (Fallow)			*Astercantha longifolia* soil (Water-logged)		
	Ammonia	Nitrites in p.p.m.	Nitrates in p.p.m.	Ammonia	Nitrites in p.p.m.	Nitrates in p.p.m.	Ammonia	Nitrites in p.p.m.	Nitrates in p.p.m.
2 days ..	*P	5	†nil	P	nil	nil	P	nil	nil
4 ,, ..	P	38	4	P	5	nil	P	nil	nil
6 ,, ..	P	130	7	P	45	nil	P	36	nil
8 ,, ..	P	182	13	P	68	6	P	75	12
10 ,, ..	P	284	18	P	105	10	P	130	14
12 ,, ..	P	370	18	P	228	15	P	*148*	19
14 ,, ..	P	445	49	P	255	20	nil	110	30
16 ,, ..	nil	*522*	78	P	310	65	nil	56	67
18 ,, ..	nil	248	125	nil	395	74	nil	nil	*78*
21 ,, ..	nil	nil	*185*	nil	460	106	—	—	—
24 ,, ..	‡—	—	—	nil	*500*	125	—	—	—
27 ,, ..	—	—	—	nil	305	150	—	—	—
30 ,, ..	—	—	—	nil	185	155	—	—	—
34 ,, ..	—	—	—	nil	nil	172	—	—	—

* P = Estimated and found present (Spot test).
† Nil = Estimated but found absent.
‡ — = denotes that no estimations were made on that day.

From the above Table and the method described, it will be evident that by estimating all the three products of nitrification at short intervals, an idea of complete nitrification is obtained instead of knowing only its end product, namely the nitrates. By this method, we have not only appreciably curtailed the incubation period but also fixed the period of incubation for each soil. This is done by stopping the experiments in each case on the day the nitrites completely disappear from the medium and measuring the nitrates on that day. Moreover, it has further shown that by the detailed study of the process, the main error of judging the nitrifying capacity by measuring the nitrates after a fixed period of incubation is eliminated, as it could be seen from our results that the period of incubation varies with the soil-types.

Thus, for the soil of *Amarantus spinosus*, the incubation period is 21 days whereas for the fallow soil of *Eleusine indica* it is 34 days. On the contrary, for the water-logged soil of *Astercantha longifolia* it is 18 days only.

From these results it can be seen that the nitrifying capacity of a soil can be judged from the number of days it takes to complete the nitrite cycle. Shorter the period, greater is the capacity to nitrify. In short, the rule may be made that the period of incubation goes in inverse proportion to the nitrifying capacity of a soil; *i.e.*, greater the incubation period lesser is the

nitrifying capacity. However, it must be pointed out that this rule can only apply to *ærobic* soils like those of *Amarantus* and *Eleusine*. To an *anærobic* water-logged soil this rule is not applicable, because according to Shreenivasan,[10] under water-logged soil conditions, the oxygen supply being limited, ammonification proceeds much faster than nitrification, so that ammonia accumulates in the medium and very little of nitrites and nitrates are formed. Also, in such soils, plants absorb nitrogen in the form of ammonia instead of nitrates and hence very little of ammonia gets transformed to nitrates. Due to these reasons, water-logged soils show very poor capacity to nitrify.

Hence, the number of days taken for nitrites to disappear from the medium, *i.e.*, the incubation period, is one criterion of judging the nitrifying capacity of a soil.

To this criterion may be added two additional ones which support the first one. They are the average nitrite and nitrate rise per day. These two factors are important because the amounts of nitrites and nitrates formed and the number of days it takes to attain them vary with the soils.

The average nitrite rise per day is calculated in the following way:

$$\frac{\text{The maximum amounts of nitrites formed}}{\text{Period of incubation}} = \text{Average } NO_2 \text{ per day.}$$

Similarly, the average nitrate rise per day is defined as:

$$\frac{\text{Amounts of nitrates formed}}{\text{Period taken for nitrites to disappear}} = \text{Average } NO_3 \text{ per day.}$$

Working on the above basis with the data from Table I, we obtain the following values for the three soils:—

TABLE II

Soils	Average Nitrite rise per day	Average Nitrate rise per day
From Amarantus spinosus ..	$\frac{522 \text{ p.p.m.}}{16 \text{ days}} = 32{\cdot}6$ p.p.m.	$\frac{185 \text{ p.p.m.}}{21 \text{ days}} = 8{\cdot}8$ p.p.m.
From Eleusine indica ..	$\frac{500 \text{ p.p.m.}}{24 \text{ days}} = 21{\cdot}0$ p.p.m.	$\frac{172 \text{ p.p.m.}}{34 \text{ days}} = 5{\cdot}0$ p.p.m.
From Astercantha longifolia ..	$\frac{148 \text{ p.p.m.}}{12 \text{ days}} = 12{\cdot}4$ p.p.m,	$\frac{78 \text{ p.p.m.}}{18 \text{ days}} = 4{\cdot}3$ p.p.m.

From the results cited in Table II, it is apparent that the soil of *Amarantus spinosus* shows the highest value for these factors whereas the water-logged soil of *Astercantha longifolia* shows the lowest value. But,

the fallow soil of *Eleusine indica* is midway between these two extremes, *i.e.*, shows lower value than *Amarantus* but higher than *Astercantha.*

Hence, it may be concluded that to judge the nitrifying power of a soil, all the three products of nitrification should be estimated at short intervals and the experiment be stopped on the day the nitrites completely disappear from the medium. This will not only curtail the long duration of experimentation but will also show the true picture of nitrification of various soils. Thus by following this method and by the proper use of the three criteria, the "nitrifying capacity" and hence the "fertility index" of any soil could be easily and correctly judged.

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