

NITROPHILY IN RELATION TO NITRIFICATION *)

(with 8 figs. and 11 tables)

by

F. R. BHARUCHA and K. C. SHERIAR

(Botany Department, Institute of Science, Bombay, India)

INTRODUCTION.

It is apparent to all who are familiar with the modern methods of plant sociology that the definition of a plant association is arrived at purely from a statistical study of the vegetation (BRAUN-BLANQUET, 1932). The very fact that this statistical method has been accepted internationally makes it irrefragable. Nevertheless it may be open to criticism from the biochemical point of view, and in 1945 BHARUCHA suggested that a biochemical proof should be brought forth to test the validity of the statistical method of the plant sociologists. An investigation was started in this laboratory on plant associations which are influenced primarily by one 'dominating edaphic' factor, namely, the nitrophilous plant association which is governed by the nitrates of the soil or the calcicolous association which is conditioned by the calcium carbonate of the soil (BHARUCHA, 1949).

During the investigation of the nitrophilous associations of Bombay the suggested biochemical theory was tried by methods based upon the following two premises:

1. that the nitrate-concentration in the cell sap of the characteristic exclusive species of that association must be high; and
2. that their soil must also show a high nitrate-concentration.

Beginning this work with the soil factor it was soon found that the nitrate-content of the soil was not an indication of the nitrate-tolerance of a characteristic species of a plant association (OLSEN, 1921), for the soil nitrates fluctuate according to the local changes. Being very soluble they are easily leached out (RUSSELL, 1915).

The other factor, that of the nitrate-content of the cell-sap, gave some promise of being useful but it also showed variations with local conditions and hence the statistical and the biological data could not be correlated.

BHARUCHA and DUBASH (1951a) suggested therefore that for the measurement of nitrophily of a plant, three factors should be considered, namely, a. the frequency of a species, b. the constancy of nitrates and c. the average nitrate-content. These 3 factors were multiplied to give a resultant value for nitrophily. On the basis of this formula they prepared a list of plants according to their degree of nitrophily from nitrate-positive to nitrate-negative plants.

But, it has been confirmed by various workers (OLSEN, 1921; and BAUER, 1938) that the plants showing nitrates in their tissues can only absorb them from the soil and hence we cannot escape the influence of this factor upon the vegetation. It was therefore suggested (OLSEN, l.c.; BHARUCHA and SHERIAR, 1952) to look for it from another point of view, namely, "the capacity of a soil to nitrify or the rate of nitrification of a soil".

OLSEN (l.c.) in his 'Ecology of *Urtica dioica*' grades *U. dioica* as the 'nitrate-plant', since the rich growth of that plant always coincides with an intensive capacity of nitrification in the soil. BRAUN-BLANQUET (l.c.) also

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regards the *Silybum-Urtica pilulifera* association of the Mediterranean region as nitrophilous. Hence our present investigations were aimed at finding out whether a soil from the root region of a nitrophilous plant shows a higher rate of nitrification than those under non-nitrophilous plants.

We have also attempted to ascertain whether there is any correlation between the statistical (floristic survey) and the chemical (rate of nitrification) factors. On the basis of these factors we have attempted to grade several plants in their order of nitrophily i.e. in terms of the rate of nitrification of their soils.

METHODS.

1. Floristic survey.

The statistical study of the plant associations growing on soils which were either littered with rubbish, or soiled with human and animal excreta (nitrate-high soils) or those growing on fallow, dry or water-logged soils (nitrate-negative soils) were made according to the standard method of BRAUN-BLANQUET and PAVILLARD (1930). A number of relevés were made all over Bombay and, for the purpose of comparison, they were grouped according to the plants which dominated them. We thus have:

1. *Amaranthus spinosus* Stand.
2. *Trianthema monogyna* „
3. *Argemone mexicana* „
4. *Boerhavia diffusa* „
5. *Hibiscus tetraphyllus* „

6. *Sida acuta* „
7. *Eleusine indica* „

8. *Astercantha longifolia* „

From the survey of the nature of the habitat of the relevés it was seen that the first five plants were mostly confined to fouled nitrogen-rich habitats. Out of these, *Amaranthus spinosus* is a characteristic exclusive species of a nitrophilous association whereas the other four plants occurred in similar habitats only in lesser and lesser degree according to their descending order as shown in the previous list.

Sida acuta and *Eleusine indica* are the characteristic species of the fallow land whereas *Astercantha longifolia* grows only under water-logged conditions.

Table No. I (A and B) shows a complete sociological survey of various plant communities.

TABLE I — B.

Showing the nature of the habitat on which the 8 dominant plants grow.

No.	The dominant plants of the associations	Nature of the habitat
1.	<i>Amaranthus spinosus</i>	Soils usually covered with rubbish and human and animal excreta. The sources of nitrates being manifold, they are termed 'Nitrate-high' or 'Nitrate-positive' soils.
2.	<i>Trianthema monogyna</i>	—do—
3.	<i>Argemone mexicana</i>	Soils not as much fouled as in the previous cases but usually covered by cow-dung and other manures.
4.	<i>Boerhavia diffusa</i>	—do—
5.	<i>Hibiscus tetraphyllus</i>	Very rarely occurring with the above mentioned four plants and growing in least-manured places.
6.	<i>Sida acuta</i>	Growing in exposed dry fallow soils usually subjected to trampling. They are regarded as 'Nitrate-low' or 'Nitrate-negative' soils because of least availability of nitrates.
7.	<i>Eleusine indica</i>	—do—
8.	<i>Astercantha longifolia</i>	Dominates the muddy, water-logged, anaerobic soils where plants absorb nitrogen in the form of ammonia and hence they are also called 'Nitrate-negative plants'.

After a thorough floristic survey, the soils from the vicinity of the root systems of the dominant plants were collected, brought to the laboratory in a closed container and tested for their nitrification value.

2. Nitrification of soil.

The nitrifying capacity of a soil was estimated by allowing it to nitrify in OMELIANSKY's (1899) nutrient medium for a definite period of 25 days and the amount of nitrates formed during that period was measured. The larger the amount of nitrates formed in a soil the higher is its nitrifying power (OLSEN, 1921). But in our previous work we have modified the technique of estimating the nitrifying power of a soil (BHARUCHA and SHERIAR, 1952). We have shown experimentally that in order to measure the power of nitrification we must take into consideration various criteria such as the average nitrite and nitrate formation per day, curtailment of the nitrification cycle etc. which vary with the soil-types. We have also shown the importance of the consideration of the nitrite-nitrogen during the process (BHARUCHA, DUBASH and SHERIAR, 1951c) and of the occurrence of the time-lag between the exhaustion of ammonia and the formation of the optimum nitrites (BHARUCHA and SHERIAR, 1951), so that we get a complete idea as to what is happening during the process.

Ammonia in the nitrified solution was estimated by the qualitative spot test method with NESSLER's reagent as recommended by FIEGEL (1939), and nitrites were measured quantitatively by GRIESS-LOSWAY's colorimetric method (CUMMING and KAY, 1939). The nitrates were detected by the colorimetric phenol-disulphonic acid method as recommended by HARPER (1914).

TABLE II.

Showing alternate day nitrification values (with their graphs) of soils from 8 dominant plants from varied habitats.

TABLE II — A.
Amaranthus spinosus Soil.
(Relevé No. 16)

Incubation period in days	Ammonia (by Spot Test)	Nitrogen as Nitrites in p.p.m.	Nitrogen as Nitrates in p.p.m.
2	* P	10	Nil
4	P	42	Nil
6	P	112	12
8	P	164	30
10	P	260	35
12	P	390	45
14	P	495	67
16	± Nil	530	89
18	Nil	327	126
19	Nil	165	+ —
20	Nil	Nil	180

* 'P' denotes estimated & found present.
± 'Nil' denotes estimated but found absent.
+ '—' denotes that no estimations were made on that day.

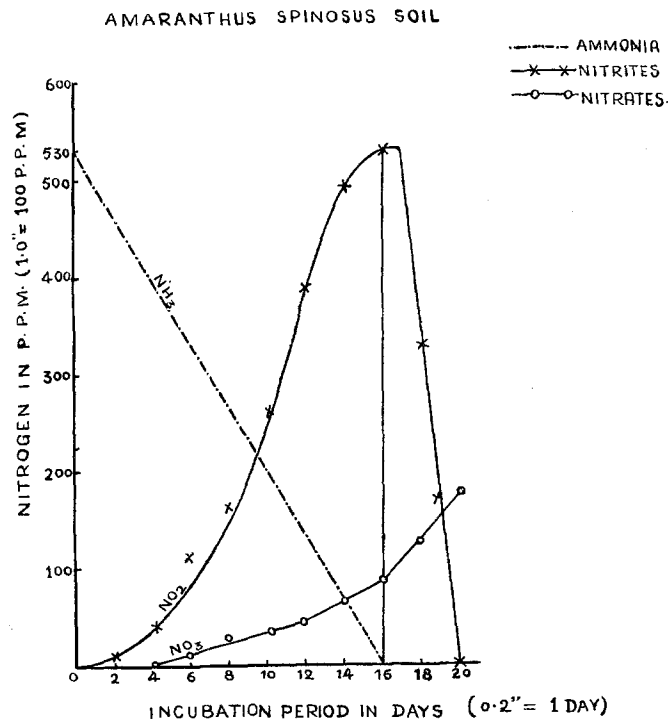


Fig. 1.

TABLE II — B.
Trianthema monogyna Soil.
 (Relevé No. 26)

Incubation period in days	Ammonia (by Spot Test)	Nitrogen as Nitrites in p.p.m.	Nitrogen as Nitrates in p.p.m.
2	P	15	Nil
4	P	50	Nil
6	P	95	Nil
8	P	136	9
10	P	260	25
12	P	395	48
14	P	500	65
16	Nil	520	70
18	Nil	405	75
20	Nil	165	120
21	Nil	60	—
22	Nil	Nil	178

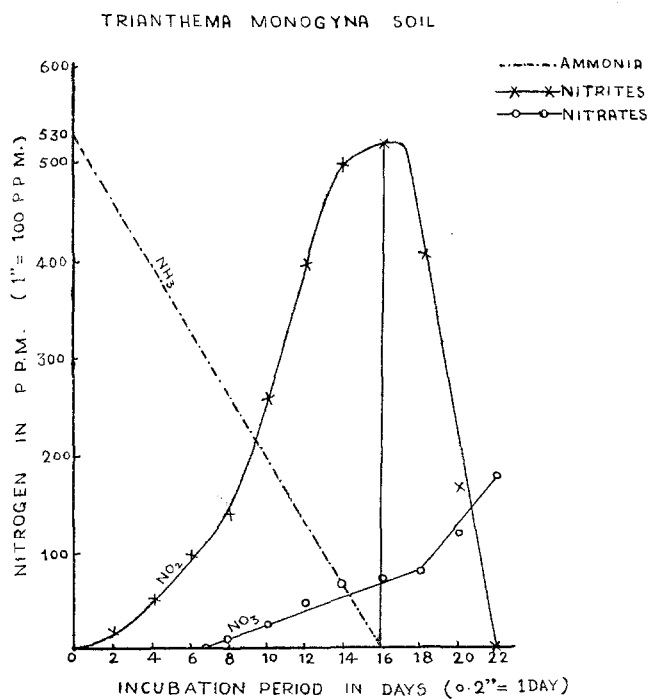


Fig. 2.

TABLE II — C.
Argemone mexicana Soil.
 (Relevé No. 5)

Incubation period in days	Ammonia (by Spot Test)	Nitrogen as Nitrites in p.p.m.	Nitrogen as Nitrates in p.p.m.
2	P	Nil	Nil
4	P	25	Nil
6	P	60	5
8	P	120	12
10	P	178	15
12	P	300	25
14	P	440	30
16	P	460	40
18	Nil	490	72
20	Nil	320	102
22	Nil	90	155
23	Nil	30	—
24	Nil	Nil	180

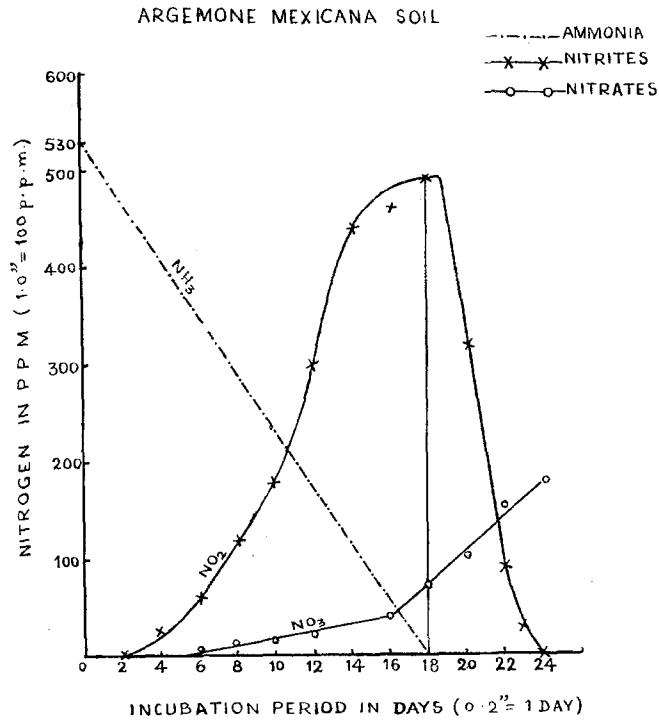


Fig. 3.

TABLE II — D.
Boerhavia diffusa Soil.
 (Relevé No. 11)

Incubation period in days	Ammonia (by Spot Test)	Nitrogen as Nitrites in p.p.m.	Nitrogen as Nitrates in p.p.m.
2	P	Nil	Nil
4	P	20	Nil
6	P	58	Nil
8	P	100	Nil
10	P	140	Nil
12	P	250	5
14	P	338	12
16	P	405	15
18	Nil	460	25
20	Nil	310	70
22	Nil	155	135
23	Nil	25	—
24	Nil	Nil	160

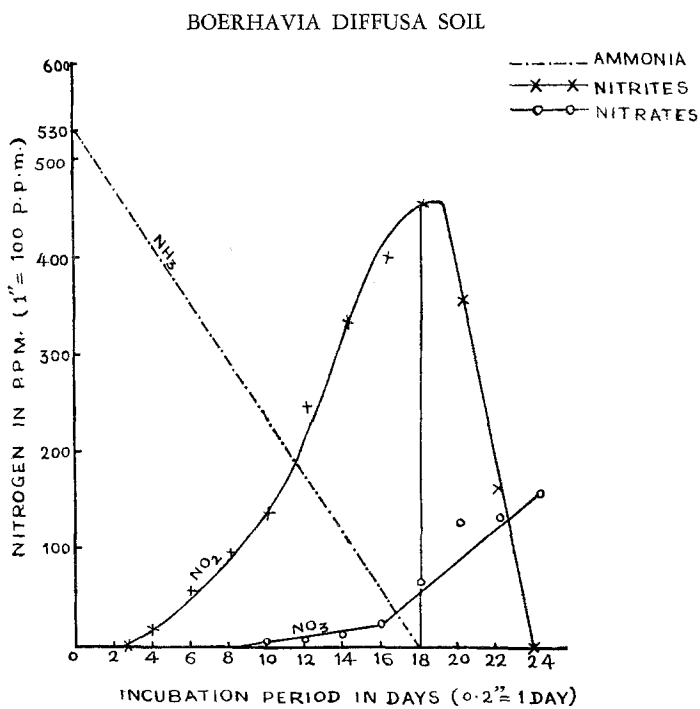


Fig. 4.

TABLE II — E.
Hibiscus tetraphyllus Soil.
 (Relevé No. 29)

Incubation period in days	Ammonia (by Spot Test)	Nitrogen as Nitrites in p.p.m.	Nitrogen as Nitrates in p.p.m.
2	P	Nil	Nil
4	P	Nil	Nil
6	P	35	Nil
8	P	62	5
10	P	105	15
12	P	125	25
14	P	220	40
16	P	300	45
18	Nil	385	45
20	Nil	425	60
22	Nil	460	80
24	Nil	290	125
26	Nil	85	160
27	Nil	Nil	162

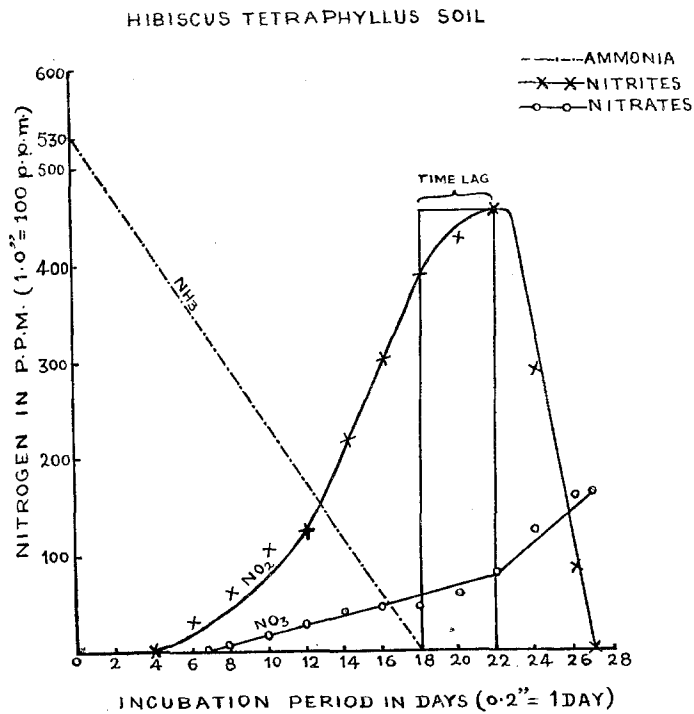


Fig. 5.

TABLE II — F.
Sida acuta Soil.
 (Relevé No. 22)

Incubation period in days	Ammonia (by Spot Test)	Nitrogen as Nitrites in p.p.m.	Nitrogen as Nitrates in p.p.m.
2	P	Nil	Nil
4	P	Nil	Nil
6	P	62	Nil
8	P	125	Nil
10	P	186	10
12	P	230	15
14	P	300	30
16	P	355	35
18	Nil	370	35
20	Nil	420	40
22	Nil	465	60
24	Nil	485	60
26	Nil	390	85
28	Nil	126	125
29	Nil	18	—
30	Nil	Nil	148

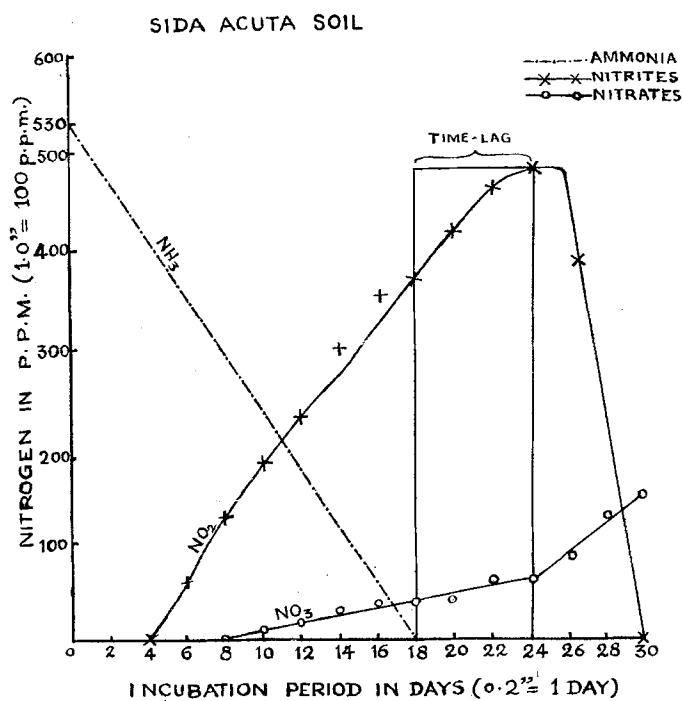


Fig. 6.

TABLE II — G.
Eleusine indica Soil.
 (Relevé No. 21)

Incubation period in days	Ammonia (by Spot Test)	Nitrogen as Nitrites in p.p.m.	Nitrogen as Nitrates in p.p.m.
2	P	Nil	Nil
4	P	Nil	Nil
6	P	5	Nil
8	P	26	Nil
10	P	70	5
12	P	90	10
14	P	210	15
16	P	265	30
18	Nil	310	30
20	Nil	395	35
22	Nil	420	50
24	Nil	470	85
26	Nil	508	90
28	Nil	295	95
30	Nil	35	—
31	Nil	Nil	148

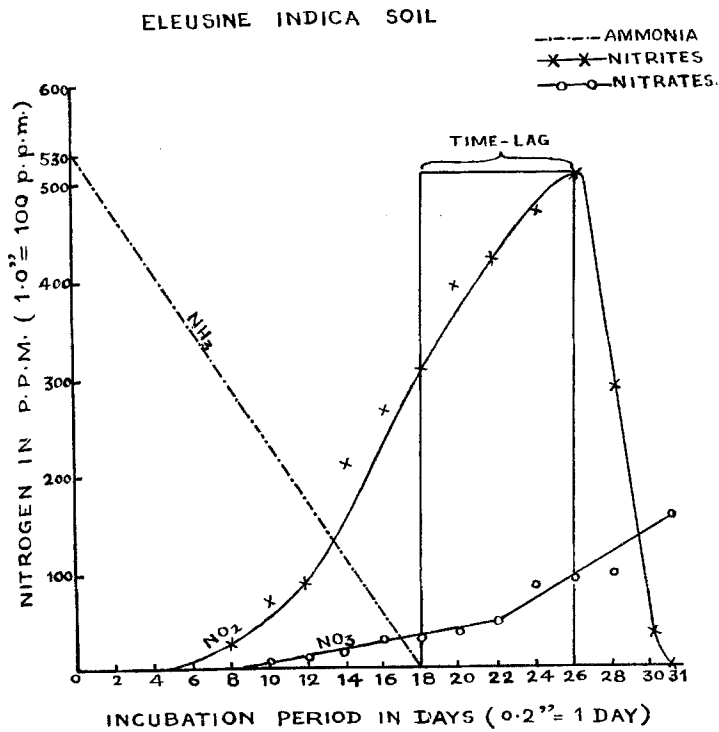


Fig. 7.

TABLE II — H.
Astercantha longifolia Soil.
 (Relevé No. 2)

Incubation period in days	Ammonia (by Spot Test)	Nitrogen as Nitrites in p.p.m.	Nitrogen as Nitrates in p.p.m.
2	P	Nil	Nil
4	P	5	Nil
6	P	55	Nil
8	P	100	5
10	P	152	20
12	P	180	40
14	P	105	45
16	Nil	60	—
17	Nil	15	—
18	Nil	Nil	78

ASTERCANTHA LONGIFOLIA SOIL

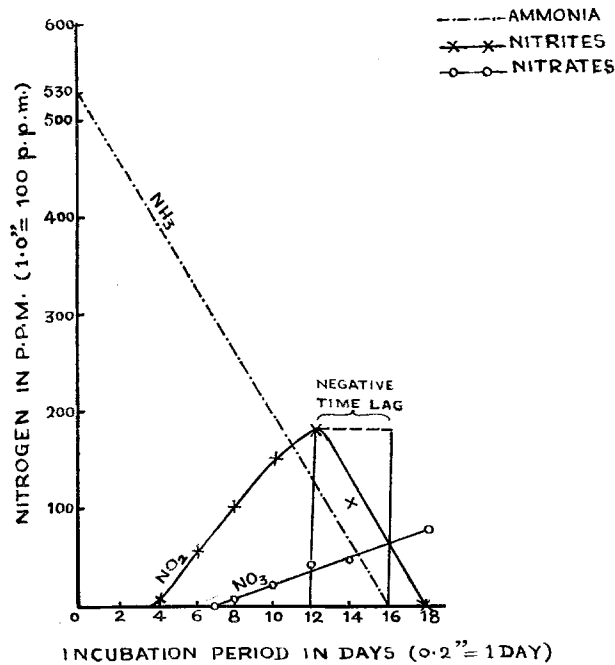


Fig. 8.

In Table I a complete floristic survey of all the relevés is given. Table II shows the alternate day results of the products of nitrification and hence a complete picture of nitrification of each type of soil is given by the aids of graphs. According to these graphs, the nitrites rise in a typical 'S' form, i.e. in an autocatalytic curve (BHARUCHA

Table III

Showing the nitrification values of soils from the root regions of the dominant plants from the 31 Relevé's surveyed.

SOIL-TYPE	Relevé Nos.	Period for Ammonia Exhaustion	Maximum NO_2 Incubation Period	Average NO_2 per day in p.p.m.	Average Nitrites of all Relevé's (of No. 4)	Maximum Nitrates NO_3 disappearance per Day	Average NO_3 per day in p.p.m.	Average Nitrites of all Relevé's (of No. 6)	"Time-lag" duration	Period for Nitrification
1	2	3	4		5	6		7	8	9
AMANTHUS SPINOSUS	1	16 Days	$\frac{620 \text{ p.p.m.}}{16 \text{ Days}} = 38.8$	} 33.8	} 9.7	$\frac{210 \text{ p.p.m.}}{21 \text{ Days}} = 10.0$	} 9.7	} 9.7	Nil	21 Days
	3	"	$\frac{570 \text{ p.p.m.}}{16 \text{ Days}} = 35.6$			$\frac{186 \text{ p.p.m.}}{21 \text{ Days}} = 8.8$			"	"
	6	"	$\frac{557 \text{ p.p.m.}}{16 \text{ Days}} = 34.4$			$\frac{180 \text{ p.p.m.}}{21 \text{ Days}} = 8.5$			"	"
	9	"	$\frac{535 \text{ p.p.m.}}{16 \text{ Days}} = 33.5$			$\frac{220 \text{ p.p.m.}}{21 \text{ Days}} = 10.4$			"	"
	16	"	$\frac{530 \text{ p.p.m.}}{16 \text{ Days}} = 33.1$			$\frac{180 \text{ p.p.m.}}{20 \text{ Days}} = 9.0$			"	20 Days
	23	"	$\frac{495 \text{ p.p.m.}}{16 \text{ Days}} = 31.0$			$\frac{240 \text{ p.p.m.}}{21 \text{ Days}} = 11.4$			"	21 Days
	27	"	$\frac{480 \text{ p.p.m.}}{16 \text{ Days}} = 30.0$			$\frac{196 \text{ p.p.m.}}{20 \text{ Days}} = 9.8$			"	20 Days
TRANTHENA MONOCYNA	17	16 Days	$\frac{540 \text{ p.p.m.}}{16 \text{ Days}} = 33.8$	} 30.7	} 8.3	$\frac{220 \text{ p.p.m.}}{22 \text{ Days}} = 10.0$	} 8.3	} 8.3	Nil	22 Days
	14	18 Days	$\frac{510 \text{ p.p.m.}}{18 \text{ Days}} = 28.3$			$\frac{190 \text{ p.p.m.}}{22 \text{ Days}} = 8.6$			"	"
	26	16 Days	$\frac{520 \text{ p.p.m.}}{16 \text{ Days}} = 32.5$			$\frac{178 \text{ p.p.m.}}{22 \text{ Days}} = 8.0$			"	"
	30	"	$\frac{456 \text{ p.p.m.}}{16 \text{ Days}} = 28.5$			$\frac{158 \text{ p.p.m.}}{21 \text{ Days}} = 8.0$			"	21 Days
ARGEMONE MEXICANA	4	18 Days	$\frac{520 \text{ p.p.m.}}{18 \text{ Days}} = 28.8$	} 27.0	} 7.2	$\frac{168 \text{ p.p.m.}}{23 \text{ Days}} = 7.3$	} 7.2	} 7.2	Nil	23 Days
	5	"	$\frac{490 \text{ p.p.m.}}{18 \text{ Days}} = 26.2$			$\frac{180 \text{ p.p.m.}}{24 \text{ Days}} = 7.5$			"	24 Days
	7	19 Days	$\frac{495 \text{ p.p.m.}}{19 \text{ Days}} = 26.0$			$\frac{155 \text{ p.p.m.}}{23 \text{ Days}} = 6.8$			"	23 Days
BOERHAAVIA DIFFUSA	10	19 Days	$\frac{496 \text{ p.p.m.}}{19 \text{ Days}} = 26.0$	} 26.2	} 6.9	$\frac{145 \text{ p.p.m.}}{24 \text{ Days}} = 6.0$	} 6.9	} 6.9	Nil	24 Days
	11	18 Days	$\frac{460 \text{ p.p.m.}}{18 \text{ Days}} = 25.5$			$\frac{160 \text{ p.p.m.}}{24 \text{ Days}} = 6.6$			"	"
	12	18 Days	$\frac{480 \text{ p.p.m.}}{18 \text{ Days}} = 26.5$			$\frac{176 \text{ p.p.m.}}{23 \text{ Days}} = 7.7$			"	23 Days
	13	"	$\frac{485 \text{ p.p.m.}}{18 \text{ Days}} = 26.6$			$\frac{172 \text{ p.p.m.}}{24 \text{ Days}} = 7.1$			"	24 Days
HIBISCUS TETRAPHYLLUS	18	18 Days	$\frac{497 \text{ p.p.m.}}{22 \text{ Days}} = 22.5$	} 21.7	} 6.3	$\frac{165 \text{ p.p.m.}}{27 \text{ Days}} = 6.0$	} 6.3	} 6.3	4 Days	27 Days
	28	19 Days	$\frac{478 \text{ p.p.m.}}{22 \text{ Days}} = 21.7$			$\frac{172 \text{ p.p.m.}}{26 \text{ Days}} = 6.6$			"	26 Days
	29	"	$\frac{460 \text{ p.p.m.}}{22 \text{ Days}} = 20.9$			$\frac{162 \text{ p.p.m.}}{27 \text{ Days}} = 5.8$			"	27 Days
SIDA ACUTA	19	18 Days	$\frac{520 \text{ p.p.m.}}{25 \text{ Days}} = 20.8$	} 20.4	} 5.15	$\frac{160 \text{ p.p.m.}}{30 \text{ Days}} = 5.3$	} 5.15	} 5.15	7 Days	30 Days
	22	19 Days	$\frac{485 \text{ p.p.m.}}{24 \text{ Days}} = 20.0$			$\frac{148 \text{ p.p.m.}}{30 \text{ Days}} = 5.0$			5 Days	"
ELFUSINE INDICA	31	18 Days	$\frac{490 \text{ p.p.m.}}{26 \text{ Days}} = 18.8$	} 19.4	} 4.5	$\frac{135 \text{ p.p.m.}}{32 \text{ Days}} = 4.1$	} 4.5	} 4.5	8 Days	32 Days
	15	"	$\frac{480 \text{ p.p.m.}}{25 \text{ Days}} = 19.2$			$\frac{155 \text{ p.p.m.}}{32 \text{ Days}} = 4.8$			"	"
	21	19 Days	$\frac{508 \text{ p.p.m.}}{26 \text{ Days}} = 19.5$			$\frac{145 \text{ p.p.m.}}{31 \text{ Days}} = 4.7$			"	31 Days
	25	"	$\frac{502 \text{ p.p.m.}}{25 \text{ Days}} = 20.0$			$\frac{139 \text{ p.p.m.}}{32 \text{ Days}} = 4.4$			6 Days	32 Days
ASTERCANTHA LONGIFOLIA	2	16 Days	$\frac{180 \text{ p.p.m.}}{12 \text{ Days}} = 15.0$	} 14.0	} 4.3	$\frac{78 \text{ p.p.m.}}{18 \text{ Days}} = 4.3$	} 4.3	} 4.3	Negative-Lag 4 Days	18 Days
	8	"	$\frac{162 \text{ p.p.m.}}{12 \text{ Days}} = 13.5$			$\frac{80 \text{ p.p.m.}}{18 \text{ Days}} = 4.4$			Negative-Lag 4 Days	"
	20	15 Days	$\frac{156 \text{ p.p.m.}}{12 \text{ Days}} = 13.0$			$\frac{80 \text{ p.p.m.}}{18 \text{ Days}} = 4.3$			Negative-Lag 3 Days	"
	24	16 Days	$\frac{175 \text{ p.p.m.}}{12 \text{ Days}} = 14.6$			$\frac{72 \text{ p.p.m.}}{17 \text{ Days}} = 4.2$			Negative-Lag 4 Days	17 Days

DUBASH and SHERIAR, 1951c). The nitrates are not formed during the first few days of incubation but they suddenly rise after ammonia is exhausted from the medium. This, according to WAKSMAN (1927), is due to the fact that the presence of ammonia is toxic to the nitrate-forming organisms and hence the nitrate-formers, though present in the culture flask, cannot function till all ammonia is exhausted.

Ammonia, which is only qualitatively estimated by the spot-test method, is shown by an imaginary straight line.

In Table III the entire chemical data (i.e. nitrifying power) of the soils from all the 31 relevés studied, is tabulated according to the criteria (BHARUCHA and SHERIAR, 1952) suggested previously.

From these results, the nitrifying powers of the first seven types of *aerobic* soils show the following characteristics:

1. Average nitrite and nitrate contents per day decrease with decreasing nitrifying power.
2. The length of the period of nitrification increases with decreasing nitrifying capacity.
3. Time-lag, the occurrence of which is regarded as due to the formation of some intermediate compounds between the ammonia and the nitrite stages (BHARUCHA and SHERIAR, 1951), appears only in poorly nitrifying soils.
4. Period for ammonia exhaustion, i.e. its complete transformation into nitrites, is shorter for fertile soils which show faster nitrification.

On the basis of the above mentioned criteria, the plants are graded according to their nitrifying capacity. Accordingly, *Amaranthus spinosus* which is usually confined to the dirty, nitrate-high habitats (BHARUCHA and DUBASH, 1951b), shows the greatest nitrifying capacity (i.e. the values of all its stands are higher than of others) closely followed by *Trianthema monogyna*, which is mostly associated with the former in similar habitats. The next three plants (*Argemone*, *Boerhavia* and *Hibiscus*) which occur only frequently in nitrophilous as well as in other habitats, come lower in the descending order of nitrophily.

Sida acuta and *Eleusine indica*, from dry fallow soils showing a very low power of nitrification, stand lower in the list. Also the floristic survey of the relevés dominated by these two plants show totally different species from the relevés with either *Amaranthus* or *Trianthema* present.

Also according to OLSEN (1921), *Urtica dioica* was not found in localities, the soils of which showed either an absence of or a too weak nitrification. The localities dominated by *U. dioica* showed a greater quantity of humus substances which are the main sources of ammonia.

Thus, according to our investigations (Tables I & III) the highest nitrification is met with in soils covered with night soil, urea, rubbish etc., which are usually dominated by *Amaranthus spinosus* and frequently by *Trianthema monogyna*.

On the other hand, the water-logged anaerobic soils of *Asterantha longifolia* give quite curious results for nitrification, which is exceedingly poor.

It has been observed by JOST (1913) that on account of poor aeration in uncultivated soils there is very weak nitrification and hence ammonia is the main source of nitrogen to the plants. Also, according to SHREENIVASAN (1937), under water-logged soils, the oxygen supply being limited, ammonification proceeds much faster than nitrification, so ammonia accumulates in the medium and very little nitrite and nitrate is formed. Also, according to CORBET (1935), in such soils, plants absorb nitrogen in the form of ammonia instead of nitrates and ammonia instead of being oxidised to nitrites is transformed to hydroxylamine and then lost as free nitrogen. For

these reasons nitrification is very poor in water-logged soil and ammonia persists for a longer time than nitrites resulting in "negative time lag" (BHARUCHA and SHERIAR, 1951).

Thus, on the basis of Tables I & III, these 8 plants, which grow in varied habitats, are graded according to their order of nitrophily and nitrification e.g. soil from *Amaranthus spinosus* shows the best results when considered according to Table III, whereas *Asterantha longifolia* gives the lowest value for these factors; so the former tops the list of nitrate-positive plants followed by other plants.

In a previous paper, BHARUCHA & DUBASH (1951b) have graded plants according to a new formula in which the phytosociological and the chemical data (i.e. nitrates of the cell sap) are combined. We have also been able to combine the statistical (floristic survey) and the chemical (soil nitrification) factors associated with the plants growing in nitrate-high localities and compared them with those growing in fallow and water-logged soils.

Thus, we have been able to show the correlation between the nitrophily of a plant and the nitrification of its soil.

Finally we should like to thank our colleague Mr. P. J. DUBASH, M.Sc., for his help.

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