

THE BIO-CHEMICAL ACTIVITY OF SOILS

A NEW METHOD OF ASSESSMENT

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Introduction

Many methods have been devised in order to obtain a picture of the relatively broad spectrum of micro-organisms present in the soil (Waksman, 1932; Löhns, 1938; Beijerinck, 1946; Winogradsky, 1949). Techniques for direct microscopic examination, including contact slide methods, were developed by a number of research workers (Rossi, 1921, 1936; Conn, 1928, 1929; Cholodny, 1930; Kubierna, 1932) and have since been used with good results (Jensen, 1935). Studies have also been carried out on the chemical activity of microbes in the soil. Thus the influence of microbes on soil fertility has been examined by Waksman (1922, 1923), Waksman and Dubos (1926), Waksman and Starkey (1923, 1924a, 1924b), van Schuchtelen (1910). The TTC dehydrogenase* activity of these organisms has been examined by Lenhard (1956, 1957), Stevenson (1959), Roth (1963, 1964), Hirte (1963) and Schaefer (1963).

Stains have been used to enable living and dead tissue to be differentiated by direct observation with a microscope (Stugger, 1949). Among these, violamin has been used, as this stains active hyphae purple in colour, while dead hyphae turn blue (Romell, 1934). Other research workers (Jones & Mollison, 1948) have recommended using acetic aniline blue for staining fungal mycelium and a number of different stains have been recommended by Waksman (1952). In recent years, some investigators have used fluorescent dyes to distinguish between bacteria and dead soil particles. For this purpose acridine-orange was used to stain soil smears and these were studied under a fluorescent microscope using ultraviolet light (Burrichter, 1953; Lehner & Nowak, 1957; Lehner *et al.*, 1958). With this technique, it was found, that although living cells normally stain green in colour and dead cells turn red, the actual colour developed depends on such factors as the concentration of the dye, the relative activity of the microbes and temperature.

The number and types of micro-organisms in soils are usually ascertained by culture techniques. These, unfortunately, all suffer from the same limitation, namely, the restriction of populations to the particular group of soil organisms which will grow on the medium selected. It has in fact been shown by direct

microscopic counts, that only 10% to 20% of the total population of living soil micro-organisms can be obtained using culture techniques, and it is assumed that many micro-organisms, because of their diverse nutritional requirements, are not isolated from the soil in pure culture. It is well known that a weak parasite will, when necessary, grow on a saprophytic medium, but many of our more virulent plant pathogens cannot be artificially cultured. For this reason soil dilution techniques tend to provide a misleading picture of the total and individual numbers of micro-organisms per gram of soil.

Soil dilution and plate culture techniques are used for counting and isolating organisms in pure culture and data obtained in this way provides an estimate of the population of micro-organisms. These methods, although they cannot be depended on to give an accurate assessment of the numbers of micro-organisms in the soil, do have the advantage of supplying soil microbiologists with culture of a number of different organisms which can then be identified.

The purpose of the present investigation is to initiate comparison between the TTC dehydrogenase technique and the common techniques of soil microbiology, as methods of studying living organisms and estimating their abundance and activity in different soils, also, to discover whether the TTC technique could be standardised for use in soil microbiological studies.

Materials and Methods

For this study, soils were taken from different sources in the cane belt and samples subjected to normal pH determinations and mechanical analyses. Similar samples were used for direct microscopic examination using methods described by Conn (1928, 1929) and modified by Jones and Mollison (1948). The dilution and plate technique modified by Bunt and Rovira (1955), has also been used as a comparison.

In addition to these methods a comparative study of soils was carried out using 2.3.5-triphenyl tetrazolium chloride (TTC). This technique, which was used and developed by the writer while studying different citrus and avocado soils during 1961/62, was discussed at the First National Symposium on Soil

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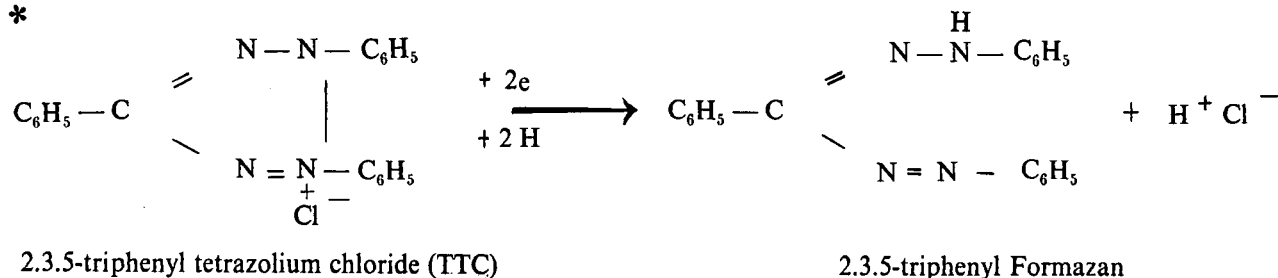


TABLE 1

Percentage light absorption and specific dominant wavelength peaks expressed in m μ , when bacteria in liquid media are exposed to TTC.

*MEDIUM	PERCENTAGE ABSORPTION				SPECTRAL ABSORPTION PEAKS			
	EXPERIMENT IA Incubation Time		EXPERIMENT IB Incubation Time		EXPERIMENT IA Incubation Time		EXPERIMENT IB Incubation Time	
	48 hrs.	96 hrs.	48 hrs.	96 hrs.	48 hrs.	96 hrs.	48 hrs.	96 hrs.
A	2	1	0	1	510	510	510	510
B	1	0	0	0	510	510	510	510
C	0	1	0	1	510	510	510	510
D	7	5	2	4	510	510	510	510
E	75	78	78	70	436/450	436/450	436/765	436/765
F	82	77	70	71	436/750	436/750	495/450	495/450
G	14	25	45	47	510	510	510	510
H	53	49	77	75	475	475	440/750	440/750
I	88	91	83	76	450	450	436/750	436/750
J	24	19	14	11	510	510	510	510
K	39	44	82	78	510	510	445/765	445/765
L	92	91	76	85	510	510	450/765	450/765
M	75	90	73	91	565/755	565/755	565	565/725
N	85	84	77	89	565	565	565/725	565/725
O	94	90	81	79	565	565	565/755	565/755
P	95	97	87	91	535/765	535/765	425/750	425/750

- A. Sterilised tap water in test tube and closed with cotton wool plug.
- B. Sterilised tap water with peptone; test tube closed with cotton-wool plug.
- C. Sterilised tap water with beef extract; test tube closed with cotton-wool plug.
- D. Tap water.
- E. Tap water with peptone.
- F. Tap water with beef extract.
- G. Water from a beaker, in which sugarcane setts were rooted, the water being changed every day.
- H. Water as from (G), but with peptone.
- I. Water as from (G), but with beef extract.
- J. Water from a beaker, in which sugar cane setts were rooted, but in this case the water was not changed (stagnant water).
- K. Water as from (J) with peptone.
- L. Water as from (J) with beef extract.
- M. Water from a beaker, in which sugarcane setts were rooted. This water was colonised mainly by one genus of spore forming bacteria, obtained when sugarcane setts are kept under anaerobic conditions during rooting.
- N. Water as from (M) with peptone.
- O. Water as from (M) with beef extract.
- P. Tap water plus peptone and beef extract.

Biology held in Potchefstroom in December, 1963. Since then the method has been improved, but has not yet been finalised.

Description of the TTC Technique

Three grams of each soil sample were mixed with 0.1 gram of CaCO₃ and placed in a test tube, to which 15 cc. of a 0.5 per cent TTC-solution was added. This volume of TTC-solution is sufficient to provide excess of TTC after reaction is completed. The test tubes were then shaken several times for periods of 10 seconds at two-hourly intervals. The suspension was

allowed to settle, and the test tubes were kept under laboratory conditions and temperatures of approximately 25 to 30° C., for a period of 4 days. After incubation, the supernatant liquid was decanted and the soil examined microscopically. 20 cc. of carbon tetrachloride (CCl₄) were then added as a dye solvent. Plates I, a to d show the various organisms which have been stained by TTC according to their rate of activity. To separate the red colour from the soil complex the test tubes were shaken 3 times; if they are shaken more often than that, the solvent will be held fast by the soil.

Five seconds after shaking, the dye solvent is decanted into another test tube and the colour intensity (Plate II) can be read at 475 m μ , using a colorimeter. These readings are shown in the tables as a percentage of light absorption. Thus the darker the colour in the solvent, the higher the degree of light absorption. The dye, which was not removed from the soil by CCl₄, can then be extracted by adding 10 cc. of ethanol or methanol to the same sample. The contents of the test tubes are shaken several times over a period of 24 hours. The alcohol is then filtered off and a second colorimetric reading made at a wavelength of 510 m μ .

The use of colour measurements to determine the activity and thereby the population of microbes, is complicated by the fact that the reddish formazan is in some cases formed at the exterior of the microbes and in other cases inside them. The former is extracted using CCl₄, but to determine the latter, measurement is by difference, the total formazan being extracted using methanol. To provide a direct colour comparison, the methanol extract is shaken with CCl₄ plus water (4:1:4). The two solvents separate but the colour is taken up completely by the CCl₄. Thus, direct comparison of partial and total extraction in the same solvent is obtained, and may be measured in terms of colour intensity.

Results

Experiment 1

In a series of 6 trials, involving the use of sugarcane setts which are rooted in beakers in order to create a bacterial complex, it was established that active bacteria are responsible for the formation of formazan from TTC. A correlation was obtained between colour absorption capacity and the activity of micro-organisms, in liquid media. In the presence of TTC, no discoloration occurred when sterilised water was stored either alone, or with peptone or beef extract. However, where conditions for bacterial development exist, a

reddish colour appears and this gradually deepens in intensity. This can be seen quite clearly in the data for treatments E to P in Table 1, the significance of the letters A to P being explained in the footnote.

The results in table 1 show clearly that, in the treatments without peptone and beef extract, the percentage colour absorption is much lower than in those with this ideal nutrient supply. The highest value is to be found in treatment P, which consisted of tap water plus peptone and beef extract.

In addition to the percentage of light absorbed, there was a very interesting effect on the spectral absorption peaks. From the results of the two trials, which are demonstrated in table 1, there were specific wavelength peaks for the different treatments. In some treatments there were two maxima, but one was always higher than the other. The peaks were in the spectral range 400 to 800 m μ , and 13 different wavelength peaks were obtained, caused apparently by the specific kind of bacteria which first invaded the medium. Artificial cultures made from the different treatments have shown that in most cases the bacteria present were specific for the wavelength peaks. Further studies are to be carried out to determine the different genera in the bacterial complex and to find the specific peaks for each.

Soil Studies

A group of trials were carried out using different soils from the sugar belt in Natal. Each of these experiments is described separately.

Experiment 2

From the chemical department of the S.A.S.A. Experiment Station at Mount Edgecombe, 26 different soil samples were obtained. These samples were used originally to study the release of mineralisable nitrogen in the soils (Wood, 1964, 1965). The same samples were used in a preliminary test, using the TTC-technique, to find out if there were, perhaps, correla-

TABLE 2
Percentage light absorption in CCl₄, when soil samples were exposed to TTC

SOIL GROUPS DERIVED FROM	CULTIVATED SOIL			VIRGIN SOIL		
	pH	CCl ₄	C ₂ H ₅ OH	pH	CCl ₄	C ₂ H ₅ OH
Middle Ecca	5.20	11	—	6.05	63	—
Lower Ecca	5.80	22	—	6.10	88	—
T.M.S. (Mist belt)	4.85	38	—	5.70	77	—
Red Sand	4.80	29	—	5.90	86	—
Grey Sand	5.50	67	—	5.60	63	—
Black dolerite	5.90	92	—	6.20	88	—
Red dolerite	5.45	47	—	6.40	53	—
Granite	5.10	47	—	5.95	62	—
Alluvium	5.50	56	—	5.60	84	—
Dwyka tillite	5.40	50	—	6.60	81	—
Beaufort sand	6.15	42	—	6.75	42	—
Tugela schist	5.80	74	—	6.10	71	—

tions between nitrogen release and bacterial activity. The following results were obtained:

Comparing cultivated soil samples with virgin soil it is obvious that the virgin soil shows a higher percentage colour absorption. As far as soil type was concerned, the highest values for both cultivated and virgin sites were obtained from black soils derived from dolerite. Among cultivated soils those derived from Middle Ecca, Lower Ecca and Beaufort Sandstone provided the lowest figures in terms of light absorption. On the other hand, the Grey Sand showed a relatively high percentage light absorption. Microscopic examinations have indicated that the Grey Sand

has a high bacterial activity. Further studies will have to be undertaken to provide an explanation of the results presented in table 2.

Experiment 3

This experiment, involving the use of the TTC technique, was designed to investigate the percentage of light absorbed in relation to the organic matter, pH-value and texture classes of soils. Soil samples were provided by the Agronomy branch of the S.A.S.A. Experiment Station as they wished to obtain information about the influence of herbicides on the dehydrogenase and bacterial activity of soils. The results are presented in table 3.

TABLE 3
Percentage light absorption in CCl₄ and Methanol, when soil samples, treated with herbicides, were exposed to TTC

SOIL SERIES	SITE	PROFILE DEPTH (inches)	TEXTURAL Class	pH VALUE	ORGANIC MATTER (%)	PERCENTAGE OF LIGHT ABSORPTION IN	
						CCl ₄	Methanol
Fernwood	Tugela	0-3	S	8.1	1.12	75	76
Fernwood	Tugela	3-6	S	8.0	1.12	69	74
Clansthal	La Lucia	0-3	LS	8.1	1.74	53	46
Clansthal	La Lucia	3-6	LS	5.3	1.19	45	12
Rosehill	Mtunzini	0-3	SL	5.6	2.02	64	90
Rosehill	Mtunzini	3-6	SL		2.09	54	88
Cartref	Doornkop	0-6	LS	8.1	1.60	73	71
Williamson	Chaka's Kraal	0-3	SL	5.9	2.23	82	69
Williamson	Chaka's Kraal (Post)	3-6	SL	6.1	2.30	58	91
Williamson	Chaka's Kraal	0-3	SL	5.9	2.44	54	69
Williamson	Chaka's Kraal (Pre.)	3-6	SL	5.7	2.72	78	85
Waldene	Mount Edgecombe	0-3	SCL	5.8	3.90	27	86
Waldene	Mount Edgecombe	3-6	SCL	5.4	3.42	10	70
Rydalvale	Mount Edgecombe	0-3	CL	6.0	5.51	28	83
Rydalvale	Mount Edgecombe	3-6	CL	5.3	5.16	26	80
Inanda	Doornkop	0-6	SL	5.8	10.80	59	50

In this experiment methanol was used to extract all the colour which remained in the soil complex after treatment with CCl₄. For comparative purposes the figures for both treatments are presented in table 3. It is obvious that, in some soil samples, there is a drastic change in the percentage of light absorbed after extraction with CCl₂, compared with the figures obtained with methanol. In this case, therefore, an explanation has also to be found through further studies.

Experiment 4

In this experiment 57 soil samples of different profile depths have been tested in order to determine if there is a change from one profile to the other or from one soil type to another. Samples were provided by the Soils department at the Experiment Station. From

table 4 it can be seen that, with few exceptions, the top-soil down to approximately 20 in. has the highest dehydrogenase and bacterial activity. There is, however, a wide range of variation between soil types.

Comparing the two columns in table 4 which show the percentage of light absorbed following treatment with CCl₄ and methanol, it is obvious that the figures are related. Three soil series were also used for plate counts the results of which will be described later. The pH-values and organic matter content are provided for comparison with these figures and the percentage light absorbed.

Experiment 5

Further trials designed to obtain more detailed information about soils and their variation in terms

TABLE 4
Percentage light absorption in CCl₄ and Methanol, when soil samples are exposed to TTC

SOIL SERIES	TEST-PIT SERIAL No.	PROFILE DEPTH (inches)	pH VALUE	ORGANIC MATTER (%)	PERCENTAGE LIGHT ABSORPTION IN	
					CCl ₄	Methanol
Mayo	1619	0-7	5.4	3.89	80	59
		7-30	5.6	3.01	57	49
		30-42	6.1	0.58	34	46
		42-72	6.6	0.22	52	54
Dansland	1702	0-9	6.0	8.68	76	72
		9-22	5.8	6.60	76	42
		22-32	6.0	3.96	56	27
		32-60	6.2	0.75	58	34
Solferino	1696	0-10	6.0	1.39	83	56
		15-24	6.6	0.66	71	79
		24-33	6.6	0.44	30	65
		33-60	6.8	0.29	27	59
Inanda	1393	0-7	5.3	10.56	82	56
		7-20	4.8	5.28	32	16
		20-43	5.0	1.76	20	14
		43-64	5.1	0.80	40	21
Cartref	1685	0-10	5.4	0.80	29	16
		10-20	5.4	0.73	77	33
		20-40	5.5	0.29	68	51
		40-52	5.6	0.29	58	35
Milkwood	1604	0-7	6.1	3.96	85	64
		7-24	5.6	4.40	46	36
		24-28	6.3	2.13	89	67
		28-36	—	0.73	82	53
Waldene	72	0-10	6.1	2.27	85	35
		10-18	7.1	1.32	78	33
		18-32	7.5	1.25	83	45
		32-60	7.4	0.29	35	43
Windermere	1700	0-8	5.4	5.80	71	66
		8-18	5.6	4.40	50	59
		18-30	6.1	2.06	31	46
		30-43	6.8	0.73	63	44
Avoca	1616a	0-9	5.1	1.17	40	47
		9-20	5.5	0.66	38	47
		20-26	6.3	0.29	49	57
		26-37	6.3	—	52	80
Shortlands	1597	0-7	6.0	4.77	76	46
		7-28	5.1	2.94	14	5
		28-40	5.2	1.47	18	6
		40-72	5.3	0.73	13	6
Clansthal	208a	0-12	5.9	0.80	44	17
		12-40	6.2	0.44	50	21
		40-72	6.6	0.73	31	15
Haig	1639	0-9	6.9	9.40	82	70
		9-20	7.0	4.68	58	37
		20-48	7.5	0.29	22	13
		48-72	7.8	0.07	21	11
Sprinz	1238	0-9	5.1	12.26	75	17
		9-22	5.2	8.59	54	27
		22-34	5.4	2.13	37	3
		34-72	5.6	0.88	44	18
Rydalvale	82	0-10	5.8	7.05	70	60
		10-22	6.1	1.17	58	27
		22-54	6.8	0.51	53	20
Fernwood	1525	0-7	5.9	0.95	73	30
		7-28	6.4	0.36	64	32
		28-72	6.2	0.14	61	19

of light absorption using the TTC-technique, were carried out, with samples of four soil series. The samples, which were obtained from the Soils Department, had been taken at different depths but, in con-

trast to the soils used in other tests, which had been stored for more than a year, these were air dried and exposed to TTC only a few days after being taken. The results are set out in table 5.

TABLE 5
Percentage light absorption in CCl₄ and Methanol, when soil samples are exposed to TTC

SOIL SERIES	SAMPLE No.	PROFILE DEPTH (inches)	pH VALUE	ORGANIC MATTER (%)	PERCENTAGE OF LIGHT ABSORPTION IN	
					CCl ₄	Methanol
Cartref	1a	0-7	5.3	2.36	45	31
	1b	7-20	5.4	2.21	40	56
	1c	20-36	5.5	2.44	39	65
Williamson	2a	0-7	5.9	3.66	61	32
	2b	7-20	6.2	4.13	47	42
Milkwood	3a	0-7	5.6	9.45	35	26
	3b	7-20	5.8	6.05	40	35
Clansthal	4a	0-7	6.0	1.02	54	39
	4b	7-20	5.4	0.31	41	71
	4c	20-36	5.2	0.17	66	79
	4d	36-48	5.0	0.15	68	79

In some instances measurement of colour absorption indicated higher TTC dehydrogenase activity than was expected. In addition, microscopic examination of samples showed that the soil with the lowest percentage of organic matter might contain the highest number of bacteria (Clansthal Series).

Experiment 6

Soil samples used in this experiment were provided by the Soil Science department of the S.A.S.A. Experiment Station and they were taken from different parts of one sugar farm. Four different soil series were involved, namely Williamson, Cartref, Trevanian and Inanda. As can be seen in table 6, the soils differ not only in their pH-values but also in the percentage organic matter which, in one sample of the Inanda series, was as high as 14.04 per cent, and reached a minimum in samples No. 4 and 6, both of which were derived from the Cartref Series.

As can be seen in table 6, the figures in the CCl₄ column are relatively low compared with those in the column for methanol. Low figures for CCl₄ and high figures for methanol are obvious in samples No. 1, 2, 19 and 20, all belonging to the Williamson Series. A fairly high percentage light absorption was obtained for both solvents (CCl₄ and methanol) in soil samples No. 13 and 14, which belong to the soil series Inanda and Trevanian, respectively. The lowest figures in both solvents were obtained for samples No. 4, 5 and 7 of the Cartref and Trevanian series. The results in table 6 show also very clearly that great differences are to be found between samples and within soil series, and a lot of work will need to be done to explain these differences from microbiological and biochemical points of view.

Experiment 7

To compare the number of micro-organisms in soil stored over a long period and freshly collected samples

using the TTC light absorption technique, a few soil samples were selected for artificial culture studies. Results of counts of the main groups, bacteria, actinomycetes, and fungi, are presented in table 7.

It is obvious from the results in table 7 that populations of the different groups of micro-organisms are still very high, even after long storage. The soil sample with the Serial No. TP 72 has been stored for many years, but despite this a large number of bacteria are present, most of which belong to the spore-forming group. Indeed, with storage, the actinomycetes seem to increase, whereas the fungi seem to be fewer in number in the lower horizons. Examination of numbers of bacteria and actinomycetes in the 4th horizon of the Serial No. 1619, indicates that these are higher than in horizon 3 immediately above it. This agrees with the light absorption values recorded, indicating a degree of correlation. In this sample, microscopic examinations revealed very high microbial activity.

Among the three fresh top-soils investigated (T.M.S., Dwyka and Granite) Dwyka showed the highest bacterial population with 93 million bacteria per gram soil, followed by Granite with 32 million. The lowest figure was obtained from T.M.S. with 7.9 million bacteria per gram soil. However, counts of actinomycetes indicated that the soil derived from granite contained the highest number with 14 million, followed by Dwyka with 4.8 million and then T.M.S. soil with 3.5 million. In the group of fungi, T.M.S. showed the largest population with 740 thousand, followed by Dwyka with 170 thousand and Granite with 59 thousand. The highest percentage light absorption was shown by Dwyka soil, which had the highest number of bacteria and a relatively high number of actinomycetes.

TABLE 6
Percentage light absorption when soil samples are exposed to TTC

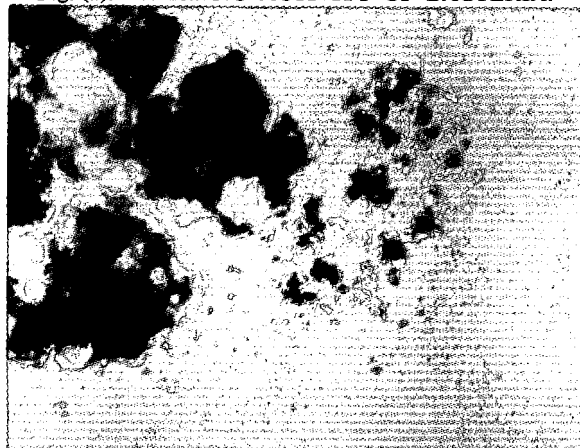
SOIL SERIES	SAMPLE No.	PROFILE DEPTH (inches)	pH VALUE	ORGANIC MATTER (%)	PERCENTAGE ABSORPTION	
					CCl ₄	Methanol
Williamson	1	0-7	6.0	3.25	19	75
Williamson	2	0-7	5.8	3.03	22	60
Cartref	3	0-7	6.1	2.14	23	65
Cartref	4	0-7	5.3	0.81	24	23
Cartref	5	0-7	5.1	2.29	30	41
Cartref	6	0-7	5.4	0.88	45	51
Trevanian	7	0-7	5.4	1.20	31	45
Inanda	8	0-7	5.3	3.56	43	75
Inanda	9	0-7	4.6	6.20	45	76
Inanda	10	0-7	5.0	8.84	47	73
Inanda	11	0-7	5.2	11.80	39	74
Inanda	12	0-7	5.2	14.04	45	68
Inanda	13	0-7	5.5	5.48	54	78
Trevanian	14	0-7	5.1	4.44	48	75
Trevanian	15	0-7	5.4	2.52	41	60
Trevanian	16	0-7	4.7	3.72	27	46
Trevanian	17	0-7	5.5	2.96	26	77
Cartref	18	0-7	5.3	2.44	29	61
Williamson	19	0-7	5.8	3.47	23	72
Williamson	20	0-7	5.5	4.72	24	77

TABLE 7
Number of soil micro-organisms relative to percentage light absorption, when soil samples are exposed to TTC

SERIAL NO. OR SOIL GROUP	PROFILE DEPTH (inches)	MICRO-ORGANISMS PER GRAM OF SOIL			% LIGHT ABSORPTION	
		BACTERIA	ACTINOMYCETES	FUNGI	CCl ₄	METHANOL
TP 1619 Mayo	0-7	3.4 x 10 ⁷	6.3 x 10 ⁸	5.9 x 10 ⁵	80	59
	8-30	1.6 x 10 ⁷	9.5 x 10 ⁸	2.9 x 10 ⁵	57	49
	31-42	7.4 x 10 ⁶	8.2 x 10 ⁸	7.8 x 10 ⁴	34	46
	43-72	1.2 x 10 ⁷	9.7 x 10 ⁸	5.3 x 10 ³	52	54
TP 72 Waldene	0-10	6.9 x 10 ⁶	4.5 x 10 ⁷	5.1 x 10 ⁴	85	35
	10-18	4.5 x 10 ⁶	5.1 x 10 ⁷	3.2 x 10 ⁴	78	33
	19-32	7.9 x 10 ⁶	9.8 x 10 ⁸	6.5 x 10 ³	83	35
	33-60	2.1 x 10 ⁶	7.7 x 10 ⁸	2.8 x 10 ³	35	43
T.M.S.	0-7	7.9 x 10 ⁶	3.5 x 10 ⁸	7.4 x 10 ⁵	27	32
Dwyka	0-7	9.3 x 10 ⁷	4.8 x 10 ⁸	1.7 x 10 ⁵	86	89
Granite	0-7	3.2 x 10 ⁷	1.4 x 10 ⁷	5.9 x 10 ⁴	50	55
Compost	—	9.4 x 10 ⁷	4.6 x 10 ⁷	6.2 x 10 ⁵	97	89

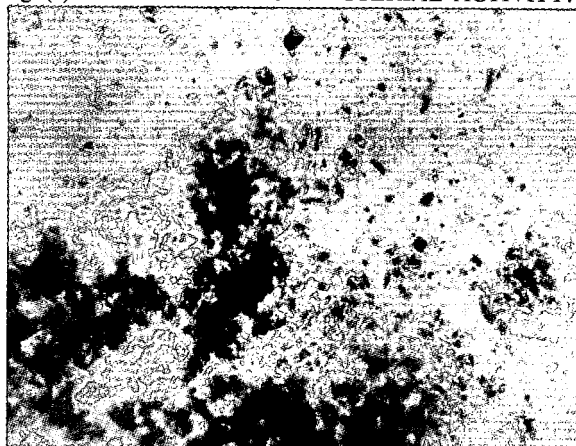
PLATE I. Dehydrogenase bacterial activity in soils.

Fig. (a). VARYING ACTIVITY OF BACTERIA.



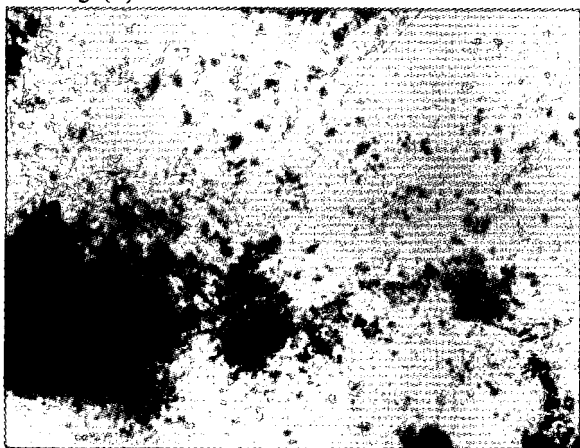
(a) Activity of microbes at an early stage in the decomposition of organic matter (red spots = bacteria; black = organic matter). $\times 450$.

Fig. (b). COMPARISON OF BACTERIAL ACTIVITY.



(b) Activity of microbes at an advanced stage of organic matter decomposition. The fine particles are cemented together by organic compounds produced by micro-organisms, and the red colour indicates activity of microbes. $\times 500$.

Fig. (c). BACTERIAL CONCENTRATION.



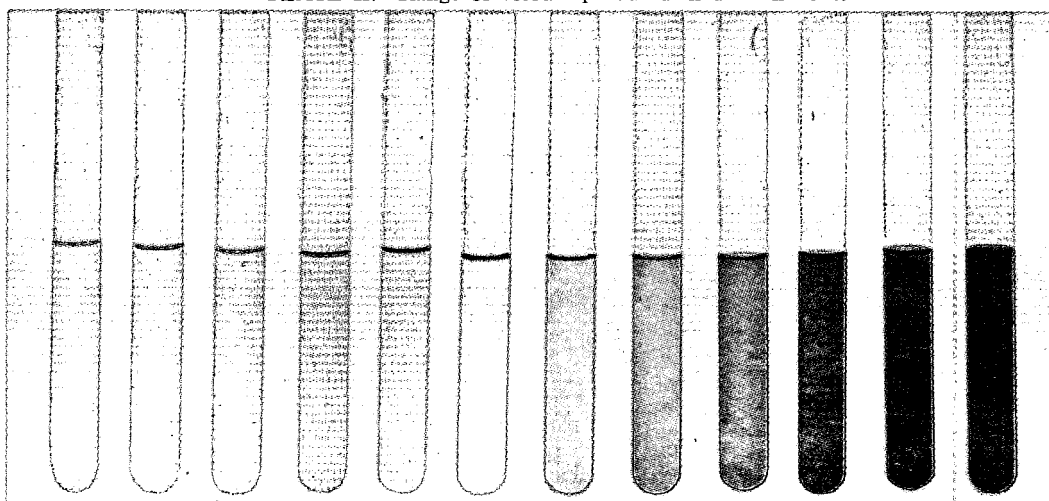
(c) Very active micro-organisms obtained from soil. The TTC. has been reduced by bacterial enzymes to yield the reddish formazan. $\times 450$.

Fig. (d). BACTERIAL AND FUNGAL ACTIVITY.



(d) Bacteria and fungi stained red in colour following exposure to TTC. and subsequent incubation for 72 hours. $\times 450$.

PLATE II. Range of colours produced in different soils.



Varying degrees of bacterial activity illustrated by extracting reddish formazan in CCl_4 . Formazan develops in the soil following treatment with TTC. solution and subsequent incubation; — the intensity of colour varying with the activity and therefore the enzyme production of micro-organisms. The supernatant fluid is then decanted and the colour absorbed from the soil with CCl_4 . The activity of bacteria in the soil can then be determined by measuring the light absorbed by the coloured solution, using a colorimeter. In the example illustrated, colour intensity in CCl_4 varies left to right from 0 to 92% light absorbed.

These results give only a very rough picture of the situation and fundamental studies are planned to obtain more information on the different groups of micro-organisms in sugarcane soils. Subjects for investigation include numbers and activity of spore-forming bacteria, actinomycetes and fungi, as well as the "specialists" in soils such as cellulose decomposers, nitrate reducers, nitrifiers (to NO_2 and NO_3), denitrifiers and micro-organisms which develop in environments rich in CO_2 . The effect of different environmental conditions including waterlogging, aeration and nutrition as well as differing depths of soil, should also be studied. The high number of microbes and percentage of light absorbed in compost is associated with a high level microbial and enzymatic activity.

Discussion and Conclusions

Soil fertility is closely associated with its biological status, and this, in terms of soil micro-organisms, represents a very wide field for research. In recent years enzymatic tests have increased in importance both as an indicator of microbiological activity of soils and as a means of evaluating the biochemical status of the various soil micro-organisms. Unfortunately no method has yet been developed which provides a quantitative estimate of overall microbiological activity.

During the last few years enzyme tests have been used to determine dehydrogenase activity in soils as a measure of microbiological activity and one such method is described in this paper.

It would seem, as a result of direct microscopic examination of soil, the use of dilution and plate count techniques and finally the TTC-techniques that the last named method might, with some modification, be developed for microbiological and biochemical studies of soil. Thus, in experiment 1 it has been very clearly shown that TTC dehydrogenase activity is related to bacterial activity in the media used, being greatest where nutrient supply is most plentiful. Micro-organisms growing under waterlogged conditions show much lower TTC dehydrogenase activity.

The vital dye, 2,3,5-triphenyl tetrazolium chloride remains colourless when in contact with dead material but, when it is exposed to living cells and some of their products, or to proteins, it is rapidly reduced by enzymatic processes to formazan, so turning carmine-red in colour.

The TTC dehydrogenase activity of soils can therefore be studied by measuring production of formazan. Used in this way, TTC is an indicator of active enzymatic processes in living tissue, and different forms of TTC may also be used as electron acceptors for many pyridine nucleotide linked enzyme systems. They are also used for histochemical demonstrations of such oxidative enzymes as cytochrome oxidase, cysteine desulphurase, succinic dehydrogenase, diphosphopyridine nucleotide (DPN) diaphorase and triphosphopyridine nucleotide (TPN) diaphorase.

The results described in this paper represent only the beginning of a fundamental examination of soil

groups which is designed to provide more information about the very complicated microbiological-biochemical soil complex. An understanding of the many important agricultural properties of a soil must depend not only on knowledge of planting, fertiliser use and harvesting but also such factors as fertility, tilth, concentration of colloids, silt, clay and organic matter content (humus) of the soil, water-air-soil relationships and finally the whole soil microbiological complex.

Some of the results presented in this paper are at this stage difficult to explain and they are certainly open to criticism. However, there can be no doubt that using the TTC technique very marked changes can be seen between soil types.

Summary

Experiments were conducted to study the dehydrogenase activity of an unknown bacterial complex. This was accomplished by measuring the light absorption capacity of the colour produced in liquid media to which has been added a few drops of a 1 per cent solution of TTC. The spectral reflection curves of the liquid media were identical when the samples were freshly prepared but with the development of different bacteria, a specific dominant wavelength was found for each. Some of the results indicate two maxima, one always being higher. All measurements were made within the range of 400 to 800 $\text{m}\mu$. The following peaks were observed: 425 $\text{m}\mu$, 436 $\text{m}\mu$, 440 $\text{m}\mu$, 445 $\text{m}\mu$, 450 $\text{m}\mu$, 475 $\text{m}\mu$, 495 $\text{m}\mu$, 510 $\text{m}\mu$, 535 $\text{m}\mu$, 565 $\text{m}\mu$, 740 $\text{m}\mu$, 750 $\text{m}\mu$, and 765 $\text{m}\mu$.

Various soil groups in the cane belt have been studied using the TTC technique. Marked differences, in their dehydrogenase activity have been recorded, with light absorption figures varying from 0 to 97%. In some experiments close correlation was observed between the TTC dehydrogenase activity and the number of bacteria in the soil. This was not the case in other studies and further work will be necessary in order to determine why these variations occur within soil types.

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Dr. Dick: It is obvious that to Dr. Roth the soil is not merely a complex of particles of various minerals of variable size with certain chemical and physical properties. Rather, it is a substratum for life.

While most of us have long appreciated the importance of life in the soil, this aspect has often been left out of our discussions by reason of its imponderability.

Some of us, on the biological side, have given a certain amount of thought and experimentation to the development of techniques for measuring various components of the biological complex of the soil. In this paper Dr. Roth has introduced what appears to be a valuable technique for estimating an important part of this biological complex in a quantitative manner.

Just over a year ago, as part of a symposium on soil biology held at Potchefstroom, Dr. Roth hinted at the possibility of a quantitative estimate of soil microflora by means of the staining technique discussed more fully today. The interest aroused by this suggestion may be judged by the fact that he had enquiries for further details on the technique from as far abroad as Alaska.

Mr. Eicker: There are a number of methods for determining the biotic components of the soil, but those based on dilution techniques or direct microscopic methods suffer from a disadvantage that, although the organisms can be counted, it is seldom possible to identify them. On the other hand, methods based on culturing of the organisms are selective and favour members of a particular group of soil organisms which will grow on the medium chosen. As yet no single method has been devised that will yield a true random selection of the total population of living soil micro-organisms. It would therefore appear that some form of combination of the two lines of approach would be the best course to follow. I would thus consider that the T.T.C. dehydrogenase technique of Dr. Roth, combined with one of the direct microscopic examination techniques, would be of great value if supplemented by culture techniques for identification of the organisms concerned.

I also feel that it would be very valuable if Dr. Roth in his own sphere of research on soil fungi could develop and standardise the T.T.C. technique so that it could also be used for research on fungi.

We know that the advance of knowledge in any branch of science is both dependant upon and conditioned by the precise nature of the techniques

specifically devised, and that the truth of any scientific generalisation in biology is rarely established by merely one set of observations, however many times it may be repeated. A closer approximation to the truth will be achieved only by the production of fresh evidence of an entirely different kind. The more numerous and the more different the approaches to a problem, the closer will become the proximation to certainty.

If I may continue further in this vein, I would like to point out that an original technique is a concrete expression of a fresh mental approach to a problem. A technique is of value if it enables something to be done that could not be done before, or if it secures results with a greater economy of effort. In my opinion Dr. Roth has achieved that aim.