

INVESTIGATIONS ON THE ^AROLE OF ORGANIC MATTER IN PLANT NUTRITION.

Part VII. Economy of Carbon during Decomposition of Cane Molasses in the
Swamp Soil.

BY G. NARASIMHAMURTHY, M.Sc.,

AND

V. SUBRAHMANYAN, D.Sc., F.I.C.,

Department of Biochemistry, Indian Institute of Science, Bangalore.

Received January 26, 1935.

ALTHOUGH it is generally recognised that organic manures increase the production of crop from swamp soils, yet the precise manner in which they function is still comparatively obscure. The available evidence would indeed show that a considerable part of the added organic matter is converted into gases (Harrison and Ayyar, 1913, 1914, 1916, 1919, 1920) and organic acids (Subrahmanyam, 1929), which do not probably play any direct part in plant nutrition. Some of the products are even toxic to plant growth (Onodera, 1923). It would be of considerable interest, therefore, to determine the quantities of the various products formed, the manner of their distribution in the soil and the extent to which they persist at different stages in the life of the crop.

In the course of an enquiry on the chemical and biological changes attendant on the decomposition of cane molasses in the swamp soil (Bhaskaran, Narasimhamurthy, Subrahmanyam and Sundara Iyengar, 1934), it was observed that there was copious production of gases and organic acids attended by dissolution of minerals, chiefly iron and aluminium. These observations suggested that, under normal field conditions, a considerable part of the added organic matter would be lost from the soil system and that the fertiliser would have practically no residual value. Since a number of previous workers (*vide* Subrahmanyam, 1933) have reported phenomenal increase in crop yield—especially of rice—consequent on the application of molasses, it appeared probable that the products of decomposition might persist long enough to produce the beneficial effect: a part might also pass into the insoluble condition and add to the humus content of the soil. In view of the above, it was considered desirable to carry out a systematic study of the distribution of carbon under such conditions. It was also hoped that the enquiry would

throw fresh light on the mechanism of plant nutrition under the conditions of the swamp soil.

Experimental.

Materials and Methods.—Four soils representing different types commonly met with in India were used in the present study.—(1) alluvial soil from Travancore, (2) black cotton soil from Sholapur, (3) *Kalar* (alkali) soil from Sindh, and (4) laterite soil from Bangalore. The samples were air-dried and ground to pass a 100-mesh sieve. The total carbon and carbonate contents of the soil, as determined according to Subrahmanyam, Narayanayya and Bhagvat (1934), were as follows (Table I).

TABLE I.

Form of carbon	Alluvial Soil	Black Cotton Soil	<i>Kalar</i> Soil	Laterite Soil
Total (per cent.) ..	1.81	1.31	1.26	0.60
Carbonate (per cent.) ..	Nil	0.41	0.66	Nil

The molasses used for treating the soils was a solidified product obtained from the local distillery. Its total carbon content was 30.7 per cent.

The changes in organic matter were followed by determining total carbon at each stage. This procedure was considered to be the most satisfactory, previous experience having shown that (a) quantitative estimation of the individual products of decomposition would, in addition to being tedious, be also incomplete; (b) methods of extraction with different solvents followed by ignition or estimation of carbon in each fraction would be only arbitrary and could not throw much fresh light on the distribution of organic matter. The estimations of carbon were carried out by the method of Subrahmanyam *et al.* (*loc. cit.*), which, in addition to being simple, also yielded highly accurate results.

The distribution of organic matter in each sample was studied under the following heads.—(a) soil system as a whole, (b) supernatant layer of liquid containing a large part of the water soluble constituents, and (c) soil sediment including the major part of humus and other insoluble forms of organic matter. The total carbon in the original system being known, it was possible, from the above determinations, to follow the gain or loss of carbon at each stage.

Owing to the uneven distribution of organic matter under field conditions (Sreenivasan and Subrahmanyam, 1934), it was considered necessary to conduct the trials with small, uniform samples of soils, the entire quantity being used in each case for the estimation of carbon. The error of random

sampling was thus avoided. The procedure adopted for the study of the distribution of carbon may be outlined as follows.—The sample for examination was placed on a rack and an aliquot (10—15 c.c.) of the supernatant pipetted out directly into the apparatus for carbon estimation, care being taken not to disturb the soil sediment. The estimation of total carbon in the liquid was then carried out in the usual way. It was sometimes necessary to dilute the fluid with water so that sufficient steam may be generated to displace all the carbon dioxide resulting from the oxidation. In every case, care was taken to add at least twice the volume of concentrated sulphuric acid as that of the fluid mixture to be digested (including the added water) so that the oxidation of carbon may be complete. The soil sediment, together with the residual supernatant, was then digested separately, the details of estimation being the same as those recommended for the estimation of carbon in swamp soils (Subrahmanyam *et al.*, *loc. cit.*). Since the total volume of supernatant in each sample was known (and maintained constant throughout the present study), the total carbon in the entire supernatant was easily calculated from the first estimate. The total carbon in the soil sediment alone was also calculated in a like manner. The sum of the two estimates gave the total carbon in the soil system. At each stage, the estimations were carried out in duplicate and the averages recorded in the various tables and figures.

There are three important stages in the cultivation of the swamp soil which may be normally expected to affect the distribution of organic matter—(A) the puddling stage during which the soil is essentially water-logged and when the added organic matter undergoes active fermentation; (B) the flooding period when the irrigation water (or rain or seepage water from a higher level depending on the situation of the land and mode of cultivation) flows gently over the land and which would, to a large extent, carry away the water soluble constituents present in the soil system; (C) the desiccation stage which commences with the ripening of the crop. The irrigation is stopped and the soil allowed to dry. This condition does in fact continue—allowing for wetting by occasional rains—until the soil is puddled preparatory to the sowing (or planting) of the next crop. The present study was accordingly divided into three stages each one of which was further sub-divided into short intervals of 4 days when the samples were examined in the manner described already.

Influence of the Nature of Soil on the Distribution of Carbon.—Samples (10 g.) of the different soils were weighed out into short (height, 10 cm.) wide-necked (diam., 3 cm.) tubes. They were then treated with solution of molasses in quantities corresponding to 30.7 mg. of carbon each. It was reckoned that

the molasses thus applied would approximate to 10 tons per acre on the field. Sufficient water was then added to the suspension so as to make the proportion of soil to solution as 10 to 25 (*vide* Bhaskaran *et al.*, *loc. cit.*). The tubes were then plugged with cotton wool and incubated at 30° over a period of 65 days. At short intervals (4 days in the early stages, and 7 or 8 in the later ones), tubes representing each soil were taken out and the distribution of carbon studied in the manner outlined already.

As usually done in field practice, the initial fermentation of molasses was allowed to proceed for 25 days. This would correspond to the period of puddling (stage A). After this, the tubes were flooded with water and the excess allowed to flow out, care being taken to see that the layer of soil was not disturbed by the process. The flooding was thus continued at short intervals over a period of 15 days, the samples being incubated at 30° at other times. This would correspond to stage (B) previously described. When it was found that further flooding produced no perceptible change in the distribution of carbon in the soil system, the contents of the tubes were allowed to dry at 30°. It was expected that this treatment would correspond to drying prior to harvest (stage C). Three samples were taken during the period of drying and the attendant changes studied. The observations were not further extended as it was found that the subsequent changes were very slow.

The results have been presented in Figs. 1-4.

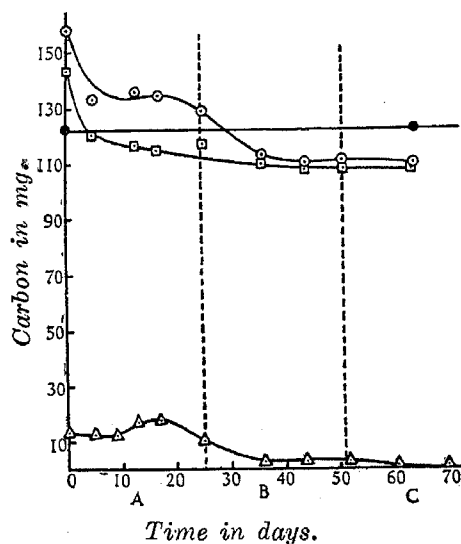


FIG. 1. Alkali soil.

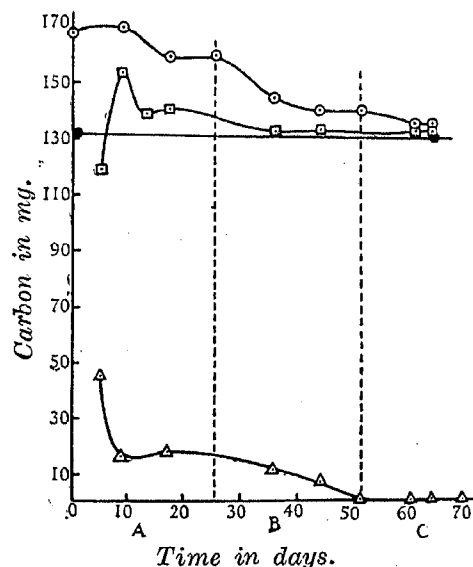


FIG. 2. Black cotton soil.

It may be noted that there was marked fall in total carbon after the first few days. The decline then slackened and the carbon content became more or less stationary at the end of three weeks. The commencement

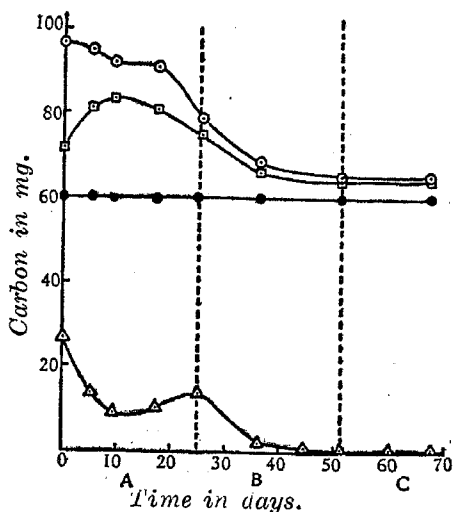


FIG. 3. Laterite soil.

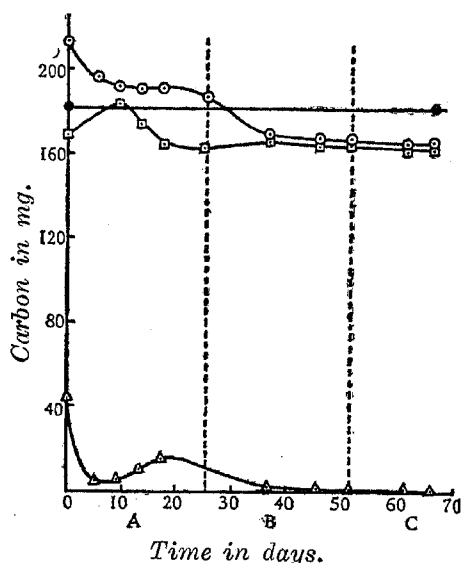


FIG. 4. Alluvial soil.

FIGS. 1—4. Influence of the nature of soil on the distribution of Carbon.

- Total carbon in the system.
- Carbon in the soil sediment.
- △—△ Carbon in the supernatant.
- Carbon in the original soil.

of flooding on the 25th day caused a further fall and a new low level was attained by about the 35th day. In two of the soils (the black cotton and the laterite), the total carbon content was, by then, reduced to about the same level as in the original soil, while, in the other two (*Kalar* and alluvial), a still lower figure was attained. Extension of the period of flooding did not cause any further lowering in the carbon content. During the period of drying, the level of organic matter remained more or less the same, thereby showing that there was no further change in the soil system.

In all the cases, the supernatant suffered an initial loss thus showing that a part of the molasses (which accounted for the bulk of soluble organic matter) was being either converted into insoluble forms or lost altogether from the soil system. The former was obviously the case, since during the same interval, there was a corresponding rise in the carbon content of the soil sediment. In the latter stages, however, there was marked loss of carbon, presumably in the form of gas. The greatest loss was observed during the period of flooding when the carbon content became practically nil. During drying, there was steady diminution in the volume of the supernatant which thus became more concentrated but there was little organic matter in solution.

As already indicated, the soil sediment gained in the early stages through a part of the organic matter in the supernatant passing into insoluble forms. The subsequent changes followed the same course as that of total carbon. The maximum loss occurred during flooding. Since there was no organic

matter in the supernatant, the level of carbon in the soil sediment ultimately became identical with that of total carbon in the soil system.

It may be seen from the foregoing observations that practically all the added carbon (and in two cases even a part of that originally present in the soil) was lost when the land was flooded. If it is reckoned that, in field practice, rice is transplanted about three weeks after application of molasses and that the irrigation water is then allowed to flow over the land, it would follow that the bulk of the added organic matter would be lost from the soil system even before the crop can establish itself. It may, however, be reasonably expected that the removal of soluble forms under field conditions will not be so complete as that obtained with the small quantities of soil used in the present study. It is, indeed, probable that a small part of the soluble products will still persist in the soil and be taken up by the growing crop. It should, nevertheless, be admitted from the present evidence that a considerable part will be normally lost from the soil system and should, if possible, be retained for the benefit of the subsequent crop.

It has been recorded by a number of previous workers (*vide* Subrahmanyam, *loc. cit.*) that the immediate products of fermentation of molasses are toxic to plant growth. It is not clear however whether the adverse effect is due to any direct action or to some of the mineral constituents (such as ferrous iron and aluminium) brought into solution during fermentation. Since the toxicity is known to wear off at the end of about three weeks of puddling, it would appear that subsequent removal of organic matter by the irrigation water or through drainage is unnecessary. In fact, if the carbonaceous matter present at that stage can be converted into insoluble forms, they will continue to remain in the system adding to the humus content of the soil. The crop which will then be putting on active vegetative growth will benefit from the added organic matter. The fertiliser will also acquire a residual value.

In the cases of the black cotton and the laterite soils, it is clear that the various transformations related primarily to the added molasses and that after its removal, partly by fermentation and partly by flooding, the soil got back to its original level of organic matter. There would no doubt be changes in the soil structure, especially in the surface layers (Sreenivasan and Subrahmanyam, 1934), but no other marked change may be reasonably expected within the period of observation. The position was rather different with regard to the alkali and alluvial soils in which some of the original carbon was also affected. In the former case, additional loss of carbon may be explained by the interaction between the soil carbonate (0.66 per cent.) and the organic acids produced during the fermentation. A similar explanation

Economy of Carbon at Different Concentrations of Molasses.—The previous observations having shown that the fermentation follows about the same course in all the soils, the present experiments were conducted only with the laterite specimen. The samples (10 g. each) were weighed out in three different batches which were treated with molasses in quantities corresponding to 0.5, 1.0 and 2.0 per cents. respectively. The details of subsequent treatments such as incubation, sampling and analysis were similar to those of the previous series. The results have been presented in Table II.

As may be naturally expected, the carbon contents of the supernatant and the soil sediment increase with the quantity of molasses. With the progress of the fermentation, the samples receiving 2 per cent. of molasses suffer the greatest loss of carbon, first from the supernatant and then from the sediment. When the soil was flooded, practically all the added carbon was lost. A small quantity (equivalent to 7 mg. of carbon for every 10 g. of soil) did however persist in the specimens treated with 2 per cent. molasses. It continued to remain there even after drying for a fortnight, so that it may be regarded as a gain to the soil system. In the other two cases, the added carbon was completely lost and the soil returned to its original level of organic matter.

The foregoing observations show that with increasing concentrations of molasses, there was correspondingly greater loss of carbon during flooding. Heavy applications of molasses would no doubt bring large quantities of minerals in solution and even bring about marked changes in the ultimate soil structure, but only a small part of the added organic matter would persist in the soil system.

Influence of Temperature on the Distribution of Organic Matter.—Since the utilisation of cane molasses as a fertiliser will be largely in the tropics, the present experiments were carried out at temperatures prevailing under such conditions. As in the previous series, the trials were confined to the laterite soil. The samples (10 g. each) were treated with molasses in quantities corresponding to 1 per cent. on the weight of the soil and divided into three batches which were incubated at 30°, 37° and 45° respectively. At stated intervals, representative specimens were taken out and analysed for their distribution of carbon. The results obtained at 45° and 37° have been presented in Figs. 5 and 6 respectively. The data obtained at 30° were the same as those given in Fig. 3.

It may be observed that the course of decomposition was about the same in all the cases. The loss of carbon proceeded most rapidly at 45°, there being steady decline both from the soil system, as a whole, and from the

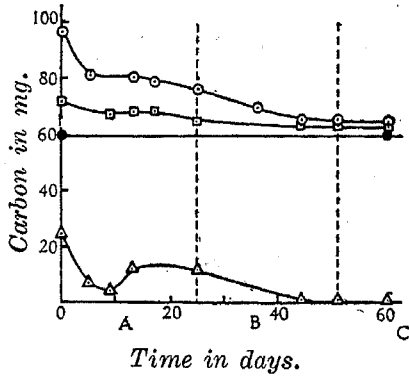


FIG. 5. Temperature, 45°.

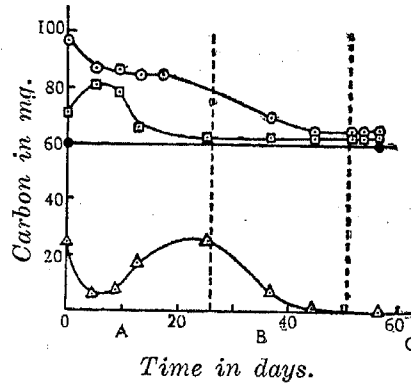


FIG. 6. Temperature, 37°.

FIGS. 5-6. Influence of temperature on the economy of carbon.

- Total carbon in the soil.
- Carbon in the soil sediment.
- △—△ Carbon in the supernatant.
- Carbon in the original soil.

sediment. There was a momentary rise in the organic matter content of the supernatant, but that too suffered rapid fall after the commencement of flooding. At the lower temperatures the loss of carbon from the soil system proceeded more slowly: the supernatant gained to a marked extent after the 5th day, though, after the 25th, all the soluble forms were removed by flooding. It would thus be seen that the rise in temperature hastened the various changes, especially the loss of carbon during puddling: but after the soil was flooded, the organic matter dropped to the level of the original soil in all the cases.

Effect of Surface on the Economy of Carbon.—Although the foregoing observations represent broadly the types of changes that attended the application of molasses to soil, they do not yet present a quantitative picture of the distribution of carbon under field conditions. This is largely due to the fact that the experiments were conducted in tubes which were comparatively narrow and did not therefore permit of free access of air as would normally be the case on the field. With a view to determining the effect of surface on the distribution of carbon, some experiments were conducted weighing out the soil into open, shallow dishes and, after adding molasses and water as usual, comparing the attendant changes with those in the previous experiments. A control series was also carried out, incubating suspensions of untreated soil (without molasses) at 30°. The results have been presented in Table III.

A comparison with the figures in Table II would show that in shallow dishes the fermentation and consequent loss of carbon during puddling proceeded very much more rapidly than in the narrow tubes. Except for a momentary rise in the soil sediment, there was steady loss of carbon from

TABLE III.

Effect of Increased Surface on the Economy of Carbon.

Time in days	Carbon in mg.						
	Untreated soil (control)			Molasses (1 per cent.) in shallow dishes			
	Super-natant	Sediment	Total	Super-natant	Sediment	Total	
A. PUDDLING							
0	..	2.4	58.0	60.4	24.8	71.9	96.7
5	..	9.5	51.7	61.2	5.4	75.8	81.2
9	..	0.9	59.2	60.1	6.3	73.5	79.8
13	4.2	70.5	74.7
17	..	0.3	60.5	60.8	4.9	68.3	73.2
25	1.9	70.9	72.8
B. FLOODING							
36	..	0.9	59.0	..	4.0	61.7	65.7
44	..	1.0	58.2	59.9	4.3	58.2	62.5
51	..	1.1	57.0	58.1	0.5	63.1	63.6
C. DRYING							
63	..	0.0	57.2	57.2	0.0
64	..	0.0	60.0	60.0	0.0	59.7	59.7
67	..	0.0	59.0	59.0	0.0	60.2	60.2

the former and by the time the specimens were flooded, the major part of the added carbon had passed away in the gaseous form. There was further loss on flooding with water and on the 36th day, the organic matter content of the soil came down to nearly the same level as the original soil. There was no further change during drying.

Since the foregoing transformations proceeded in presence of liberal supply of air, it may be assumed that the attendant changes were largely of the nature of oxidation. Since the chief gaseous product formed under

such conditions is carbon dioxide (Bhaskaran *et al.*, *loc. cit.*; Narasimhamurthy, unpublished data) it may be inferred that the added carbon was mostly lost in that form.

The control experiments with the untreated soil would show that there was comparatively little change during a period of two months. In the early stages, there was slight increase in the carbon content of the supernatant: the forms thus brought into solution soon became insoluble and passed again into the sediment. The original conditions were thus restored. The subsequent transformations, both during flooding and drying, were negligible.

Influence of the Degree of Submergence.—Under normal conditions, the rice field is submerged under water to the extent of only a few inches. There are other conditions, however, such as high level in the irrigation channel, and heavy rains or floods when the depth of water is greatly increased. It would be of much practical interest therefore to determine the effect of varying proportions of water on the progress of fermentation and distribution of organic matter. Some experiments were accordingly conducted with the laterite (Bangalore) soil. After treating the samples (10 g. each) with 1 per cent. of molasses, varying quantities of water were added, so that the proportion of soil to solution were as 10 : 10, 10 : 25 and 10 : 50 respectively. The incubations were at 30° as in the previous trials. The results obtained at ratios of 10 : 10 and 10 : 50 have been presented in Figs. 7 and 8, while those secured at 10 : 25 were the same as those given in Fig. 3.

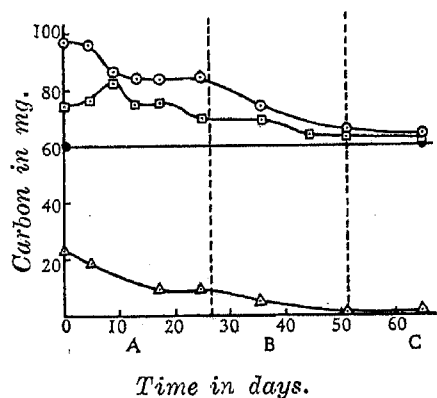


FIG. 7. Proportion of water to soil. 10 : 10 (by weight)

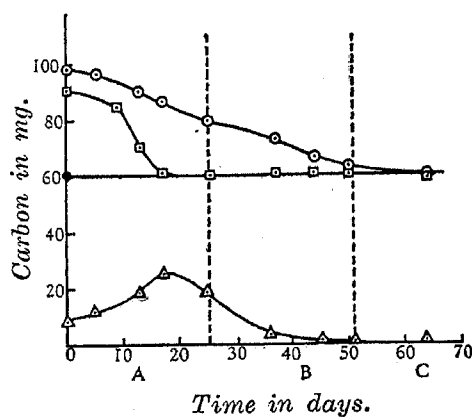


FIG. 8. Proportion of water to soil. 50 : 10 (by weight)

Figs. 7—8. Effect of changing the proportion of water to soil on the distribution of carbon.

- Total carbon in the soil.
- Carbon in the soil sediment.
- △—△ Carbon in the supernatant.
- Carbon in the original soil.

It may be noted that the quantities of organic matter passing into the supernatant generally tended to increase with the proportion of soil to water.

When the ratio was 10 : 50, almost the entire quantity of added carbon passed into the supernatant and sediment came to the level of the original soil even before the end of the puddling period. The supernatant retained a large part of the organic matter until the flooding period when the entire quantity passed out into the drainage. When the ratio of soil to water was narrowed, the sediment tended to retain a useful part of the organic matter and continued to do so throughout the period of observation. There was, no doubt, some loss during puddling and further depletion after flooding, but a small part of the added organic matter was always present in the soil system.

The chemical nature of the substances involved in the foregoing transformations is still awaiting systematic enquiry. It has already been shown that the sugars disappear completely within the first three days (Bhaskaran *et al.*, *loc. cit.*). It may be assumed therefore that the subsequent changes relate largely to the non-gaseous products of fermentation—chiefly organic acids and their salts. These being mostly water soluble, it may be reasonably expected that a considerable part will pass into drainage and thus be lost from the soil system.

Discussion.

The present enquiry has brought into relief certain important features in regard to the economy of carbon in the swamp soil. They show that, in addition to the possible losses in the gaseous form, there is a still greater danger of the removal of a considerable part of the added organic matter during flooding.

The problem of conservation of organic matter in the swamp soil would naturally resolve itself into one of (a) reducing the loss of carbon in the gaseous form and (b) obtaining, as far as possible, products, which would not be easily leached out by water but which would, nevertheless, remain in the soil system and be available to plant nutrition. Some useful work has already been done on the first aspect but practically nothing is known regarding the second one.

It is well known that when substances with wide C-N ratios are applied to the soil, there is considerable loss of carbon in the gaseous form until the ratio attains a low level of about 10—1. If, to such substances, nitrogen in some readily available form is added, the loss of carbon is greatly retarded. These principles have been successfully applied to the conservation of organic matter in the dry cultivated soil, but comparatively little is known regarding their extension to swamp conditions. The more recent work of Sreenivasan and Subrahmanyam (1935) would, indeed, show that while the loss of nitrogen from the readily fermentable material, can, to some extent, be retarded by suitable adjustment of the C-N ratio, the economy of carbon could not be

ensured by such a treatment. Further systematic work is needed, therefore, to clarify the position in regard to this important aspect of the problem.

The influence of the C-N ratio of organic manures on the yield of crop from the swamp soil is still obscure. Itano and Arakawa (1927) studied the effect of applying different organic materials to rice fields in Japan but could not find any definite correlation between the C-N ratio and the yield of crop. Osugi, Yoshie and Komatsubara (1931) adduced evidence to show that transformations of organic matter in the swamp soil are determined not by the gross quantities but by the availabilities of different forms of carbon and nitrogen. It would thus appear that (a) the chemical nature of added organic materials would determine, to a large extent, the economy of carbon in the swamp soil and (b) adjustment of the C-N ratio of a manure so as to avoid loss of either of those constituents may not always lead to the benefit of the crop that follows immediately after the application. The above observations will apply particularly to the use of molasses as fertiliser because (a) that product is easily fermentable and will require a highly reactive form of nitrogen to retain the products in the soil system and (b) as already indicated (Bhaskaran *et al.*, *loc. cit.*) the fertilising action of molasses is largely due to the indirect action of the products of fermentation. A useful procedure will therefore be to first take advantage of the beneficial effects of the fermentation and then to modify the conditions in such a manner that the water soluble forms will be converted, through microbial agency, into insoluble products. The latter will add to the humus content of the soil and thus impart a residual value to the fertiliser.

Further work along these and allied lines is in progress and will form the subjects of later communications.

Summary.

(1) When molasses was applied to the swamp soil, a part (about 30 per cent.) of the added organic matter passed into the soil sediment and the rest into the supernatant. During puddling, there was much loss of carbon in gaseous forms, the supernatant suffering more heavily than the sediment. There was still greater loss immediately after flooding, almost the entire quantity of the added organic matter being carried away by the water. There was no appreciable change in the carbon content of the soil system during the short period of drying that followed.

(2) The transformations of organic matter followed about the same course in all the four types (laterite, alluvial, black cotton and alkali) of soil that were examined. In two of the cases, the stage of equilibrium, reached after flooding, corresponded to that of the original soil, while, in the other two, a still lower level was reached.

(3) The distribution of carbon in the soil system was not appreciably influenced by the concentration of the molasses. Flooding removed the bulk of the added organic matter in all the cases, but there was a small, non-leachable residue left at the highest concentration (2 per cent.) that was tried.

(4) Increase of temperature hastened the rate of fermentation during the puddling period. There was also greater loss of carbon, chiefly from the supernatant.

(5) Fermentation in shallow, open vessels led to greater loss of carbon than that in narrow tubes. The loss was almost exclusively from the supernatant. The organic matter of the soil sediment continued practically unaffected until the flooding period when that too was rapidly reduced to the original level.

(6) With increasing proportions of water to soil, larger quantities of organic matter passed into the supernatant. There was not much loss of carbon during puddling, but, as in the other cases, flooding removed almost the entire amount of added organic matter from the soil system.

(7) The practical significance of the foregoing and allied observations has been discussed. Attention is drawn to the need for converting the major part of the added organic matter into insoluble forms before flooding period. Certain lines of work leading to that end have been indicated.

REFERENCES.

- Bhaskaran, T. R., Narasimhamurthy, G., Subrahmanyam, V., and Sundara Iyengar, B. A. *Proc. Ind. Acad. Sci.*, 1934, 1B, 155.
- Harrison, W. H., and Ayyar, P. A. S. *Mem. Dept. Agric. India, Chem. Ser.*, 1913, 3, 65; *Ibid.*, 1914, 4, 1; *Ibid.*, 1916, 5, 1; *Ibid.*, 1916, 5, 173; *Ibid.*, 1920, 5, 183.
- Itano, A., and Arakawa, S. .. *Ber. Ōhara Inst.*, 1927, 3, 331.
- Onodera, I. *Ber. Ōhara Inst.*, 1923, 2, 361.
- Osugi, S., Yoshie, S., and Komatsubara, I. *Mem. Coll. Agric. Kyoto*, 1931, 12, 1.
- Sreenivasan, A., and Subrahmanyam, V. *J. Indian Inst. Sci.*, 1934, 17A, 113.
- Sreenivasan, A., and Subrahmanyam, V. *Proc. Ind. Acad. Sci.*, 1934, 1B, 123.
- Sreenivasan, A., and Subrahmanyam, V. *J. Agric. Sci.*, 1935, 25, 6.
- Subrahmanyam, V. *J. Agric. Sci.*, 1929, 19, 627.
- Subrahmanyam, V. *Curr. Sci.*, 1933, 2, 195.
- Subrahmanyam, V., Narayana, Y. V., and Bhagvat, K. *J. Indian Inst. Sci.*, 1934, 17A, 197.