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EFFECT OF SALINITY ON ^{14}C-LABELLED MICROBIAL BIOMASS AND ITS CONTRIBUTION TO SOIL ORGANIC MATTER*

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Abstract

Estimation of soil microbial biomass using fumigation technique revealed a decrease in its content with increase in soil salinity and the microbial biomass was found to be directly proportional to the CO_2 evolution from soil. Biomass was estimated to be 4-5% of the residual organic carbon. ^{14}C component derived from labelled plant material ranged from 31-63% of the total biomass; maximum being in normal soil and minimum in saline-sodic soil. The results of organic matter fractionation revealed a higher ^{14}C activity in fulvic and humic acid extracted from fumigated than that extracted from untreated soil. This difference has been taken as an evidence of direct contribution of microbial biomass to humic compounds.

Introduction

Microbial activity in saline soils plays an important role in the amelioration of salt affected soils (Malik, 1978). Sandhu & Malik (1975) proposed a plant succession scheme on such soils with the object of raising the biological activity by growing salt tolerant plants and then ploughing them under as a source of organic matter. Decomposition of plant residues allows release of CO_2 which helps in the solubilization of $CaCO_3$ already present in these soils. During this process, the stable organic matter fraction which is so vital for soil fertility is increased resulting in the improvement of soil chemical properties.

The involvement of soil microorganisms in the decomposition and humification of organic residues and their transformation to stable organic matter fractions in soil is well known (Kononova, 1966). Some of these microogranisms are also known to synthesize certain phenols which polymerize to form large polymers of humic compounds

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(Haider & Martin, 1967; Visser, 1968). Microbial melanins have also been found to be similar to humic acids as regards their stability (Haider *et al.*, 1974; Filip *et al.*, 1974; Meuzelaar *et al*;, 1977; Linhares & Martin, 1978) thus indicating their direct contribution to the stable organic matter fraction.

Such melanoid microorganims especially fungi are of quite common occurrence in soil (Warcup, 1967) and their role in the decomposition of plant residues in saline soils has been previously reported (Malik & Sandhu, 1973a; Malik *et al.*, 1979). Hence, quantitative estimation of soil microbial biomass can indicate its contribution towards stable organic matter fraction. Moreover such information can be useful in determining the potential nutrient supplying capacity of the soil.

Various physiological methods have been used recently in which the respiratory activities of substrate-supplemented habitats are used to estimate relative proportion of metabolizing biomasses in soil (Wright & Hobbie, 1966; Anderson & Domsch, 1978). However, another method proposed by Jenkinson & Powlson (1976) seems to be simple, easy to handle and gives the most direct estimation of actual weight of microbial biomass. In this method the carbon bound in the microbial biomass is released by microbial mineralization and thereby provides a mean for calculating its weight. Therefore, this method was used in the present investigation.

The objective of the studies reported here was, therefore, to estimate the microbial biomass originating from the decomposition of uniformly ^{14}C labelled Kallar grass (*Diplachne fusca* Linn. (Beauv.) a salt tolerant grass used as primary colonizer of salt affected soils) and to determine the transformation and distribution of ^{14}C activity in various organic matter fractions both due to the humification of the plant residues and due to that of microbial biomass.

Materials and Methods

The soil samples were collected from top 15 cm of three different fields around NIAB. All these soils were loamy in texture (Table 1). Classification of salinity as proposed in the USDA Handbook 60 (1960) has been followed. Soil samples were passed through a 2 mm sieve before use.

Uniformly ^{14}C labelled Kallar grass was obtained by growing it in air tight perspex field canopy securely sealed on to a 10 cm high stainless steel frame which was driven into the soil to a depth of 7 cms. The $^{14}CO_2$ generated by adding a solution of $Na_2{}^{14}CO_3$ to lactic acid. was circulated by a double diaphragm pump through a closed system which included the $^{14}CO_2$ generating flask, the canopy and a counting chamber where the radioactivity is constantly monitored and kept at a certain level by adding more labelled carbonate to the acid. The stainless steel frame was internally lined with copper

Table 1. Chemical properties of soils.

Soil	Electrical conductivity (Sm^{-1})	pH	Na	K	Ca+Mg	Sodium adsorption ratio	Exchangeable sodium percentage	% Organic C
			——meq/l——					
Normal	0.40	7.9	18.0	1.3	31.8	4.5	5.12	1.055
Saline	0.99	8.4	28.7	0.6	34.3	6.9	8.17	0.538
Saline-sodic	0.86	8.6	61.3	3.8	26.2	16.9	19.16	0.511

tubing through which chilled water was circulated and the inside temperature thremostatically controlled. Plants were grown for 4 weeks after which these were harvested, dried, powdered and a sample fractionated for confirming the uniformity of the label.

The sieved and air dried soil in 150 g portions was taken in 250 ml Erlenmeyer flasks and mixed thoroughly with 1.0% ^{14}C-labelled powdered Kallar grass plant material (sp. activity 10.10 uCi/gC; C. 42%). The soil was brought to 60% WHC and the flasks attached to an aeration system providing CO_2-free and humidified air. Three replicates were kept for each soil. Incubation was carried out for 60 days at a temperature of 27-30°C. CO_2 evolved was absorbed in 10% NaOH solution which was regularly replaced by fresh volume. This was analysed for total CO_2 by titrating the excess alkali against HCl and $^{14}CO_2$ by taking 1 ml of alkali in scintillation vial and adding 2 ml of H_2O and 7 ml of Dimilume scintillator (Packard, Frankfurt). Radioactivity was measured in a liquid scintillation counter (Packard Tri-Carb 3320).

After 60 days of incubation, soil from three replicate flasks was mixed together and used for microbial biomass estimation (Jenkinson & Powlson, 1976). Soil in 100 g quantities from each treatment, in duplicate, were fumigated with chloroform. The flasks were attached to the aeration system as explained above. Similarly unfumigated soil was also aerated and at the end of the incubation period of 10 days, CO_2 and $^{14}CO_2$ was estimated. The microbial biomass was estimated by the formula B=F/K where B=soil biomass carbon mg/100 g soil; F=CO_2 evolved by fumigated soil minus that evolved from unfumigated soil incubated for the same time under the same conditions; K=the fraction of the biomass-C mineralized to CO_2 during the incubation following fumigation. Value of K has been determined experimentally by Jenkinson (1976) equal to 0.5 (i.e. 50% mineralization during the first 10 days following fumigation). For calculating biomass, an assumption was made that 'K' is same in different soils, provided the conditions are not adverse to aerobic microbial activity in the period after fumigation.

After the termination of incubation period, portions of soils from both fumigated and unfumigated treatment were fractionated into humic acid, fulvic acid and humins (Kononova, 1966). Radioactivity in all these fractions was estimated by using wet combustion method (IAEA, 1976).

Results

The results of the rate of mineralization of ^{14}C-labelled Kallar grass plant material in saline-sodic (S3), saline (S2) and normal soil (S1) are summarized in Fig. 1. The rate of total CO_2 evolved (including $^{14}CO_2$) is also presented (Fig. 1). In normal soil, about 40% ^{14}C was lost as $^{14}CO_2$ after 60 days of incubation. In case of saline soil (S2) and saline-sodic soil (S3) 30% and 20% of ^{14}C was lost as $^{14}CO_2$ after an incubation period of 60 days. However, total CO_2 was always more than $^{14}CO_2$ in all the treatments.

After the termination of incubation period, estimation of microbial biomass was made. The results of the estimation based on $^{14}CO_2$ evolution are summarized in Table 2 whereas that based on total CO_2 evolution are presented in Table 3.

Maximum biomass ^{14}C amounting to 25.22 mg/100 g soil was estimated in case of normal soil whereas in case of saline and saline-sodic soil it was 12.88 mg and 9.08

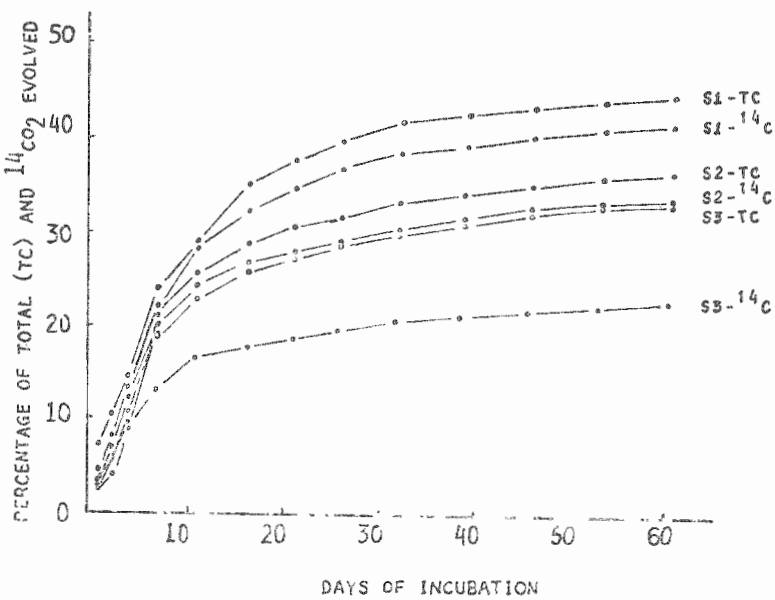

Fig. 1. Decomposition of ^{14}C labelled Kallar grass during incubation in soil. S1 = Normal soil; S2 = Saline soil; S3 = Saline sodic soil; TC = Total carbon.

Table 2. ^{14}C-labelled biomass in three soils calculated from the increase in $^{14}CO_2$ evolution caused by fumigation with $CHCl_3$.

Soil	$^{14}CO_2$ evolved/100 g soil: Untreated soil 0-10 days (A)	Fumigated soil 0-10 days (B)	Flush of decomposition* (F=B-A)	Biomass ^{14}C** mg/100 g soil (F/K)	Residual soil ^{14}C in biomass	^{14}C labelled biomass, % of total biomass
	—dpm x 10^5—					
Normal	13.35	16.18	2.83	25.22	12.71	63.15
Saline	14.11	15.55	1.44	12.88	4.65	40.35
Saline-sodic	18.37	19.38	1.01	9.08	2.47	31.69

*$^{14}CO_2$ evolved by fumigated soil in 0-10 days period less that evolved by untreated soil during the same period.

**Calculated from flush using a K factor of 0.5 and the equivalence:

1 mg ^{14}C = 22424 dpm.

Table 3. Biomass content of three soils calculated from the increase in CO_2 evolution caused by fumigation with $CHCl_3$.

Soil	CO_2-C evolved/100 g soil					
	Untreated soil 0-10 days (A)	Fumigated soil 0-10 days (B)	Flush of decomposition* mg CO_2-C/100 g soil (F=B-A)	Biomass** mgC/100 g soil (F/K)	Soil organic C in biomass %	C content before fumigation mg/100g soil.
Normal	38.91	58.90	19.99	39.98	4.84	826.00
Saline	30.12	46.08	15.96	31.92	5.37	594.00
Saline-sodic	28.20	42.54	14.34	28.68	4.74	605.00

*CO_2-C evolved by fumigated soil in 0-10 days period less that evolved by untreated soil during the same period.
**Calculated from flush using a K factor of 0.5.

mg/100 g soil respectively. The pattern was thus similar to that of $^{14}CO_2$ evolution. The values when calculated on the basis of residual ^{14}C present in the soils, showed a maximum of 12.71% ^{14}C in biomass in case of normal soil and 4.65% and 2.47% in case of saline and saline-sodic soils respectively.

The biomass results based on total CO_2 evolution (Table 3) showed a similar trend but the biomass C values were much higher. The values of percentage organic carbon as biomass did not show much difference as the original organic carbon in three soils was not the same. However, the component of biomass derived from ^{14}C-labelled plant material was as high as 63.15% of the total biomass in normal soil and it decreased to 40.35% and 31.69% in case of saline soil and saline-sodic soil respectively.

The percentage distribution of ^{14}C activity in humic and fulvic acid fractions after incubation of fumigated and unfumigated soils is presented in Table 4. Relatively high ^{14}C activity was observed in humic and fulvic acid fraction of normal soil and was then followed by saline and saline-sodic soil. However, the ^{14}C activity in these fractions was always more in fumigated treatment than in unfumigated soil. This difference in percentage ^{14}C activity was more in case of fulvic acid fraction. Again, maximum difference was observed in the normal soil.

Discussion

Present investigations show that soil salinity has a depressing effect on the mineralization of labelled plant materials also previously observed (Malik *et al.*, 1979; Laura, 1974). In the present investigtion the effect of salinity and sodicity on the microbial biomass as estimated by fumigation method (Jenkinson & Powlson, 1976) has been observed. The soil biomass in the Rothamstead soils as estimated by Jenkinson & Powlson (1976) ranged from 1.7-3.7% of soil organic carbon. Similarly Ayanaba *et al.* (1976) also reported soil biomass in Nigerian soils, formally under tropical forest, to be around 2% of the total soil carbon. Lynch & Panting (1980) reported 1.6-2.7% soil biomass in cultivated soils in England. Oades & Jenkinson (1979) working in Australia, established a direct correlation between soil ATP and microbial biomass. In these studies as well the microbial biomass was around 2% of the soil organic carbon.

All these estimations of soil microbial biomass have been done on soils which have attained equilibrium regarding the organic matter dynamics. However, there are quantitative variations in soil biomass as a result of cultivation, plant growth and other ecological factors. Soderstrom (1977) used floursecein diacetate (FDA) staining technique to estimate active fungal biomass in pure forest soil in Sweden over a period of 27 months. The FDA-active biomass ranged from 0.5 and 2.4 g d.w.m^2. Recently Zafar (1980) estimated microbial biomass from different agricultural and forest soils which ranged from 1-5% of the organic C present in those soils.

Table 4. Per cent distribution of soil ^{14}C in humic and fulvic acid after incubation of fumigated and untreated soil for 10 days.

Soil	Humic acid			Fulvic acid		
	Fumigated (A)	Untreated (B)	A-B	Fumigated (A)	Untreated (B)	A-B
Normal	19.70	17.40	2.30	25.30	18.10	7.20
Saline	10.50	8.60	1.90	15.80	10.70	5.10
Saline-sodic	7.60	5.90	1.70	11.00	7.50	3.50

In the present studies, microbial biomass was found to be 4-5% of the soil residual organic carbon. Such relatively high values are due to the addition of plant material to the soil and then incubation for 60 days. However, the transformation of added plant material to the microbial biomass as determined by ^{14}C activity is much higher as it ranges from 9-25% of the ^{14}C-labelled plant material added to the soil.

Soil salinity has adversely affected the transformation of ^{14}C labelled plant material to microbial biomass. This correlates well with the rate of $^{14}CO_2$ evolution from the three soils amended with labelled plant material (Fig. 1). In the total biomass, ^{14}C-labelled component ranged from 31% to 63%; minimum being in case of saline-sodic soil and maximum in normal soil. Hence, it is evident that greater the CO_2 evolution, greater will be the microbial biomass.

The results relating to the organic matter fractionation of the fumigated and unfumigated soils after incubation of 10 days (Table 4) have indicated a direct contribution of microbial biomass to be humic and fulvic acid fractions. In our studies (Malik & Haider, 1980) with ^{14}C-labelled melanins of some dematiaceous fungi, the contribution to humic acid was in the range of 2-3%. In the persent report, ^{14}C activity in the humic and fulvic acid fraction was always higher in fumigated than in unfumigated treatments. This consistent difference can only be attributed to the transformation of killed microbes (due to fumigation) to humic and fulvic acid fractions during incubation period. Such transformation is not possible in case of unfumigated soils as microbial activity is normal in these soils and no new substrate (in the form of microbial debris) has been introduced.

The role of microbes in humification processes is quite well known. The participation of microbial melanins in the formation of humic compounds has also been reported (Haider & Martin, 1967, 1979; Visser, 1968; Martin & Haider, 1969) but there is not much information available on the fate of microbial debris in the soil and its capability to release nutrients. Recently Ladd *et al* (1977) have studied the release of nitrogen from soil biomass using fumigation technique. Ayanaba *et al.*, (1976) also demonstrated that fumigation and incubation of a range of Nigerian soils released mineral-N in amounts directly related to soil biomass-C in the ratio 1:8.

Based on some of the results presented here it can be expected to find a reasonably positive correlation between the humus content, the microbial biomass and decomposition rates in various types of soils. Further studies in this regard would help in estimating potential nutrients locked up (immobilized) in microbial bodies which can be made available (mineralizable) to the plants.

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