

## Relationship Between Microbial Numbers and Other Microbial Indices in Soil

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Pesticides reaching the soil may affect non-target organisms and their activities (Tu and Miles, 1976). To examine the effect of pesticides on microbial growth and activity, various parameters like microbial numbers, respiration, soil enzymes etc. are studied to draw definite inferences. Invariably the effect of pesticides are at first studied on total microbial counts and then later extended to one or two parameters. However, no one parameter has been found to be acceptable owing to complex and divergent activities of the microorganisms. The objective of the present work was to find out the relationship between the microbial numbers and that of other microbial parameters like CO<sub>2</sub> evolution and soil enzymes. Rice straw was used as an amendment as it is known to increase microbial activity in general and this will enable to discern the various activities in obvious manner.

### MATERIALS AND METHODS

Four different soil types were used and the characteristics are given in Table 1. Throughout the experiment the water level in soils was maintained at 60% m.h.c. of each soil. Two treatments were maintained for each soil: 1) unamended (control) soil, 2) rice straw amended soil. Soil was amended with 1% dried rice straw powder.

Table 1. Soil characteristics

Site of collection	Texture	pH	Organic C (%)
Trombay	Clay (black)	7.2	1.26
Trombay	Sandy loam (red)	6.8	0.46
Hatkanangale	Loam	8.2	1.32
Ratnagiri	Clay	6.1	0.52

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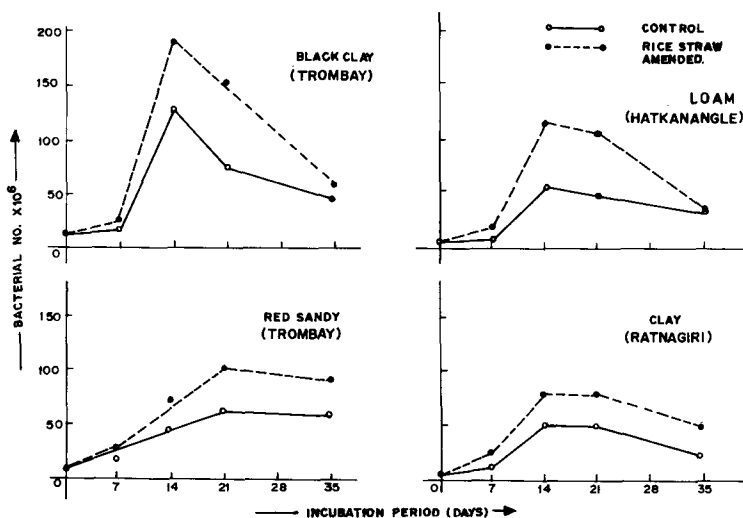


Figure 1. Viable plate counts of bacteria

Soil bacteria and fungi were enumerated by soil dilution plate technique (Johnson et al. 1958). For bacteria, the nutrient medium was used while for fungi Martin's medium (Rose bengal - streptomycin agar) was used. The plates were incubated at 28-30°C and the counts were taken after 4-6 days.

50 g of soil was used for measuring the evolution of CO<sub>2</sub> in a 250 ml conical flask. The flask was closed with a rubber stopper and they were loosened for a brief period every day to provide fresh air. A vial containing 3 ml of 1 N KOH was also kept in the same flask for trapping CO<sub>2</sub>. The trapped CO<sub>2</sub> was estimated titrimetrically (Anderson, 1982) at periodic intervals, and new vials containing fresh aliquots of KOH were introduced into the flask every time.

The method of Casida et al. (1964) was followed for measuring the soil dehydrogenase. The acid and alkaline phosphatases were studied using the method of Bremner and Tabatabai (1969). The pH was measured by soil : water mixture (1:2) using pH meter.

## RESULTS AND DISCUSSION

The number of bacteria that were enumerated are shown in Fig. 1. The soils were kept in moist conditions throughout the experiment which eliminates to some extent the dormancy factor. Rice straw amendment stimulated the bacterial numbers and the effect was continued for a period of five weeks. The stimulation ranged anywhere between 1.5 to 2.2 fold with rice straw amendment with only exception of Hatkanangale loam soil. The fungal numbers were also stimulated by rice straw amendment in general although random fluctuations were noticed (results not presented).

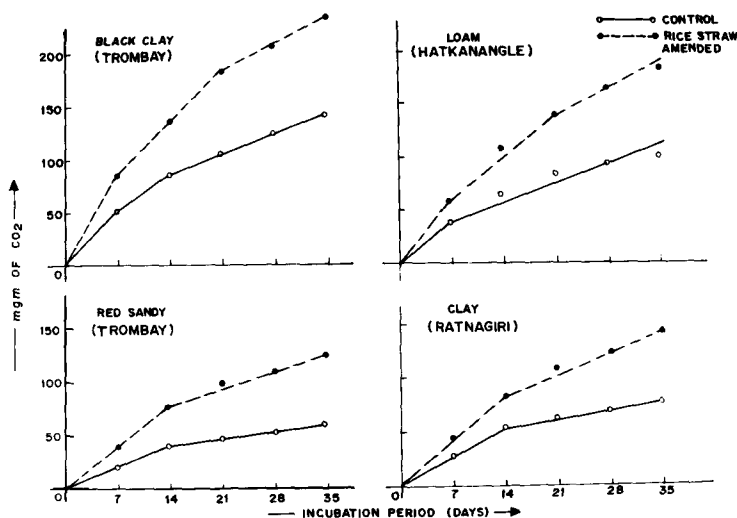


Figure 2. Soil respiration

Soil respiration as an indicator of biological activity in soils had been suggested by many workers (Frankenberger and Dick, 1983). Rice straw amendment stimulated soil respiration in all the soils ranging from 1.53 to 2.15 fold over a period of five weeks (Fig. 2). It is indeed interesting to note that stimulation of soil bacterial numbers also showed similar trend.

Soil dehydrogenase activity was greatly stimulated by rice straw amendment. In red sandy loam soil (Trombay) the formazan values were below the detectable limits ( $1.0 \mu\text{g}/10 \text{ g soil}$ ) under the assay conditions (Fig. 3).

Rice straw amendments also stimulated acid and alkaline phosphatases from anywhere between 1.1 to 2.1 fold in all the soils (results not presented). Eivazi and Tabatabai (1977) showed that acid phosphatase activity was more related to acid soils but in the present experiments there was no such correlation seen. However, alkaline phosphatase activity was more in alkaline soil of black clay (Trombay) and loam (Hatkanangale) as compared to other two soils.

A regression analyses of microbial numbers on other parameters of acid and alkaline phosphatases, dehydrogenase and  $\text{CO}_2$  evolution is presented in Table 2.

The microbial numbers were significantly correlated to acid phosphatase in amended Trombay (black) soil, to alkaline phosphatase in amended Ratnagiri and to dehydrogenase in Trombay (black and red) unamended soils but without any significant correlation to all other soils with or without amendment. Attempts were made by earlier investigators to correlate microbial numbers to enzyme activity but significant correlation could be accounted only in few cases. This is confirmed by the present experiments using four different soils with or without rice straw amendment.

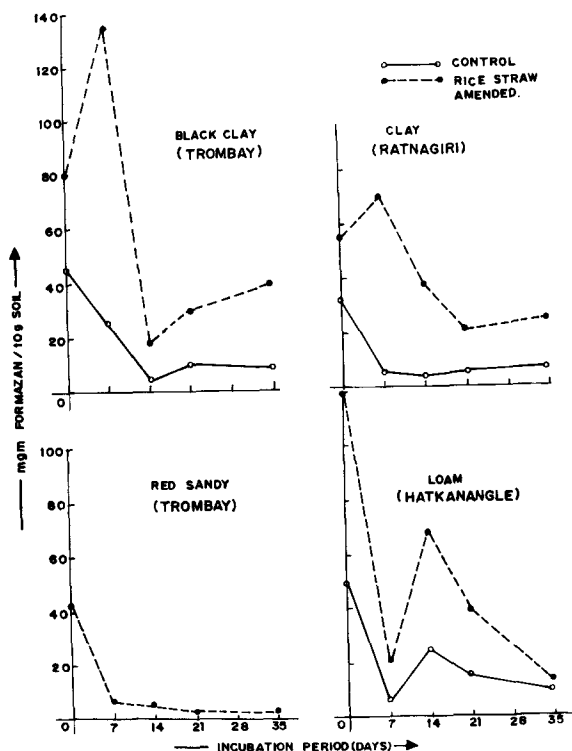


Figure 3. Soil dehydrogenase activity

Table 2. Regression analyses of microbial numbers on other microbial activities

Soil & Treatments		Acid phosphatase	Alkaline phosphatase	Dehydrogenase	CO <sub>2</sub> evolution
Trombay (black)	U	-0.5795	0.2030	0.8070*	0.5083**
"	A	-0.7065*	0.1159	-0.7323*	0.4360
Trombay (red)	U	0.366	0.348	-	0.047*
"	A	0.326	0.193	-0.766	0.932**
Hatkanangale	U	0.155	0.236	0.657	0.695*
"	A	0.116	0.255	0.366	0.535*
Ratnagiri	U	0.472	0.278	0.789	0.595*
"	A	0.617	0.706*	0.831	0.767*

U = unamended, A = amended with rice straw. \*P<0.05, \*\*P<0.01

However, significant correlation between microbial numbers and  $\text{CO}_2$  evolution was seen in all cases except in amended Trombay (black) soil (Table 2). Oxygen uptake and  $\text{CO}_2$  evolutions are the most frequently used and considered the most reliable methods for assessing the activity of microbial populations (Gray & Williams 1971). The estimation of microbial numbers is dependent on the media used. It may be difficult to get a significant correlation between enzymatic activity and microbial numbers as enzyme activity is subjected to random fluctuations. Many of the studies carried out to find the effect of xenobiotic chemicals on soil microflora involve soil microbial numbers and other parameters like  $\text{CO}_2$  evolution, soil enzymes etc. Since there is a significant correlation between the microbial numbers and  $\text{CO}_2$  evolution, the evolution of  $\text{CO}_2$  may serve as an indicator of the effect of these xenobiotics. Moreover,  $\text{CO}_2$  evolution could be conveniently and quantitatively estimated.

#### REFERENCES

- Anderson JPE (1982) Soil respiration. In: Page AL (ed) Methods of soil analysis. Part 2 No. 9 Agronomy Series. Ame Soc Agron Inc, Madison, p 831
- Bremner JM, Tabatabai MA (1969) Use of p-nitrophenyl phosphate for the assay of soil phosphatase activity. Soil Biol Biochem 1: 301-307
- Casida LE Jr, Klein DA, Dick WA (1964) Soil dehydrogenase activity. Soil Sci 98: 371-376
- Eivazi F, Tabatabai MA (1977) Phosphatases in soils. Soil Biol Biochem 9: 167-172
- Frankenberger WT Jr, Dick WA (1983) Relationship between enzyme activities and microbial growth and activity indices in soil. Soil Sci Soc Am J 47: 945-951
- Gray TRG, Williams ST (1971) Soil microorganisms. Longmann, New York
- Johnson LF, Curl EA, Bond JH, Fribourg HA (1959) Methods for studying soil microflora-plant disease relationships. Burgess Pub. Co. Minneapolis Minn USA
- Tu CM, Miles JRW (1976) Interaction between insecticides and soil microbes. Res Rev 64: 17-65

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