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Effect of Gross Pollution by Kerosine Hydrocarbons on the Microflora of a Moorland Soil

THE microbiology of the degradation of hydrocarbons in soil has been studied in some detail[1–4]; as well as the many bacteria able to oxidize alkanes, certain fungi are thought to be involved to a significant degree in moorland soil[5].

We have investigated the situation in two upland moorland sites called *A* and *B* in North Wales, which were polluted by the dumping of large quantities of an acidic sludge containing kerosine hydrocarbon. For analysis we used the method of Ramsdale and Wilkinson[6]. At site *A* the *p*H of contaminated soil was 2·0 and of uncontaminated soil was 4·6; at site *B* values were 1·0 and 4·2 respectively. The soil at site *A* was drier (moisture content, 71–175 per cent dry weight) than at site *B* (325–600 per cent dry weight). The sites were investigated during 7–10 months after the estimated date of pollution by methods based on those used previously[5,7]. Samples were taken at the same level inside and outside the polluted zones; numbers of viable bacteria and fungi were estimated standard media for plate counting. Hydrocarbon oxidizing microorganisms were estimated by membrane filtration of a suitably diluted soil sample followed by incubation of the filter at 10° C on a sterile glass fibre filter pad, soaked in a mineral salts medium to which either *n*-dodecane or crude oil diluted in *n*-hexane had been added. Plates were counted after 8, 14 and 16 days. The samples were analysed in a Gilson respirometer at 10° C. The results are the means of three samples at each site.

The microflora of the two sites responded differently to pollution by the kerosine. At site *A* bacterial and fungal counts declined significantly ($P = 0.05$), whereas at site *B* there was no significant difference in bacterial counts, and fungal counts increased ($P = 0.05$) (Table 1). Estimates of hydrocarbon oxidizers (Table 2) showed the same trend with a more marked response to hydrocarbon pollution at site *B*. Fungi able to metabolize hydrocarbons increased most here ($P = 0.01$) regardless of the hydrocarbon test substrate used. Oxygen consumption was not affected at site *A* (Table 3) but was stimulated to some extent by pollution at site *B*. The possibility of anomalous results due to a requirement for CO_2 by certain microorganisms was overcome by determination of total gas exchange. The calculated CO_2 evolution confirmed the stimulation of respiration in the polluted zone of site *B*. The addition of an emulsion of *n*-dodecane in distilled water to unpolluted soils inhibited respiration at site *A* but stimulated it at site *B*, indicating the ability of a proportion of the latter population to metabolize hydrocarbons. With soils from polluted zones results were variable and contradictory, and we are developing more reliable methods for estimating rates of hydrocarbon metabolism.

Estimates of soil respiration due to hydrocarbon metabolism have been extremely variable[8]. Some of the difficulties in interpretation may be a consequence of a limitation on mineral nutrients which resulted from removal to the laboratory, for upland peats such as these derive much of their mineral nutrients from rainfall. There is indirect evidence for this in the lack of response of microflora at the drier site and the pronounced adaptation in numbers of hydrocarbon oxidizers at site *B*, which is well flushed with water and presumably with mineral nutrients. The low *p*H of the oil spillage has presumably played some part in the response of the fungi.

The frequency of spillages on land in recent years led us to try a simple qualitative test for the response of fungi in soils. Small soil samples (about 5–10 g wet weight) were placed in sterile disposable Petri dishes and 0·5 ml. of test hydrocarbon was pipetted on to the soil, and the dishes were sealed. After various test conditions had been tried, we incubated this soil for 10 days at 20° C with *n*-dodecane as substrate. No fungal hyphae grew on site *A* soils (polluted or clean) but there was heavy growth on the clean soil of site *B*, indicating the ability of the fungal population at this site to adapt to hydrocarbon oxidation, and the polluted soil of site *B*, indicating the absence of inhibition by the spillage. There was no growth on control soil samples to which hydrocarbon had not been added.

Table 1. ESTIMATES OF VIABLE BACTERIA AND FUNGI AT SAMPLING SITES

Sample	Fungal counts $\bar{x}$/g dry wt	Bacterial counts $\bar{x}$/g dry wt
Site *A* clean	41×10^4	640×10^4
Site *A* polluted	11×10^4	34×10^4
Site *B* clean	76×10^4	640×10^4
Site *B* polluted	420×10^4	590×10^4

Table 2. ESTIMATES OF HYDROCARBON OXIDIZING BACTERIA AND FUNGI $\bar{x}$/g dry wt

Sample	Fungal counts		Bacterial counts	
	n-Dodecane	Crude oil	*n*-Dodecane	Crude oil
Site *A* clean	54×10^3	56×10^3	$15{\cdot}2 \times 10^3$	$25{\cdot}4 \times 10^3$
Site *A* polluted	$8{\cdot}2 \times 10^3$	10^3	$5{\cdot}5 \times 10^3$	$5{\cdot}5 \times 10^3$
Site *B* clean	46×10^3	21×10^3	$8{\cdot}4 \times 10^3$	10^3
Site *B* polluted	385×10^3	252×10^3	14×10^3	7×10^3

Table 3. RATES OF RESPIRATION OF SOIL SAMPLES

Sample	Mean rates (μl. of gas/h/g dry wt)		Percentage inhibition (−) or stimulation (+) in respiration on addition of *n*-dodecane
	O_2 uptake	CO_2 evolution	
Site *A* clean	13–22	6– 9	−12 to −60
Site *A* polluted	12–30	4– 9	− 5 to + 5
Site *B* clean	20–46	6–13	+ 7 to +20
Site *B* polluted	50–80	12–21	− 1 to − 8

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