Pentachlorophenol (PCP) has been used extensively worldwide as a preservative by the timber and textile industries because of its antimicrobial properties. This has resulted in large areas of land being contaminated (Crosby et al., 1981; Sato, 1983), which can affect soil microbial communities and, thereby influence the turnover of nutrients and soil fertility (Domsch et al., 1983; Welp and Brümmer, 1992). Most organic compounds are not perceived to be a threat to soil microbial processes (Wild et al., 1994). However, many xenobiotic organic compounds found in the environment, are known biocides and may negatively affect the soil microflora and its activities (Welp and Brümmer, 1992; Harden et al., 1993; Chaudri et al., 1996).

The aim of this paper is to determine whether the indigenous soil microbial populations can survive and utilize PCP as a carbon source. In this study, non-contaminated soil was amended with known concentrations of PCP. The soil microbial biomass-C content and the total number of aerobic heterotrophic bacteria were determined after chronic exposure. In addition, we investigated the possible use of PCP when presented as the sole carbon source, by the microbial populations that survived in the PCP-amended soils.

The soil used was a sandy loam (pH 7.0, 14% clay, 1.48% organic carbon, 0.15% total N) of the Cottonham series (Typic Udipsamment) from a long-term ley-arable field experiment at Woburn, southeast England. The plot sampled was under continuous grass for 8 yr during which no nitrogen fertiliser was added. Prior to this, farm yard manure at 38 t ha⁻¹ was added 35 yr ago. The heavy metal concentrations in the soil were (mg kg⁻¹): 30 Zn, 4 Cu, 17 Ni, <0.2 Cd, 28 Cr and 31 Pb. Moist soil was separated initially into 1 kg portions (dry weight basis), before amending with 25, 50, 75, 100, 175 and 200 mg kg⁻¹ PCP. The PCP (Sigma pure grade) was dissolved in methanol, as it is relatively insoluble in water. The treatments included two controls with either water or methanol (10 ml kg⁻¹) added, to ensure that the effects observed on the microorganisms were not due to the solvent. During and after treatment, the soils were thoroughly mixed using an electronic food mixer, then placed in plastic bags, and their moisture contents adjusted to 50% water holding capacity. The bags were loosely tied and stored at 20°C for 6 months in the dark.

Soil microbial biomass-C was determined using the method of Vance et al. (1987). Briefly, the same soil was split into two equal portions of known weight (on an oven dry basis), both at 50% water holding capacity. One portion was extracted with 0.5 M K₂SO₄ (1:4 w/v) before fumigation with chloroform and the other after fumigation for 24 h. The dissolved organic carbon in the K₂SO₄ extracts was measured using a high temperature carbon analyser (TOC 200, UV Developments, UK). The soil microbial biomass-C was then determined from the C flush (Vance et al., 1987). Total aerobic heterotrophic bacteria (TSBA) were counted, after 10-fold serial dilutions of the soil using sterile deionised distilled water, and tryptone soy broth agar (TSBA, Difco, UK) as the plating medium. TSBA colonies were counted at the appropriate dilution after 48 h incubation at 28°C.

PCP utilization by microorganisms in the stored treated soils were measured in vitro using microtitre plates. Either 40 μl methanol (plate/methanol control) or methanol containing dissolved PCP (final concentration in the assay of 12.5 mg PCP l⁻¹) were added to the wells in the microtitre rows; a row of wells was left...
without additions as a plate/water control. The methanol was allowed to evaporate completely for 12 h under vacuum, before adding 40 µl tetrazolium dye (0.1% w/v) and 120 µl sterile water to each well. Then 40 µl of a 1:100 (w/v) soil to water suspension of each of the PCP-amended soils was added to one column on the plate, giving 1 replicate well of each control and 6 replicates for each soil treatment. The bacterial counts in the 1:100 soil to water suspensions were standardised to 8000 cells ml⁻¹. The tetrazolium dye is light yellow, but it reacts with electrons produced by microbial respiration to give a violet colouration of the solution in the well. The colour change is proportional to the amount of respiration. If no electrons are produced, due to a lack of metabolic activity, then there is no or very little colour change. Any other effects, apart from respiration, of the soil in the assay were accounted for by the control wells which contained no carbon source. The microtitre plates were read approximately every 12 h for 220 h after inoculation using a Multiskan RC microtitre plate reader (Labsystems, UK).

Fig. 1 shows the effects of different concentrations of PCP in the soil after 6 months incubation on the soil microbial biomass-C and on the numbers of total aerobic heterotrophic bacteria. The methanol control contained 23% more biomass-C compared to the water control after 6 months. The soil microbial biomass-C was reduced by 56, 91, 77, 84, 69 and 22% at soil PCP concentrations of 25, 50, 75, 100, 175 and 200 mg kg⁻¹, respectively, compared to the methanol control (one-way ANOVA: variance ratio 190; P < 0.001). The increase in microbial biomass-C with methanol conflicts with the known biocidal effects of methanol. It is possible that the methanol initially killed a portion of the microorganisms leading to partial sterilisation and subsequent release of carbon and nutrients. These could then be utilized by the surviving organisms to make more biomass, hence the overall increase after 6 months. However, the increase in biomass-C cannot be explained by this release concept alone, and therefore, it is possible that some of the methanol remained in the soil and was utilized as a carbon source by the remaining population. Alternatively the surviving population was more able to utilize soil carbon sources which they could not do when in competition with the organisms killed by the methanol treatment. However, this effect of methanol was not seen in the PCP treatments, which all showed significant reductions in biomass-C (P < 0.001) compared to methanol alone. The soil microbial biomass-C in the 25 and 175 mg PCP kg⁻¹ soils was not significantly different to each other, but it was significantly greater than the 50, 75 and 100 mg kg⁻¹ soils, which did not differ significantly. The biomass-C in the 200 mg kg⁻¹ treatment was greater than all other PCP treatments. It seems that the toxic effects of PCP were so great as to annul any stimulating effects of methanol as a carbon source. Surprisingly, the lower concentrations of PCP were extremely deleterious to the soil microbial biomass.

Other workers have also found that small concentrations of PCP applied to soil significantly decrease the soil microbial biomass (Zeitz et al., 1987; Schönborn and Dumpert, 1990). For example, 12 applications of 5 g PCP m⁻² (calculated to be equivalent to approx. 25 mg PCP kg⁻¹ per application) every 2 months over 24 months in the field reduced the soil microbial biomass to such an extent that it took two years for it to fully recover (Schönborn and Dumpert, 1990). Schönborn and Dumpert (1990) suggested that the metabolites of PCP were likely to be more toxic to soil microorganisms. The half-life in soil of PCP has been determined to be 50 d in degradation studies, but the bacteria that degrade this compound are particularly sensitive to it (Sato, 1983). This may explain the lack of recovery by the microbial biomass seen in our experiments at lower concentrations, whereas the higher concentrations may have selected for microbial populations resistant to PCP. These could then grow with much decreased competition, and dominate and increase in number by utilising the PCP as a carbon source. Although the surviving microbial biomass was showing signs of recovery in soil containing the largest concentration of PCP (200 mg kg⁻¹) it was still 22% lower then the methanol control and as such, accord-
According to Domsch et al. (1983), it can be regarded as ecologically critical.

The total aerobic heterotrophic bacterial counts (TSBA) followed a similar pattern to that of the soil microbial biomass-C (Fig. 1), with a marked decrease at 50, 75 and 100 mg PCP kg$^{-1}$ soil. However, at 25, 175 and 200 mg PCP kg$^{-1}$ soil, the counts were the same as the control soils.

In our study, the PCP utilization assay tested the ability of the soil microorganisms from the PCP spiked soils, after 6 months incubation, to utilize PCP as a carbon source. When soil suspensions from the control soils (water and methanol only) were added to the water and methanol control microtitre wells containing no PCP or other carbon sources, there was very little change in optical density with time showing that there was no carbon available for microbial respiration (Fig. 2a). However, with soil suspensions from control soils, (incubated without PCP), but given PCP in the assay, small increases in absorbance occurred, showing that the soil microorganisms were capable of using PCP (Fig. 2b). With smaller soil concentrations (25, 50 and 75 mg kg$^{-1}$) of PCP, there were only small increases in the optical density suggesting that the soil microorganisms were not particularly effective at utilizing PCP (Fig. 2c). With soil suspensions from soils incubated with higher concentrations of PCP (i.e. 100 mg kg$^{-1}$ and above), the optical density readings increased substantially, suggesting greater PCP utilization (Fig. 2d). The largest optical density increase occurred with soil suspensions from soils containing 175 and 200 mg PCP kg$^{-1}$, confirming the use of PCP as a carbon source by microorganisms in these soils. Regression analysis (Genstat 5 Committee, 1987) carried out on PCP utilization patterns, showed there were no significant differences within the following groups of treatments: water and methanol controls; soil water and soil methanol controls; 25, 50 and 75 mg PCP kg$^{-1}$, and 100, 175 and 200 mg PCP kg$^{-1}$. Hence, a single regression line could be drawn through each group (Fig. 2). However, further analysis of the areas under the utilization curves, using one-way ANOVA (Genstat 5 Committee, 1987) showed PCP utilization between the treatment groups to be significant different ($P < 0.001$).

In conclusion, the negative impacts of PCP at relatively low concentrations (50–100 mg kg$^{-1}$ PCP in soil) on the soil microbial biomass-C and on TSBA numbers were severe. At higher concentrations, it would seem that a part of the microbial population resistant to the effects of PCP proliferated and were able to utilize the carbon from the PCP, as well as the carbon liberated by the death of the sensitive populations.

The soil used in this study has no previous history of PCP use as a pesticide, and therefore the surviving microbial populations in the PCP treated soils must have been intrinsically resistant to its effects. This is important as it shows that a soil when presented with an organic contaminant may have microbial populations that will, to some extent, ameliorate the effects of the organic compound. Hence, over time, soils contaminated with organic compounds may be detoxified through soil microorganisms degrading and/or metabolising these compounds. However, this process of natural attenuation and recovery may take a long time, and will certainly depend on the soil type. This attenuating capacity of the soil should not be taken as a reason for allowing unrestricted entry of pollutants into soils.

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References


