EFFECTS OF PENTACHLOROPHENOL ON ASYMBIOTIC NITROGEN FIXATION IN SOIL

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Abstract. The effect of 50, 100, 150, and 400 μg sodium pentachlorophenate (Na-PCP) per gram soil was studied in nonsterile soil incubated under aerobic and anaerobic conditions, and in sterilized soil inoculated with *Azotobacter sp.* isolated from the soil. N_2 fixation was determined by acetylene reduction. Pentachlorophenate at a concentration of 50 μg g^{-1} had an inhibitory effect in nonsterile soil incubated aerobically while strong inhibition of dinitrogen fixation in nonsterile soil occurred in the presence of 100 μg g^{-1} and above. The EC_{50} values for the inhibition of nitrogenase activity in nonsterile soil incubated aerobically and anaerobically and in sterilized soil inoculated with *Azotobacter sp.* suspensions were 49.8 ± 1.4 μg Na-PCP g^{-1}, 186.8 ± 2.8 μg Na-PCP g^{-1}, and 660.8 ± 29.3 μg Na-PCP g^{-1}, respectively.

1. Introduction

Recent research on the effects of pesticides on microbial activities in soil has been concerned with pesticide effects on nontarget microorganisms, which are responsible for nutrient and mineral cycling in agricultural soil, and secondly, on some microorganisms which can serve as biological indicators for environmental contamination.

Pentachlorophenol (PCP), particularly its sodium salt (Na-PCP), is a widely used pesticide (Cirelli, 1978; Pierce and Victor, 1978). It is versatile because it is used as a herbicide in sugar cane and rice paddy fields (Kaufman, 1976; Watanabe, 1977) and as a fungicide for wood preservation (Spencer, 1968).

With regards to its effects on soil microorganisms, PCP has been reported to affect some microbial activities in soils (Ishizawa *et al.*, 1961). Carbon dioxide production from microbial degradation of cellulose in soil was inhibited by PCP (Murthy *et al.*, 1979). In addition, oxidative phosphorylation and ATPase activity in *Micrococcus denitrificans* cultures have been shown to be strongly inhibited by PCP at very low concentrations (Imai *et al.*, 1967). Although agricultural soils treated with PCP become enriched with PCP-decomposing microorganisms (Watanabe, 1977; 1978), the rate of PCP degradation in soil is relatively slow (Suzuki and Nose, 1970; Watanabe and Hayashi, 1972). The persistence of PCP in soil has previously been documented (Brown, 1978; Wong and Crosby, 1978) especially in soils with low organic matter contents (Murthy *et al.*, 1979). It has been reported that after 160 days of incubation of PCP in soil, 7 and 80% of PCP was degraded under anaerobic and aerobic conditions, respectively (Baker and Mayfield, 1980). As far as the nitrogen economy of agricultural soil is concerned, the possibility that PCP in soil may have an adverse effect on soil nitrogenase activity could

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be of considerable importance. Babak (1968) has shown that pure cultures of *Azotobacter* were sensitive to a number of herbicides at concentrations used in routine field application.

We have previously reported the inhibitory effect of Na-PCP on nitrogenase and respiratory activities in pure cultures of *A. vinelandii* (Tam and Trevors, 1981). The present report describes the effects of Na-PCP on nitrogenase activity in soil under aerobic and anaerobic conditions. In addition, a nitrogen-fixing *Azotobacter sp.* was isolated from the same soil and the effect of Na-PCP on nitrogenase activity in sterilized soil inoculated with this microorganism was examined.

2. Materials and Methods

2.1. Soil samples

Sandy loam was collected from the top 10 cm of an agricultural soil near Floradale, Ontario, Canada. Soil was sieved through a 2-mm mesh screen and stored at 4 °C in the dark before use. Various characteristics of the soil were measured by the techniques described previously (Tam *et al.*, 1981) and are presented in Table I.

TABLE I

Characteristics of the soil

Characteristic	Soil sample[a]
Combustible matter (% dry weight)	10.2
Sand (%)	56
Silt (%)	34
Clay (%)	10
Carbohydrate	54.4 mg g^{-1}
NO_2^--N	0 ng g^{-1}
NO_3^--N	81.2 ng g^{-1}
NH_4^+-N	2.5 µg g^{-1}
pH	6.5

[a] Wherever applicable, values are expressed on a soil dry weight basis.

The number of aerobic and facultative anaerobic heterotrophs in the soil was enumerated as previously described (Tam *et al.*, 1981). The N-free media and the procedures used for the enumeration of aerobic (*Azotobacter*) and anaerobic (*Clostridium*) diazotrophs in the soil were as described by Brouzes *et al.* (1971). The population of heteorotrophs, *Azotobacter,* and *Clostridium* was 1.3×10^7, 324 and 567 bacterial cells per gram dry weight of soil, respectively.

2.2. Nonsterile soil incubations

Each 10-g sample was placed in a 50-ml Erlenmeyer flask and 0.5 ml of distilled water was added (50 to 60% of field capacity). For aerobic incubation, flasks were left air-filled

and closed with serum stoppers (Suba Seal, Barnsley, England). For anaerobic incubation, flasks were capped with serum stoppers, evacuated and back-filled with pure nitrogen three times. Each flask was amended with 0.1% (w/w) glucose and 0.2-ml amounts of appropriate concentrations of sodium pentachlorophenate dissolved in water (Fluka, AG, Switzerland) were injected. A 0.1 atm of the gas phase from each flask was replaced with pure C_2H_2. In flasks incubated aerobically, the O_2 concentrations of the gas phase were analyzed daily and pure O_2 was added to replace that consumed in respiration. All flasks were incubated in the dark at 20 °C for 12 days.

2.3. Gas analysis

At appropriate intervals, a 1.0-ml syringe equipped with a Mininert valve (Precision Sampling Corp., Baton Rouge, LA.) was used to withdraw 0.2 ml of the gas phase from each flask which was then analyzed for C_2H_4, C_2H_2, and O_2 by gas chromatography (GC) as previously described (Brouzes *et al.*, 1971; Tam and Knowles, 1979; Tam *et al.*, 1981). All GC data are reported as the average of triplicate flasks.

2.4. Pure culture studies in sterile soil

A nitrogen-fixing *Azotobacter sp.*, isolated from the soil using the procedures described by Brouzes *et al.* (1971), was used as a representative of one of the commonly occurring groups of soil aerobic diazotrophs. This *Azotobacter sp.* was grown in 100 ml of the Ashby's N-free medium (Ashby, 1907). N-free agar was prepared by adding 1.5% of Difco agar to the above medium.

To investigate the effects of Na-PCP on nitrogenase activity of *Azotobacter sp.* in soil, flasks containing 10 g of soil were autoclaved at 121 °C for 1.5 h on each of two successive days. Each flask was then inoculated with 1.0 ml (containing 150 μg cell protein) of cell suspensions of the microorganism followed by addition of mannitol to a final concentration of 2.0% (w/w).

3. Results

3.1. Soil nitrogenase activity under aerobic and anaerobic conditions

At concentrations of 50, 100, 150, and 400 μg Na-PCP g^{-1}, the amount of total C_2H_4 production under anaerobic conditions was decreased by 4, 26, 31, and 79% respectively (Figure 1). The amounts of total C_2H_4 produced in aerobic soil (Figure 2) was less than in anaerobic soil. However, nitrogenase activity in aerobically incubated soil was more sensitive to Na-PCP (Figure 2). At concentrations of 50, 100, and 150 μg Na-PCP g^{-1}, the amount of total C_2H_4 production was inhibited by 67, 79, and 96%, respectively.

3.2. Nitrogenase activity in sterile soil inoculated with *Azotobacter sp.*

To study the effects of Na-PCP on a commonly occurring diazotroph, sterilized soil was inoculated with an *Azotobacter sp.* which was isolated from the soil. The effect of Na-PCP on the nitrogenase activity of this bacterium (Figure 3) was less pronounced than that

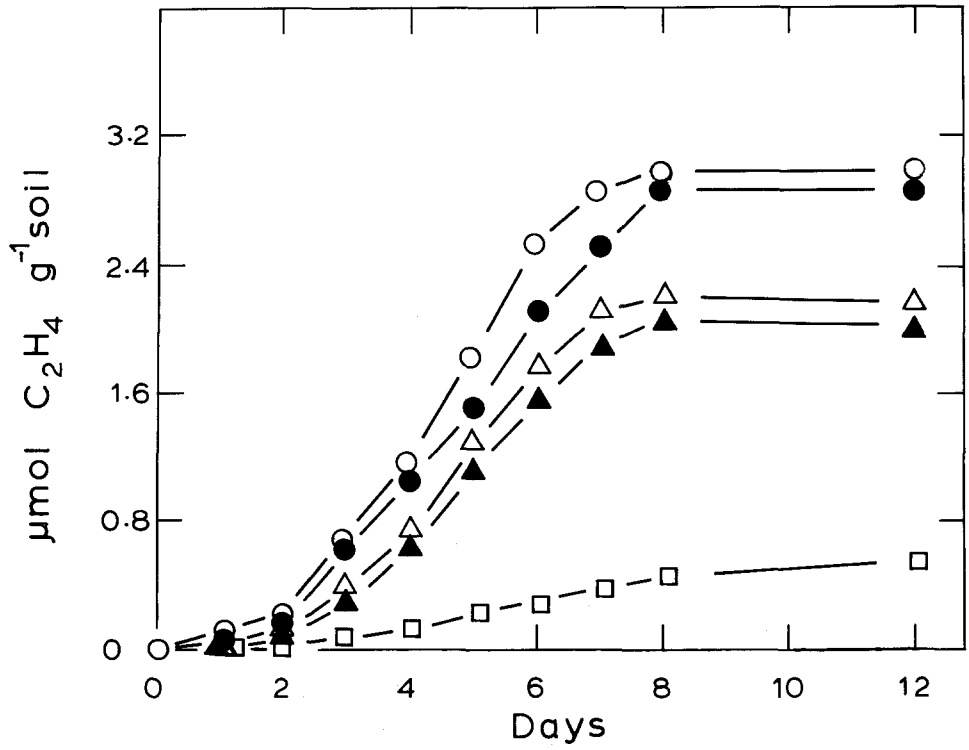

Fig. 1. C_2H_4 production by nonsterile soil incubated anaerobically. Flasks contained Na-PCP at the following concentrations (μg g^{-1}): 0 (○), 50 (●), 100 (△), 150 (▲), and 400 (□).

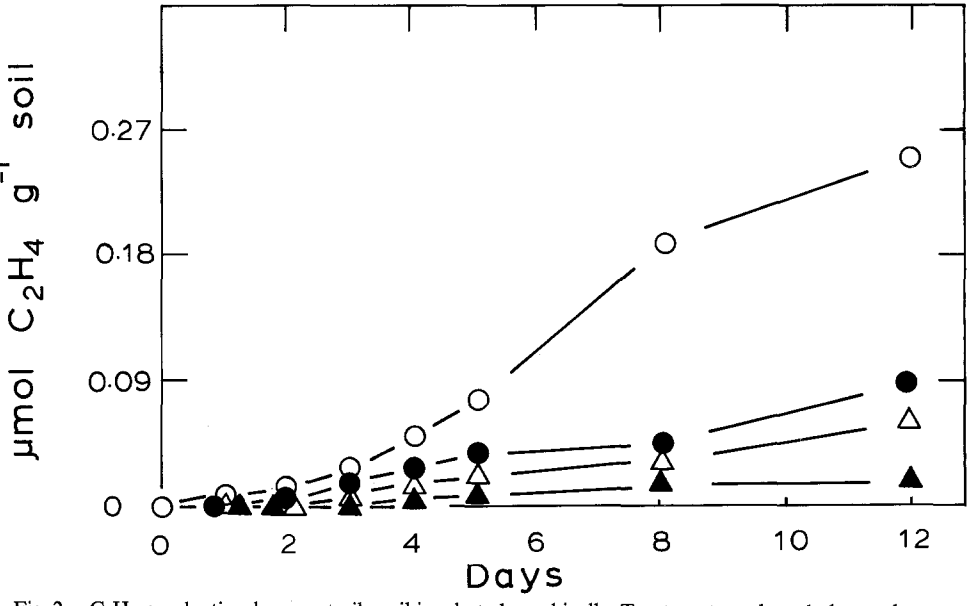

Fig. 2. C_2H_4 production by nonsterile soil incubated aerobically. Treatments and symbols are the same as for Figure 1.

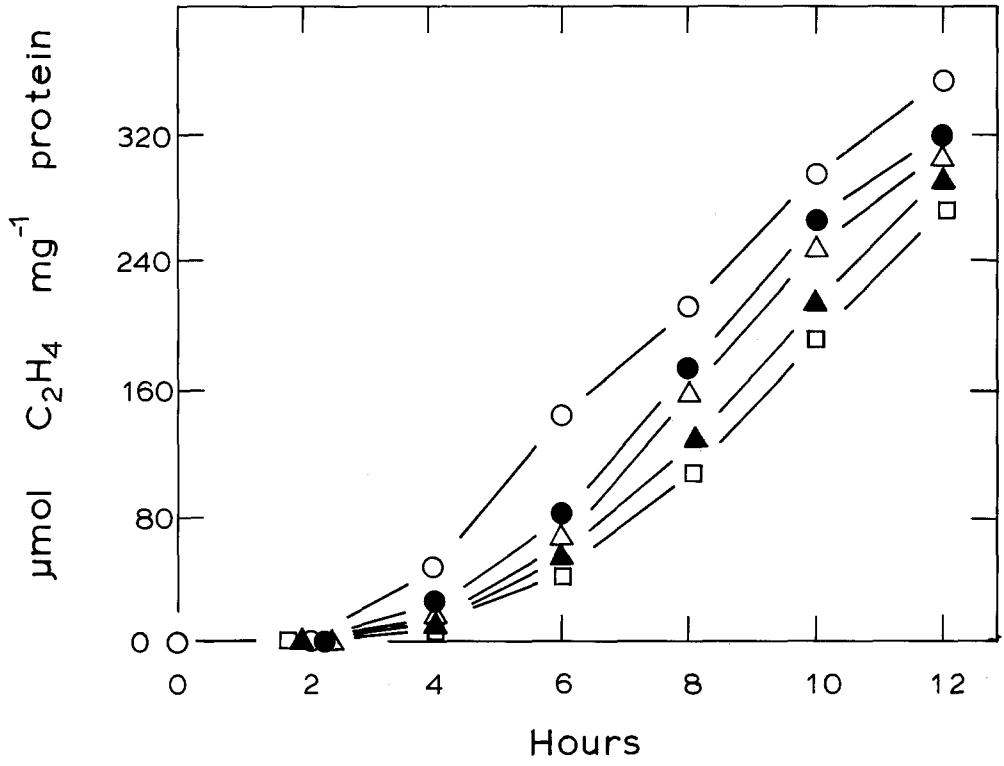

Fig. 3. C_2H_4 production by sterile soil inoculated with *Azotobacter sp.* Flasks contained Na-PCP at the following concentrations (µg g^{-1}): 0 (○), 100 (●), 200 (△), 300 (▲), and 400 (□).

in the nonsterile soil. At a concentration of 400 µg Na-PCP g^{-1}, C_2H_4 production by this microorganism was inhibited by only 22.3% (Figure 3).

3.3. EC_{50} VALUES UNDER VARIOUS CONDITIONS

The EC_{50} (the concentration of Na-PCP that decreased 50% of the total C_2H_4 production) was calculated using the 'probit' procedure of the Statistical Analysis System (SAS Institute Inc., 1979). The EC_{50} value for the inhibition of nitrogenase activity was lower in aerobically incubated soil than in anaerobically incubated soil (Table II). This

TABLE II

EC_{50} values for the inhibition of C_2H_4 production by Na-PCP in nonsterile soil and in sterilized soil inoculated with *Azotobacter sp.*

	Nonsterile soil		Sterilized soil with *Azotobacter sp.* (µg g^{-1})
	Anaerobic (µg g^{-1})	Aerobic (µg g^{-1})	
EC_{50}	186.8 ± 2.8	49.8 ± 1.4	660.8 ± 29.3

suggested that Na-PCP was less inhibitory to soil nitrogenase activity under anaerobic than aerobic conditions.

4. Discussion

PCP has been shown to be a potent inhibitor of oxidative phosphorylation and ATPase activity in pure cultures of *Micrococcus denitrificans* (Imai *et al.*, 1967). The lower nitrogenase activity in the presence of higher concentrations of Na-PCP could possibly be attributed to either a decrease in energy (ATP) supply for nitrogenase activity or an increase in mortality rate of diazotrophs, or both of these mechanisms.

The present findings suggest that the use of Na-PCP at normal field application rates (1 to 5 $\mu g\, g^{-1}$ soil, according to Brown, 1978), may not have adverse effects on nitrogenase activity in soil. On the other hand, the present data also showed the potentially inhibitory effects of Na-PCP, when present at high concentrations, on nitrogenase activity in nonsterile soil.

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