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Toxicity of Pentachlorophenol to *Azotobacter vinelandii*

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As the use of pesticides in agricultural soils has increased in recent years, the influence of pesticides on microbial activities in soils is of concern. Under the Toxic Substances Control Act, one of the proposed microbiological assays requires microorganisms involved in nitrogen cycling to be tested for their sensitivity to environmental toxicants (STERN 1980). Among various microbial activities in soil, dinitrogen fixation by diazotrophs contributes significantly to the overall nitrogen economy of agricultural soils. N_2-fixing Azotobacter spp. are widely used diazotrophs for testing the effect of pesticides on N_2 fixation. BABAK (1968) reported that Azotobacter was sensitive to a number of herbicides. Strong inhibition of nitrogenase activity in pure cultures of A. vinelandii and in soil caused by the herbicide, 4,6-dinitro-o-sec-butylphenol, has also been reported (VLASSAK 1976).

Pentachlorophenol (PCP), particularly its sodium salt (Na-PCP), is a widely used pesticide (CIRELLI 1978; PIERCE and VICTOR 1978). It is used both as a herbicide to remove weeds in rice paddy fields and as a fungicide for wood preservation (SPENCER 1968; WATANABE 1977). The possibilty that PCP may have an adverse effect on soil nitrogenase activity could be of considerable importance.

We report here the potentially inhibitory effect of Na-PCP on N_2 fixation (C_2H_2 reduction), CO_2 evolution and O_2 consumption by pure cultures of A. vinelandii.

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MATERIALS AND METHODS

Azotobacter vinelandii ATCC 12837 was grown in Ashby's N-free medium (ASHEY 1907) on a reciprocal shaker (120 strokes/min) for 18 h at 30°C. After three successive transfers of the culture to fresh medium, cells were harvested by centrifugation (5,500 x g at 4°C) and resuspended in sterile phosphate buffer (150 mM, pH 7). Appropriate dilutions were made in sterile buffer to yield 1.1×10^{11} cells/mL, which was equivalent to 150 µg protein/mL as determined by the method of BRADFORD (1976). Aliquots (1.0 mL) of the diluted cell suspensions were transferred to 50-mL Erlenmeyer flasks each containing 10 mL of the fresh medium. Flasks were left air-filled and closed with serum stoppers (Suba Seal, Barnsley, England). Sodium pentachlorophenate (Fluka, AG, Switzerland) was dissolved in distilled water and filter-sterilized. Appropriate concentrations of Na-PCP (0.2 mL) were added to flasks with a sterile syringe. Pure acetylene to give 10.12 kPa was injected into each flask with a sterile syringe after an equivalent amount of the gas phase had been removed. The oxygen concentration in each flask was measured by gas chomatography (GC) and adjusted every hour by injecting pure oxygen into each flask to replace the amount consumed. All flasks were incubated at 30°C in the dark on a reciprocal shaker (120 strokes/min) for 12 h.

At appropriate intervals, gas samples (0.2 mL) were withdrawn with a 1.0-mL syringe equipped with a Mininert valve (Precision Sampling Corp., Baton Rouge, LA) and analyzed for C_2H_2, C_2H_4, CO_2 and O_2 by GC as previously described (TAM and KNOWLES 1979; TAM et al. 1981). All GC data are the means of triplicate flasks and are corrected for gas leakage and solubility.

RESULTS AND DISCUSSION

Sodium pentachlorophenate at a concentration of 50 µg/mL inhibited C_2H_2 reduction by A. vinelandii (Figure 1). The degree of inhibition of C_2H_2 reduction increased as the concentrations of the Na-PCP increased. The presence of 100, 200, and 250 µg Na-PCP/mL, respectively caused 18, 33 and 51% inhibition of total C_2H_4 production when compared to the controls (Figure 1). The low C_2H_2-reducing activity in the presence of Na-PCP could possibly be due to the inhibitory effect of Na-PCP on oxidative phosphorylation and ATPase activity (IMAI et al. 1967), hence less energy was available for

N_2 fixation by the microorganism. The present data indicated that high concentrations of Na-PCP could adversely affect nitrogenase activity.

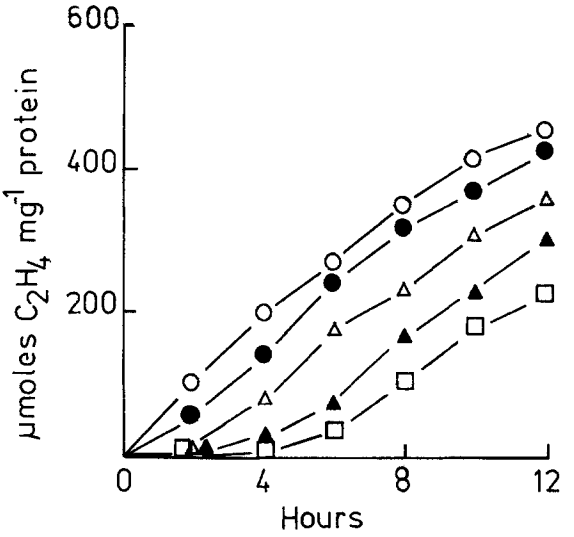

Figure 1. C_2H_4 production by A. vinelandii in presence of various concentrations of Na-PCP. Symbols are : (O) 0, (●) 50 µg/mL, (△) 100 µg/mL, (▲) 200 µg/mL, and (□) 250 µg/mL.

The patterns of Na-PCP inhibition of CO_2 evolution and O_2 consumption by A. vinelandii (Figures 2 and 3) were similar to that of C_2H_4 production. Na-PCP at a concentration of 50 µg/mL inhibited both CO_2 evolution and O_2 uptake by this microorganism. As the concentrations of Na-PCP were increased to 100, 200 and 250 µg/mL, the per cent inhibition was, respectively, 17, 27 and 54% for total CO_2 production (Figure 2) and 10, 27 and 37% for total O_2 uptake (Figure 3). The present data indicated that Na-PCP, when present at high concentrations, could inhibit microbial respiration. Inhibition of microbial CO_2 evolution in soil by PCP has been reported (MURTHY et al. 1979). In addition, the electron transport activity in the Na-PCP treated cell suspensions, as measured by the amount of p-iodonitrotetrazolium violet (INT) reduced to formazan (KENNER and AHMED 1975b; ZIMMERMAN et al. 1978), decreased as the concentrations of Na-PCP present increased (unpublished data). The correlations between O_2 uptake and electron transport activity in some organisms have been established (KENNER and AHMED 1975a).

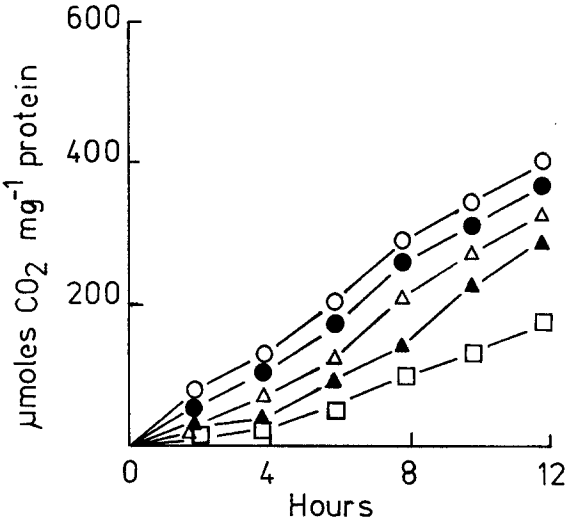

Figure 2. CO_2 evolution by A. vinelandii in the presence of various concentrations of Na-PCP. Treatments and symbols are the same as for Figure 1.

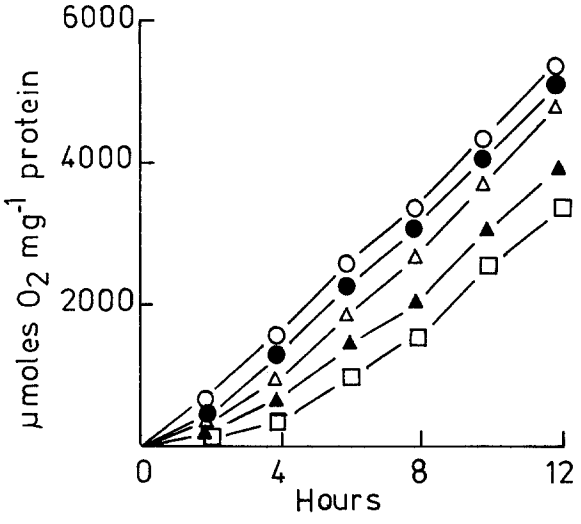

Figure 3. O_2 uptake by A. vinelandii in the presence of various concentrations of Na-PCP. Treatments and symbols are the same as for Figure 1.

Agricultural soil treated annually with Na-PCP showed a slightly higher number of PCP-decomposing microorganisms compared to untreated soil (WATANABE 1977; 1978). However, PCP decomposition under field conditions is relatively slow (MURTHY et al. 1979; SUZUKI and NOSE 1970; WATANABE and HAYASHI 1972). In fact, PCP may persist in soil for periods up to 3 months (BROWN 1978) suggesting that frequent applications of Na-PCP in agricultural soils may result in accumulation of high levels of this compound with consequent inhibition of nitrogenase and respiratory activities. In addition, other non-target microorganisms responsible for nutrient and mineral cycling in agricultural soils may also be adversely affected by high concentrations of Na-PCP.

REFERENCES

ASHBY, S. F.: J. Agric. Sci. 2, 35 (1907).

BABAK, N. M.: Mikrobiologiya. 37, 338 (1968).

BRADFORD, M. M.: Anal. Biochem. 72, 248 (1976).

BROWN, A. W. A.: Ecology of Pesticides. Wiley, New York (1978).

CIRELLI, D. P.: In Pentachlorophenol (RAO, K., ed). Plenum Press, New York (1978).

IMAI, K., A. ASANO and R. SATO: Biochim. Biophys. Acta. 143, 462 (1967).

KENNER, R. A. and R. A. AHMED: Mar. Biol. 33, 119 (1975a).

KENNER, R. A. and R. A. AHMED: Mar. Biol. 33, 129 (1975b).

MURTHY, N. B. K., D. D. KAUFMAN and G. F. FRIES: J. Environ. Sci. Health Part B 14, 1 (1979).

PIERCE, R. H. and D. M. VICTOR: In Pentachlorophenol (RAO, K., ed). Plenum Press, New York (1978).

SPENCER, E. Y.: Chemicals Used in Crop Protection—Guide. Canadian Department of Agriculture Publication, Ottawa, Canada (1968).

SUZUKI, T. and Y. NOSE: Noyaku Seisan Gijutsu. Japan. 22, 27 (1970).

STERN, A. M.: In Microbiology—1980. (SCHLESSINGER, D., ed). American Society for Microbiology, Washington, D.C. (1980).

TAM, T.-Y. and R. KNOWLES: Can. J. Microbiol. 25, 1133 (1979).

TAM, T.-Y., C. I. MAYFIELD and W. E. INNISS.: Can. J. Microbiol. 27, 555 (1981).

VLASSAK, K., K. A. H. HEREMANS and A. R. VAN ROSSEN.: Soil Biol. Biochem. 8, 91 (1976).

WATANABE, I.: Soil Biol. Biochem. 9, 99 (1977).

WATANABE, I.: Soil Biol. Biochem. 10, 71 (1978).

WATANABE, I. and S. HAYASHI: J. Sci. Soil Manure. Japan. 43, 119 (1972).

ZIMMERMAN, R., R. ITURRIAGA and J. BECKER-BIRCH: Appl. Environ. Microbiol. 36, 926 (1978).

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