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Effect of Pentachlorophenol on Electron Transport System Activity in Soil

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Recent research on the effects of environmental pollutants on microbial activities in soil has focused on the detrimental effects on nontarget microorganisms which are involved with mineral and nutrient cycling, and secondly on test organisms which can act as biological indicators for a particular environment (TAM & TREVORS 1981).

Pentachlorophenol (PCP) and its sodium salt (Na-PCP) are widely used as a pesticide (CIRELLI 1978) because of its versatility. It has also been used as a fungicide for the preservation of wood and fabrics. TAM and TREVORS (1981) reported that the use of Na-PCP at normal field application rates may not have adverse effects on nitrogenase activity in soil. However, microorganisms other than those capable of fixing nitrogen were not tested for their sensitivity to Na-PCP. The present report describes the effects of Na-PCP on microbial electron transport system (ETS) activity using the quantitative reduction of 2-(p-iodophenyl) -3- (p-nitrophenyl) -5- phenyl tetrazolium chloride (INT) to iodonitrotetrazolium formazan (INT-formazan). This enzyme assay measures the general activities of a large part of the microbial community in soils or other ecosystems (TREVORS et al. 1982), and therefore may be useful for assessing the effects of environmental toxicants in a relatively short period of time.

MATERIALS AND METHODS

Soil Samples

Sandy loam soil was collected from the top 10 cm of an agricultural field near Floradale, Ontario, Canada. The soil was sieved through a 2-mm mesh screen and stored at 4°C in the dark. Various characteristics of the soil were measured as previously described (TAM & TREVORS 1981) and are presented in Table 1. The number of aerobic heterotrophs was enumerated by the spread plate technique using soil extract agar as the growth medium. Triplicate plates were incubated at 20°C for 7 days, at which time colony forming units were enumerated. Total microscopic counts were carried out using epifluorescence microscopy with acridine orange as the fluorescent dye.

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Measurement of ETS activity in soil

A 10-g sample of soil was placed in a sterile 50-mL Erlenmeyer flask and incubated at 20°C for 24 h at which time the ETS activity was measured. Sterile controls were prepared by autoclaving flasks containing soil for 1.5 h at 121°C on two consecutive days. Non-amended soil received 1 mL of sterile distilled water. Flasks amended with glucose or yeast extract (Difco) received 1 mL of a 1% (w/v) sterile solution. Sodium pentachlorophenol (Fluka, AG, Switzerland) was dissolved in distilled water, filter sterilized and added to flasks as a 0.2-mL volume. Each flask then received a 1.5 mL of a 0.4% (w/v) aqueous solution of filter sterilized INT (Sigma Chemical Co., St. Louis, Mo.). The soil was mixed with sterile glass rod and the flask capped with a sterile serum stopper. After 24 h a subsample of soil (approximately 0.5 g dry weight) was removed from each flask and placed in a test tube with 10 mL of methanol. The contents were vortexed for 1 min and then filtered through a Whatman No. 5 filter. If the extraction was not complete, more methanol was added to complete the extraction. All values are expressed on a per gram dry weight of soil basis.

The INT-formazan in the methanolic extract was measured spectrophotometrically at 480 nm against a methanol extract of soil containing no INT. The INT-formazan concentration was derived from a standard curve of INT-formazan (Sigma Chemical Co., St. Louis, Mo.) in methanol. Sodium pentachlorophenol does not interfere with the measurement as it can only be detected in the ultraviolet range of the spectrum. A more detailed procedure has been described by TREVORS et al. (1982).

RESULTS AND DISCUSSION

At concentrations of 50, 100 and 200 µg/g of Na-PCP, the amount of total INT-formazan produced in non-amended soil was decreased by 0, 0, and 5.8% respectively (Table 2). In fact, at concentrations of 50 and 100 µg/g, Na-PCP stimulated ETS activity (Table 2). When used at concentrations of 50, 100 and 200 µg/g in either glucose or yeast extract amended soil, Na-PCP was more inhibitory than in non-amended soil. At a concentration of 200 µg/g, ETS activity was decreased 24.8 and 27.6% respectively, in the glucose and yeast extract amended soil. Sterile controls displayed no ETS activity, indicating that the INT dye was not subject to chemical reduction.

The stimulation of ETS activity by low concentrations of Na-PCP is not unusual. TAM & TREVORS (1981) reported that concentrations of Na-PCP at normal field application rates were not inhibitory to soil nitrogenase activity. WATANABE (1977, 1978) also reported that agricultural soils treated with PCP can become enriched with PCP-decomposing microorganisms.

Table 1

Characteristics of the Soil

Characteristic	Soil Sample*
pH	6.5
Sand (%)	56
Silt (%)	34
Clay (%)	10
Moisture (%)	25
Total microscopic count	5.6×10^9
Viable plate count	2.0×10^7

* Values are expressed per gram dry weight of soil wherever applicable.

Table 2

Effect of Na-PCP on ETS activity in soil incubated aerobically for 24-h

Na-PCP (µg/g)	Soil amendment	ETS activity* (µg formazan/g soil)	Percent inhibition
0	none	15.7 ± 0.5	control
0	glucose	46.4 ± 6.4	control
0	yeast extract	47.7 ± 3.6	control
50	none	26.8 ± 5.2	0
50	glucose	43.2 ± 9.1	7.0
50	yeast extract	65.1 ± 8.6	0
100	none	22.1 ± 2.8	0
100	glucose	39.4 ± 0.9	15.1
100	yeast extract	46.5 ± 4.3	2.4
200	none	14.8 ± 1.8	5.8
200	glucose	34.9 ± 1.7	24.8
200	yeast extract	34.5 ± 3.2	27.6

* Mean ± standard error of the mean (n = 3).
Sterile controls containing Na-PCP and no Na-PCP demonstrated no ETS activity.

Other applications of the ETS activity method are possible. Modifications in the environmental conditions of incubation (such as temperature variation, pH, changes in pO2 or pCO_2 levels) would provide information on the effects of different parameters on ETS activity in the absence and presence of environmental toxicants.

The effects of nutrients on the microbial activity was investigated in the present study. What is apparent, is that non-amended soil has relatively low activity and is not affected by relatively high concentrations of Na-PCP. Other aspects can also be studied, such as the effects of different combinations and concentrations of any water soluble or water insoluble toxicants. This would allow one to determine additive, antagonistic, or synergistic interactions in a relatively short period of time.

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