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Effects of Paraquat and Glyphosate on Growth, Respiration, and Enzyme Activity of Aquatic Bacteria

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Herbicides often exert stimulatory or inhibitory effects on growth and other activities of many microorganisms both in nature and in culture (Grossbard and Davies 1976). The effects of herbicides on soil microorganisms have been studied extensively (Andus 1964; Grossbard 1976; Tu and Bollen 1968). Herbicides may enter freshwater by run-off from terrestrial application, effluents from herbicide manufacturing plants and irresponsible disposal by the users. Leaching of herbicides from terrestrial application could give rise to highly measureable residues in bodies of water (Wojtalik et al. 1971). However, one finds in the literature a paucity of information on the effects of herbicides on aquatic bacteria (Beckmann et al. 1984)

The herbicides paraquat (N,N'-dimethyl bipyridylium dichloride) and glyphosate (N-phosphonomethyl glycine) are among the most commonly used herbicides. Paraquat has been known to interfere with the biosynthesis of macromolecules in E. coli (Davison and Papirmeister 1971). Glyphosate is a non-selective postemergence herbicide which inhibits the activity of enzymes involved in the biosynthesis of tryptophan, phenylalanine and tyrosine (Amrhein et al. 1980; Steinrucken and Amrhein 1980). In addition, glyphosate has been reported to inhibit the activities of phospho-2-oxo-3-deoxyheptonate aldolase and 3-dehydroquinate synthase, as well as Fe^{2+} transport in several bacteria (Barton et al. 1982; Roisch and Lingens 1980). The present paper reports the results of a study on the effects of paraquat and glyphosate on growth, respiration and enzyme activity of aquatic bacteria.

MATERIALS AND METHODS

The experimental pools A, B and C were located at the Lam Tseun River in Hong Kong. All these pools were gravel pits with surface areas of 25, 23 and 21 m^2, and mean depths of 0.8, 0.6 and 0.54 m, respectively. The water flows slowly into pool A and then branches into two yet smaller streams where pools B and C were respectively located. All three pools had similar sur-

roundings and supported abundant growth of eel grass (Vallisneria spiralis), water milfoil (Myriophyllum sp.), water hyacinth (Eichhornia crassipes), canadian pondweed (Hydrilla verticillata), and species of the algal genera Nitella, Cladophora and Spirogyra, and numerous species of diatoms.

Paraquat (GRAMOXONE®, 1,1'-dimethyl-4,4'-bipyridinium dichloride with 24% active ingredient), obtained from Imperial Chemicals Inc., U.K., was applied to pool B so a concentration of 20 ppm was obtained. Glyphosate (ROUNDUP®, isopropylamine salt of N-phosphonomethyl glycine containing 356 g l^{-1} glyphosate) from Monsento Co., U.S.A. was applied to pool C so that a concentration of 200 ppm was acheived. Pool A was used as the control. Viable cell counts of the pool waer were determined at 2-day intervals by using the spread plate method with Nutrient Agar (Difco) for a total of 30 days after application of the herbicides. All agar plates were incubated at 25°C for 48 hr before the number of bacterial colony forming unit (CFU) was counted. Two bacteria of most frequent occurrence were isolated into pure cultures. They were identified as Aeromonas hydrophila C-1 which was a facultatively anaerobic strain, and Pseudomonas chlororaphis L-1 which was a strictly aerobic strain according to the description of Buchanan and Gibbons (1974). These two bacteria were used in subsequent experiments.

A. hydrophila C-1 and P. chlororaphis L-1 were grown in pure cultures in a defined medium (pH 6.8) which consisted of Na_2HPO_4 (10.5 g), $(NH_4)_2SO_4$ (2 g), KH_2PO_4 (13.6 g), $MgSO_4.7H_2O$ (0.2 g), $FeSO_4.7H_2O$ (5 mg), NaCl (0.029 g), L-lysine (10 mg), L-proline (100 mg), thiamine (1 mg), glucose (910 mM) and 1000 ml of distilled water. The inoculum size was 5 x 10^5 cells ml^{-1}, and all cultures were incubated at 25°C. All cultures were set up in triplicate and the figures reported represented the means of these. Bacterial cells from mid-log phase of growth were transferred to fresh medium containing either paraquat or glyphosate at specific concentrations. Counts of CFU of these cultures were carried out daily for 6 days by spread plate method using Nutrient Agar plates.

A separate experiment was performed to detect the effect of the aromatic amino acids phyenylalanine and tyrosine on reversing the glyphosate action on bacterial growth. Millipore-filter sterilized solution containing either phenylalanine or tyrosine, or a mixture of these two each at a concentration of 50 mM was added to the bacterial cultures treated with 1500 ppm (A. hydrophila C-1) or 200 ppm (P. chlororaphis L-1) of glyphosate on the 3rd day of herbicide treatment. CFU count was made daily for 6 days.

Oxygen uptake by the bacterial cultures treated with the two herbicides was measured with a Gilson Differential Respirometer. Readings of O_2 uptake in µl were taken at 10 min intervals for 50 min.

Enzyme assays were carried out with log phase cells which were disrupted by sonification at 0°C. Dehydrogenase activity was

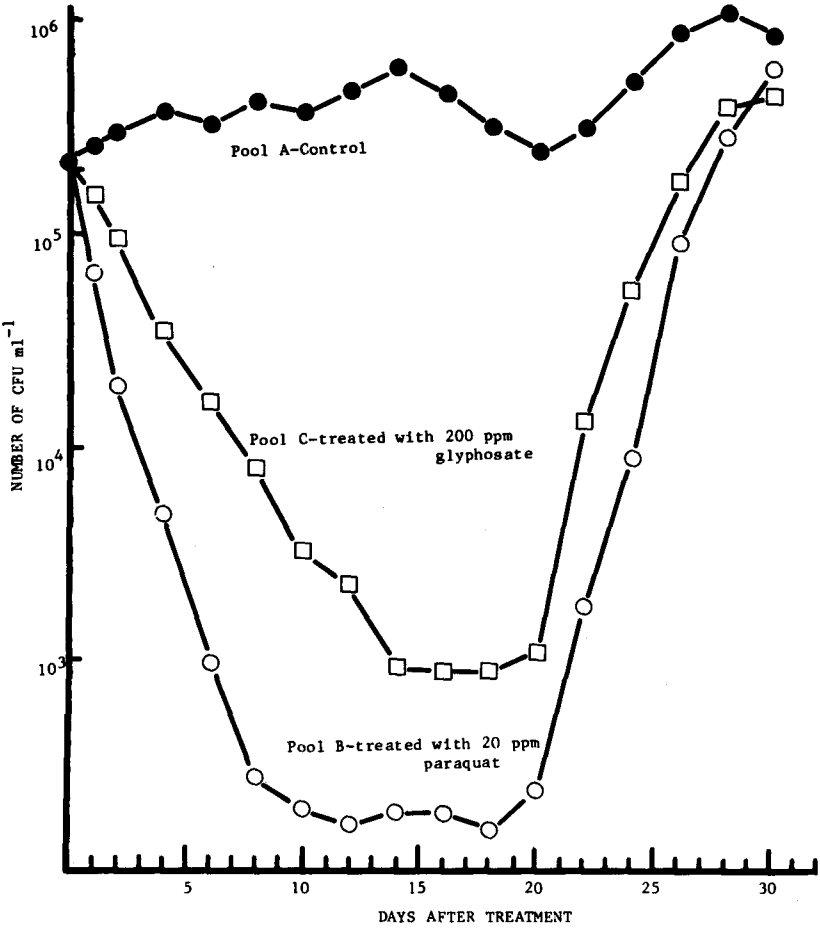

Figure 1. Changes of colony forming units (CFU) in the water of pool A (Control), pool B (20 ppm of paraquat) and pool C (200 ppm of glyphosate)

determined by the method of Casida et al. (1964), that of protease according to the method of Jensen et al. (1980). The activity of 5-enolpyruvyl-shikimic acid-3-phosphate synthase was determined according to Steinrucken and Amrhein (1980).

RESULTS AND DISCUSSION

Both paraquat and glyphosate contain very soluble active components (Calderbank and Slade 1976; Hoagland and Duke 1982) which diffuse rapidly through the water and therefore affect the aquatic biota. In pool B, the CFU decreased immediately after the addition of paraquat, and the number dropped from 2×10^5 ml^{-1} to 1×10^2 ml^{-1} in 10 days (Fig. 1). After bing stationary at this level for 8 days, the number of CFU increased again to a level comparable to that of the original population (Fig. 1). The bacterial population in pool C showed similar pattern of response to glyphosate treatment (Fig. 1). However, the effect of glyphosate was less severe than that of paraquat at the tested concentrations.

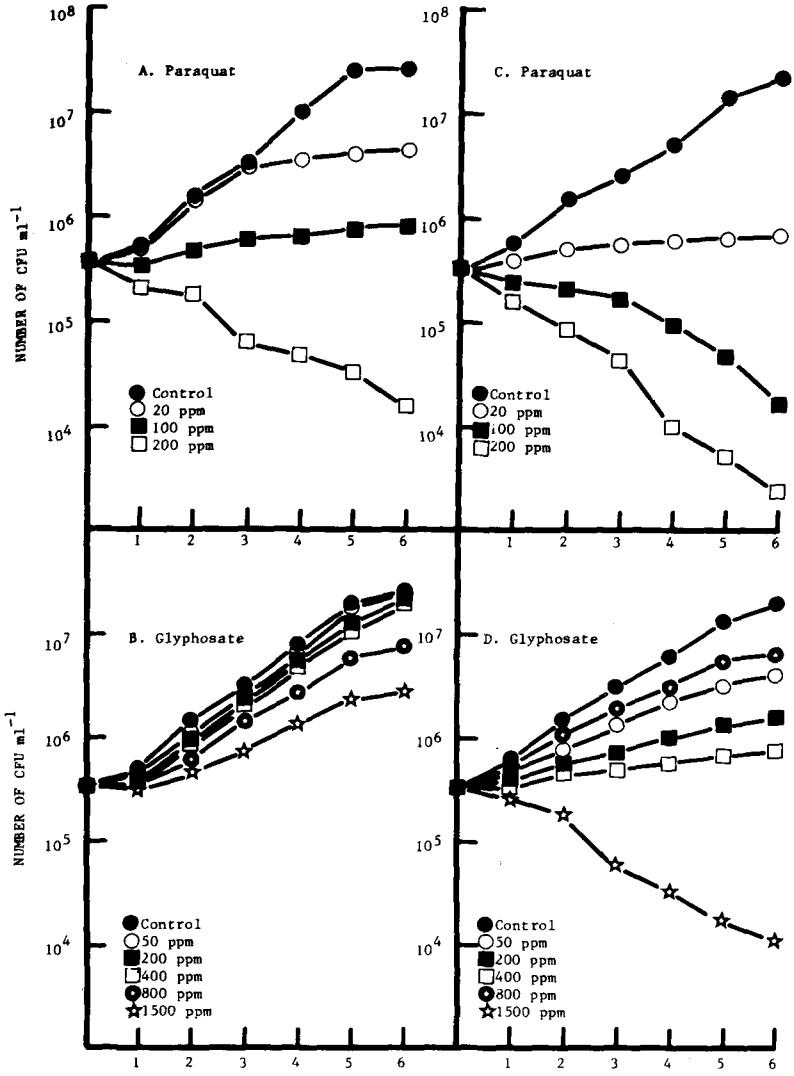

Figure 2. Changes of colony forming units (CFU) in A. hydrophila C-1 treated with paraquat (A) and glyphosate (B), and in P. chlororaphis L-1 treated with paraquat (C) and glyphosate (D)

The lowest level of CFU in pool C was 1 x 10^3 ml^{-1} and it took only 6 days for the bacterial population to start to build up again (Fig. 1). Apparently the natural bacterial populations in the pools were not completely killed by these herbicides at the tested concentrations, and the effects of these herbicides were only transient. It is conceivable that this was due either to the dilution effect of the stream water or that the concentrations of herbicides used were only sublethal to most of the bacterial species in the water, or could be due to both. Bacteria which survived the herbicidal action were able to grow rapidly again once the herbicide residues were removed by water current.

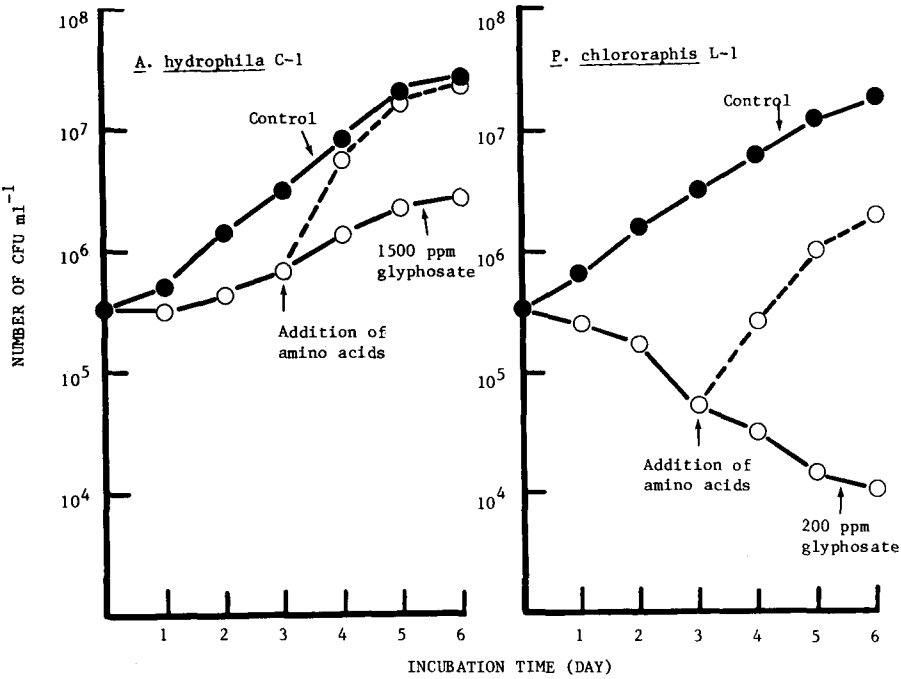

Figure 3. Effect of amino acid solution (50 mM of phenylalanine + 50 mM tyrosine) on changes of colony forming units (CFU) in cultures treated with glyphosate

Growth of A. hydrophila C-1 was found to be inhibited by paraquat at all tested concentrations (Fig. 2A). Growth of the culture treated with 20 ppm of paraquat was not affected until the 3rd day of treatment, which resulted in a lower yield as compared with the control. The inhibitory effect of 100 ppm paraquat was detected immediately upon incubation, and the yield was only 3% of that of the control (Fig. 2A). Paraquat at 200 ppm was lethal to cells of A. hydrophila C-1. P. chlororaphis L-1 was more susceptible to paraquat than A. hydrophila C-1. Paraquat at 100 and 200 ppm was lethal to the cells while 20 ppm strongly inhibited growth of the cultures (Fig. 2B). Cells of A. hydrophila C-1 were more resistant to glyphosate action as compared with that of paraquat. The yield of the culture treated with 1500 ppm of glyphosate was approximately 10% of that of the control (Fig. 2C). Growth of P. chlororaphis L-1 was strongly inhibited by glyphosate at concentration as low as 50 ppm (Fig. 2D). Glyphosate at 1500 ppm was lethal to the cells of P. chlororaphis L-1.

Breazeale and Camper (1972) reported that relatively low concentrations of paraquat were sufficient to inhibit the growth rates of Erwinia carotovora, Pseudomonas fluorescens and a Bacillus sp..

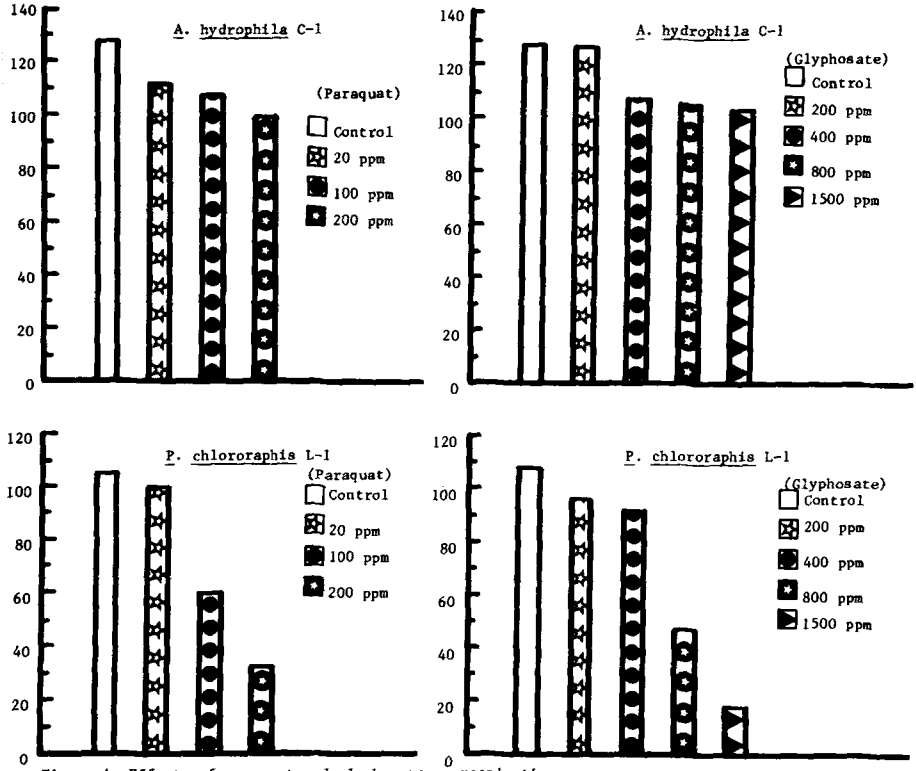

Figure 4. Effects of paraquat and glyphosate on respiration

Growth of human bacteria was also reported to be inhibited by paraquat at concentrations ranging from 256 to 2560 ppm, although most of these tested bacteria was not killed by this condentration range of paraquat (Peterson et al. 1981)

The addition of a solution containing phenylalanine and tyrosine to the bacterial cultures can eliminate the inhibitory action of glyphosate on bacterial growth. Complete reversal of growth inhibition was observed with A. hydrophila C-1 (Fig. 3), while only partial reversal of the glyphosate inhibition was observed with P. chlororaphis L-1 (Fig. 3). Phenylalanine or tyrosine alone did not have any effect. The fact that phenylalanine and tyrosine acted synergistically to reverse growth inhibition caused by glyphosate indicates that glyphosate interferes with the formation of the common precursor of these aromatic amino acids by probably inhibiting the activity of 5-enolpyruvyl-shikimate-3-phosphate synthase (Amrhein et al. 1980; Steinrucken and Amrhein 1980) of these bacterial isolates.

Respiration of A. hydrophila C-1 was only slightly inhibited by the two herbicides (Fig. 4). Paraquat at 100 and 200 ppm and

Table 1. Effects of paraquat and glyphosate on activities of dehydrogenase (DH), protease (PRO) and 5-enolpyruvyl-shikimate-3-phosphate synthase (EPS)

	Enzyme activity (% control)					
	A. hydrophila C-1			P. chlororaphis L-1		
	DH	PRO	EPS	DH	PRO	EPS
Paraquat (ppm)						
Control	100	100	100	100	100	-
20	100	100	-	76	81	-
60	97	76	-	53	54	-
100	95	51	-	38	33	-
Glyphosate (ppm)						
Control	100	100	100	100	100	100
200	100	91	87	88	75	78
800	96	73	71	63	58	55
1500	94	42	47	41	36	39

glyphosate at 800 and 1500 ppm significantly inhibited oxygen uptake of P. chlororaphis L-1 (Fig. 4). Davison and Papirmeister (1971) concluded that paraquat failed to damage the bacterial membrane and that the primary action of paraquat was on processes involved in energy metabolism. In addditon, inhibition of Fe^{2+} transport by glyphosate as reported by Barton et al. (1982) might also affect the electron transport system, which in turn may lead to the observed growth inhibition on the two aquatic bacteria.

Both herbicides did not affect the dehydrogenase activity of A. hydrophila C-1 significantly even at high concentrations (Table 1). However, that of P. chlororaphis L-1 was strongly inhibited (Table 1). Paraquat at 20 ppm did not affect the protease of A. hydrophila C-1, however paraquat and glyphosate at all tested concentrations inhibited the protease activity of P. chlororaphis L-1. The activity of 5-enolpyruvyl-shikimate-3-phosphate synthase was found to be inhibited by glyphosate in both bacteria (Table 1). The fact that dehydrogenase activity was not seriously affected by the herbicides might account for the observation that respiration was not affected by these two herbicides in A. hydrophila C-1. The ability of paraquat to form metal chelates (Davison and Papirmeister 1971) may be a factor leading to the inactivation of certain respiratory enzymes in P. chlororaphis L-1.

The present results indicate that aquatic bacteria in general are more susceptible to paraquat than the soil microflora and human bacteria in terms of paraquat concentration applied to the aquatic environment. Since in situ study confirms the inhibitory effects of these two herbicides on aquatic bacterial populations and it is conceivable that communities of higher trophic level in the aquatic environment might also be affected. Consequently, consideration must be given to the possibility of contamination of non-target environment such as streams, ponds and lakes when applying paraquat and glyphosate in the field.

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