

# Degradation of the Urease Inhibitor Phenyl Phosphorodiamidate in Solutions and Floodwaters

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The degradation of the urease inhibitor phenyl phosphorodiamidate (PPDA) in buffered and unbuffered solutions followed first-order reaction kinetics, and the rates were greatly affected by the buffering salts. The rate of PPDA degradation increased linearly with increasing buffer concentrations. At pH values where the salts had no buffering ability, they did not affect the rate. Degradation of PPDA in floodwaters overlying soils depended on the daytime pH of the water, and there was no evidence that biological or adsorption-catalyzed degradation was important. The PPDA failed to be an effective urease inhibitor shortly after its concentration dropped to 0.5 ppm. It was difficult to reduce the floodwater pH with organic salts. Algicides were the most effective means of decreasing the base hydrolysis of PPDA.

Urea N has emerged as the most important source of solid fertilizer, particularly in the developing countries. In South and Southeast Asia, urea presently accounts for at least 63% of the total N fertilizer used (Martinez and Diamond, 1984). Ammonia volatilization has been shown to be an important mechanism for loss of nitrogen from urea applications in flooded soils (Fillery and Vlek, 1986). One proposal for decreasing  $\text{NH}_3$  volatilization losses from urea applications is by use of urease inhibitors (Vlek et al., 1980). When the enzymatic hydrolysis of urea to  $\text{NH}_3$  is inhibited, the amount of  $\text{NH}_3$  eligible for such loss is reduced. In addition to decreasing N losses, urease inhibitors have been proposed to reduce  $\text{NH}_3$  toxicity to newly germinating seeds (Martens and Bremner, 1984) or growing plants (Rogers et al., 1987) and to reduce  $\text{NO}_2^-$  toxicities.

A potent urease inhibitor that has been extensively evaluated is phenyl phosphorodiamidate (PPDA). It was originally found to reduce  $\text{NH}_3$  volatilization losses on sandy upland soils (Heber et al., 1979; Matzel et al., 1979) although in other studies it was found to have little effect (Broadbent et al., 1985; Schlegel et al., 1986), possibly because actual losses through  $\text{NH}_3$  volatilization were low rather than because the inhibitor performed poorly. When applied to flooded rice soils, PPDA generally increased N uptake by the plants but rice yields were not increased (Byrnes et al., 1983; Fillery et al., 1986; Simpson et al., 1985). The inhibition of urease by PPDA, based on urea concentrations in the floodwater, was lost very suddenly (Vlek et al., 1980; Byrnes et al., 1983), and urea hydrolysis then resumed at rates similar to those in the uninhibited system. This loss of inhibition was thought to be due to the poor stability of PPDA. Soil incubation experiments at different temperatures support this contention (Martens and Bremner, 1984).

Research by Austin et al. (1984) established a high-performance liquid chromatography (HPLC) method for PPDA analysis and showed that PPDA can be hydrolyzed by both acid and base catalysis. Whereas the base-catalyzed hydrolysis produces phenol and diamidophosphoric acid, the acid-catalyzed reaction proceeds by a sequential deamination, producing phenyl phosphoramidate and then phenyl phosphate.

Factors affecting the degradation of PPDA have not been evaluated in flooded rice systems. The pH of the

floodwater of flooded soils is normally basic; the pH rises to 9 or 10 during the day, because of algal uptake of  $\text{HCO}_3^-$  (Bouldin, 1986) and thereby tends to favor rapid base-catalyzed hydrolysis of PPDA during the day. Adsorbed protons on soil particles may affect the rate of degradation, or adsorption reactions may catalyze degradation, as has been found for pesticides (Bowman and Sans, 1977). Decomposition in soils is thought to be caused by a non-biological heterogeneous catalysis on clay minerals (Bremner and Martens, 1987; Byrnes, 1988). It has not been established whether the floodwater pH alone affects PPDA degradation or if soil or biologically mediated reactions are also important.

These studies were conducted to determine the effects of various pH buffers and floodwater additions on PPDA degradation and to evaluate the possibility of lowering the pH of the floodwater to decrease PPDA degradation rates and thus prolong its effectiveness.

## MATERIALS AND METHODS

**Degradation of PPDA in Solutions.** *Buffered Solutions.* The degradation of PPDA in buffered solutions and in a nonbuffered pH-stat was studied in order to evaluate the effects of buffering substances on the degradation rate of PPDA. Solutions were made of the individual constituents  $\text{KH}_2\text{PO}_4$ ,  $\text{H}_3\text{BO}_3$ , and  $\text{CH}_3\text{COOH}$  at concentrations of 0.024, 0.075, 0.15, and 0.30 M. The pH was adjusted to 5.0 by addition of KOH. Similarly, solutions at pH 7.0 and 9.0 were made. At pH 9.0, a carbonate buffer made from  $\text{K}_2\text{CO}_3$  was used instead of acetate, and the pH 7.0 solutions were made with  $\text{K}_2\text{HPO}_4$ ,  $\text{H}_3\text{BO}_3$ , and  $\text{CH}_3\text{COOK}$ . The PPDA stock solutions were made from PPDA recrystallized from ethanol and dissolved by use of a sonic bath.

An aliquot of PPDA stock solution was added to each of the buffer solutions to produce a concentration of approximately 25 ppm PPDA. These solutions were kept at room temperature (approximately 20 °C) and analyzed for PPDA at hourly intervals by reversed-phase HPLC by a method similar to that of Austin et al. (1984). A stainless steel column (0.8 cm (i.d.)  $\times$  30 cm) of 5- $\mu\text{m}$   $\text{C}_{18}$  resin was used; a mobile phase of 50/50 (volume/volume) methanol and water was pumped at a rate of 0.7 mL/min with a Kontron LC Model T-414 pump. A Kontron model MS 1660 automatic sampler and a Uvikon Model 720 LC spectrometer, which measured absorption at 200 nm, were used. A Shimadzu Model C-R1B integrator calculated and recorded the PPDA concentrations.

To determine the functional form of the degradation kinetics, permutations of PPDA concentration were plotted versus time. The data were found to fit the form of

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## Chart I. Treatments Used in Soil Experiment

- 1 no inhibitor (control, urea alone)
- 2 9% PPDA, based on the weight of urea
- 3 PPDA + 10 g of dried and ground cattle manure mixed into soil before submergence
- 4 PPDA + 0.6 g of  $\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$  into floodwater (0.0037 M)
- 5 PPDA + 0.126 g of  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  into floodwater (0.0017 M)
- 6 PPDA + 0.136 g of  $\text{CH}_3\text{COONa}$  into floodwater (0.0055 M)
- 7 PPDA + 0.062 g of  $\text{H}_3\text{BO}_3$  (0.0033 M)
- 8 PPDA + 10 g of cattle manure +  $\text{Al}_2(\text{SO}_4)_3$
- 9 PPDA + 0.016 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (20 ppm Cu in floodwater)

first-order kinetic equations, as previously shown for similar studies on PPDA degradation (Austin et al., 1984).

The pH of the solutions was measured with a combination pH electrode at the completion of the studies (normally after 48–90 h) to verify that the pH was within 0.1 pH unit of the desired value.

**Nonbuffered Solutions.** Studies on the degradation of PPDA in nonbuffered solutions used a Radiometer pH titration system as a pH-stat system. This system consisted of a PHM62 pH meter, a TTT60 titrator, and an ABU13 autoburet. A water-jacketed 100-mL reaction vessel, stirred by magnetic stirrer and kept at 20 °C, was used. Appropriate concentrations of either KOH or HCl were used to maintain the pH during the incubations. The 100-mL volume of PPDA solution was not changed by more than 2% during the study periods (up to 4 days). The pH-stat apparatus was also used for the lowest concentration of phosphate and borate buffers at pH 9 to maintain the proper pH. With no buffer in solution, 0.1 N KCl was used to ensure completion of the two half-cells in the pH measurement.

The HPLC instrumentation used to measure PPDA concentration in studies conducted with the pH-stat and the soil studies was as described by Austin et al. (1984), except that the data were acquired and processed with an IBM Model 100 personal computer using Nelson analytical software.

**Effects of Buffers, Acids, and Tillage in Flooded Soil Systems.** Two soil systems were used, 300 g of Crowley silt loam and 60 g of Crowley soil mixed with 260 g of coarse washed sand, to allow the study of the inhibitors in a soil of high urease activity and in a system of much lower urease activity when sand was added.

The soil or soil plus sand was placed in plastic containers (11.5-cm diameter  $\times$  7-cm depth). The Crowley silt loam is a Typic Albaqualf, with a water pH of 5.2. Manure was added to two treatments (3 and 8), and the soil was flooded and puddled with a spatula and kept flooded at 3-cm depth in the greenhouse for 3 weeks. Then, the acids and algicide additions were made to the floodwater, and urea and the urease inhibitors were added as solutions to the floodwater. The urea was added at a rate of 35 mg of N, which made the initial concentration approximately 200 ppm urea N. The PPDA made the floodwater approximately 40 ppm, or 9% of the urea on a weight to weight basis. This initial concentration of the inhibitors was necessary to obtain reliable HPLC results; it is about 4 times higher than previously used (Byrnes et al., 1983; Simpson et al., 1985). The urea and ammonium N concentrations, daytime pH, and PPDA concentrations were monitored daily for 26 days after the urea addition. The floodwater was analyzed by AutoAnalyzer (Technicon, 1973, 1974), and the pH was determined by a combination pH electrode.

The urease inhibitor and soil amendments are listed in Chart I.

The amounts of salts added were the amounts necessary to decrease the floodwater pH to about 4.5 for  $\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$  and 5.5 for the other salts, as determined by

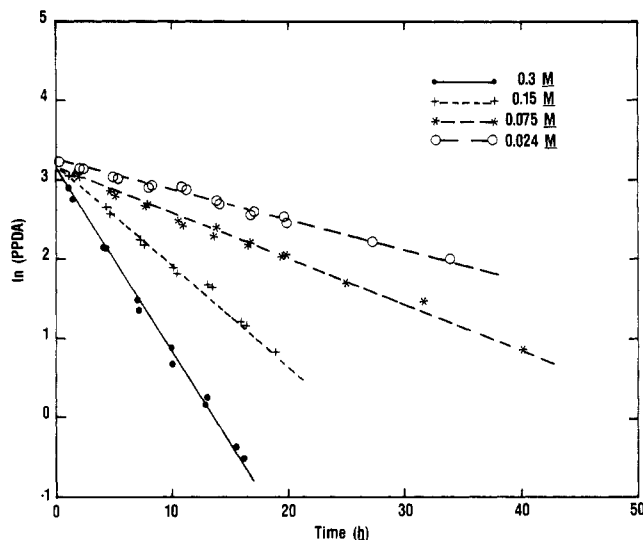


Figure 1. First-order plots of PPDA degradation at four phosphate buffer concentrations, pH 9.

laboratory experimentation with distilled water. The  $\text{CuSO}_4$  was added at sufficient amounts to act as an algicide.

The experiment was conducted in duplicate, and the soil systems were either mixed before the addition of the urea, inhibitors, and other amendments or not mixed. Thorough mixing was done with a spatula, and the soil was allowed to settle for 1 h before the additions were made. The tillage variable was to determine whether mixing the biologically active soil surface would change the rate of urea hydrolysis and the pH conditions of the floodwater and thus affect the inhibitor performance.

## RESULTS AND DISCUSSION

**Solution Studies.** Austin et al. (1984) found that the overall rate constant for the degradation of PPDA is given by

$$K_{\text{PPDA}} = 10^{-\text{pH}K_A} + 10^{(\text{pH}-14)K_B}$$

where  $K_A$  and  $K_B$  are the second-order rate constants for acidic and basic pH, respectively. The constants were calculated from the rates at the ranges pH 2–5 and 10–12. Their experiments, conducted with approximately 0.06–0.08 M phosphate buffers, showed a faster rate than calculated by the equation in the pH 5–9 range.

The concentration of PPDA was found to decrease according to first-order kinetics in the various buffers and buffer concentrations (example shown in Figure 1). The rate constant ( $k$ ) increased with increasing phosphate concentration (Figures 2–4). At pH 5, acetate concentration also had a large effect, whereas borate had none (Figure 2); at pH 7, borate and acetate concentrations had little effect (Figure 3). At pH 9, borate had a larger effect than phosphate and carbonate had an intermediate effect (Figure 4). Increasing the salt concentrations shortened the half-life of PPDA to as little as one-eighth its half-life without a buffer.

When the degradation of PPDA was studied without buffers in the pH-stat system, the rate constants were in good agreement with the values obtained by extrapolating the buffer data to zero-buffer concentration (Figures 2–4).

These data show that at pH values where the salts act as buffers the concentrations of buffer affect the degradation rate and the rate constant is not just pH dependent. At these pH's the species can act as an acid or base, in accordance with the concept of a Bronsted–Lowry acid and base. From the  $\text{p}K_a$  values for the buffers used, acetate

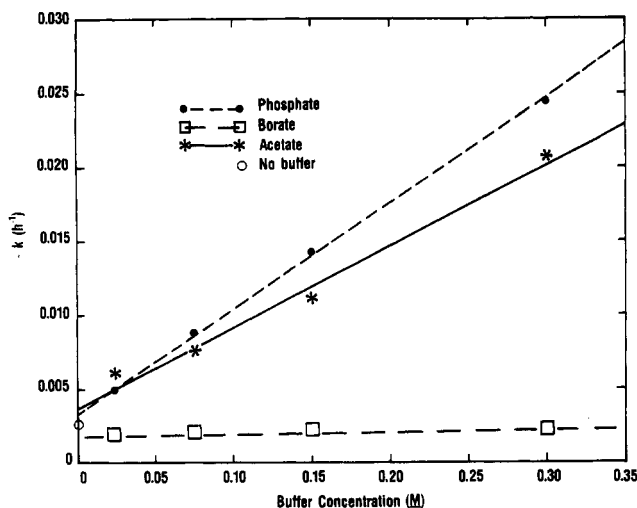


Figure 2. Effect of buffer concentration of PPDA degradation constant, pH 5.0.

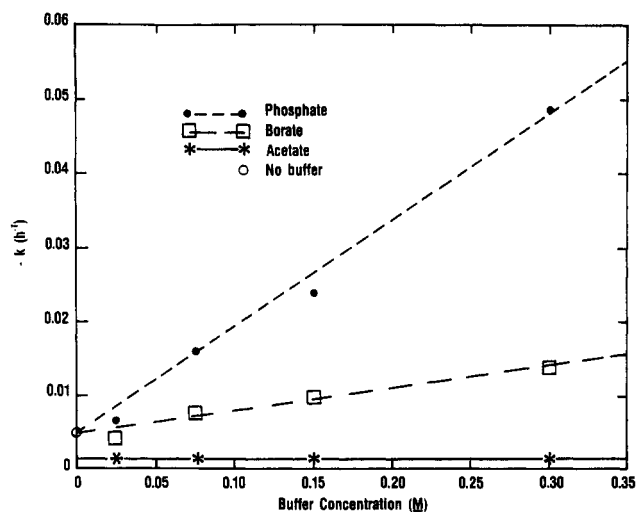


Figure 3. Effect of buffer concentration of PPDA degradation constant, pH 7.0.

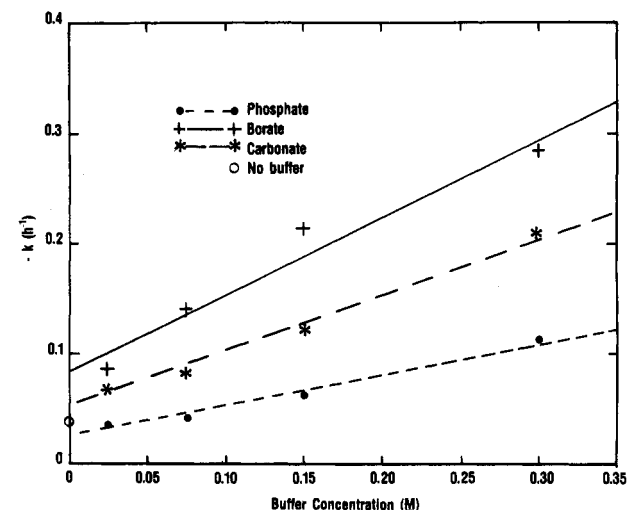


Figure 4. Effect of buffer concentration of PPDA degradation constant, pH 9.0.

( $pK_a = 4.76$ ) would be expected to influence the hydrolysis of PPDA at pH 5 but not at pH 7. Borate ( $pK_a = 9.19$ ) would be expected not to affect the rates at pH 5, to have little effect at pH 7, and to have a large effect at pH 9. Similarly, carbonate ( $pK_a = 6.46$  and  $10.22$ ) would be expected to have an effect at pH 9 (Figure 5), and phosphate

would be expected to have an effect at all the pH values studied (Figures 2–4). Nonbuffering salts would not be expected to affect the hydrolysis rates, and no effect was found (data not presented).

Totally protonated or unprotonated species would not be expected to have significant effects in that they exhibit little exchange of protons, and the  $H^+$  and  $OH^-$  concentrations become high relative to the concentration of the buffer species. This explanation of the effect of buffer activity was found to be in agreement with other work showing that buffers at concentrations above 0.001 M become potentially significant in increasing acid-base catalysis (Perdue and Wolfe, 1983). Calculation of the coefficient for the buffer effect was found to be less in all cases than the maximum effect predicted by Perdue and Wolfe (1983).

These data indicate that, at the high daytime pH of floodwaters, the presence of carbonate or  $NH_4^+$  ( $pK_a = 9.24$ ) due to any urea hydrolysis may enhance PPDA degradation. Although carbonate concentrations are normally low during daytime photosynthesis, there may be enough carbonate to act as a buffer at other times. Any loss of inhibition due to PPDA degradation may accelerate further PPDA decomposition as carbonate is released from urea hydrolysis. This may explain the sudden loss of inhibition by PPDA in flooded soil systems.

**Degradation in Flooded Soil Systems.** Because PPDA has more potential to reduce  $NH_3$  volatilization losses from flooded soils if its stability can be increased, efforts focused on decreasing the floodwater pH. This was done by adding acidifying salts and by controlling algae growth, which is responsible for the very high daytime pH of paddy floodwaters (Bouldin, 1986). Efforts were also made to evaluate the influence of organic matter additions, which affect the biological activity of the soil.

The addition of acidifying agents lowered the pH of the floodwaters, particularly the addition of  $Al_2(SO_4)_3$ , which reduced the pH to about 4 in both soil systems (Table I). This pH caused rapid degradation of PPDA, and PPDA was essentially undetectable in 3 or 4 days. When the floodwater was highly alkaline, as with the manure and the  $CH_3COONa$  additions, degradation was also rapid. When the floodwater pH was not below pH 5, as in most of the rest of the treatments in the Crowley sand mixture, the PPDA remained in the floodwater for 9–12 days.

Although the algicide reduces the daytime pH of the floodwater, it also affects other aspects of the biology of the system, making it difficult to unequivocally distinguish effects on biological degradation from simple pH effects. Daytime pH values for the acetate addition and the manure addition in the Crowley soil mixed with sand were very similar, and the PPDA disappeared at the same rate in the two treatments (Table I).

The degradation rates in the floodwaters are in rough agreement with the data of Austin et al. (1984), particularly at the extremes of pH. A close agreement would not be expected because of the buffer concentration effect and, probably more importantly, because of the large diurnal changes in pH and temperature (25–34 °C) that occurred in the floodwaters. Despite the inability to quantify the pH effects and the lack of data for values from pH 5.5 to 8.0 in Crowley soil, the resulting data give no indication that biological factors, other than those that influence floodwater pH, are important in PPDA degradation. These results agree with other work with heat-sterilized and chemically sterilized soils that showed that PPDA degradation is principally through nonbiological reactions (Bremner and Martens, 1987; Byrnes, 1988).

**Table I. Pseudo-First-Order Degradation Constants (*k*) and Half-Life of PPDA and Afternoon Floodwater pH**

	treatment	soil <sup>b</sup>	Crowley sand mixture			Crowley soil		
			av daytime pH	$-k, \text{h}^{-1} \times 10^2$	$T_{1/2}, \text{h}$	av daytime pH	$-k, \text{h}^{-1} \times 10^2$	$T_{1/2}, \text{h}$
2	none	M	8.0	1.33	52	8.4	4.62	15
		S	8.0	1.67	42	9.4	6.75	10
3	manure	M	9.1	6.67	10	8.5	5.63	12
		S	8.8	7.88	8.8	8.5	7.17	10
4	$\text{Al}_2(\text{SO}_4)_3$	M	4.1	7.50	9.2	4.3	6.67	10
		S	4.3	10.5	6.6	4.3	8.79	7.9
5	$\text{CaH}_4(\text{PO}_4)_2$	M	5.0	0.875	79	8.2	4.25	16
		S	5.2	0.750	92	8.2	4.00	17
6	$\text{CH}_3\text{COONa}$	M	9.1	6.75	10	9.0	6.71	10
		S	9.1	5.21	13	9.0	6.88	10
7	$\text{H}_3\text{BO}_3$	M	7.1	1.13	61	8.1	3.21	22
		S	7.0	1.13	61	8.3	4.46	16
8	manure and $\text{Al}_2(\text{SO}_4)_3$	M	4.3	5.17	13	4.8	3.29	21
		S	4.3	5.42	13	5.5	2.67	26
9	$\text{CuSO}_4$	M	6.0	1.25	56	8.1	3.29	21
		S	5.9	1.00	69	8.0	3.50	20

<sup>a</sup> Half-life. <sup>b</sup> Key: M is mixed soil; S is the settled or undisturbed soil.

Although the phosphate buffer worked well to maintain the pH in the Crowley sand mixture, it did not work well with Crowley soil alone. Apparently phosphate was removed by algae, stimulated its growth, and further raised the pH. Algicide addition with the phosphate may work well, but the algicide probably should be an organic herbicide and not unchelated Cu, which would tend to precipitate. The other acidifying additions performed much better with the algicide addition than they did by themselves to maintain a lower pH.

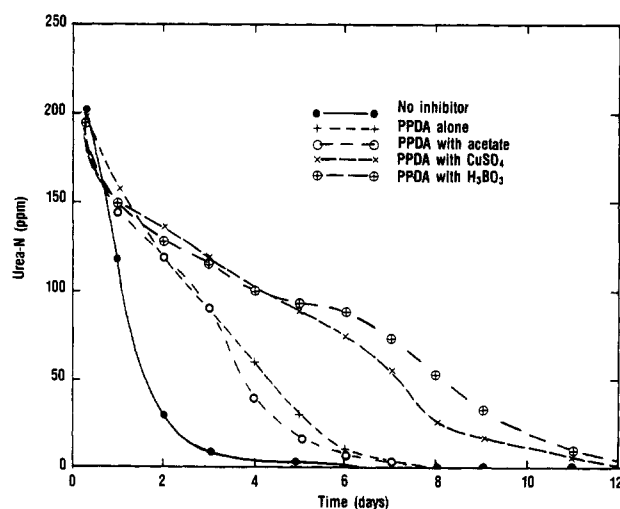
Unlike the Crowley soil-sand mixture, there was an effect of soil mixing with the Crowley soil on the floodwater pH when PPDA was used alone (treatment 2). Because of reduced biological activity, the pH of the mixed system was 1 pH unit less than when the surface layer was undisturbed, and the PPDA concentration was maintained above 0.5 ppm for an additional 1 day. The manure addition resulted in pH's similar to that with PPDA alone, and there was a similar effect on the PPDA concentrations in the mixed and settled systems.

In the Crowley soil, the algicide reduced the pH of the floodwater by 0.3–1.4 pH units (Table I) and slowed the rate of PPDA disappearance.

No increase in the rate of degradation was observed as a result of the added buffers, possibly because the concentrations were too low. The use of a buffering salt would probably be impractical unless an algicide was used.

On the first day after PPDA addition, phenol was found in the floodwaters of some treatments at concentrations up to 9 ppm, which is equivalent to the degradation of practically half of the added PPDA. Quantitative accounting for PPDA degradation would not be expected from the phenol concentrations because the compound is volatile and reactive in soils (Dobbins et al., 1987). Phenol was found even in the treatments whose floodwaters were very acidic; apparently the acid hydrolysis products were further hydrolyzed to phenol, which would not be predicted by previous work (Austin et al., 1984). The enzyme phosphatase may cause catalysis of phenyl phosphate to phenol and phosphoric acid, as in the tests for phosphatase activity with *p*-nitrophenyl phosphate (Tabatabai, 1982).

Because the differences in the urea concentrations due to soil mixing were generally small, the average concentrations of the mixed and settled treatments are presented in Figure 5. This figure demonstrates the rapid urea hydrolysis that occurred when no inhibitor was used and the rapid hydrolysis that occurred for the PPDA treatments within 1 day of when the PPDA concentration decreased to less than 0.5 ppm. The exception to this was



**Figure 5.** Average floodwater urea concentrations of the Crowley soils.

the boric acid treatment, which worked exceptionally well in the Crowley soil. This inhibition could not be attributed to PPDA, because the pH during the day was slightly above 8.0 and PPDA degradation would be expected to be rapid and PPDA degradation occurred in this treatment at the same rate as others with floodwaters of similar pH (Table I). The urea concentrations were maintained better in this treatment than in any of the other treatments (Figure 5), indicating that the boron acted as a urease inhibitor (Tabatabai, 1977).

## CONCLUSION

The degradation rate of PPDA is greatly affected by buffering substances, and this effect should be considered in hydrolysis studies involving buffers. It is likely, however, that the concentrations of buffering substances are high enough to have a significant effect in natural floodwaters only due to urea hydrolysis following urea application, and even then the effect is likely to be small, since  $\text{NH}_4^+$  N and carbonate concentrations would not normally be above 0.004 M in the floodwater (Byrnes et al., 1983). A similar conclusion as to the lack of buffer effects in acid-base catalysis in natural waters was reached by Perdue and Wolfe (1983). The decomposition of PPDA in floodwaters was affected by changing the pH of the waters, particularly in a soil-sand mixture. To control the pH of the system with soil alone was much more difficult, and the addition of an algicide had a very large effect on the disappearance

of PPDA and hydrolysis of urea. The algicide addition with PPDA in Crowley soil prolonged its effectiveness for about 3 days more than without the algicide.

Generally, the daytime floodwater pH gave an indication of the degradation rate of PPDA. At daytime pH's of approximately 9, the half-life of PPDA was only 10 h; at neutral to slightly acidic pH's, the half-life could be extended to about 25 h with the Crowley soil and up to about 90 h with the soil-sand mixture. The absorption of PPDA may be confounding the apparent degradation rates, in that the rate of PPDA disappearance in the Crowley soil was much faster than for the soil-sand mixture at similar daytime pH's. There is little evidence from the treatments with organic matter additions that PPDA decomposition was biologically mediated other than through the effects of biological activity on floodwater pH.

**Registry No.** PPDA, 7450-69-3;  $\text{Al}_2(\text{SO}_4)_3$ , 10043-01-3;  $\text{CaH}_4(\text{PO}_4)_2$ , 7758-23-8;  $\text{CH}_3\text{COONa}$ , 127-09-3;  $\text{H}_3\text{BO}_3$ , 10043-35-3;  $\text{CuSO}_4$ , 7758-98-7; urease, 9002-13-5.

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Received for review March 1, 1988. Accepted August 16, 1988.