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Dissipation of Phloxine B and Uranine in Sediment and Water at a Kauai Spill Site

Q. X. Li, C. J. Voisinet Bender,* J. P. Alcantara-Licudine

Department of Environmental Biochemistry, University of Hawaii at Manoa,
Honolulu, HI 96822, USA

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The development of insecticides based on a photochemical mechanism that requires light activation as an integral part of the toxic action is currently under study. Attempts are being made to develop and register phloxine B (2',4',5',7'-tetrabromo-4,5,6,7-tetrachlorofluorescein disodium salt) and uranine (fluorescein disodium salt) developed as a product called "SureDye" to control a variety of insect pests. SureDye is a 1:1 molar mixture of phloxine B (69% by weight) and uranine (31% by weight) which is included as 1% concentration in a protein bait (USEPA 1995). Environmental fate of phloxine B and uranine has not been reported and only predicted with laboratory and mathematical models (Bergsten 1995). Recent studies examined effects of various environmental factors on photolysis of phloxine B fortified in different solutions and water under laboratory conditions (Li et al. 1997a; Wang et al. 1998).

Phloxine B and uranine commonly used as color additives in drugs and cosmetics have been recently studied to control Mediterranean fruit flies in coffee fields in Hawaii (Liquido et al. 1997). The aerial application rate of SureDye was 0.56 oz (0.39 oz of phloxine B and 0.17 oz of uranine) of active ingredient (a.i.) per acre for the coffee fields in Hawaii. An accidental spill of the SureDye bait occurred on the staging area of the Haiku agricultural landing strip (Figure 1) near the test field on Kauai Island on October 18, 1996. Immediately after the spill, the contractor washed down the runway and apron which are on a slight incline. The total amount of spilled SureDye a.i. was about 1.2 kg of phloxine B and 0.54 kg of uranine. This study investigated the dissipation and fate of phloxine B and uranine in water and sediment in the spill site. Distribution coefficients (Koc) of phloxine B and uranine were determined.

MATERIALS AND METHODS

Phloxine B and uranine were purchased from ICN Biochemicals (Cleveland, OH). Phloxine B was purified by silica gel column chromatography as previously reported (Alcantara-Licudine et al. 1997). HPLC grade ammonium acetate

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*Present address: Department of Chemistry, United States Military Academy, West Point, NY 10996, USA

Correspondence to: Q.X. Li

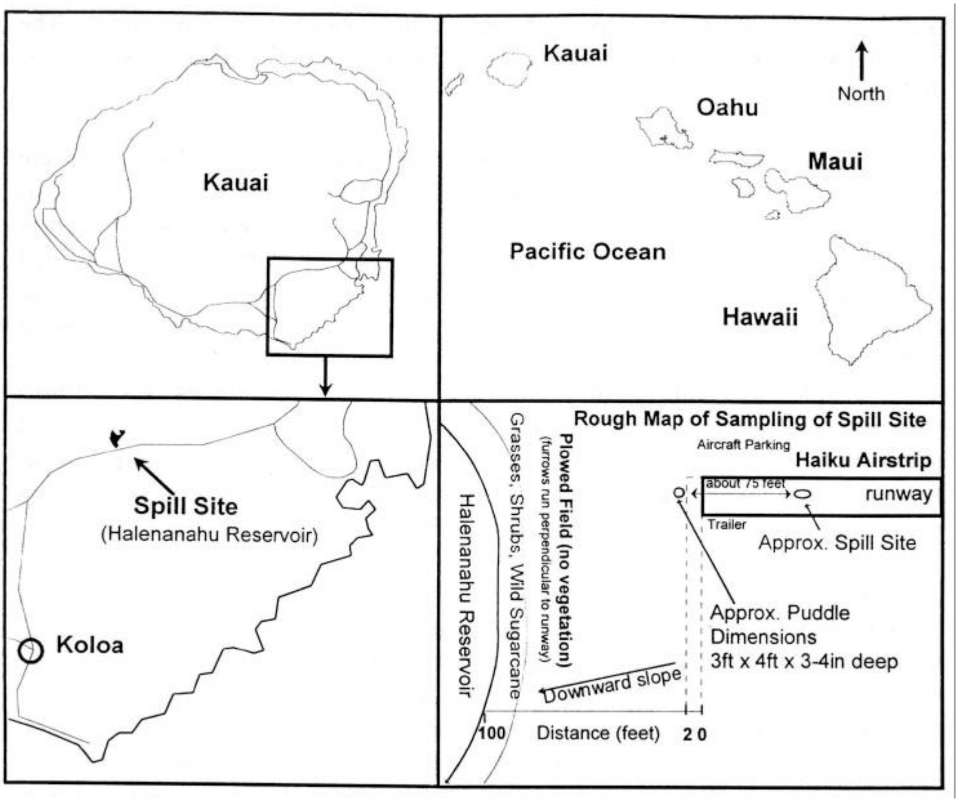

Figure 1. Map of the spill site.

(NH_4OAc), *n*-butyl amine (*n*-BA), optima grade methanol (MeOH) and acetonitrile (ACN) were obtained from Fisher Scientific (Pittsburgh, PA). The solid phase extraction (SPE) cartridges were obtained from J.T. Baker Inc. (Phillipsburg, NJ).

Standard stock solutions were 1 mg/mL of phloxine B or uranine in MeOH. Working standard solutions of phloxine B or uranine were diluted with deionized water. All standard solutions were covered with aluminum (Al) foil and stored in the dark at 4°C.

The water and sediment samples were collected 12, 26, 82, 123, and 284 days after the spill. Three water samples were taken from Halenanahu reservoir along the shore and about 20 feet apart between sites at the first sampling date (October 30, 1996) (Figure 1). Two water and three sediment samples were collected from the puddle on each sampling date. The sediment samples were taken from the puddle about two inches deep and were approximately one foot apart. The samples were covered with Al foil, frozen within 5 hrs after collection and stored at -20°C.

The water (25 mL) from Halenanahu reservoir and tap water (100 mL) were separately fortified by adding 5 mL of the 2 µg/mL standard solution to 1 L of water for recovery determination. The spike samples were stirred for 20 min in the dark. No fortification was a control. The analytes in water were extracted with SPE phenyl cartridges according to the procedure described by Li et al. (1997b). The columns were activated with MeOH (2x5 mL) and then washed with deionized water (20 mL) at a flow rate of 4-6 mL/min. After pH value of water samples was adjusted to < 3 using 0.2 M HCl, the samples (25-100 mL) were passed through the columns using a Baker-10 SPE™ system at a flow rate of about 5 mL/min. The analytes were eluted with 10 mL MeOH containing 2 µL *n*-BA, and the eluent was collected in 15-mL test tubes. The extracts were concentrated to 2 mL under nitrogen stream for quantitative HPLC analysis.

Preparation and analysis of the sediment samples followed the procedure of Alcantara-Licudine et al. (1997). The sediment samples were air-dried, sieved through 20 mesh and stored in sealed jars at room temperature. After the moisture was adjusted to about 10%, Na_4EDTA (100 mg) was added and thoroughly mixed. The samples were covered with paraffin film and kept in the dark overnight. Samples were quantitatively transferred to a 2.5-mL extraction vessel. MeOH (1 mL) and *n*-BA (0.05 mL) were added on top of the soil. The extraction vessel was placed in the extraction chamber for 5 min for temperature equilibration, then statically extracted for 5 min with supercritical CO_2 at 60°C/272 atm. Dynamic extraction followed, allowing 50 mL of supercritical CO_2 to pass through the sample at 4-8 mL/min. Samples were extracted again with supercritical CO_2 at 60°C/476 atm after a second addition of *n*-BA (0.05 mL) and MeOH (1 mL). The extracts were collected in MeOH (10 mL) in the same vial covered with Al foil. SFE extracts were degassed by sonicating in a lukewarm bath for 0.5 min to remove dissolved CO_2, concentrated and filtered through a Gelman 0.45 µm acrodisc for HPLC analysis.

The distribution of phloxine B and uranine between water and soil systems using a batch equilibrium adsorption procedure was determined as follows. Ten milliliters of aqueous solution of phloxine B or uranine were added to one gram of air-dried soil or sediment in 40-mL teflon centrifuge tubes. The tubes were capped and shaken vigorously in a Burrell shaker twice a day for 30 min within 72 hr equilibration period in a dark room. Spike concentrations of phloxine B or uranine ranged from 1.25 to 20,000 µg/mL. Each soil treatment was replicated at least three times. Following equilibration, the samples were centrifuged for 10 min at 10,000 rpm in a Sorvall Superspeed RC 2-B rotor. Aliquots of the supernatant solution were diluted for accurate determination of concentrations by capillary zone electrophoresis (CZE) (Li et al. 1997b). Soil (0-5 cm depth) and sediment samples were collected from the spillsite and blank samples were analyzed for phloxine B and uranine.

HPLC analysis was performed with a Hewlett-Packard (HP) HPLC Series 1050 equipped with an autosampler, diode array detector, and HP 3396 Series II integrator. The HPLC column was Waters Nova Pak C_{18} (10 cm x 8 mm i.d., 4 µm particle size). Gradient elution was a mixture of ACN and 50 mM NH_4OAc. ACN was increased from 10 to 30% in 5 min (4%/min), to 60% in 10 min (6%/min), and to 90% in 12 min (12%/min). After the mobile phase remained at 90% ACN and 10% NH_4OAc for 2 min, it was changed to 10% ACN and 90% NH_4OAc for 2 min. The detection wavelength was initially set at 493 nm (0-6 min) and changed to 546 nm after 7 min. The flow rate was 2 mL/min. The injection volume was 50 µL.

CZE was performed in an uncoated fused silica capillary of 50 cm x 75-µm i.d. with an effective separation length of 44 cm. The capillary electrophoresis apparatus was a Dionex CES I (Dionex, Sunnyvale, CA) equipped with an autosampler and ultraviolet-visible and fluorescence detectors. Samples were introduced into the capillary by gravity at a height of 100 mm for 10 sec. A constant voltage of 20 kV was applied to the capillary during electrophoresis in the anionic mode. The separation medium was 10 mM $Na_2B_4O_7$-50 mM H_3BO_3 with pH adjusted to 8.5. Absorptions of phloxine B and uranine were monitored at 546 and 493 nm, respectively. Samples and standards were in aqueous solutions.

RESULTS AND DISCUSSION

Phloxine B and uranine were spiked at a concentration of 10 ppb each in tap water and water samples collected from the puddle at the Kauai spill site. Recoveries averaged 93-103% for uranine and 65-73% for phloxine B in tap water using MeOH alone (10 mL) to elute the analytes from the SPE column. Average recoveries were 106-113% for uranine and 100% for phloxine B spiked in the Kauai water samples using 10 mL of MeOH mixed with *n*-BA (0.02%) to elute the analytes. *n*-BA improved the elution of the analytes adsorbed on the SPE column. The concentration immediately after the spill was estimated to be 1000 ppm of phloxine B and 500 ppm of uranine in the puddle water (Table 1). Phloxine B and uranine dissipated rapidly in the puddle water. Phloxine B concentration in the water was 76, 8, 4 and 0.3 ppb after 12, 26, 82, and 123 days, respectively. Levels of uranine in the puddle decreased from 55 ppb on the 12th day to 3 ppb on the 26th day after the spill. No detectable levels of phloxine B and uranine were found in the water samples collected from Halenanahu reservoir which is about 100 feet away from the puddle.

No uranine was detected in all the sediment samples from the puddle (Table 1). Phloxine B was sorbed by the sediment, and was consistently detected over a 284-day period. Phloxine B concentration was 1420, 1540, 2340, 791, and 485 ppb in the sediment after 12, 16, 82, 123 and 284 days of the spill, respectively. The high data fluctuation among sediment samples indicates a variation in the sampling and/or the nature of samples. The SureDye was unevenly spilled in the puddle. The sediment samples were randomly collected about two inches deep from three sites in the puddle. Those all may introduce sample variation and thus the levels of the analytes in the sediment fluctuated.

Table 1. Concentrations of phloxine B and uranine detected in sediment and water samples from the puddle at the Kauai spill site[a].

Sample type	Sampling date	Days after spill	Concentration, ppb	
			Phloxine B	Uranine
Water	10/18/1996	0	(~1000 ppm)	(~500 ppm)
	10/30/1996	12	76.3 ± 1.1	55.1 ± 2.8
	11/13/1996	26	7.6 ± 0.3	3.1 ± 0.1
	01/08/1997	82	3.8 ± 0.4	ND[b]
	2/19/1997	123	0.3 ± 0.2	ND
	07/30/1997	284	ND	ND
Sediment	10/30/1996	12	1,420 ± 610	ND
	11/13/1996	26	1,540 ± 660	ND
	01/08/1997	82	2,340 ± 80	ND
	02/19/1997	123	791 ± 211	ND
	07/30/1997	284	485 ± 90	ND

[a] No phloxine B and uranine were detected in the water samples collected from Halenanalu reservoir on Oct. 30, 1996.

[b] ND = not detected.

Phloxine B and uranine are nonvolatile and water soluble aromatic compounds. The octanol-water partition coefficient is 0.62 for phloxine B at 25 °C. The octanol-isotonic salt solution partition coefficient is 0.72 for uranine at 25 °C (Bergsten 1995). Sorption experiments showed that uranine tends to stay in the aqueous phase at low concentration (<5 ppm) which explains the fast dissipation of the dye in the field due to photolysis or it may have been washed out by rain (Figure 2). Adsorption of phloxine B in Lihue silty clay and sediment collected from the spill site was stronger than that of uranine as shown by their Koc values (Table 2). This result is consistent with the degree of halogenation in the xanthene dye molecule, phloxine B being highly halogenated. Depletion of the dyes in water by fast photolysis drives the equilibrium toward desorption from soil particles. Strong adsorption of phloxine B on soil particles limits the desorption, and thus phloxine B degraded relatively slower than uranine. It was also observed that adsorption coefficients of phloxine B and uranine were higher in soil than in sediments although the organic carbon content of the sediment is higher than soil. Saturation points for either phloxine B or uranine were not attained up to a concentration of 20,000 µg/mL. The increased adsorptive capacity of the soil for these dyes could be attributed not only to organic carbon but also to high mineral content of the soil as shown in Table 2. This phenomenon was also observed in the adsorption of 2,4-D and methyl parathion (Reddy and Gambrell 1987) and other herbicides in the Hawaiian soils (Hilton and Yuen 1963).

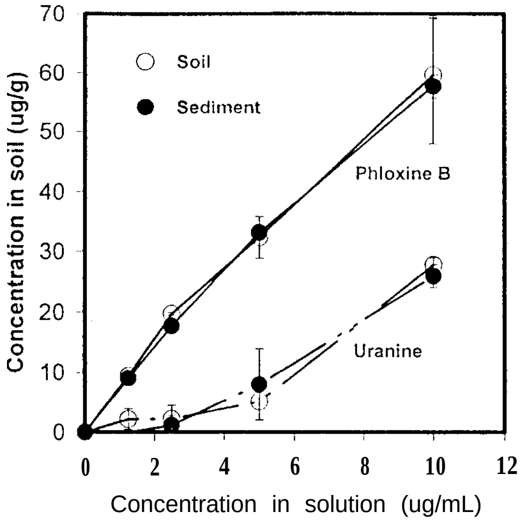

Figure 2. Adsorption of phloxine B and uranine in a water-soil slurry system.

Table 2. Properties of soil and sediment samples in Kauai spill site.

Sample	pH	Fe %	Ca µg g^{-1}	Mg µg g^{-1}	OC[a] %	K_{oc} Phloxine B	 Uranine
Sediment	8.0	18.7	2398	109	3.94	145.15	69.43
Lihue silty clay	7.0	18.6[b,c]	7640[c]	1632[c]	3.10	188.55	88.81

[a] OC = organic carbon.

[b] It was analyzed as free iron oxide (Fe_2O_3).

[c] Data obtained from USDA Soil Conservation Service (1972).

In conclusion, phloxine B and uranine are potential photoactive insecticides being studied for fruit fly control in coffee fields in Hawaii. An accidental spill of the insecticides occurred. Water and sediment samples were collected from the spill site for a 284-day period after the accident. Concentrations of phloxine B and uranine in water decreased over time. Data showed that phloxine B and uranine degraded fast in water at the spill site. However, phloxine B was more persistent than uranine in the puddle, particularly in the sediment. Distribution coefficients (Koc) of phloxine B were 145.15 and 188.55 in sediment and in Lihue silty clay, respectively. Koc of uranine was 69.43 in sediment and 88.81 in Lihue silty clay. Rapid degradation and strong sorption of phloxine B and uranine to Kauai soils indicated a decreased potential for groundwater contamination if they are used as insecticides. Kauai field conditions such as high temperature, frequent rainfall, and strong sunlight, accelerated the dissipation of these dyes in the spill site and minimized possible harmful effect in the environment.

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