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Effect of Suspended Bentonite Clay on the Acute Toxicity of Glyphosate to *Daphnia pulex* and *Lemna minor*

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Inorganic suspended sediment is a common component of many aquatic ecosystems. In aquatic environments, pesticides may be partitioned between the dissolved and particulate phases of the system, depending on the physical and chemical properties of the water, the type of suspended sediment present, and the molecular nature of the pesticide itself (Sharom et al. 1980). This partitioning affects the availability of the pesticide to different aquatic organisms and hence it's potential for having adverse effects.

To evaluate the hazard of any pesticide to non-target aquatic organisms under natural conditions, one must consider both bioavailability and toxicity. The purpose of this study was to demonstrate the effect of a common type of inorganic suspended sediment (bentonite clay) on the acute toxicity of the herbicide Roundup (glyphosate) to an aquatic invertebrate (Daphnia pulex) and an aquatic plant (Lemna minor). Glyphosate was chosen because it is soluble in water (Holland 1977), readily adsorbed onto inorganic particles (Sprankle et al. 1975), moderately toxic to several aquatic organisms (Johnson and Finley 1980), and has potential for use in and near aquatic environments (Holland 1977).

MATERIALS AND METHODS

Tests were conducted between April 1979 and May 1980 at the Yankton, South Dakota, Field Research Station, of the Columbia National Fisheries Research Laboratory. Daphnia pulex were initially collected from the Missouri River, about 15 km downstream from Yankton, in January 1979 and stock cultures (4 L) were maintained in well water (pH, 7.6; hardness, 282 mg/L as $CaCO_3$) at 15^{o} C, according to the method described by Martin and Novotny (1975) for Chydorus sphaericus. Lemna minor was obtained in October 1979 from a spring on the Missouri River floodplain, near Yankton, and stock cultures (1 L) were maintained under continuous illumination (2000 lux) at 22^{o}C in the synthetic medium described by Miller et al. (1978). A liquid formulation of Roundup (41% glyphosate) was purchased locally in April 1979 and stored at room temperature. All concentrations of glyphosate reported in this study are based on calculated concentrations of active ingredient.

The toxicant was mixed by combining glyphosate with test medium on the day each test was begun. No organic solvents were used. Inorganic sediment, Ardmore Bentonite (South Dakota Geological Survey Reference Number JCH 69-151B), was obtained from the University of South Dakota Geology Department. A suspended sediment concentration of 50 mg/L (particle size < 2 μm) was used in all test media containing suspended sediment. Glyphosate was added to test media containing suspended sediment and mixed vigorously with a magnetic stirrer for at least 2 hours before the test organisms were introduced. Adsorption of glyphosate in soil-water mixtures approaches maximum in less than 1 hour (Sprankle et al. 1975).

Acute toxicity tests with D. pulex were conducted in well water (pH, 7.6; hardness, 282 mg/L as $CaCO_3$) at 15°C according to methods described for the BASIC STATIC TEST by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Ten mature daphnids were randomly placed into 400 mL beakers containing 200 mL of toxicant solution. Twelve concentrations of glyphosate, ranging from 0 to 20 mg/L, were used in four replicate tests. In each test, identical concentrations of glyphosate and controls were prepared with and without suspended sediment and tests run concurrently. The results of the four tests were combined, and the 48-h EC50 was calculated according to Litchfield and Wilcoxon (1949).

Acute toxicity tests with Lemna minor were conducted under continuous illumination (2000 ± 10% lux) in synthetic medium at 22°C according to methods described by Walbridge (1977), except that static, rather than flow-through conditions were used. Tests were initiated by placing five, actively growing two-frond plants in 500 mL Erlenmeyer flasks containing 100 mL toxicant solution. Six toxicant concentrations (0 to 10.0 mg/L) and controls, were used both with and without suspended sediment. The number of new fronds produced in each test concentration was determined after 14 days, and results of four replicate tests were combined to calculate ED50 values according to Walbridge (1977).

An additional experiment was conducted to simulate the effects of a single dose of glyphosate (with and without suspended sediment) on populations of D. pulex. Twenty-four 4 L cultures of D. pulex were allowed to reach stable population densities at 15°C by using the methods of feeding and harvesting described by Martin and Novotny (1975). Before treatment, cultures were sampled (by harvesting one-fourth of the total population) at weekly intervals for 7 weeks to establish pretreatment population characteristics. Three replicate cultures were then treated to obtain final concentrations of 0, 1.0, 2.0, and 4.0 mg/L glyphosate (both with and without suspended sediment). The short-term effects and subsequent recovery of the populations were observed for an additional 9 weeks post-treatment by continued weekly harvesting and feeding. An analysis of variance combined with the Waller-Duncan, K-Ratio, t-test was used to compare population parameters in the treatment and control cultures.

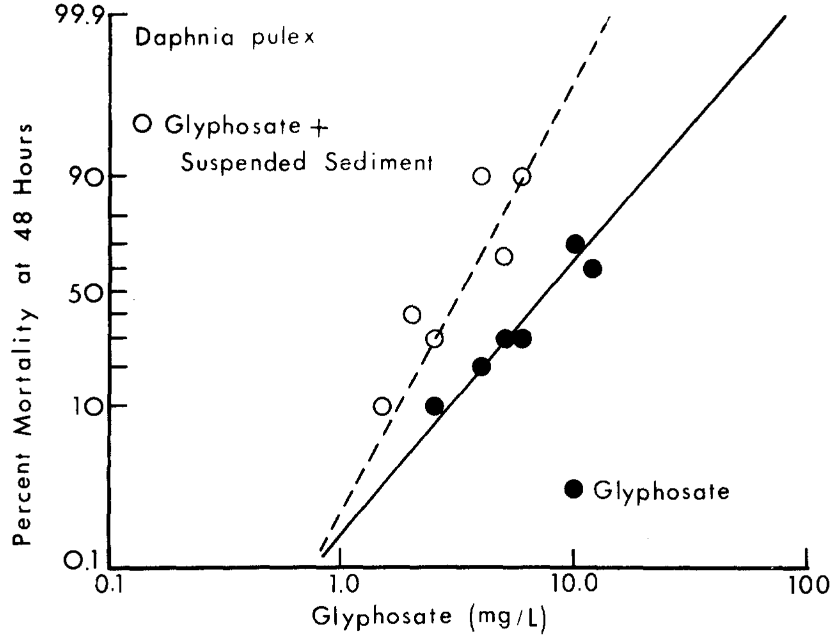
RESULTS AND DISCUSSION

In the BASIC STATIC TEST, the calculated 48-h EC50 for Daphnia pulex at 15°C was 3.2 mg/L with suspended sediment (95% Confidence Interval (CI), 3.0-3.4 mg/L) and 7.9 mg/L without suspended sediment (95% CI, 7.2-8.6 mg/L). Johnson and Finley (1980) reported a 48-h EC50 of 3.0 mg/L (95% CI, 2.6-3.4 mg/L) with first instar Daphnia magna (without suspended sediment) at 22°C. The slightly increased sensitivity to glyphosate shown in their experiments may have been due to species differences, to their use of immature daphnids, or to the higher test temperature used. In our tests, suspended sediment apparently increased the acute toxicity of glyphosate more than twofold. The dose-response curves (Fig. 1) fitted by using Chi-square procedures of Litchfield and Wilcoxon (1949), indicated that mortality was negligible at about 0.75 mg/L with or without suspended sediment. As concentrations of glypohsate increased, daphnid mortality increased significantly in the presence of suspended sediment. There was no overlap of the 95% confidence limits for the two calculated EC50 values.

The experiment simulating a single dose of glyphosate to D. pulex populations showed that suspended sediment increased the short-term toxicity of glyphosate at all concentrations (Table 1). In the pretreatment cultures, total standing crop of daphnids ranged from 52 to 56/L. Immature organisms composed 65 to 75% of the total population, and egg-bearing adults made up 7 to 15%. The average clutch size of gravid adults ranged from 3.1 to 3.7 eggs.

One week after the cultures were treated, total population numbers were significantly ($P<.05$) reduced at all glyphosate concentrations, both with and without suspended sediment; however, total standing crops were reduced less in the otherwise equivalent glyphosate solutions that lacked suspended sediment. The reduction in all glyphosate treatments was primarily a result of selective toxicity to immature organisms. The percentage of immature organisms in control cultures remained at pretreatment levels (73%), whereas in the glyphosate treatments there was a marked shift in the population age structure toward adult organisms. This shift was significantly greater in the treatments with suspended sediment. The effect of glyphosate (both with and without suspended sediment) on egg production was inconclusive. There was an increase in the percentage of egg-bearing adults in populations treated with glyphosate and suspended sediment, but this increase was due to a greater reduction of immature than of mature individuals in the total population and not to a significant increase in the total number of egg-bearing individuals. Average clutch size appeared to be slightly reduced in all of the glyphosate treatments, but reduction was not statistically significant, and did not appear to be in proportion to the treatment concentration. It should be noted that treatment with suspended sediment alone did not result in a significant change in any of the population characteristics measured.

Schober and Lampert (1977) demonstrated that continuous exposure to sublethal concentrations of atrazine without sediment had a



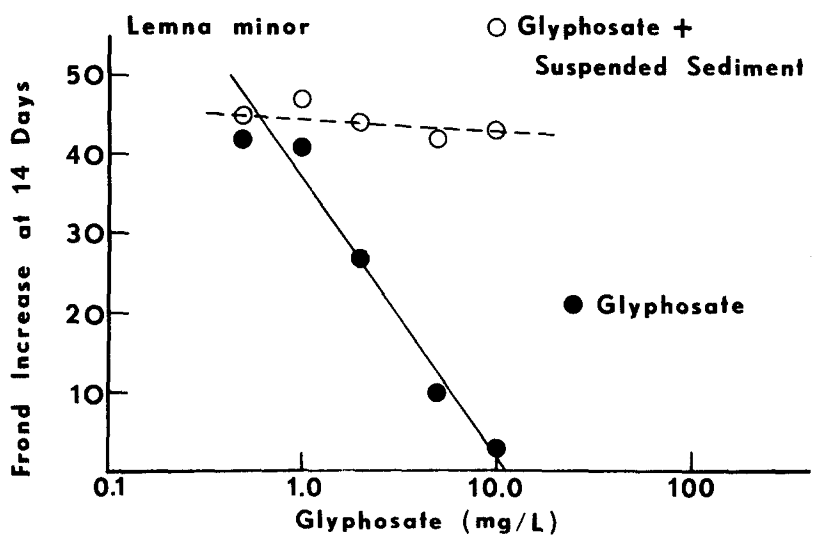


Figure 1. Toxicity of glyphosate and glyphosate plus suspended sediment to Daphnia pulex (top) and Lemna minor (bottom).

Table 1. Effects of a single dose of glyphosate and glyphosate plus suspended sediment on continuous cultures of *Daphnia pulex*. Values given are average population parameters from weekly sampling of three replicate cultures for each treatment. Standard deviations are in parentheses.

	Glyphosate				Glyphosate plus suspended sediment			
Time and glyphosate concentration (mg/L)	Total population (No./L)	Immature (No./L)	Adults with eggs (No./L)	Clutch size[1]	Total population (No./L)	Immature (No./L)	Adults with eggs (No./L)	Clutch size[1]
7 weeks pretreatment								
0.0 (Control)	54(8)	38(8)	6(4)	3.4(1.8)	56(10)	42(6)	4(4)	3.4(1.6)
1.0	52(8)	36(8)	6(4)	3.3(1.7)	52(8)	34(8)	8(4)	3.4(1.9)
2.0	52(10)	34(8)	6(2)	3.5(1.8)	52(8)	34(6)	8(4)	3.3(1.9)
4.0	56(10)	38(8)	4(4)	3.1(1.8)	56(10)	38(10)	6(4)	3.7(1.9)
1 week posttreatment[2]								
0.0 (Control)	52(2)^{a}	38(4)^{a}	6(2)^{a}	3.3(1.5)^{a}	52(6)^{a}	38(6)^{a}	4(4)^{a}	3.0(1.0)^{a}
1.0	42(2)^{b}	30(2)^{b}	4(4)^{a}	2.1(1.1)^{a}	22(4)^{c}	10(2)^{d}	4(4)^{a}	2.4(0.5)^{a}
2.0	44(6)^{b}	28(2)^{b}	4(4)^{a}	2.0(0.7)^{a}	20(2)^{c}	8(2)^{d}	4(2)^{a}	2.2(0.4)^{a}
4.0	26(4)^{c}	14(2)^{c}	4(2)^{a}	3.0(1.6)^{a}	6(2)^{d}	0^{e}	2(2)^{a}	2.2(0.5)^{a}
2–9 weeks posttreatment[3]								
0.0 (Control)	54(4)	38(4)	6(2)	3.2(1.4)	50(4)	36([illegible]	6(4)	2.8(1.4)
1.0	52(6)	38(4)	6(2)	3.0(1.4)	52(6)	38[illegible]	6(2)	2.9(1.4)
2.0	52(6)	38(6)	4(2)	2.8(1.3)	50(8)	36(6)	6(2)	3.6(1.8)
4.0	52(6)	38(4)	6(2)	3.1(1.1)	48(8)	34(6)	6(2)	3.6(1.6)

[1] *Number of eggs per gravid adult*
[2] Different superscripts indicate significant differences at 95% confidence level
[3] No significant difference among treatments for any population parameter

marked effect on the production of young in *D. pulex* cultures. Such an effect would inhibit the recovery of a natural population following a dose of this pesticide in a field situation. In our experiment, however, there was no apparent lasting effect of the single dose of glyphosate, either with or without suspended sediment. Populations recovered by the end of the second week, and maintained average weekly population characteristics comparable with those during the pretreatment period. Total number of daphnids ranged from 48 to 54/L; immature organisms composed 70 to 73% of the total; and adults with eggs ranged from 8 to 12%. Average clutch size among gravid adults was 2.8 to 3.6 eggs.

On the basis of this experiment, it would appear that a single dose of glyphosate greater than 1 mg/L resulted in a significant reduction in a population of daphnids, that this decrease was primarily due to mortality of immature life stages, and that the decrease was greater in the presence of suspended sediment; however, given an adequate number of surviving adults, the population recovered within a relatively short time.

In the acute toxicity tests conducted with *Lemna minor*, glyphosate with suspended sediment was clearly less toxic than comparable concentrations of glyphosate alone (Fig. 1). The calculated ED50 for glyphosate was 2.0 mg/L, whereas concentrations of up to 10.0 mg/L glyphosate with suspended sediment had little effect. Suspended sediment alone had no effect on the growth of the plant.

Results of this study indicate that suspended sediment influences the acute toxicity of glyphosate to the organisms tested. The specific mechanisms of this observed effect were not delineated. One possible explanation is that adsorption of glyphosate by particulate matter alters the availability of the toxicant to the organisms tested. Sprankle et al. (1975) showed that glyphosate was readily bound to the type of clay used in the present experiments, and that the rate of microbial degradation of glyphosate in soil was inversely related to the quantity adsorbed. They concluded that adsorbed glyphosate was not available to the microorganisms present. Adsorption of glyphosate on suspended sediment may similarly render it unavailable to *Lemna minor*, a plant that removes dissolved constituents from the aqueous phase of the medium in which it grows. Adsorbed glyphosate may be ingested along with particulate matter by the filter-feeding *D. pulex* and be more toxic by this route of exposure. The importance of particulate matter on uptake or toxicity of a variety of chemical contaminants on various forms of aquatic organisms has been demonstrated by other investigators (Brungs and Bailey 1967, Hongve et al. 1980, Pesch and Morgan 1978). However, none of these investigators reported increased bioavailability or enhanced toxicity with suspended sediment, as suggested by our results with *D. pulex*.

The Committee of Scientists of the Agricultural Research Service USDA (1975), listed 169 agricultural pesticides currently in use in the United States, along with their principal mode of transport into aquatic environments. From this list, nearly two-thirds were shown to be potentially adsorbed onto suspended sediment. In evaluating the hazard of these chemicals to aquatic biota, information on bioavailability and toxicity of adsorbed chemicals is needed. This is especially important if adsorption increases bioavailability, and may be particularly relevant to aquatic invertebrates that obtain their food by ingesting or filtering large quantities of fine particulate matter.

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