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Uptake and Phytotoxicity of Soil-Sorbed Atrazine for the Submerged Aquatic Plant, *Potamogeton perfoliatus* L.

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Abstract. The photosynthetic inhibitory effect of atrazine-sorbed soil placed on the leaf surfaces of *Potamogeton perfoliatus* was investigated under laboratory conditions. Leaves simultaneously exposed to atrazine both in solution and sorbed to soil exhibited a similar uptake of atrazine and associated photosynthetic reduction as did leaves exposed to the same concentration of atrazine in solution only. A small quantity of atrazine (0.19 μg/gdw leaf) was found in leaves treated with atrazine-sorbed soil at 120 μg/kg whereas a significantly larger amount (3.57 μg/gdw leaf) was present in leaves treated with dissolved atrazine at a concentration of 100 μg/L. It is concluded that atrazine sorbed to soil on leaf surfaces is less available for uptake by aquatic plants than atrazine in solution. Of greater physiological concern is the physical presence of the soil on the leaves and the resultant reduction of light.

The ecological effects of herbicide runoff into estuarine aquatic environments, as in the Chesapeake Bay, have received considerable attention recently with special emphasis toward the impact on submerged macrophyte vegetation (Stevenson and Confer 1978; Correll *et al.* 1978). Triazine herbicides such as atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine) are relatively mobile in the soil (Wauchope and Leonard 1980) and move from agricultural fields. Approximately 1% of the atrazine applied to a field may ultimately enter nearby aquatic systems (Muir *et al.* 1978; Triplett *et al.* 1978; Wu 1980), most of which occurs during the first major storm event following application (Muir *et al.* 1978; Triplett *et al.* 1978; Wauchope 1978). This runoff can result in dissolved atrazine concentrations ranging from less than 1 to 100 μg/L in adjacent waters (Frank *et al.* 1979; Frank and Sirons 1979; Wu 1980; Hershner *et al.* 1981).

The partitioning of atrazine between the dissolved phase and the soil-sorbed phase in the run-off component may be of key importance as to the availability of the herbicide for uptake by submerged macrophyte species. Although the partition coefficient (Kd = sorbed concentration ÷ dissolved concentration at equilibrium) for atrazine is variable due to soil parameters (most notably organic matter, pH, and clay content), average values for many agricultural soil types are usually between 1–5 (Talbert and Fletchall 1965). Partition coefficients of 1–4 for atrazine have also been reported for estuarine sediments (Means *et al.* 1981). However, substantially higher Kd values (5–260) for atrazine in suspended sediment in run-off have been calculated (Correll and Wu 1982). Since the Kd values for atrazine are routinely greater than 1, there is the potential for concentration of atrazine within the suspended particulate fraction of field run-off material. The accumulation of this atrazine-sorbed sediment produces a microenvironment on leaf surfaces that theoretically could result in a high concentration of atrazine in the interstitial water of that sediment. Therefore, it has been suggested that atrazine sorbed to suspended particulates may, in effect, result in the exposure of submerged macrophytes to elevated herbicide concentrations by sedimentation of this material onto leaf surfaces (Correll and Wu 1982).

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This research was initiated to examine the magnitude of atrazine uptake by the submerged macrophyte *Potamogeton perfoliatus* L. when soil-sorbed atrazine is placed on plant leaves. Atrazine uptake and the resulting photosynthetic depression in *P. perfoliatus* leaves were investigated under the following conditions: 1) atrazine in solution and on the overlying soil 2) atrazine in solution only 3) atrazine on the overlying soil only.

Materials and Methods

Sorption-Desorption of Atrazine on Soil

The soil was Mattapex silt-clay collected from an agricultural field that had not been tilled for four years. Two particle size classes were tested for their sorptive properties. One class contained particles which passed through a 105 μm sieve, but not through a 74 μm sieve. The other contained particles which would pass through a 74 μm, but not a 53 μm sieve. These sizes were chosen because they could be suspended in a column of water and yet would settle out within five min, suggesting that when suspended in an aquatic environment, these particles might easily settle on the leaves of submerged macrophytes.

Sorption of atrazine was examined at a water:soil ratio of 5:1 using five concentrations (10, 50, 100, 500, 100 μg/L) of uniformly ring-labelled ^{14}C-atrazine (s.a. 1.85 MBq/mg) in methanol. Two-gram soil samples (triplicated) were added to 15 × 150 mm glass screw cap tubes to which 10 ml of atrazine solution was added. The soil samples were allowed to equilibrate for 6 hr on a mechanical shaker at room temperature, after which they were centrifuged. One-ml supernatant water samples were taken from each tube and placed into 10 ml of Aquasol-2 (New England Nuclear) for counting on a Packard Tri-Carb Model 460C Liquid Scintillation spectrometer. The difference between the amount of atrazine originally in solution and the amount remaining in solution after incubation was assumed to be the amount sorbed to the soil.

After water samples were removed from the tubes, the remaining supernatant was aspirated off and replaced with 10 ml of deionized water. The tubes were shaken for 2 hr, centrifuged, and sampled as before to determine the amount of atrazine which had desorbed from the soil. This entire procedure was repeated to determine second-degree desorption. Wet weights and then dry weights of the soil samples were taken to determine the interstitial water volume which was used to correct desorption values.

Plant Preparation

Potamogeton perfoliatus (L) plants were collected from shallow waters of the Choptank River estuary (salinity of 12 g/L) just prior to each experiment. Epiphytes and sediments were removed from the leaves manually and selected leaves from the terminal 20 cm of at least 10 different plants were removed from the stems and placed in filtered (.45 μm) Choptank water.

Leaves were arranged in four rows on a rack consisting of a ring of PVC pipe (150 mm inside diameter, 43 mm high) with a black plastic mesh (2.75 mm) bottom with three supporting legs (15 mm high). Four strands of monofilament line, with copper wire hooks at each end, were used to secure the leaves. These racks were used in conjunction with flat-bottom glass bowls (230 mm o.d., 68 mm deep, 2-L volume).

Application of Atrazine and Analyses

Potamogeton leaves were subjected to atrazine and sediment according to the following scheme. Treatment #1 consisted of applying sediment, to which atrazine had been sorbed, to the upper surface of the leaves and incubating the preparation in Choptank water also containing atrazine at the appropriate Kd. This was accomplished by weighing out 2.0 g of sediment and then adding 2.0 ml of 0.12 μg/ml ^{14}C-atrazine in methanol to the sediment (120 μg/kg), mixing, and allowing the methanol to evaporate leaving the sediment dry. One gram was applied to the leaves on the rack with a spatula. Fall-through sediment was collected, weighed, and subtracted from the original weight. The control consisted of leaves without applied sediment. For both treatment and control, glass incubation bowls were prepared by adding 10 ml of 10 μg/ml ^{14}C-atrazine in methanol to GF/C filtered water for a total volume of 1-L (final atrazine concentration 100 μg/L).

The treatment and control racks containing the leaves were gently lowered into the bowls and were incubated for 4 hr at 25°C with constant illumination of 115 μEin/m^2/sec from fluorescent bulbs. Treatment #2 was identical to Treatment #1 except the treatment bowl contained no aqueous atrazine. In Treatment #3, atrazine was present in solution only; methanol alone was applied to the soil and placed on the leaves as above.

At the end of the incubation period, the racks containing the leaves were removed and placed over a second bowl where surface sediment and atrazine were removed by a stream of river water from a squeeze bottle. The sediment was subsequently dried to constant weight at 80°C. Leaves were washed a second time by removing them from the rack with forceps and placing them into a third bowl containing 500 ml of filtered Choptank water. They were removed quickly, blotted dry, and placed into glass petri dishes. The leaves were dried overnight at 80°C, separated into three replicate groups, and then each group ground to a fine powder with mortar and pestle. Subsamples (15–40 mg) of each replicate group were placed into glass screw cap tubes and the ^{14}C-atrazine extracted with nitric acid according to the method of Lewis *et al.* (1982). One ml aliquots of the digested material were placed into 10 ml of Aquasol-2 and counted as above. Counting efficiency was determined by the external standard channels ratio method, and was always greater than 80%.

Placement of Soil on Leaves and Photosynthetic Measurement

Soil was applied to detached leaves in varied amounts of 0.25, 0.50, 0.75, and 1.0 g and the leaves incubated for 2 hr as above with 15 μci/L of [^{14}C]-sodium bicarbonate (s.a. 24.86 MBq/mg) to determine photosynthetic rate. In Treatment #1, the experimental system contained leaves exposed to 1 g surface soil with 0.12 μg/g adsorbed unlabelled atrazine and 100 μg/L unlabelled atrazine in solution. To test for the effect of aqueous atrazine only, another bowl containing 100 μg/L atrazine with no applied soil was used. The photosynthesis control bowl contained no soil and no atrazine.

In Treatment #2 leaves were exposed to 120 μg/kg atrazine on the soil with no atrazine in solution. The other two bowls

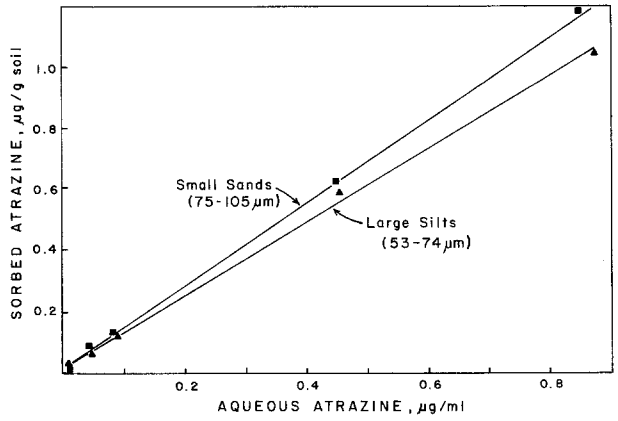

Fig. 1. Atrazine sorption on 2 particle sizes (53–74 µm, 75–105 µm) of Mattapex silt-clay at a water:soil of 5:1

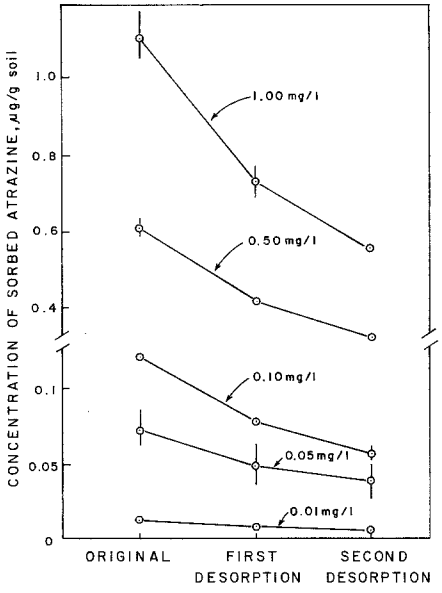

Fig. 2. Atrazine desorption from Mattapex silt-clay at a water:soil of 5:1. Error bars represent range between the particle sizes used (53–74 µm and 75–105 µm)

were identical to those in Treatment #1. In Treatment #3, soil was applied without sorbed atrazine (methanol served as a vehicle control); however, the water contained 100 µg/L atrazine. Again, the other bowls were identical to Treatments #1 and #2.

Results and Discussion

The two particle size classes of Mattapex silt-clay examined for use in this study exhibited similar adsorptive (Figure 1) and desorptive (Figure 2) characteristics. Ultimately, the larger size class (<105 µm, >74 µm) was chosen over the smaller (<74 µm, >53 µm), because the former has a greater tendency to remain settled on *P. perfoliatus* leaves during the experimental procedures. This larger

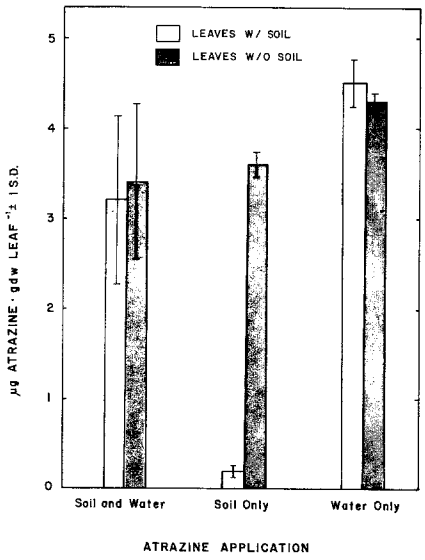

Fig. 3. Atrazine uptake in leaves of *P. perfoliatus* from atrazine-sorbed soil and from solution ($\bar{x}$ ± S.D.)

size class has a partition coefficient (K_d) for atrazine of 1.2 (r^2 = .99).

The importance of achieving a uniform distribution of soil over the leaves of a plant and the necessity of being able to apply and recover known quantities of soil was addressed by the use of detached leaf experiments. Atrazine uptake rates (in dissolved phase) by attached and detached leaves of *P. perfoliatus* were not significantly ($p > 0.05$) different. The mean uptake of atrazine (µg atrazine/gdw leaf) at a dissolved concentration of 120 µg/L for attached leaves was 6.31 ± 1.32 ($\bar{x}$ ± S.D.) and 5.97 ± 1.51 for detached leaves. However, attached leaves did exhibit a higher mean photosynthetic rate (10.74 ± 2.01 mgC/gdw/hr) than did detached leaves (6.94 ± 1.93 mgC/gdw/hr). Since only relative photosynthetic differences among treatments was considered to be of importance, this photosynthetic rate difference was of no consequence.

The results of experiments on atrazine uptake by leaves of *P. perfoliatus* from sorbed-soil *vs* aqueous solution indicate the relative low availability of soil-sorbed atrazine for plant uptake (Figure 3). In Treatment 1, leaves that were exposed to atrazine-sorbed soil and dissolved atrazine simultaneously (at the proper K_d = 1.2) exhibited no significant difference ($p > .01$) in uptake from leaves exposed to dissolved atrazine only (3.32 ± 0.31 *vs* 3.58 ± 0.21 µg atrazine/gdw leaf, respectively). When leaves were exposed to atrazine-sorbed soil only (Treatment 2), uptake was minimal (0.19 ± .03 µg/gdw) as compared with uptake by leaves without soil exposed to an equal concentration of dissolved atrazine (3.57 ± .11 µg/gdw). The physical presence of soil over-

lying the leaves did not impede or promote the uptake of atrazine from solution (Treatment 3). The uptake by leaves with applied soil (no atrazine) and exposed to dissolved atrazine alone did not significantly differ ($P > .01$) from the atrazine uptake by leaves without soil exposed to dissolved atrazine ($4.55 \pm .013$ and $4.31 \pm .06$ μg atrazine/gdw, respectively).

Forney and Davis (1981) in their study of 4 Chesapeake Bay submerged macrophyte species and atrazine also found that atrazine uptake from the water was the main mode of entry of the herbicide into the plant. They exposed the plants to atrazine dissolved in the water and also, by using a root-shoot isolation scheme, exposed the roots only to atrazine-sorbed soil. They found that atrazine concentrations in the soil of less than 100 μg/L did not inhibit growth of the plants through 25 days.

The results obtained in the atrazine-sorbed soil treatments are consistent with reported atrazine adsorption-desorption kinetics in relation to water:sediment ratios. As this ratio decreases, atrazine sorption to the same sediment (same K_d) increases. The water:sediment ratio in the microenvironment of pore-waters between adjacent sediment particles accumulated on submerged macrophyte leaf surfaces would be extremely low causing the atrazine to remain sorbed to the sediment. However, the upper surfaces of the accumulated sediment would be exposed to the larger volume of water in the system and the atrazine would desorb accordingly.

The photosynthetic response of *P. perfoliatus* leaves to shading by varied amounts of applied soil (0 to 9.93 g soil/gdw leaf) was quite variable and non-linear, appearing not to increase past about 4 g soil/gdw leaf at which point the photosynthetic reduction was 27%. When atrazine and surface soil are present in a system, the resulting photosynthetic reduction is due to both physical shading of the leaves by the soil and to the presence of atrazine (dissolved and/or sorbed to the soil). To calculate an estimate of the reduction in photosynthesis due to the presence of atrazine on the soil only, the 27% photosynthetic reduction brought about by surface soil at 4 g soil/gdw leaf was subtracted from the total photosynthetic reduction determined for each treatment (Table 1). When atrazine was present in solution, the resulting photosynthetic reduction ranged from 52 to 69%. Additional atrazine sorbed to the surface soil did not result in any additive photosynthetic reduction. Also, when atrazine was introduced on the soil only, very little photosynthetic reduction resulted, again indicating that atrazine-sorbed soil was not taken up by the leaves of *P. perfoliatus*.

Table 1. Percent photosynthetic reduction in *P. perfoliatus* leaves caused by applied surface soil and atrazine (aqueous and/or soil-sorbed)

Application of atrazine[a]		Percent Photosynthetic Reduction		
		Due to atrazine	Due to soil	Total
Water	100	52	27	79
Soil	120			
Water	0	8	27	36
Soil	120			
Water	100	55	27	83
Soil	0			
Water	100	69	—	69
No Soil				

[a] μg/L and μg/kg for water and soil, respectively

Apparently, the most important effect of soils (with or without atrazine) on plant leaves is attributable to the physical presence of sediment on leaf upper surfaces. Published data on the photosynthetic inhibitory effects of sediment on submerged macrophyte leaf surfaces is sparse. However, data on epiphyte biomass on leaves are available. Epiphytes exert a similar influence on submerged macrophyte species as does settled soil in that they attenuate light. It has been shown that light to the leaf surface of *Zostera maximum* can be reduced by 90% by natural epiphytic growth (Phillips *et al.* 1978, Borum and Wium/Andersen 1980) and photosynthesis in *Zostera* can be reduced by as much as 31% (Sand-Jensen 1977) by overlying epiphytes.

In conclusion, it appears from the data that soil-sorbed atrazine is relatively unavailable for uptake by *P. perfoliatus* and that the greatest reduction in photosynthesis is caused by the settled soil. Desorption of atrazine from soil is rapid, and therefore, it is likely that the soil coming from the land or resuspension and then deposited on submerged macrophyte leaves would have an atrazine concentration in equilibrium (K_d) with the water. Furthermore, atrazine degrades more rapidly to hydroxyatrazine (nonphytotoxic) when it is in close proximity to soil surfaces (Armstrong and Chesters 1968) so that the soil on leaf surfaces help to detoxify atrazine.

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