

## **Performance of Meadow Voles from Sewage Sludge-Amended Fields in Swim-Escape Behavior Trials**

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Disposal of treated sewage sludge in land application programs can effectively fertilize and condition soil (KELLING et al. 1977, STUCKY and NEWMAN 1977, HINESLY et al. 1978, POMARES-GARCIA and PRATT 1978). However, the presence of elevated concentrations of toxic substances in the sludge has led to concern over its potential hazard to plants and animals, and ultimately to humans (CHANEY 1973, C.A.S.T. 1976, BAKER et al. 1979).

In 1977 a long-term field study was undertaken at Miami University to determine the effects of land application of sewage sludge on agricultural and old-field ecosystems (ANDERSON and BARRETT 1981; ANDERSON et al. 1981). A major aspect of the study has focused on effects on meadow vole (Microtus pennsylvanicus) populations. This report presents the results of behavior toxicology assays on voles from sludge-amended, fertilized, and control fields. The objective was to detect behavioral alterations, if any, due to food-chain uptake of toxicants from sludge.

### STUDY AREA

The study was conducted at the Miami University Ecology Research Center located on the Bachelor Wildlife Reserve near Oxford, Ohio. A grid system of 16 0.1 ha enclosures of galvanized steel walls surrounds 1.6 ha of experimental grassland in two community-types.

Eight enclosures were planted in winter wheat (Triticum aestivum var. Ranger) in October 1977. These were allowed to go fallow, and were in the first year of old-field succession during the period of this behavior study (1979).

The remaining eight enclosures were planted in bluegrass (Poa pratensis) and fescue (Festuca elatior) in 1974. They were in the fifth year of old-field succession in 1979.

### MATERIALS AND METHODS

Enclosures in each community-type were randomly selected as either sludge, fertilizer, or control treatments (two controls and three each of sludge and fertilizer in each

community-type). The sludge used was Milorganite (6-2-0, N-P-K), an anaerobically digested, heat-dried municipal sewage commercially processed and marketed by the City of Milwaukee, Wisconsin. Sludge-treated enclosures received five monthly applications (May to September) at recommended N-fertilizing rates (SHEA and STOCKTON 1975) of 1793 kg/ha each application. This constituted a total of 8963 kg/ha/yr.

Fertilized enclosures were treated on the same days as sludge treatments. A commercial urea-phosphate fertilizer (6-2-0) was applied at 314 kg/ha each application, for a total of 1569 kg/ha/yr. This applied an N-P-K nutrient subsidy equivalent to the sludge applications. Control enclosures were untreated.

Five breeding pairs of meadow voles were released into each enclosure in mid-June. Populations were monitored by semi-weekly live-trapping through October. In mid-November voles were removal-trapped, and some were taken into an on-site laboratory for behavior testing.

All voles were acclimated to laboratory conditions for at least 0.5 and not more than 2 wks prior to testing. Voles were tested by the swim-escape method (BARRACO et al. 1978) for possible behavioral changes due to sludge treatment. Sub-adult voles (20 to 34 g) in good health were used. When possible, an equal number of males and females was used in each treatment. Non-lactating females were used to avoid confounding performance due to pregnancy. Use of sub-adult voles assured that the animals had lived their entire lives in a particular treatment. Ten voles per treatment were tested for each community-type except the first-year old-field control, in which only two were available.

A number 3 galvanized washtub (60 cm diameter, 27 cm high) filled with 16°C water to a depth of 17 cm was the test arena. A 1.5 cm diameter hemp rope, weighted at the bottom, was suspended into the center of the tub.

Each test session involved placing a vole in the water facing away from the rope at a fixed point along the tub. Voles typically swam around the periphery of the tub for several seconds, then began to criss-cross the tub until the rope was discovered to be a means of escape. Time (latency) was recorded with a stop-watch until the vole grasped the rope with all four feet.

Each vole was tested twice. The first (naive) exposure was a training period. Comparison of training latencies between treatments tested for impairment of learning ability. A second exposure was made 4 days later to test for retention (memory) impairment. Treatment effects were compared statistically by analysis of variance.

## RESULTS

Most voles learned to use the rope for escape within about 1.5 min of the training sessions. However, one female from the first-year old-field fertilizer treatment had a training latency of more than 6 min, and had not climbed the rope after 10 min had elapsed in the retention trial. This individual was replaced with another female from this treatment, and the data from the first was discarded. All other voles exhibited effective learning; mean retention latencies for all treatments were significantly lower ( $p < 0.05$ ) than training latencies (Table 1).

No significant treatment differences ( $p > 0.05$ ) were detected in training or retention latencies. Neither community (first-year vs fifth-year old-field) nor sex was a significant source of variation ( $p > 0.05$ ). Thus, there was no evidence that treatment, community, or sex differences produced behavioral alterations in the voles.

TABLE 1. Results of swim-escape behavior trials on meadow voles.

	Number of voles	Latency (sec) <sup>a</sup>	
		training	retention
First-year old-field			
control	2	158±37	43±30
fertilized	10	97±10	20±2
sludge	10	86±12	29±6
Fifth-year old-field			
control	10	71±11	22±5
fertilized	10	92±20	29±7
sludge	10	94±8	16±2

<sup>a</sup> Mean ± standard error

## DISCUSSION

The swim-escape test did not detect behavioral alterations in voles from sewage sludge- or fertilizer-amended fields. This lack of evidence of treatment differences could be due to either of two causes. It may be that 1.) these doses of sewage and fertilizer do not cause behavioral changes in meadow voles through food-chain uptake of toxicants, or 2.) the test used was not sensitive to a particular type of behavioral response elicited by the treatments.

It is quite likely that the results in fact reflect a lack of consequential behavioral impairment. In detailed vole population dynamics analyses made during the treatment period (ANDERSON and BARRETT 1981), no disruptions of normal population functioning could be attributed to treatment. Thus it is apparent that the various behavioral responses necessary for normal survival functioning in semi-natural conditions were unimpaired.

The swim-escape test measured a generalized behavioral response which is relevant to survival functioning. Components of the response included immediate reaction (swimming) to avoid drowning, exploration of surroundings, perception and recognition of a means of escape, negotiation of the escape route, and memory of the experience for future reference. Compromise of any of these components due to toxicosis could have serious consequences for survival in the field. This important behavioral repertoire was adequately assessed in this test.

It was concluded that sewage sludge or fertilizer treatment did not produce consequential behavioral alterations in the meadow voles. These results, however, are from a short-term (2-yr) land application study. Research is continuing at Miami University to determine whether long-term land application of sludge will result in significant environmental effects.

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