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Survivorship of Meadow Voles, *Microtus pennsylvanicus*, from Sewage Sludge-Treated Fields

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Recently, land application has become increasingly attractive as a a method of disposal of treated sewage sludge. The application of sludge as a fertilizer and soil conditioner can effectively enhance plant productivity and increase available nutrient levels (Pomares-Garcia and Pratt 1978; Magdoff et al. 1980). However, since sewage sludge contains elevated concentrations of toxic elements (Furr et al. 1976), land application may be potentially hazardous to plants and animals (Baker et al. 1979). Numerous investigators have documented the accumulation of trace elements by sludge-treated soils and plants (Varanka et al. 1976; Soon et al. 1980; Williams et al. 1980; Soon 1981). Laboratory studies have demonstrated that animals feeding on plants fertilized with sewage sludge accumulate toxic elements in livers, kidneys, and other vital organs (e.g., Hansen and Hinesly 1979; Williams et al. 1978; Hansen et al. 1981). Unfortunately, there is a paucity of information on the effects of sludge application on toxic metal accumulation and survivorship of animals in field situations.

A long-term field study was begun in 1977 at Miami University to evaluate the effects of land application of sewage sludge on experimental old-field communities (Anderson and Barrett 1982; Anderson et al. 1982). The effects of sludge application on toxic metal concentrations in meadow vole (Microtus pennsylvanicus) organs during the first two years of the study have been reported elsewhere (Anderson et al. 1982). During the first two years of sludge application, no detrimental effects were observed in vole survivorship as a result of sludge treatment (Anderson and Barrett

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1982). The current report analyzes vole survivorship and toxic element concentrations during the third and fourth years of sludge application on the experimental old-fields.

MATERIALS AND METHODS

The study was conducted at the Miami University Ecology Research Center located on the Bachelor Wildlife Reserve near Oxford, Ohio.
Two experimental old-field communities, each consisting of eight 0.1-ha enclosures, served as the study area. Enclosure walls were constructed of galvanized steel which extended 60 cm above ground and 45 cm into the soil. Previous studies determined that this type of enclosure is effective in restricting small mammal movements (Stueck and Barrett 1978; Spencer and Barrett 1980).

One set of eight enclosures was planted in winter wheat (*Triticum aestivum* var. Ranger) in October 1977. This community was in the second and third years of secondary succession in 1980 and 1981 and will be referred to as the two-year and three-year old-field. The remaining set of eight enclosures was seeded in bluegrass (*Poa pratensis*), rye grass (*Lolium perenne*) and fescue (*Festuca elatior*) in 1974. This community was in the sixth and seventh years of succession in 1980 and 1981 and will be termed the six-year and seven-year old-field.

Enclosures within each old-field were randomly selected as either sludge, fertilizer, or control treatments in 1978. Since then three enclosures in each old-field have received applications of Milorganite (6-2-0, N-P-K), an anaerobically-digested, heat-dried municipal sewage sludge which is processed and marketed by the City of Milwaukee, Wisconsin. Milorganite is characterized by a high cadmium content relative to other sewage sludges (Furr et al. 1976) and has been shown to be detrimental to mice in laboratory feeding studies (Chaney et al. 1978). Sludge-treated enclosures received five monthly (May through September) applications at recommended N-fertilizing rates (Shea and Stockton 1975) of 1792 $kg \cdot ha^{-1} \cdot mo^{-1}$ for a total of 8960 $kg \cdot ha^{-1} \cdot yr^{-1}$. This application rate represented an annual metal addition of 0.65 $kg \cdot ha^{-1}$ for Cd, 2.99 $kg \cdot ha^{-1}$ for Cu, 3.67 $kg \cdot ha^{-1}$ for Pb, and 8.45 $kg \cdot ha^{-1}$ for Zn (Maly and Barrett 1984).

Three enclosures in each old-field received an equivalent nutrient subsidy consisting of commercial urea-phosphate fertilizer (34-11-0, N-P-K). Fertilizer application at the rate of 314 $kg \cdot ha^{-1} \cdot mo^{-1}$ was conducted on the same dates as the sludge treatment and totaled 1570 kg•ha^{-1}•yr^{-1}. Two enclosures in each old-field served as untreated controls.

Five non-juvenile (>20 g) pairs of meadow voles (Microtus pennsylvanicus) were released into each enclosure in June of each year of the study. Prior to release, enclosures were trapped to remove all voles remaining from the previous year. Vole populations were censused by live-trapping from June until November. Trapping was conducted once or twice weekly, depending on population densities (Spencer and Barrett 1980). Captured voles were toe-clipped for identification and released at the site of capture.

Vole life spans were computed as the number of days after introduction or first capture that an animal continued to be trapped. Survivorship was compared among treatments, years, and old-fields for both stocked (introduced) and recruited voles (animals born in the enclosures). Four-way analysis of variance was used to test for effects of sex and of different treatments, years of study, and old-field ages on vole life spans. Where effects were statistically significant ($P \leq 0.05$), multiple comparisons of means were performed using Fisher's Protected Least Significant Difference.

Trapping efficiencies were computed as the ratio of the number of animals caught on each trapping date to the number of animals known to be present in the population on that date (based on subsequent captures). Efficiencies were compared between sexes, treatments, years and old-fields using four-way analysis of variance in order to evaluate biases in the trapping regime which could have affected vole survivorship comparisons.

During November of each year, enclosures were trapped and voles were collected for analysis of toxic element concentrations. A total of 390 voles were sacrificed and lungs, livers, kidneys, and gonads were removed and frozen for storage. Organ samples were later digested using a wet-ash technique employing nitric (HNO_3) and hydrochloric (HCl) acids (Maly and Barrett 1984). Samples (1 g each) were first digested in 10 ml HNO_3 and then the acid was evaporated at 120 C. Samples were subsequently dissolved in 5 ml HNO_3, which was then evaporated; this step was repeated twice. Residues were then dissolved in 2 ml fresh aqua regia (3 parts HCl: 1 part HNO_3) and diluted to standard volumes. Digests were analyzed for cadmium, copper, lead and zinc concentrations using flame atomic absorption spectroscopy with an IL 157 spectrophotometer. Results were analyzed using analysis of variance and Fisher's Protected Least Significant Difference where appropriate.

RESULTS AND DISCUSSION

Life spans of stocked voles did not differ significantly ($P>0.10$) between years, treatments or sexes.
There was a significant ($P\leq0.05$) difference between different age old-fields in stocked vole life spans. Voles introduced into the six- and seven-year old-field ($\bar{X}$=81.7 days, SE=3.3 days) survived significantly longer (9.1 days) than comparable animals in the two- and three-year old-field ($\bar{X}$=72.6 days, SE=3.5 days).

Analysis of variance in life spans of recruited voles (Table 1) indicated two significant sources of variation. First, a significant ($P\leq0.05$) interaction occurred between the effects of old-field age and year of the study. In 1980, voles from the six-year old-field survived an average of 12.5 days longer than voles from the younger, two-year old-field. In 1981, the trend was in the opposite direction since voles from the older, seven-year old-field lived an average of 6.7 days less than voles from the three-year old-field. Recruited vole life spans did not differ significantly ($P>0.20$) between sexes.

Table 1. Mean life spans (days) ± SE of meadow voles recruited into enclosures. Numbers in parentheses indicate sample sizes.

Old-field	Control	Fertilizer	Sludge
1980			
2-year	38.4±7.7 (11)	41.1±4.7 (37)	32.3±4.3 (40)
6-year	59.9±4.4 (46)	46.3±5.2 (44)	43.0±4.1 (56)
1981			
3-year	33.6±7.8 (14)	57.9±7.2 (15)	42.8±4.5 (28)
7-year	46.8±5.9 (9)	44.7±8.1 (14)	30.6±5.4 (25)
Mean	50.9 (80)	45.9 (110)	38.0 (149)

Life spans of recruited voles differed significantly ($P\leq0.05$) with respect to treatment. Animals from fertilizer-treated enclosures did not exhibit significantly lower life spans ($\bar{X}$=45.9 days, SE=3.0 days) than did control animals ($\bar{X}$=50.9 days, SE=3.3

days). However, sludge-treated voles had significantly shorter life spans (X=38.0 days, SE=2.3 days) than control voles. Thus sludge treatment appeared to have had a detrimental effect on recruited meadow vole survivorship. This effect, while significant in the four-way analysis of variance, did not occur in all experimental combinations of old-field age and year of the study. In 1981 in the three-year old-field, vole life spans were lowest in control enclosures.

Trapping efficiencies did not differ significantly ($P>0.10$) between sexes, treatments, years or old-fields. Therefore life spans calculated from trapping data were not biased by differential trappability of any experimental group. Mean trappability for 1980 and 1981 combined was 32.4%.

Concentrations of copper, lead and zinc in vole organs did not exhibit consistent significant differences with respect to treatment. These data are summarized by Maly (1982). Cadmium concentrations in vole livers and kidneys (Table 2) were significantly ($P\leq0.05$) elevated by sludge treatment relative to control and fertilizer treatments in each old field during both years of the study. No significant trends were observed with respect to treatment in lung and gonad cadmium concentrations.

Survivorship of both stocked and recruited meadow voles varied with respect to either the age of the old-field community or an interaction between old-field age and year of the study. Differences in vole survivorship between old-fields may have resulted from differences in vegetative cover between old-fields reported by Maly and Barrett (1984). During the first year of this study, vole survivorship was lower in treatments with minimal vegetative cover (Anderson and Barrett 1982). This result is consistent the observations of Birney et al. (1976) who found vegetative cover to be significant in determining vole population characteristics.

Meadow voles recruited into sludge-treated enclosures exhibited significantly reduced survivorship relative to control and fertilizer treatments. This suggests that land application of sludge represents a potential hazard to resident animal populations. Because vole survivorship was not reduced by sludge treatment in earlier years of this study (Anderson and Barrett 1982), it appears that detrimental effects of sludge may occur only after long-term application and accumulation in old-field communities.

Although vole liver and kidney cadmium concentrations were significantly elevated by sludge treatment, other

Table 2. Cadmium concentrations in meadow vole organs.[1]

Old-field	Control	Fertilizer	Sludge
		Liver	
1980			
2-year	0.05 (19) a	0.13 (34) a	1.19 (35) b
6-year	0.06 (23) a	0.06 (37) a	0.70 (43) b
1981			
3-year	0.08 (17) a	0.21 (29) a	1.82 (30) b
7-year	0.16 (20) a	0.14 (37) a	1.24 (52) b
		Kidneys	
1980			
2-year	0.56 (7) a	0.90 (12) a	4.76 (12) b
6-year	0.20 (8) a	0.57 (13) a	3.41 (15) b
1981			
3-year	0.50 (7) a	1.06 (14) a	9.34 (13) b
7-year	0.47 (10) a	0.38 (15) a	6.27 (23) b

[1]Means of treatment replicates ($ug \bullet g^{-1}$ wet wt); the number of samples is in parentheses; means within each row followed by the same letter are not significantly different (P>0.05; Fisher's Protected Least Significant Difference). Data for 1981 are taken from Maly and Barrett (1984).

elements studied (Cu, Pb, Zn) did not accumulate in sludge-treated voles in excess of control levels. Although some release of elements (particularly Zn and Cd) from the galvanized steel walls of the enclosures into adjacent soils could have occurred, these should have been equal among enclosures and thus could not have generated the observed differences in tissue cadmium levels.

The mechanism through which sludge application resulted in lower vole survivorship is uncertain. Since survivorship of recruited and not stocked voles was decreased by sludge application, it appears that the detrimental effects of sludge may occur during vole development. Voles from sludge-treated enclosures were observed to accumulate significantly greater concentrations of cadmium in liver and kidney tissue than fertilizer-treated and control voles. Liver and kidney accumulation was greater in the third and fourth years of sludge application than in previous years (Anderson et al. 1982). Since significant sludge-related effects on vole survivorship occurred in the third and fourth years and not in previous years (Anderson and Barrett 1982), it seems possible that vole cadmium concentrations have exceeded a threshold above which toxic effects begin to appear. However this relationship is only correlative at best and no cause and effect relationship can be inferred from the results in light of the fact that one of the experimental old-fields (three-year) did not exhibit sludge-related depression of life spans in 1981. Nevertheless, these results do suggest that long-term field studies are necessary in order to evaluate adequately the impact of sludge application on animals living in terrestrial communities.

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