The Effect of Spent Mushroom Substrate Land Applications On Adjacent Surface Water Using Aquatic Macroinvertebrates as Bio-Indicators

INTRODUCTION

Mushroom growers generate about 0.5 tons of spent mushroom substrate (SMS) from each ton of spawned mushroom compost used in cultivating *Agaricus* mushrooms. The Pennsylvania mushroom industry generated over 550,000 tons of SMS in 1991 (Wuest and Fahey 1992). Because the density of mushroom farms is quite high in southeastern Pennsylvania, short term on-farm storage of SMS may always be necessary. SMS is spread onto the land and allowed to weather. Weathering allows salts and nitrates to leach from the SMS. There are many beneficial uses for SMS such as mulch for ornamental plants, as a soil amendment for horticultural and agronomic crops, as potting soil and landscaping material. Recently, the environmental benefits of using SMS in bioremediation have been explored (Buswell 1993).

The population of southern Pennsylvania has increased 35% between 1970 and 1990. As the density of neighbors increases so do the number of complaints against mushroom growers. The most frequent complaints involve noise, odor, and water runoff. Some complaints from nonfarming neighbors express a concern for the quality of adjacent surface waters and could lead to new ordinances affecting mushroom farmers (Kelsey & Singletary 1997). This research was designed to examine the potential impact of SMS on surface waters.

EFFECTS ON WATER QUALITY

Wuest and Fahy (1991) reported that SMS land applications may impact water quality. They quantified the impact of SMS on both surface and subsurface waters in southeastern Pennsylvania. Corn plots were planted with 6, 3, 1.5, and 0 inches of SMS incorporated into the soil. Tall fescue containing 20% rye grass was planted in soil surrounding the corn plots and a diversion berm was constructed above the plots. Storm water collection devices were installed down-gradient from the edge of the plots. To measure the influence of SMS storage pile dimensions on surface water runoff, a runoff collector was placed down-gradient of a 30 x 40 foot, two foot deep, pile of SMS. The next year a new SMS storage pile of equal size was added to the experiment. Surface runoff samples from corn plots and the SMS piles were analyzed. Runoff samples from the corn field were very low in chlorides, ammonia, nitrogen, and carbonaceous biochemical oxygen demand (CBOD). The incorporation of less than six inches of SMS into the soil did not contaminate surface runoff as nutrients were held in the SMS matrix. Runoff from both the aged and the new storage piles was low in chlorides, ammonia nitrogen, and CBOD. They also reported that the slope of the land was directly related to the concentration of nutrients in runoff. A slope of less than 5% may allow nutrients to percolate down rather than running out of an area. Berms may also reduce the velocity of flow of runoff and divert it away from areas of concern such as streams (Wuest and Fahy 1992). Stroud Water Research Center (SWRC) in Avondale, PA studied the impact SMS land applications had on water quality at four study sites in 1995. One SWRC study site was located on the same mushroom farm as the stream used in this research. Soilwater lysimeters and ground water monitoring wells were installed. Samples were analyzed for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), ammonia, chloride, nitrate, phosphate, sulfate, aluminum, cadmium, calcium, chromium, copper, lead, magnesium, mercury, nickel, potassium, silicon, sodium, and zinc. They also tested for the pesticides methomyl, dimethoate, hexazionone, atrazine, diuron, and permethrin. Although no pesticide residues were found in the ground water at the mushroom farm, high concentrations of salt were detected. Concentrations of ammonium, nitrate, chloride, sulfate, calcium, magnesium, sodium, and potassium were elevated as were concentrations of DOC and DON (Kaplan *et al.* 1995). Nitrate levels in soil water were higher below bulk stockpiles of SMS. There may have been a cumulative effect of successive SMS land applications. Monitoring lysimeters and ground wells showed a downward movement of nitrates. Nitrate levels may have been elevated by bacterial action that convert ammonia to nitrate as it moved through the soil.

We were interested in the potential effects of SMS bulk storage on adjacent streams. We asked the following questions.

(1) Does the community structure of stream insects differ at sites upstream, adjacent to, and below an SMS land application?

(2) Are there components of SMS that are directly toxic to the insect fauna of a stream?

(3) Would a pulse of SMS extract that simulates storm runoff have effects on stream insects?

To answer these questions, the effect of SMS on a tributary of the White Clay Creek was examined from three different directions.

(1) A survey of insects in a stream with adjacent SMS land applications.

(2) A bioassay measuring the mortality incurred by mayfly larvae exposed to increasing concentrations of SMS extract.

(3) A behavioral study involving insect drift in a stream.

This approach provides baseline data that can be used to monitor the condition of this stream and of similar streams, and to monitor the effect of new SMS land applications on the study site.

Many diversity indices have been developed to summarize insect collection data and to determine the quality of streams. Use of diversity indices assumes that high diversity values indicate a balanced, stable community. Shannon's diversity index, H', incorporates both the number of species (species richness) and their proportion in a community. A high value of H' reflects a community in which each species is equally abundant. Another index used to determine diversity of macroinvertebrates is Margalef's index, Dmg (Pielou 1975).

Drifting behavior is an important aspect of aquatic insect life cycles in running waters. Most aquatic insect larvae, as well as some adults, drift downstream with the current at some stage in

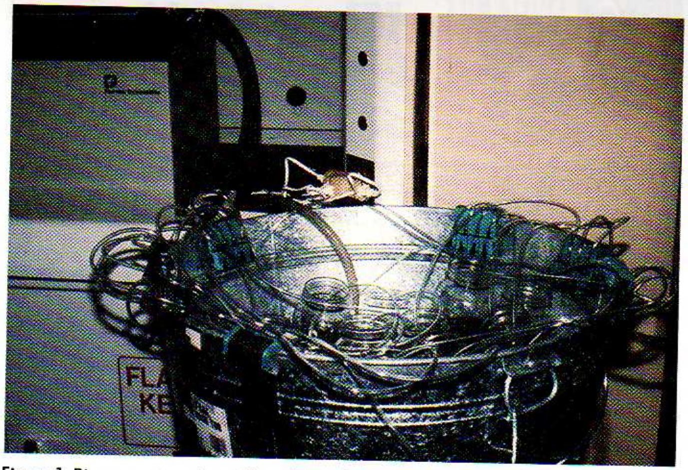


Figure 1. Bioassay apparatus with cooling tub, aeration pumps, tubing and cooling bath.

their lives and tend to fly back upstream as adults. Four categories of drift have been recognized: (1) distributional drift, a method of dispersal; (2) behavioral drift occurring at night; (3) background drift, which represents low numbers of insects that have been accidentally dislodged; and (4) catastrophic drift associated with flood conditions or pollutants. Our experiment was designed to evaluate catastrophic drift due to runoff from SMS storage areas.

METHODS & MATERIALS

Study Sites

Experiments were conducted in a stream near a mushroom grower's property in Avondale, PA. The stream was part of the White Clay Creek Watershed. The stream was located in a shallow valley adjacent to two SMS land applications. SMS has been trucked out of adjacent mushroom houses and dumped on either side of the stream for decades. The SMS was about 20 m from the water's edge. The SMS was then spread and graded and allowed to age on the hillsides, which had a slope of 5.8%. The SMS pile was slightly vegetated.

Three sites were established that were downstream (A), adjacent (B), and upstream (C) from the SMS land application. The streambed was cobble and sand with many shallow riffles at all three sites. Sites A, B, and C were used in a macroinvertebrate survey. A fourth site was established just above site B to conduct the in-stream drift experiment.

Macroinvertebrate Survey

Insect collections were made at sites A, B and C. Riffles were sampled with a Hess sampler in a replicated design. Rocks larger than five cm were scrubbed within the sampler with a small, soft nylon brush to remove any attached larvae. In most cases, insects were identified to genus and species using dichotomous keys in Merritt and Cummins (1996) and Peckarsky *et al.* (1990). The three sites were sampled in June and August 2000 with three samples taken at each site.

Bioassays

We hypothesized that certain concentrations of aqueous extracts of SMS could cause mortality in stream insects. A mayfly larvae, *Stenonema modestum* (Banks) was exposed to different concentrations of SMS extract in two laboratory bioassays.

Stenonema modestum was selected as the test organism because larvae were readily available in the test stream and no species of *Stenonema* is considered tolerant of gross organic pollution, pH changes greater than 2 units, and/or temperatures above 25°C for extended periods. In our bioassays, we maintained a pH of 7.3 and a temperature below 25°C for the length of the experiment.

The SMS used in both bioassays was collected as a mushroom house was being emptied. An extract was prepared by mixing and soaking 20 kg of SMS in 10 liters of stream water. This extract was strained by pouring the liquid portion of the mixture through 1 mm^2 mesh screen. In the first bioassay, dilutions of 100%, 50%, 25% and 12.5% of the primary extract were prepared. There were 4 treatments with 4 replications. Controls for both bioassays were 100% stream water. One-liter Mason jars were used to contain the extract treatments and were half submerged inside a tub of water. A pebble (ca. 1 cm^3) was placed inside each Mason jar. Five air pumps were suspended from the outside of the tub to supply aeration to each jar through an air stone (Fig. 1).

About 300 mayflies were collected from the test stream. The collection was made upstream from the adjacent SMS land application. The insects were returned to the University of

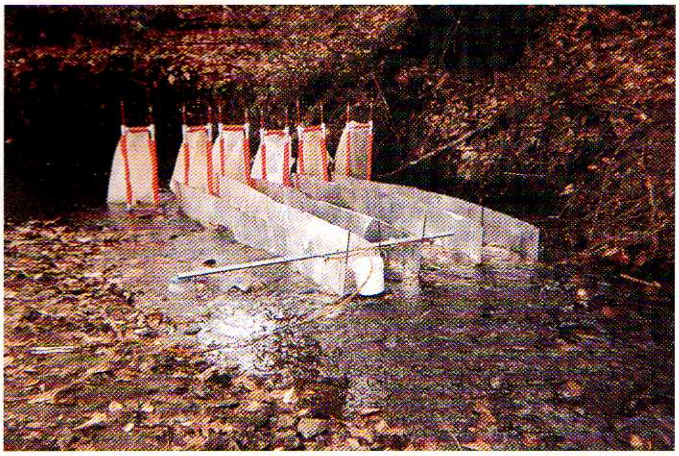


Figure 2. Corridors used in drift experiment with treatment boom and drift nets at the end of each corridor. Current flow was toward the drift nets.

Delaware Insectary. Five *Stenonema* were placed in each Mason jar. The insect larvae were exposed to the dilutions of SMS extract for 72 hours. Mortality was determined by removing the larvae and inspecting them under a dissecting microscope for movement. This process was repeated for the second bioassay, but the exposure time was shortened to 48 hours and the 100% SMS treatment was eliminated. Treatments of 50%, 25%, and 12.5% and a control were used. Four replications were performed in the second bioassay.

Drift Experiment

In this experiment, the impact of simulated runoff from SMS land applications on drifting behavior of aquatic insect larvae was evaluated. A pulse of SMS extract was used to simulate a rain event where run-off through a SMS land application carries components into the adjacent stream. SMS extract was produced by trickling water through SMS at a rate of three liters per minute. Approximately 200 kgs of SMS was collected from a conveyor belt emptying a mushroom house and transported to the University of Delaware Insectary. It was stored outside under a tarp for 6 months. Fifteen kgs of SMS were packed into the body of a funnel. Fifty liters of tap water were run through the SMS and collected in a plastic drum. This was repeated three times using fresh SMS each time. Forty five kg of SMS were extracted with 150 liters of water.

The stream was divided into six corridors by securing five metal dividers made from aluminum flashing into the streambed. Each corridor was 3 m long. The dividers were held in place with 1m long reinforcement rods that were hammered into the streambed. Drift nets, made out of 250 μm mesh nylon, were positioned at the down-stream exit of the corridors between two PVC pipes. The PVC pipes were 1 m long and 5 cm in diameter. The nets were held in place by driving two 1.5 m reinforcing bars into

Table 1. Numbers of aquatic insects by taxa, collected in the first macroinvertebrate survey[1].

TAXON	Site A				Site B				Site C			
	Sample				Sample				Sample			
	1	2	3	Total	1	2	3	Total	1	2	3	Total
Ephemeroptera												
Heptageniidae												
Stenonema modestum		3	2	5						1		1
Baetidae												
Baetis tricaudatus	28	2	7	37	48	5	6	59	10	5	21	36
Baetis sp.1	25	11	19	55	1			1	2	1	2	5
Baetis sp.2	92	41	59	192	5		1	6	1	4	7	12
Isonychidae												
Isonychia sp.		2	2	4		8	3	11				
Ephemeridae												
Ephemera sp.			1	1								
Plecoptera												
Perlidae												
Acroneuria sp.												
Leuctridae												
Leutra sp.	1			1								
Megaloptera												
Corydalidae												
Corydalus sp.												
Coleoptera												
Psephenidae												
Psephenus sp.	5			5					2	2		4
Elmidae												
Optioseruus sp.	3	3	14	20		119	125	244	7	26	16	49
Dubiraphia sp.	2	3	2	7			3	3	1	4	1	6
Trichoptera												
Hydropsychidae												
Hydropsyche sp.	84	50	47	181	199	102	84	385	22	50	35	107
Cheumatopsyche sp.	24	26	21	71	31	17		48	7	6	3	16
Hydroptilidae												
Leucotrichia	2	2		4	1		3	4				
Philopotamidae												
Chimarra sp.		5	3	8		24		24		2		2
Glossosomatidae												
Diptera												
Tipulidae												
Tipula sp.	2	1	1	4	4	23	14	41	14	5	2	21
Chironomidae	85	64	66	215	45	36	8	89	45	48	63	156
Simuliidae												
Simulium sp.	1	2	15	18	1	2	15	18	2	4	18	24
Empididae												
Hemerodromia sp.		6	1	7	4			4		3		3

1. Macroinvertebrates were collected using a Hess sampler during June. Three samples were taken at each of the three study sites. Site A was located downstream from the spent mushroom soil (SMS) land application. Site B was adjacent to SMS land application, Site C was upstream from the SMS land application.

the streambed at the end of each corridor and sliding the PVC pipes over them.

A delivery system was assembled using a sprayer boom without restriction in the nozzles. The sprayer boom was 2.5 cm aluminum stock and was 3 m long. It was positioned at the upstream end of the corridors with spray nozzles positioned over each corridor. It was suspended over the corridor entrances by clamping it to the flashing support rods (Fig. 2). The boom and the three SMS drums were connected with 30 m of plastic hose. The system was gravity fed as the drums were placed on the stream bank two and half meters above the boom.

This experiment was conducted twice. In the first experiment, three treatments and three controls were conducted simultaneously. The application of the SMS extract took two hours and 10 minutes to apply 150 liters of SMS extract to three corridors. In the second experiment in August, four controls were run and then four treatments were run for the same amount of time. The outside corridors were eliminated because the first experiment showed differences in drift in the corridors near the stream bank. It took fifty-five minutes to apply 150 liters to four corridors in the second experiment. After applications of SMS extract were made to the corridors, the drift nets were removed from the stream and their contents were stored in alcohol. Samples of stream water and SMS extract were sent to the University of Delaware Bioresources Engineering Laboratory for analysis.

SMS Extract, Stream Water, and Rain Runoff Analysis

Stream water, surface runoff during rain, and SMS extracts were tested for chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), hardness, alkalinity, and acidity at the Bioresources Engineering Laboratory at the University of Delaware. Standard methods were used for the analysis of these samples (Clesceri *et al.* 1995).

Statistics

In the macroinvertebrate survey, Shannon and Margalef's diversity indices were calculated for each sample. Calculated indices were compared between sites within each survey using analysis of variance (ANOVA) (SAS Institute 1989). Mean mortality in the bioassays were plotted against SMS concentration, and COD, TKN, and hardness of SMS extracts adjusted for exposure time. Mean macroinvertebrate counts in control and SMS treatments in the drift experiments were compared using ANOVA. Means are reported with associated standard errors.

RESULTS

Macroinvertebrate Survey

Distributions of different species were observed between sampling sites (Tables 1 and 2). Mayflies in the family Baetidae were more abundant at Site A in the first survey (34%) in June compared to the second survey in August (5%). There were 247 riffle beetles, Elmidae, (27%) in the first survey at Site B compared to 27 at Site A (7%), and 54 at Site C (20%). In the first survey, there were 252 caddisfly larvae, Hydropsychidae, (27%) at site A, 433 (47%) at site B and 123 at site C (46%). In the second survey, there were 5 black fly larvae, Simuliidae (1%) at site A, none were collected at site B, and 132 (50%) at Site C. Crane fly larvae, Tipulidae, were present at all three sites. There were 66 tipulid larvae (4%) collected in the first survey and 98 (8%) collected in the second survey. There were only four stone flies, Perlidae, collected in the first and second surveys.

Data from the collected insect larvae were analyzed to determine any differences in diversity between surveys and between collection sites. Pooled Dmg diversity values from sites A, B, and C of the first survey were not significantly different from those from the second survey. Mean H' diversity of the first survey was significantly higher than mean H' of the second survey. To examine homogeneity of the insect community between sites A, B, and C, diversity values from both surveys at each site were compared. We found no significant differences between mean Dmg diversity of sites A, B and C. Mean H' diversity was higher at site A, adjacent to the SMS storage area, than at both sites B and C. There was no difference in H' diversity between sites B and C. The higher H' at site A suggests that the SMS land application did not negatively impact the insect community. The similarity between Dmg diversity values for sites A, B, and C also supports the contention that SMS land applications have not significantly impacted insect communities at the three sites on this stream.

Table 2. Numbers of aquatic insects by taxa collected in the second macroinvertebrate survey[1].

TAXON	Site A				Site B				Site C			
	Sample				Sample				Sample			
	1	2	3	Total	1	2	3	Total	1	2	3	Total
Ephemeroptera												
Heptageniidae												
Stenonema modestum					2	5	4	11	2	1		3
Baetidae												
Baetis tricaudatus	3	7	1	11					1	4	2	7
Baetis sp. 1												
Baetis sp. 2	2	1		3						4	3	7
Ephemeridae												
Ephemera sp.												
Isonychidae												
Isonychia sp.			1	1	3	3	1	7			1	1
Plecoptera												
Perlidae												
Acroneuria sp.							1	1		3		3
Leuctridae												
Leutra sp.												
Megaloptera												
Corydalidae												
Corydalus sp.											1	1
Coleoptera												
Psephenidae												
Psephenus sp.		2	2	4		3	3	6				
Elmidae												
Optioseruus sp.	4	30	10	44	7		30	37	3	2		5
Dubiraphia sp.	1	4	2	7	1		3	4		1		1
Trichoptera												
Hydropsychidae												
Hydropsyche sp.	13	65	23	101	22	41	40	103	21		15	36
Cheumatopsyche sp.	2	4	1	7	2	4	2	8	8		3	11
Hydroptilidae												
Leucotrichia		1	1	2	1		3	4				
Philopotamidae												
Chimarra sp.		10	3	13	2	3	3	8				
Glossosomatidae					2			2				
Diptera												
Tipulidae												
Tipula sp.	3	14	3	20		37	13	50	11	17		28
Chironomidae	6		33	39	62	14	15	91	39	156	102	297
Simuliidae												
Simulium sp.			1	5					1	104	27	132
Empididae												
Hemerodromia sp.		1	1	2	14	46	1	61			8	8

1. Macroinvertebrates were collected using a Hess sampler during August. Three samples were taken at each of the three study sites. Site A was located downstream from the spent mushroom soil (SMS) land application. Site B was adjacent to SMS land application, Site C was upstream from the SMS land application.

Bioassays

In the first bioassay, 0%, 12.5%, 50%, and 100% concentrations of SMS extract produced 5%, 0%, 5%, 45%, and 85% mean mortality, respectively. In

Table 3. Water analyses[1] performed by the University of Delaware Bioresources Engineering Laboratory on samples of stream water from macroinvertebrate survey sites, surface runoff collected from the stream bank during a heavy rain event, and from spent mushroom soil (SMS) extracts used in the second drift experiment and the two bioassays.

Sample	BOD mg/L	COD mg/L	TKN ppm	Hardness mg/L	pH	Alkalinity mg/L	Acidity mg/L
Stream water							
Survey site A	1.983	0	0.365	122.86	7.80	57.14	2.39
Survey site B	2.470	0	0.156	119.64	8.01	52.63	1.91
Survey site C	1.869	0	0.678	81.01	7.30	28.88	1.91
Surface runoff	–	43.01	1.747	595.52	7.56	154.08	9.55
SMS extracts							
Drift experiment	458.268	4340.54	131.332	2441.08	7.83	921.70	214.92
First bioassay	–	378.01	8.394	1073.00	7.40	153.62	28.66
Second bioassay	–	1251.33	62.720	456.03	7.17	283.96	66.86

1. BOD-biological oxygen demand, COD-chemical oxygen demand, TKN-total Kjeldahl nitrogen and hardness were measured using standard methods for waste water analysis (Clesceri *et al.* 1995).

the second bioassay, 0%, 12.5%, 25%, and 50% concentrations of SMS extract produced mean mortalities of 0%, 10%, 10%, and 25%. The 100% concentration of SMS extract was eliminated, as it was clear that this concentration of SMS extract caused high mortality in mayfly nymphs. Mortality increased as SMS extract concentration increased. The extracts used in the first and second bioassays were dramatically different (Table 3). COD, TKN alkalinity and acidity were much higher than the extract used in the second bioassay. However, the hardness of the extract used in the first bioassay was more than twice that of the extract used in the second bioassay. To correlate the first and second bioassay data, mortality data was corrected for exposure time to SMS extracts and plotted against TKN, COD and hardness for the first and second bioassays. Mortality rates plotted against TKN for the first and second bioassays diverged as did mortality plotted against COD (Figs. 3 and 4). Mortality rates plotted against hardness for the first and second bioassays converged (Fig. 5) indicating that hardness of the SMS extract had a major effect on mortality.

Drift Experiment

In the first drift experiment, the control corridors C1, C2, and C3 had insect counts of 2, 21, and 27 respectively with a mean of 16.67 ± 7.54 insects in each drift net. The treatment corridors T1, T2, and T3 had counts of 27, 54, and 8 with a mean of 29.67 ± 13.35 insects. There was no significant difference between the control corridors and the treatment corridors. The drift counts in the two outside corridors, C1 and T3, were lower than the counts from the center of the stream. When these two outside corridors were eliminated from the analysis, a significant difference was found between the controls and treatments.

In the second drift experiment, the control corridors C1, C2, C3, and C4 captured 4, 2, 17, and 1 insect respectively. The treatment corridor T1, T2, T3, and T4 captured 11, 34, 12, and 4 insects.

There were no significant differences between insect drift in the control and treatment corridors.

DISCUSSION

Macroinvertebrate Survey

In general, the majority of insect species in the Ephemeroptera, Plecoptera, and Trichoptera are considered sensitive to pollution (Lenat 1993). In the first survey, Site A had a large number of Baetidae, including *Baetis tricaudatus*, a pollution intolerant species (Lenat 1993). Mayflies in the family Baetidae are fine-particle detritivores. The SMS land application may be a source of mild nutrient enrichment and provide a suitable habitat for *Baetis*. The 283 *Baetis* found at site A in the first survey accounted for 34% of the larvae in the sample. In the second survey, two months later, the number of Baetidae decreased to only 5%. This may have been due to emergence of these insects during the late summer months.

The hydropsychid caddis flies were a dominant taxon at sites A, B, and C in the first survey. These insect larvae construct cases made of organic and mineral fragments. They use a silk net at the entrance of these retreats to filter fine particulate organic material out of the current (Mackay 1979). The SMS land application may have contributed fine particulates to the stream.

The large proportion of midge larvae, Chironomidae, at site C also indicated that there has been an impact on the stream at this site. Chironomid larvae are tolerant of pollution (Lenat 1993). They were 55% of the total sample at site C in the second survey as compared to and 23% at site B and 15% at site A.

Black fly larvae were in high abundance at site C and are moderately tolerant to pollution (Lenat 1993). Black fly larvae are also colonial. Hundreds of simuliids were clumped on single stones in the streambed. These larvae are filter feeders and position themselves on stones at ideal spots in the current for oxygen uptake. Placing a sampler on one of these aggregations can produce a disproportionately large number of black fly larvae in one sample.

Tipulidae larvae were found at all three sites in both surveys. These larvae burrow in the detritus and leaf packs in a stream. They thrive in habit that has large amounts of course particulate organic materials. Especially high counts were found at site B. At site B, large amounts of leaf litter entered the stream. An open area with large trees was exposed to the wind and often blew leaves over the stream bank and into the water. This provided the ideal habitat for tipulid larvae and may have accounted for their abundance.

There were very few perlid stone flies found in either survey. This is similar to macroinvertebrate surveys done in other

Figure 3. Relationship of *Stenonema modestum* (Banks) (Ephemeroptera: Heptageniidae) mortality to TKN exposure per hour in the first and second bioassays. Exposure was calculated by multiplying the concentration of spent mushroom soil extract by the measured TKN (ppm) in full strength extract, divided by time (hours). Insects were exposed for 72 hours in the first bioassay and 48 hours in the second.

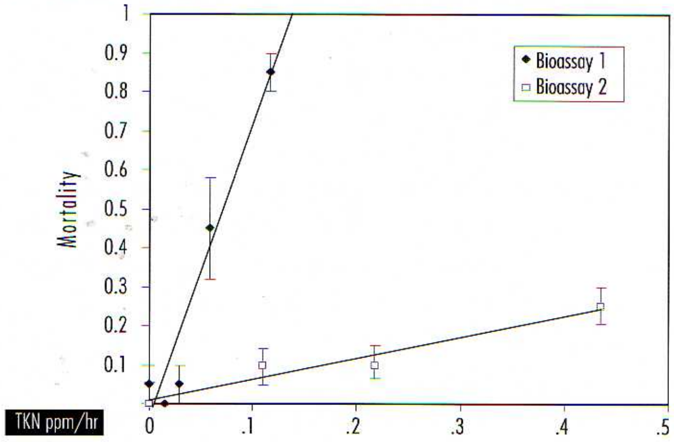


Figure 4. Relationship of *Stenonema modestum* (Banks) (Ephemeroptera: Heptageniidae) mortality to COD exposure per hour in the first and second bioassays. Exposure was calculated by multiplying the concentration of spent mushroom soil extract by the measured COD (mg/L) in full strength extract, divided by time (hours). Insects were exposed for 72 hours in the first bioassay and 48 hours in the second.

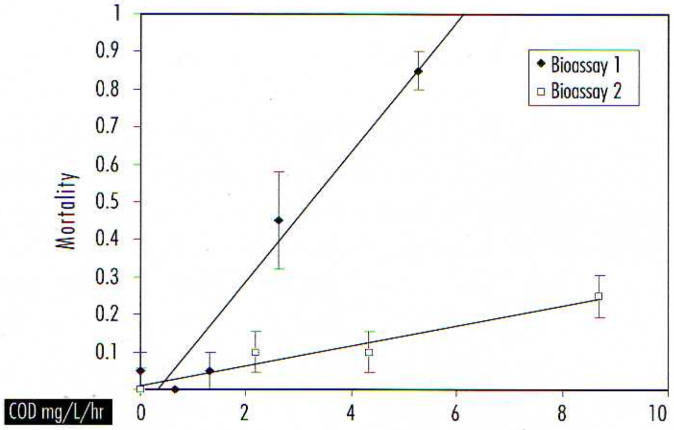
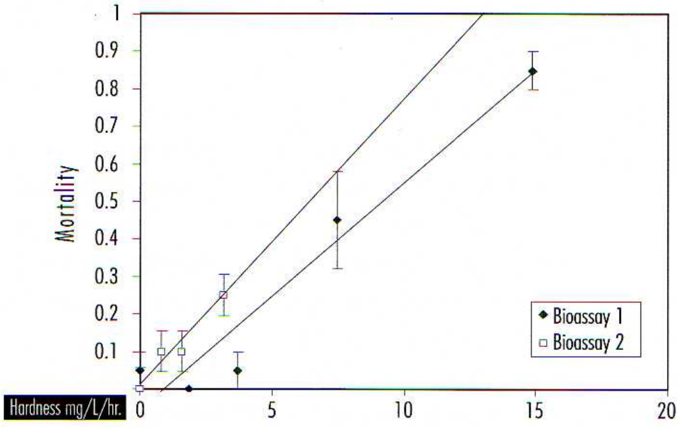


Figure 5. Relationship of *Stenonema modestum* (Banks) (Ephemeroptera: Heptageniidae) mortality to hardness exposure per hour in the first and second bioassays. Exposure was calculated by multiplying the concentration of spent mushroom soil extract by the measured hardness (mg/L) in full strength extract, divided by time (hours). Insects were exposed for 72 hours in the first bioassay and 48 hours in the second.



streams in the White Clay Creek Watershed. Perlidae are very intolerant to pollution (Lanet 1993) and are in low numbers in many moderately impacted streams in the White Clay Creek Watershed (George Fox, Ashland Nature Center Stream Watch, personal communication).

We hypothesized that if SMS runoff affected stream quality, insect diversity indices would differ relative to the location of the SMS. Both diversity indices (Dmg and H') showed little or no difference in diversity between sites A, B, and C suggesting that runoff from nearby SMS applications did not significantly impact the stream insect community. Shannon's diversity index (H') was slightly higher below the SMS land application at site A. This was probably a result of nutrient enrichment by runoff at that location. Although we tried to select sites that were comparable, habitat in this stream improved downstream from its source. The variability in the stream habitat may be greater than the effect, if any, of the SMS land applications.

Because of the high cost of the quantitative techniques used to monitor water quality, a rapid assessment approach has been adopted by organizations such as SWRC. A section of the White Clay Creek running behind SWRC with a high macroinvertebrate diversity has been used as a reference site to compare other sites in the White Clay Creek drainage. SWRC has used four measures to evaluate the condition of sites relative to the reference station: richness, abundance, community density and similarity indices (Resh and Jackson 1993). Although the SWRC rapid assessment protocol was not followed, comparison to a reference stream can help characterize the stream that we used in our experiments.

Our sites were not as deep as the site behind SWRC and we used a slightly different sampler. Site A was similar to many other study sites along the White Clay Creek. Site A, downstream from the SMS storage area, had an average of 8 families of insects compared to 19 families found at SWRC (unpublished data, SWRC). This is 42% of the reference site and gives the stream a rating of 3 out of 6 according to the Rapid Bioassessment Protocol (unpublished data, SWRC). A rating of 3 suggests that the stream is moderately impaired, similar to other SWRC study sites on the White Clay Creek.

Bioassays

With no significant differences in our insect surveys, it was important to establish the concentration of components of SMS extract that could have a negative effect on *Stenonema modestum*, a representative insect. Determining what specific component or group of SMS components caused mortality in *Stenonema* larvae will require further study. Wuest and Fahey (1992) reported that potassium, calcium, and magnesium accounted for about 80 - 90% of the dissolved salts in leachate from SMS. High potassium and sulfate levels in SMS have also been reported (Chang and Hayes 1978). Rather than isolating specific ions, we considered three characteristics of SMS, chemical oxygen demand, total nitrogen, and hardness.

The first and second bioassays both had an increase in mortality with increasing SMS extract concentration but both showed divergent rates of mortality when graphed against TKN and COD after adjustment for exposure time. Hardness was much higher in the extracts used in the first bioassay whereas TKN and COD were both higher in the second bioassay. Plots of mortality rates against hardness of SMS extracts from the first and second bioassays converged indicating that hardness of the extracts may have influenced mortality of *Stenonema* larvae.

Hardness is a measure the number of disassociated ions in a solution. Most often these ions are calcium and magnesium. Calcium oxalate is a metabolic byproduct of mushroom growth. Mushroom hyphae accumulate calcium oxalate crystals during spawn run. (Latche and Piquemal 1975). Oxalate limits the development of larvae of a major mushroom pest, *Lycoriella mali* (Diptera: Sciaridae) (Binns 1980). The bioassays indicated that increased mortality in *Stenonema* was associated with increased hardness due to calcium released from the dead hyphae of mushrooms. Oxalate may have been correlated with hardness. Additional bioassays will be necessary to determine if hardness is a critical component of SMS that is detrimental to mayfly larvae at high concentrations.

During a storm, when rainwater was moving quite rapidly through the SMS land application, runoff water was collected as it cascaded over the stream bank. The hardness of this sample was 595.52 mg/L. This was notably high. The hardness of undiluted SMS extract used in the second bioassay was 456.03mg/L (Table 3).

Drift Experiment

Although no statistically significant increase in catastrophic drift was detected in our corridor experiments, there was a slight difference when outside corridors were eliminated in the first experiment. The microhabitats on the outside corridors were not well suited for insect larvae. Outside corridors were located along the stream edges and had lower water levels, slower current, and less cobble than the interior riffle areas. We used only the four center corridors in the second drift experiment to minimize this effect. SMS extracts failed to produce a differential drift response from insect larvae in this experiment.

By considering the application rate of SMS extract in the corridor experiment, the mortality causing concentrations of SMS in the bioassays and the analysis of runoff collected during a rain event we can infer the effect SMS land applications might have on insect larvae. For example, the COD concentration in the extract used in the corridor experiment was 100 times greater than the COD concentration found in the runoff during a severe rain. However, when diluted by the volume of water moving through each corridor, the concentration of COD was 5.98 x 10^{-4} mg/L. This concentration of COD is much less than that found in the runoff, 43.01 mg/L, and also less than COD concentrations in the first and second bioassays, 378.01 mg/L and 1251.33 mg/L respectively. The diluting capability of the stream would prevent SMS concentrations from reaching even our lowest bioassay concentration of 12.5 % SMS. **MN**

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