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Monitoring Stream Water Quality with Mouse Cell Culture and On-Site Allium Tests

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The intent of this study was to determine the effects that sediment influx and storm drain effluent have on stream water quality. The stream of interest is located within the Westside Nature Preserve of Western Connecticut State University, in Danbury, Connecticut. Since 1994, when adjacent woodlands were cleared for athletic fields, this stream has been markedly impacted with sediment. A storm drain conduit discharges additional sediment and effluent near the headwater of the stream, thereby compounding the existing problem.

The stream within the Westside Nature Preserve is a second order stream which flows into Kohanza Brook; it is located within the watersheds of the Kohonza Brook and Still River. The surface water which drains into both of these watersheds is classified as B/A and A respectively (Harris 1993).

MATERIALS AND METHODS

Stream water quality was evaluated at three sites (i.e., Sites 1, 2 and 3) using two bioassays: *Allium* (onion) root tips and fetal mouse cell cultures. Concurrent *Allium* and cell culture studies were conducted in June and August of 1997.

Individual batches of cell culture media were prepared with stream water collected from each of the three sampling sites. Using 200 mls of a given water sample, 2.5 gms of McCoy's powered medium (Sigma M-4892) and 0.44 gm of tissue culture tested $NaHCO_3$ (Sigma 5761) were brought into solution; the media were filter sterilized and transferred into sterile, capped, plastic flasks. Commercially prepared, McCoy's medium (Sigma M-8403) was used for the control. To each flask of medium, 20 ml of newborn calf serum (Sigma N-4762) and 0.1mg of gentamicin (Sigma G-1272) per ml of medium were added. The pH of all media was observed, using phenol red, to be 7.2.

A quantified, one ml inoculum of fetal mouse cells (from primary cultures) was transferred into each of two T- 25 vented flasks. The flasks contained 3 ml of culture medium prepared with stream water from one of the sample sites or commercially prepared medium (i.e., control). This resulted in two cultures set up for each sampling site and the control. All cultures were placed in a humidified, CO_2 (5% CO_2, 95% air) incubator set at 37° C.

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The percentage of confluency for each culture was recorded daily; the duration of culture was determined by the number of days it took for control cultures to become confluent. All cultures were subcultured when the controls were confluent and the number of cell/ml was quantified for each culture at that time.

For the August study, the above procedure was modified as follows: (1) each culture was inoculated with 125, 000 cells and (2) upon completion of the second subculture, each culture was fixed and stained using LeukoStat (Fisher Scientific CS430D); the numbers of giant, multinucleated and highly vacuolated cells observed in a 2 cm square (drawn on the bottom of each T-flask), were tallied.

Root tips were examined macroscopically for signs of toxicity; this involved inspecting for the presence of hook-shaped root tips, tumor formation and stunted growth of roots (Fiskesjo 1985a). Additionally, the length of each root was measured, directly on the bulb and means and standard deviations of root length values were calculated for both control and test onions.

After assessing macroscopic parameters, two root tips, approximately 1 cm in length, were fixed and prepared for microscopic evaluation with the squash. technique. Observation for the presence of chromosomal aberrations (e.g., c-mitosis, vagrant and fragmented chromosmes), micronuclei and abnormal cell morphology in nondividing cells were made. Additionally, mitotic indices were obtained by counting the number of dividing cells per thousand cells viewed in each root tip meristem. Analysis of variance for *Allium* tests was conducted with SPSS Software

RESULTS AND DISCUSSION

Both subcultures were confluent in three days for the June fetal mouse cell culture study. Based on the time required to attain 100% confluence, the percentages of confluence of the cultures maintained in medium prepared with stream water were comparable to those maintained with commercially prepared medium (control). The data from the August cell culture studies (Table 1) indicate that cells cultured in media using water from Site 1 or 2 were confluent in the same number of days as the control cultures. However, cells cultured in medium prepared with water from Site 3 maintained, for the duration of the study, 25% confluence. Interestingly, these cultures had the highest percentages of highly vacuolated cells and giant cells (Table 2, Figure 1).

Table 1. Percent confluence for August fetal mouse cell cultures.

Site	Day 1	Day 3	Day 5	Day 6
1	25	40	50	90
2	25	40	50	95
3	25	25	25	25
Control	25	40	50	100

Table 2. August fetal mouse cell culture results.

Site	Norm	Multi-nucleated	%	Highly Vacuolated	%	Giant	%
Site 1	1,086	160	14.7	11	1	3	0.3
Site 2	868	127	14.6	5	0.6	2	0.2
Site 3	722	151	21	37	5	7	1.0
Control	1,225	125	10	14	1.1	0	0

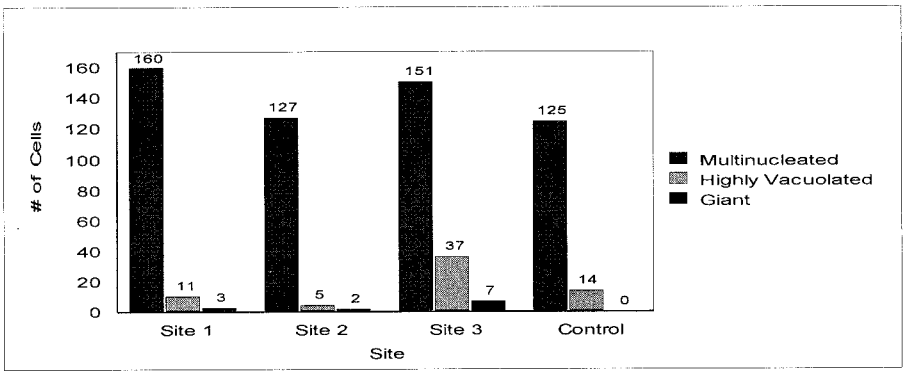

Figure 1. The number of abnormal cells observed in August fetal mouse cell cultures.

Mean root tip lengths and standard deviations for all *Allium* tests are located in Table 3; ranges of root tip lengths are located in Figures 2. and 3. Results for the June study show that the root tip lengths are comparable to or exceed the control onions. August results show that there are no statistically significant differences between test or control onions.

Despite inherent fluctuations in the number of dividing cells that occurred in each study, the onions cultivated at Site 3 consistently had less dividing cells than did the other test and control onions (Tables 4 and 5). The number of dividing cells in onions cultivated at Site 3 was 393 and 591 for June and August, respectively; control results were 557 and 712, respectively. The difference in the number of dividing cells observed in Site 3 onions in comparison to all test and control onions was found to be statistically significant ($p = 0.04$). Overall, the number of chromosome aberrations is low with respect to the total number of dividing cells. Although the total number of aberrations is, generally, lowest in the control onions and highest in onions cultivated at Site 3 (Table 6) the statistical significance, if any, of such differences requires a larger study.

The lowest mitotic indices consistently occurred in Site 3 onions (Tables 5 and 6). The mitotic indices for Site 3 were 20 and 29 for June and August, respectively; for the control they were 28 and 36, respectively.

Overall, the number of chromosome aberrations is low with respect to the total number of dividing cells. The total number of chromosome aberrations is lowest in the controls; however, the highest numbers of aberrations consistently occurred in onions cultivated at Site 3 (Table 6).

Table 3. *Allium* test mean root tip lengths (cm) and standard deviations (STD) for June and August.

Month	June				August			
Site	1	2	3	Control	1	2	3	Control
Mean	1.96	4.42	2.06	2.55	1.9	1.4	1.2	1.84
STD	0.64	1.06	0.84	0.59	0.92	0.61	0.52	0.53

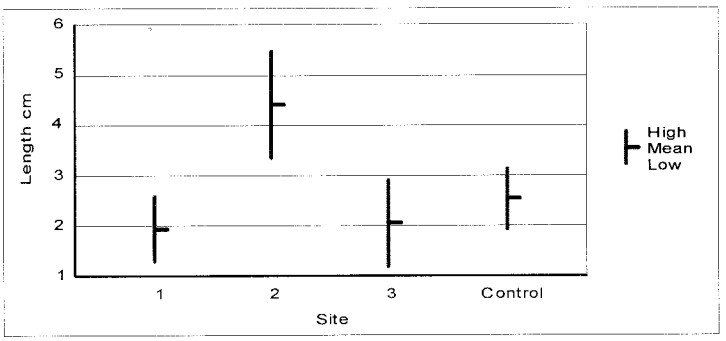

Figure 2. Range of root tip lengths (cm) for June.

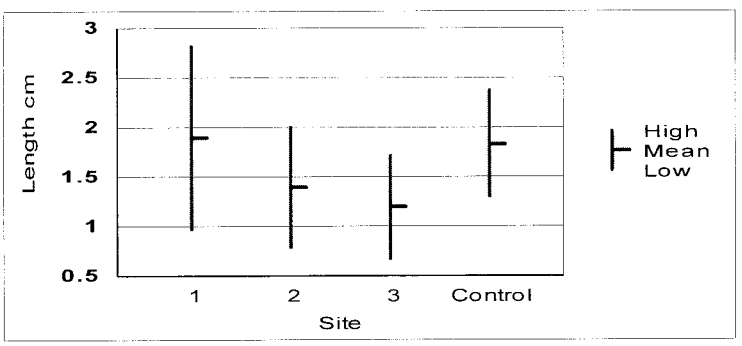

Figure 3. Range of root tip lengths (cm) for August.

Table 4. June *Allium* test results per two thousand cells counted.

Dividing Cells	Site 1	Site 2	Site 3	Control
Prophase	442	339	325	465
Prometaphase	19	3	12	20
Metaphase	45	34	28	50
Anaphase	22	22	18	14
Telephase	32	15	10	8
Total # Dividing Cells	560	413	393	557
Mitotic Index	28	21	20	28
Nuclear Blebs	2	0	2	1
Vagrant Chromosomes	1	2	3	0
Fragments	1	2	2	0
C- Mitosis	1	1	1	1

Table 5. August *Allium* test results per two thousand cells counted.

Dividing Cells	Site 1	Site 2	Site 3	Control
Prophase	579	587	479	607
Prometaphase	21	27	25	20
Metaphase	52	38	20	25
Anaphase	30	30	25	32
Telophase	26	45	27	22
Total # Dividing Cells	708	727	576	706
Mitotic Index	35	36	29	35

Table 6. The total number of aberrations observed in *Allium* tests.

Month	Site 1	Site 2	Site 3	Control
June	5	5	8	2
August	8	5	15	6

The results of both the fetal mouse cell culture studies and *Allium* tests conducted during August, may indicate that the water quality at Site 3 had degraded sometime after June. This is evidenced by the fact that the highest numbers of highly vacuolated cells and giant cells occurred in the Site 3 fetal mouse cell cultures. Also, the confluency rates of these cultures remained unchanged from the inoculum density. It has been determined that changes in cell morphology and/or the absence of cell proliferation in culture can be induced by exposure to toxins (Barile 1994; Frazier 1992; Freshney 1987). Additionally, cell vacuolization may be attributed to the presence of toxins at noninhibitory concentrations (Ekwall 1983). Site 3 *Allium* tests had the lowest mitotic indices and the highest number of aberrations. This too is indicative of exposure to toxins (Fiskesjo 1979, 1985b, 1985c; Grant 1978, 1982).

Although Site 3 is located the furthest from the site of the sediment loading; it is located next to a paved road. Therefore, it is possible that contaminants such as hydrocarbons and petroleum compounds may have entered the stream in the form of runoff. These substances can adversely affect water quality (Doenges JM, Allan CP, Benson J and Jontos RJ 1994). In addition to *Allium* tests and fetal mouse cell cultures, subsequent studies of the water quality at Site 3 should include chemical analysis for hydrocarbons.

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