A Preliminary Study on Trophic Level Analysis for Heavy Metals in Aquatic Macroinvertebrates in Two Streams in Columbia, PA

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Abstract

Two steams in Lancaster County were studied for the heavy metal concentration in the aquatic macroinvertebrates and fish of the steam. Sample sites were located above and below a discharge pipe on the impact stream and were compared to a site on a control stream. Sites were monitored on a monthly basis and sampled for water, macroinvertebrate, and fish analysis of heavy metals. Downstream from the discharge pipe compared to the above and control sites a significant rise in the concentration of the heavy metals iron, nickel, and chromium occurred. These increases in heavy metal concentration occurred across all trophic levels of macroinvertebrates and fish. The areas above the impact point and the control site showed no sign of bioaccumulation of the heavy metals. Diversity of the macroinvertebrates also indicated the area downstream from the impact point has been compromised.

Introduction

Aquatic ecosystem degradation occurs when a waterway becomes less able to support life by stresses such as nutrient or contaminant loads and thermal pollution. Waterways are degraded in two ways, point source and non-point source. For purposes of the Clean Water Act, “Point Source” is defined as any discernible, confined, and discrete conveyance, including but not limited to, any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, landfill, leachate collection system, vessel or other floating craft from which pollutants are or may be discharged (EPA, 1998). There is no doubt that the insults to aquatic ecosystems are increased along with urban, industrial, agricultural, and other development. As a result, many aquatic ecosystems have become severely degraded and are in need of some drastic corrective measures (Allan, 1995).

Biomagnification is the *accumulation* from food, whereas the food chain effect refers to the *transfer* of contaminants through the food web. Bioconcentration, i.e. the accumulation from the surrounding medium, in some lower trophic levels forms the basis of any biomagnification or food chain effect (Suedel, 1994). In Germany a study of (what?) showed the presence of cadmium in algae accumulated in the aquatic insects of the stream (Shaffer, 1998). Another study showed an increase of four orders of magnitude in the concentration of methylmercury in higher trophic levels, from algae to herbivores and detritus feeders (e.g. zooplankton, mollusks) (Langston and Spence, 1995). The use of freshwater benthic macroinvertebrates to monitor such phenomenon has been demonstrated to be an accurate indicator of the presence of toxic chemicals such as heavy metals and pesticides (Buikema and Voshell, 1993). Benthic macroinvertebrates (those that live at the bottom of a stream) have been attractive targets of biological monitoring efforts because they are a diverse group of long lived, sedentary species that react strongly, and often, predictably to human influences on aquatic ecosystems (Cairns and Pratt, 1993).

Benthic macroinvertebrates have been separated into function feeding groups based on the association between a limited set of feeding adaptations found in freshwater invertebrates and their basic nutritional resource categories (Merritt and Cummins, 1996). Limitations on food acquisition mechanisms left these groups relatively fixed through evolutionary time. That is, these organisms are separated via the morphological/behavioral adaptations that allow them to feed on a diverse array of food resources. The functional feeding groups include: 1) shredders, which feed mainly on coarse particulate organic matter (>1mm thick) such as woody debris; 2) collectors, which feed mainly on fine particulate organic matter (<0.5mm thick); 3) scrapers, which feed mainly on algae attached to rocks and wood; and 4) predators, which feed on other aquatic invertebrates.

The use of macroinvertebrates to determine diversity in streams is commonly used as a method of toxicity testing (EPA, 1998). Along with their availability as a parameter, they can be used to test for specific toxins, including heavy metals. Macroinvertebrates, because they are sensitive to changes in the ecosystem as well as being long lived, cannot easily escape changes in the water quality and they can be collected very easily from most aquatic systems with inexpensive or homemade equipment (Klem, 1990). In prior tests, macroinvertebrates, as compared with water and other biological samples, were the most analytically sensitive to a survey of 40 major toxins commonly found in fresh waterways (Buikema and Voshell, 1993). The most sensitive organisms to pollution in studies have been stone flies, may flies, and hellgrammites (Mayer and Ellersieck, 1986; Larson and Hyland, 1987). Those that seem to survive better in polluted waters have been aquatic worms, midge flies, and black flies (Giesy et al., 1980). These organisms have been used to test for toxicity in the past and have been shown to successfully indicate the presence of pollutants such as heavy metals as early as the 1930's (Anderson, 1958). Macroinvertebrates are good indicators of heavy metal accumulation and were the focal point of this investigation (Kohler, 1992). Heavy metals in the environment are those metals that have aqueous forms that can adversely affect the waterway, including but not limited to iron, lead, mercury, arsenic, chromium, and nickel. These metals have been known to cause a variety of health problems, and are considered to be carcinogenic.

The objectives of this project include characterization of the water chemistry and physical attributes at each site; identify and compare the macroinvertebrate communities from each site;

determination of presence/absence of heavy metals bioaccumulating up trophic levels at these sites.

Methods

Study Site

Shawnee Run, a first order stream, is located adjacent to a proposed “Rails to Trails” restoration. These projects utilize the abandoned railroad and adjacent habitat as a recreational area with a nature friendly trail, and in some cases, signs indicating the trails history and significance. This property is adjacent to a long-time zoned industrial sector. A five-foot concrete false riverbed turns the creek 90° toward the company, forming a tributary. Sixty meters downstream, another concrete wall returns the tributary to the original path of the Shawnee Run. This unnamed tributary to the Shawnee (UNTSR) is the focus of this project.

ANVIL has a lagoon at the edge of their property, which discharges effluent directly into the tributary. The ANVIL plant, formerly Grinnell, is just one of the companies that has been located in the immediate area of the railroad, which currently includes a metal scrap yard. The Shawnee Run parallels the unused train track and is intersected by UNTSR (Figure 1). Preliminary monitoring data indicate that the water quality of the connecting tributary to Shawnee Run may have been compromised. The control site is located approximately 0.5 miles from UNTSR, along the Shawnee Run.

It is bordered by very little riparian buffer vegetation and by residential and urban land. The above/upstream site, the Shawnee Run, is along the unused railroad, one hundred meters from the suspected impact point. The below/downstream site, UNTSR is located 40 meters from the impact point. The above and below sites are surrounded by a larger riparian buffer than the control site.

<u>Figure 1</u>. Map of the Three Sites and the Surrounding Area in Columbia, PA

Experimental Design

The Before/After, Control/Impact, or BACI sampling approach was utilized in the collection of samples. To survey for heavy metal presence in the stream, samples were collected from sites above the impact point of the lagoon (Point 1A, Figure 1), below the impact point (Point 2, Figure 1), and from the control stream (Point 3, Figure 1). The above site is approximately 40 meters further from the impact point than the below site, because a secondary water source coming from ANVIL, and not coming from the lagoon, was directly upstream from the suspected impact point. In July and October, the above site had dried up from lack of rain. In October, the above site sampled was moved much closer to the discharge source. For water and trophic level analyses, samples collected from September to November at the above site were from Point 1B in Figure 1. The samples collected were of a wide variety of macroinvertebrates and other organisms so that a possible pattern could be developed showing heavy metal accumulation up the trophic levels of the food chain.

The physical analysis of the stream included flow rate measurements, which were done with a March McBirney Flow Meter. Flow rates were taken (three replicates) in both pools and riffle areas of the stream at each site. The discharge was calculated using the following formula:

$$\text{Discharge} = \text{Stream Area x Velocity}$$

Stream area is the product of width times depth (m^3) and the velocity equals (m/s). The chemical properties of the sites were determined using a Hydrolab H_2O Water Quality Multiprobe. These properties included the concentration of dissolved oxygen, the specific conductivity, and the temperature. The pH was determined in the lab prior to heavy metal analysis using a pH probe.

Macroinvertebrates were collected from each site with kick seines. The samples were sorted in the field and transported to the lab for identification and heavy metal analysis.

Insectivorous fish, such as the Blacknose Dace, were collected from each site with a D-Frame net or electro fish shocker. Fish were sorted in the lab and frozen at 4°C prior to analysis for heavy metal concentration. The samples were exposed to the least amount of air possible to avoid contamination. The samples were then frozen at 4°C until the analysis was completed. The macroinvertebrates were separated into functional feeding groups for trophic level comparisons from each site and for heavy metal analysis.

Figure 2. A Schematic of the AAS

The Atomic Absorption Spectrometer (AAS) was used to test for the presence of heavy metal concentrations of these organisms. AAS is one of the most commonly used instruments in the analysis of heavy metals, along with an ICP. AAS uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace. In their elemental form, metals will absorb ultraviolet light when they are excited by heat. Each metal has a characteristic wavelength that will be absorbed. The AAS instrument looks for a particular metal by focusing a beam of

UV light at a specific wavelength through a flame and into a detector. The sample of interest is aspirated into the flame. If that metal is present in the sample, it will absorb some of the light, thus reducing its intensity. The instrument measures the change in intensity. Concentration measurements are usually determined from a working curve derived from a set of standards run on the instrument prior to the samples being analyzed (Skoog, 1998). This has been demonstrated to be the most efficient and reliable way to test for heavy metals (Soster, 1990).

Once thawed, the samples were weighed on an analytical balance ± .0001 g to 1 g (availability of some samples was limited and the procedure altered to accommodate this by simply changing calculations). The one-gram analyte was mixed with 10 mL of a 1:1 solution of sulfuric acid and deionized water (DI) in a covered 100 mL beaker. This solution was heated on a hot plate in a fume hood for 10 minutes and cooled. An aliquot (5 mL) of concentrated nitric acid was added and the solution was covered and heated for 30 minutes. If brown fumes were emitted indicating oxidation of the sample by nitric aid, the step was repeated until the fumes ceased. The same amount of nitric acid was added to each sample for quality assurance of the samples. Once this is reached, the solution is heated for 2 hours. After cooled, 2 mL of DI water and 3 mL of 30% hydrogen peroxide were added. Hydrogen peroxide (1 mL aliquots) was added to the sample until effervescence subsided (no more than 10 mL of hydrogen peroxide was added to each sample). The sample was heated for another 2 hours, cooled, and concentrated hydrogen acid (10 mL) was added. The solution was quantitatively transferred to a 100 mL volumetric flask using DI water. The volumetric was then filled with DI water and the solution analyzed with the AAS.

The standards were prepared by analytically weighing out the equivalent of 0.1, 0.2, 05., 1.0, 1.5, 2.0, 3.0, 5.0, and 10.0 ppm of each compound being tested. For this test iron, lead, nickel, chromium, copper, and arsenic were tested.

Results

Water Physio/chemical Analysis

In May the water temperature was greater from the below site compared to the above and control sites. The dissolved oxygen levels were comparable at each of the three sites. The conductivity at all three sites was similar (Table 1). The stream velocity was greater at the control stream than at either the upstream and downstream sites. In June the temperature was again higher below the impact point (Table 1). The dissolved oxygen was slightly higher at the below site than at either the above site or the control site.

Table 1. Chemical/Physical Data from the Control Site (Shawnee Run) and the Above (Point 1A/Below Discharge Sites on the Impact Tributary.

		May			June	
Parameter	**Control**	**Above**	**Below**	**Control**	**Above**	**Below**
Temperature (°C)	12.4	12.4	15.6	16.2	18.5	23.5
Conductivity (Ms/cm)	5.3	3.1	5.5	5.7	4.9	5.5
Dissolved oxygen (mg/L)	7.7	8.0	7.6	7.5	3.5	7.2
Mean Velocity (m/s)	0.2	0.1	0.1	0.2	0.1	0.1

In July, due to a lack of precipitation, the above site was a dry riverbed. Water flow resumed further downstream from the above site. The water temperature was higher at the below site than the control site. The dissolved oxygen and conductivity were similar at both sites. The water discharge at the below site was greater than that of the above site. In July, we observed a 31% increase in water volume contributed from the discharge pipe/groundwater supply. There was no water flow at the above site (Point 1A), so no analysis was done in July for the above site. In the month before the August sample date there was enough precipitation that the above site was flowing once again. The site that was possibly contaminated was directly above the impact point and was now used for the above site so that some data could be drawn from it. The temperature of the water at the below site was higher than the above and the below and the control were comparable (Table 2). The specific conductivity was approximately the same for all three sites. The discharge of the above site was significantly less than after the ANVIL outflow. In August, we found that 98% of the total water volume contributed from either groundwater discharges or from the discharge pipe on ANVIL property.

Table 2. Chemical/Physical Data from the Control Site (Shawnee Run) and the Above/Below Discharge Sites on the Impact Tributary. (The above site in August was redesignated as Point 1B in Figure 1.)

		July			**August**	
Parameter	**Control**	**Above**	**Below**	**Control**	**Above**	**Below**
Temperature (°C)	15.6	*	21.9	17.3	15.21	25.1
Conductivity (Ms/cm)	5.3	*	4.9	6.5	5.6	6.5
Dissolved oxygen (mg/L)	7.7	*	6.4	7.8	3.6	5.9
Mean Velocity (m/s)	0.5	*	0.6	0.5	0.05	0.7
Discharge (ft^3/sec)	1.76	(0.53)	0.77	1.5	0.02	1.6

* Indicates section of stream without water to obtain measurements

( ) Indicates that measurement was taken above discharge pipe but not from sample area a few days after July's samples were collected

There was no precipitation during September. Water temperature at the below site was again higher than at either the control or the above sites (Table 3). The dissolved oxygen and discharge are higher below the impact point than above (Point 1B), a direct result of the amount of water added to the stream from ANVIL in conjunction with the possible groundwater supply. The conductivity was similar at all three sites. As in the July samples, the differences in the physical properties were very little with exception to the temperature. The temperature was now higher at the above site (Point 1B, Figure 1) when throughout the rest of the project the below site had been higher.

Table 3. Chemical/Physical Data from the Control Site (Shawnee Run) and the Above/Below Discharge Sites on the Impact Tributary. (The above site refers to Point 1B in Figure 1.)

Parameter	September			November		
	Control	**Above**	**Below**	**Control**	**Above**	**Below**
Temperature (°C)	14.04	12.6	18.3	8.2	14.4	10.5
Conductivity (Ms/cm)	6.07	6.54	6.52	6.3	4.87	6.8
Dissolved oxygen (mg/L)	8.08	3.47	6.91	8.7	6.96	6.93
Mean Velocity (m/s)	0.5	*	*	0.10	N/A	0.14
Discharge (ft^3/sec)	1.76	*	*	0.01	N/A	0.01

* Indicates section of stream without water to obtain measurements

( ) Indicates that measurement was taken above discharge pipe but not from sample area a few days after July's samples were collected

Macroinvertebrate Analysis

Figure 2. Macroinvertebrate population of the three sites for the sampling period.

Except for May, the control site was the most diverse throughout the sampling period compared to the above and below sites on UNTSR (Figure 2). At the control site, the number of functional feeding groups present was greater than the other sites throughout the sampling period. It appears that diversity was highest for all three sites in August. There is no value for the above site diversity in July due to a dry sample site (Figure 3).

In May the analysis of the macroinvertebrates diversity was similar to all three sites, with the above site having less diversity (Appendix A1). The diversity of functional feeding groups was dominated by Shredder species at all three sites (Appendix A2). Collector-gatherers population was less than Shredders in the above site and below site. Shredders at all three sites dominated the population of macroinvertebrates in June. Also, the variety of species was greater at the below site than at the above site (Appendix B1).

The macroinvertebrate diversity at the control was dominated by Shredders in July, but the below site was dominated by predators (Appendix C2) and there were very few Shredders. Shredders again dominated the macroinvertebrates populations of the control and above site in August (appendix D2). In November the below site was represented mainly by only 3 functional feeding groups (predators, collector-filterers, and collector-gatherers) (Appendix D1).

The macroinvertebrates population diversity at the control site was dominated by Shredders, as was the above site in September (Appendix E2). The below site was dominated by both predators and collector-gatherers. In November, Shredders dominated the diversity at the control. The new above and the below were both dominated by collector-gatherers, with Shredders very scarce at both sites (Appendix F2).

Heavy Metal Analysis

In May the heavy metal concentration across all trophic levels was greater at the below site. At both the above site and the control site there was little to no heavy metal accumulation. The metals that were present at the below site were iron, chromium, and nickel (Table 4). The level of heavy metal concentration was not showing food chain effects, which was demonstrated in previous studies (Suedel, 1994). The metal analysis in June showed that at the below site across all trophic levels there were higher concentrations than at the other sites. As in May, nickel and iron concentrations were in the highest amounts observed. Both metals appeared in the highest concentrations in the Shredder functional feeding group (Table 4).

Figure 3. *Nickel concentrations for the sampling period of carnivorous and herbivorous macroinvertebrates and insectivorous fish at the below site. Observed nickel to increase overall from May to November for both herbivorous and carnivorous macroinvertebrates. Found similar levels of nickel in the fish. Nickel concentration was highest in July and November.*

Analysis of heavy metals from the representative functional feeding groups of macroinvertebrates in the control site (Shawnee Run) and the Above/Below sites from UNSTR stream for May and June 2001. Heavy metal units are expressed in ppm (parts per million), some sites did not record and were labeled N/A (not available).

Table 4. Metal concentration for May and June

Metal	Control		Above		Below	
	May	June	May	June	May	June
Lead (Pb)						
Shredder	0	0	0	0	0	0
C-G	0	0	0	0	0	0
C-F	0	N/A	0	N/A	0	N/A
Predator	0	0	0	0	0	0
Iron (Fe)						
Shredder	0	0	0	0	3.55	2.10
C-G	0.88	N/A	N/A	N/A	1.11	N/A
C-F	0	N/A	0	N/A	0	N/A
Predator	0	0	0	0	0.88	1.40
Chromium (Cr)						
Shredder	0	0	0	0.31	14.44	1.70
C-G	0	0	N/A	0	N/A	N/A
C-F	0	0	0	N/A	0.10	0.86
Predator	0.22	0	0	0	18.88	0
Nickel (Ni)						
Shredder	0	0	0	0	4.64	7.00
C-G	0	0	N/A	N/A	N/A	N/A
C-F	0	N/A	0	N/A	0	N/A
Predator	0	0	0	0	0.78	4.47

C-G = Collector—Gatherers
C-F = Collector—Filterers

In July the heavy metal concentration across all trophic levels at the control site was again almost zero in most cases (no above site measurements made because of lack of samples). At the below site the highest levels of heavy metals were detected for the entire project (Figures 3, 4, 5). Almost no accumulation of heavy metals was observed in the tissues of the macroinvertebrates at both the control and the above sites. The concentration at the below site was much higher in comparison with the above site across all trophic levels (Table 5). There was no biomagnification, but higher trophic levels were bioaccumulating metals. The metal concentration in August was less than that of the same functional feeding groups from July (Figure 4).

Figure 4. *Iron concentrations for the sampling period of carnivorous and herbivorous macroinvertebrates and insectivorous fish at the below site.*

The concentration was at its highest value in July. The fish showed a trend of increasing iron concentration from August to November. We observed that the concentration of iron was consistent from May to November, excluding July.

Table 5. *Analysis of heavy metals from the representative functional feeding groups of macroinvertebrates in the control site (Shawnee Run) and the Above/Below sites from the impacted tributary stream for July and August, 2001. Heavy metal units are expressed in ppm (parts per million). Some sites did not record and were labeled N/A (not available).*

Metal	**Control**		**Above**		**Below**	
	July	**August**	**July**	**August**	**July**	**August**
Lead (Pb)						
Shredder	0	0	N/A	0.11	0.9	1.05
C-G	0.06	0	N/A	0	2.28	1.26
Predator	0	0	N/A	0	1.07	0.85
Fish	0.06	0.11	0	0.18	0.98	2.20
Iron (Fe)						
Shredder	0	0	N/A	0	1.41	0.92
C-G	0	0	N/A	0.07	3.61	2.63
Predator	0.07	0	N/A	0	2.63	1.41
Fish	0.05	0	0	0.20	4.34	2.87
Chromium (Cr)						
Shredder	0.02	0	N/A	0	4.28	3.96
C-G	0	0	N/A	0.13	12.62	10.94
Predator	0.23	0	N/A	0	13.74	10.65
Fish	0.12	0	0	0.11	11.49	13.61
Nickel (Ni)						
Shredder	0.15	0	N/A	0.14	9.86	7.84
C-G	0.14	0.09	N/A	0.20	6.64	9.28
Predator	0	0.1	N/A	0.13	6.80	4.53
Fish	0	0.15	0.14	0.29	7.65	8.46
Copper (Cu)						
Shredder	0	0	N/A	0	0	0
C-G	0	0	N/A	0	0	0
Predator	0	0	N/A	0	0	0.10
Fish	0	0	0	0	0.16	0.07
Arsenic (As)						
Shredder	0	0	N/A	0.16	1.32	0.85
C-G	0	0	N/A	0	1.06	0.85
Predator	0	0	N/A	0.34	2.04	1.14
Fish	0	0	0	0	2.24	0.83

C-G = Collector—Gatherers

In September, at the control site and the above site, almost no sign of bioaccumulation in the macroinvertebrates tissue of heavy metals was present at the control and above sites (Table 6). However, at the below site there were significant accumulations of heavy metals across all trophic levels especially for nickel, iron, and chromium. The levels of metal concentration did not biomagnify in higher trophic levels. The heavy metal analysis showed that the control site had only very minimal accumulation of heavy metals. The results for the new above site indicated heavy metals were present. The metals it showed were nickel, iron, and chromium (Table 6). These same metals also appeared at the below site. These are indeed the same metals that have shown significant quantities of contamination and accumulation at the below site throughout the project. The quantities were less than the below site, but were much higher than the control.

Figure 5. *Chromium concentrations for the sampling period of carnivorous and herbivorous macroinvertebrates and insectivorous fish at the below site*

Chromium concentrations were consistent over the sampling period. The highest concentrations observed were in May. Concentrations were consistent June through November.

<u>Table 6</u>. *Analysis of heavy metals from the representative functional feeding groups of macroinvertebrates in the control site (Shawnee Run) and the Above/Below sites from the impacted tributary stream for September and November, 2001. Heavy metal units are expressed in ppm (parts per million). Some sites did not record and were labeled N/A (not available).*

Metal	**Control**		**Above**		**Below**	
	September	**November**	**September**	**November**	**September**	**November**
Lead (Pb)						
Shredder	0	0	0	0	0	0
C-F	0	0	0.13	0.54	2.34	3.78
Predator	0	0	0	0.38	1.17	2.31
Fish	0	0	0.13	0.76	0.98	1.54
Iron (Fe)						
Shredder	0	0	0.14	0	1.36	0
C-F	0	0	0	3.02	3.71	3.65
Predator	0	0	0	1.04	2.65	3.67
Fish	0	0	0.14	2.29	4.25	6.03
Chromium (Cr)						
Shredder	0	0	0.11	0	4.23	0
C-F	0	0	0	4.87	8.20	10.35
Predator	0	0	0.14	3.54	9.41	11.27
Fish	0	0	0.4	5.26	8.0	6.57
Nickel (Ni)						
Shredder	0.29	0.20	0	0.23	6.54	0.21
C-F	0	0.21	0	6.51	9.01	11.20
Predator	0	0.23	0.22	1.08	2.24	6.28
Fish	0	0.20	0	4.24	6.47	8.46
Copper (Cu)						
Shredder	0	0.05	0	0.23	0	0
C-F	0	0.25	0	0.23	0	0.23
Predator	0	0.28	0	0.28	0	0.23
Fish	0	0.38	0	0.33	0.11	0.33
Arsenic (As)						
Shredder	0	0	0	0	0.54	0
C-F	0	0	0	0	1.20	1.40
Predator	0	0	0	0	0	0
Fish	0	0	0	0	0.11	0.83

C-F = Collector—Filterers

The highest levels of heavy metals were found in the months of July and November which were when the above site had dried up (Figure 3, 4, 5). A trend of increasing heavy metal concentration during the sample period occurred.

Table 7. Concentration of Iron in Water Samples, July – November, 2001

Iron			
Month	**Control**	**Above**	**Below**
July	0	0	0.23
August	0	0.05	0.09
September	0	0.06	0.12
November	0	0.11	0.21

The amount of iron in the water is shown in Table 7. There is no iron present at the control site and significant at the above site only in November. Each month there is a significant amount at the below site.

Table 8. Concentration of Nickel in Water Samples, July – November, 2001

Nickel			
Month	**Control**	**Above**	**Below**
July	0	0	0.21
August	0	0.02	0.11
September	0	0.07	0.13
November	0	0.09	0.57

The amount of nickel in the water is shown in Table 8. There is no nickel present at the control site and only in November does it appear at the above site. Each month nickel is present in the water at the below site.

Table 9. Concentration of Chromium in Water Samples, July – November, 2001

Chromium			
Month	**Control**	**Above**	**Below**
July	0	0	0.2
August	0	0	0.09
September	0	0.03	0.29
November	0	0.16	0.33

The amount of chromium in the water is shown in Table 9. There is no chromium present at the control site and only in November does it appear at the above site. Each month, chromium is present in the water at the below site.

Discussion

Throughout the sampling period, excluding November, water temperature at the below site was greater compared to the other sites. This increase in temperature at the below site may either be attributed to the outflow from the ANVIL lagoon or from some other contribution via over land flow or groundwater upwelling. Temperature has been indicated to act as a temporal or spatial isolating agent for some species of stone flies (Lillehammer, 1975). This may have affected the diversity of the macroinvertebrates in these streams.

Each month stream discharge was greater at the below site than the above site, possibly indicating additional ground water seepage or significant discharge from the retention pond upstream. The stream discharge from the below site is undeterminable without taking groundwater measurements. The increased flow had several implications on the Shawnee and its unnamed tributary. Increased flow should show a more heavily populated and diverse waterway than one with less discharge (Dunne, 1995). Although the below site had a larger discharge than the above site, macroinvertebrate abundance was lower downstream compared to the other sites. We found that macroinvertebrate diversity samples after August in the above site were greater than the below site even though discharge was less above stream. For most months, the macroinvertebrate diversity and abundance was greatest at the control site. This site had greater water flow then either the above or below sites. A normal diversity distribution indicates a clean stream showing no signs of interference with the normal amount of biota (Cleveland, 1998). The below site was dominated by Shredders (herbivores) in May and June. Throughout the rest of the study period collector-gatherers and predators, functional feeding groups, dominated the populations collected. The control site reflected a FFG diversity of a normal stream and 2 systems with a riparian buffer and was a good basis with which to compare the above and below sites.

The heavy metal concentration was considerably higher in the below site than the above site. In all months, except November, there was little to no bioaccumulation of heavy metals at either the control or the above sites at any trophic level. In November, when the above site was changed to an area much closer to a secondary impact point from ANVIL, the heavy metal concentration was much higher than the control site across all trophic levels. The same three metals (nickel, iron and chromium) showed the highest concentrations each month. The concentration of nickel increased throughout the study, reaching a high point in November. Iron concentrations were at the highest in July and were consistent over the period excluding July. Chromium was at its highest point in May and was consistent over the rest of the period. There was no sign of biomagnification of heavy metal contaminants, since the concentrations did not significantly increase in higher functional feeding groups. In similar studies of another stream biomagnification had occurred, and although the heavy metals appear to be traveling up trophic levels, a large increase in their concentrations is not occurring (Shaffer and Ratte, 1998). Because a considerable amount of the discharge at the below site was presumably due to the outflow from ANVIL, possibly the heavy metal contaminants may be coming from the ANVIL lagoon. As the amount of water coming from the above site diminished, the bioaccumulation of heavy metals at the below site increased proportionally. Water flowing from above the discharge pipe could have been acting as a diluting agent. Excluding chromium in May, in both months when the above section had dried up, July and November, the heavy metal concentrations across all trophic levels reached their highest points. The source of the heavy metal contaminants lies at or near the discharge pipe, and if it were released at a constant rate, the increased above site's discharge would dilute its downstream concentration.

The water had small amounts of the three heavy metals, nickel, iron, and chromium each month at the below site. None was present at the control site and a significant amount appeared only in November at the above site. The same metals that were accumulating in the macroinvertebrates at the below site were also present in the water. EPA regulations for water quality allow the amounts of the heavy metals that were determined to be in the water. Although the water may not be the only contributor of the heavy metals, they are present after the discharge pipe.

In conclusion, UNSTR, which is diverted to the property line of ANVIL, may have been compromised. It appears that pollutants may be entering the stream in the area surrounding the discharge pipe. The pollutants found have been determined to be the heavy metals (chromium, iron, nickel, and to a lesser extent lead). The preliminary data presented is an indicator of the tributary's properties, but a secondary professional endorsement of the values must be made to corroborate the data presented. Core substrate samples would give a better picture of the full effects of this accumulation of heavy metal sin the surrounding soils.

Appendices

Appendix A. Macroinvertebrate Data for May

1. Species List
2. Functional Feeding Group Population Percentages

Appendix B. Macroinvertebrate Data for June

1. Species List
2. Functional Feeding Group Population Percentages

Appendix C. Macroinvertebrate Data for July

1. Species List
2. Functional Feeding Group Population Percentages

Appendix D. Macroinvertebrate Data for August

1. Species List
2. Functional Feeding Group Population Percentages

Appendix E. Macroinvertebrate Data for September

1. Species List
2. Functional Feeding Group Population Percentages

Appendix F. Macroinvertebrate Data for November

1. Species List
2. Functional Feeding Group Population Percentages

Appendix A

1. List of macroinvertebrates and their corresponding common and functional feeding group names for May, 2001.

Site	Family Name	Common Name	Functional Feeding Group
Control	Gammaridae	Scuds	Shredder
	Oligochaete	Aquatic Worms	Shredder
	Asellidae	Aquatic Pill Bugs	Shredder
	Ephemeridae	Mayfly	Collector-Gatherer
	Hydropsychidae	Caddisfly	Collector-Filterer
	Chironomidae	Midge	Collector-Gatherer
	Assorted Fish		Predator
Above	Gammaridae	Scuds	Shredder
	Physidae	Snail	Scraper
	Capniidae	Stonefly	Shredder
	Simuliidae	Black Fly	Collector-Filterer
	Dytiscidae	Diving Beetle	Predator
Below	Amphipoda	Scuds	Shredder
	Oligochaete	Aquatic Worms	Shredder
	Tipulidae	Crane Fly	Shredder
	Cambaridae	Crayfish	Shredder
	Chironomidae	Midge	Collector-Gatherer
	Hydropsychiade	Caddisfly	Collector-Filterer
	Coenagrionidae	Damselfly	Predator
	Dytiscidae	Diving Beetle	Predator
	Assorted Fish		Predator

2. Macroinvertebrate population distribution for May.

Appendix B

1. List of macroinvertebrates and their corresponding common and functional feeding group names for June, 2001.

Site	Family Name	Common Name	Functional Feeding Group
Control	Gammaridae	Scuds	Shredder
	Oligochaete	Aquatic Worms	Shredder
	Asellidae	Aquatic Pill Bugs	Shredder
	Ephemerellidae	Mayfly	Collector-Gatherer
	Chironomidae	Midge	Collector-Gatherer
	Gerridae	Water Strider	Scavenger/Predator
	Calopterygidae	Damselfly	Predator
	Assorted Fish		Predator
Above	Tipulidae	Crane Fly	Shredder
	Physidae	Snail	Scraper
	Gerridae	Water Strider	Scavenger/Predator
	Dytiscidae	Diving Beetle	Predator
	Assorted Fish		Predator
Below	Amphipoda	Scuds	Shredder
	Tipulidae	Crane Fly	Shredder
	Cambaridae	Crayfish	Shredder
	Chironomidae	Midge	Collector-Gatherer
	Coenagrionidae	Damselfly	Predator
	Assorted Fish		Predator

2. Macroinvertebrate population distribution for June.

Appendix C

1. List of macroinvertebrates and their corresponding common and functional feeding group names for July, 2001.

Site	Family Name	Common Name	Functional Feeding Group
Control	Oligochaete	Aquatic Worms	Shredder
	Asellidae	Aquatic Pill Bugs	Shredder
	Hydropsychidae	Caddisfly	Collector-Filterer
	Elmidae	Beetle	Scraper
	Chironomidae	Midge	Collector-Gatherer
	Calopterygidae	Damselfly	Predator
	Assorted Fish		Predator
Above	* No invertebrate samples were collected because streambed was dry.		
	Assorted Fish		Predator
Below	Oligochaete	Aquatic Worms	Shredder
	Asellidae	Aquatic Pill Bugs	Shredder
	Chironomidae	Midge	Collector-Gatherer
	Coenagrionidae	Damselfly	Predator
	Assorted Fish		Predator

2. Macroinvertebrate population distribution for July.

Appendix D

1. List of macroinvertebrates and their corresponding common and functional feeding group names for August, 2001.

Site	Family Name	Common Name	Functional Feeding Group
Control	Gammaridae	Scuds	Shredder
	Oligochaete	Aquatic Worms	Shredder
	Tipulidae	Crane Fly Larva	Shredder
	Asellidae	Aquatic Pill Bugs	Shredder
	Hirudinea	Leeches	Plant Piercer
	Elmidae	Beetle	Scraper
	Chrionomidae	Midge	Collector-Gatherer
	Gerridae	Water Strider	Scavenger/Predator
	Calopterygidae	Damselfly	Predator
	Coenagrionidae	Damselfly	Predator
	Dytiscidae	Diving Beetle	Predator
	Assorted Fish		Predator
Above	Amphipoda	Scuds	Shredder
	Cambaridae	Crayfish	Shredder
	Physidae	Snail	Scraper
	Hydrophilidae	Beetles	Collector-Gatherer
	Chironomidae	Midge	Collector-Gatherer
	Gerridae	Water Strider	Scavenger/Predator
	Dytiscidae	Diving Beetle	Predator
	Salamanders		Predator
	Assorted Fish		Predator
Below	Amphipoda	Scuds	Shredder
	Cambaridae	Crayfish	Omnivore
	Elmidae	Beetle	Scraper
	Chironomidae	Midge	Collector-Gatherer
	Hydropsychiade	Caddisfly	Collector-Filterer
	Calopterygidae	Damselfly	Predator
	Coenagrionidae	Damselfly	Predator
	Assorted Fish		Predator

2. Macroinvertebrate population distribution for August.

Appendix E

1. List of macroinvertebrates and their corresponding common and functional feeding group names for September, 2001.

Site	Family Name	Common Name	Functional Feeding Group
Control	Gammaridae	Scuds	Shredder
	Oligochaete	Aquatic Worms	Shredder
	Ephemerellidae	Mayfly	Collector-Gatherer
	Chironomidae	Midge	Collector-Gatherer
	Gerridae	Water Strider	Scavenger/Predator
	Calopterygidae	Damselfly	Predator
	Assorted Fish		Predator
Above	Tipulidae	Crane Fly	Shredder
	Physidae	Snail	Scraper
	Gerridae	Water Strider	Scavenger/Predator
	Dytiscidae	Diving Beetle	Predator
	Assorted Fish		Predator
Below	Cambaridae	Crayfish	Shredder
	Chironomidae	Midge	Collector-Gatherer
	Coenagrionidae	Damselfly	Predator
	Assorted Fish		Predator

2. Macroinvertebrate population distribution for September.

Appendix F

1. List of macroinvertebrates and their corresponding common and functional feeding group names for November, 2001.

Site	Family Name	Common Name	Functional Feeding Group
Control	Oligochaete	Aquatic Worms	Shredder
	Elmidae	Beetle	Scraper
	Chironomidae	Midge	Collector-Gatherer
	Calopterygidae	Damselfly	Predator
	Assorted Fish		Predator
Above (New)	Oligochaete	Aquatic Worms	Shredder
	Chironomidae	Midge	Collector-Gatherer
	Ephemerellidae	Mayfly	Collector-Gatherer
	Coenagrionidae	Damselfly	Predator
	Assorted Fish		Predator
Below	Tipulidae	Crane Fly	Shredder
	Chironomidae	Midge	Collector-Gatherer
	Coenagrionidae	Damselfly	Predator
	Assorted Fish		Predator

2. Macroinvertebrate population distribution for November.

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