

Ecological Health Assessment of Laurel Fork  
through Water Conditions, Metals Analysis, and Fish Integrity

Ashby Gale

Environmental Toxicology BIO3542-101/5542-101

February-May 2012

In collaboration with:

Caroline Crouse, Michael Anderson, Anthony Mayen

Donna Lisenby, Watauga Riverkeeper

Shea Tuberty, ASU Dept. of Biology

I have not violated the Appalachian State University Academic Integrity Code.

---

## ABSTRACT

Streams and rivers serve as important ecosystems supporting a wide array of aquatic organisms and creating niches for plants, trees, and land animals. Disruption of these ecosystems can have a cascading effect, starting at the microbial level. Factors such as turbidity, pH, water conductivity, and metal concentration in the water and sediments all can impact the diversity of fish, benthic macroinvertebrates, and neighboring plant species. This study monitored the health of Laurel Fork running along HWY 105 south from Boone. Methods involved analyzing metal concentrations in sediment and water on an Inductively Coupled Plasma spectrometer, chlorine, nitrate, and phosphate levels measured on an Ion Chromatograph, fish shocked for an Index of Biotic Integrity, water conditions collected off a portable meter, and water tested for Coliform cultures. The fish IBI returned a value of acceptable species diversity, but with a lower than normal abundance value for each species. Metal concentrations in water support this finding with silver concentrations of around 0.3 parts per billion, nearly five times higher than the set NC standard. Such high levels have been shown to impair juvenile trout, and quicken the hatch rate before fry reach the normal stage for hatching.

## INTRODUCTION

Monitoring the health of a stream is important for both the organisms living in the water, and the humans that interact with the system, whether indirectly or directly. Techniques used for quantifying a stream's health include visual surveys, water quality indices, biological inventories, and flow rate surveys. Current work being done in the field of water quality assessment is focused on release of endocrine disruptors from municipal waters, groundwater contamination from hydraulic fracturing, and recycling rainwater before it reaches creeks and

rivers. Other studies may assess a waterway in the aftermath of a toxic spill, such as research done in Kingston, TN after a catastrophic coal ash spill from the TVA coal fired power plant in 2008. Research of the catfish in the area of the spill showed significant concentrations of selenium at levels high enough in the ovaries and testes to cause reproductive failure. Other metal concentrations in the area such as lead and arsenic occurred beneath the toxic threshold levels, but still high enough in catfish for concern when considering bioaccumulation (Tuberty et al., 2008).

Turbidity is another factor of concern in waterways, affecting the livelihood of fish and benthic macroinvertebrates. One study published in the Water Quality Research Journal of Canada reviewed the effectiveness of riparian buffers in reducing water turbidity from agriculture as well as removing nitrates from runoff. The researchers found up to a 99% reduction in groundwater nitrate levels with a 25 meter buffer, and up to a 90% reduction in surface water turbidity from wood lot buffers (Hickey et al., 2004). Another study performed in England has monitored eutrophication effects of sewage discharge from a wastewater treatment plant, along with the proliferation of *C. riparius* from conditions high in nitrates and phosphates. Tests displayed nitrate levels two to three times higher than those found naturally occurring, leading to eutrophic conditions—those not suited for native fish species (Gower et al., 1978). Through the results of these three studies, it is clear that water quality assessments can cover a wide range of issues that waterways face.

The purpose of this study was to determine the overall health of Laurel Fork through assessment of metal concentrations, chloride, nitrate, phosphate and sulfate levels, *E. coli* occurrence from sewage effluent, water turbidity, pH, conductivity, and a fish Index of Biotic Integrity (IBI) survey. The initial prompt for this experiment came from a series of public

complaints about increased water turbidity in Laurel Fork, the stream turning a milky white at times. The source of this problem was traced to a discharge pipe from the Vulcan Materials Company rock quarry; the drainage pipe led from a sediment pond on site where rock dust was collected and stored until the pond was emptied. After catching the public's eye, Watauga Riverkeeper Donna Lisenby started to take action on the matter. Initial concern about increased turbidity in Laurel Fork made me hypothesize that due to increased suspended sediment levels, adverse effects on the aquatic life in the stream could include decreased biodiversity, increased dissolved metal concentrations, and other unknown detrimental effects.

## METHODS

Research on Laurel Fork began in March 2012 with initial water samples collected for Ion Chromatograph (IC) analysis. All water samples were obtained as grab samples 0 – 12 inches beneath the surface of Laurel Fork and the Watauga River. Nonreactive Nalgene I-Chem jars were used for water collection. All water for IC analysis was treated with 1% concentration of nitric acid and refrigerated until tested. Water collected for Inductively Coupled Plasma (ICP) spectrometer readings in April was stored in the I-Chem jars and refrigerated until processed for metals analysis. Sediments for metal concentrations were collected in April, stored in nonreactive plastic sediment bags, and dried for analysis by a Labconco Freezone 4.5 freeze-drying apparatus.

Data analyzed further in the lab setting included ICP analysis of sediment and water, and IC analysis of water. After freeze-drying sediments to remove all water, triplicate 0.5 gram samples were weighed for each site, and processed in a CEM Mars 230/60 digester. Water samples were measured to 10 mL volumes, added to 40 mL of 100% Omnitrac nitric acid, and

digested in the same cycle as sediments. After digestion, triplicates of each sampling site were loaded into IC syringe filters and hand injected into the Ion Chromatograph machine. Once results were stored on computers connected to the IC and ICP, data was processed and reduced in Microsoft Excel and Google Document spreadsheets.

Coliform water samples were collected and processed in accordance with 3M Petrifilm<sup>TM</sup> procedure (3M, 2006). One milliliter of tested water was placed in the center of each Petrifilm, and incubated at 37 °C for 72 hours. Water conditions (turbidity, pH, conductivity) obtained during collection of IC and ICP samples were measured on a DRT 15CE Turbidimeter-Portable pH/ISE/Conductivity/DO meter; all values recorded from 0 – 12 inches beneath surface water levels. All fish collected were shocked with a Smith-Roof Inc. LR24 Electrofisher set to generate pulses of optimal shocks based on the current water conditions. One person ran the Electrofisher while three others netted fish on the left, right, and center of the shocking apparatus; on occasion and where feasible, two other volunteers obstructed the downstream path with two kick-seines. Fish were measured in the field with wet measuring boards and portable scales; all weights recorded in the results section are wet weights. Fish biodiversity values were calculated as described by Raytheon Employees Wildlife Habitat Committee (REWHC 2000). From these calculated values, an overall Index of Biotic Integrity (IBI) value was calculated following the North Carolina Department of Environment and Natural Resources (NCDENR) Standard Operating Procedure for Biological Monitoring (NCDENR 2001).

## RESULTS

General water conditions for pH, conductivity, and turbidity are listed in Table 1, and ordered from the furthest upstream of Vulcan, to the confluence of Laurel Fork and the Watauga River. Conductivity values ranged from 44.4  $\mu\text{S}/\text{cm}$  at the confluence of Laurel Fork and the Watauga River, to 530  $\mu\text{S}/\text{cm}$  released from the apartment container sewage treatment system. There is a degree of uncertainty in the pH values obtained from erratic calibration of the water meter; these pH values ranged from 8.8 down to 6.5. Turbidity values in the first column were obtained on May 3, while the second column indicates sampling on March 29, only collecting data from four sites. The overall range of turbidity was from 2.8 Nephelometric Turbidity Units (NTUs) in the Watauga River, to 1224 NTUs from the Vulcan discharge pipe.

**Table 1. Laurel Fork Water Quality Measurements**

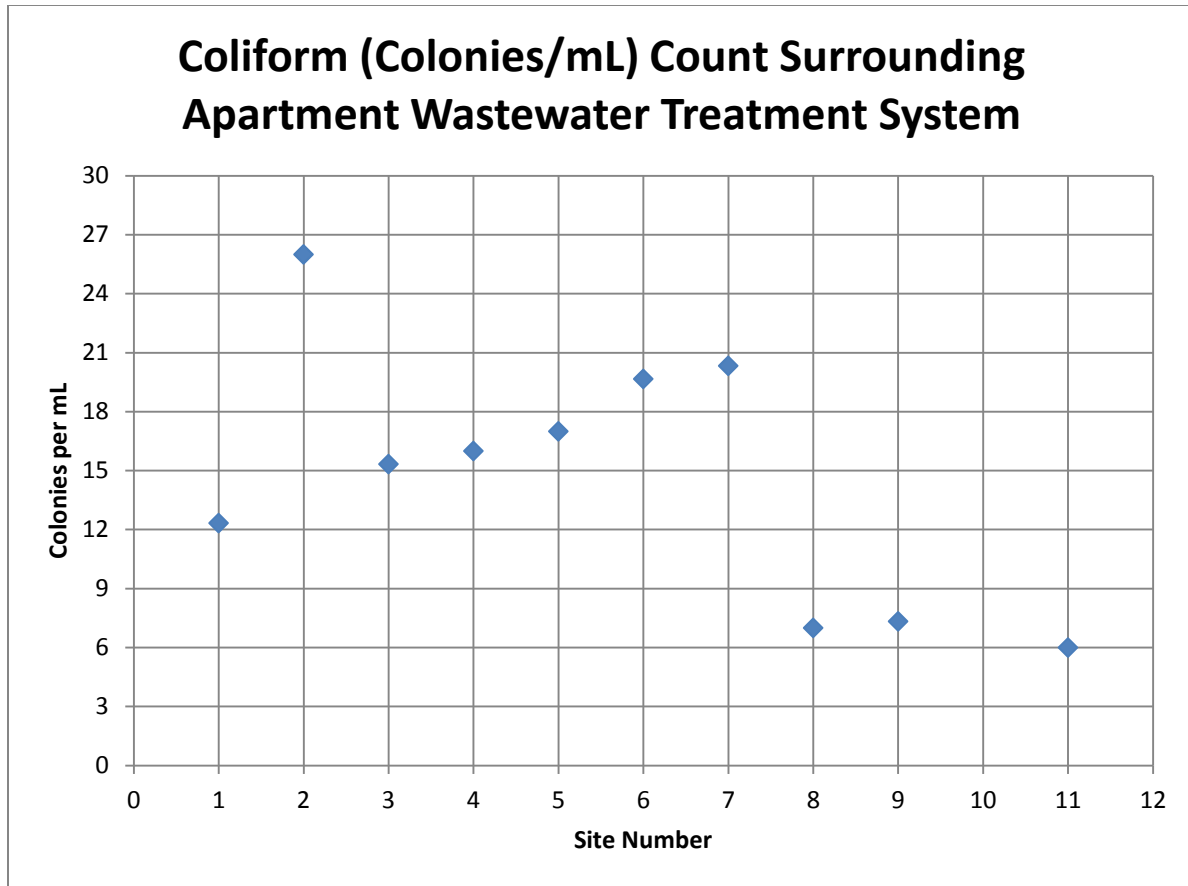
Site Name	Conductivity ( $\mu\text{S}/\text{cm}$ )	pH	Turbidity NTU (5/3)	Turbidity NTU (3/29)
Upstream of Vulcan Pipe	101	8.8	7.8	34
Vulcan Drainage Pipe	109	7.9	22	1224
20' Below Vulcan Confluence	102	7.9	9	50
Downstream of Vulcan	100	7.9	8	48
Apartment Upstream	104.1	7.01	NA	NA
Apartment Effluent	530	6.85	NA	NA
Apartment Downstream	104.8	7.1	8.8	NA
RV Park Flintlock Campground	101.2	6.67	7.7	NA
Upstream Baird's	101.3	6.75	8.9	NA
Downstream Baird's	88	6.66	8.4	NA
Old Danner Rd.	86.2	6.63	6.2	NA
HS: Upper Watauga	44.4	5.92	NA	NA
HS: Confluence	45.7	6.5	NA	NA

Coliform and *E. coli* data are shown in Table 2 and Figure 1; values are organized from the farthest collection point upstream, to the last collection point downstream. Site numbers in

Figure 1 reference the corresponding numbers in Table 2. The final value at the bottom of Table 2 indicates a water sample with a dilution factor of 1:100 as a safeguard for exceedance that would yield tightly clustered colonies. Note the sustained elevated levels of Coliform after the Apartment effluent empties into Laurel Fork as evident in Figure 1. Coliform levels range from 7 colonies per mL at the Ham Shoppe (HS) Confluence of Laurel Fork and the Watauga River, to 26 colonies per mL directly from the discharge pipe of the container sewage treatment system. *E. coli* levels did not show as clear results with steady concentrations around 0.33 to 2 colonies per mL of tested water.

**Table 2. *E. coli* and Coliform Colony Concentrations**

<b>Site Name &amp; Number</b>	<b>Coliform Colonies/mL</b>	<b>E. Coli Colonies/mL</b>
(1)Apt. Upstream	12.33	1
(2)Apt. Effluent	26	0.33
(3)Apt. Downstream	15.33	0.33
(4)RV Park	16	2
(5)Upstream Baird's	17	1.33
(6)Downstream Baird's	19.67	0.67
(7)Old Danner Rd.	20.33	0.67
(8)HS: Confluence	7	0
(9)HS: Upper Watauga	7.33	0
(10)Apt. Effluent (1:100 dilution)	6	0



**Figure 1. Colony per mL Count of Coliform Levels near Apartment Wastewater Treatment System**

Fish Indices of Biotic Integrity returned values of 28 IBI upstream, 26 IBI downstream, and an overall IBI rating of 34 for the total 1400 ft expanse of Laurel Fork shocked for fish. The Upstream category covers the section of Laurel Fork the runs from the Vulcan Effluent pipe down to the Apartment Effluent Pipe. The Downstream section is a three hundred foot stretch immediately after the Old Danner Rd sampling site.

**Table 3. Condensed Fish IBI Values**

<b>Location Shocked</b>	<b>IBI Values</b>
Upstream	28
Downstream	26
Total	34



Ion Chromatography data returned values significant to the apartment wastewater treatment system and aquatic life downstream, as displayed in Table 4. All values shown have a degree of uncertainty resulting from delayed turnaround time in processing. Apartment Effluent values are bold to signify point-source discharge, sampled directly from the treatment's output pipe. Laurel Fork chloride levels range from 34 ppm to 1.5 ppm, sulfates range from 10.1 ppm to 2.8 ppm, phosphate only returned the effluent value, and nitrate levels range from 9.7 ppm to 1.5 ppm and below detection limit (BDL). Secondary peaks can be noted from the Vulcan Discharge Pipe.

**Table 4. Ion Chromatography Results listed in ppm**

<b>Sample Name</b>	<b>Chloride</b>	<b>Sulfate</b>	<b>Phosphate</b>	<b>Nitrate</b>
Upstream of Vulcan	33.9993	6.0187	BDL	5.1503
Vulcan Drainage Pipe	32.4144	10.1287	BDL	9.7335
20' Below Vulcan Confluence	30.1075	5.7184	BDL	4.8853
Downstream of Vulcan	29.7048	5.7344	BDL	4.1386
Apartment Upstream	23.1719	6.7145	BDL	3.978
<b>Apartment Effluent</b>	<b>47.073</b>	<b>68.0281</b>	<b>11.5832</b>	<b>62.242</b>
Apartment Downstream	19.3435	6.4131	BDL	3.6285
RV Park Flintlock Campground	18.6079	6.4641	BDL	3.512
Upstream Baird's	17.8257	5.9993	BDL	3.3731
Downstream Baird's	12.1274	7.4951	BDL	2.5934
Old Danner Rd.	1.5472	2.767	BDL	1.5372
HS: Upper Watauga	5.8389	5.6058	BDL	BDL
<b>Action Level</b>	<b>230</b>	<b>NA</b>	<b>0.1</b>	<b>3.1</b>

Table 5 displays results from sediment metals analysis, while Table 6 shows metals concentrations (ppm) in the water. Sediments were run as triplicates from each site, with standard deviations shown beside metal averages. Bolded values in each average column indicate sites with the highest levels of each metal, comparable to the maximum levels determined by the state of North Carolina. Zinc values returned especially high from the Vulcan Discharge Pipe, nearing around half of the allowable sediment concentration. Table 6 values reflect metals concentration (ppb) in Laurel Fork surface water; silver concentrations are bolded showing exceedance of state standards for both surface and trout waters by a factor of five and two, respectively. Zinc data was excluded from water analysis due to an incorrect standard curve from which to base results. All other metal levels are well below the state levels, with cadmium at the next closest level to cause concern—one order of magnitude beneath the standard.

## DISCUSSION

Through the overall water quality and conductivity of Laurel Fork, it was found that as the distance from the town of Boone increased, water conductivity decreased. A supporting theory could be that free ions in solution are binding to available elements and sediments, decreasing the salinity of the water; even the apartment effluent did not raise the conductivity in any measurable amount. However, while conductivity is decreasing, pH is decreasing and becoming more acidic. This is interpreted as an accumulation of all compounds and oils off of the roadway magnifying as the water progresses. Most any stream followed from its origination to successive confluences will note an increase in xenochemical or foreign substance concentrations.

Turbidity results also display an interesting point for concern—rainy days, or previous rain events show a dramatic increase in the turbidity of Laurel Fork, especially water collected from Vulcan's drainage pipe. As evident in the two different turbidity collections, higher values were collected two days after a rain event; moreover, qualitatively, the pipe discharge did not appear to be as turbid as previous reports from 2005. Yet, values retrieved from this study showed Laurel Fork turbidity over NC trout water standards by a factor of 3 to 120—two days after a medium sized rainfall. Based on these results, we are recommending more research be done specifically on turbidity, with more data to show levels during rain events.

NC Administrative Codes also address the acceptable levels of fecal Coliform as a geometric mean of 200count/100mL samples; given this standard, our results returned the highest values of 2000count/100mL in regular Coliform loads. Additionally, *E. coli* counts showed insignificant levels, especially with the treated sewage effluent emptying straight into

Laurel Fork. It would still be advised that additional monitoring is necessary, especially during peak periods of usage during football weekends and other holiday gatherings.

Ion Chromatography data suggest that chloride, nitrate, phosphate, and sulfate levels are all highest near the town of Boone, with the exception of the apartment effluent. Our research suggests that potential causes for higher levels near Boone result from fertilizer and pesticide use, applications of road salt during the winter season, and general proximity to constant human activity. Laurel Fork is additionally fed by Bairds Creek, which receives runoff from a golf course and gated community upstream; this is a likely contributor to nitrate and phosphate levels in the summer months of application, explaining the lower levels during the spring period of data collection. Qualitative analysis of the water conditions beneath the apartment effluent pipe supported the possibility of nitrification and eutrophication, by excess amounts of algae and anaerobic tolerant microbes spotted in a stagnant pool where the discharge is released.

The fish integrity testing (IBI) was perhaps one of the most surprising data yielding tests performed for the overall study. IBI diversity values showed acceptable levels for mountain streams, especially in the daylighted section labeled Downstream. However, the abundance values for each species were incredibly low. Standard protocol as described by NCDENR directed that our team of three researchers netting and one researcher shocking should have been more than adequate to return the necessary amount of fish from the stream; NCDENR standards dictate that one netter and one shocker are sufficient. Even accounting for visible fish that escaped our team of netters and those that were not observed, but implied by the numbers yielded in the nets, our abundance values were still too low. It was not until processing the metals concentration data that our team discovered the cause for decreased fish abundance.

Metals concentrations in sediment showed no real cause for concern until zinc levels were processed; Vulcan Drainage Pipe levels were over half of the advised state level. Possible sources of zinc include galvanized metals, roofing, and farm equipment. These levels should be studied further with benthic macroinvertebrates and bioaccumulation surveys done in these organisms, along with the fish consuming them. The levels may reach high enough in trout to show additional harm to the fish.

However, the main cause was not the high levels of zinc in sediment, but rather, metal concentrations in the main body of water. Silver levels showed a fivefold increase of the allowable maximum level in NC surface waters, and a twofold increase of the limit set to protect trout hatching and development. In 1978, an US EPA study measured the affects of silver and the 96-h LC<sub>50</sub> in rainbow trout. It was shown that silver concentrations of 0.17 µg/L or greater caused premature hatching of eggs, and reduced fry growth rates. Eggs exposed to silver were completely hatched within 10 days of silver exposure, whereas the control eggs were completely hatched after a normal 42 day cycle (Davies et al., 1978). Additionally, the fry that hatched prematurely were not fully developed and often died soon after hatching. Dr. Abbott, a mineralogy professor at Appalachian State University stated that a potential source for silver in the area may be a mineral called sphalerite; this mineral is present in the Grandfather Mountain Formation, the very same formation that Vulcan is quarrying for material.

## CONCLUSION

This study originally set out to “catch” the Vulcan Materials Company for releasing sediments in excess of the NC trout water turbidity standards. From the initial reports back in 2005, Vulcan has definitely decreased the rate at which they release sediment from the property

into Laurel Fork. Photo and eyewitness evidence can attest to the once white, milky body of water that Laurel Fork would become after Vulcan released sediments. With this set of data, we feel confident enough about its validity to claim that at one point in time, Vulcan Materials Company did have a negative impact on the ecosystem of Laurel Fork. From this point forward, it will be important to revisit the site and have adequate follow through on data collection and analysis to monitor whether the health of Laurel Fork is increasing, decreasing, or remaining the same. Multiple studies are necessary to become better acquainted with the dynamics of Laurel Fork, along with collaboration in the public sector and increasing public awareness of this issue. Those who enjoy the Laurel Fork include trout fisherman, residents along the stream, seasonal campers at the RV Park, outdoor enthusiasts, and other ecologically minded individuals. The health of this stream should be a priority in the minds of all Watauga county residents, especially taking into consideration those who live downstream from Boone.

## REFERENCES

- [3M] 3M Petrifilm Environmental Listeria Plate. 2006. 3M Materials Page, <[http://www.3m.com/intl/kr/microbiology/p\\_listeria/tech1.pdf](http://www.3m.com/intl/kr/microbiology/p_listeria/tech1.pdf)>. Accessed 2012 April 20.
- [NCDENR] North Carolina Department of Environment and Natural Resources. 2001. PDF Document, <<https://docs.google.com/viewer?>>. Accessed 2012 April 25.
- [REWHC] Raytheon Employees Wildlife Habitat Committee Biodiversity Measures. 2000. REWHC Biodiversity Page, <<http://rewhc.org/biomeasures.shtml>>. Accessed 2012 May 6.
- Davies, P.H., Goettl, J.P. Sr., and Sinley, J.R. (1978) Toxicity of silver to rainbow trout (*Salmo gairdneri*). p. 113-117.
- Gower, A.M., and Buckland, P.J. (1978) Water quality and the occurrence of *Chironomus riparius* Meigen (Diptera: Chironomidae) in a stream receiving sewage effluent. p. 153-164.
- Hickey, B.C., and Doran, B. (2004) A Review of the Efficiency of Buffer Strips for the Maintenance and Enhancement of Riparian Ecosystems. p. 311-317.
- Tuberty, S.R., Babyak, C., Carmichael, S., George, A., Lisenby, D., Jackson, D., and Sakamach, Y. (2008) Effects from the Catastrophic Rupture of a Coal Fly Ash Settling Pond in Kingston, TN. Poster.