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Poster Session

Kentucky Water Resources Research Institute, University of Kentucky

THE NEW KENTUCKY NITROGEN AND PHOSPHORUS RISK ASSESSMENT TOOL TO PROTECT WATER QUALITY

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ABSTRACT

Agricultural systems require nitrogen (N) inputs to maximize yields and economic returns for farmers, but when N is applied at higher rates than necessary, there is potential for increased N and phosphorus (P) losses that can negatively impact groundwater quality (N), air quality (N), and surface water quality (N and P). New tools are needed that can be used by nutrient managers and conservationists to quickly assess the risk of N and P losses and determine alternative management practices that could reduce off-site losses of these nutrients. A new N and P Index for Kentucky was developed to enable quick assessments of the effects of management practices on the risk of N and P losses. The N Index component of this tool has been compared with experimental field data and been shown to estimate the effects of management practices on N loss pathways ($P < 0.001$). Nitrate-nitrogen (NO_3-N) leaching losses estimated by the tool correlate with measured NO_3-N leaching values ($P < 0.001$). Results for the P Index component of the tool suggest that its estimations of P loss risk correlate with measured risk values.

Tools like this are of key importance. A study released in September 2011 by the USDA found that only about a third of U.S. cropland is applying all of three best management practices (BMPs) for N in terms of application rate, time, and method, and that it costs billions of dollars annually to remove nitrate from drinking water (http://www.ers.usda.gov/Publications/ERR127/). To help reduce negative impacts to the environment the new National 590 Nutrient Management Standard was released in January 2013. The standard requires the development of state specific N risk assessment tools if N leaching and runoff from agricultural land presents a resource concern in the state. It also gives instructions for updating existing P risk assessment tools, focusing on the transport factor of P entering surface waters from crop fields.

Tools like the Kentucky N and P tool will help implement conservation on the ground to minimize environmental impacts from nutrient losses. The Kentucky N and P Index for laptop and desktop computers can be downloaded from the USDA-ARS-SPNR webpage at http://www.ars.usda.gov/Services/docs.htm?docid=20334. Additionally, the

N Index component of the tool is already available in the mobile application, and the P Index component will be released in the near future. This new tool developed for Kentucky is a new, cutting-edge prototype that is being used by the USDA Natural Resources Conservation Service (NRCS) in Kentucky as a conservation planning tool to enhance efforts to reduce non-point source nutrient pollution in the state that is generated from animal manure and commercial fertilizer applications on crop fields.

GROUNDWATER PHOSPHORUS IN KENTUCKY RELATIVE TO KARST, GROUNDWATER SENSITIVITY, AND GROUNDWATER PHYSIOGRAPHIC REGION

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The Kentucky Division of Water is charged with protecting and enhancing the quality of its waters. As part of this effort, the Division monitors groundwater for a number of parameters, including phosphorus. Phosphorus naturally occurs in water, but high levels result in eutrophication. A better understanding of the relationship between phosphorus levels in groundwater and karst, groundwater sensitivity category, and groundwater physiographic region was sought.

Groundwater total phosphorus concentrations from 2003 through 2012 were used to examine these relationships. Nonparametric methods were used to evaluate the data. Data were analyzed using SAS® v. 9.3 to determine if significant differences existed based on the presence or absence of karst, groundwater sensitivity region, or groundwater physiographic region. Descriptive statistics were produced to summarize the three parameters. Tables were produced that include the median, minimum, maximum, number of samples, estimates of the interquartile range as determined by Kaplan Meier analysis, and the percent of the group that was censored. Because the data did not follow a normal distribution and were highly censored, the Wilcoxon rank sum test was used to determine if groups were significantly different. The analysis examined the groups as a whole, and then by pairs within each group.

The Wilcoxon Rank Sum test showed significant differences existed between groups for all three parameters tested.

Karst

For the comparison between karst pairs, significant differences existed when comparing the moderate karst regions with the well developed and null regions. No difference was found when comparing the well developed region with the null region.

Groundwater Sensitivity

Groundwater sensitivity category 1 was not included in the pair comparisons because of its small sample size. Significant differences were found between all sensitivity categories with the exception of the comparison between categories 2 and 4.

Groundwater Physiographic Regions

The Commonwealth has six groundwater physiographic regions. When comparing the Bluegrass region to the other 5 regions, significant differences were found with the Jackson Purchase, Mississippian Plateau, and the Ohio River Alluvium. Significant differences were found between the E. Coal Field and Jackson Purchase, Mississippian Plateau, and Ohio River Alluvium regions. The Jackson Purchase and W. Coal Field were significantly different, as were the Mississippian Plateau and W. Coal Field.

COMPARISON OF IRRIGATION SCHEDULING BASED ON DAILY WATER USE OR PLANT WATER DEMAND OF CONTAINER GROWN NURSERY PLANTS

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Introduction

Container nursery production depends on irrigation considering the small volume of growing media and its low water holding capacity. Irrigation water management is a key consideration in ornamental crop production and for reducing the impact of fertilizer and pesticide runoff on local water resources (Beeson et al., 2004). The nursery industry tends to schedule irrigation on time intervals rather than the available irrigation scheduling methods based on crop water use and/or environmental conditions. Irrigation scheduling based on daily water use (DWU) was reported as a better way to improve water use efficiency of nursery plants. Substrate moisture sensors were used to measure how much water was removed through evapo-transpiration in a day and to schedule irrigation cycles to meet plant water demand (Warsaw et al., 2009). A recently proposed plant demand-based irrigation system considers that photosynthetic rate is a sensitive indicator of the water status of the plant. It assumes that growth would not be compromised when an irrigation set point was used based on the substrate water content where photosynthesis begins to decline due to water stress. This demand-based irrigation system was developed under controlled environments using *Hibiscus rosa-sinensis* 'Cashmere Wind' (Fulcher et al., 2012). It was found that water use efficiency (WUE) could be improved using this system without reducing plant biomass and plant quality. The current study was designed to evaluate this demand-based irrigation scheduling system under outdoor nursery conditions and to compare it to its WUE with a daily water use-based irrigation system.

Methods

The study was conducted at the University of Kentucky Horticulture Research Farm in Lexington in conjunction with Tennessee in 2012. *Buxus microphylla* 'Green Ice' plants were obtained as 4-inch liners from Spring Meadow Nursery (Grand, Haven, MI). Plants were potted into 1-gal containers with 85% pine bark: 15% peatmoss (vol:vol) (Renewed Earth, Inc., Kalamazoo, MI). After transplanting, plants were fertilized with 19.0N–2.2P–7.5K controlled release fertilizer with micronutrients (HFI Topdress Special; Harrell's Inc.) at the high, medium low rate (8 g per container). Irrigation zones were 10 square feet with 18 plants per replicate. There were three replicate zones per treatment. Each treatment replicate was controlled by a Rain Bird 13DE04K solenoid valve (Rain Bird Corporation). Irrigation was applied through four overlapping Toro 570 Shrub Spray Sprinklers (The Toro Co., Riverside, CA) per irrigation zone. Emitters were mounted on 1.3-cm diameter risers at a height of 66 cm. The pH and electrical conductivity of

leachate was monitored during the study. Volumetric water content was measured using Echo-5 probes (Decagon Devices, Pullman, WA) inserted into two containers per irrigation zone. Daily water use (Warsaw et al., 2009) was calculated based on the average soil moisture readings of the two ECHO-5 probes per plot and irrigation was applied daily at 9 am. The demand-based irrigation system (Fulcher et al., 2012) was designed to apply irrigation to return the moisture to container capacity (0.53 cm^3) after substrate moisture set point (0.28 cm^3) has been reached. Acquisition and control were monitored using a data logger (CR 1000, Campbell Scientific, Logan, UT). Plant WUE was estimated by dividing total dry weight at the time of harvest by total water volume applied (irrigation plus precipitation; L per container). Year 2012 was one of the hottest and driest in the weather history of Lexington. Plant growth index was calculated [(height+width1+width2 perpendicular to width1)/3] to determine plant performance under the different irrigation regimes.

Results

The average growth index and average plant dry weight at the end of study were not statistically different among plants grown in DWU and demand-based irrigation treatments. Plant physiological parameters such as leaf water potential, photosynthetic rate, transpiration rate and stomatal conductance were not significantly different among plants in both the treatments. Total irrigation water applied was significantly (35%) more for the DWU based treatment than the on-demand irrigation treatment. Plants under on-demand treatment significantly increased irrigation WUE by 31% than plants in the DWU treatment. The pH and electrical conductivity of leachate were similar between the treatments and were within the acceptable range during the study. These results suggest that irrigation based on plant physiological parameters can significantly reduce water use compared to using DWU based methods. In addition, for woody plants with lower water requirement such as boxwood, DWU methods may significantly increase water usage by irrigating every day compared to a plant demand-based water application.

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ARSENIC SPECIES IN BROILER (*Gallus gallus domesticus*) LITTER, SOILS, MAIZE (*Zea mays* L.), AND GROUNDWATER FROM LITTER-AMENDED FIELDS

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Manure and bedding material (litter) generated by the broiler industry (*Gallus gallus domesticus*) often contain high levels of arsenic (As) when organoarsenical roxarsone and/or p-arsanilic acid are included in feed to combat disease and improve weight gain of the birds. This study was conducted to determine As levels and species in litter from three major broiler producing companies, and As levels in soils, corn tissue (*Zea mays* L.), and groundwater in fields where litter was applied. Total As in litter from the three different integrators ranged between <1 and 44 mg kg^{-1}. Between 15 and 20% of total As in litter consisted of arsenate, with smaller amounts of roxarsone and several transformation products that were extractable with phosphate buffer. Soils amended with litter had higher levels of bioavailable As (extractable with Mehlich 3 solution). Arsenic concentrations in plant tissue and groundwater, however, were below the World Health Organization thresholds, which was attributed to strong sorption/precipitation of arsenate in Fe- and Al-rich soils used in the study. Ecological impacts of amending soils with As-laden litter will depend on the As species in the litter, and chemical and physical properties of soil that affect As mobility and bioavailability in the environment.

NOTES

CYBERINFRASTRUCTURE AND ENVIRONMENTAL SENSORS IN KENTUCKY LAKE: A VIRTUAL OBSERVATORY AND ECOLOGICAL INFORMATICS SYSTEM (VOEIS)

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A collaborative KY EPSCoR RII cyberinfrastructure grant (VOEIS – 2009-2013) has enabled Hancock Biological Station (HBS) to deploy four automated water quality and meteorological sensor systems on fixed and floating platforms on Kentucky Lake and two sub-watersheds (Ledbetter and Panther creeks). Data are transmitted from each location every 15 minutes to a database server at HBS (data from the fixed site are available to the public at http://www.murraystate.edu/qacd/cos/hbs/WQ.cfm).

High-frequency monitoring of water quality and meteorological conditions on Kentucky Lake and tributaries allow scientists to understand limnological and watershed processes at resolutions not previously available. For example, high-frequency data from stream monitoring sites give estimates of sediment and nutrient fluxes to Kentucky Lake from sub-watersheds. High-frequency weather data demonstrate the influence of meteorological conditions on water quality. High-frequency measurements of dissolved oxygen, temperature, wind speed and solar radiation are used to assess the balances among heterotrophy and autotrophy and lake productivity.

High-frequency meteorological data such as a) precipitation, b) barometric pressure, and c) air temperature allow observations of storm event impacts on solute concentrations and other water quality parameters not observable from less frequently collected long-term monitoring data.

High-frequency dissolved oxygen and water temperature demonstrate diel changes in lake metabolism, a metric that characterizes the trophic status of the lake and describes the biological relationships between O_2 production and O_2 consumption. Gross primary production (GPP), net primary production (NPP), and respiration (R) can be calculated from O_2 concentrations providing a better understanding of biological activity.

High-frequency measurements of chlorophyll *a* are being used to assess environmental drivers of phytoplankton biomass in Kentucky Lake and other lakes and reservoirs. Examples of high frequency Chl *a* data show fluorescence differences between daylight and night. Daylight signals are quenched by high light intensities while nightly signals are considered unquenched and more indicative of actual Chl *a* concentrations. Wavelet analysis deconstructs the time series data to time scales where Chl *a* signals rapidly increase over time showing strong daily activity from July through September and strong seasonal activity from March through October. The information increases our understanding of controls on phytoplankton blooms and the relationships among water quality variables and phytoplankton seasonal succession.

NOTES

MOLECULAR ENGINEERING OF AQUAPORIN

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Over 30% of the world's population lives in countries facing water shortage. This figure is predicted to double by 2025. The overall fresh water storage on our planet is a largely fixed number. The only methods that can increase the usable fresh water supply beyond what is available from the water cycle are desalination of sea water, purification of ground water, and recycling of waste water. All these methods depend on the availability of efficient techniques to separate water from solute as well as insoluble substances co-existing with water.

Figure 1. Ribbon diagram of aquaporin tetramer. Each subunit has a water conducting channel in the center of the helix bundle.

Separation involving membranes with high water permeability and selectivity is the method of choice. Such membranes require no chemical additives or thermal input, are convenient to use, and require no regeneration of spent media. The development of advanced and innovative membranes by incorporating protein nano-channels in a synthetic matrix will drive the progress of finding a technological solution to global water shortages. Water channel proteins called aquaporins confer high water permeability and selectivity to biological membranes. In this study, we will develop molecular engineering strategies to improve the stability of the water channel protein aquaporin. Aquaporin Z (AqpZ) is an integral membrane protein discovered as an effective water channel in *Escherichia coli* decades ago. *In vitro* studies showed that it is selectively permeable to water molecules, but excludes other molecules including glycerol, salt, and even protons. Due to its superb specificity and high permeability, AqpZ has been incorporated into artificial membranes used for water filtration and desalination. Obtaining an artificial membrane and protein which are chemically and mechanically stable over extended periods of time under high pressure and high salinity is an obstacle for applications involving biomimic membranes.

Our goal is to improve the intrinsic stability of AqpZ (Figure 1). AqpZ is a tetramer, but its water permeability is independent of the quaternary structure. AqpZ is functional in the monomer state. Hence, we focus on the improvement of stability of AqpZ monomer.

We proposed to use two methods: introduction of proline into β-turns and introduction of disulfide bond to AqpZ monomer by site directed mutagenesis. Proline is commonly found in β-turns. Due to its rigid backbone structure, more energy is usually required to unfold protein containing Pro, which leads to protein stabilization. Several studies have shown that introduction of proline to β-turns is an effective way for stabilizing protein tertiary structure, especially when proline was introduced at the secondary site (*i + 1*) in a β-turn (Figure 2A). AqpZ monomer contains eight β-turns. Three of these eight β-turns already contain Pro at the desired position. We will replace the (*i + 1*) site in the other five β-turns with Pro one at a time to improve protein stability. The introduction of disulfide bonds to protein is another traditional and effective way to improve the stability of protein. First, we will take advantage of an existing free thoil group C9 and mutate two residues from the neighboring helix (A87 and G91) to cystein in order to form disulfide bond with C9 (Figure 2B). In parallel, we will engineer Cys pair into the periplasmic loops of AqpZ at locations that are within disulfide bonding distance, including G28-G33, S118-V193, and A111-F196. Since none of the proposed mutation sites involves conserved residues or locate in the water translocation pathway, we expect that mutations will not affect the water transportation of AqpZ. Activity and stability of mutant AqpZ will be examined using stopped-flow spectroscopy and circular dichroism spectroscopy.

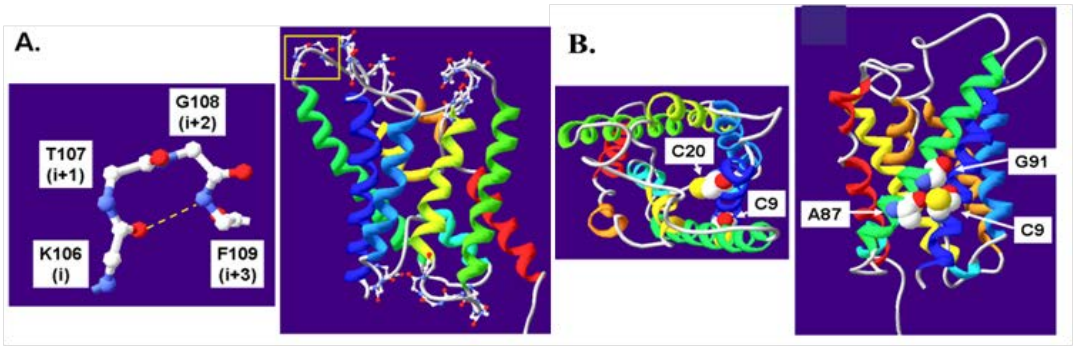

Figure 2. **A.** β-Turns and their presence in AqpZ structure. The ribbon diagram of an AqpZ monomer (1RC2.pdb). Backbone trace was shown for stretches that form β-turns, with the β-turn formed by K106 to F109 highlighted in a box. **B.** Head view of the structure with positions of the two intrinsic Cys highlighted using space filled model (left). Side view of the structure with positions of C9 and two residues close to C9 in space, A87 and G91, highlighted (right).

TRAPPING OF FLUVIAL SEDIMENT WITHIN GRAVEL AND COBBLE SUBSTRATES

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Deposition of Fine Particulate Organic Matter (FPOM) to streambed substrates can a have significant effect on hyporheic exchange and adversely impact aquatic ecosystem functioning when present in excess or if carrying contaminants. The trapping and flushing of FPOM within streams remain a sediment process that requires further research in order that recommendations can be made to the stream restoration community. The process is characterized by downward gravity and fluid pumping forces upon FPOM. Near the streambed, FPOM can be resuspended, transported through the bed framework to the lowermost layer, or clogged in the bed interstices. The transport of FPOM in the bed is not currently well predicted but is recognized to be dependent upon the bed substrate geometry, bed thickness, intragravel flow, freestream turbulent shear, and the size of FPOM relative to the bed substrate. The present study uses two recirculating laboratory flumes, one with a representative gravel streambed and the other with a representative cobble streambed, to isolate the physical processes responsible for FPOM trapping. The trapping efficiency of each bed is investigated under a range of engineered fluvial sediment sizes, sediment concentrations, and hydraulic conditions. In our initial testing, it was found that the sediment passed through the each streambed without forming a seal within the bed, and the sediment in each flume filled the bed from the bottom. This can be attributed to our relatively high bed to grain ratio whereby gravity settling dominates over clogging in the subsurface. But, sediment trapping was lower than anticipated due to hydraulic pumping, which remobilized the fines. Ongoing results are being collected to better isolate the above processes under a wider range of flow conditions.

NOTES

EFFECTS OF ATRAZINE ON CHEMCIAL ALARM CUE RESPONSE IN CENTRARCHID SPECIES

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The chemical alarm cue response is a well established phenomenon in fish whereby prey species exhibit antipredator behavior and predatory species exhibit increased foraging behavior when exposed to pheromones in fish skin extract. Atrazine is the most widely used herbicide in the United States and therefore one of the most common contaminants found in the ground and surface waters of the U.S. The present study will examine the effects of the herbicide atrazine on the chemical alarm cue response in centrarchid species longear sunfish (*Lempomis megalottis*) and smallmouth bass (*Micropterus dolomieu*). Previous studies in our laboratory have demonstrated response to chemical alarm cues in predatory but not prey fish. Smallmouth bass exhibited a significant increase in time spent moving when exposed to longear sunfish, but not smallmouth bass extract. The current study will investigate whether atrazine exposure disrupts this behavior in centrarchid species as has been demonstrated in other fish species such as goldfish and Chinook salmon. Fish will be collected from local watersheds and acclimated in the laboratory. Skin extracts from both longear sunfish and smallmouth bass will be obtained and fish movement will be quantified for 5 minutes prior to and after extract exposure in the presence and absence of atrazine at concentrations of 4, 7, and 10 mg/L. A significant change in chemical alarm cue response between treatment and control groups will be indicative of a potential disruption of this response by a widely used herbicide.

NOTES

THE FISHES OF THE HICKMAN AND JESSAMINE CREEK WATERSHED

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Fish are important indicators of aquatic ecosystem health. We are surveying the fish populations of the Hickman and Jessamine Creek watersheds, located in the Kentucky River Basin and occupying the greater portion of Jessamine and much of Fayette County, Kentucky. Nearly 80 % of the land in each watershed is agricultural with lesser percentages of residential and industrial use land in each. Both the Hickman and Jessamine Creek watersheds support a remarkable diversity of flora and fauna although data concerning fish distribution in these watersheds is currently relatively limited. This study will provide a detailed list of the species present and their distribution within each watershed. Twenty-one fish species have been collected to date including four species of darters (rainbow, *Etheostoma caeruleum*; fantail, *E. flabellare*, greenside, *E. blennioides*; and logperch, *Percina capodes*), five species of sunfish (green, *Lepomis cyanellus*; bluegill *L. macrochirus*; longear, *L. megalotis*; warmouth, *L. gulosus*; and rockbass, *Ambloplites rupestris*), two species of black bass (smallmouth, *Micropterus dolomieu*; and largemouth, *M. salmoides*), and one madtom (*Noturus flavus*). The results of this study provide baseline data on the fish communities present in these relatively undisturbed systems and provide heuristic data for future study of the fishes of the Jessamine and Hickman Creek watersheds.

NOTES

GENE EXPRESSION IN SUNFISH AS A BIOMARKER OF CONTAMINANT EXPOSURE

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The use of biomarkers allows researchers to assess physiological responses to pollution and thereby enables pollutant detection and contributes to an understanding of the biological significance of contamination. We sequenced and examined the expression of three pollutant sensitive genes (cytochrome P4501A1, metallothionien, and uridine 5'-diphospho-glucuronosyltransferase) in green sunfish, a widely distributed species of North American sunfish. Fish were collected from both reference and contaminated sections of streams surrounding the Paducah Gaseous Diffusion Plant (PGDP) in western Kentucky. The streams surrounding the PGDP that are the focus of this study have a long and well documented history of contamination by both organic and inorganic contaminants. Sediment PCB concentrations in contaminated sites were between 10.09 and 14.29 μg/Kg while PCB concentrations as high as of 0.473 μg/g total PCBs were observed in fish tissue. Aqueous copper concentrations in contaminated sites were as high as 9.18 μg Cu/L, a level consistent with the induction of the metal sensitive protein metallothionien in similar studies. These results provide valuable information linking contaminant levels to biomarker response in resident organisms.

NOTES

PRELIMINARY ASSESSMENT OF WATER QUALITY IN A RESTORED SECTION OF THE CANE RUN WATERSHED, LEXINGTON, KY

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Riparian areas are vegetated borders, either naturally occurring or manually planted, that separate a stream or other water body from an upland land use. Both the amount and type of vegetation growing in a riparian area influence water quality. There is increasing evidence that restoring native vegetation to riparian buffers beside streams improves water quality and the overall stability of the streams. The taller plants and trees near a stream provide shady areas that lower water temperature, which can increase the dissolved oxygen and positively influence the macroinvertebrate community. Riparian buffers also stabilize stream banks and lower nonpoint source pollution by improving infiltration of runoff.

This preliminary assessment was conducted concurrently with a riparian buffer restoration project on a channelized tributary of the Cane Run Watershed in Lexington, KY. The concurrent project focuses on the maintenance of the riparian area as it relates to soil properties such as infiltration rate, soil aggregate size, root density, and nutrient content to maximize the function of the riparian area. The riparian buffer restoration project consists of 10 replicates of four treatments (Intense Mow, Moderate Mow, No Mow, and Native Grass) in plots 10 m wide (parallel to the tributary) extending 15.25 m away from the tributary. The intense mowing treatment was also used as a control because this is the previous plant growth management practice in this area of the tributary. The total reach of the tributary utilized for these 40 plots was 540 m. Due to agricultural and geological features along the tributary, sample area was divided into three blocks creating an incomplete block design. Water samples were taken at the tributary edge on the downstream end of the plots within each block in July and December of 2012. Channel depth and width, pH, electrical conductivity (EC), nutrient content, fecal coliforms, and biological oxygen demand (BOD) were measured.

	Depth (cm)	Width (m)	pH	EC (mS cm^{-1})	BOD (mg L^{-1})	NH_4 (ppm)	NO_3 (ppm)	NO_2 (ppm)	PO_4 (ppm)	Fecal Coliforms (CFU 100 mls^{-1})
7/18/12										
Range	1.27-13.97	1.40-7.70	7.24-8.10	0.44-0.53	0.86-7.47	0-0.66	0.05-0.3	0-0.01	0.01-0.4	3-369
Mean (Std dev)	7.13 (3.2)	3.43 (1.19)	7.68 (0.2)	0.49 (0.02)	3.69 (1.98)	0.07 (0.1)	0.1 (0.04)	0(0)	0.15 (0.1)	76 (90.87)
12/6/12										
Range	6.35-21.59	2.20-9.40	7.77-8.48	0.53-0.59	1.2-5.37	0-0.28	0.5-0.87	0-0.01	0.05-0.14	50-290
Mean (Std dev)	13.48 (3.55)	4.05 (1.50)	7.96 (0.13)	0.57 (0.01)	2.62 (0.97)	0.03 (0.06)	0.70 (0.07)	0.01 (0)	0.1 (0.02)	148 (48.2)

The tributary changed in depth and width along the reach and as a consequence of the season. pH was routinely alkaline, which is consistent with flow over the underlying Ordovician limestone, and along with EC was the most static of the water quality parameters measured regardless of season. Nutrient concentration was usually, but not consistently higher in July than December. The exception was NO_3 concentration in December. Fecal coliform concentration occasionally exceeded primary water contact standards each sampling period. BOD was extremely variable within each transect, with the coefficient of variation exceeding 54% in July and 37% in December. Preliminary analysis suggests that there are differences in fecal coliform and BOD values that can be detected in a 10 m section of the tributary in response to the riparian buffer management. However more data will be needed to determine if clear patterns appear.

GROUNDWATER MONITORING OF DRINKING WATER SOURCES IN KENTUCKY, 2000-2012: COMPARISON OF PHYSIOGRAPHIC REGIONS

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Groundwater data was collected for twelve years from drinking water wells across Kentucky and analyzed for up to sixteen metals (Al, Ag, Ba, Be, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sr, Tl, Zn) and four metalloids (As, B, Se, Sb). Results were grouped by physiographic region (Eastern Coalfield, Bluegrass, Mississippi Plateau, Western Coal Field and Jackson Purchase) and compared to National Drinking Water Standards (MCLs, SDWR, and Health Advisory levels) to determine if regional differences in the potential for health effects were present. Although levels of metals and metalloids were above National Standards in some well samples, regional median levels remained 5-40 times lower than the health standards. Many individual well samples indicated that the concentrations were below the analytical detection limits for the constituent. No regional human health effects from drinking water well sampling were indicated, and no regional differences were distinguished for any metal or metalloid.

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PRELIMINARY RESULTS FOR THE STATEWIDE GROUNDWATER PATHOGENS STUDY

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The Kentucky Division of Water (DOW) is conducting a statewide study of pathogens in groundwater as a part of a nonpoint source assessment funded by the Clean Water Act Section 319(h). The goals of the study are to investigate human impacts to groundwater and inform private water well owners about the state of their wells and proper maintenance. Kentucky does not regulate private drinking water sources and this project is an opportunity for DOW to evaluate whether Kentucky's domestic well user are being exposed to pathogens in their drinking water. It is also a chance for interested groundwater users to obtain water quality data on their well and/or spring water. This project represents the first attempt at a statewide, systematic assessment of these water quality parameters in private groundwater sources. Historically, DOW has only collected this particular suite of samples on an infrequent basis, typically in response to citizen complaints. Without a systematic approach it is often difficult to distinguish between actual groundwater contamination and bacteria growth confined to the well bore due to poor construction or a lack of maintenance.

To date, roughly 200 domestic water wells and springs have been sampled throughout the state, with priority on those used as private drinking water sources. Samples were analyzed for total coliform, *E.coli*, Iron Related, Sulfate Reducing, and Slime-Producing bacteria (using BART© kits) as well as caffeine. Many factors can influence the presence of bacteria in water wells, including poor well construction and maintenance, failing onsite sewage disposal systems, animal feedlot operations and a variety of nonpoint sources. Wells that test positive with BART© kits or for total coliform but not *E.coli* or caffeine may simply illustrate the need for regular well maintenance. Positive *E.Coli* results indicate impacts to groundwater from human or animal waste and a positive caffeine result would indicate that the well is being impacted by human activities.

This study has greatly expanded our knowledge of the occurrence of these parameters in groundwater across Kentucky. Preliminary results show a plausible link between maintenance and disinfection frequency, well construction (specifically seal type and integrity), and source type (well vs. spring) to bacteria presence.

A secondary goal of this study is outreach and education for citizens using private groundwater sources as drinking water supplies. All participants were informed about the importance of routine water well and septic tank maintenance. Copies of DOW's Water Well Maintenance Guide and generic Groundwater Protection Plans for septic systems and water wells to use for record keeping were presented at each site. Participants also received results for samples collected from their wells/springs along with a letter of explanation regarding the quality of their drinking water. Future studies could include expanded monitoring and follow-up samples of wells after they have been disinfected properly.

ARSENIC DETECTION USING QUARTZ CRYSTAL MICROBALANCE

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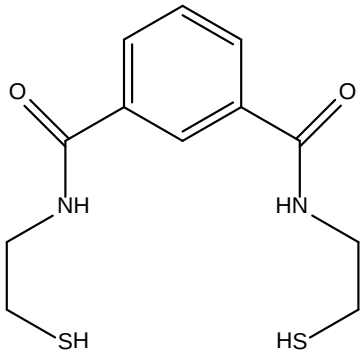
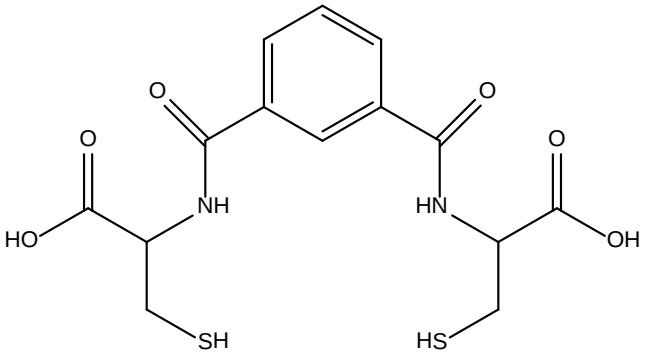
Most of the current methods for the detection of selenium and arsenic require preconcentration or digestion prior to analysis on a laboratory instrument, such as inductively coupled plasma mass spectrometry (ICP-MS). The current methods do not allow onsite detection and can have long turnaround time for analysis reports. The results obtained in this study will allow the determination of optimized conditions for real time selenium and arsenic detection with very low limits of detection using a quartz crystal microbalance. In the quartz crystal microbalance a quartz crystal oscillates at its resonance frequency and any changes in mass on the crystal causes a change in the resonant frequency, which is measured and the change in mass calculated. The sensitivity of this detection method can measure changes in mass as small as 100ng[1]. It is believed that attaching a ligand to the surface of the quartz crystal will allow for detection of metal and metalloid ions in solution based on their interaction with the receptor ligand.

Figure 1 shows the structure of the receptor that is attached to the quartz crystal which is a carboxylic acid derivative (abbreviated AB9) of the parent compound N, N'-bis(2-mercaptoethyl)isophthalamide (abbreviated B9). B9 and AB9 have been shown to remove arsenic from water and the bonding to the ligands has been established. This work focused on investigating the reaction mechanism and final products of the reaction of the dithiol ligand B9 with selenite. It is well known in the literature that small alkylthiol compounds undergo a series of reaction steps, including formation of alkylthioselenic acid, bis(alkylthio)selenoxide, sulfenic acid, and thiosulfonate before eventually producing elemental selenium and disulfide[2]. Based on pH and other factors, the reaction can be stopped at various points along the reaction steps. To the best of our knowledge no studies with selenite have been performed with a dithiol ligand and this was addressed in the current research. Reaction products were characterized using infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectrometry, differential scanning calorimetry, and thermo gravimetric analysis.

Figure 1: Structure of B9 and AB9:



B9



AB9

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SPECTRAL CHARACTERIZATION OF 2-D WATERBODIES FROM LEAF-OFF MULTISPECTRAL IMAGERY

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Agricultural ponds are numerous and ubiquitous in Kentucky and account for a fairly substantial portion of the open water land cover class (USGS, 2013). These two-dimensional (2-D) waterbodies undergo changes in water depth, open water area, shoreline morphology and degree and type of pollution throughout the year. A systematic visual examination was undertaken of several image tiles composing the 4-band, 0.3048 m resolution, digital aerial orthophotography datasets acquired by the Kentucky Aerial Photography and Elevation Program (KYAPED, 2013). Spectral statistics were extracted for the known, mapped features present in the National Hydrography Dataset, 2-D waterbodies layer as well as for those additional features that had not been accounted for (USGS, 2013). The spectral information was compared to that acquired during 2010 and 2012 as leaf-on, summer multispectral imagery from the National Agricultural Imagery Program (NAIP) (Kentucky Geoportal, 2013).

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NOTES

EVALUATION OF TRICHLOROETHYLENE DECHLORINATION AND SELENIUM CAPTURE IN AQUEOUS PHASE USING ZERO-VALENT IRON NANOPARTICLES SYNTHESIZED IN SITU FROM EXHAUSTED CATALYST OF IRON-BASED REDOX POLYMERIZATION

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Water pollution is mainly made by man and is a serious concern in all the aspects of society including development, politics, economy, and science and technology. Most of the pollutants have two main origins: organic compounds and heavy metals. Two of these contaminants have high interest because of their persistence in nature and the difficulties related with their treatment and/or elimination. These compounds are an organic compound Trichloroethylene (TCE) and Selenium a heavy metal.

TCE is a liquid chemical artificially synthesized and used as a solvent to remove grease in metal cleaning processes. Although a large part of TCE volatilizes in air, small quantities can go to soils and contaminate aquifers. TCE is heavier than water, it sinks below the ground water and is released over time making it very persistent in nature with a half life between months and decades depending on the releasing and storage times.

The effects of TCE have been proven carcinogenic in experimental animals. TCE is distributed in general in all tissues of the body; it is metabolized in the liver and excreted through the urine but small quantities can be stored in body fat. This pollutant in water may cause damage in the liver and the kidney, harm fetal development and affect immune system function as well.

Selenium (Se) on the other hand is a metal found in mineral deposits. Its uses go from electronic and printing components to metal alloys, textiles, dyes, and pigments.

Selenium causes in the short term damage in the peripheral nervous system, fatigue and irritability; hair and fingernail changes; and in the long-term damages to kidney and liver tissue, nervous and circulatory systems and the loss of hair and fingernails. The American Cancer Society states that Se could be good as a nutrient that may help prevent cancer.

To capture the Selenium and to reduce TCE, extensive studies have shown the effect of zero-valent iron nanoparticles (nZVI). These nanoparticles have a very high surface area (which is related to reactivity) about 30 times higher than iron powder. However, the

nanoparticles in solution phase treatment cannot be recovered due to their minute size, thus it is necessary to make a support that helps in their immobilization and possible recycling.

The creation of a support like polymer hydrogel or a membrane pore domain for the immobilization of nanoparticles (nZVI) prevents their loss and reduction of active surface area. The novelty of this work is that the nanoparticles are synthesized using the metal catalysts from the polymerization of PAA hydrogel.

This work describes the preparation of a polymer of acrylic acid (AA) crosslinked with *N*,*N'*-Methylenebisacrylamide (MBAm) by redox polymerization and the use of the exhausted metal based catalysts (ferric ion) of the process to synthesize zero-valent iron nanoparticles for water remediation applications. The polymerization is done by free radicals in a reaction between a catalyst ($FeCl_2$) and an initiator (Potassium persulfate). This redox polymerization is the source of the metal ions that are going to be reduced and immobilized simultaneously in the polymer.

The objective in the present work is to show the characterization of different formulation of poly(acrylic acid) hydrogel samples through simple methodologies followed by characterization and utilization of the iron nanoparticles within as reducing agent of Trichloroethylene (TCE) and capture of Selenium in aqueous solution. Morphological, spectral and thermal properties of the produced hydrogel were examined with and without the nanoparticles formed.

WATERSHED BASED PLANNING IN THE URBAN WOLF RUN WATERSHED

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The objective of this presentation is to present a case study of the development of a watershed based plan in an urban environment. In August 2010, the Lexington-Fayette Urban County Government (LFUCG) contracted with Third Rock Consultants and Friends of Wolf Run, to complete a watershed plan for Wolf Run Creek. The project was funded through a U.S. Environmental Protection Agency 319(h) grant awarded by the Kentucky Division of Water (KDOW) to LFUCG.

The Wolf Run Watershed is Lexington's most highly urbanized watershed with about 40% of the surface covered with impervious material. The 13.5 miles of perennial streams and tributaries in the watershed drain an area of 10.18 square miles. The watershed has significant karst development including Preston's Cave, McConnell Springs, and Kenton Blue Hole among other features which complicates the hydrology by redirecting groundwater from the adjacent basins. Wolf Run Creek is listed as impaired on the 303(d) list for nonsupport of primary and secondary contact recreation and partial support of warmwater aquatic habitat with cause including fecal coliform, nutrient / eutrophication biological indicators, and specific conductance from a number of suspected sources. The goal of the watershed plan was to identify the sources of pollution and the remediation efforts necessary to return the stream to its designated uses.

The Wolf Run Watershed Council was formed in December 2010 to receive input from the community on citizen desired goals and objectives for the watershed, to provide local knowledge on specific issues within the watershed, and to provide review and feedback on the plan progress.

All available data was compiled and reviewed to evaluate the additional data necessary to complete the plan. A plan was developed in April 2011 and executed from May 2011 to May 2012. Tasks included gathering data on the macroinvertebrate community, stream habitat, hydrogeomorphology, hydrology, and chemical water quality. The data was collected through a successful cooperative effort between of Friends of Wolf Run volunteers and Third Rock biologists and engineers.

The monitoring indicated that the aquatic macroinvertebrate community ranged from "poor" to "very poor" according to macroinvertebrate biotic index scores assessed at seven sites due to extremely low numbers of absence of mayflies, stoneflies, and caddisflies. The habitat, assessed at 33 reaches according to the Rapid Bioassessment, ranged from 50 to 153, but with only 2 of the 33 reaches achieving a "fair" narrative criteria and all others "poor." Contributing factors to the poor scores included narrow riparian zone width, lack of pools and available cobble habitat, embeddedness, and poor

base flow levels. Hydrogeomorphic assessments, conducted at nine sites, indicated the streams are generally over-widened and entrenched with significant channel alteration, bank armoring and bedrock substrate on many reaches. Sedimentation deposition and aggradation was noted downstream of Preston's Cave. Stage-discharge curves, developed for five locations in the watershed, indicate that streams were extremely flashy during storm events, but also sustain frequent and prolonged periods of dry or low flows.

Water quality monitoring results, sampled at 12 locations over 10 months, indicate nitrogen, phosphorus, dissolved oxygen, ammonia, specific conductance, suspended solids, and *E. coli* each exceed benchmarks for one or more events. Annual pollutant loads and reduction goals are calculated for nitrogen, phosphorus, suspended solids, and *E. coli*. Wet weather contributions to the annual loading are the most significant for *E. coli*, phosphorus, and suspended solids but less significant for nitrogen. Load reductions of over 90% are required to reach the regulatory levels for recreational use. Significant load reductions in suspended solids and phosphorus are necessary in some subwatersheds and only slight load reductions in nitrogen are needed. A watershed-wide specific conductance survey (373 measurements in 8 days) indicates the highest concentrations were in the headwaters of Wolf Run, Vaughn's Branch, and the Big Elm Tributary.

Based on these results, the Wolf Run Watershed Council devised general goals and objectives for the watershed, and recommended the types of Best Management Practices (BMPs) and locations for implementation. An Outreach Campaign Subcommittee was organized to develop an education and outreach plan for the watershed and a Water Quality BMP Technical Subcommittee was organized to review the Council recommendations and develop an implementation strategy with prioritized projects.

In January 2013, a comprehensive implementation plan was developed based on these efforts and presented for public comment. The plan identifies 138 BMP project opportunities in the watershed, 61 high priority, 33 medium priority, and 44 low priority projects. These implementation projects include 18 BMPs targeted to address the *E. coli* load and sanitary sewer, 14 education and outreach BMPs, 39 green infrastructure BMPs, 16 trash and debris cleanup BMPs, a Neighborhood Association BMP Program, and several target locations for Streets and Roads BMPs. Additional stream and habitat improvement opportunities include 3.5 miles of stream restoration, 5.6 miles of riparian buffer restoration, and approximately 850 feet of bank stabilization. Wetland creation or expansion is proposed for approximately 20 acres and enhancements are proposed at two springs.

Implementation has been initiated or is planned for the near future on about 40 of these projects. Next steps include implementation of other identified opportunities, ongoing monitoring of the water quality improvements and implementation status, and adapting the plan to address the changing needs of the watershed.

OCCURENCE OF ESTROGENIC COMPOUNDS IN SURFACE WATER, SOURCEWATER AND DRINKING WATER IN THE BARREN RIVER WATERSHED

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Estrogenic compounds have been detected in the environment and can elicit biological effects on humans and wildlife. The objectives of this study were designed to assess the occurrence of estrogenic compounds in the Barren River Watershed. A major component of the study was to adapt EPA method 539M for determination of hormones in drinking water by solid phase extraction and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS) to the Barren River Watershed sample matrices. Solid phase extraction was accomplished by filtering samples through C18 discs, eluting with methanol, and reconstituting samples for analysis. This procedure was followed to determine if detectable concentrations of estrogenic compounds exist in the Barren River upstream and downstream of Bowling Green, KY, and in the municipal drinking water supply. Results of the study indicate that estrogenic compounds are detectable in the Barren River downstream from a wastewater effluent discharge. This project serves as a catalyst for future research on the occurrence, distribution and fate of estrogenic compounds in receiving streams of Kentucky.

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ANALYZING RAINFALL BASED CLIMATE INDICES IN KENTUCKY AND INDIANA

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Rainfall based climate indices are popularly used to examine the changes in behavior of rainfall pattern. In this study, six different indices were used to examine the changes in the recent 30 years to that of the previous 30 years. Indices, namely maximum 1 day precipitation, maximum 5 day precipitation, number of days when recorded rainfall is more than 10 mm (R10), number of days when recorded rainfall exceeds 20 mm (R20), consecutive dry days (CDD), and consecutive wet days and very wet days were considered in this analysis (Teegavarapu *et. al.*, 2012).

For the analysis, Indiana and Kentucky were divided into four zones each and the regional behaviors were compared. Historic daily rainfall data were downloaded from the NOAA web site and were organized for this analysis (1950 to 2010). Using the point rainfall stations, the shifts in maximum one day precipitation, maximum 5 day precipitation and consecutive dry days in the recent 30 years and previous 30 years were compared. The results indicated a marginal shift indicating increased daily and 5 day precipitation indices.

To study the usefulness of these indices in drought analysis, consecutive dry days and SPI index (Wilhite and Glantz, 1985) were compared. All the indices were constructed for six month span. In this analysis, for example, for the consecutive dry days, number of consecutive dry days in a six month span was captured for the 60 year span. For comparison, the SPI values were also calculated for same time span. The indices that correlated very well with the SPI were examined initially. R10 and CDD had good correlation with SPI. To study the patterns, for each region, frequency of CDD were calculated and Cumulative Distribution Function (CDF) was prepared for 1950 to 1980 and 1981 to 2010. The CDF of the two time periods were compared on the same plot to

see the variations. As an example, Northwest Kentucky CDD data analysis is presented in Figure 1 which clearly indicates decreased probability of getting CDD in recent years.

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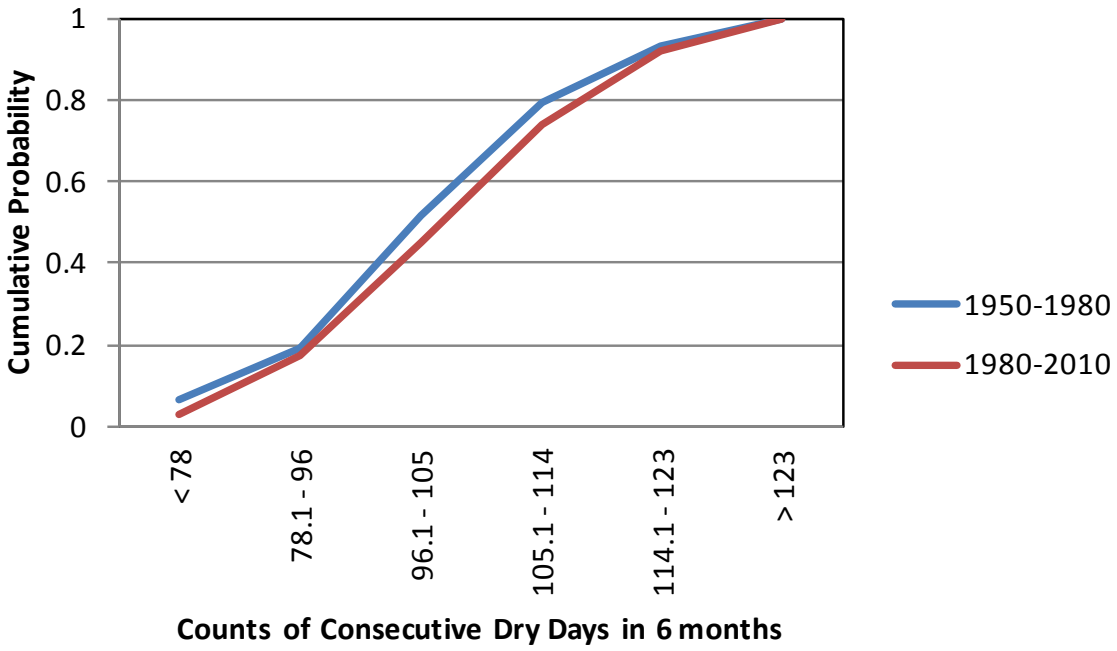

Figure 1. Variation of CDF for CDD counts in the two time periods

ENGAGING PARTNERS IN THE CANE RUN WATERSHED

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The Cane Run Watershed (CRW) project management team, composed of the University of Kentucky, College of Agriculture, Environmental and Natural Resources Initiative, and the Department of Biosystems and Agricultural Engineering staff, is involved in an innovative, comprehensive program that engages K-12 students and teachers and citizens within the watershed and is designed to increase understanding of water quality issues and promote urban stream restoration. The two goals of the project, one for each major audience, include the following:

Goal 1: Engage K-12 and community partners in an urban watershed, the Cane Run Watershed, in an innovative education project to improve water quality in the watershed

Goal 2: Engage all citizens within the watershed in the urban-stream restoration project.

The K-12 portion of the program involves the formation of partnerships between community groups and three schools to develop a geographic education tool – a map of Cane Run Environment & Watershed. The map uses a GIS platform and is accessible from an Internet portal at UK. When completed, the map will include water quality data, demographics, geographic information, photos and video intended to illustrate the environmental health of the watershed. In the process of making the map, students will interact with environmental science professionals to learn about career opportunities and the technical knowledge required for these professions. In addition to creating the map, students will make presentations at community and local government events to demonstrate their learning.

A second component of the program involves an upcoming urban stream restoration project. The restoration project involves a section of the Cane Run Creek located in a city-owned park adjacent to a newly built 12-mile streamside walking/biking path; the project location presents a unique opportunity for citizens to become involved in watershed issues. The UK management team will not only educate citizens regarding this restoration project but will also create opportunities for community input (including students, teachers, and their newly-developed map) into the planning process.

Participants who attend this session will learn about the planned education and community activities and progress to date. Students involved in the program will be invited to the Symposium to present their findings and talk about their maps.

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