Indiana Water Resources Research Center
Annual Technical Report
FY 2009

Introduction

This report covers the activities of the Indiana Water Resources Research Center (IWRRC) for the period March 1, 2009 to February 28, 2010 and is reported by Ronald F. Turco, Director of the center. The report is provided to meet requirements and obligations under the 104 (B) of the USGS water centers program. The objectives of the fiscal year 2009 program of the IWRRC have been: (1) to continue to engage the water community in the State of Indiana as related to water research and education with a major focus on the Wabash River and Wabash River Basin; (2) foster a research program that encompass several water issues related to emerging contaminants (pharmaceuticals, personal care products, nanomaterials) primarily focused on the Wabash River; (3) continue to support an outreach program related to water and water quality (in particular rural water protection/safety and agriculture) and (4) to strengthen interactions with State regulatory agencies and Federal Agencies (largely through grant applications).

In the last year we have supported externally reviewed 104(B) projects, closed out a USDA-CSREES facilitation grant (EPI-Net.org) participated in a visioning sessions on the future of Indiana's water resources (both quality and quantity), maintained a functional website (www.iwrrc.org) been involved in the development, submission and management of number of grant proposals. In terms of web resources we are currently digitizing all back issues of water center reports and will make them available by fall 2010. We have had a large number of request for older project information and think this will be a welcome addition. We continue to help with a 104(G) project on Nutrient and carbon delivery to streams in artificially drained landscapes of the Midwest: continued to work on a Middle Reach of the Wabash River. We have also work on a CEAP grant that was funded four years ago and this has lead to a number of interactions and secondary projects. We have worked with Cites of Lafayette West Lafayette, Indiana and the Wabash River Enhancement Corporation (WREC) to facilitate discussions on long-range planning for Wabash River Redevelopment. We have continued to work on a 319 effort entitled: Developing a watershed management plan (WMP) for the Middle Wabash-Little Vermillion Basin (HUCs 05120108-010, 05120108-020, and 05120108-030). Most importantly, we have convened and developed the "Wabash River Research Consortium" which is an effort to organize research on the Wabash River. We have also been active in establishing the Purdue University water community (PWC) and have facilitated a number of campus wide meetings to engage this group. We are currently developing a strategic plan for the PWC that includes interactions with the IWRRC. International, we are now working with Purdue's office of global engineering on a number of water projects and have help facilitate work with France.

For this reporting period, we continue the strategic outreach alliance with the Purdue Pesticide Program office for the development of document and educational materials on methods to prevent water contamination. By leveraging our funds with the Purdue Pesticide Program office's core efforts we are using the opportunity to include the IWRRC in many of their programs. Our efforts have established a constant and vital outreach effort that is associated with prevention rather than remediation of environmental problems. In the future we are increasing our support of the PPP office. The recent title: Plan today for Tomorrow's Flood: A flood response plan for Agricultural Retailers.

Research Program Introduction

Project 01: Program Administration and State Coordination

The administrative portion of the project has been used to support the management of the IWRRC's research projects and to facilitate the development of other research projects. We have also stepped up our efforts to coordinate campus level interactions with state and federal agencies. All of these efforts have the ultimate goal of improving the quality of water resources in the State of Indiana. We have used a limited amount of money on the administrative portion but it has allowed the IWRRC director some means to invest time in the efforts to integrate with state and federal agencies. Most of IWRRC funds are used for projects and the directors time is contributed to the project. The IWRRC director has worked with state and federal environmental agencies, the governments of Indiana's cities and counties and key citizen groups on water education and water resources planning activities. In this way, the results from the research projects can be transferred to interested individuals in the state. The IWRRC director will participate in important national and international meetings related to water and environmental protection.

Projects Areas

1. The Wabash River runs some 764 km (475 mi), is situated across five 8-digit Hydrologic units (HUC), crosses 19 counties and at its full distance stretches from the Ohio border in the Northeast corner of the state to the Southwest corner where it combines with the Ohio River below Mount Vernon on its way to the Mississippi River. In the counties associated with the HUCs, the population is estimated at 2,388,658, fully one-third of the total population of the state. Working with John Shuey and Kent Wamsley from The Nature Conservancy (TNC) and Mark Pyron Ball State University we have established the Wabash River Research Consortium. The group met for the first time on November 11, 2008 and has meeting each semester since its inception. The group is developing a position paper on the Wabash River as a Natural Resource investment and proposing an action plan to implement a coordinated research agenda. This Wabash River Research Consortium is an extremely diverse group with representation from the Indiana Department of Natural Resources, Indiana Department of Environmental Management, Indiana State Department of Agriculture, NRCS, Fish and Wild Life, Purdue University, The Rivers Institute at Hanover College, Ball State University, DePauw University, The Wabash River Heritage Corridor Commission (WRHCC), Wabash River Enhancement Corporation (WREC) established by a grant from North Central Health Services; and IUPUI. Our goal is simple: develop a coordinated research and management agenda for work on the Wabash River. The long-term goal of the effort is to help re-establish the Wabash River as a healthy water body that provides quality recreation and economic value to the state.

2. Continued work with the “State Water Monitoring Council”. (https://engineering.purdue.edu/~inwater/conference/) leading to an online inventory of projects https://engineering.purdue.edu/~inwater/.

3. Working with Dr. Fred Whitford and the Purdue Pesticides Program Office to establish an outreach effort centered on water protection emphasizing pesticide and farmstead management. We are undertaking efforts to enhance this interaction.

4. Continued interactions with a number of consulting firms related to water quality issues.

Grant Applications Submitted thorough/with IWRRC:

a. (Funded) USDA-AFRI Tracking the survival and distribution of Mycobacterium avium subsp paratuberculosis in the agroecosystem. $375,000. E. Rizaman, C. Wu and R. Turco.

b. (Funded) Project: Optimization of biomass productivity and environmental sustainability for cellulosic feedstocks: Land capability and life cycle analysis. $875,000 S.M. Brouder, PI, R.F. Turco, J.J. Volenec, D.R. Smith and G. Ejeta CoPIs

c. (Funded and ongoing) IDEM 319: Wabash River: Lafayette-West Lafayette Reach of the Wabash River Watershed Management Plan. Submitted in conjunction with the Wabash River Enchantment Corporation $700,000. L. Prokopy, L. Bowling, K. Wilson and R. Turco.

d. (Funded and ongoing) USDA NRI, Managed Ecosystems. Ecological services of agro-biofuels: productivity, soil C storage, and air and water quality. $399,999. Submitted Dec. 2007. S.M. Brouder, PI, R.F. Turco, J.J. Volenec, D.R. Smith and G. Ejeta CoPIs.

e. (Funded and ongoing) USDA Conservation Effects Assessment Program. $660,000. Watershed-Scale Evaluation of BMP Effectiveness and Acceptability: Eagle Creek Watershed, Indiana. Developed with Jane Frankenberg, Lenore Tedesco, Jerry Shively, Linda Prokopy. This was an outgrowth of an effort submitted last year to EPA but note funded: Creating sustainable drinking water supplies for Central Indiana: Innovations to achieve reductions in watershed and reservoir nutrient levels.

f. (Continued Funding) USEPA $350,000. Fate of hormones in tile-drained fields and impact to aquatic organisms under different animal waste management practices. Linda Lee, S. Brouder, C. Jafvert, M. Sepulveda and R. Turco.

g. (Continued Funding) IDEM-319—Development and Demonstration of Outcomes-Based Evaluation Framework for the Indiana Nonpoint Source Program. Developed with Jane Frankenberger, and Linda Prokopy

External Board of Advisors Membership: Dr. Lenore Tedesco, Director Center for Earth and Environmental Science, Indianapolis IN Dr. Jack Wittman, President, Wittman Hydrosciences, Bloomington IN Dr. Bill Guertal Director, USGS Indiana Water Science Center, Indianapolis IN Mr. Jeff Martin, USGS Indiana Water Science Center, Indianapolis IN Dr. Linda Lee, Associate Director Center for the Environment, Purdue University Ms. Martha Clark-Mettler, Director Watersheds Program IDEM, Indianapolis IN

Faculty Advisory Committee: Dr. Linda Lee, Professor and Director of ESE Dr. Jane Frankenberger, Agriculture and Biological Engineering Dr. Larry Nies, Civil and Environmental Engineering Dr. Inez Hua, Civil and Environmental Engineering

Nutrient and carbon delivery to streams in artificially drained landscapes of the Midwest: matrix flow, overland flow or macropore flow?

Basic Information

Title:	Nutrient and carbon delivery to streams in artificially drained landscapes of the Midwest: matrix flow, overland flow or macropore flow?
Project Number:	2007IN227G
Start Date:	4/1/2008
End Date:	3/31/2011
Funding Source:	104G
Congressional District:	7
Research Category:	Ground-water Flow and Transport
Focus Category:	Solute Transport, Surface Water, Hydrology
Descriptors:	
Principal Investigators:	Philippe Gilles Vidon, Nancy T. Baker, Jeffrey W Frey

Publications

1. Cuadra, P.E., P. Vidon, 2009. Natural Variability in Dissolved Organic Carbon and Dissolved Organic Nitrogen Transport in Artificially Drained Landscapes of the U.S. Midwest. Abstract#B34A-04. American Geophysical Union Joint Assembly, Toronto, ON, Canada, May 2009
2. Vidon, P. and P.E. Cuadra*, 2010. Impact of precipitation characteristics on soil hydrology in tile-drained landscapes. Hydrological Processes, DOI: 10.1002/hyp.7627 (Online – Early View).
3. Cuadra*, P.E., and P. Vidon. Storm nitrogen dynamics in tile-drain flow in the US Midwest. Biogeochemistry (in review).
4. Vidon, P., and Cuadra*, P.E. Phosphorus dynamics in tile-drain flow during storms in the US Midwest. Agricultural Water Management (in review).

Progress Report for Award # 08HQGR0052 - Nutrient and carbon delivery to streams in artificially drained landscapes of the Midwest: matrix flow, overland flow or macropore flow? – **YEAR 2**

04/08-04/11, (PI) P. Vidon, (Co-PIs) J.W. Frey, N.T. Baker. USGS-NIWR National Competitive Grant Program (Award # 08HQGR0052). Title: Nutrient and carbon delivery to streams in artificially drained landscapes of the Midwest: matrix flow, overland flow or macropore flow? $129,042

Abstract / Summary

Understanding the processes controlling the delivery of nitrogen, phosphorus and carbon to streams in artificially drained landscapes of the Midwest is of critical importance to developing comprehensive nutrient management strategies at the watershed scale. Most nutrient and carbon losses in artificially drained landscapes of the Midwest occur during precipitation events through tile drain flow and overland flow. In addition, recent research has identified preferential flow through soil macropores as an important export mechanism contributing to tile drain flow. There is nevertheless a lack of empirical data documenting the relative importance of overland flow (OLF), matrix flow (MF) and preferential flow through soil macropores (PF) on nitrogen, phosphorus and dissolved organic carbon (DOC) losses to streams. For this project, a team of USGS scientists has teamed up with the PI (Vidon) to measure the relative importance of OLF, MF and PF during 6-8 storms over a two-year period in an artificially drained Midwestern watershed, and to identify the changes in the nature of in-stream nitrogen (nitrate, ammonium, total Kjeldahl nitrogen (TKN)), phosphorus (soluble reactive phosphorus (SRP), total phosphorus (TP)), and DOC (aromaticity) during storms.

Fieldwork is taking place in a small first order watershed, which is continuously monitored by the U.S. Geological Survey as part of the National Water Quality Assessment Program (NAWQA) for the White River, Great, and Little Miami River Basins. Water quality data have been collected in precipitation and at 2-4 hour intervals during 7 storms in overland flow, tile flow and the stream. Data analysis is underway and the PIs will used a two phase (tile + stream) multi-tracer (chloride, cation, oxygen-18) approach to independently estimate the relative importance of tile drain flow, overland flow, precipitation and seepage in the stream, and the relative importance of matrix flow and preferential flow through soil macropores in tile flow. The potential of DOC and DOC specific UV absorbance (SUVA) as potential hydrologic tracers to identify water sources in a watershed context will also be evaluated.

By providing a direct quantification of the relative importance of each water delivery pathway to NPC transport to streams for a variety of storms and crop development conditions, data collected as part of this project provide an increased understanding of the processes controlling NPC delivery to streams, and provide tools to better target best management practices (BMP) to minimize the impact of agriculture on raw rural water quality in the Midwest.

Problem

Phosphorus, nitrogen and carbon losses to streams affect aquatic productivity, food web structure, and water quality (Martin et al., 1999; Dalzell et al., 2005). Understanding the processes controlling the delivery of these solutes to streams is therefore of paramount importance in order to develop comprehensive watershed nutrient management strategies.

It is well established that most nutrient exports occur during episodic high flow periods (Royer et al., 2006) and that nutrient concentration in streams, hydrological processes and flowpaths often change rapidly during precipitation events in response to variations in precipitation intensity/duration and pre-event moisture conditions (Creed and Band, 1998; Sidle et al., 2000; Hangen et al. 2001; Wigington et al. 2003; Inamdar et al., 2004). The nature of dissolved organic carbon (DOC) (aromaticity, relative abundance of humic/non-humic substances) in streams also often varies during storms, indicating a change in the sources of DOC as a function of discharge (Katsuyama and Ohte, 2002; Hood et al 2006).

High nutrient losses and quick changes in nutrient and carbon concentration/nature during storms stress the importance of conducting research aimed at thoroughly understanding nutrient dynamics and flowpaths during storms. This will increase our ability to predict nutrient and carbon losses at the watershed scale with more precision in the years to come. It is especially important to address this issue in artificially drained landscapes of the Midwest, as agricultural states like Indiana, Ohio and Illinois have been identified as major contributors to excess nutrients in the Mississippi River (Goolsby et al., 2000; Royer et al. 2006).

Recent research has identified preferential flow through soil macropores as an important transport mechanism for solute transport during precipitation events in artificially drained landscapes of the Midwest (Kung et al., 2000a; Stone and Wilson, 2006). Nutrient losses via overland flow in artificially drained landscapes have also been shown to influence the dynamics of NPC losses to streams (Kurz et al., 2005; Royer et al., 2006). Nevertheless, there is a dearth of empirical data documenting the relative importance of overland flow (OLF), matrix flow (MF), and preferential flow through soil macropores (PF) during storms and/or the relative importance of each of these processes on the delivery of nutrients and carbon to streams in artificially drained landscapes of the Midwest.

Research Objectives

Primary objective 1: Identify the relative importance of overland flow, stream bank seepage, matrix flow and preferential flow through soil macropores to streamflow during storms in artificially drained landscapes of the Midwest.

Primary objective 2: Identify the relative importance of each of these water delivery pathways on nitrogen, phosphorus and carbon delivery to streams during storms. Particular attention will be given to characterizing the changes in the nature of N (nitrate, ammonium, total Kjeldahl nitrogen (TKN)), P (soluble reactive phosphorus (SRP), total phosphorus (TP)), and dissolved organic carbon (DOC) (aromaticity) losses to the stream during the storms studied.

Achieving these objectives will help manage raw rural water quality and quantity by allowing landscape managers to better target BMPs, as BMPs often influence soil moisture and water infiltration in soil, and therefore the relative importance of overland flow, matrix flow, and preferential flow through soil macropores. This broad objective is identified as an area of high priority in the RFP FY2007 of the Water Resources Research National Competitive Grant Program, section 104G (page 4).

Two corollary objectives will also be addressed as part of this project:

Corollary objective 1: By monitoring tile drain flow in two tile drains draining two fields under till and no-till, respectively, we will assess the impact of this best management practice (BMP) on raw rural water quality in the watershed.
Corollary objective 2: Assess the potential of using DOC and DOC Specific UV Absorbance (SUVA) to identify the relative contribution of various sources of water to the stream during storms. This objective will contribute to the development of better techniques to assess various components of the water cycle, which is a priority area for the 104G program in 2007 (RFP FY 2007, page 4).

Methodology

The project is field based in nature and is taking take place in the headwaters of Sugar Creek Watershed, in a small watershed (7.2 km^2), locally known as Leary Weber Ditch (LWD). Soils in LWD are suited for row crop agriculture such as corn and soybeans but require artificial drainage to lower the water table, removing ponded water, adding nutrients and ensuring good soil tilth. LWD is representative of many watersheds in the Midwest where poorly drained soils dominate and where artificial drainage is commonly used to lower the water table.

For this project, we quantified water and nutrient fluxes and delivery pathways in LWD for a total of 7 storms in years 1 and 2. These storms varied in duration and intensity and 3 of them generated significant amounts of overland flow. For each storm, a stream water mass balance will be performed (in progress). This approach will allow the team of PIs to identify the relative contribution to discharge of overland flow, tile flow, stream bank seepage, and direct interception of precipitation by the stream. Hydrological tracers (cation, oxygen-18, chloride) will be used to differentiate the relative contribution of new water (event water) and old water (pre-event water) to the stream during each storm, and to differentiate the relative importance of new water and old water in tile drain flow. In tile drains, old water will be considered equivalent to matrix flow (MF) and new water equivalent to preferential flow through soil macropores (PF) (Stone and Wison, 2006). Nitrogen (nitrate, ammonium, total Kjeldahl nitrogen (TKN)), phosphorus (soluble reactive phosphorus (SRP), total phosphorus (TP)) and dissolved organic carbon (DOC) will be measured in overland flow, tile drain flow, streamflow and precipitation to identify the relative importance of each water delivery pathway to nutrient and carbon losses to the stream. The change in the nature of DOC during each storm will be monitored spectrometrically to determine the usability of DOC as a tracer and characterize changes in the sources of DOC to the stream during storms.

Results

Analysis of data for the whole watershed is currently underway. However, three manuscripts looking at water, N and P dynamics in tile drains only (for now) are in various stages of publication (See list below). A summary of the findings presented in these manuscripts is shown here.

In spring, although variations in antecedent water table depth imparted some variation in tile flow response to precipitation, bulk precipitation was the best predictor of mean tile flow, maximum tile flow, time to peak and runoff ratio. The contribution of macropore flow to total flow significantly increased with precipitation amount, and macropore flow represented between 11% and 50% of total drain flow, with peak contributions between 15% and 74% of flow. For large storms (>6 cm bulk precipitation), cations data indicated a dilution of groundwater with new water as discharge peaked. Although no clear dilution or concentration patterns for Mg^{2+} or K^+ were observed for smaller tile flow generating events (<3 cm bulk precipitation), macropore flow still contributed between 11% and 17% of total flow for these moderate size storms.

Bulk precipitation amount had little impact on solute median concentrations in tile-drains during storms, but clearly impacted NO_3^- concentration patterns. For large storms (> 6 cm of bulk precipitation), large amounts of macropore flow (43-50% of total tile-drain flow) diluted NO_3^- rich groundwater as discharge peaked. This pattern was not observed for NH_4^+ and DON or for smaller tile-flow generating events (< 3cm) during which macropore flow contributions were limited (11-17% of total tile-drain flow). Precipitation amount was positively ($P<0.01$) correlated to NO_3^- and NH_4^+ export rates, but not to DON export rates. Limited variations in antecedent water table depth in spring had little influence on N dynamics for the storms studied. Although significant differences in flow characteristics were observed between tile-drains, solute concentration dynamics and macropore flow contributions to total tile-drain flow were similar for adjacent tile-drains. Generally, NO_3^- represented >80% of N load during storms, while DON and NH_4^+ represented only 2-14% and 1-7% of N load, respectively.

Depending on the storm, median concentrations varied between 0.006-0.025 mg/L for SRP and 0.057-0.176 mg/L for TP. For large storms (> 6 cm bulk precipitation), for which macropore flow represented between 43-50% of total tile-drain flow, SRP transport to tile-drains was primarily regulated by macropore flow. For smaller tile-flow generating events (<3 cm bulk precipitation), for which macropore flow only accounted for 11-17% of total tile-drain flow, SRP transport was primarily regulated by matrix flow. Total P transport to tile-drains was primarily regulated by macropore flow regardless of the storm. Soluble reactive P (0.01-1.83 mg/m^2/storm) and TP (0.10-8.64 mg/m^2/storm) export rates were extremely variable and positively significantly correlated to both mean discharge and bulk precipitation. Soluble reactive P accounted for 9.9-15.5% of TP fluxes for small tile-flow generating events (<3 cm bulk precipitation) and for 16.2-22.0% of TP fluxes for large precipitation events (>6 cm bulk precipitation). Although significant variations in tile-flow response to precipitation were observed, no significant differences in SRP and TP concentrations were observed between adjacent tile-drains.

Major Conclusions and Significance

Results presented above significantly increase our understanding of the hydrological functioning of tile-drained fields in spring, when most N losses to streams occur in the US Midwest. In particular, results stress the non-linear behavior of N export to tile drains during spring storms in artificially drained landscapes of the US Midwest, at a critical time of the year for N management in the MRB. For P, results stress the dominance of particulate P and the importance of macropore flow in P transport to tile-drains in the US Midwest. This brings critical insight into P dynamics in tile-drains at a critical time of year for water quality management.

Publications (* = graduate students)

Vidon, P. and P.E. Cuadra*, 2010. Impact of precipitation characteristics on soil hydrology in tile-drained landscapes. *Hydrological Processes*, DOI: 10.1002/hyp.7627 (Online – Early View).

Cuadra*, P.E., and P. Vidon. Storm nitrogen dynamics in tile-drain flow in the US Midwest. *Biogeochemistry* (in review).

Vidon, P., and Cuadra*, P.E. Phosphorus dynamics in tile-drain flow during storms in the US Midwest. *Agricultural Water Management* (in review).

Presentations

Vidon, P, P.E. Cuadra*, 2010. Phosphorus dynamics in tile-drain flow during storms in the US Midwest. Annual meeting of the American Water Resources Association, Philadelphia, PA, November 2010 (Forthcoming)

Hennessy*, M, P. Vidon, 2009. Constraining nitrogen, phosphorus and carbon exports in a Midwestern Agricultural Watershed. American Geophysical Union Joint Assembly, Abstract#H71B-07. page 5, Toronto, ON, Canada, May 2009.

Cuadra*, P.E., P. Vidon, 2009. Natural Variability in Dissolved Organic Carbon and Dissolved Organic Nitrogen Transport in Artificially Drained Landscapes of the U.S. Midwest. Abstract#B34A-04. Page 103, American Geophysical Union Joint Assembly, Toronto, ON, Canada, May 2009.

Grant Submissions n/a

Students

Graduate students: 3
Undergraduate Researchers: 4

Grant No. 08HQGR0007 Web-Based Low Impact Development Decision Support and Planning Tool

Basic Information

Title:	Grant No. 08HQGR0007 Web-Based Low Impact Development Decision Support and Planning Tool
Project Number:	2007IN263S
Start Date:	10/15/2007
End Date:	9/30/2009
Funding Source:	Supplemental
Congressional District:	04
Research Category:	Water Quality
Focus Category:	Water Quality, Management and Planning, None
Descriptors:	
Principal Investigators:	Ron F. Turco, Bernard Engel

Publications

1. Hunter, J.G., Engel B.A. and Quansah, J.E. "Web-based Low Impact Development Decision Support and Planning Tool" 2009 AWRA annual Water Resources Conference
2. Hunter, J.G. "Preliminary Assessment of Stormwater Constructed Wetland and Low Impact Development Practices for Pendleton, IN". Town of Pendleton and Central Indiana Water Resources Partnership – Watershed Initiative. February 06, 2009. Pendleton, IN.
3. Engel, B.A., Hunter, J.G., and Quansah J.E. "Web-Based Decision Support and Planning Tools for Watershed Management". Enhancing our Understanding of Erosion and Sediment Control in the Great Lakes Basin: Developing a Web-Based Toolkit. MN/MI/WI Lake Superior SWCDs Webinar, January 28, 2009.
4. Hunter, J.G. "Web-based Low Impact Development Decision Support and Planning Tool" Web Planning Tools - Hands on Training. Lake Michigan Watershed Academy Conference. Chicago Metropolitan Agency for Planning. Purdue University-Calumet. May 20 - 22, 2008. Hammond, IN.

IWRRC Report

Title: 08HQGR0007 Web-Based Low Impact Development Decision Support and Planning Tool

Funding Period: October 2007 – September 2009

Problem: Mitigating the adverse water quantity/quality impacts of urban development is of national importance. Low Impact Development (LID) practices are increasingly recognized as critical to reduce the impacts of urban and suburban development on water resources, infrastructure costs, aesthetics, biodiversity, and carbon emissions. Increasingly, stormwater best management practices (BMPs) and LID practices have been shown to improve hydrology and water quality on a localized scale. Unfortunately, communities have been slow to adopt these more innovative approaches to urban and suburban development. Significant obstacles to the adoption and acceptance of LID practices by federal, state, and local agencies stem from the lack of tools to quickly and easily predict the impact of LID practices within an area.

Research Objectives: The overall objective of this project is to develop and disseminate a web-based decision support system for LID BMP assessment. The tool enables regulatory and voluntary organizations and watershed practitioners to quickly assess: (1) the impact of proposed development (land use change) on average annual runoff volume and nonpoint source pollution, and (2) the potential stormwater reduction benefits of proposed LID BMPs.

In response to increasing demands for information about LID and tools for comparing LID practices and systems to traditional stormwater systems, we proposed to:

- Develop a postscript for L-THIA that summarizes the results of computations for estimation of the average annual runoff volume change due to LID;
- Modify L-THIA to include LID practices and systems (e.g., create L-THIA/LID);
- Demonstrate the application of L-THIA/LID in case studies;
- Disseminate L-THIA/LID via the internet as freeware for use throughout the nation.

Methodology: The L-THIA/LID model employs the NRCS (formerly SCS) curve number method and event mean concentration (EMC) methods for simulation of surface runoff and NPS loading. The L-THIA (Long-Term Hydrologic Impact Assessment) model and decision support capabilities estimate long-term average annual runoff and NPS pollutants for a land use configuration based on 30-year climate data, soils, and land use data for an area.

As in the existing L-THIA program, users are required to identify the area where land use is expected to change, select a land use-LID combination, and request an L-THIA run. Before and after water quantity and water quality results of the proposed land use change will be displayed in tables, bar charts, and pie charts.

Principal Findings: Models and decision support tools are needed to help quantify the site specific impacts of LID practices to allow the identification of the most appropriate

BMPs.   L-THIA/LID is an easy to use tool developed to readily provide data for such needed analysis.

Summary: L-THIA/LID has been developed as a simple to use screening tool to evaluate the benefits of LID practices. As in the existing L-THIA program, users identify the area of land use change, select a land use-LID combination, and request an L-THIA run. Water quantity and water quality results of the proposed land use change and proposed LID scenarios will be displayed in tables, bar charts, and pie charts. Case studies are currently being used to demonstrate the model's ability to assess the impact of LID practices within a watershed. The model's results will enable decision makers to formulate an effective watershed management plan to achieve desired stormwater management and water quality goals. Our experience with similar decision support tools suggests that this tool will be well received and would have a significant user base nationally, thereby facilitating the promotion of LID practices. The tool, along with its supporting documentation, is available online as freeware at the Purdue University website.

Results and Significance: To meet the principle decision making needs of individuals and organizations looking to utilize L-THIA/LID, two interrelated screening levels (basic screening and lot level level screening) were developed.

L-THIA/LID consists of a "basic" screening analysis, where users can make adjustments at the watershed and site level to reflect the following "basic" LID concepts to:

- Reduce impervious surfaces (in percent area) for developed land use
- Conserve infiltratable soils
- Conserve hydrologically functional/sensitive landscape
- Minimize land disturbances

The "basic" screening input allows the user to create pre-development and post-development scenarios to employ the conversation, minimization, and reduction of imperviousness LID concepts. Once the scenarios are chosen, the user can adjust the percent impervious surface for the developed land use to account for percent area reductions related to sidewalks, driveways, parking lots, streets, and roofs.

L-THIA/LID also allows the user to customize landuse attributes at the "lot level" based on adjustments to landscape features to reflect LID practices at the microscale. Provided is a list of the LID practices is been incorporated in L-THIA/LID to simulate "lot level" LID practices and analysis: The tool allows users to add or remove different LID practices of interest and adjust the design measurement of these practices to evaluate the optimal runoff and NPS pollution reduction potentials. The LID practices included are:

- Preservation of natural features / conservation of functional landscape
- Reduction in compaction
- Green roofs
- Narrowing impervious areas (sidewalks, driveways, roads)
- Porous Pavement
- Bioretention (i.e. rain gardens)
- Rain barrels/cisterns - these devices are for water harvesting

- Disconnectivity of downspouts to sewers
- Conservation - Forest, Wetlands
- Drainage swales
- Reduce imperviousness
- Conservation of A&B soils

Major Conclusions: The primary goal of this project was to develop and disseminate a stakeholder-driven web-based tool for evaluating the performance of LID practices. L-THIA/LID decision support tool and supporting materials will enable decision makers to formulate effective watershed management plans to achieve desired stormwater management and water quality goals. Stakeholders will be able to quickly identify critical land use issues, anticipate pollutant types and levels, analyze potential improvement from implementation of LID practices and prioritize watershed management and water quality goals.

L-THIA/LID supports "sustainable" and "green" urban and neighborhood design by helping easily and quickly quantifying the location specific impacts of LID practices. By getting this tool to builders, developers, and local planning officials, we believe the LID practices will become more widely understood, accepted, and implemented. L-THIA/LID intends to bridge the gap between researchers, engineers, and the development community.

Publications: https://engineering.purdue.edu/mapserve/LTHIA7/lthianew/lidIntro.htm

Hunter, J.G., Engel B.A. and Quansah, J.E. "Web-based Low Impact Development Decision Support and Planning Tool" 2009 AWRA annual Water Resources Conference

Hunter, J.G., Quansah, J.E., and Engel, B.A. (2009) "Web-based Low Impact Development Decision Support and Planning Tool". in preparation for submission.

Hunter, J.G. "Preliminary Assessment of Stormwater Constructed Wetland and Low Impact Development Practices for Pendleton, IN". Town of Pendleton and Central Indiana Water Resources Partnership – Watershed Initiative. February 06, 2009. Pendleton, IN.

Engel, B.A., Hunter, J.G., and Quansah J.E. "Web-Based Decision Support and Planning Tools for Watershed Management". Enhancing our Understanding of Erosion and Sediment Control in the Great Lakes Basin: Developing a Web-Based Toolkit. MN/MI/WI Lake Superior SWCDs Webinar, January 28, 2009.

Hunter, J.G. "Web-based Low Impact Development Decision Support and Planning Tool" Web Planning Tools - Hands on Training. Lake Michigan Watershed Academy Conference. Chicago Metropolitan Agency for Planning. Purdue University-Calumet. May 20 - 22, 2008. Hammond, IN.

Students: (1) Graduate Students: Prathima Rao

Award No 08HQGR0079 Web-Based Load Duration Curve for TMDL

Basic Information

Title:	Award No 08HQGR0079 Web-Based Load Duration Curve for TMDL
Project Number:	2008IN294S
Start Date:	4/10/2008
End Date:	4/9/2009
Funding Source:	Supplemental
Congressional District:	4
Research Category:	Climate and Hydrologic Processes
Focus Category:	Models, Management and Planning, Agriculture
Descriptors:	flow and load duration curves TMDL
Principal Investigators:	Bernard Engel

Publications

1. A straight forward user manual/documentation and demonstration video was developed and placed on the tool webpage page for free access by stakeholders and other users to easily follow to use the tool: https://engineering.purdue.edu/~ldc
2. Eckhardt K., 2005. How to Construct Recursive Digital Filters for Baseflow Separation. Hydrological Processes 19(2): 507-515.
3. USEPA, 2007. An Approach for Using Load Duration Curves in the Development of TMDLs, EPA 841-B-07-006, August 2007. Watershed Branch (4503T), Office of Wetlands, Oceans and Watersheds, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave. NW . Washington, DC 20460. http://www.epa.gov/OWOW/TMDL/techsupp.html
4. Robert L. R., G. C. Charles, and A. C. Timothy, 2004. Load Estimator (LOADEST): A Fortran Program for Estimating Constituent Loads in Streams and Rivers. Techniques and Methods Book 4, Chapter A5.
5. SJRWI, 2006. St. Joseph River watershed management plan; three states, six counties, one watershed. SJRWI, Fort Wayne, Indiana. SJRWI, Fort Wayne, Indiana.

IWRRC Report

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Title: 08HQGR0079 - Web-based Load Duration Curve (LDC) Tool for TMDL

Funding Period: April 10, 2009 – September 30, 2009

Problem:

While flow and load duration curves are increasingly used in the development of TMDLs, the complexity and time requirement of developing these curves, using EPA developed excel spreadsheets, serve as a limitation for most stakeholders in using load duration curve approach to TMDL development. Further, flow and load duration curves are useful for other watershed analyses.

Research Objectives:

The goal of this project was to develop a simple web-based tool to assist stakeholders with easy and rapid development of flow and load duration curves that can be used in support of TMDL development. The specific objectives were to:

1. Develop a web-based prototype tool that creates flow duration curves as described in a slideshow titled Flow Duration Curves – The Basics: Guide to Use of Spreadsheet created by Bruch Cleland. The web-based tool will use WHAT capabilities to obtain stream flow data. New capabilities will be created to perform flow duration analysis, graph the results, allow the user to upload water quality data, and create load duration curves.
2. Obtain feedback on the prototype web-based tool and use this feedback to create the final tool.
3. Develop documentation describing web-based flow and load duration tool.
4. Conduct a workshop for state TMDL personnel.
5. Make the web-based tool and supporting documentation available to potential users through a freely accessible web site.

Methodology:

The Web-based Load Duration Curve (LDC) system overview is shown in Figure 1. To generate a LDC, the flow duration curve (FDC) is first generated using flow data, which is then used with water quality standard to develop an LDC. All procedures described in Figure 1 are automated with Perl/CGI, GNUPLOT, and JavaScript programming as well as Google Maps API scripts. The web-based tool interface can be accessed at https://engineering.purdue.edu/~ldc

Development of Flow Duration Curve (FDC) Module

The Web-based LDC system module reads the daily stream flow data and water quality data, either prepared by a user or retrieved directly from a USGS remote server using the Google Map interface. The Google Map-based interface was developed and integrated with the Web-based LDC system for collecting stream flow data from USGS gauging stations. The Google Map interface of the Web-based LDC system links the USGS data server and the data stored at the remote server for automatic retrieval of stream flow and water quality data to generate the FDC and the LDC. The user entered or USGS retrieved daily flow data are then sorted from the highest to the lowest to calculate percentage of days these flows are exceeded to generate the FDC data and graph (USEPA, 2007) using GNUPLOT scripting and CGI programming (Figure 3). In areas of interest with no gauging station but with available water quality data, users can enter drainage area ratio in the input interface to calculate flow data from a nearby gauging site.

The flow data are grouped into 5 flow regimes or zones, namely High-Flows, Moist-Conditions, Mid-range Flows, Dry-Conditions, and Low-Flows, represented by 0-10 %, 10-40%, 40-60%, 60-90%, 90-100% of flow duration intervals, respectively. These 5 zones can be used to explain the watershed characteristics and flow patterns according to hydrologic condition.

The digital filter method and LOADEST model were also added to the Web-based LDC system to separate surface runoff from the streamflow in calculating the daily pollutant loads.

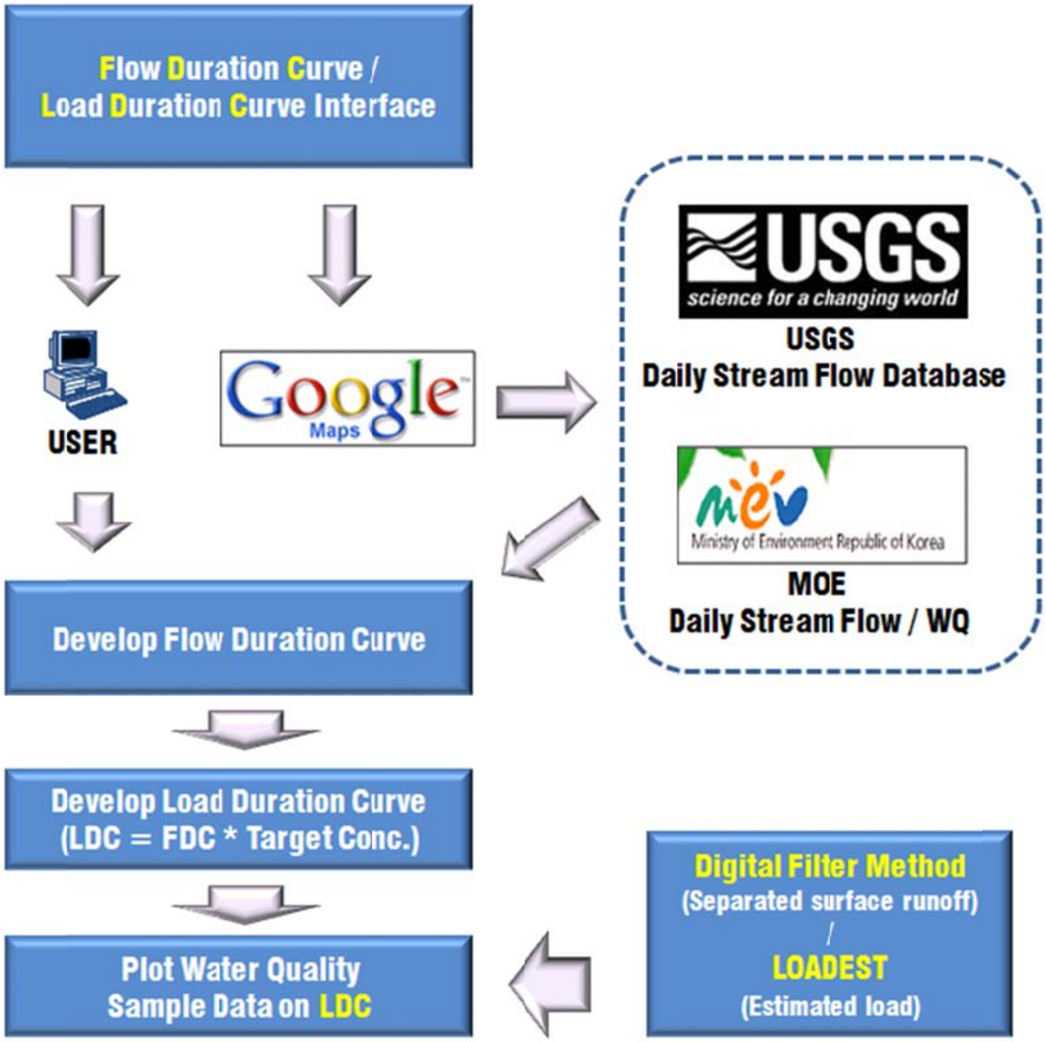

Figure 1. Overview of the Web-based Load Duration Curve System

Development of Load Duration Curve (LDC) Module

The pollutant load data to generate the Load Duration Curve (LDC) (Figures 4 and 5) are calculated by multiplying the daily stream flow data from FDC by the water quality standard value entered by users in the input interface (Figure 1). The observed pollutant loads are plotted on the Load Duration Curve in order to help the users compare measured water quality data with the load duration curve (Figure 4.) This plotted water quality data explain exceedance of the water quality standards and the associated allowable loading. In addition, box-whisker plots on the LDC provide water quality distribution for each flow duration regime. The section of box-whisker plots were placed at the center of the High-Flow, Moist-condition, Mid-range flow, Dry-condition, and Low-Flow zones at the 5th, 25th, 50th, 75th, and 95th percentiles, respectively. The box-whisker plots for each flow duration curve zone can be used to interpret the water quality condition and more easily understand watershed characteristics.

To better interpret the hydrologic condition and watershed characteristics, a surface-runoff separation module, i.e., baseflow separation from the stream flow, was developed in the tool to provide more information in the FDC and LDC using Perl/CGI programming and GNU scripting. The surface-runoff separation module separates the surface runoff component from the stream flow and determines the surface runoff ratio by dividing the surface runoff by the stream flow data. If the surface runoff exceeds 50% of the total stream flow, the surface runoff separation module plots the load data in different symbol to provide pollutant loads characteristics under various flow conditions. The digital filter method, developed Eckhardt (2005) for separating surface-runoff considering a digital filter parameter and BFI_{max} (maximum value of long-term ratio of base flow to total stream flow), was used for surface-runoff separation module of the Web-based Load Duration Curve system.

A pollutant load estimation module, using the LOADEST model as a core engine (Robert et al., 2004), was also developed and integrated with the Web-based LDC system with Perl/CGI programming and GNUPLOT scripts to provide daily pollutant load for comparison of estimated loads with the observed load. Daily pollutant load data, are thus, calculated using the LOADEST model, and plotted over the load duration curve to provide general pollutant load characteristics over the various flow regime. This allows daily load estimation plotted on the LDC to be compared with measured pollutant load data plotted on the LDC.

Dr. Indrajeet Chaubey presented the tool in a workshop for state TMDL personnel within US EPA Region 5. Feed back from the workshop was incorporated into the final web-based LDC tool to improve usability and clarity.

Principal Findings

Summary

The web-based LDC tool provides a very quick means of generating FDC and LDC for analyzing the potential level to which water quality standards within a watershed are in or out of compliance. The estimation of pollution load will serve as a useful tool for the development of TMDLs for different pollutants of concern and other watershed analyses.

Results and Significance

The tool generates simple understandable LDC and FDC Graphs and downloadable tables for easy, yet significant analysis for TMDL development. The tool is online and assessable to all stakeholders, research institutions, and watershed planners across the country and globally.

The project team fields several questions each month regarding the application of the tool. Anecdotal evidence indicates users in contact with the project team find the tool to be helpful in their various efforts.

Major Conclusions
Since the tool requires water quality data for the generation of LDC, it will be very useful to develop the tool to directly upload water quality data from various gauge stations across the country. Potentially the tool could be linked to US EPA STORET WQ dataset. The tool could also be upgraded with capabilities for delineating watersheds to compute drainage area ratios or to delineate watersheds with EPA STORET and USGS gauging stations to compute the watershed area ratios.

Publications
A straight forward user manual/documentation and demonstration video was developed and placed on the tool webpage page for free access by stakeholders and other users to easily follow to use the tool: https://engineering.purdue.edu/~ldc

A draft journal paper entitled "Development of Web-based Load Duration Curve System for Analysis of TMDL and Water Quality Characteristics" have been completed for further review and publication.

Students
Joseph Quansah and James Hunter, both post doctoral associates, assisted with the project effort. Graduate students within Dr. Chaubey's group assisted with testing of the tool.

Graphical Demonstration of the Web-based Load Duration Curve Tool

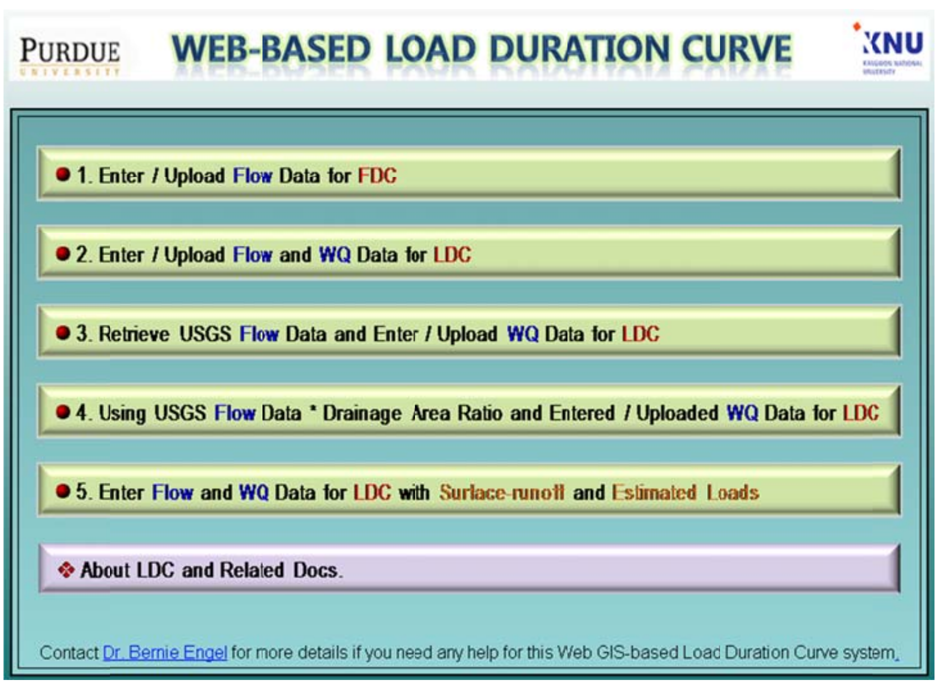

Figure 2. Web-based Load Duration Curve tool interface

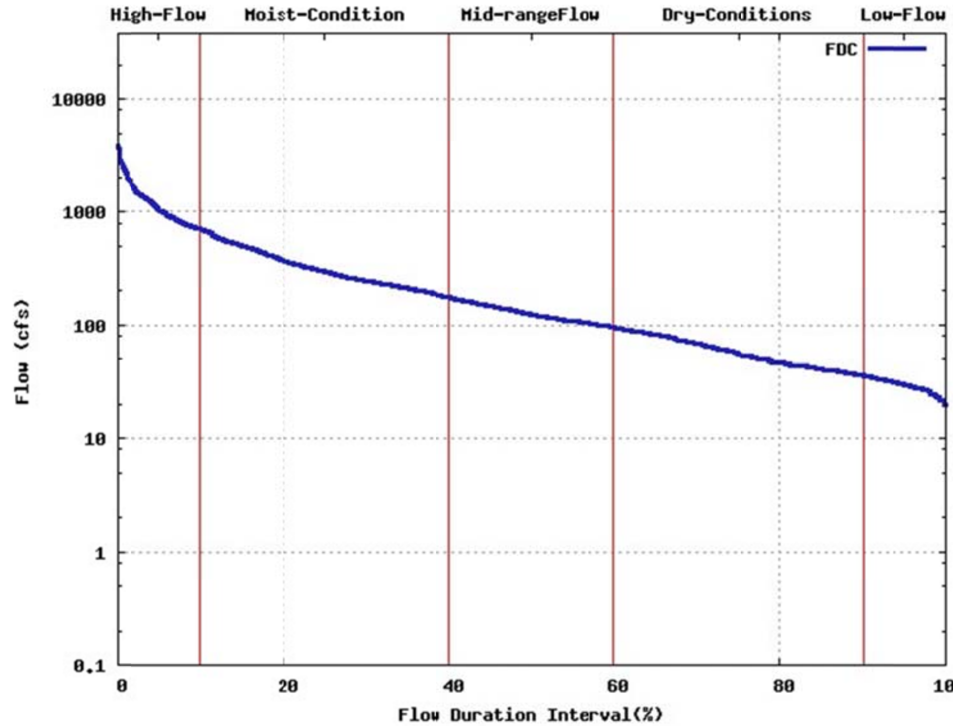

Figure 3. Flow Duration Curve (2002-2007) for USGS Gauge 04180000, Cedar Creek, IN

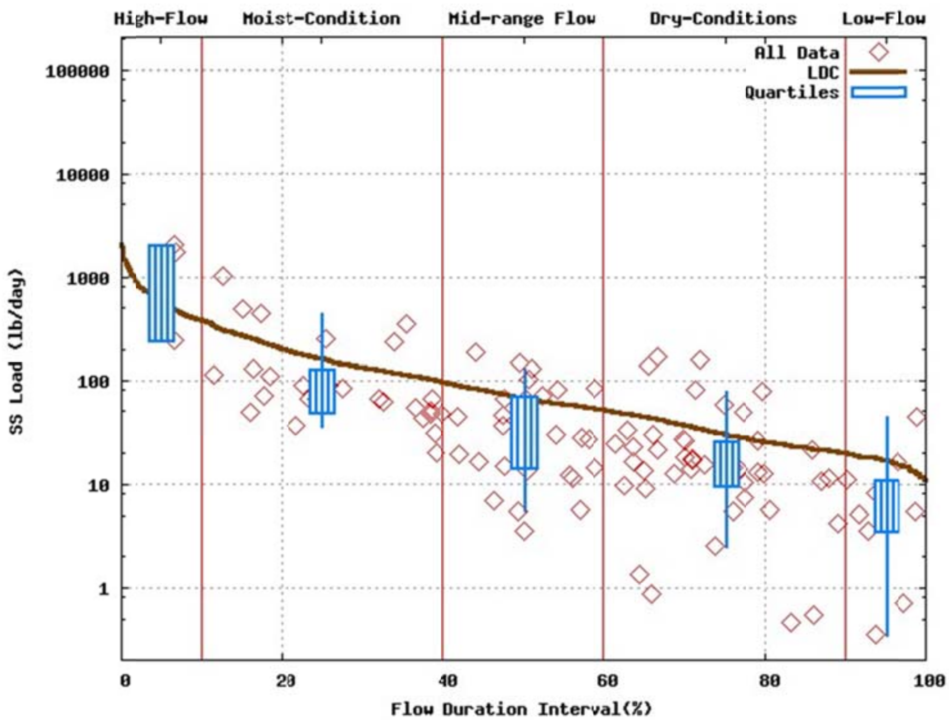

Figure 4. LDC (2002-2007) for Ammonia at USGS Gauge 04180000, Cedar Creek, IN

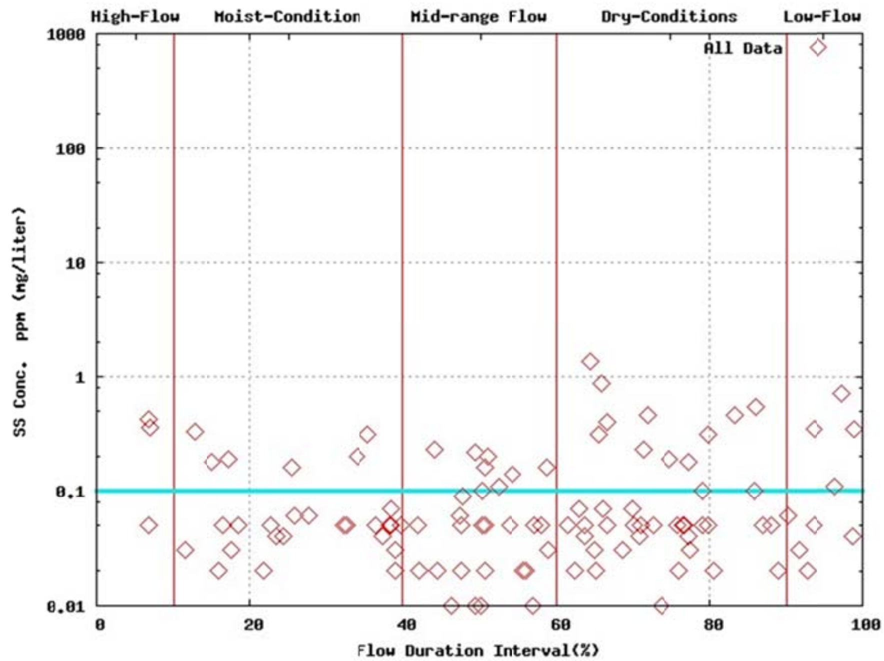

Figure 5. Target Concentration Graph for Ammonia at USGS Gauge 04180000, Cedar Creek, IN

Eckhardt K., 2005. How to Construct Recursive Digital Filters for Baseflow Separation. Hydrological Processes 19(2): 507-515.

USEPA, 2007. *An Approach for Using Load Duration Curves in the Development of TMDLs*, EPA 841-B-07-006, August 2007. Watershed Branch (4503T), Office of Wetlands, Oceans and Watersheds, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave. NW . Washington, DC 20460. http://www.epa.gov/OWOW/TMDL/techsupp.html

Robert L. R., G. C. Charles, and A. C. Timothy, 2004. Load Estimator (LOADEST): A Fortran Program for Estimating Constituent Loads in Streams and Rivers. Techniques and Methods Book 4, Chapter A5.

SJRWI, 2006. St. Joseph River watershed management plan; three states, six counties, one watershed. SJRWI, Fort Wayne, Indiana. SJRWI, Fort Wayne, Indiana.

Remote Sensing of Water Quality Indicators in the Wabash River

Basic Information

Title:	Remote Sensing of Water Quality Indicators in the Wabash River
Project Number:	2009IN219B
Start Date:	3/1/2009
End Date:	2/28/2010
Funding Source:	104B
Congressional District:	4
Research Category:	Water Quality
Focus Category:	Agriculture, Hydrology, Management and Planning
Descriptors:	
Principal Investigators:	Keith Cherkauer, Indrajeet Chaubey

Publications

There are no publications.

IWRRC 2009 Project Reports (Information needed by May 20, 2010)

State: IN **Project Number:** 2009IN219B
Title: Remote Sensing of Water Quality Indicators in the Wabash River **Project Type:** Research
Focus Category: Agriculture, Hydrology, Management and Planning
Keywords: water quality, remote sensing, sediment transport, Wabash River
Start Date: 3/1/2009 **End Date:** 2/28/2010
Congressional District: 4 **PI:** Cherkauer, Keith Assistant Professor, Purdue University email:cherkaue@purdue.edu phone:765-496-7982 **Co-PI(s):** Chaubey, Indrajeet Associate Professor, Purdue University email:ichaubey@purdue.edu phone:7654945013

Report Format

Abstract / Summary

Water quality is a major issue for rivers in the agriculturally dominated Midwestern United States. Most monitoring of water quality relies on the installation of expensive real-time monitoring stations, or the selection of permanent sampling sites where samples are collected by hand. The former results in good temporal resolution but is limited by cost to relatively few locations, the latter method allows the spatial coverage of the samples to increase at the expense of collecting samples less frequently, on a monthly basis for example. Remote sensing imagery has been used to monitor water quality on lakes, reservoirs and costal zones, providing high spatial resolution with relatively regular sampling frequencies interrupted only by clouds, however, their applicability in determining river water quality is not well tested. This project applies existing techniques for extracting water quality information from remote sensing images, to images of the Wabash River to test the ability of satellite-based sensors to monitor water quality in this and other Midwestern river systems. In-situ water samples and spectral measurements are collected coincident with satellite overpasses to develop a database of spectral signatures related to current flow and water quality conditions within the river channel. This information will be used to calibrate and evaluate the extraction of water quality parameters, such as chlorophyll-a (chl-a), total suspended solids (TSS) and dissolved organic matter (DOM), from remote sensing images. We expect this project to result in a better understanding of which image processing algorithms are effective and what sensor characteristics are required to produce high-quality maps of the spatial distribution of regional water quality. This will eventually lead to the development of near real-time maps for the evaluation and monitoring of water quality in the region.

Problem:

Excess concentrations of sediment and nutrients are two primary sources of water quality impairment in the rivers of the Midwestern United States (National Research Council, 2008). Non-point source pollution originating from agricultural activities is the dominant source of sediment and nutrients to these rivers (Alexander et al., 2008). With the increased emphasis on biomass for biofuel production, significant land use and land management changes are occurring at a rapid pace in Midwestern watersheds. For example, increased corn production to meet grain-based ethanol production in the near future and increased removal of biomass from the landscape for cellulosic ethanol conversion in the long-term, can potentially increase sediment and nutrient delivery to receiving water bodies, thus exacerbating the current water quality problems in this area. Midwestern rivers, especially in Indiana, are also major contributors to downstream problems in water quality, especially the hypoxic zone in the Gulf of Mexico (Burkhart and James, 1999), which has increased by 300% since 1970 (Goolsby et al., 2001).

Assessing water quality is critical for its management and improvement. In-situ measurements of stream characteristics (e.g. stream width, depth, substrate quality) and the collection of water samples for subsequent laboratory analyses are accurate but expensive and time consuming. Because of these limitations, stream habitat and water quality assessments have been limited to less than 40% of rivers in the USA (EPA, 2007). Even for those streams that have been evaluated using in-situ data, knowledge of stream conditions remains fragmented (Marcus and Fonstad, 2007). Remote sensing could be an alternative means of monitoring optically active constituents (OAC) of water quality over a greater range of temporal and spatial scales. Remote sensing data could also be a valuable source for the retrieval of historic water quality information from unmonitored sites.

Research Objectives:

Our primary objective for this research is to evaluate existing algorithms for the assessment of water quality in oceans, lakes and reservoirs as applied to river systems in the Wabash River using satellite remote sensing and in-stream sample analysis. Specific research questions that will be addressed are:

1. *How well do current remote sensing methods do at obtaining water quality measurements for Midwestern Rivers in agriculturally dominated environments, especially when sediment load is the primary pollutant?*
2. *Can we observe inter-seasonal changes in spatially distributed water quality, and how does that differ from what is measured from in-situ observations?*

Methodology:

In-situ sampling, Task 1

We have collected *in-situ* observations of water quality and water temperature from several sites along the Wabash River between Delphi and Attica, Indiana and its primary tributaries. These have been collected primarily by lowering a sampling chamber into the river from crossing bridges. When the sample chamber reaches a depth of 20-30 cm the doors are triggered trapping a water sample. Samples have been collected from both sides of each bridge to coincide with five over flights by the Landsat 5 TM and Landsat 7 ETM+ sensors. All samples were analyzed for nutrients (e.g. NO_3-N, NH_4-N, PO_4-P, and TP), and Major optically active constituents (OAC) of water that are predictable using remote sensing: chlorophyll-a (chl-a), total suspended solids (TSS) and dissolved organic matter (DOM). Water samples have also been collected from a boat in the Wabash

River, allowing more detailed collection of spatial water quality information.

Spectral sampling, Task 2

Spectrometers will be used to collect water surface reflectance for a variety of conditions by sampling from the sides of bridges, in conjunction with the water samples taken in Task 1. Measurements will be used to establish a baseline of reflectance values.

Remote sensing data collection, Task 3

Ground truth collection, Task 3.1

We will arrange to have project participants work to collect both *in-situ* (Task 1) and spectrometer data (Task 2) coincident with scheduled image acquisitions from satellite-based sensor platforms. We have now established nine bridges from which samples can be collected for the Wabash and Tippecanoe Rivers within a 2 hour window of image acquisition.

Satellite-based observations, Task 3.2

We continue to collect 30 m resolution Landsat 5 and 7 images. Between the two satellites a sensor passes over the region on average every eight days, though days with cloud cover or low river flows are not useable. When the weather and river conditions are suitable for a good image, we conduct the *in-situ* observations described in Task 1.

Analysis of remote sensing imagery, Task 3.3

We will use various commercial software packages to extract water quality components from the remote sensing imagery. These estimates will be compared with spectrometer and *in situ* measurements for the same time period to determine which equations and sensor platforms produce the most accurate estimates under different flow conditions.

Results

Despite collecting ground truth for all days with a satellite overpass and mostly clear skies, we succeeded in obtaining only two usable images during the summer of 2009, one of which is still partially obscured by cloud cover. We are therefore continuing our efforts in the spring and summer of 2010. We did, however, leverage some funds from this project to obtain additional support from the Laboratory for Applications of Remote Sensing (LARS) to get a couple of lines flown over the Wabash River from a airplane mounted hyperspectral imager that was in the area for another project. The preliminary data for parts of both reaches is show below. The overflight was coordinated with the collection of 18 water samples from a boat on the Wabash and Tippecanoe Rivers. We are expecting the remote sensing images to be available in the next few weeks along with the results of the water quality analysis. In addition, as we've waited for good images of the Wabash River, we have been practicing the analysis techniques that we will need on a collection of images acquired by Dr. Cherkauer for the Green, Cedar and Yakima Rivers in Washington.

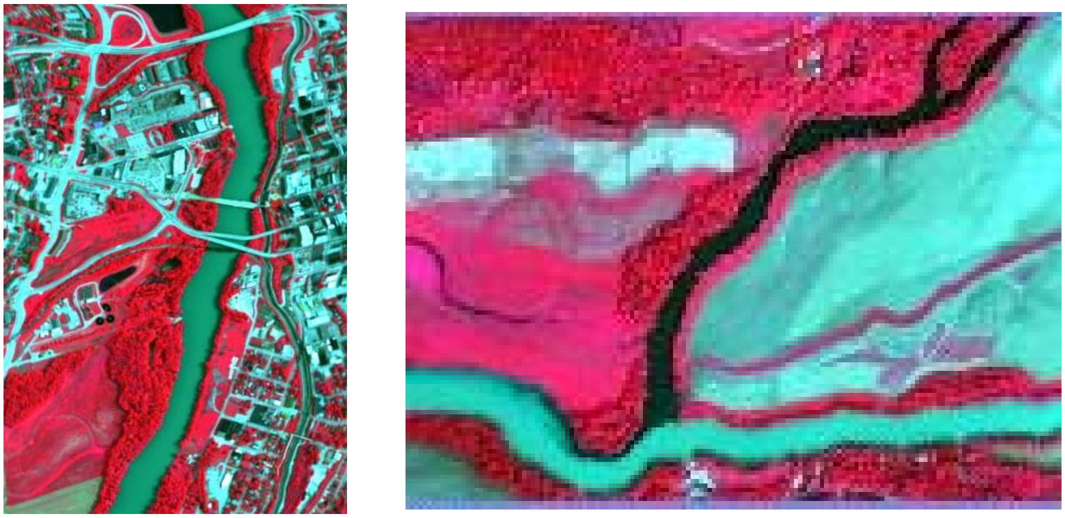

Figure 1. Quick look images extracted from hyperspectral data collected over two reaches of the Wabash River: (left) between Lafayette and West Lafayette, and (right) at the confluence with the Tippecanoe River showing the clear (dark) water from the Tippecanoe River mixing with the turbid water from the Wabash as it mixes moving downstream (to the left in the picture).

Major Conclusions and Significance

Water quality is a major concern on the Wabash River and other agriculturally dominated river systems in the United States and globally. Understanding the sources of water quality problems, and how water quality varies temporally and spatially at resolutions higher than is typically collected using in-situ sampling are important for monitoring existing problems and the effectiveness of best management practices (BMPs) currently being employed. Significant differences in water quality between the Wabash River and the Tippecanoe River (a major tributary), are easily visible from remote sensing imagery. What still remains to be determined is if water quality indicators can be related to the OACs visible by remote sensing, and if satellite remote sensing is a useful tool for such a job.

Publications

http://www.agry.purdue.edu/hydrology/Projects/WabashWaterQuality.asp

Grant Submissions:

IL-IN Sea Grant, *Pending*, Co-PIs: I. Chaubey (ABE), C. Troy (CIVL), "Monitoring episodic river inflow plumes using in-situ and remote sensing data."

NASA NEWS-WQ, *Not Funded*, Co PIs: I. Chaubey (ABE), C. Bachmann (NRL), "Remote Sensing of Water Quality in Midwestern Rivers."

Students

Jing Tan, Master's student, ABE

Laurent Ahiablame, Ph.D., ABE
Joshua Seidner, undergraduate, ABE

Fish Responses to Sediment Associated with Increasing Biofuel-related Crop Production in the Wabash River Watershed

Basic Information

Title:	Fish Responses to Sediment Associated with Increasing Biofuel-related Crop Production in the Wabash River Watershed
Project Number:	2009IN242B
Start Date:	3/1/2009
End Date:	2/28/2010
Funding Source:	104B
Congressional District:	4
Research Category:	Biological Sciences
Focus Category:	Agriculture, Conservation, Ecology
Descriptors:	
Principal Investigators:	Reuben R Goforth

Publications

There are no publications.

IWRRC 2009 Project Report

State: IN **Project Number:** 2009IN242B
Title: Fish Responses to Sediment Associated with Increasing Biofuel-related Crop Production in the Wabash River Watershed
Project Type: Research
Focus Category: Agriculture, Conservation, Ecology
Keywords: Biofuels, Stream fish ecology, Suspended sediments, Respirometry, Central Stoneroller, *Campostoma anomalum*, Blacknose Dace, *Rhinichthys atratulus*, Mottled sculpin, *Cottus bairdii*, Raindow darter, *Etheostoma caruleum*
Start Date: 3/1/2009 **End Date:** 2/28/2010
Congressional District: 4 **PI:** Goforth, Reuben R email:rgoforth@purdue.edu

Abstract / Summary

Among the potential environmental changes expected to result from shifts in agricultural practices to favor biofuel raw materials is increased sedimentation of streams. Sediment levels above those that naturally occur in streams are known to negatively affect many stream fish species, although threshold responses of stream fish to suspended sediment levels are unknown. We have begun testing the acute responses (i.e., 3 hr exposure) of representative stream fish to increased levels of suspended sediment in experimental chambers designed to measure the fishes' oxygen consumption as a proxy of stress. To date, our results indicate that blacknose dace (*Rhinichthys atratulus*) exhibit significantly greater oxygen consumption when exposed to chamber sediment levels of 0.5 and 1.0 g/L. Central stonerollers (*Campostoma anomalum*) exposed to 0.5 g/L sediment did not exhibit higher oxygen consumption, although they did exhibit significantly greater oxygen consumption in 1.0 g/L treatments compared to the sediment free controls. Mottled sculpin (*Cottus bairdii*) >5.0 g somatic wet weight did not exhibit increased oxygen consumption rates when exposed to 1.0 g/L sediment concentrations, and this was also true for mottled sculpin <5.0 g somatic weight. However, smaller mottled sculpin were exposed to a wider variety of sediment concentrations and exhibited significantly higher oxygen consumption when exposed to sediment levels of 1.75 g/L. Interestingly, small mottled sculpin exposed to 2.5 and 5.0 g/L sediment did not exhibit significantly greater oxygen consumption rates compared to no-sediment controls, suggesting that this species may decrease metabolic activity once a sediment threshold has been exceeded. Finally, rainbow darters (*Etheostoma caeruleum*) did not exhibit different oxygen consumption between no-sediment controls and 1.0 g/L sediment concentrations. Our results provide the proof of concept to support continued experimentation with these species to determine potential threshold responses over a wider range of sediment levels. The implications thus far are that stream fish tend to exhibit increased respiration rates when exposed to increased sediment concentrations. Based on the small mottled sculpin trials, there is also the potential that fish respond to levels above a threshold by lowering their metabolism to avoid exposing their gills to the damaging effects of suspended sediments. Managing landscapes to prevent sediment levels from surpassing these thresholds could help to protect fish assemblages of streams in intensively managed landscapes such as those used for biofuel cropping.

Problem

Agriculture has long been critical for sustaining human populations over local, regional, national, and global scales. Additional agricultural demands for raw materials beyond food products are rapidly emerging, especially with regard to biofuels. Indiana has great production capacity to provide raw materials for the biofuel industry. As a result, the Indiana State Department of Agriculture has identified maximization of Indiana's competitive advantage in agriculturally derived energy as one of seven key initiatives in its strategic plan. The potential for biofuels development in Indiana is huge, and there is great need to develop a sustainable framework for providing raw materials for this industry. Among the components critical for this sustainable development is consideration of the potential effects of biofuel cropping on stream resources.

Agriculture, like other human land uses, has undeniably altered stream ecosystems at both local and landscape scales (Richards and Host 1994, Allan et al. 1997, Richards et al. 1997, Wang et al. 2003). Management practices such as crop rotation and no-till agriculture have been successful in at least moderating these impacts. While these improved land use practices have the potential to protect stream biodiversity, growing demands for biofuel-related raw materials and animal products may be intensifying the environmental effects of agricultural land uses. For example, farmers are expected to alter crop rotation cycles and tillage practices, increase nutrient and pesticide applications, and convert more land, both existing agricultural and non-agricultural, into corn production in response to growing biofuel demands. Past research has demonstrated that such changes in land management can influence the hydrology, sediment yields, nutrient regimes and biological communities of streams within these landscapes (Newcombe and MacDonald, 1991; Sigler et al., 1983; Reid et al., 2003; Zimmerman et al., 2003; Vincent et al., 2004). These physicochemical changes have great potential to generate a cascade response within aquatic ecosystems, whereby biological communities and ecological functions become altered over multiple spatial scales (e.g., Richards et al. 1996, Allan and Johnson 1997, Roth et al. 1997, Goforth and Bain *in review*, Goforth et al. *in prep*). The resulting changes may have substantial environmental consequences for water resources ranging from local biological community structure and ecosystem function to cumulative impacts such as Gulf of Mexico hypoxia (e.g., Royer et al. 2006). It is therefore critical that we evaluate the potential stream changes that may accompany shifts in agricultural production to favor raw materials for the biofuel industry so that strategies for maximizing agricultural production while protecting stream resources can be developed.

Elevated sediment concentrations in streams, particularly fine suspended sediments, are a highly noticeable consequence of widespread agricultural land use. In fact, many people familiar with the Wabash River refer to it as a "dirty river" due to its chronic and highly visible suspended sediments. These sediments are a product of both existing bed materials in the river and fine particles contributed from tributary streams. It is the interface between small streams and agricultural lands that is likely to be the greatest source of suspended sediments in the main stem of the Wabash River based on sheer numbers of river miles represented by the smaller streams. It is therefore important to consider not only the water quality of the Wabash River itself, but also its tributaries in management efforts to improve water quality of this big river ecosystem. Thus, setting biologically relevant regulatory criteria is dependent upon understanding the sensitivity of representative biota from headwaters to the main stem of the river.

Research Objectives:

1) *Evaluate the specific responses of four fish species to increased sediment loads projected to accompany increased biofuel-related crop production.* The working hypothesis is that stream fish exhibit specific responses to land use-related environmental changes in streams, the responses vary among fish species, and thresholds exist for these responses that may have little current regulatory significance but high biotic relevance. I am working with four undergraduate research technicians to develop and implement research to attempt to identify such thresholds in four fish species common to tributaries (i.e., mottled sculpin, *Cottus bairdi*; rainbow darter, *Etheostoma caeruleum*; blacknose dace, *Rhinichthys atratulus*; and central stoneroller, *Campostoma anomalum*) of the Wabash River in the vicinity of West Lafayette, Indiana.
2) *Use the results of this project to contribute to sustainable Indiana biofuel development.* My students and I will communicate the results of this project to Purdue Extension colleagues, State of Indiana agency personnel, and other stakeholders to help inform efforts to achieve biofuel competitive advantage within the State while promoting environmental sustainability. Products will include poster presentations, at least one peer-reviewed journal article, extension fact sheets, a project website, and other outreach publications.

Methodology

We are using microcosm experiments to evaluate the responses of fluvial specialist species (i.e., those species that require moving water habitats to complete their life histories and thus persist within a stream) to simulated sediment regimes. Fluvial specialist species serving as subjects in the respiration trials include mottled sculpin (*Cottus bairdii*), rainbow darter (*Etheostoma caeruleum*), blacknose dace (*Rhinichthys atratulus*), and central stoneroller (*Campostoma anomalum*). All fish used in experimental trials were measured for fork or total length (± 1 mm) and weighed (±0.01 g). Acute fish responses to suspended sediments are being measured based on oxygen consumption (i.e., via respirometry) in custom-designed and constructed respirometers. These respirometers are closed systems with an integrated water pump for unidirectional flow that maintains fine sediment (i.e., <250 µm particle size) in suspension during the trials. We are currently using small and medium chambers with volumes of 0.80 L and 1.28 L, respectively. The small chambers are used for fish < 5.0 g somatic weight, and the medium chambers are used for fish >5.0 g somatic weight. The chambers are fitted with a Jenway 2000 dissolved oxygen probe (±0.01 mg/L) to monitor oxygen levels during the trials. Individual fish are sealed in the chambers with predetermined suspended sediment concentrations, and the chambers are incubated in larger aquaria maintained at 12 ºC for the duration of the trials. Trials last 3.5 hr, and oxygen consumption is determined based on the starting (30 minutes after the trial begins) and ending (210 minutes after the trial begins) concentrations in the chamber. Oxygen consumption is calculated as mg O_2/g-fish to account for size differences among individuals used in the trials.

Results

To date, we have conducted 29 rainbow darter, 30 central stoneroller, 39 blacknose dace, and 65 mottled sculpin trials, for a total of 163 trials across all species. Mean (± 1 standard error) somatic weight of blacknose dace used across all trials was 3.06±0.25 g, respectively. Blacknose dace exhibited significantly greater oxygen consumption in trials with 0.5 and 1.0 g/L sediment compared to sediment-free controls ($F=9.21$, $p=0.001$) (Figure 1). Mean wet weight of central stonerollers used in experimental trials was 9.69±0.66. Central stonerollers also exhibited significant differences in oxygen consumption among sediment treatments ($F=4.51$, $p=0.028$), and post hoc analysis using Tukey's method indicated that oxygen consumption was significantly lower in the sediment-free control vs. the 1.0 g/L treatment (Figure 2). However, mean oxygen consumption by central stonerollers exposed to 0.5 g/L sediment was not statistically different from the means for fish in the control and 1.0 g/L treatments. Mean wet weight of mottled sculpin <5.0 g wet somatic weight (i.e., small mottled sculpin) was 2.46±0.11, and mean wet weight of mottled sculpin >5.0 g wet somatic weight (i.e., large mottled sculpin) was 8.10±1.03. Small mottled sculpin were subjected to the widest range of sediment concentrations, and exhibited different oxygen consumption rates among treatments ($F=4.40$, $p=0.005$) (Figure 3). Post hoc analysis using Tukey's method indicated that small mottled sculpin mean oxygen consumption was significantly lower in the sediment-free control, although it was not significantly different from any of the other treatment means. Similar to the small mottled sculpin, large mottled sculpin did not exhibit a significant difference in mean oxygen consumption between control and the 1.0 g/L sediment treatment ($t=-1.85$, $p=0.08$) (Figure 4). Mean wet weight of rainbow darters used across all trials was 2.99±0.22, and there was no significant difference in oxygen consumption between rainbow darters in sediment-free controls vs. 1.0 g/L sediment treatments ($t=-0.95$, $p=0.35$) (Figure 5).

Major Conclusions and Significance

The results presented here remain preliminary, although they do provide the proof of concept to support continued experimentation with stream fish to identify potential sediment thresholds for informing management and regulatory activities. The sediment concentrations in the chambers do not directly reflect suspended sediment concentrations, and we need to determine the effective suspended sediment levels for each chamber size and sediment trials. For example, the 1.00, 1.75, 2.50, and 5.00 g/L sediment concentrations in the small chamber equal suspended sediment levels of 32, 183, 478, and 837 mg/L, respectively. However, we have not determined the effective suspended sediment concentrations for all sediment levels in the medium chamber as yet. Relating stress in the selected fish species to effective suspended sediment concentrations will be key for demonstrating responses of these species to regulatory criteria.

While we still have much work to do to complete this project, we are well on the way to demonstrating explicit thresholds for stress response in representative stream fish to elevated suspended sediment levels. Once we have completed this work, we expect that the results will help to inform efforts to more sustainably regulate and manage lands adjacent to streams to help maintain sediment levels that will be tolerable by a range of resident fish species.

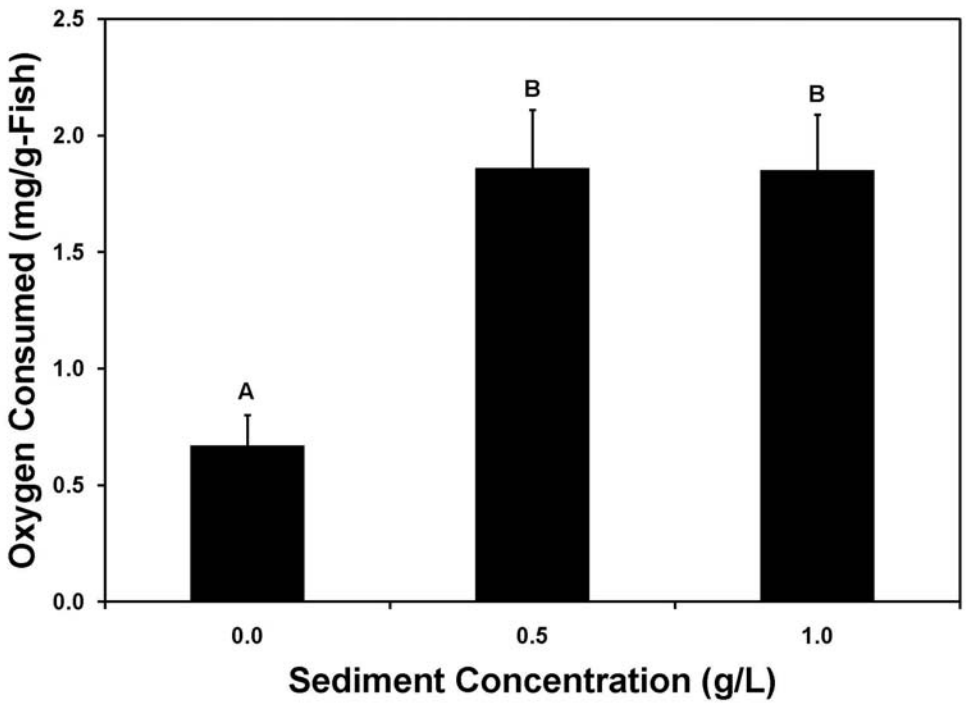

Figure 1. Mean (±1 standard error) amount of oxygen consumed by blacknose dace (*Rhinichthys atratulus*) exposed to various levels of sediment in experimental respirometry chambers over a 3 hr period. Bars with the same letter designations were not significantly different at alpha = 0.05.

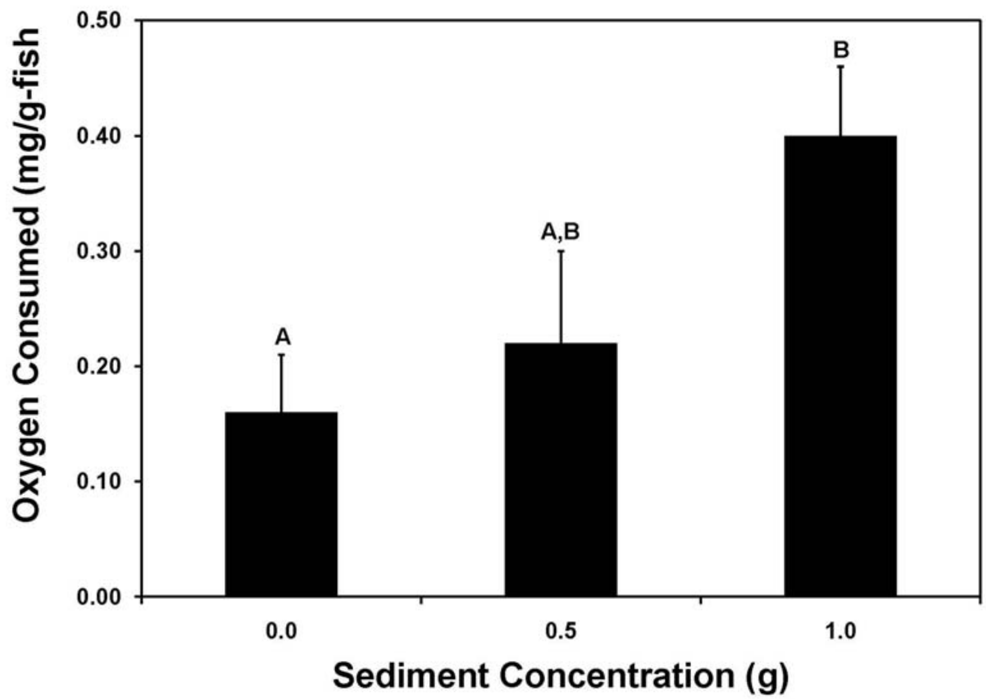

Figure 2. Mean (±1 standard error) amount of oxygen consumed by central stonerollers (*Campostoma anomalum*) exposed to various levels of sediment in experimental respirometry chambers over a 3 hr period. Bars with the same letter designations were not significantly different at alpha = 0.05.

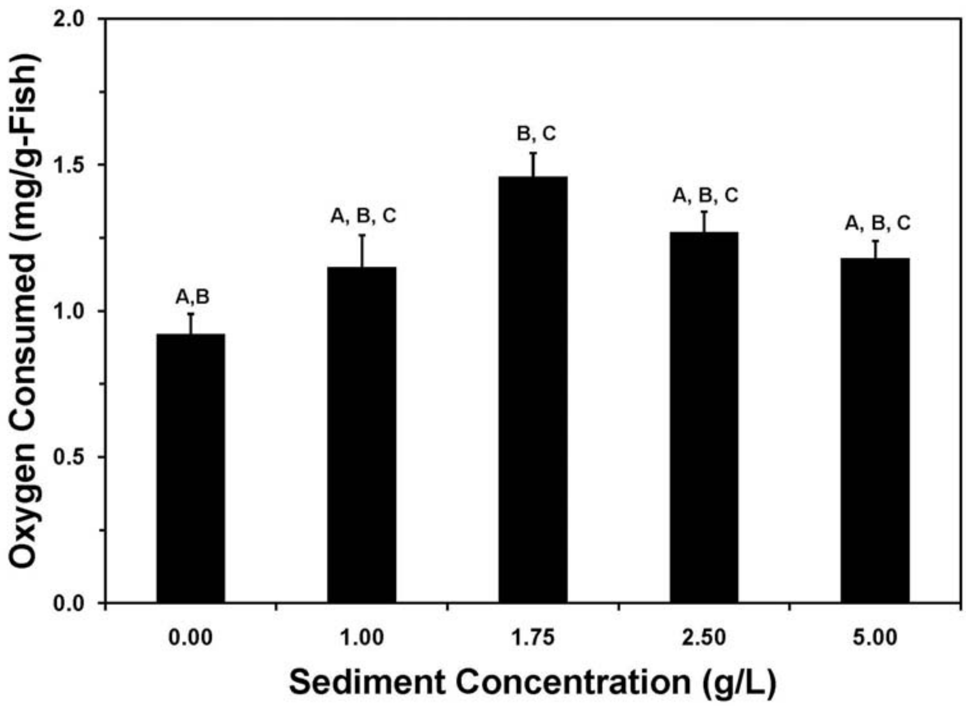

Figure 3. Mean (±1 standard error) amount of oxygen consumed by mottled sculpin (*Cottus bairdii*) <5.0 g exposed to various levels of sediment in experimental respirometry chambers over a 3 hr period. Bars with the same letter designations were not significantly different at alpha = 0.05.

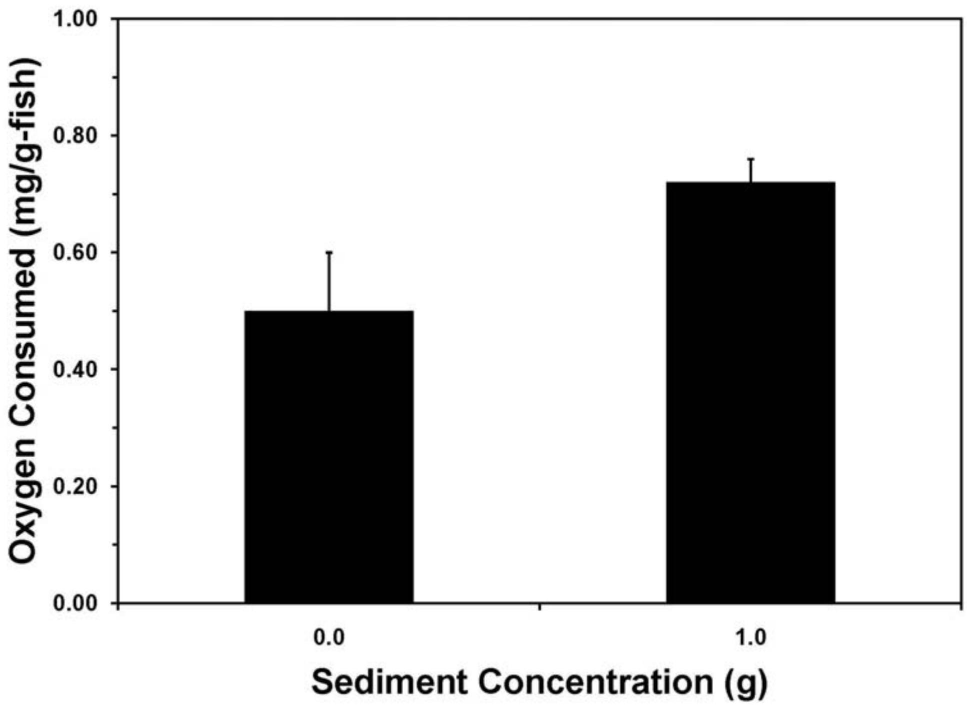

Figure 4. Mean (±1 standard error) amount of oxygen consumed by mottled sculpin (*Cottus bairdii*) >5.0 g exposed to various levels of sediment in experimental respirometry chambers over a 3 hr period. Means for the two exposures were not significantly different at alpha = 0.05.

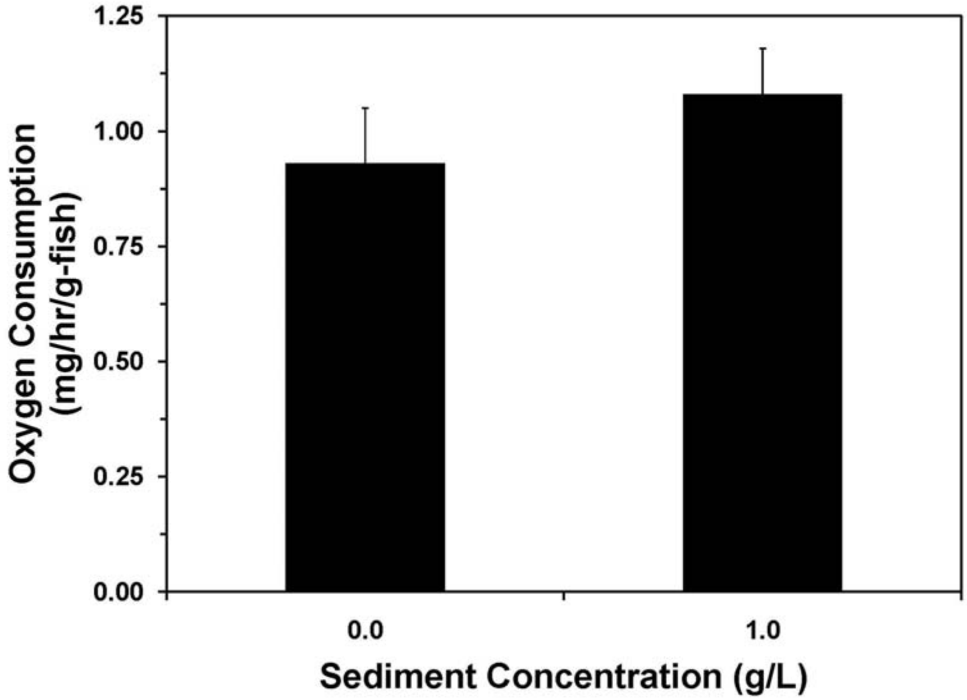

Figure 5. Mean (±1 standard error) amount of oxygen consumed by rainbow darters (*Etheostoma caeruleum*) exposed to various levels of sediment in experimental respirometry chambers over a 3 hr period. Means for the two exposures were not significantly different at alpha = 0.05.

Publications

This work has not been published to date due to the preliminary nature of the results. However, the results to date will serve as the basis for a poster presentation to be given at the annual meeting of the American Fisheries Society (pending acceptance of the abstract)

Grant Submissions

This work has served as part of the basis for submission of several internal grants at Purdue University. To date, $83,000 in funding has been secured related to this project, including a Purdue College of Agriculture Mission-Oriented Grant, a Purdue Agricultural Research Programs Graduate Assistantship, a Purdue College of Agriculture Undergraduate Research Scholarship, and support from the Purdue Department of Forestry and Natural Resources Partnering for Land Use Sustainability Area of Expertise.

Students

Five Purdue undergraduate students have been involved in this project to date, including Robert Johns, Melissa Bartlett, Gary Hoover, Megan Gunn, and Kristen Ruhl.

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Environmental Implications of Manufactured Nanomaterials: Silver, Gallium and Indium

Basic Information

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Environmental Implications and Ecotoxicological assessment of Manufactured Nanomaterials: Gallium and Indium

Abstract/Summary

Potential release of manufactured nanomaterials, such as fullerenes, fullerols, carbon nanotubes, and nanometals to the environment as spills or emissions from manufacturing or as loses from finished products is concerning and foreseeable. At the same time, the potential positive use of nanomaterials for environmental improvements or protection is also inevitable and desirable. Like other environmental introductions the interactions between the introduced materials and the environmental matrix should be assessed before any widespread introductions of the materials occurs. While studies to evaluate the effects of manufactured nanomaterials in aqueous environments and pure culture systems have been undertaken, only limited work has been reported that scrutinizes the effects of manufactured nanomaterials on soil-biological and water systems. This is surprising given the fact that soil is routinely used for disposal of municipal and industrial biosolids both of which could contain nanomaterials and water is routinely used to dispose of treated waste water. It is clear, however that the impact on soil systems should be considered as studies have indicated a toxic effect of some nanomaterials on both prokaryotic and eukaryotic organisms. In this report, the impact of the nanometals Gallium (Ga) and Indium (In) on the microbial functions that underpin soil processes is discussed. Briefly, over the last few years our work shows carbon materials to be mostly benign while the environmental profile for nanometals is more complex and warrants a more detailed investigation and evaluation.

Problem

Nanotechnology is a relatively new area that has introduced novel materials at the nano-scale, which have high commercial value due to their enhanced properties. The application of these engineered nanoparticles has rapidly increased over the last decades, and nowadays there are approximately 475 different nanotechnology products in the market (1). Although these materials have been subjected to investigation for several years, there is not a clear understanding about the fate and effects of such materials in the environment.

Even though little work has been done to examine the behavior and impacts of nanomaterials in the environment, it has been suggested that the unique characteristics of these novel compounds might enable them to interact with living organisms in a way that larger materials cannot (i.e. more reactive and toxic) (2). Therefore, the assessment of the potential environmental impact of nanomaterials is warranted.

Research overview

Currently, one of the principal and increasing applications of nanomaterials is in the semiconductor industry. Gallium (Ga) and Indium (In) compounds are the major components of semiconductors, and their consumption has almost double in the last decades (3). It has been reported that these ultra trace elements have potential toxicity as heavy metals (4-5). Exposure and health risk studies have been conducted to investigate de effects of these rare materials on semiconductor industry workers. Chen (6-7) found workers exposed to Ga and In compounds may suffer of serious health effects. The potential bioaccumulation of these metals has also been demonstrated. Suzuki *et al*. (8) found high concentrations of Ga, In, As, Cd and Tl (primary materials used in the semiconductor industry) in Formosan squirrels of Taiwan and Japan. Such findings suggest squirrels were directly exposed to the metals either via discharges from a semiconductor company or via atmospheric exposition. However, little work has been done regarding the effect of Gallium and Indium based nanomaterials on soil microbial activity and development and growth of plants.

This study aims to investigate the effects of Ga and In, due to their increasing application in the semiconductor industry, on key biological soil activities and the seed germination and root growth of Lettuce and Corn (one of the recommended species for ecotoxicity analisys by USEPA, (9). The metals are studied as granular forms, at different concentrations. Two soils with different structure and organic matter content are examined. Results from this study may provide essential information of the impact of Gallium and Indium based nanomaterials on microbial activity and plant growth, in order to assess their ecotoxicology.

Methodology

1.1 Soil microbial activity assessment

1.1.1 Soil collection

Aggregate samples of two surface soils with different physicochemical properties were used for this study. Drummer soil (silty clay loam, 4% organic matter, pH 6) was collected from the Purdue Agriculture Research Education Center (northwestern of the Purdue campus). Tracy soil (sandy loam, 1.5% organic matter, pH 5) was collected from the Pinney-Purdue Agriculture Center (county line between Indiana's Porter and LaPorte counties). Samples were sieved to 4mm and pebbles, residues and macrobiota were removed.

1.1.2 Soil Basal Respiration

Soil basal respiration is one of the most common tests used to gauge microbial activity and substrate decomposition in soils (10). This analysis provides information about soil microbial populations, soil biomass and the relative contributions of living organisms to total carbon flux from the soil. In this case, the soil basal respiration is conducted using biometer flask microcosms (11). Samples of the two different soils were weighed (50 g dry weight) into 250 mL biometer flask and then preincubated at 23°C for 3 days. After the preincubation period, subsamples of each soil were conditioned at their -0.03 Pa water content and granular GaN or In (1,10,100 and 1000 ug g^{-1} soil) was added to microcosms and mix with soil. Treatments were done in triplicates, including soil controls and no-soil blanks. CO_2 evolved from microcosms was trapped in 10 mL of 1M KOH and titrated with HCl. Soil basal respiration was measured for up to 30 days.

1.1.3 Substrate Induced Respiration

As shown by Anderson and Domsch (12), soil microbial activity can be determined by measuring susbstrate-induced respiration (SIR). This method consists on estimating the maximal respiration rate induced by a substrate (usually glucose) over a short time period. SIR has been widely used for toxicity tests and it has been suggested to be a very sensitive indicator (13-15). Glucose induced-respiration was conducted at the end of 30 days of incubation for 10 and 1000 ppm treatments for each of the nanometal compounds. Subsamples (50 g dry weight equivalent) were removed from each treatment and mixed with glucose. Preliminary analyses showed that 500 µg g^{-1} of glucose are the amount of substrate which gives maximal respiration, and 3hrs were found to be the optimum time for CO_2 measurement after glucose application for both Drummer and Tracy soils. The level of CO_2 evolved in 3hrs was determined using biometer flaks microcosms, exactly the same way as in the soil basal respiration experiment.

1.1.4 Enzymatic activity

Enzymes carry out important functions in the soil and their inhibition could have important consequences for soil sustainability. Therefore, soil enzyme activities have been suggested to be suitable indicators of soil quality (16-19). Enzyme activities for β –glucosidase and urease were selected to be measured in this study due to their main role in the nutrient cycling of C and N, respectively (20-22).The enzymes in soil were evaluated using 10 and 1000 ug/g of Ga and In compounds. β-glucosidase was determined according to Gianfreda *et al*. (23). Urease measurement is based on Gianfreda et al (23) method, but modified by Tong et al (24).

1.2 Plant growth assessment

1.2.1 Seeds

Seeds of *Lactuca sativa* (lettuce- loose head type) and *Zea mays* (yellow sweet corn) were purchased from Burpee Co., USA. These plant species have been used in several phytotoxicity studies, and they are among the recommend species by USEPA (25) for the determination of ecological effects of toxic substances on plants. A preliminary study showed that the average germination rate is greater than 80% for both plant species.

1.2.2 Germination and root growth

Higher plants have been recognized to be good indicators of effects of environmental chemicals. Traditionally, seedling emergence and plant growth have been the recommended test to assess contaminants toxicity in soils (25-27). Even though seed germination is considered to be a less sensitive indicator of toxicity in soils, a lot of studies have use this parameter along with root growth to assess metal and engineered nanoparticles toxicity (28-31).

For this experiment, seeds were soaked in a 10% sodium hypochlorite solution for 15 min and then rinsed three times with distilled water to ensure surface sterility (25). Seeds were placed on Nobel agar slants containing 10, 100, 250, 500, 750 and 1000 ppm of Ga and In respectively. Tubes were placed in a growth chamber at 20°C on a 12h photoperiod. Controls were formulated using Nobel agar only. Fifteen replicates of each treatment were done. After an incubation period of 13 days for lettuce, and 8 days for corn, plants were harvested from the agar using forceps. Percentage of germination, dry biomass (oven-dried at 65°C), shoot and root lengths were measured for each treatment. Statistical differences of experimental data were examined by the Tukey's test. Each of experimental values was compared with its corresponding control. All the statistical analysis was implemented using JMP 7.0.

2 Results and discussion

2.1 Soil basal respiration

Soil basal respiration was monitored 11 times over a 30 days period following treatment with either distilled water (control), GaN or InN. Results are presented as cumulative CO_2 production (Figure 1). Noncumulative respiration data collected at each time point were subjected to Tukey's test for the treatment effects and showed no significant differences ($P > 0.05$) between any treatments and the control. From the literature, the rate of soil basal respiration would be expected to be reduced when microbial community is exposed to highly toxic materials (32-34). Even though, no significant differences were found between treatments and the control, the respiration rate tended to decrease for the 1000 ppm In treatment.

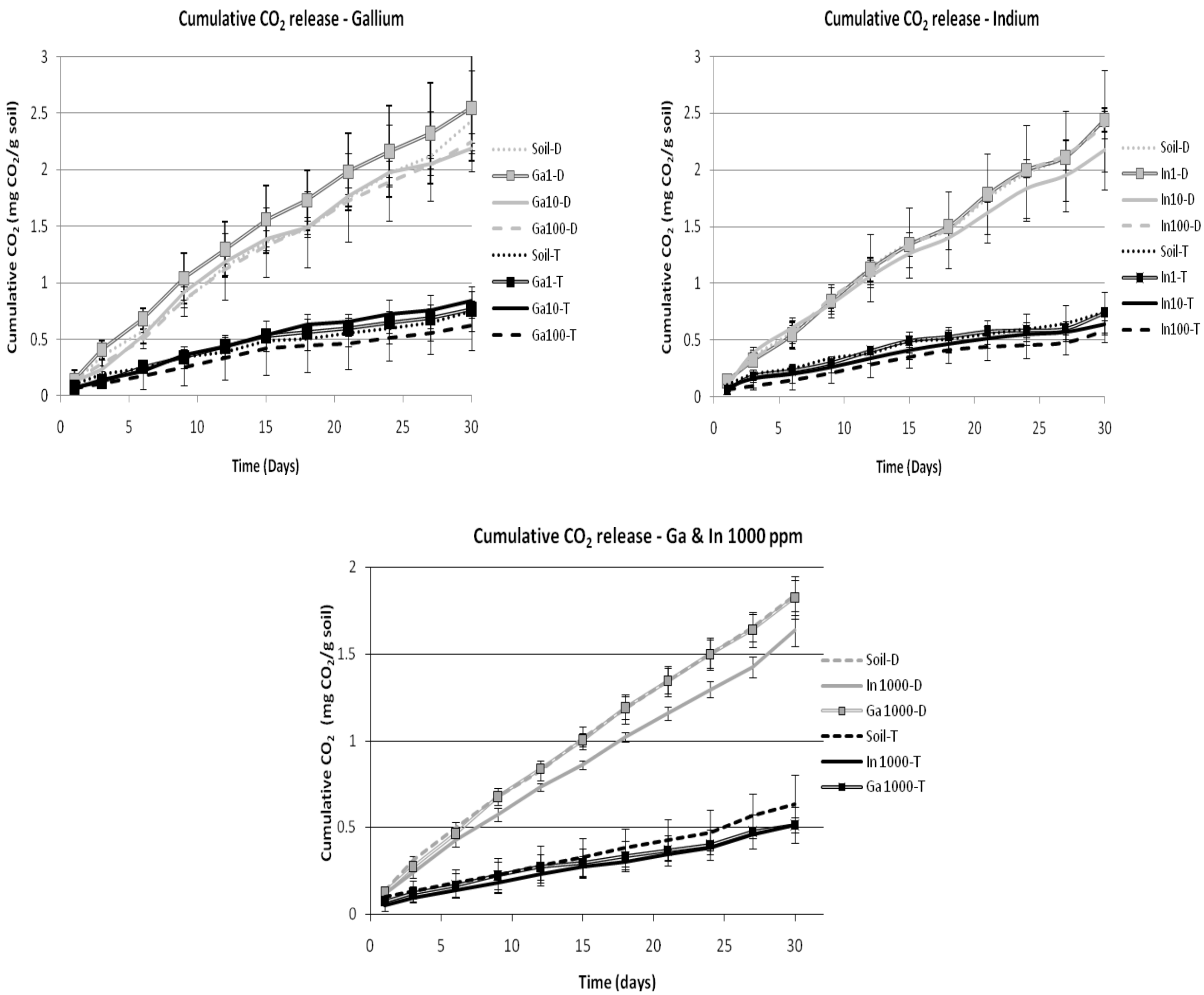


Figure 1 Cumulative CO_2 release from a control or soil treated with 1,10,100 and 1000 µg g-1 of GaN or In, for Drummer (D) and Tracy (T) soils. Data are mean values ± SD, n=3.

2.2 Substrate induced respiration

Subsamples from soil microcosms treated with either distilled water, GaN or InN, were taken every week for SIR analysis. Evolved CO_2 at each time point were subjected to Tukey's test and show no significant differences ($P > 0.05$). After 30 days of incubation, the level of CO_2 production ranged from 8.4 to 14.4% of the applied C-glucose in Drummer, and from 3.8 to 8.3% in Tracy (Figure 2).

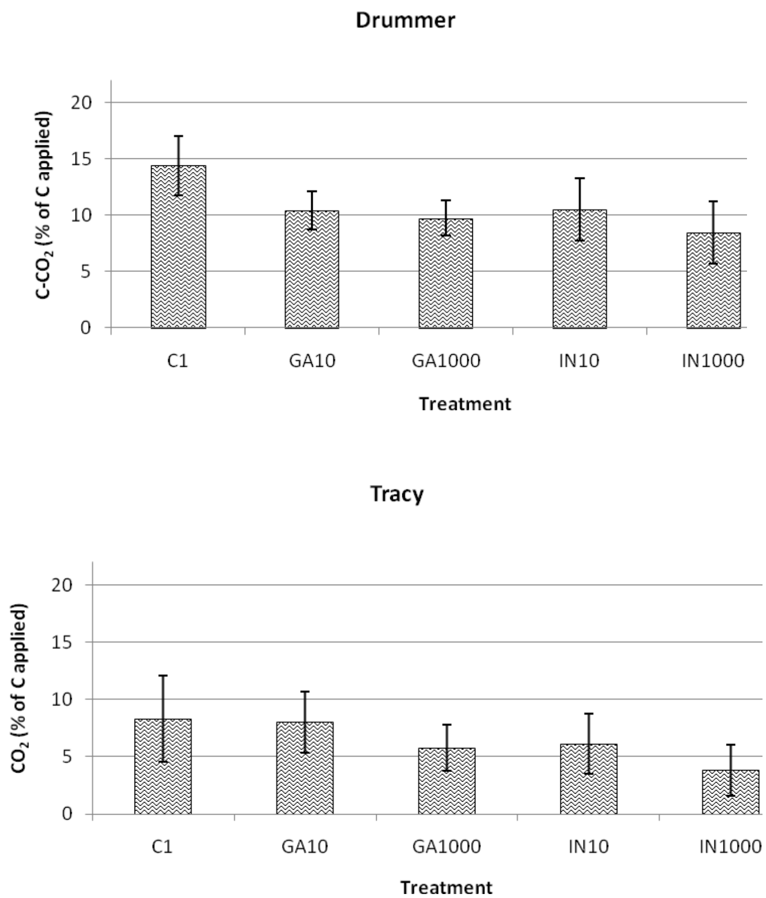


Figure 2 Respiration response estimated by total C-CO_2 released (% of C applied) 3 hours following C-glucose amendment after 30 days incubation for soils treated with GaN, In or soil control. Data are mean values ± SD, n=3.

2.3 Enzymatic activity

Measurement of enzyme activity has also been used as an indicator to assess soil quality (16-19). When a soil system is exposed to toxic conditions, it is also expected for the enzyme activities to decrease. Using subsamples of soil microcosms treated with either distilled water, GaN or In for up to 30 days β-glucosidase and urease enzyme activities were determined. Enzyme assays results are summarized in Figure (3). Some variation was observed among treatments, particularly with β-glucosidase. Compared to the soil controls, In treatments (10, 1000 ug g^{-1}) have a significant increase in β -glucosidase activity ($P < 0.05$) for Drummer soil. In the Tracy soil, β -glucosidase activity was also significantly higher for In treatments, after the 18th day ($P < 0.05$).

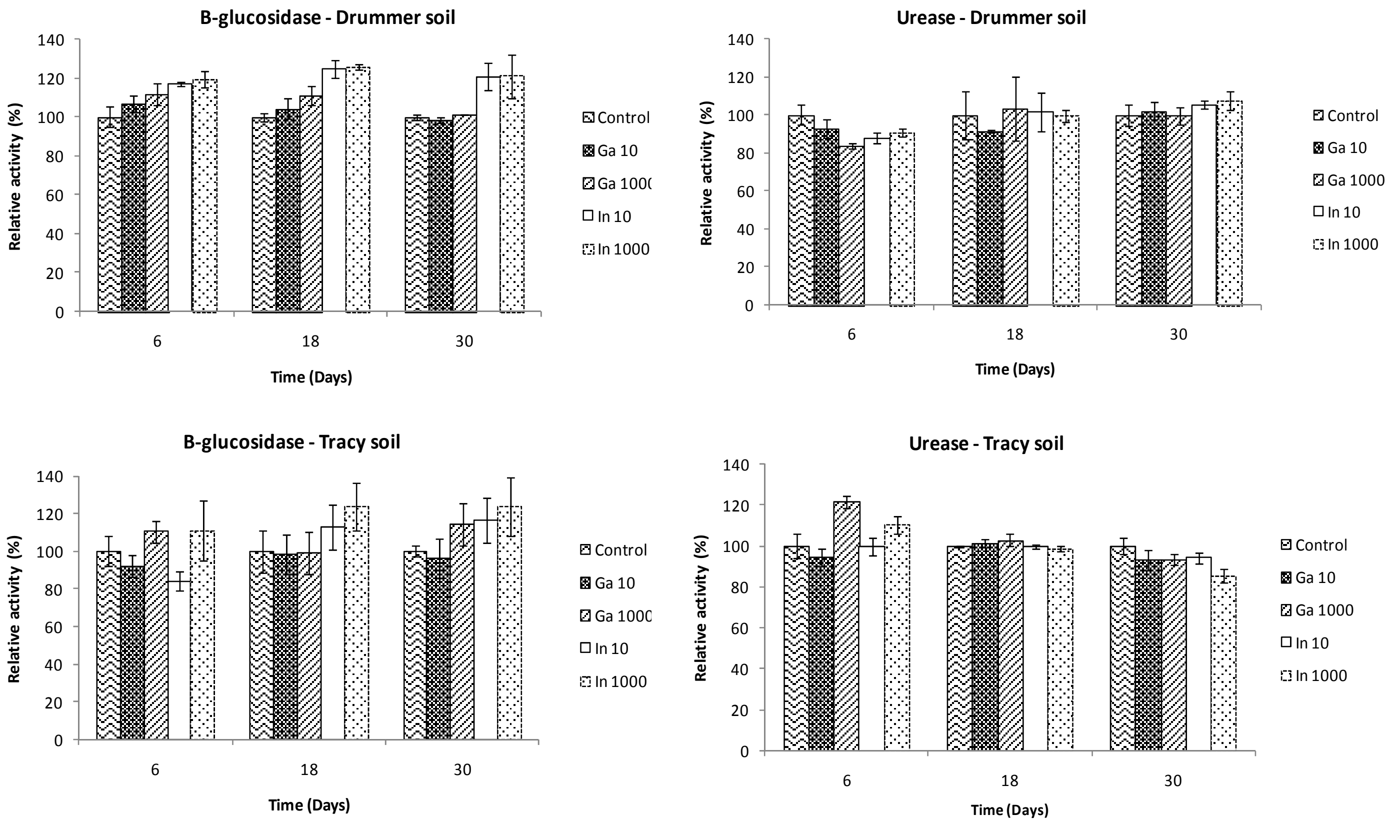


Figure 3 Relative activities of β-glucosidase and urease of samples treated with GaN and In (at 10 or 1000 ppm) compared with soil controls (set to 100%) at times up to 30 days, for Drummer and Tracy soils. Data are mean values ± SD, n=3.

Drummer soil had a significant increase in urease activity during the first week for samples treated with GaN-10 g g^{-1}(P ‹ 0.05). Nevertheless, no significant differences between treatments and controls were found after the first week (P › 0.05). In Tracy soil, treatments with Ga and In (1000g g^{-1}) were significantly higher compared to the controls (P ‹ 0.05) in the first week. No significant differences were found in the 3rd week (P › 0.05). After 30 days of exposition, urease activity significantly decreased for treatment with In-1000ug g^{-1} (P ‹ 0.05). As mentioned before, enzyme activities have been shown to be good indicators of stress. From these results, no adversely effects due to the exposition to Ga or In compounds are observed.

2.4 Germination and root growth

Percentage of germination, dry biomass (oven-dried at 65°C), shoot and root lengths were measured for each treatment after an incubation period of 13 and 8 days for lettuce and corn respectively.

Seed germination was not affected at the tested concentrations for each metal used in this experiment. Indeed, the percentage of germination for both, lettuce and corn, was almost constant (>80%) for all treatments. Germination percentages for each treatment compared to the control were not statistically significant in any case.

Table 1 shows seedling biomass (dry weight), shoot and root growth, expressed as means and their statistical differences from the control, in Ga and In treatments for both lettuce and corn species. From Tukey's multiple comparisons test, plant biomass was not statistically significant (P>0.05) for any treatment under Ga and In exposition, for both lettuce and corn species respectively. Shoot and root growth showed statistically significant differences for seedlings exposed to Ga and In, for both lettuce and corn species.

Table 1 Effect of Gallium and Indium on plant response

	Lettuce			Corn		
Treatment	**Shoot length (cm)**	**Root length (cm)**	**Plant Biomass (g DW)**	**Shoot length (cm)**	**Root length (cm)**	**Plant Biomass (g DW)**
Control	2.073 a	6.017 a	0.0010 a	9.820 a	13.620 a	0.199 a
Ga-10	1.920 ab	6.607 a	0.0012 a	8.571 ab	13.786 ab	0.196 a
Ga-100	2.079 a	5.357 a	0.0011 a	7.467 ab	10.017 abc	0.192 a
Ga-250	2.387 a	5.813 ab	0.0009 a	8.200 ab	10.630 abc	0.194 a
Ga-500	1.820 ab	4.173 b	0.0015 a	5.322 ab	8.111 bc	0.204 a
Ga-750	1.253 bc	1.213 c	0.0012 a	5.078 b	8.633 abc	0.180 a
Ga-1000	1.047 c	1.547 c	0.0012 a	6.306 ab	7.082 c	0.194 a
Control	2.073 a	6.017 a	0.0010 a	9.820 a	13.620 ab	0.199 a
In-10	1.087 b	4.780 ab	0.0012 a	10.100 a	14.913 a	0.199 a
In-100	1.387 ab	3.533 b	0.0011 a	8.913 ab	8.725 bc	0.176 a
In-250	1.687 ab	1.623 c	0.0015 a	7.556 ab	7.989 c	0.196 a
In-500	1.772 ab	1.693 c	0.0009 a	5.310 ab	9.090 bc	0.184 a
In- 750	1.507 ab	1.087 c	0.0019 a	5.489 ab	8.600 c	0.183 a
In-1000	1.478 ab	1.122 c	0.0010 a	5.037 b	3.644 d	0.194 a

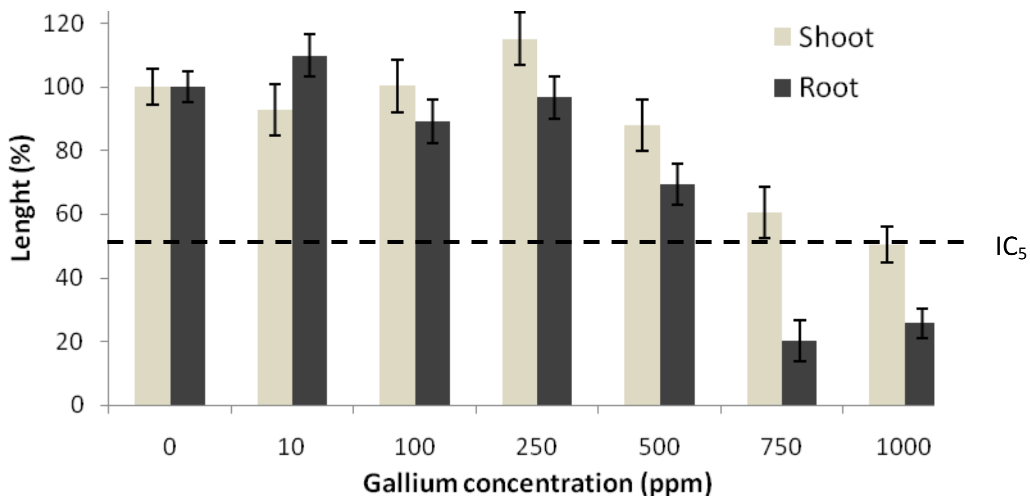
Data were analyzed using Tukey's test. Means with different letters are significantly different from each other (P < 0.05).

Figure 4 and 5 show shoot and root growth for both, lettuce and corn species, expressed as a percentage of the mean of the control treatment, in Ga and In treatments, respectively.

Root growth of lettuce seedlings was clearly inhibited (less than 50% root growth) when exposed to concentrations greater than 750 ppm of Gallium, and 250 ppm of Indium, respectively (Figure 4). 50% reduction of the shoot growth of lettuce was just observed under 1000 ppm of Gallium.

Corn seedlings were less sensitive to Gallium and Indium exposition. 50% reduction of root growth was only observed at 1000 ppm of Ga and In, respectively. Shoot growth was inhibited (less than 50% growth) at 1000 pp, of Ga (Figure 5).

A



B

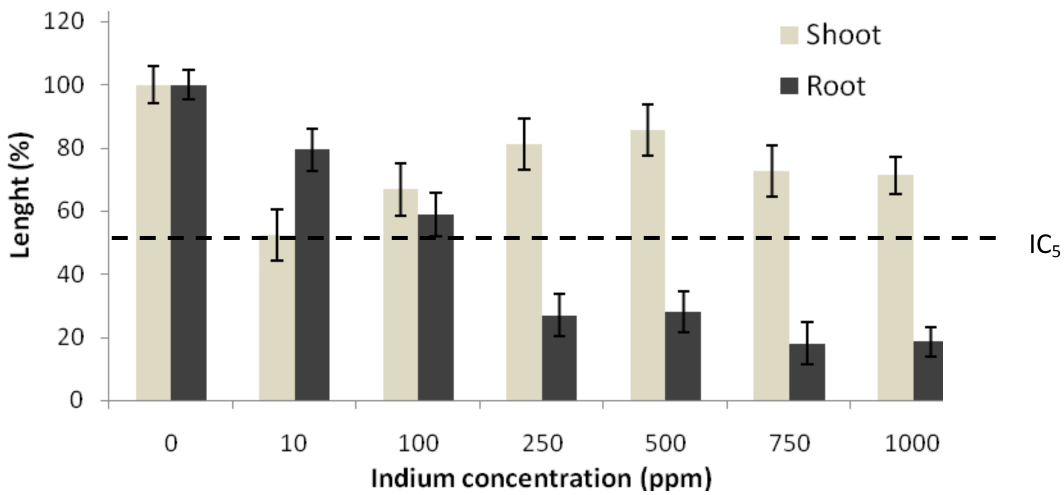


Figure 4 Shoot and root growth in lettuce plants as a percent of control (no metal added) after 13 days of incubation at different concentration of Gallium (A) and Indium (B), respectively. IC_{50} corresponds to the 50% shoot/root growth inhibitory concentrations.

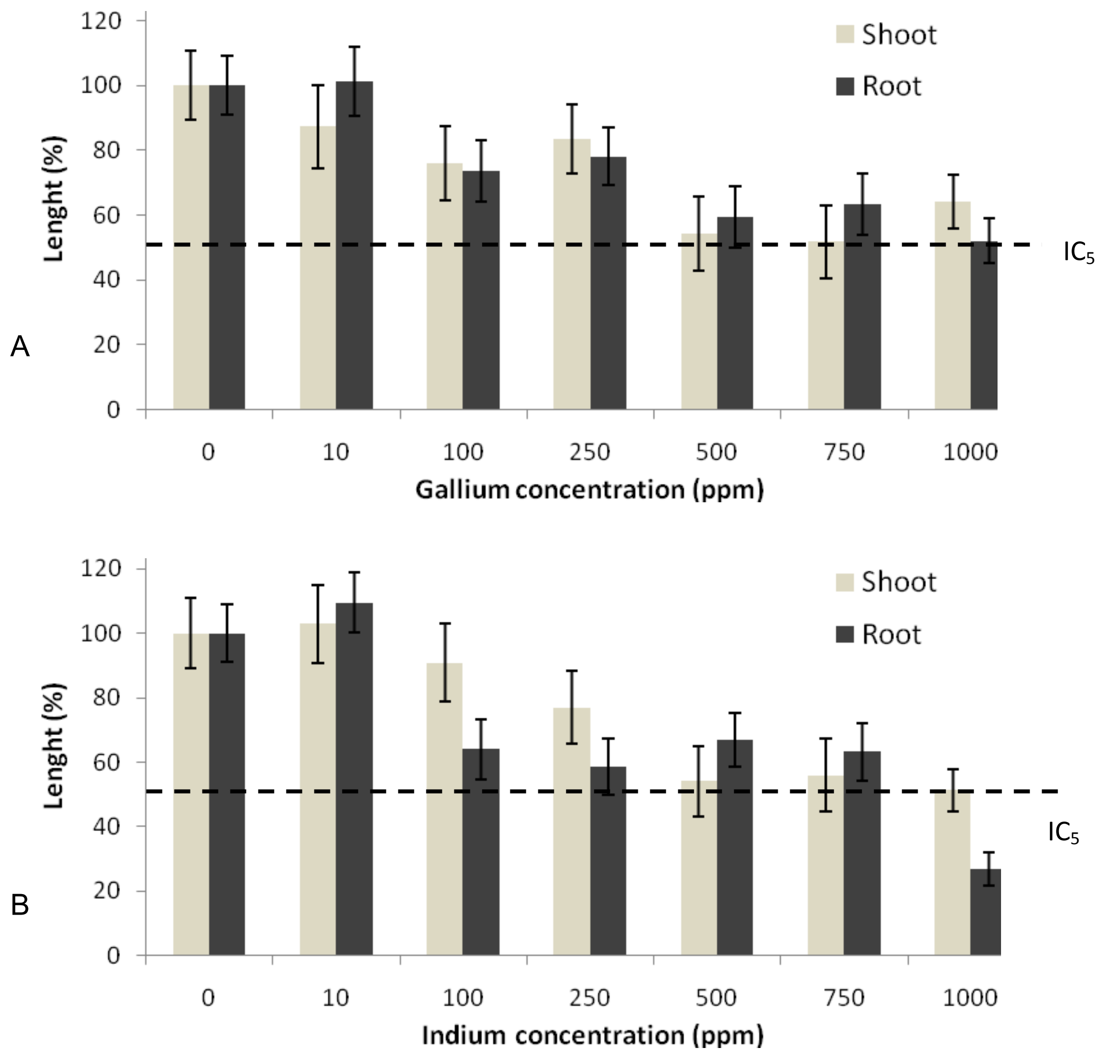


Figure 5 Shoot and root growth in corn plants as a percent of control (no metal added) after 8 days of incubation at different concentration of Gallium (A) and Indium (B), respectively. IC_{50} corresponds to the 50% shoot/root growth inhibitory concentrations.

Figures 6, 7, 8 and 9 show seedling growth for both corn and lettuce species respectively. Visible manifestations of toxicity were observed in lettuce and corn under exposure of Ga and In. Brown to black spots appeared on the leaves of lettuce when exposed to 1000 ppm of Ga and In, respectively. Root tips of lettuce seedlings appeared darker than those of control plants under 500 to 1000 ppm of Ga.

Secondary roots were hardly found in corn under 1000 ppm of both, Ga and In.

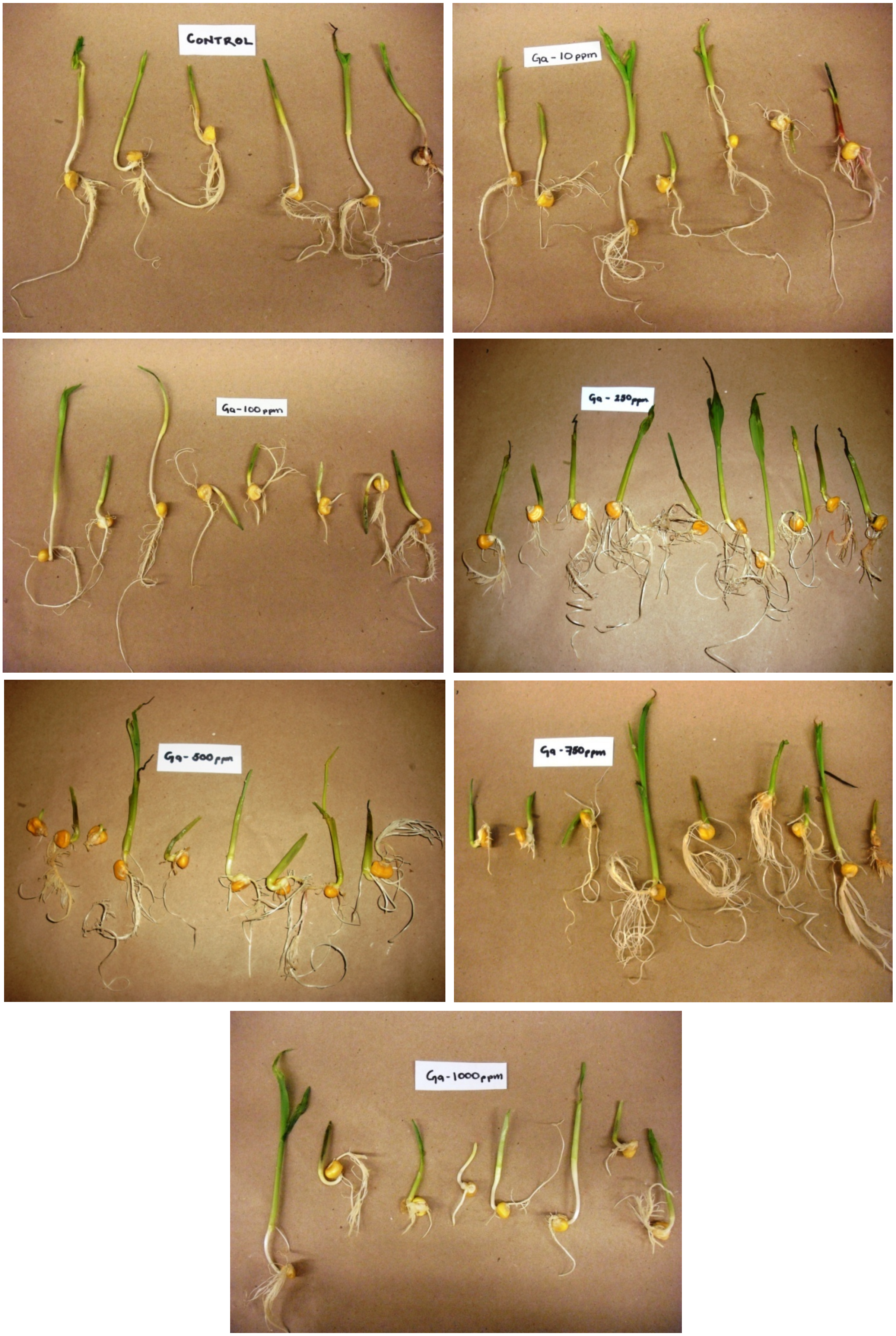


Figure 6 Control and Gallium-exposed corn plants.

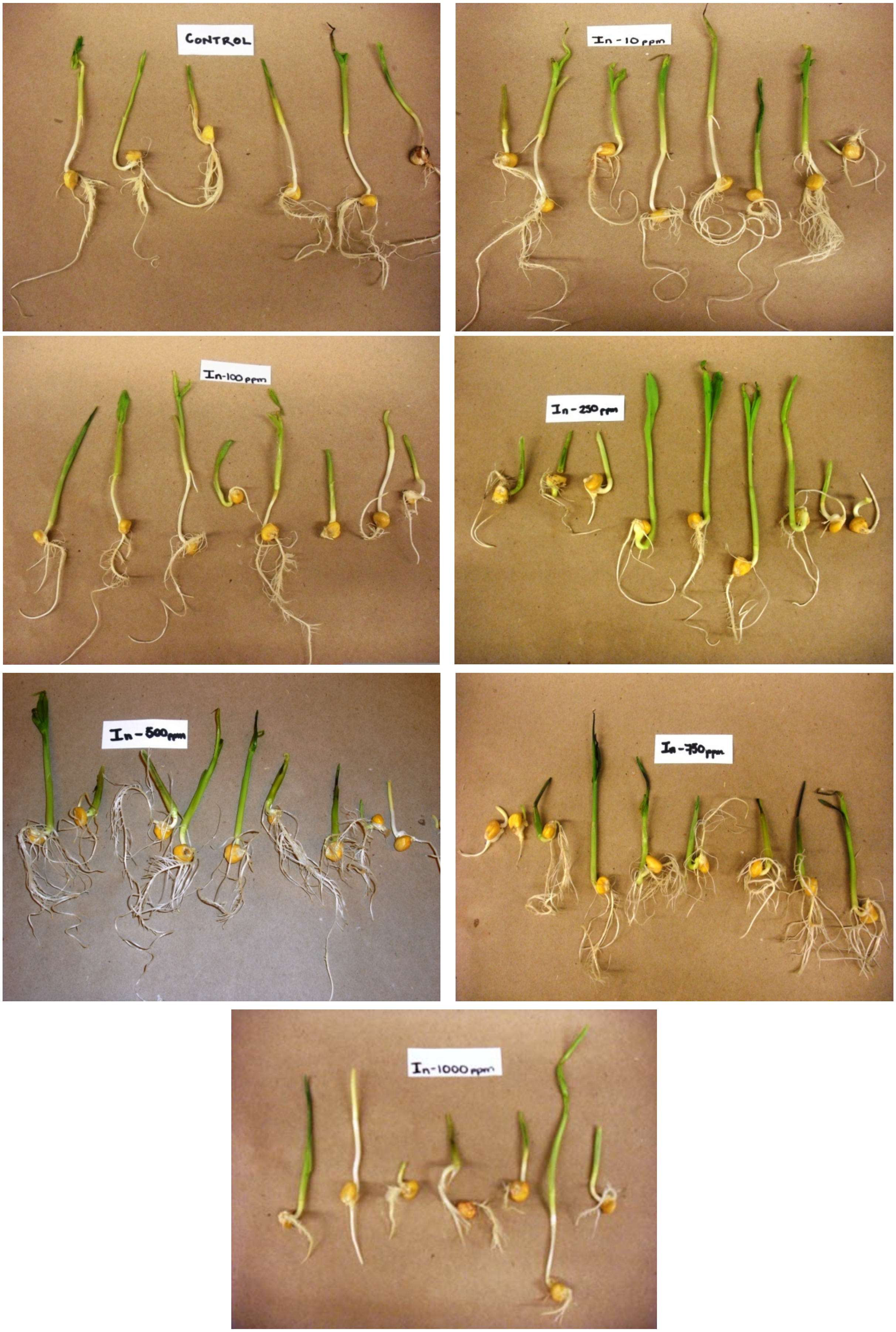


Figure 7 Control and Indium-exposed corn plants.

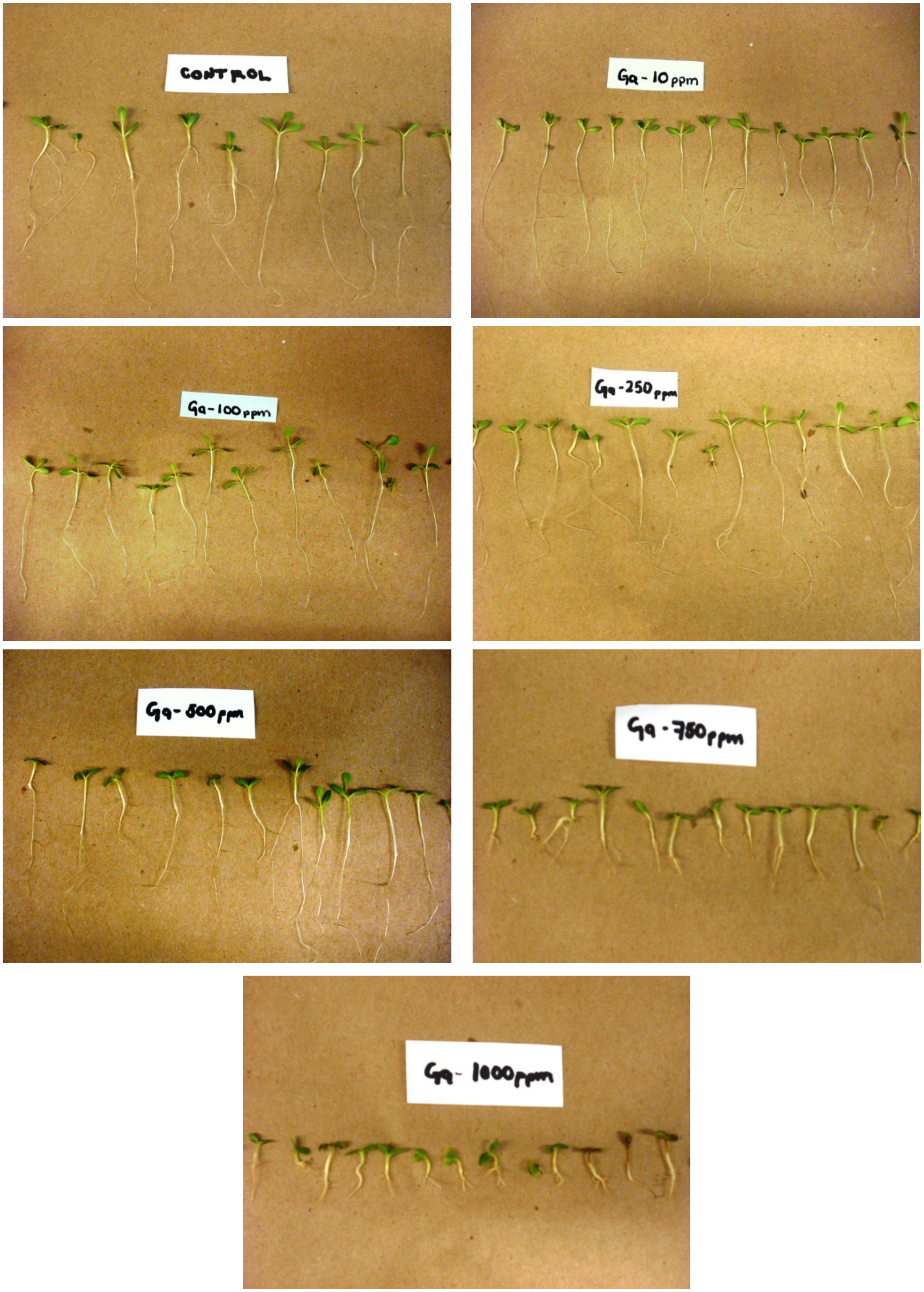


Figure 8 Control and Gallium-exposed lettuce plants.

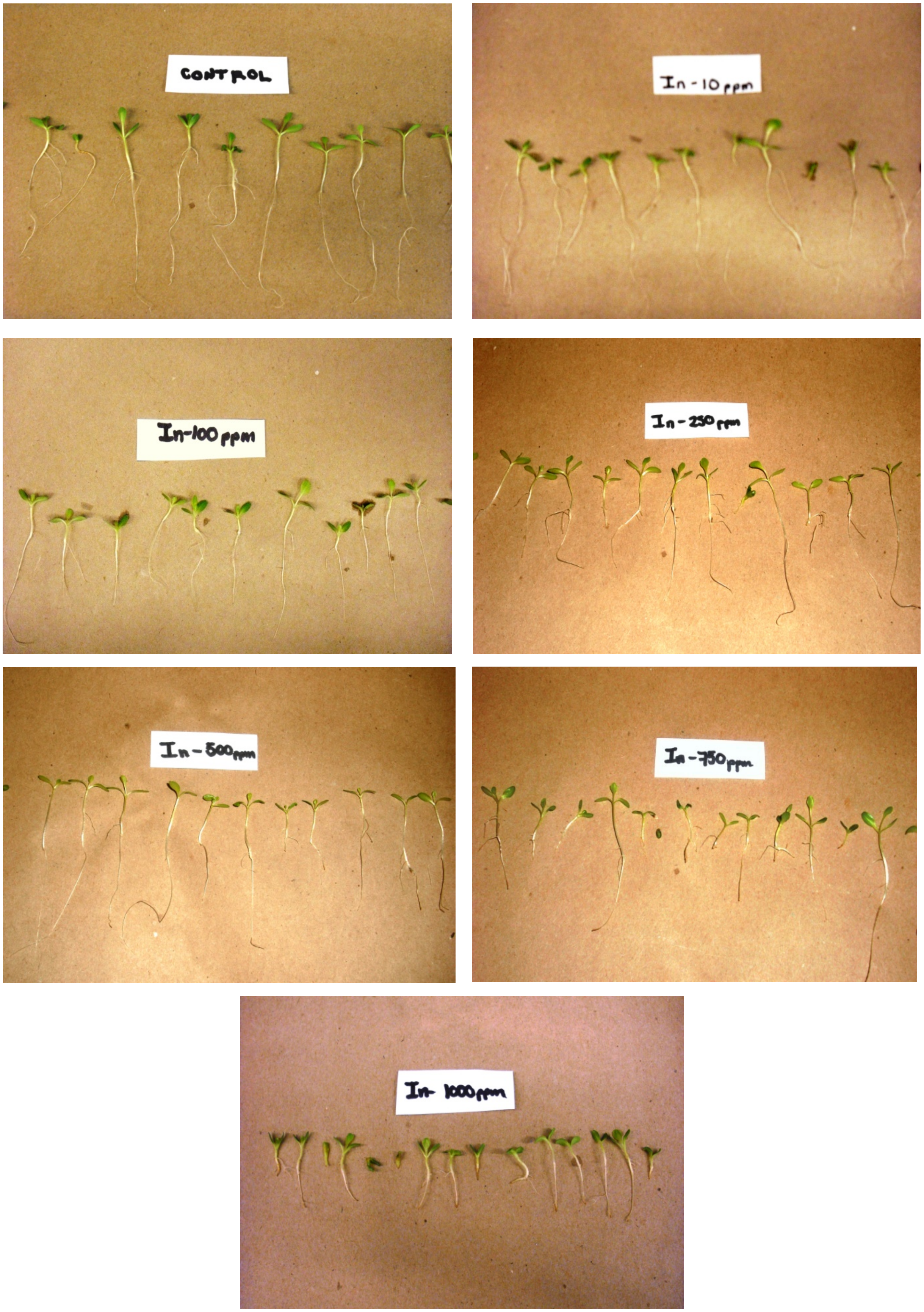


Figure 9 Control and Indium-exposed lettuce plants.

Major Conclusions and Significance In this study, the potential effects of Gallium and Indium on soil microbial activity and higher plant species were investigated. One of the hypotheses of this project was that microbial activity, measured as SBR, SIR and enzyme activity, would be affected by the exposition to Gallium and Indium; nevertheless, no effects on the majority of the treatments were observed. On the other hand, growth of higher plant species (lettuce and corn) was indeed affected by Ga and In exposition. There was a clear tendency on the severity of the response on root growth related to the increasing metal concentrations. 50% reduction of root growth was only observed at 1000 ppm of Ga and In, respectively for corn seedlings, and at 750 to 1000 ppm of Ga, and 250 to 1000 ppm of In for lettuce seedlings. The metals have little effect on plants at low dosage, but would be toxic at relatively high doses. Therefore, the environmental effects of these metals should be concerned. This study is the first approach to understanding ecotoxicity of nanoparticles of Gallium and Indium. More studies are needed concerning microbial activity and their community composition when exposed to Ga and In, as well as bioaccumulation of such metals on plants. Such information might be useful for future ecological assessments.

3 References

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Publications:

Laban, G., L.F. Nies, R.F. Turco, J.W. Bickham and M. S. Sepúlveda 2010. The effects of silver nanoparticles on fathead minnow (Pimephales promelas) embryos. Ecotoxicology (On line: DOI 10.1007/s10646-009-0404-4)

Grant Submissions:

Support: Pending
Project/Proposal Title: Understanding how *insitu* processes alter the environmental fate and impacts of manufactured nanotubes
Source of Support: Increasing Scientific Data on the Fate, Transport and Behavior of Engineered Nanomaterials in Selected Environmental and Biological Matrices. EPA-G2010-STAR-N1
Project Cost: $600,000
Location of Project: Purdue University; Person-Months Per Year Committed to the Project **0**.5 ACAD

Students:

Ericka Espinosa , MS completed May 2010

The Influence of Nonprescription Pharmaceuticals on Aquatic Ecosystems: Direct Toxicity and Indirect Trophic Interactions

Basic Information

Title:	The Influence of Nonprescription Pharmaceuticals on Aquatic Ecosystems: Direct Toxicity and Indirect Trophic Interactions
Project Number:	2009IN244B
Start Date:	3/1/2009
End Date:	2/28/2010
Funding Source:	104B
Congressional District:	6
Research Category:	Biological Sciences
Focus Category:	Non Point Pollution, Solute Transport, Surface Water
Descriptors:	
Principal Investigators:	Melody J. Bernot

Publications

There are no publications.

IWRRC 2009 Project Reports

State: IN **Project Number:** 2009IN244B
Title: The Influence of Nonprescription Pharmaceuticals on Aquatic Ecosystems: Direct Toxicity and Indirect Trophic Interactions
Project Type: Research
Focus Category: Non Point Pollution, Solute Transport, Surface Water
Keywords: wastewater, nonpoint pollution, streams, pharmaceuticals, toxicology
Start Date: 3/1/2009 **End Date:** 2/28/2010
Congressional District: 6 **PI:** Bernot, Melody J. email:mjbernot@bsu.edu

Report Format

Abstract / Summary

Pharmaceuticals in freshwater is increasingly becoming a concern due to potential problems associated with drinking water contamination and freshwater habitat degradation. However, little data is available on the effects of trace concentrations of pharmaceuticals on aquatic ecosystems. Further, only a few studies have quantified the range and variability of pharmaceuticals in freshwater. We quantified the abundance of pharmaceuticals in the Upper White River watershed of central Indiana and the influence of environmental concentrations of pharmaceuticals on freshwater sediment microbial activity. Descriptive surveys of pharmaceutical concentrations in freshwater found higher concentrations of pharmaceuticals in more rural areas of central Indiana indicating leaking septic tanks may be important contributors of pharmaceutical contaminants to freshwaters. Pharmaceutical concentrations were correlated positively with agricultural land use in the sub-watershed as well as nitrate concentrations. Pharmaceuticals were negatively correlated with urban land use in the sub-watershed. Additionally, pharmaceutical concentrations in freshwater were orders-of-magnitude higher in winter. Previous assessments of pharmaceuticals in freshwater have focused on sampling conducted in spring and summer potentially underestimating overall exposures to these trace contaminants. Pharmaceuticals had variable effects on biotic activity depending on response variable measured and pharmaceutical compound. Caffeine, cotinine, and nicotine significantly influenced sediment microbial respiration, nitrification, and nutrient uptake whereas ibuprofen and acetaminophen did not influence microbial activity. These data indicate that pharmaceuticals are ubiquitous in central Indiana freshwaters and may affect aquatic ecosystems even at trace concentrations.

Problem:

Pharmaceuticals in freshwater is increasingly becoming a concern due to potential problems associated with drinking water contamination and freshwater habitat degradation. However, little data is available on the effects of trace concentrations of pharmaceuticals on aquatic ecosystems and only a few studies have quantified the range and variability of pharmaceuticals in freshwater. Without more comprehensive data to guide management programs, we may incorrectly assume that these programs are adequate for maintaining or improving water resources.

Research Objectives:

Research conducted addressed two primary objectives:

- Quantify the spatial and temporal variability of pharmaceuticals in the Upper White River watershed of central Indiana.
- Quantify the influence of environmental concentrations of pharmaceuticals on biotic activity via *in vitro* experiments.

Methodology:

Water samples were collected at sites within a sub-watershed of the Wabash River, the Upper White River watershed (UPWR) in central, Indiana. Sites were selected to represent a range of land uses in the surrounding drainage area. A total of 12 sites were sampled for pharmaceutical analysis with both comprehensive spatial assessments conducted in August and comprehensive temporal measurements conducted monthly on two sites. At each site and for each sampling event, hydrology, chemistry, and biology was measured in conjunction with pharmaceutical concentrations. Pharmaceutical samples were collected into baked amber-glass bottles and analyzed by the Iowa Hygienic Laboratory and United States Geological Survey National Water Quality Laboratory. Measurements of surface water discharge were made using a Marsh-McBirney flow meter for cross-sectional measurements of flow every 10m for a 100m stream reach. Water-column nutrients were measured by taking replicate grab samples of stream water in rinsed, acid washed bottles and subsequently filtering for analysis of dissolved nutrients (nitrate, phosphate, ammonium, sulfate) via ion chromatography. Filters were retained for analysis of suspended particulate material and chlorophyll *a* concentrations using hot-ethanol extraction. Ancillary measurements of water temperature, pH, specific conductivity, and dissolved oxygen (O_2) concentrations were also collected at all sites at every sampling event using a thermometer, pH probe, and Hydrolab minisonde equipped with an LDO sensor. Sediment samples were collected using a sediment corer and subsequently dried, ground, and analyzed on an elemental analyzer for N and carbon (C) content as well as 15N content. Channel and riparian characteristics as well as benthic organic matter standing stocks were measured once at each site during base flow conditions.

Laboratory mesocosm experiments were conducted to test the direct effects of target pharmaceuticals on microbial activity. Laboratory experiments utilized a completely randomized split-plot design geared at determining toxicity of pharmaceuticals. Experiments used stream sediment (as microbial inoculum) and water in 250mL Ehrlenmeyer flasks. Microbial assays determined changes in nutrient uptake as differences between pre- and post- measures of nutrient concentrations, differences in respiration with dehydrogenase enzyme activities, and differences in nitrification using the nitrapyrin-inhibition technique in response to addition of trace concentrations of pharmaceuticals based on descriptive measurements of environmental concentrations.

Results

Several notable results were found in conjunction with this research that add to our current understanding of pharmaceutical abundance and potential effects on the environment:

1. At base flow, pharmaceutical concentrations in the UWR are higher in areas dominated by agriculture (as opposed to urban areas including Indianapolis, Anderson) and were correlated with nitrate concentrations in water.
2. Pharmaceutical concentrations in the UWR are on the higher end of concentrations observed in other environmental studies across the country (much higher when considering winter samples) and frequency of detection in this study was higher for many compounds (cotinine, acetaminophen, DEET, carbamazepine, ibuprofen, gemfibrozil, caffeine, lincomycin, trimethoprim) than any other previous documentation.
3. Pharmaceutical concentrations are significantly higher in the winter relative to the rest of year. Many compounds are regularly detected (e.g., carbamazepine, caffeine, DEET, ibuprofen, sulfamethoxazole, gemfibrozil) and similar patterns across sites were observed.
4. Trace concentrations of pharmaceuticals measured in central Indiana streams did affect sediment microbial activity (as respiration, nutrient uptake, and nitrification). Effects varied with measure of activity and individual pharmaceutical compounds. Some pharmaceuticals increase rates of microbial activity up to a saturation point whereas other pharmaceuticals resulted in linear declines in microbial activity even at trace concentrations tested.

Major Conclusions and Significance

Data indicate pharmaceutical concentrations are highest in rural areas of central Indiana, relative to more urban areas, potentially due to differences in wastewater treatment. Thus, effects of pharmaceuticals on freshwater ecosystems and potential for human exposure may be greatest in less urbanized areas. Pharmaceuticals were also found to be highest in winter, a relatively understudied time period for assessments of pharmaceutical contamination. Increases in pharmaceuticals during winter are likely due to lower photo- and microbial degradation of these compounds. Further, trace concentrations of pharmaceuticals measured did yield changes in microbial activity indicating that potential effects may be observed at larger scales.

Publications

1. Bunch, AR and MJ Bernot. *In Review*. Pharmaceutical abundance in central Indiana streams and effects on microbial activity. *Aquatic Toxicology.*
2. Bunch, AR. August, 2009. Pharmaceutical abundance in central Indiana streams and effects on microbial activity. MS Thesis. Ball State University.
3. Listman, L, & MJ Bernot. 2010. Antibiotic resistance associated with stream sediment microbial communities influenced by different land use. BSU Student Symposium. March.
4. Veach, AM, & MJ Bernot. 2010. Pharmaceutical Abundance and microbial degradation in central Indiana streams. BSU Student Symposium. March.
5. Porter, T, & MJ Bernot. 2010. The influence of lithium on microbial activity. BSU Student Symposium. March.
6. Bourdon, L, & MJ Bernot. 2010. The influence of testosterone and estrogenic compounds on microbial activity. BSU Student Symposium. March.
7. Listman, L, & MJ Bernot. 2009. Antibiotic resistance associated with stream sediment microbial communities influenced by different land use. Indiana Academy of Sciences. October.
8. Veach, AM, & MJ Bernot. 2009. Pharmaceutical Abundance and microbial degradation in central Indiana streams. Indiana Academy of Sciences. October.
9. Sajjad, S, and MJ Bernot. 2009. Influence of non-prescription pharmaceuticals on pond sediment nitrification. North American Benthological Society. May.
10. Bunch, AR, and MJ Bernot. 2009. Abundance of nonprescription pharmaceuticals in streams and effects on sediment microbial activity. North American Benthological Society. May.

Grant Submissions:

1. Bernot, RJ and MJ Bernot. 2010-2012. National Science Foundation. RUI: Human and Disease Impacts on Aquatic Communities: Effects of Trematodes and Nanomaterials on Freshwater Benthic Interactions. Total Award: $135,000.
2. Bernot, MJ. 2010-2011. Indiana Water Resources Research Consortium. Transport, Fate, and Effects of Pharmaceuticals derived from Animal Feeding Operations. Total Award: $46,714.
3. Bernot, MJ. 2010-2011. BSU Faculty Research Grant. Developing bioindicators for pharmaceuticals in freshwater ecosystems. Total Award: $6,000.
4. Bernot, MJ. 2010-2011. BSU Immersive Learning Provost Initiative Grant. Immersive Learning in Freshwater Ecosystems: Building collaborations with the Indiana State Department of Health for immersive undergraduate education in water resources. Total Award: $30,590.
5. Bernot, MJ. BSU Reprint Funding $200.
6. Veach, A and MJ Bernot. 2010-2011. Quantifying microbial degradation of pharmaceuticals in freshwater ecosystems. Indiana Academy of Sciences Senior Research Grant Program. Total Requested: $2,582.

7. Veach, A. 2010. Relationships between pharmaceuticals and d15N signatures in freshwaters. BSU Graduate Student Grant Competition. Total Award: $500.
8. Listman, L. 2009. Antibiotic resistance associated with stream sediment microbial communities influenced by different land use. Undergraduate Research Award. Total Award: $538.
9. Bragg, R. 2009. Examining effects of multiple pharmaceuticals on amphibian development. BSU Undergraduate Student Grant. Total Award: $300.
10. Bernot MJ and C Chatot. *To be submitted June 2010*. Teratogencity of pharmaceuticals in drinking water. NIEHS.

Students

A total of 2 graduate students and 7 undergraduate students were involved with this project.

Hydrology, Substrates and Fish Assemblages of the Wabash River

Basic Information

Title:	Hydrology, Substrates and Fish Assemblages of the Wabash River
Project Number:	2009IN248B
Start Date:	3/1/2009
End Date:	2/28/2010
Funding Source:	104B
Congressional District:	6
Research Category:	Biological Sciences
Focus Category:	Non Point Pollution, Solute Transport, Surface Water
Descriptors:	
Principal Investigators:	Mark Pyron

Publication

1. Pyron, M., J. Beugly, Pritchett, J., Jacquemin, S., Lauer, T. and J. Gammon. 2010. Long-term fish assemblages of inner bends in a large river. River Research and Applications 26:1-9.

State: IN **Project Number:** 2009IN248B
Title: Hydrology, Substrates and Fish Assemblages of the Wabash River
Project Type: Research
Focus Category: Non Point Pollution, Solute Transport, Surface Water
Keywords: Fish assemblages, river habitat, hydrology
Start Date: 3/1/2009 **End Date:** 2/28/2010
Congressional District: 6 **PI:** Pyron, Mark Associate Professor, Ball State University
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Report Format

Abstract / Summary: The local substrate composition of large rivers varies with local current velocity and high flow events. We evaluated effects of hydrology on local substrate variation for 28 Wabash River sites from 2005-8, and subsequent variation in fish assemblages using multivariate analyses. Sites were 500-m in length and fish collections were by boat electrofisher. Hydrologic variation was analyzed with the Indicators of Hydrologic Alteration (IHA) software and tested for temporal hydrology effects on substrate variation and for effects of substrate variation on taxonomic and functional fish assemblages. The analyses resulted in significant relationships for hydrologic variation with annual variation in substrates and for hydrologic variation with fish assemblage structure. Mantel tests were used to test for concordance among hydrology, local substrate variation, and fish assemblage structure. Pairs of the variables hydrology, local substrate variation, and fish assemblage structure had significant concordance in years 2005, 2006, and 2008, but not in 2007. These results demonstrated that Wabash River fish assemblages respond to substrate variation and substrate variation is controlled largely by hydrology.

Problem: The Wabash River includes the longest free-flowing stretch in the eastern U.S. and as such is a unique system for study. This project has been funded historically as a monitoring project to assess the effects of electric generator plants and pharmaceutical laboratories on fish assemblages in the Wabash River. These efforts have resulted in a good quality longterm dataset for the same sites over more than 25 years. The current proposal is to include detailed substrate samples at the same sites. I will test for variation in substrate size categories at the 28 sites that differ from 2008 and test for substrate associations with fish assemblages.

A large literature documents the responses of fishes to habitat variation and habitat quality (Matthews 1998, Simon 1999). One of the primary influences on the habitats of fishes and other stream biota are hydrological flow patterns (Poff et al. 1997, Allan 2004). Humans have altered flow regimes through a wide variety of impacts including reservoir construction, navigation, water removal by municipalities, agriculture, and industry, and altered drainage from agricultural fields and urbanizations. The result of altered flow regimes impacts stream organisms through multiple direct and indirect effects. Stream organisms have evolved life histories in response to natural flow regimes that include predictable high and low flow events and predictable variations in flow. Altered flow regimes frequently result in decreased recruitment, or even local extirpations of native populations that require natural flow regimes (Poff et al. 1997). In addition, altered hydrologic regimes result in indirect negative changes in substrata through scouring and deposition patterns that result in absences of appropriate substrata for benthic or other organisms with specific habitat requirements. In the Wabash River, effects from changes in the hydrologic regime were observed over 100 years ago (Gammon 1998). Water clarity changed from clear to turbid from 1845 to 1900, likely a response to the magnitude of basin effects from increasing agriculture within the watershed, and during the 20^{th} century, other related human impacts.

Pyron and Lauer (2004) suggested that fish assemblages were responding to an altered hydrologic regime in the Wabash River. Further analyses of Gammon's 25-year dataset from 1974 to 1998 showed gradual, directional changes in the fish assemblage of the Wabash River at the scale of the 230-km reach that appear to have hydrological relationships (Pyron et al. 2006). At this river scale, the fish assemblages of the Wabash River have been impacted by humans, and impacts appear to be driven largely by hydrologic alteration. Hydrologic alterations include moderated seasonal flows in upstream reaches from reservoir releases, and increased flow variability in downstream reaches, largely from agricultural water drainage patterns. These hydrological alterations are still present and appear to be augmented by increased precipitation over the past century (Pyron and Neumann 2008).

At 28 sites fishes will be sampled in a single pass along a 500-m reach located on the outer river bends. Gammon (1998) demonstrated that the 500-m site length is where species richness reached an asymptote. All fishes will be collected by boat electrofisher (Smith-Root 5.0 GPP) using DC voltage (600 V, 6-8 A) and two netters using dip nets with 6.25-mm_2 mesh. Individual fish will be identified to species, measured (mm), weighed (g), evaluated for DELT (deformities, erosions, lesions, and tumors) anomalies, and released. I will calculate IBI scores for each of the samples using the methods of Gammon & Simon (2000). Three substrate samples will be collected at each site and processed in the laboratory.

Daily discharge hydrology data will be downloaded from five mainstem Wabash River USGS gaging station websites. Daily discharge data for year 2008 will be used to calculate 16 hydrology variables that potentially influence substrates, using the Indicators of Hydrologic Alteration (IHA) software (Richter et al. 1996). These hydrology variables are in two IHA parameter groups: magnitude and duration of annual extreme water conditions, and frequency and duration of high and low pulses. These 16 hydrology variables will be used in predicting substrate variation. I will examine variation in substrate size categories among the 28 sites and test for the ability of this variation to predict species composition among the sites. The responses of the fish assemblages will include an upstream-downstream gradient. This project will demonstrate the effects of river hydrology on habitat quality, with a longterm goal for hydrologists and/or engineers to perform hydrological restoration that will enhance the quality of the river.

Research Objectives: This project will result in: 1) collections of fishes and detailed substrate data at 28 Wabash River sites; 2) effects of the annual hydrology regime on substrate variation; 3) fish assemblage responses to variation in substrate; 4) final report to funding agency; 5) publication in suitable peer-reviewed journal; 6) presentations at local and national conferences.

Methodology: Site description. The Wabash River is the longest free-flowing North American river east of the Mississippi River. One mainstem reservoir is located at river km 662 and creates J. Edward Roush Reservoir. The upper river above Lafayette, IN has a mean gradient of 0.45 m/km and the downstream reaches have a mean gradient of 0.12 m/km (Gammon 1998). The surrounding geology is glacial deposits with limestone outcrops (Thompson 1998). Landuse in the watershed is approximately two-thirds agriculture and one-third forest, pasture, and other landuse types (Gammon 1998).

Annual fish collections in 2005-8 were by boat electrofisher (Smith-Root 5.0 GPP) with DC voltage and two netters. Fish were sampled at 28 sites that were 500-m long and located on outer river bends (Figure 1). The 500-m site length was based on an earlier analysis by Gammon (1998) that showed species richness reached an asymptote at this distance. Fish were identified, measured, and released at the site. Collections were in summer months of years 2005-8 when discharge was less than 143 m^3/s at the Montezuma, IN USGS gaging station (waterdata.usgs.gov). Substrates were quantified and depths were recorded at each site following a method developed by Ohio River Valley Sanitation Commission (ORSANCO) and used by Mueller and Pyron (2010). The 500-m site distance was divided into five 100-m transects perpendicular to the shoreline. The dominant sediment size was estimated with a 6-m long copper pole that was plunged to the bottom at 3-m increments from the shore, including a shoreline observation. Substrate size categories of boulder, cobble, gravel, sand, fines, and hardpan were from Wentworth (1922). Depth was simultaneously recorded to the nearest 0.1 m at each pole plunge location. Current velocity at 1-m depth was quantified at the end of each transect with a Marsh-McBirney Flow Meter.

Fish assemblage data were analyzed for spatial and temporal variation using taxonomic identities (species) and functional groups. Functional groups were assigned using Poff and Allan's (1995) trophic, habitat, and tolerance categories. Abundances of species that were equal to or less than 1% of the total catch of all years combined were considered rare and were deleted (Mueller and Pyron 2010). Functional group abundances were converted into percentages and transformed (arcsine square root), and taxonomic data were transformed (log + 1) for normalization (Ter Braak and Smilauer 1998). Fish assemblage data were analyzed by year using Principal Components Analysis (PCA) in PC-ORD (McCune and Mefford 1999) with significant PCA axes identified by the broken-stick model (Jackson 1993). Substrate frequency values were converted into percentages and transformed (arcsine square root) for normalization. PCA analyses were used to reduce substrate data into fewer uncorrelated variables for further analyses by year.

Hydrologic data were obtained from five U.S. Geological Survey (USGS) gaging stations that were assigned to collection locations based on proximity (Figure 1). Mean daily discharge data for years 1940-2008 were downloaded from the USGS website ((U. S. Geological Survey website). We used the Indicators of Hydrologic Alteration (IHA) flow-based software by Richter et al. (1996) to calculate hydrologic variables for each year 2005-8. This approach uses 33 hydrologic parameters to assess flow magnitude, timing, frequency, duration and rate of change (Richter et al. 1996). These 33 hydrology variables were then used in regression and PCA analysis.

The resulting scores from PCAs of taxonomic and functional fish data, substrate PCAs, and hydrology PCAs were compared among years by separate repeated measures ANOVA. Fish assemblage PCA axes, substrate PCA axes, and hydrology PCA axes were tested for significant concordance using Mantel tests (Douglas and Endler 1982).

Results The total abundance of all fishes was 5,918, and the five most common species were freshwater drum *Aplodinotus grunniens*, spotfin shiner *Cyprinella spiloptera*, emerald shiner *Notropis atherinoides*, gizzard shad *Dorosoma cepedianum*, and river carpsucker *Carpiodes carpio* (Table 1). Fish taxonomic PCA axes 1-3 were significantly different from random based on the broken stick model (Table 2). The first taxonomic PCA axis explained 19.2% of total variance and was associated with increased abundances of flathead catfish *Pylodictis olivaris*, blue sucker *Cycleptus elongatus*, and common carp *Cyprinus carpio* in the negative direction, and bullhead minnow *Pimephales vigilax*, longear sunfish *Lepomis megalotis*, and river shiner *Notropis blennius* in the positive direction (Figure 2). The second taxonomic PCA axis explained 10.1% of variance with freshwater drum, shorthead redhorse *Moxostoma macrolepidotum*, and blue sucker in increased abundance in the negative direction (Figure 2). Flathead catfish, smallmouth bass *Micropterus dolomieu*, and bluegill had higher abundances at sites in the positive direction on this 2nd axis. The third taxonomic PCA axis explained 8.7% of variance with gizzard shad, steelcolor shiner *Cyprinella whipplei*, and sand shiner *Notropis stramineus* in the negative direction, and flathead catfish, spotted bass *Micropterus punctulatus*, and common carp in the positive direction. Fish functional group PCA axes 1-6 were significantly different than random (Table 3). The first functional PCA axis explained 21.6% of the variance and was negatively associated with categories of silt substrate preference, benthic invertivore trophic guild, and general current velocity preference, and in the positive direction with surface/water column invertivore trophic guild, a high tolerance to silt, and slow to no current velocity preference (Figure 3). The second functional PCA axis explained 17.0% of the variance and was associated with categories of silt substrate preference, planktivore trophic guild, and small to large stream size preference in the negative direction and moderate current velocity preference, medium tolerance to silt, and rocky to gravel substrate preference in the positive direction (Figure 3). The third functional PCA axis explained 14.8% of variance with piscivore trophic guild, general substrate preference, and general current velocity preference in the negative direction, and herbivore-detritivore trophic guild, slow-none current velocity preference, and medium tolerance level in the positive direction. The fourth functional PCA axis explained 10.8% of variance with surface/water column invertivore trophic guild, small to large stream size preference, and sandy substrate preference in the negative direction. Species with silt substrate preference, omnivore trophic guild, and lentic stream size preference had higher abundances in the positive direction. The fifth functional PCA axis explained 7.7% of variance with low tolerance to silt, general invertivore, and rubble substrate preference in the negative direction and moderate current velocity preference, small to large stream size preference, and sandy substrate preference in the positive direction. The sixth functional PCA axis explained 7.0% of variance with species with general substrate preference, benthic invertivore trophic guild, and low tolerance levels in the negative direction. Species with sand substrate preference, piscivore trophic guild, and general invertivore trophic guild had higher abundances in the positive direction.

The PCA for substrate frequency resulted in two axes that were significantly different from random (Table 4). The first substrate PCA axis explained 35.9% of the variance and was associated with hardpan and fines in the negative direction and cobble and gravel in the positive direction (Figure 4). The second substrate PCA axis explained 22.3% of the variance and was associated with sand and boulder in the negative direction and hardpan and gravel in the positive direction.

The IHA analysis resulted in hydrology PCA axes 1-4 that were significantly different from random (Table 5). The first hydrology PCA axis explained 34.9% of the variance and was associated with 90-day maximum, 30-day maximum, and 7-day maximum discharge in the negative direction and base flow, fall rate and low pulse number in the positive direction (Figure 5). The second hydrology PCA axis explained 33.9% of the variance and was associated with date of minimum discharge, low pulse number, and date of maximum discharge in the negative direction and 90-day maximum discharge, high pulse length, and rise rate in the positive direction. The third hydrology PCA axis explained 13.3% of variance with number of high pulses, March mean discharge, and rise rate in the negative direction and fall rate, mean duration of high pulses, and the Julian date of 1-day minimum in the positive direction. The fourth hydrology PCA axis explained 11.6% of the variation with number of reversals, June mean discharge, and February mean discharge in the negative direction. Fall rate, November mean discharge, and October mean discharge were important variables in the positive direction.

Fish assemblage structure, substrate variation, and hydrology were significantly different among years (Table 6). The variables that differed in among years comparisons by repeated measures ANOVA were functional group PCA axes 1 and 3, substrate PCA axis 2, and hydrology PCA axes 1, 2 and 3 (Table 6). The Mantel tests were significant for four comparisons in 2005, two in 2006, none in 2007, and three in 2008 (Table 7).

Major Conclusions and Significance Wabash River fish assemblages respond to local substrate variation, which is shaped by hydrology. These analyses resulted in significant relationships for annual hydrologic variation with substrates and fish assemblage structure. Fish assemblages responded to variation in substrate type, depth, and water velocity indicating that the presence of appropriate benthic habitat and hydrology are significant predictors of fish assemblage structure. A repeated measures ANOVA results demonstrated significant differences among years for substrates, hydrology, and fish assemblages. Others have identified that fish assemblage structure is related to physical habitat at microhabitat scales (Fischer *et al*., 2009). Influences of hydrology on substrate variation and fish assemblages in riverine ecosystems have been demonstrated in small streams (Poff and Allan, 1995; Allan, 1995; Murchie *et al.*, 2008). Poff and Ward (1990) showed that physical microhabitat composition controlled the abundance and distribution of riverine biota. Our results demonstrate that similar patterns occur in a large river.

A natural flow regime produces habitat variation that native organisms respond to through life history strategies and morphologic adaptations (Poff *et al.*, 1997). These riverine habitats are a product of complex geomorphological interactions of flow and existing sediments. For example, substrate particle distribution is dependent on local hydrology. Higher current velocities result in larger particle size in local patches, such as gravel and cobble. Sand and silt are deposited in locations such as inner bends, with slower current velocities.

Hydrologic events with the potential to disturb and rearrange local substrates include large hydrologic pulse events (Leopold *et al.*, 1964). My results and the results of Mueller and Pyron (2010) indicate that the Wabash River fish assemblages respond to substrate and hydrology variation. Hydrology variables that I found to be correlated with substrate and fish assemblage variation were discharge magnitude and length of extreme water conditions, and the frequency and duration of high and low pulses. These extreme hydrologic events have strong effects at local scales and produce substrate variability from the redistribution of particles (Allan, 1995) as well as impact the river channel morphology and the distribution of substrate particles.

Hydrologic alterations negatively influence fish assemblages (Poff and Allan, 1995) partly because substrate quality influences fish assemblages (Hitt and Angermeier, 2008). In the Wabash River, a portion of environmental quality appears to be driven by hydrologic alterations, including increased mean base flow and fewer, less intense flood events and altered sediment patterns (Pyron and Lauer, 2004). Hydrologic alterations have negative effects on native organisms (Poff *et al.* 1997), which have adaptations for survival in a natural flow regime (Lytle and Poff, 2004). My study provides additional evidence of a mechanistic link for hydrologic alterations to fish assemblages and impact on substrate distribution.

Further details of Wabash River fish assemblage variation with local habitat were obtained from my functional analyses. When I categorized Wabash River fish assemblages by Poff and Allan's (1995) functional groups, I found that functional groups of substrate preferences, current velocity preference, and trophic guild categorization varied significantly with local habitat variation. Fischer and others (2009) also found that taxonomic and functional fish assemblages could be explained from local habitat characteristics. My Mantel tests of concordance using functional groups resulted in stronger relationships with hydrology and substrate composition than analyses using taxonomy (Table 6). Different results for the taxonomic and functional group analyses were not surprising (Hoeinghaus *et al.*, 2007).

The hydrology of the Wabash River mainstem is altered from a natural flow regime (Pyron and Neumann, 2008). Additional products of these hydrologic alterations are altered benthic habitats (substrate variation) and fish assemblage structure. There is potential for restoration of a component of the natural flow regime for the Wabash River from modifying upstream dam releases (Richter and Thomas, 2007), extending buffer zones, and reducing sediment runoff. Longterm monitoring is necessary to detect further modifications to the hydrology, substrates, and fish assemblages of the Wabash River watershed.

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Table 1. Rank abundances of 21 common fish species in the Wabash River, from collection years 2005-8.

Species	Latin name	Abundance
Freshwater Drum	*Aplodinotus grunniens*	929
Spotfin Shiner	*Cyprinella spiloptera*	815
Emerald Shiner	*Notropis atherinoides*	761
Gizzard Shad	*Dorosoma cepedianum*	416
River Carpsucker	*Carpiodes carpio*	345
River Shiner	*Notropis blennius*	337
Longear Sunfish	*Lepomis megalotis*	303
Bullhead Minnow	*Pimephales vigilax*	263
Bluegill	*Lepomis macrochirus*	218
Shorthead Redhorse	*Moxostoma macrolepidotum*	204
Common Carp	*Cyprinus carpio*	168
Steelcolor Shiner	*Cyprinella whipplei*	162
Flathead Catfish	*Pylodictis olivaris*	146
Silver Redhorse	*Moxostoma anisurum*	124
Smallmouth Buffalo	*Ictiobus bubalus*	124
Sand Shiner	*Notropis stramineus*	120
Channel Catfish	*Ictalurus punctatus*	115
Blue Sucker	*Cycleptus elongatus*	111
Smallmouth Bass	*Micropterus dolomieu*	99
Spotted Bass	*Micropterus punctulatus*	89
Golden Redhorse	*Moxostoma erythrurum*	69

Table 2. Fish taxonomy PCA loadings from Wabash River collections during 2005-8.

Species	PC 1	PC 2	PC
Blue Sucker	-0.004	-0.710	0.265
Bluegill	0.293	0.486	0.416
Bullhead Minnow	0.659	0.092	0.181
Channel Catfish	0.021	-0.120	0.560
Common Carp	0.445	-0.142	0.242
Emerald Shiner	0.483	0.282	-0.171
Flathead Catfish	-0.314	0.365	0.499
Freshwater Drum	0.515	-0.378	-0.003
Gizzard Shad	0.151	0.350	-0.500
Golden Redhorse	0.484	-0.246	-0.172
Longear Sunfish	0.665	-0.006	0.290
River Carpsucker	0.493	0.016	-0.119
River Shiner	0.740	0.177	-0.090
Sand Shiner	0.497	0.330	-0.206
Shorthead Redhorse	0.459	-0.500	-0.146
Silver Redhorse	0.483	-0.212	-0.036
Smallmouth Bass	0.287	0.407	0.141
Smallmouth Buffalo	0.337	-0.348	0.125
Spotfin Shiner	0.511	0.149	0.183
Spotted Bass	0.111	0.154	0.544
Steelcolor Shiner	0.245	0.162	-0.309

Table 3. Fish functional groups PCA loadings from Wabash River collections during 2005-8.

Functional Group	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Trophic Guild						
Herbivore-detritivore	-0.128	-0.158	0.462	0.358	0.130	-0.209
Omnivore	0.210	0.358	-0.392	0.493	0.217	-0.218
General invertivore	0.359	0.410	0.137	0.304	-0.490	0.381
Surface/water column invertivore	0.580	-0.157	-0.161	-0.720	0.232	0.008
Benthic invertivore	-0.627	0.313	0.371	0.001	-0.017	-0.421
Piscivore	-0.421	-0.272	-0.628	0.141	-0.036	0.424
Planktivore	-0.047	-0.604	0.019	0.134	-0.117	0.258
Current velocity preference						
Moderate	-0.437	0.490	-0.184	-0.168	0.381	0.013
Slow-none	0.727	-0.208	0.506	0.171	0.033	-0.178
General	-0.587	-0.156	-0.515	-0.080	-0.382	0.227
Substrate preference						
Rubble (rocky, gravel)	-0.256	0.669	-0.015	-0.250	-0.460	-0.086
Sand	0.487	0.330	0.240	-0.426	0.293	0.504
Silt	-0.669	-0.459	0.314	0.442	0.099	0.018
General	0.558	-0.142	-0.609	-0.010	-0.107	-0.433
Tolerance						
High	0.723	-0.270	-0.276	0.020	-0.194	-0.194
Medium	0.095	0.493	0.722	0.048	0.038	0.259
Low	-0.089	0.461	0.130	-0.277	-0.586	-0.238
Stream size preference						
Medium-large stream size	-0.464	0.489	-0.356	-0.140	0.377	-0.119
Small-large stream size	-0.207	-0.683	0.396	-0.442	-0.199	-0.066
Lentic	0.542	0.430	-0.201	0.585	-0.032	0.154

Table 4. Substrate frequency PCA loadings for 28 sites on the Wabash River in 2005-8.

Substrate type	PC 1	PC 2
Fines	-0.646	0.046
Sand	-0.296	-0.852
Gravel	0.738	0.519
Cobble	0.618	-0.284
Hardpan	-0.698	0.395
Boulder	0.483	-0.319

Table 5. Hydrology PCA loadings from Indicators of Hydrologic Alteration analyses of years 2005-8.

Hydrologic Variable	PC 1	PC 2	PC 3	PC 4
October mean	-0.212	-0.041	-0.569	0.781
November mean	-0.720	-0.194	-0.278	0.584
December mean	-0.858	-0.174	-0.425	0.174
January mean	-0.827	-0.331	0.280	0.328
February mean	-0.753	0.095	0.291	-0.564
March mean	-0.518	0.271	-0.791	0.110
April mean	-0.451	0.644	-0.561	0.201
May mean	0.104	0.962	0.098	0.007
June mean	-0.389	0.651	-0.136	-0.600
July mean	-0.355	0.813	0.079	-0.431
August mean	-0.294	0.892	-0.277	0.153
September mean	-0.162	0.940	-0.015	0.201
1-day minimum	-0.194	0.902	0.187	0.261
3-day minimum	-0.158	0.918	0.159	0.281
7-day minimum	-0.179	0.923	0.141	0.258
30-day minimum	-0.147	0.969	0.096	0.154
90-day minimum	-0.361	0.902	-0.030	-0.201
1-day maximum	-0.917	-0.193	0.270	-0.170
3-day maximum	-0.925	-0.167	0.287	-0.127
7-day maximum	-0.941	-0.120	0.276	-0.063
30-day maximum	-0.956	-0.167	0.195	0.095
90-day maximum	-0.993	-0.002	0.091	-0.025
Base flow index	0.629	0.616	0.346	0.209
Julian date of 1-day minimum	0.152	0.620	0.508	-0.148
Julian date of 1-day maximum	0.431	0.666	0.138	-0.298
Number of low pulses	0.723	0.247	-0.278	0.319
Mean duration of low pulses	-0.606	-0.614	0.155	0.296
Number of high pulses	0.140	-0.211	-0.863	-0.349

Mean duration of high pulses	-0.785	-0.373	0.450	0.083
Rise rate	-0.535	0.190	-0.642	-0.417
Fall rate	0.693	-0.300	0.404	0.467
Number of reversals	0.327	-0.419	-0.260	-0.675

Table 6. Results of repeated measures ANOVA tests for differences among years in PCAs of fish assemblages, substrates, and hydrology.

Variable	SS	DF	MS	F	P
Fish Taxonomy PC1					
Year	22.3	3	7.4	4.7	< 0.01
Error	127	81	1.6		
Fish Taxonomy PC 2					
Year	15	3	5	4.1	< 0.05
Error	98.3	81	1.2		
Fish Taxonomy PC 3					
Year	43.2	3	14.4	13.3	< 0.001
Error	87.4	81	1.1		
Fish Functional Groups PC 1					
Year	27.8	3	9.3	3	< 0.05
Error	254.2	81	3.1		
Fish Functional Groups PC 2					
Year	10	3	3.3	1.4	0.2
Error	189.2	81	2.3		
Fish Functional Groups PC 2					
Year	23.6	3	7.9	3.8	< 0.05
Error	169.5	81	2.1		
Substrate PC 1					
Year	3.4	3	1.1	2.2	0.1
Error	40.6	81	0.5		
Substrate PC 2					
Year	9.2	3	3.1	9.1	< 0.001
Error	27.5	81	0.3		
Hydrology PCA 1					
Year	344.6	3	114.9	85.5	< 0.001
Error	108.8	81	1.3		
Hydrology PCA 2					

Year	908.5	3	302.8	297.5	< 0.001
Error	82.5	81	1		
Hydrology PCA 3					
Year	421.6	3	140.5	1787.7	< 0.001
Error	6.4	81	0.1		

Table 7. Mantel r and probability values for tests of concordance of Wabash River fish assemblages (using taxonomy and functional categorizations) with substrate variation and hydrology variables for years 2005-8. Significant relationships are in bold.

Year	**Taxonomy-Substrate**	**Functional-Substrate**	**Taxonomy-Hydrology**	**Functional-Hydrology**
2005	**0.28 (< 0.01)**	**0.32 (< 0.01)**	**0.12 (< 0.05)**	**0.09 (< 0.05)**
2006	0.15 (> 0.05)	**0.33 (< 0.01)**	0.07 (> 0.05)	0.19 (> 0.05)
2007	**0.01 (> 0.05)**	0.02 (> 0.05)	0.01 (> 0.05)	0.09 (> 0.05)
2008	**0.22 (< 0.05)**	**0.17 (< 0.05)**	0.17 (> 0.05)	**0.19 (< 0.05)**

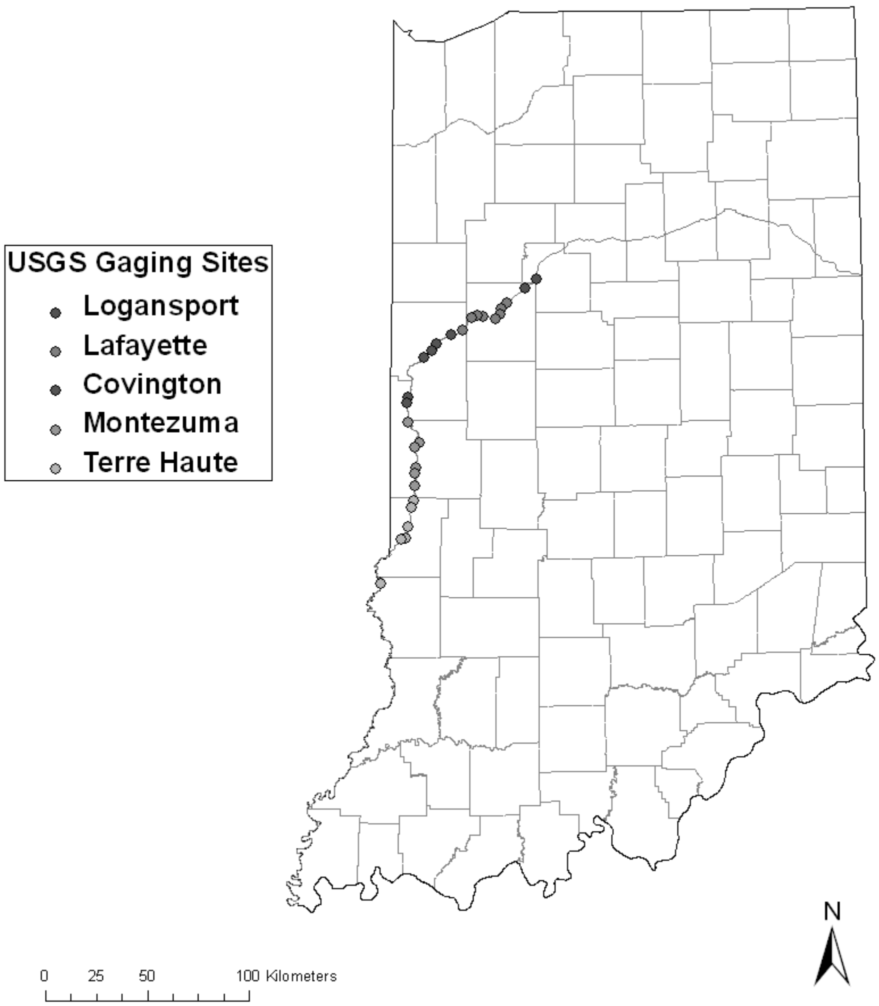

Figure 1. Sample sites and USGS gaging station assignments on the Wabash River.

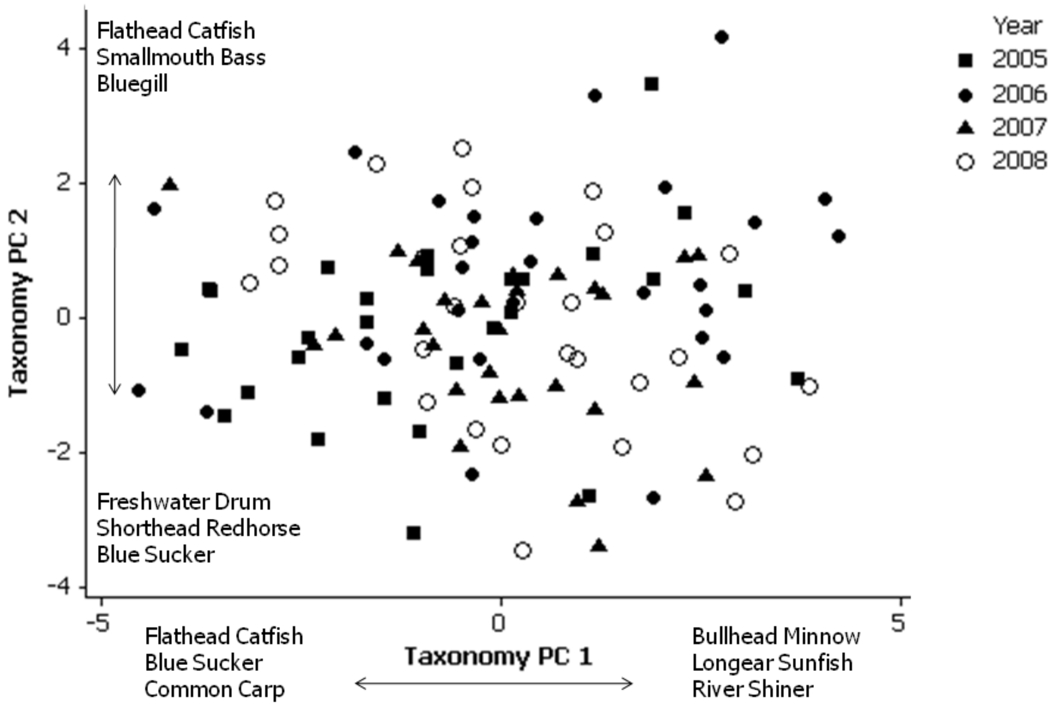

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Figure 2. Fish taxonomic PCA axes 1 and 2, years 2005-08.

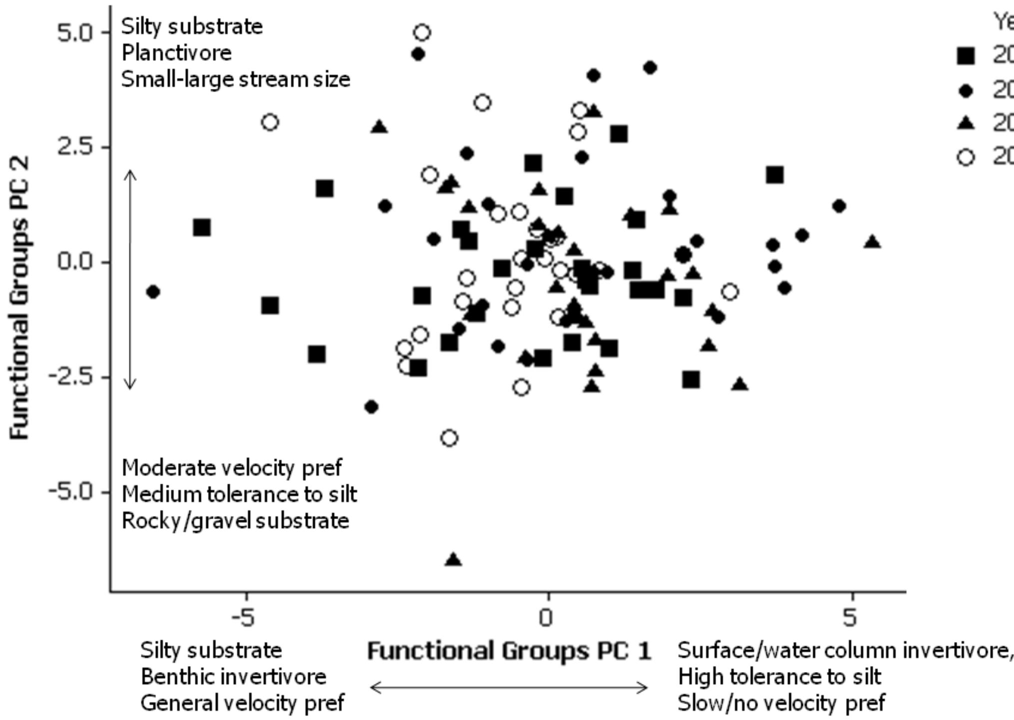

Figure 3. Fish functional groups PCA axes 1 and 2, years 2005-08.

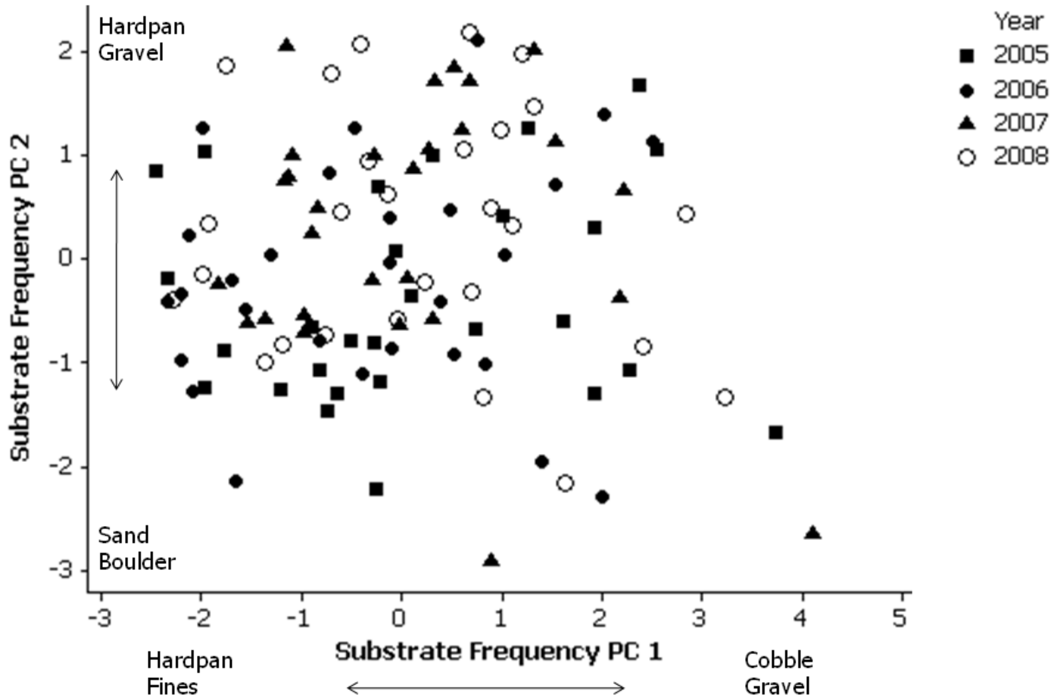

Figure 4. Substrate PCA axes 1 and 2, years 2005-08.

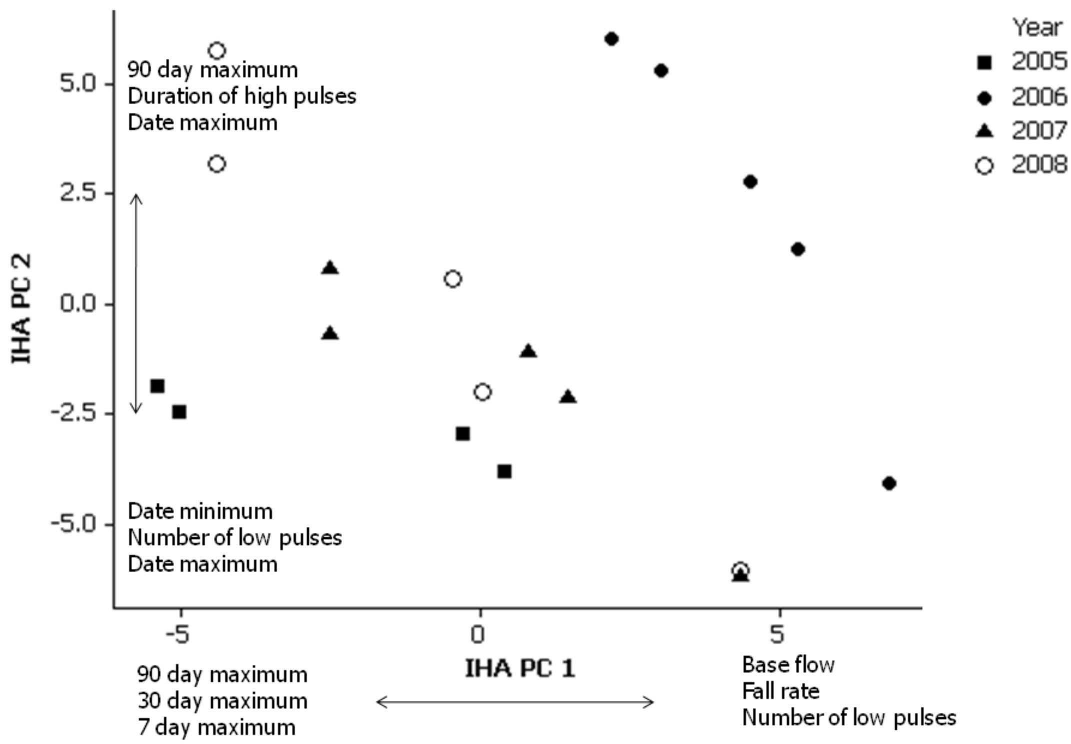

Figure 5. Hydrology PCA axes 1 and 2, years 2005-08.

Publications

Pyron, M., J. Beugly, Pritchett, J., Jacquemin, S., Lauer, T. and J. Gammon. 2010. Long-term fish assemblages of inner bends in a large river. River Research and Applications 26:1-9.

Presentations

Pritchett, J. and M. Pyron. Ecological effects of floods on the middle Wabash River. Oral presentation at Indiana Chapter of the American Fisheries Society, Elkhart, IN, Feb 2010.

Pritchett, J. and M. Pyron. Ecological effects of floods on the middle Wabash River. Oral presentation at 30th Annual Indiana Water Resources Association Conference, Columbus, IN, May 2009.

Pritchett, J. and M. Pyron. Effects of hydrology on substrate variation and subsequent fish assemblages for four years in the middle Wabash River. Oral presentation at Indiana Chapter of the American Fisheries Society, Indianapolis, IN, Jan 2009.

Pritchett, J. and M. Pyron. Effects of hydrology on substrate variation and subsequent fish assemblages for four years in the Wabash River. Poster at American Fisheries Society Conference, Nashville, TN, Sep 2009.

Grant Submissions:

Pilot Project to Collect Aquatic Habitat Physical Characteristic Data for a Two-Mile Reach of the Wabash River in Lafayette, Tippecanoe County, Indiana. The grant includes an agreement with USGS to survey substrates, bathymetry, and flow for $7,500. Wabash River Enhancement Corporation. Funded June 2009 – Dec 2009. $12,500.

Students Two graduate students

Information Transfer Program Introduction

None.

Publication 1: What Killed the Fish? Using Observations, Sampling, and Science to Solve the Mystery/Publication 2: A Field Guide for Determining the Cause of a Fish Kill

Basic Information

Title:	Publication 1: What Killed the Fish? Using Observations, Sampling, and Science to Solve the Mystery/Publication 2: A Field Guide for Determining the Cause of a Fish Kill
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Principal Investigators:	Fred Whitford

Publications

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2. Whitford, F., J. Becovitz, B. Robertson, B. MacGowan, G. Blasé, B. Avenius, J. Donahoe & D. Zimmerman. 2009. What killed the fish? PPP-79.

Titles: Publication 1: *What Killed the Fish? Using Observations, Sampling, and Science to Solve the Mystery* Publication 2: *A Field Guide for Determining the Cause of a Fish Kill* \

Focus Categories: AG, GW, LIP, NPP, SW, WQL, WS

Key Words: Pesticides, water quality, fish kill

Project Duration: March 1, 2009 to February 28, 2010

Funding Requested: $10,000

Principal Investigator: Fred Whitford, Ph.D., Coordinator, Purdue Pesticide Programs, Purdue University, 915 West State Street, West Lafayette, IN 47907-2054; Phone: 765-494-1284; Fax: 765-494-1556; Email: fwhitford@purdue.edu

Report Format

Problem: Questions arise whenever a pond owner discovers dead fish floating on the surface or lying along the shore of a lake, especially after a recent pesticide application to the pond or to neighboring property: Did the pesticide cause the fish kill? A fish kill complaint is serious and requires a quick response and a thorough investigation. This will require putting together many pieces of information collected at the site to answer the question of whether the pesticide may have contributed to the fish kill.

Outreach/Extension Objectives: The objective was to develop a publication that describes how to deal with a fish complaint, what to look for, and how to use that information in making a decision of how the fish might have died. This publication was to be incorporated into a number of state, regional, and national workshops to discuss the topic of fish kills as it relates to pesticides and other biological and chemical agents.

Principal Deliverables:

Extension Publications

- **Whitford, F.**, J. Becovitz, B. Robertson, B. MacGowan, G. Blasé, B. Avenius, J. Donahoe & D. Zimmerman. 2009. Solving the fish kill mystery…Questions to ask. PPP-80.

• **Whitford, F.**, J. Becovitz, B. Robertson, B. MacGowan, G. Blasé, B. Avenius, J. Donahoe & D. Zimmerman. 2009. What killed the fish? PPP-79.

Publication Printer and Distribution

• *A total of 10,000 copies were printed of PPP-79. Currently, 1,800 remain in inventory. A total of 3,900 copies mailed to every pesticide business in Indiana that are licensed with the Office of the Indiana State Chemist.*

• *A total of 10,000 (first run) and 4,000 (second run) were printed of PPP-80. Current inventory is at 3,700. A total of 3,900 copies mailed to very pesticide business in Indiana.*

• *Publication can be downloaded at www.btny.purdue.edu/ppp.*

Trade Journal

None

Train-the Trainer Programs

None

Indiana Presentations

• You killed my fish! 2010. Midwest Aquatic Plant Management Society Annual Conference. Indianapolis, Indiana.

• Solving the mystery of the fish kill: what questions to ask. 2010. Townsend Chemical Division Vegetation Management Pesticide Applicators Workshop. Indianapolis, Indiana.

• Mystery of the fish kill: natural or chemical. 2010. National Railroad Contractors Association. Indianapolis, Indiana.

• Fish kills. 2010. Indiana Arborist Association Conference. Indianapolis, Indiana.

• Solving the fish kill mystery. 2010. Aquatic Control Applicator Workshop. Indianapolis, Indiana.

• What killed the fish? 2010. Indiana Green Expo. Indianapolis, Indiana.

• What killed the fish? 2009. Northeast Indiana Landscape and Turf Seminars. Fort Wayne, Indiana.

• What killed the fish? 2009. Purdue University Turf and Ornamental Seminar. West Lafayette, Indiana.

• Solving the mystery of the fish kill: what questions to ask. 2009. Hoosier Energy Herbicide Seminar. Bloomington, Indiana.

• Solving the fish kill mystery. 2009. Tri-State Field Day. Columbia City, Indiana.

• The mystery of the fish kill. 2009. Madison County Private Applicator Recertification Program. Alexandria, Indiana.

- What you need to know about fish kills. 2009. Cygnet Enterprises Fifth Annual Aquatic Management Winter Workshop. West Lafayette, Indiana.

National Presentations

- Fish kills and public concerns. 2010. University of Florida Aquatic Weed Control Program. **Coral Springs, Florida.**

- Solving the fish kill mystery. 2010. West Virginia Vegetation Management Association. **Roanoke, West Virginia**.

- Mystery of the fish kill: natural or chemical. 2010. Oklahoma Vegetation Management Association Spring Conference. **Oklahoma City, Oklahoma.**

- What killed the fish? 2010. Alabama Vegetation Management Society. **Tuscaloosa, Alabama**.

- Solving the mystery of the fish kill: what questions to ask. 2010. Townsend Chemical Division Vegetation Management Pesticide Applicators Continuing Education Seminar. **Springfield, Ohio**.

- Mystery of the fish kill: natural or chemical. 2009. Oregon Vegetation Management Association. **Seaside, Oregon**.

- What killed the fish? 2009. Texas Vegetation Management Association. **Corpus Christi, Texas**.

USGS Summer Intern Program

None.

Notable Awards and Achievements