December 2012

An Evaluation of the Effect of Stormwater Treatment Ponds on Wetland and Stream Quality Indicators

Subhomita Ghosh Roy
University of Wisconsin-Milwaukee

AN EVALUATION OF THE EFFECT OF STORMWATER TREATMENT PONDS ON WETLAND AND STREAM QUALITY INDICATORS

by

Subhomita Ghosh Roy

A Thesis Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Master of Science

in Biological Sciences

at

The University of Wisconsin-Milwaukee

December 2012

ABSTRACT

AN EVALUATION OF THE EFFECT OF STORMWATER TREATMENT PONDS ON WETLAND AND STREAM QUALITY INDICATORS

by

Subhomita Ghosh Roy
The University of Wisconsin- Milwaukee,2012
Under the Supervision of Timothy Ehlinger Ph.D.

Modifications of land cover in urban areas are leading to hydrological, physiochemical and subsequent biological disturbances in the receiving aquatic ecosystems. Resulting in damage of the limited quantity of available freshwater. Based on the recognition of the value of natural wetlands in water quality improvement, constructed wetlands have been widely used for water treatment, to remove fine pollutants from catchment runoff also to control increased surface runoff from urbanization. The hypotheses of the study was that the surface water quality would improve while the sediment quality would vary moving from up-gradient to down-gradient through the interconnected wetlands, relative to precipitation, discharge rate and season. The interconnected wetlands in Pike River watershed (Racine, WI) were chosen for the study. Water quality (physical characters and nutrients) and sediment studies were performed in these three interconnected wetlands and in the stream as well. Physical parameters (including pH, specific conductivity, dissolved oxygen, turbidity) and nutrient levels (nitrogen, phosphorus) were analyzed from the water. Sediment bioassays were performed with the plant species *Sinapsis, Lepidium*, and *Sorghum* and with the invertebrate *Heterocypris* as an indicator species. Also, *Thamnocephalus* was used as an indicator for the pore water bioassay. Results showed strong indication of water quality improvement by phosphate reduction towards the down-gradient wetland, high specific conductance, turbidity and Low dissolved oxygen partly in the up-gradient wetland. Although there were some exceptions in the results, but its important to realize that these wetlands are just 10 years old and may not have their biological potential at the fullest like natural wetlands. Another important finding of the study was that the stream also performed in a comparable fashion with the wetlands. These findings suggest that a functional interconnected wetland system can discharge less polluted fresh water to its connected water body.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to thank my advisory committee: Dr. Timothy Ehlinger, Dr. James Reinartz and Dr. Peter Dunn. Dr. Ehlinger not only inspired me about the field of Wetland Ecology but also supported me in all the difficult times I had here, professional or personal. Without his help and assistance and passion this project would not been a reality. Dr Reinartz helped in forming my research questions and technical design of the study. Dr Dunn assisted me with the statistical analysis and also in the development and presentation of the study. Thank you for all of your time and help in all these difficult years.

I would also like to thank my fellow Fish Ecology lab graduate and undergraduate students: Jesse Jensen (M.S), Mathew Dellinger (Ph.D) and Andy McGuire (Ph.D candidate), Aaron Krizek (B.S.) and Kendall Behnke. Jesse, Andy and Matt assisted me during the field works, which would be not possible without them. Jesse trained me in all the Ecotoxicological experiments and also helped me in the formation of experiment designs. Andy, Matt, Jesse and Aaron was always there whenever needed them for doing field works. I will miss all those hot summer days.

This degree could never be possible, neither I would have come to a foreign country to fulfill my dreams without the support from my father Asis Ghosh Roy, my mother Uma Ghosh Roy, my sister Moumita Ghosh Roy and my uncle Dr. N Sinha. Thanks also to my friends and my fiancé Abhishek Bagchi for all your help and support throughout these difficult times.

INTRODUCTION

The process of urbanization in the United States since the mid 1900s has presented significant environmental challenges for the protection and maintenance of water resources. Increased public perception of these challenges lead to the establishment of the US Clean water Act (CWA) in 1972 for regulating discharges of pollutants into the waters of the United States and regulating quality standards for surface waters (EPA 2012, Carey and Hochmuth et al., 2012). Expanding areas of impervious surfaces result in reduced infiltration and increased storm-water runoff into receiving waterways (Bannerman et al.1993). In addition to flooding, the pollutants carried by storm-water can seriously harm water quality and biotic integrity in rivers, streams and lakes (EPA 2003).

Stormwater ponds are often constructed to mitigate the impacts of increased runoff flows, volumes and pollution loads (Tixier et al., 2011), stormwater ponds have been used extensively during the past 35 years (Chocat et al., 2001). Among the various best management practices (BMPs), stormwater management ponds have become common features of urban landscape in the USA, Canada, Australia, Denmark, France, Sweden, and UK, where tens of thousands of such ponds were built in residential, commercial and industrial urban areas, and in transportation corridors. First designed to provide stormwater storage for controlling runoff peaks and flooding, their functions soon expanded to enhancing stormwater quality by various treatments (e.g., settling, bacterial degradation) and thereby protecting receiving waters against pollution. Stormwater ponds are commonly designed to provide additional benefits including aesthetic / recreational amenities, groundwater recharge, sub-potable water supply and new habitats for wildlife (Marsalek et al., 2005a).

Within the context at assessing the functionality of storm water ponds it is important to recognize the critical role that they also play by acting as wetlands in the natural cycling of sediments and nutrients in the environment – an attribute that is hugely beneficial to human livelihoods and well-being. The role wetlands play in trapping excess sediments and preventing them from entering river and lake systems present downstream is very important. As these sediment particles are often vehicles for transporting pollutants such as nutrients (for example, nitrate and phosphate), pesticides, and heavy metals (EPA, 2001).

Many studies have investigated the retention capacity and effects on nutrient levels in wetlands (Kadlec and Wallace 2009). For example, a series of storm-water wetlands were monitored in a heavily urbanized 12.5 ha watershed in North Carolina (Hathaway and Hunt, 2010), which allowed for an examination of the diminishing returns provided by three successive BMPs of a similar type. At least 80% of the total concentration reduction for all pollutants occurred within the first wetland cell (Hathaway and Hunt, 2010).

Physical characteristics and hydrologic conditions can directly modify or change chemical and physical properties such as nutrient availability, degree of substrate anoxia, soil salinity, sediment properties, and pH (Mitsch and Gosselink 1993). Surface Inflows and Outflows, may be seasonal, are often matched with precipitation pattern or spring thaw, and can be channelized as stream flow or nonchannelized as runoff (Mitsch and Gosselink 1993, Kadlec and Wallace 2009).

The self-purification potential (SPP) of stormwater pond-wetland systems reflects the capacity of the ecosystem to assimilate all inputs (Tixier et al., 2011). Within specific boundaries set by the hydraulic needs of the system to control flooding and erosion (e.g., storage volume, detention time), the SPP represents all nutrient cycling and detoxification processes that result from synergy of biological processes (metabolic activities of all living organisms), physical factors (e.g., hydro geomorphic context, dynamics of flow exchanges between surface water and groundwater, settling of solids with associated chemicals) and chemical factors, including redox potentials, binding of pollutants by complexants, speciation of heavy metals (Tixier et al., 2011).

Other studies have examined the spatial and seasonal performance of stormwater management systems using an integrated sediment quality assessment approach that incorporate monitoring of water quality, monitoring of nutrients input and ecotoxicological bioassays and biotic characters related to precipitation, discharge rate and season. For example, Tixier et al. (2011) used detailed monitoring data for the characterization of self-purification potential of both constructed treatment ponds and natural riparian wetlands, reflecting their capacity to assimilate all inputs. This included estimating effects on physiochemical parameters (conductivity, pH, suspended sediment, dissolved oxygen) and nutrient inputs and outputs (phosphorus and nitrogen). In addition, they included ecotoxicological assays as a tool for prospective risk assessment (Chapman, 1995). The results from these and other studies demonstrate that the self-purification potential of constructed treatment pond-wetland systems varies seasonally and is contingent on patterns of precipitation, temperature and biological productivity of the system (Kadlec and Wallace 2009).

The purpose of this current study is to investigate the self-purification potential of a constructed stormwater pond-wetland system in the Pike River, located in the rapidly urbanizing watersheds of Racine and Kenosha Counties in southeastern Wisconsin (Figure 1). A flood-control plan implemented for the Village of Mount Pleasant included significant modifications in channel morphology, creation of riparian wetland-pond systems, and the installation of fish habitat along an 8 Kilometer stretch of river (Crispell-Synder, Inc. 1997, Ehlinger et al. 2002, Ehlinger and DeThorne 2004). Phase 1 of the multi-phase project included the construction of a 10 hectare riparian wetland system which included a series of 3 connected stormwater ponds, designed to capture and treat runoff from surrounding residential development (Figure 2). A field-sampling program was initiated to collect data on water quality, nutrient concentrations, and sediment ecotoxicology. These data were then examined relative to seasonal precipitation and flow patterns to determine the effectiveness of the pond system in improving water quality in the Pike River. The hypotheses of the study was that the surface water quality would improve while the sediment quality would vary moving from up-gradient to down-gradient through the interconnected wetlands, relative to precipitation, discharge rate and season.

MATERIALS AND METHODS

Study System

Three wetland ponds were chosen as sampling sites in the Pike River watershed in order to assess the effect of the interconnected wetland systems on water quality, toxicity and nutrient loading (Figure 2). Sampling sites included 3 wetland locations (1-3 in Figure 2). W1 receives water from a stormwater culvert draining a local residential subdivision. Water flows from W1 into W2 through a metal culvert, and then through an open channel into W3 (Figure 2). Water received into W3 then diffuses to the stream channel through a diffuse series of rivulet channels. All three wetland ponds also receive runoff from the surrounding farm fields and bike paths (Figure 2). The wetland ponds differed in physical dimensions with W1 being deepest and W3 being the shallowest (Table 1).

Four additional sampling sites in the stream were selected (S4-S7 in Figure 2) in order to assess stream water quality and toxicity above and below the wetland-pond system. The most upstream site (S4) also receives input from the local residential areas via a culvert.

Water Quality: Multiparameter Sondes

In the wetland sites, continuous monitoring of the water quality at 30 minute intervals was performed using YSI 6600 EDS multiparameter sondes equipped to measure the following parameters: pH, specific conductivity, dissolved oxygen, turbidity, depth and temperature from June through August

2012. Sondes were installed in each of the wetland sites for 6-week durations after which they were retrieved, the data were downloaded and a replacement set of calibrated sondes was deployed. Sondes were also installed to three of the four stream sites (S4, S5, S7) during August and September 2012 for six weeks. Sondes were placed approximately 15 centimeters above the stream or the wetland bed (see photos in Appendix F). Due to unavailability in the number of sondes the installation period was different in the wetlands and streams.

Surface Water Sampling and Nutrient Analysis

Presence of nutrients like Nitrate and Phosphate in surface water of all wetlands and the stream sites were analyzed. Water samples were collected on 11 dates during spring and summer 2012 using a US DH-81 integrated sampler. Samples were collected during dry periods (non-event) and following precipitation (event). The rainfall events were categorized as samples collected within 48 hours of precipitation falling greater than 1 cm in 24 hours at the Racine airport. The US DH-81 (Appendix E) integrated sampler was used for water sampling that enables to sample water in a 1-meter vertical column (USGS 2005, Appendix E i). In each wetland-pond, 3 water samples were collected along a vertical transect at the inflow and at the outflow and then spanning equidistant from the inflow to the outflow. For each stream site, water samples were collected from midstream thalwag. After collection, all water samples were transferred to 1000 mL Nalgene bottles and were placed on ice and transferred to the laboratory for analysis within 24 hours of sample collection.

Nitrate and ortho-phosphate were analyzed with HACH DR 2800™ spectrophotometer. For nitrate, the Cadmium Reduction method with applicability in water, wastewater and seawater and a detection range of 0.3 - 30.0 mg/L NO_3^{-}N was followed. Similarly for phosphorus the PhosVer 3 (Ascorbic Acid) method with applicability in water, wastewater and seawater and a detection range of 0.02 - 2.50 mg/L PO_4 was followed.

Sediment Sampling

An Ekman dredge was used to collect the biologically active layer of the benthic zone (near surface), or approximately top 10 cm of surface sediment from all of the wetland sites (1, 2 and 3) and three stream sites (4, 5 and 7). A steel corer was also used to collect core samples of about 40 cm from the wetland sites (1, 2 and 3) to incorporate the effect of deeper sediment accumulating layers in the wetlands. Sediment samples were collected followed southflow. Sample grabs were taken from each location along the transect and were composited in the field as one sample for each wetland. Core sampling followed the same procedure as above except that samples were composited by layer (top and bottom) yielding two samples per wetland. Approximately top 15 cm were collected as the top layer and the remainder of the total 30 cm core was taken out as the bottom layer.

Three sample grabs were collected at each stream site using the Ekman dredge. Two grabs were along the two shores and one approximately at the middle of the two. Finally all the grabs were homogenized in the field yielding one composite sample per stream site. All samples were stored in 1000 mL Nalgene bottles and placed in coolers temporarily while being transported back to laboratory. In lab, each sample was divided into 500 mL centrifuge bottles and spun at 4000 rpm for 15 minutes in Beckman J2-HS centrifuge to separate the pore water from the sediment. Both the sediment and the pore water were then frozen separately (-20^{0}C) and stored in accordance with the United States Geological Survey procedure (USGS et al 2011). Sediments and pore waters were transferred into the refrigerator (at 4^{0}C) from the freezer to thaw before at-least 48 hours prior to testing.

Ecotoxicological Bioassays

Ecotoxicological tests were used for the assessment of the total toxicity of all wetland site sediments, covering organic and inorganic pollutants. While the pore water assays generally reveal only the dissolved (bioavailable) contaminants, direct-contact tests provide a better assessment of overall ecotoxicological potential (Standard Operational Procedure, Ostracodtoxkit, Microbiotest Inc). As such, three different methods developed by MICROBIOTESTS INC. were used to evaluate ecotoxicologial properties of the samples collected: (1) OSTRACODTOXKIT™ for direct sediment contact, (2) RAPIDTOXKIT™ for pore waters and (3) PHYTOTOXKIT™ for sediments.

Ostracod, *Heterocypris incongruens*, cysts were hatched, pre-fed, and incubated for 48 hours. A subset of 10 individuals was sampled and initial body length measurements were recorded, and the remaining organisms were distributed in sets of 10 into individual wells filled with control and test sediments. Test and standardized quartz control sediments were prepared by mixing with a standardized water solution (US EPA formula for “moderately hard water”). A prepared algal solution was added to the wells to serve as food and the organisms were incubated in darkness for 6 days at 25 ^{0}C. After incubation, the contents of wells were micro-sieved and the ostracods were separated out from the sediments under microscope. The number of dead and living organisms was recorded. The surviving ostracods were placed in a fixative (lugol solution), digitally photographed, and the length of individuals was recorded using a micrometer (Appendix B). Growth inhibition and mortality rates were calculated by comparing responses of treatment sediments with controls (Standard Operational Procedure, Ostracodtoxkit, Microbiotest Inc).

RAPIDTOXKIT^{TM} tests were used to evaluate the response of *Thamnocephalus platyurus* larvae to pore waters from the wetland sites as well as stream sites by measuring feeding inhibition relative to controls. *T. platyurus* cysts were hydrated, and then incubated for 30-45 hours (Appendix A). After incubation, the hydrated cysts were then distributed into separate test tubes with either control water or test pore water and were incubated for an hour. Next, colored microspheres acting as food (dyed red for color indicator) to be taken up by the organisms were added to the test tubes and were incubated for an additional 30 minutes. The *T. platyurus* were then fixed with a lugol solution, transferred to an observation plate and examined under a stereomicroscope. The digestive tracts of the *T. platyurus* were observed and scored as either “red”, indicating that feeding had occurred (Appendix A) or as “clear”, indicating feeding inhibition (Standard Operational Procedure, Rapidtoxkit, Microbiotest Inc).

PHYTOTOXKIT^{TM} assays were used to assess the toxicity by measuring the rate of seed germination and the growth of young stems and roots of selected higher plants that are exposed to the stream and all wetland site sediments. The plants selected for the PHYTOTOXKIT included: the monocotyl *Sorghum saccharatum*, dicotyls *Lepidium sativum* (garden cress) and *Sinapis alba* (mustard). These species were selected due to their sensitivity to contaminants, rapid rates of germination, and growth of their stems or roots, allowing scoring of the results after three days or 72 hours. (Standard Operational Procedure, Phytotoxkit, Microbiotest Inc). This toxicity test utilizes a dual compartment test plate (Appendix C) where the bottom compartment of each transparent test plate (10cm square by 0.5 cm deep) was filled with 90 cm^3 of water-saturated control or test sediment. Filter paper was placed over the saturated sediment and seeds of the test plants are positioned near the middle ridge of the test plate on top of the filter paper. Test plates were covered and incubated vertically in darkness at 25 ^{0}C for three days. At the end of the incubation period, a digital image was taken of the test plates and stem and root lengths were measured using Image J software.

Pond Zooplankton Community

Zooplankton samples were collected from the wetland sites only during July 2012. Three samples were collected at random locations in each pond using a plankton net (mesh size: 20 um) with an anterior reducing cone; a posterior conical filtering net; and Dolphin™ adapter with a bucket. The plankton net was dipped in water with a tow length of 0.75 meter to 1 meter. Samples were washed through a 0.36 mm net and preserved in 95% ethanol. Organisms were identified in the laboratory under a stereomicroscope in 50x magnification up to the lowest possible taxonomic level (Balcer et al. 1984; Pennak 1978).

Vegetation survey

Vegetation surveys were performed during August 2012 in the wetland-pond sites. Eight 1m square quadrats (Appendix G) were sampled in the each wetland with half of the samples along the edge of the water and the remainder in the upper part of the bank. Dominant plant species in each location were identified and their percent cover estimated to the nearest 5 percent. This was repeated for all three wetland-ponds. Plants were identified to the lowest possible taxonomic level following the Wisconsin State Herbarium Website.

Precipitation and Discharge

Total daily precipitation data from Racine, WI (station- airport) were taken from wunderground.com from September 2011- August 2012. Pike River discharge (m^3/s) data at the USGS gauging station at Kenosha for the study period of September 2011 till first week of September 2012 were downloaded from the USGS database of National Water Information System.

Data Analyses

Data were checked for normality and transformed as necessary to meet assumptions of statistical tests. Count and length data were transformed using a log10 transformation (log10 (Y + 1)) while proportional data were transformed using an arcsine transformation (Sokal and Rohlf 1994). Statistical analyses were conducted using JMP® 10 (SAS Institute 2011). Analysis of variance (ANOVA) was used to test for differences among sites, and to examine the effects of precipitation (event, non-event) and interactions.

RESULTS

Stream Hydrological & Wetland-Pond Physical Characteristics

Daily total Precipitation and mean stream discharge for the 2012 study period are presented in Figure 3. Spring precipitation and high flows (January-March) were followed by an extended period of dry weather and low discharge (May-July). Precipitation increased in late July-August, resulting in higher stream flow. Of the 11 dates when water samples were collected for nutrient analysis (Figure 3), 4 were classified as non-event (baseflow) and 7 were classified as event samples (stormflow).

Physical characteristics of the wetland-ponds are presented in Table 1. Surface area increased and depth decreased moving from W1-W3, resulting in W1 having a volume more than 3 times the volumes of W2 and W3 (Table 1). Discharge measured at the inflows to each pond during baseflow and stormflow allowed for the calculation of average turnover rates for each pond. Turnover in

W1 was 3-4 times greater (longer retention time) compared to the other ponds during baseflow, but only 2-3 times higher during stormflow.

Water Quality

Patterns of variation in water quality collected by multiparameter sondes across the study period for wetland and stream sites are presented in Figures 4 and 5, respectively. Analysis of covariance of hourly means using precipitation as a covariate was conducted. A median filter was used to reduce random scatter in turbidity readings. This analysis showed that wetland-ponds sites differed from each other for all parameters (Table 2 - pH, Specific Conductivity, Dissolved Oxygen and Turbidity), but only specific conductance and dissolved oxygen were affected by precipitation. The same effects were observed in the stream sites, however pH levels were also affected by precipitation.

The patterns of precipitation effects differ over time. For example, during the extended dry period of June, the pH for sites varied among each other (Figure 4A). However, following extended rainy periods in late July, the pH levels for all wetland sites increased and remained high through August. Similarly to pH, dissolved oxygen in the wetland-ponds decreased during the dry month of June, then was higher during the wetter periods later in the summer (Figure 4C). Overall, specific conductance exhibited the greatest sensitivity to precipitation for both wetland-ponds and stream sites (Figure 4B, Figure 5B). The patterns of turbidity for the wetland-pond sites indicate turbidity was most often highest in W1 (Figure 4D). Although ANCOVA did not detect an effect of daily precipitation, turbidity generally increased later in the summer, associated with increased frequency of rain (see Figure 3).

Surface Water Nutrient Concentration

Nitrate and phosphate concentrations varied significantly among sites and across sampling dates for both wetland-ponds and stream sites (Figure 6 and Figure 7). Two-way analysis of variance was used to examine effect of rain events (event vs. non-event) on the differences among sites (Tables 4 and 5). The results of these analyses are presented separately for wetland-ponds (Figures 8 and 9) and stream sites (Figures 10 and 11).

For the wetland-pond system, although there is a slight indication that nitrate decreased moving down-gradient (Figure 6A), the nitrate levels did not differ significantly between ponds (Table 4, Figure 8A). However nitrate increased in all ponds during rain events compared to non-events (Table 4, Figures 8A and 8B). By contrast, phosphate levels decreased significantly moving down-gradient from W1 into W2 and W3 (Figure 6B, Figure 9A, Table 4), and was more pronounced during the event samples in July and August (Figures 6B and 9B).

The impact of rain events on nutrient concentrations was pronounced for stream sites (Figure 7). Nitrate concentrations decreased moving downstream during non-events (Figure 7A, Figure 10A, Table 5). However, nitrated levels were significantly lower during events compared to non-events for all sites (Figure 10A and 10B). By contrast, phosphate concentrations decreased moving from upstream to downstream during rain events (Figure 11A), and were higher for the upstream sites during events compared to downstream sites (Figure 11B, Table 5)

Ecotoxicological Bioassays

Ostracod toxicity measures of relative mortality and growth inhibition and RapiTox values for feeding inhibition are presented in Figure 12 and Figure 13 for wetland-pond and stream sites. Ostracod growth inhibition did not differ significantly among wetland-ponds, but was higher in October 2011 compared to March and June 2012 (Figure 12A, Table 6). Ostracod relative mortality did not vary significantly across sites or dates (Table 6), but variation was much greater in the samples collected in 2012 compared to 2011 (Figure 12B). Rapidtox feeding inhibition did not differ among sites, but did vary among seasons (Figure 12C, Table 6). For stream sites, feeding inhibition was measured for only one sampling date (Figure 13). Although there was visual trend of increasing inhibition moving downstream, the effect was not statistically significant (Table 6).

Phytotox™ toxicity test measures for root and shoot growth inhibition are presented in Figure 14 and Figure 15 where toxic effects are indicated by positive inhibition values and lack of toxic effects (conversely facilitation) are indicated by negative inhibition values. *Lepidium* exhibited no toxic effects for stems, but significant toxic effects on roots (Figure 14). Root inhibition differed by sampling date (season) with a significant interaction with site and season (Table 7). For example, W1 exhibited higher toxic effects on 2 dates compared to the other sites (Figure 14B). A similar upstream-downstream pattern for *Lepidium* was observed for the stream sites but was not statistically significant (Figure 15, Table 7).

Growth inhibition in *Sinapsis,* exhibited a qualitatively similar pattern to *Lepidium* (Figures 14 and 15). Growth inhibition in *Sorghum* was highest compared to the other plant species, indicating the greatest toxic response for both wetland-ponds (Figure 14) and stream sites (Figure 15). A difference among sites was detected for stem inhibition for wetland-pond and streams sites (Table 7). Stem inhibition increased moving from the up-gradient to down-gradient in the wetland ponds on 3 of 4 dates (Figure 14A), but decreased moving from upstream to downstream in the stream sites for both root and stems (Figure 15).

Wetland-Pond Plankton and Vegetation

Zooplankton species richness was similar among ponds, however Shannon-Weiner Diversity Index increased moving from W1-W3 (Table 1B). By contrast, plant species richness was lower in the down-gradient wetlands compared to the up-gradient wetlands and vegetation diversity in wetlands decreased from W1-W3 respectively (Table 1). A full listing of species present is included in the Appendix H.

DISCUSSION

The goal of the study was to characterize the how an interconnected system of wetlands can improve water quality, including of physical parameters, nutrients concentration along with ecotoxicological measures.

Although nutrients are essential for living organisms, excesses can cause phenomenon like eutrophication. Excessively high or low pH levels are often associated with nutrient deficiencies, metal toxicities, or other problems for aquatic life (Kadlec and Wallace 2009). Specific conductance is highly dependent on the amount of dissolved solids in the water. High specific conductance indicates high dissolved-solids concentration; dissolved solids can affect the suitability of water for domestic, industrial, and agricultural uses. (USGS 2012). For aquatic species, adequate dissolved oxygen is of prime importance to their continued survival. Turbidity is the measurement of water clarity.
Suspended sediments, such as particles of clay, soil and silt, frequently enter the water from disturbed sites and affect water quality. Suspended sediments can contain pollutants such as phosphorus, pesticides, or heavy metals. Suspended particles cut down on the depth of light penetration through the water, hence they increase the turbidity -- or "murkiness" or "cloudiness" -- of the water. High turbidity affects the type of vegetation that grows in water. Higher turbidity increases water temperatures because suspended particles absorb more heat.

Selected bioassays were used to assess the level of total toxicity in sediments and pore waters through the observation and interpretation of both lethal (i.e. relative mortality) and sub-lethal (i.e. feeding inhibition, growth inhibition) responses in organisms exposed to samples collected from wetland and stream sites. Hydrologic inputs like precipitation and water discharge from connected water bodies largely influences all these physiological parameters directly or indirectly (Mitsch and Gosselink 1993). These physiological parameters along with the hydrologic inputs can also impact the toxic character of the wetland sediments.

The pH of wetland-pond W1 was lower compared to the other wetlands, during most of the study period. This trend observed during most of the study duration, indicates the possibility of W1 having some acidic input from the nearby residential area (Figure 4A). Specific Conductance was observed to be high in the most up-gradient wetland-pond W1 that decreased towards the down-gradient indicating the improvement in water quality (Figure 4B). Low dissolved oxygen and high turbidity during portions of the study period in the up-gradient sites indicates that W1 is most polluted compared to the other sites and water quality improves towards the down-gradient wetlands (Figure 4C and D). This may be the result of the larger volume of W1 and its longer retention capacity (Table 1). But there were some exceptions to this, in W1 high DO was observed even when turbidity was highest in portions of the study. This opposite phenomenon may have resulted because W1 is highly abundant with algae and emergent macrophyte producing a lot more oxygen from photosynthesis than required. The prolonged drought (June to middle of July 2012) also influenced this due to availability of more sun energy (Figure 4C). The decrease of these water quality parameters like specific conductance, turbidity from up-gradient to down-gradient and increase in dissolved oxygen from up-gradient to down-gradient wetlands with a strong site effect indeed indicates the potentiality of the wetlands in purifying water (Table2).

The effect of rain was evidently observed on these water quality parameters. The rain events in July (Figure3) resulted in increased pH, dissolved oxygen and turbidity values in all of the wetlands and a decrease in specific conductance (Figure 4). Hence after these rain-events the amounts of dissolved solids were reduced due to dilution of the water but the amounts of suspended solids were not really affected (Table 2), although the effect of site combined with precipitation was not significant in the wetland sites.

The stream sites also showed similar trends during the study period. The pH and dissolved oxygen were low in the upstream sites whereas the specific conductivity was higher. Although Turbidity was higher in the downstream site indicating the probability of increased runoff in the downstream (Figure 5). The site effect again had a strong significance for the stream sites as well (Table 3), indicating the improvement of water quality towards the downstream as well. The effect of precipitation was also evident on pH, specific Conductivity and dissolved oxygen in these stream sites (Table3).

In the wetlands, Phosphate concentration decreased from up-gradient to down-gradient wetlands with few exceptions relating to the rain events (Figure 6B). The site effect was found to be stronger in the phosphate than the nitrate (Table 4), due in great part to the effect of W1. The non-significant probability of site effect suggests that nitrate levels are not affected significantly by the wetland series. This phenomenon suggests that each wetland site may have a high nitrate demand. Hence the nitrate is not significantly getting reduced in the down-gradient wetlands because available nitrate may be produced or mobilize in the down-gradient wetlands by the nitrogen cycle to meet the demand. On the other hand nitrate is highly driven by the event type in these wetlands (Table 4). When precipitation is higher nitrate input is higher in the overall wetland system, but not equally in all of the individual sites (Table4). However, the strong site effect on phosphate concentration suggests that the phosphate gets incorporated (or settled to the bottom) in the up-gradient wetland, producing clean water quality towards the down-gradient. Although event type do not affect have significant affect on phosphate concentration in the wetlands (Table 4).

In streams, nitrate concentration decreased towards the downstream sites, especially during the non-events and also in each site the concentration of nitrate is higher at the non-events (Figure 10), suggesting a dilution effect may be occurring after events. But unlike the wetlands the stream sites have a significant site effect indicating the decrease of Nitrate from upstream to downstream be considerable and also each site is impacted by the type of events (Table 5).

Phosphate levels decrease significantly towards the downstream with a significant site effect during events and non-events (Figure 11 and Table 5), suggesting that the phosphate in streams is driven by activity (uptake) at each site thereby reducing concentrations as water moves downstream. This phenomenon of nutrient uptake and release was likely detected in this study because it was conducted during the growing season when there is a high rate of uptake of nutrients by emergent and submerged vegetation from the water and sediments. In temperate climates, retention of certain chemicals such as nutrients is greatest in the growing season primarily because of higher microbial activity in the water column and sediments and secondarily because of greater macrophyte productivity (Mitsch and Gosselink 1993). All these variations with relative to precipitation indicate how a wetland series can perform in water quality improvement.

Ecotoxicological approaches are of paramount importance for testing the potential effect of contaminants on some biota and have been considered the best tool in prospective risk assessment (Chapman 1995). As they are based on standardized protocols, the results are well reproducible which provides the advantage of allowing comparisons and facilitating interpretation of results (Calow and Forbes 2003). The effect test from ANOVA shows the level of significance of sites on different test parameters. The probabilities of site effects (Table 6) on the relative mortality, growth inhibition of *Heterocypris*, feeding inhibition of *Thamnocephalus* and probability levels of site on root growth inhibition and stem growth inhibition of *Sinapis, Sorghum, Lepidium* were not significant. Figure 12B suggests that the relative mortality of *Heterocypris*, was lower in the down-gradient wetlands, but Figure 12A and 12C suggest the growth inhibition of *Heterocypris* and feeding inhibition of *Thamnocephalus* have and increasing trend of toxicity towards the down-gradient wetlands. The results may not be statistically significant, but the spatial trends suggest variation in sediment toxicity levels among the wetland sites that should be further explored. At the same time, seasonal effects were stronger in the wetland sites (Table 6 and 7), in part because samples for the ecotoxicological studies in wetlands were collected in different seasons. Hence it can be concluded that seasonal effects, independent of rainfall may have impacted the study.

In the stream sites however significant levels for root and stem growth inhibition of *Sorghum* were detected (Table 7). Because the stream samples were collected on a single day, no temporal effects were examined.

In a recent similar study (Tixier et al., 2011) also demonstrated spatial and seasonal toxicity in a storm water management facility, by adapting an integrated sediment quality approach. The toxicity results, performed under controlled laboratory conditions, can be difficult to extrapolate to the ecosystem level for multiple reasons, and the results should be interpreted with caution. Indeed, it is now well established that important aspects of the ecosystem are not taken into consideration by traditional ecotoxicological approaches, i.e., laboratory toxicity tests (Calow and Forbes 2003, Jansen et al. 2008). With respect to this, it could be said that these tests also lack natural conditions, which may be highly related to the character of the sediment in the ecosystem itself.

Wetland soils or sediments can have different characters; it may be organic or mineral rich in type. Again continuous nutrient transformation affects this in nature. Hence, it can be predicted that natural conditions like sediment type may have influenced the responses from the ecotoxicological stresses tested on different species. So to understand this interaction of the stress factors to different species, sediment characterization is extremely important which was not a part of this short span study. On the other hand the input materials in wetland are highly influenced by different hydrologic factors like precipitation and surface water inflows, which in turn influences the sediment character. For example, precipitation tends to contain contaminants at higher concentration when precipitation is infrequent (Mitsch and Gosselink 1993). Again, during wet periods and storm events, the water is contributed primarily by recent precipitation that enters the stream without coming in contact with soil and subsurface materials. During low flow, some or much of the streamflow originates as groundwater and has higher concentration of dissolved materials (Mitsch and Gosselink 1993).

The hypotheses of this study predicted that there would be improvement in water quality parameters from up-gradient and down-gradient in an interconnected wetland system. High specific conductivity and turbidity and low dissolved oxygen in the up-gradient W1, in addition to higher phosphorus reduction and variability in sediment toxicology between the wetland sites mostly during the study period, generally supports the hypotheses. But there are some definite exceptions that are situation-dependent. For example, high dissolved oxygen in the most up-gradient wetland and the fluctuations of specific conductivity and turbidity and non-significant site effect of nitrate. But there can several reasons for these exceptions to happen. The data collection period of the water quality parameters occurred during a prolonged drought, which may have promoted the dissolved oxygen to be higher in the up-gradient wetland due to presence of higher amount of plants and algae resulting in high DO from photosynthesis. Nitrate is driven by rain events and the demand for nitrate in the ponds is supported by each site ecosystem itself.

It is important to recognize that these wetland-ponds were constructed in 2002 and are only 10 years old. It is reasonable to assume that they will not necessarily perform as natural wetlands, but may improve over time. It is likely that these wetland-ponds have not yet developed their biological potential to the fullest. Another important finding from this study was, that the stream system performed in a similar or comparable pattern to the wetland, indicating that stream systems function as a medium to improve water quality.

CONCLUSION

Today's environmental problems are complex and increasingly pressing. One of the most important problems world-wide is the quality of freshwater. Limited supply and distribution of freshwater leads to competition between consumers, cities, states and nations and the lack of freshwater retards the development of society. Anthropogenic activities are polluting and therefore heavily influence the quality of freshwater. Contributors to pollution include several point and non-point sources. The degrading quality of scarce freshwater is a major concern to general public health.

According to the hypotheses there was strong indication of water quality. Improvement in phosphate reduction towards the down-gradient wetland, high specific conductance, turbidity and Low dissolved oxygen in portions in the up-gradient wetland proves this improvement of water quality. Although there were some exceptions.

All these relationship explain the fact that how an interconnected wetland system, with widely varying important water quality parameters, nutrient concentration and sediment toxicity can ameliorate the water quality before it is discharged to the connected fresh water bodies rivers or lakes thus providing a safe, biologically rich environment.

FIGURES

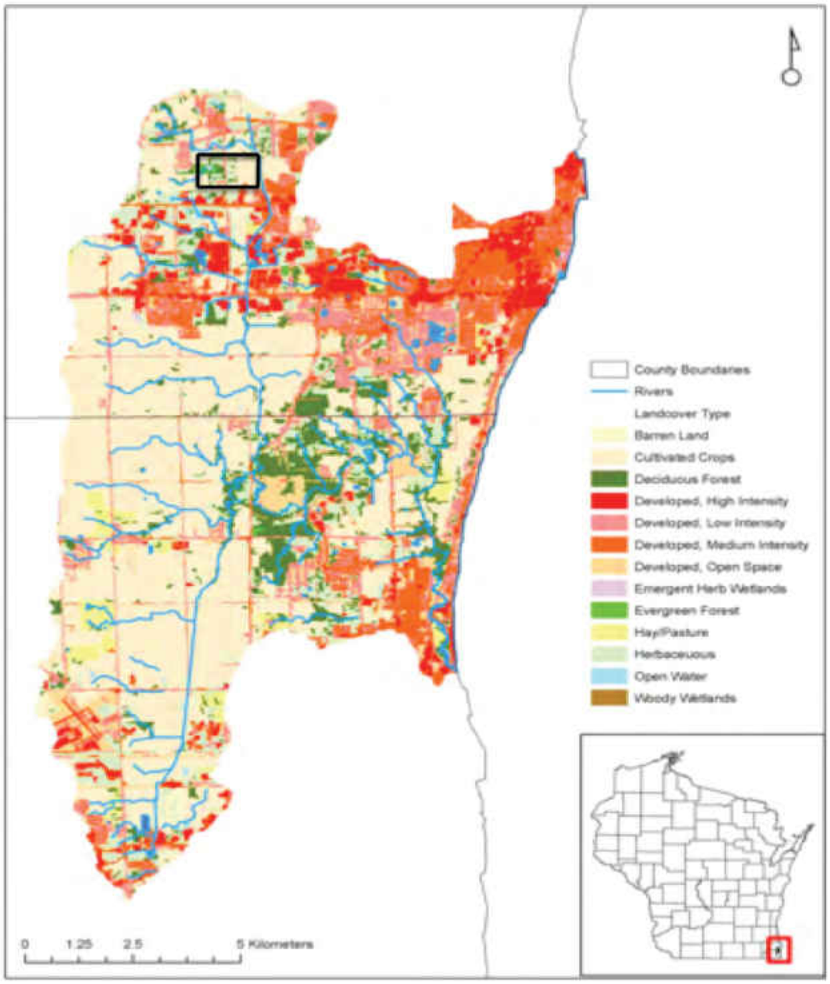


Figure 1. Land cover GIS map of the Pike River Watershed. All county, state, land cover, hydrology, and watershed shapefiles taken from the Wisconsin Department of Natural Resources (WDNR, 2005). The location of study area shown in Figure 2 is indicated by the black box.

Figure 2: Aerial Photograph (July 2011) of Pike River watershed from Google Earth, displayed with the wetland sampling stations (W1-3) and stream sampling station (S4-7) chosen for the study. Flow direction is from left to right.

.

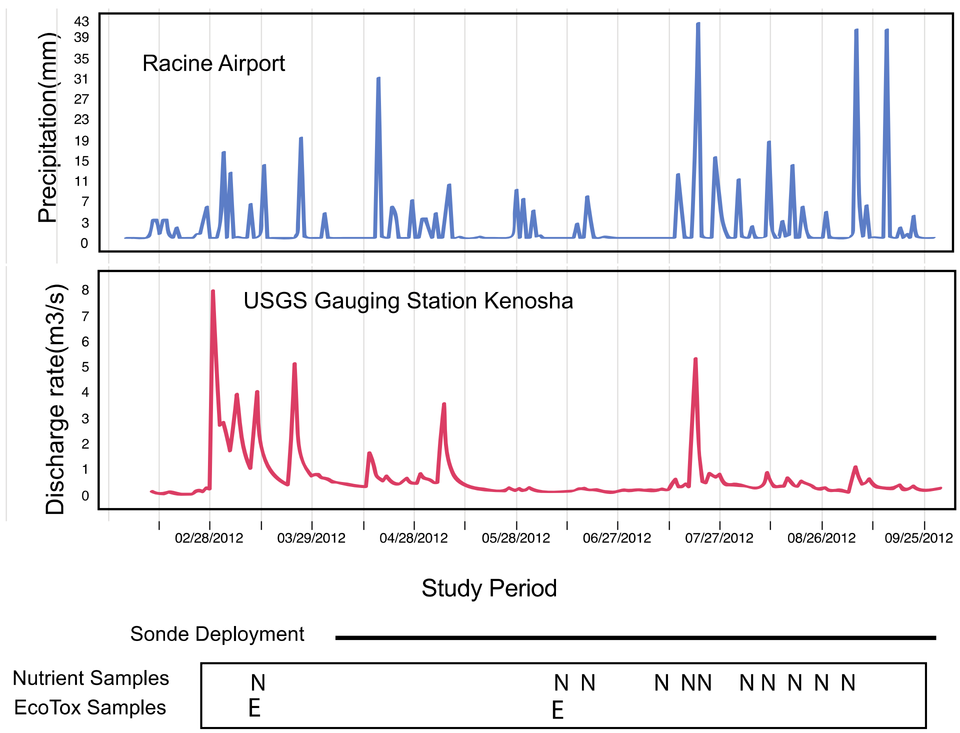


Figure 3: Variation of total precipitation (mm) and discharge rate (m^3/s) across the sampling period and dates of different test parameters.

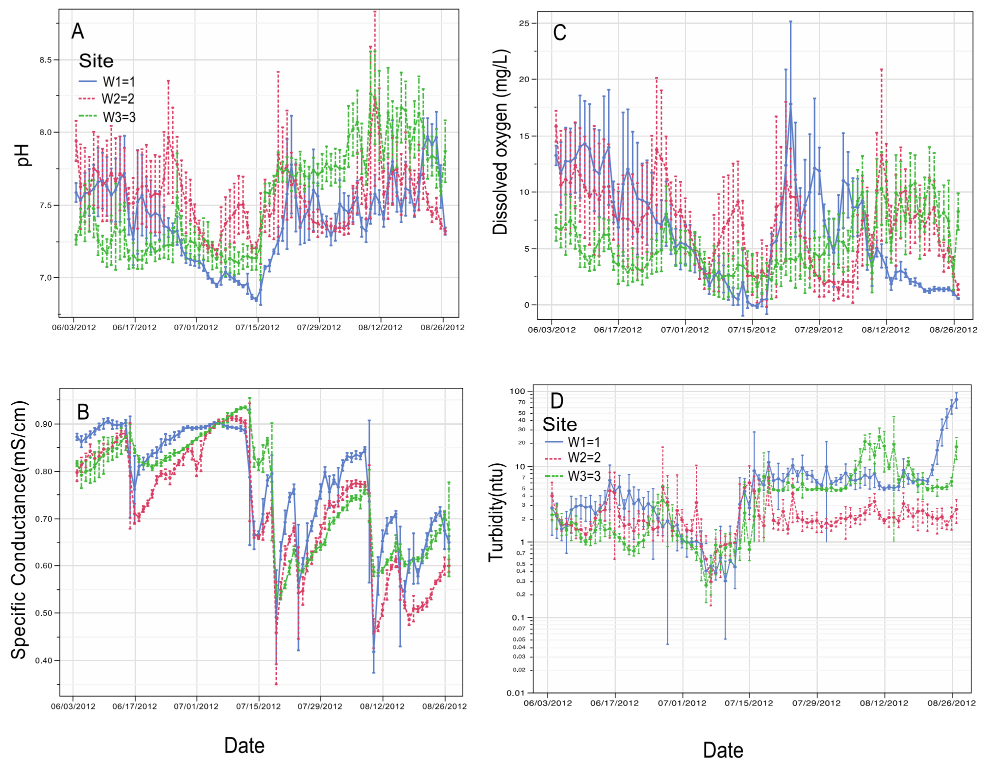


Figure 4: Variation of daily means of pH, specific conductivity, dissolved oxygen and turbidity across the wetland sampling sites (W1-3) sites during the data collection period of June 2012 to August 2012. Each error bar is constructed using 1 standard deviation from the mean.

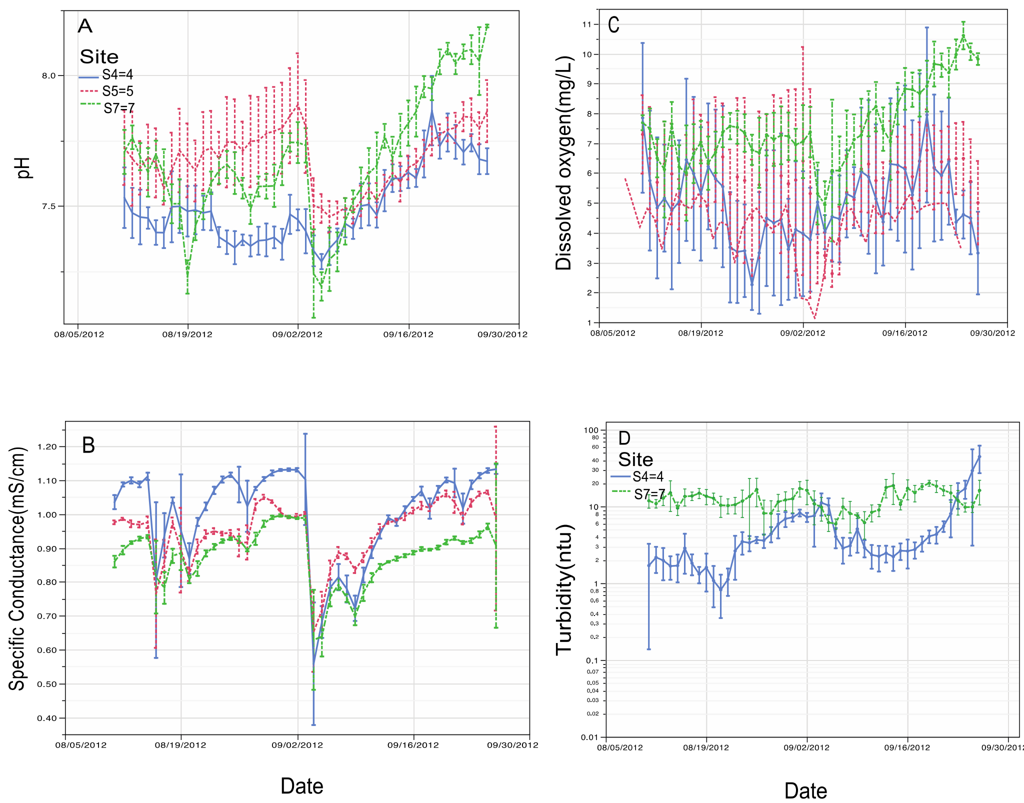
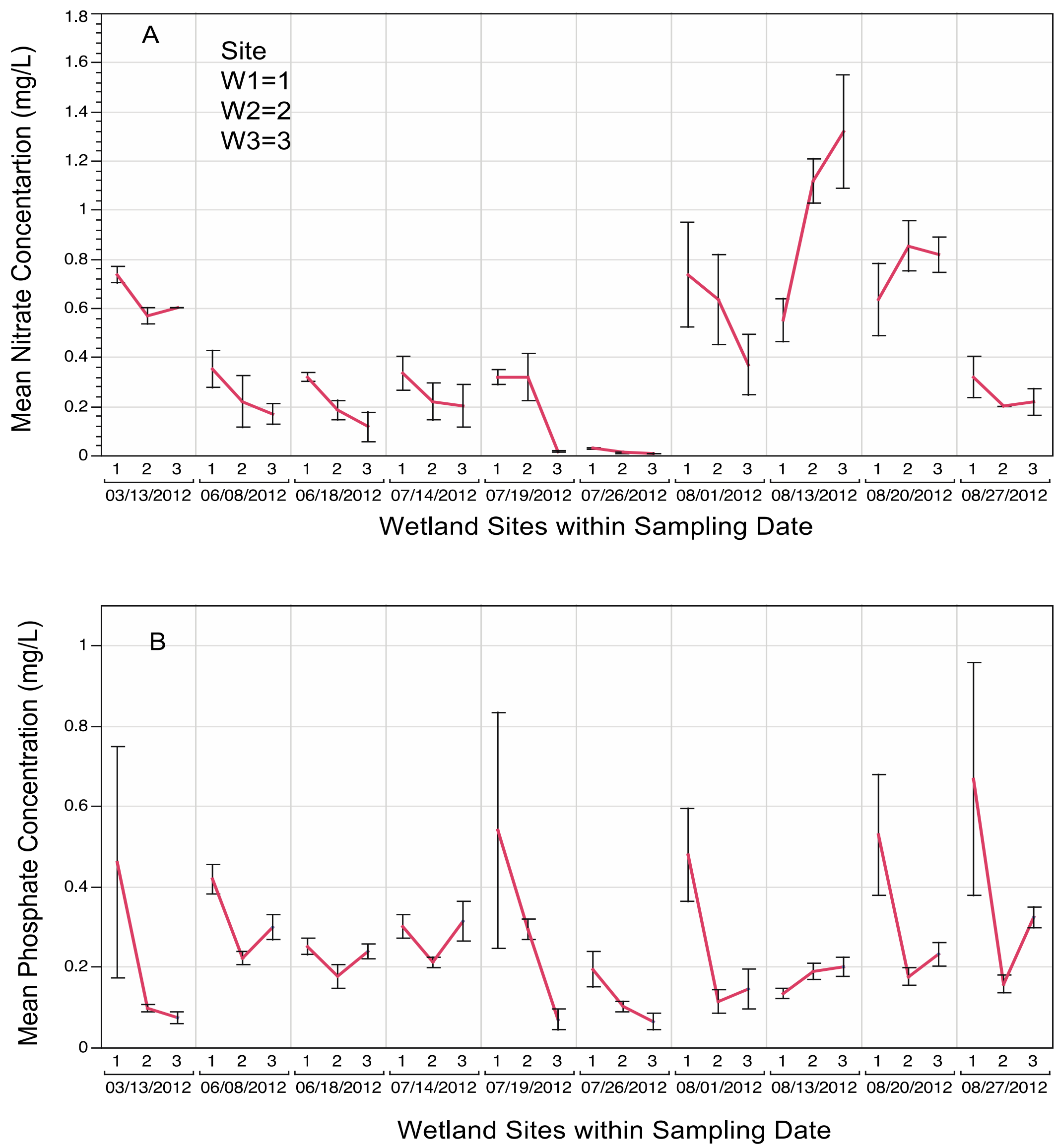
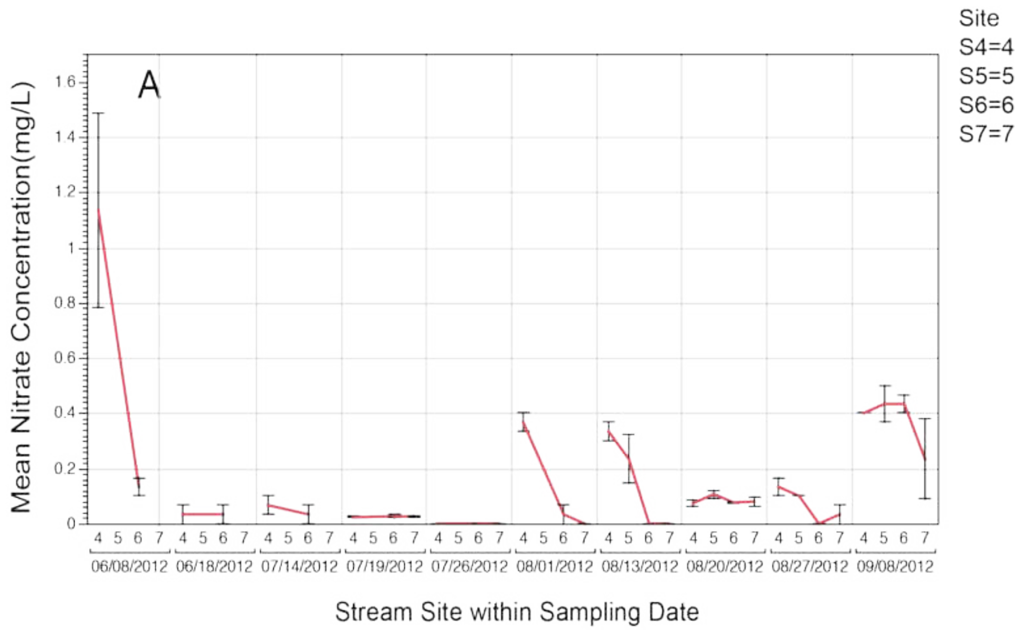


Figure 5: Variation of daily mean of pH, specific conductivity, dissolved oxygen and turbidity across the stream sampling sites (S4-7) during the data collection period of June 2012 to August 2012.Due to malfunctioning of the sonde in site S5, the data was rejected. Each error bar is constructed using 1 standard deviation from the mean.



<u>Figure 6:</u>Variation of Nitrate and Phosphate Concentration across the wetland sampling sites (W1-3) sites during the data collection period of June 2012 to August 2012.Each error bar is constructed using 1 standard error from the mean.



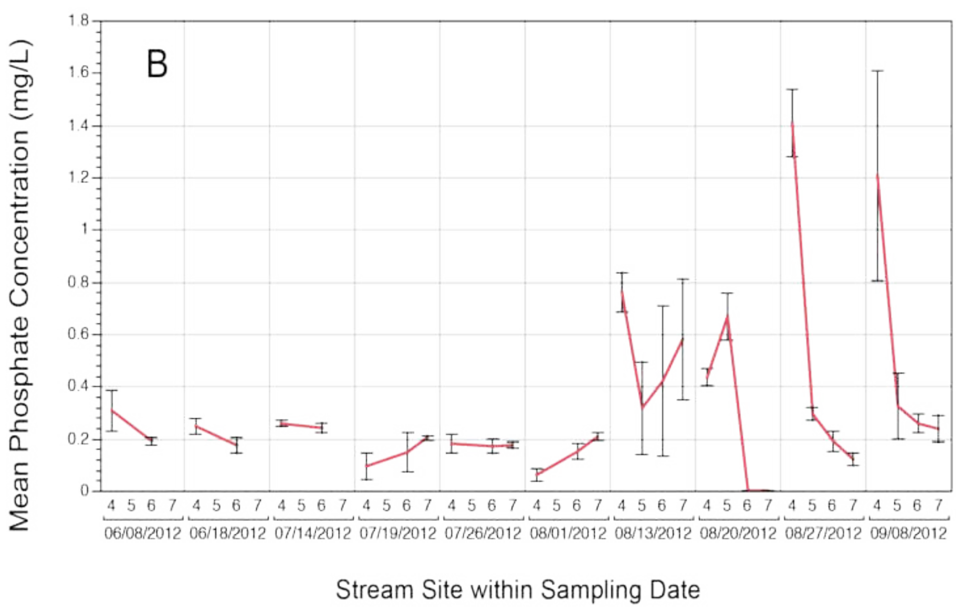


Figure 7:Variation of Nitrate and Phosphate Concentration across the stream sampling sites (S4-7) sites during the data collection period of June 2012 to August 2012. Each error bar is constructed using 1 standard error from the mean.

A. Wetland Site Effect (by Precipitation Event Type)

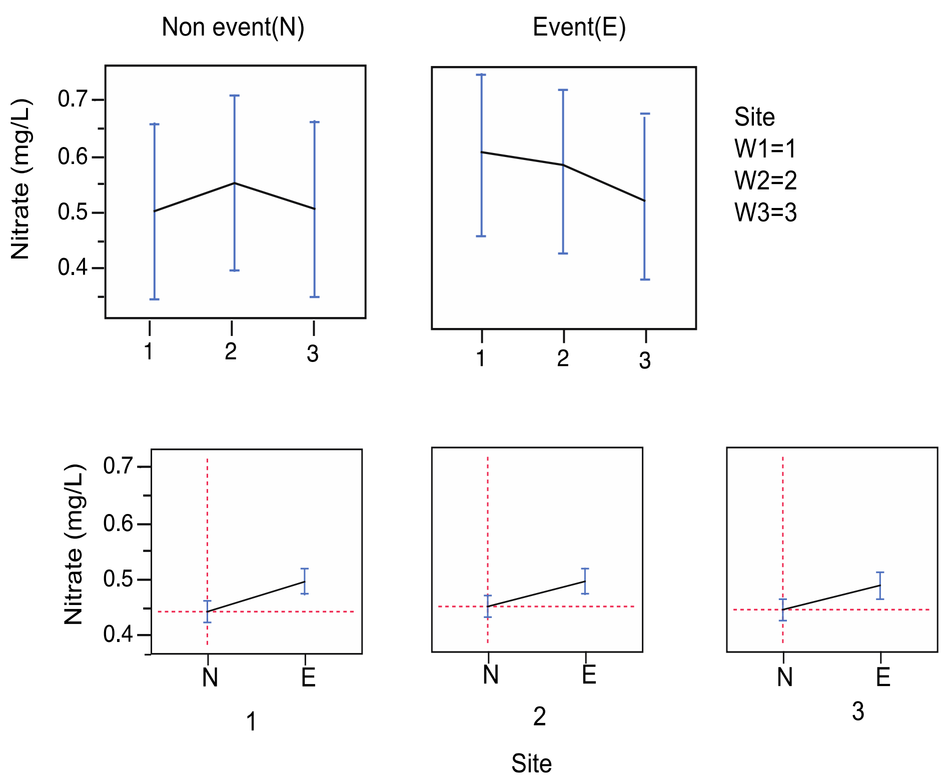


Figure 8: Variation in least square means of Nitrate concentration from ANOVA with wetland sampling sites (W1-3) relative to precipitation events and with event across the wetland sampling sites. Each error bar is constructed using 1 standard error from the mean.

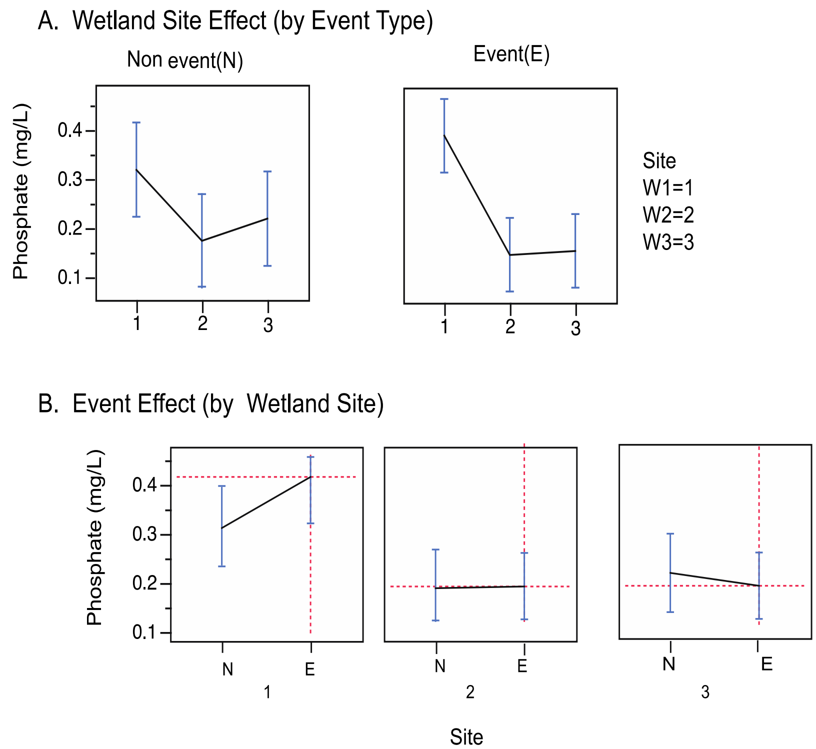
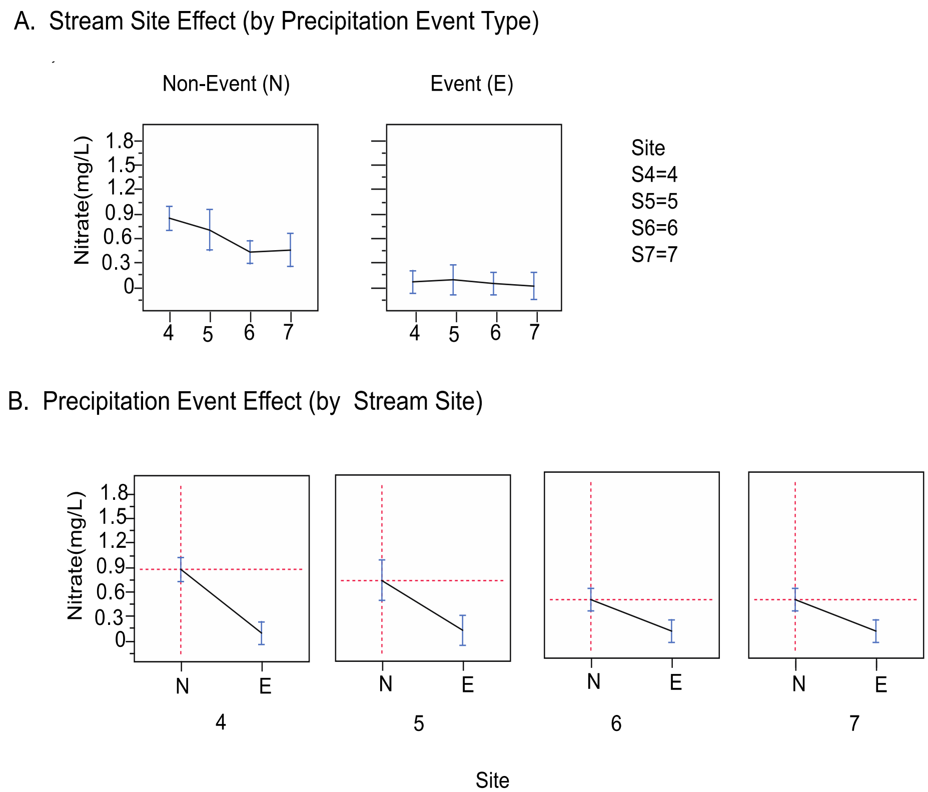
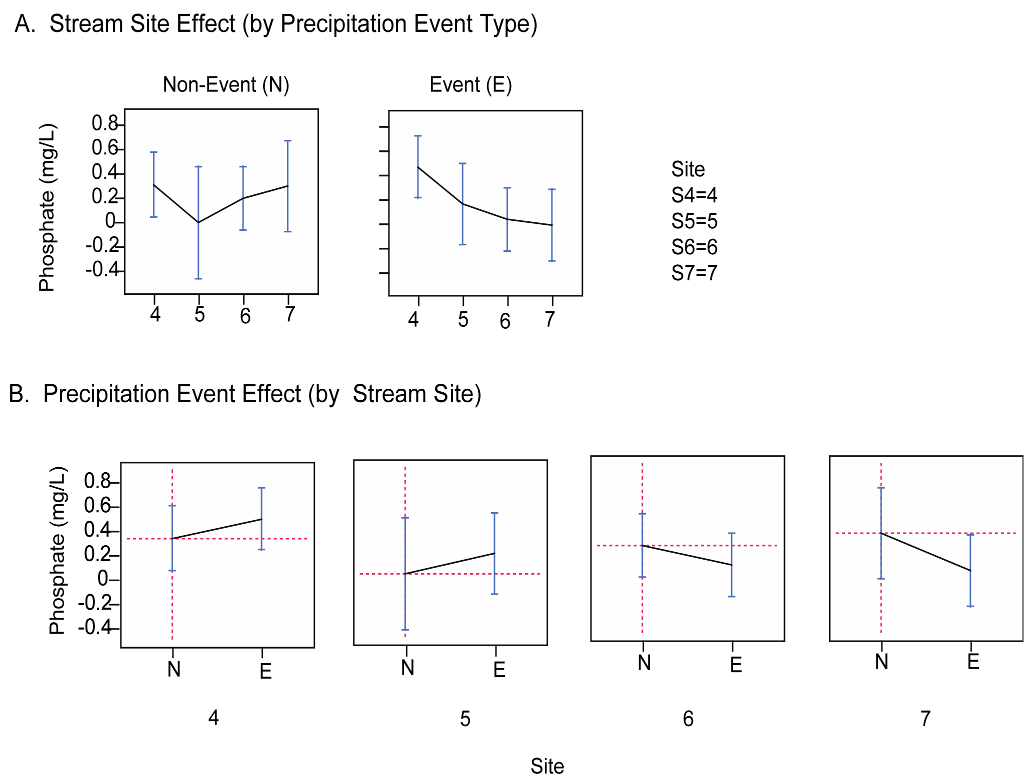


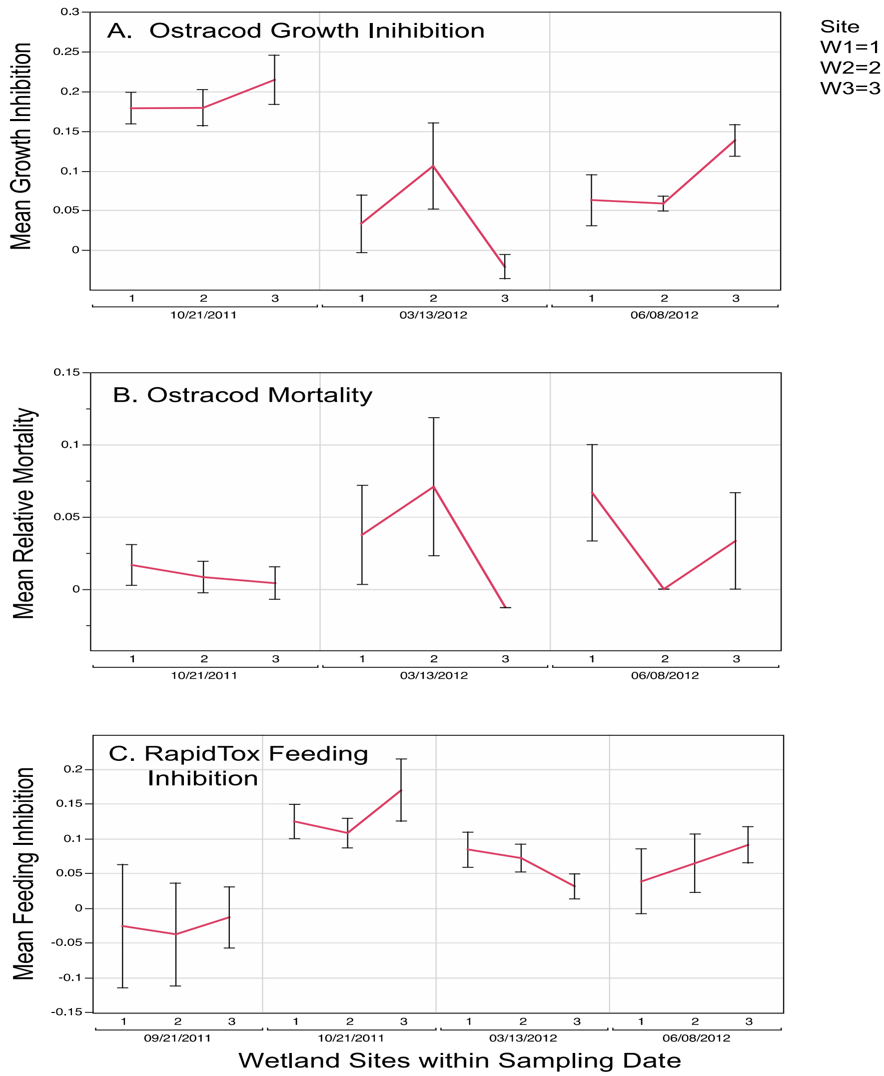
Figure 9: Variation in least square means of Phosphate concentration from ANOVA with wetland sampling sites (W1-3) relative to precipitation events and with event across the wetland sampling sites. Each error bar is constructed using 1 standard error from the mean.



<u>Figure 10:</u> Variation in least square means of Nitrate concentration from ANOVA with stream sampling sites (S4-7) relative to precipitation events and with event across the stream sampling sites. Each error bar is constructed using 1 standard error from the mean.



<u>Figure 11:</u> Variation in least square means of Phosphate concentration from ANOVA with stream sampling sites (S4-7) relative to precipitation events and with event across the stream sampling sites. Each error bar is constructed using 1 standard error from the mean.



<u>Figure 12:</u> Variation of Growth Inhibition, Relative Mortality of *Heterocypris* (Ostracod) and Feeding Inhibition of *Thamnocephalus (*Rapidtox*)* with wetland sampling sites (W1-3) within the sampling date. Each error bar is constructed using 1 standard error from the mean.

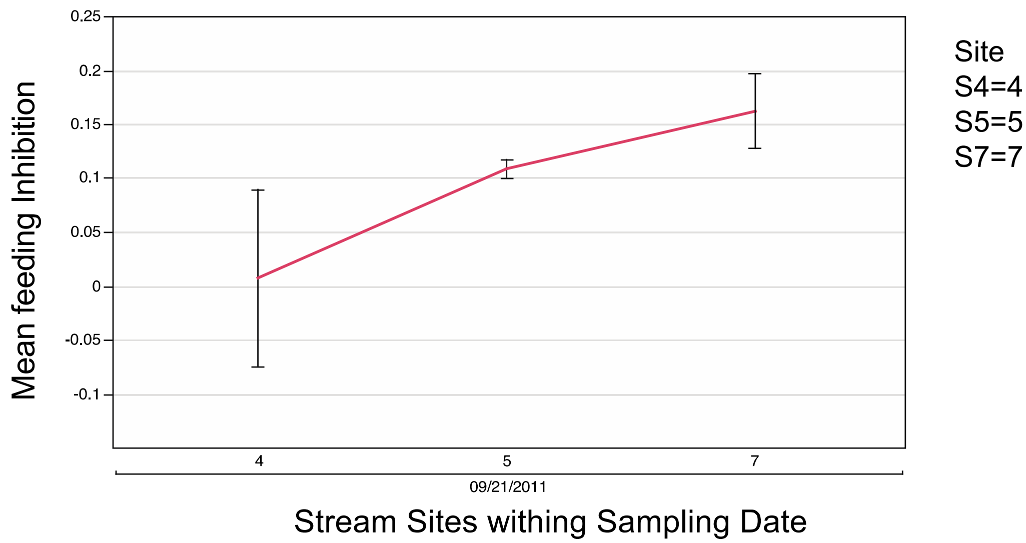


Figure 13: Variation of Feeding Inhibition of *Thamnocephalus* (Rapidtox) with stream sampling sites (S4-7) within the sampling date. Each error bar is constructed using 1 standard error from the mean.

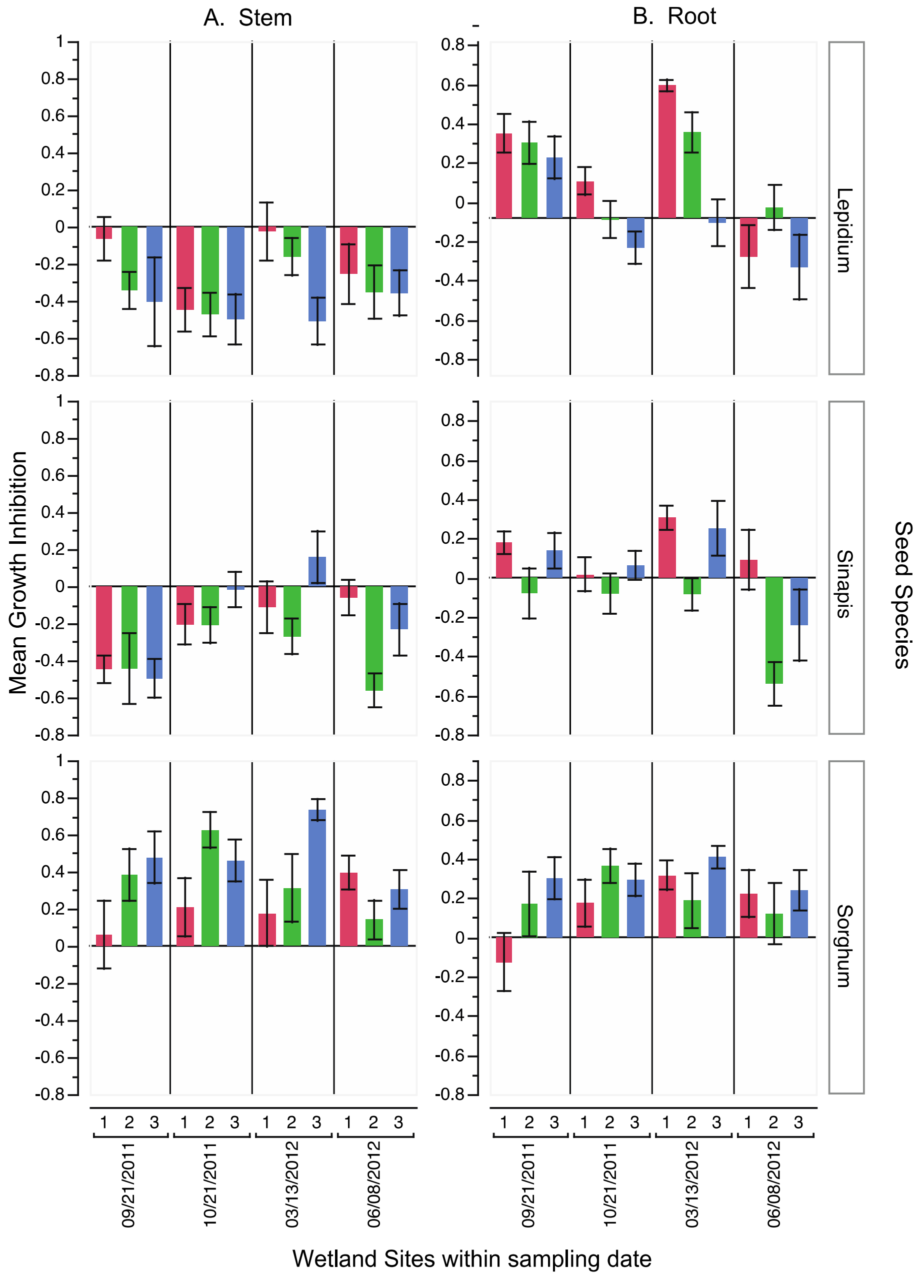


Figure 14:Variation of Stem and Root Growth Inhibition of *Lepidium, Sinapis and Sorghum (*Phytotox) with wetland sampling sites (W1-3) within the sampling date. Each error bar is constructed using 1 standard error from the mean.

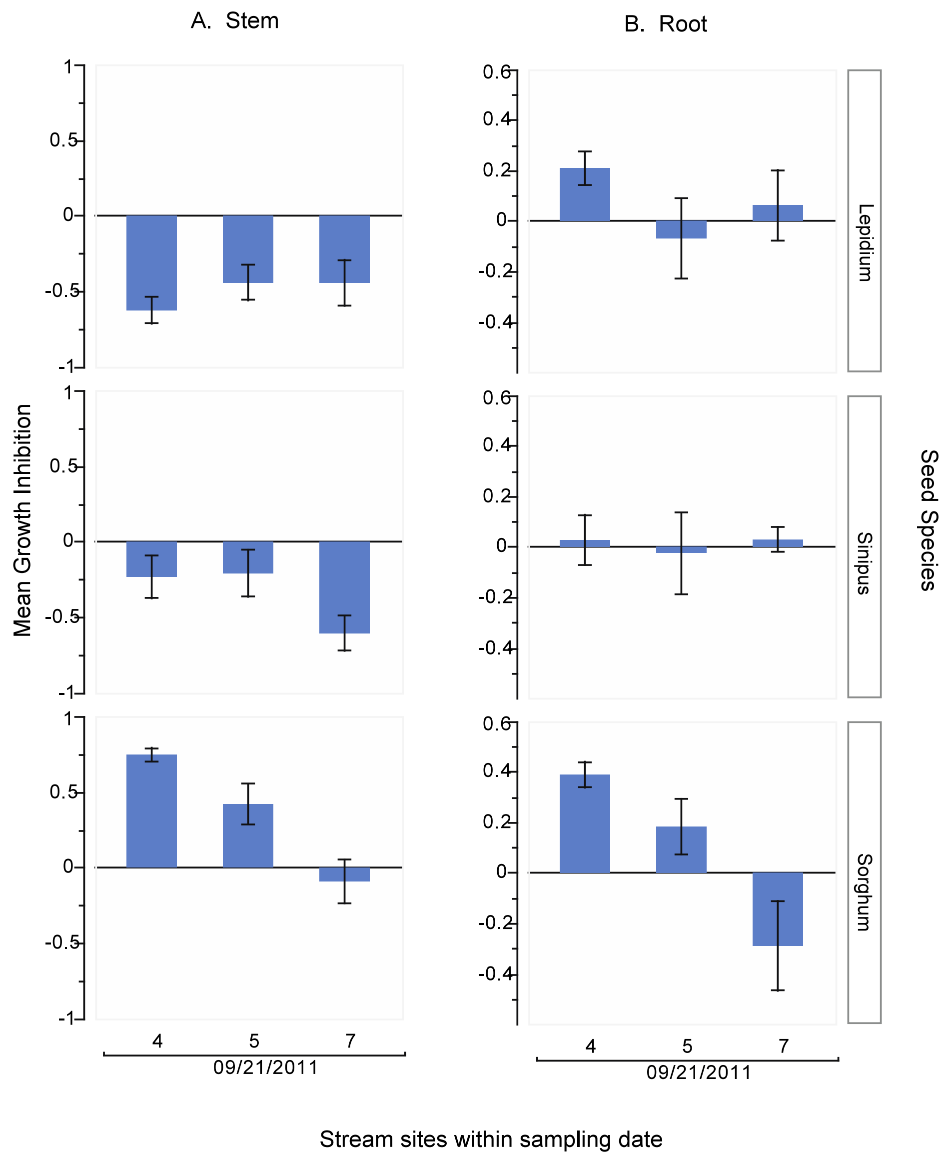


Figure 15:Variation of Stem and Root Growth Inhibition of *Lepidium, Sinapis and Sorghum* (Phytotox) with stream sampling sites (S4 -7) within the sampling date. Each error bar is constructed using 1 standard error from the mean.

TABLES

Table 1:Characteristics of the Wetland-pond systems (see Figure 2). (A) Physical and hydrological parameters measured at Baseflow (6 June 2012) and event flow (20 July 2012). (B) Biological parameters measured August 2012.

A.

	Physical Characteristics				
Wetland	Surface Area (m2)	Avg.Depth (m)	Avg. Volume (m3)	Base Flow Turnover (d)	Event Flow Turnover (d)
1	1050	3.1	3255.0	2.97	1.65
2	1260	0.6	756.0	0.74	0.48
3	1530	0.6	933.3	0.79	0.61

B.

	Zooplankton		Vegetation	
Wetland	Species Richness	Diversity Index	Species Richness	Diversity Index
1	8	0.27	30	0.47
2	10	0.63	18	0.38
3	9	1.41	16	0.28

Table2: Effect test from ANOVA showing the significance of site, precipitation and combined effect of site and precipitation on pH, Specific conductivity, Dissolved oxygen and Turbidity across all wetland sites.

Test Parameter	Source	F Ratio	Prob > F
pH	Site	9.1300	0.0001
	Precipitation	0.2500	NS
	Site * Precipitation	1.2100	NS
Specific conductivity	Site	5.0800	0.0069
	Precipitation	20.8000	<.0001
	Site * Precipitation	0.4400	NS
Dissolved oxygen	Site	4.3600	0.01
	Precipitation	6.0500	0.01
	Site * Precipitation	0.0400	NS
Turbidity	Site	8.9100	0.0002
	Precipitation	0.8900	NS
	Site * Precipitation	0.0800	NS

Table3: Effect test from ANOVA showing the significance of site, precipitation and combined effect of site and precipitation on pH, Specific conductivity, Dissolved oxygen and Turbidity across all stream sites.

Test Parameter	Source	F Ratio	Prob > F
pH	Site	15.6900	<.0001
	Precipitation	11.9200	0.0007
	Site * Precipitation	2.0100	NS
Specific conductivity	Site	20.0200	<.0001
	Precipitation	24.2200	<.0001
	Site * Precipitation	0.7900	NS
Dissolved oxygen	Site	72.6100	<.0001
	Precipitation	8.9400	0.0033
	Site * Precipitation	1.9600	NS
Turbidity	Site	36.2900	<.0001
	Precipitation	0.0019	NS
	Site * Precipitation	0.0127	NS

Table 4:Effect test from ANOVA showing the significance of site, event type, and combination of site and event type on Nitrate and Phosphate Concentration in the wetland sites.

Test Parameters	Source	Nparm	DF	SS	F Ratio	Prob>F
Nitrate	Site	2	2	0.081676	0.2725	NS
	Event Type	1	1	1.3010105	8.6819	0.0037
	Site * Event Type	2	2	0.0573485	0.1913	NS
Phosphate	Site	2	2	1.3959702	12.4624	<.0001
	Event Type	1	1	0.045791	0.8176	NS
	Site * Event Type	2	2	0.1926462	1.7198	NS

Table 5:Effect test from ANOVA showing the significance of site, event type, and combination of site and event type on Nitrate and Phosphate Concentration in the stream sites.

Test Parameters	Source	Nparm	DF	SS	F Ratio	Prob>F
Nitrate	Site	3	3	1.0108629	6.9812	0.0003
	Event Type	1	1	0.0658729	1.3648	NS
	Site * Event Type	3	3	0.7471754	5.1602	0.0025
Phosphate	Site	3	3	1.1230907	3.6059	0.0166
	Event Type	1	1	0.0027339	0.0263	NS
	Site * Event Type	3	3	0.7662974	2.4604	NS

Table 6: Effect test from ANOVA showing the significance of site, season and the combination of site and season on relative mortality, growth inhibition of *Heterocypris* (Ostracod) and Feeding inhibition of *Thamnocephalus* (Rapidtox) across the wetland and stream sites.

Test	Parameter	Stream/ Wetland	Source	Nparm	DF	SS	F Ratio	Prob>F
Heterocypris	Relative Mortality	Wetland	Site	2	2	0.00	0.15	NS
			Season	2	2	0.01	1.23	NS
			Site*Season	4	4	0.02	1.59	NS
	Growth Inhibition	Wetland	Site	2	2	0.01	0.70	NS
			Season	2	2	0.30	20.42	<.0001
			Site*Season	4	4	0.07	2.27	NS
Thamnocephalus	Feeding Inhibition	Wetland	Site	2	2	0.00	0.04	NS
			Season	3	3	0.15	6.17	0.0009
			Site*Season	6	6	0.04	0.74	NS
	Feeding Inhibition	Stream	Site	2	2	0.02	2.31	NS

Table 7 Effect test from ANOVA showing the significance of site, season and the combination of site and season on Root and Stem growth inhibition of *Lepidium, Sinapis and Sorghum* (Phytotox) across all the wetland and stream site.

Test	Stream/ Wetland	Parameter	Source	Nparm	DF	SS	F Ratio	Prob>F
Lepidium	Wetland	Stem	Site	2	2	0.59	1.37	NS
			Season	3	3	1.38	2.13	NS
			Site*Season	6	6	0.89	0.68	NS
		Root	Site	2	2	0.06	0.25	NS
			Season	3	3	5.94	15.53	<.0001
			Site*Season	6	6	1.62	2.12	0.05
	Stream	Stem	Site	2	2	0.17	0.68	NS
		Root	Site	2	2	0.28	0.93	NS
Sinapis	Wetland	Stem	Site	2	2	0.02	0.05	NS
			Season	3	3	2.75	5.60	0.0012
			Site*Season	6	6	1.48	1.51	NS
		Root	Site	2	2	0.38	1.35	NS
			Season	3	3	2.45	5.73	0.001
			Site*Season	6	6	1.07	1.24	NS
	Stream	Stem	Site	2	2	0.94	2.77	NS
		Root	Site	2	2	0.02	0.08	NS
Sorghum	Wetland	Stem	Site	2	2	0.80	1.94	NS
			Season	3	3	0.54	0.87	NS
			Site*Season	6	6	2.29	1.86	NS
		Root	Site	2	2	0.90	2.89	0.05
			Season	3	3	0.68	1.44	NS
			Site*Season	6	6	0.96	1.03	NS
	Stream	Stem	Site	2	2	3.57	13.24	<.0001
		Root	Site	2	2	2.27	8.19	0.0017

LITERATURE CITED

Awal, S. and D. Svozil (2010). "Macro-invertebrate species diversity as a potential universal measure of wetland ecosystem integrity in constructed wetlands in South East Melbourne." Aquatic Ecosystem Health & Management 13(4): 472-479.

Balcer, M. D., N. L. Korda, et al. (1984). Zooplankton of Great lakes, A Guide to the Identification and Ecology of the Common Crustacean Species, The University of Wisconsin Press.

Banerman, Owens, et al. (1993). "Sources of Pollutants in Wisconsin Stormwater." Water Science & Technology 28: 241-259.

Bay, S. M. and S. B. Weisberg (2010). "Framework for Interpreting Sediment Quality Triad Data " Integrated Environmental Assessment and Management: 589-596.

Binelli, A., S. K. Sarkar, et al. (2008). "A comparison of sediment quality guidelines for toxicity assessment in the Sunderban wetlands (Bay of Bengal, India)." Chemosphere 73(7): 1129-1137.

Calow, P. and V. E. Forbes (2003). "Does Ecotoxicology Inform Ecological Risk Assesment?" Environ. Sci. Technol.: 147-151.

Carey, R., G. J. Hochmuth, et al. (2012). "Regulatory and Resource Management Practices for Urban Watersheds: The Florida Experience." Hort Technology 22(4): 418-429.

Cohen, M. J. and M. T. Brown (2007). "A model examining hierarchical wetland networks for watershed stormwater management." Ecological Modelling 201(2): 179-193.

Covaci, A., A. Gheorghe, et al. (2006). "Levels and distribution of organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in sediments and biota from the Danube Delta, Romania." Environmental Pollution 140(1): 136-149.

Chapman, D. P. M. (1995). "Do sediment toxicity tests require field validation?" Environmental Toxicological and Chemistry 14(9): 1451-1453.

Cherry, J.A. (2011) Ecology of Wetland Ecosystems: Water, Substrate, and Life. Nature Education Knowledge2 (1): 3

Chial B, Persoone G. (2002). "Cyst-based toxicity tests XIV - application of the ostracod solid-phase microbiotest for toxicity monitoring of river sediments in Flanders(Belgium)." Environmental Toxicology 17(6): 533-537.

Chocat, B., Krebs, P., Marsalek, J., Rauch, W., Schilling, W., (2001). "Urban drainage redefined: from stormwater removal to integrated management. " Water Sci. Technol. 43, 61–68

Crispell-Synder, Inc. 1997. Executive summary of WDNR facilitation presentation for Pike River improvements by Mount Pleasant Storm Water Drainage District No. 1. Crispell-Synder, Inc. Consulting Engineers.

Czerniawska-Kusza, I., T. Ciesielczuk, et al. (2006). "Comparison of the Phytotoxkit microbiotest and chemical variables for toxicity evaluation of sediments." Environmental Toxicology 21(4): 367-372.

Ehlinger, T. J., and L. DeThorne. 2004. Monitoring of stream habitat and aquatic biotic integrity - Pike River North and South Branches, Racine and Kenosha Counties, Wisconsin, Interim Report. Department of Biological Sciences, University of Wisconsin-Milwaukee.

Ehlinger, T. J., L. DeThorne, and B. Berner. 2002. Monitoring of stream habitat and aquatic biotic integrity - Pike River North and South Branches, Racine and Kenosha Counties, Wisconsin, Interim Report. Department of Biological Sciences, University of Wisconsin-Milwaukee.

EPA (1997). Volunteer Stream Monitoring: A Methods Manual. Office of Water. Available: http://water.epa.gov/type/rsl/monitoring/stream_index.cfm

EPA (2001). Functions and Values of Wetlands. Office of Water, Office of Wetlands, Oceans and Watersheds. Available:

www.epa.gov/owow/wetlands/pdf/fun_val.pdf

EPA (2003). Protecting Water Quality from Urban Runoff. Available: http://water.epa.gov/polwaste/nps/urban_facts.cfm

EPA (2004). Wetlands Overview. Office of Water. Available:

www.epa.gov/owow/wetlands/facts/overview.pdf

EPA. World In Our Backyard, Chapter III. Available: http://www.epa.gov/region1/students/teacher/world.html

EPA (2012). Summary of the Clean Water Act. Available: http://www.epa.gov/lawsregs/laws/cwa.html

Hartzell, D., J. R. Bidwell, et al. (2007). "A comparison of natural and created depressional wetlands in central Oklahoma using metrics from indices of biological integrity." Wetlands 27(4): 794-805.

Hathaway, J. M. and W. F. Hunt (2010). "Evaluation of Storm- Water Wetlands in Series in Piedmont North Carolina." Journal of Environmental Engineering-Asce 136(1): 140-146.

Hornberger, G. M., J. P. Raffensperger, et al. (1998). Elements of Physical Hydrology. Baltimore, The John Hopkins University Press.

Kadlec and Wallace (2009). Treatment Wetlands, CRC Press Taylor & Francis Group,LLC.

Keiter, S., A. Rastall, et al. (2006). "Ecotoxicological assessment of sediment, suspended matter and water samples in the upper Danube River - A pilot study in search for the causes for the decline of fish catches." Environmental Science and Pollution Research 13(5): 308-319.

Lewis, M.A. (1995)."USE OF FRESH-WATER PLANTS FOR PHYTOTOXICITY TESTING – A REVIEW." Environmental Pollution 87(3): 319-336.

Liao, S. W. and N. L. Chang (2004). "Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan." Journal of Aquatic Plant Management 42: 60-68.

Lougheed, V. L., B. Crosbie, et al. (1998). "Predictions on the effect of common carp (Cyprinus carpio) exclusion on water quality, zooplankton, and submergent macrophytes in a Great lakes wetland." Canadian Journal of Fisheries and Aquatic Sciences 55: 1189-1197.

J.Cooper, M., D. G.Uzarski, et al. (2007). "Macroinvertebrate community composition in relation to anthropogenic disturbance, vegetation, and organic sediment depth in four lake Michigan drowned river-mouth wetlands." Wetlands 27(4): 894-903.

Marsalek, J., Rochfort, Q., Grapentine, L., (2005a). “Aquatic habitat issues in urban stormwater management: challenges and potential solutions.“ Ecohydrology and Hydrobiology 5, 269–279.

Mitsch, W. J. and J. G. Gosselink (1993). Wetlands. NY, Van Nostrand Reinhold.

Pennak, R. W. (1978). Fresh-Water invertebrates of the United StatesNY,John Wiley & Sons, Inc.

Ross, P., P. Leitman, et al. (2000). Microbiotests for contaminated soils and sediments; interpreting the data. New York, Kluwer Academic/Plenum Publishers.

SAS Institute Inc. 2011 JMP® 10

Schindler, D. W. (1987). "Detecting Ecosystem Responses to Anthropogenic Stress". Canadian Journal of Fisheries and Aquatic Sciences 44(Suppl 1): 6-25.

Schmitt-Jansen, M., U. Veit, et al. (2008). "An ecological perspective in aquatic ecotoxicology: Approaches and challenges." Basic and Applied Ecology 9(4): 337-345.

Standard Operational Procedure, Ostracodtoxkit, Microbiotest Inc, Available: http://www.microbiotests.be

Standard Operational Procedure, Rapidtoxkit,Microbietest Inc, Available: http://www.microbiotests.be

Standard Operational Procedure, Phytotoxkit. Seed germination and early growth microbiotest with higher plants, Microbiotest Inc, Available: http://www.microbiotests.be/SOPs/Phytotoxkit%20SOP%20-%20A5.pdf

Sharma, R. C. and J. S. Rawat (2009). "Monitoring of aquatic macro-invertebrates as bioindicator for assessing the health of wetlands: A case study in the Central Himalayas, India." Ecological Indicators 9(1): 118-128.

Sokal, R. R., and F. J. Rohlf. 1994. Biometry: the principles and practices of statistics in biological research, 3rd edition. W. H. Freeman, New York.

Snodgrass, J. W., R. E. Casey, et al. (2008). "Microcosm investigations of storm water pond sediment toxicity to embryonic and larval amphibians: Variation in sensitivity among species." Environmental Pollution 154(2): 291-297.

The Ramsar Convention on Wetlands: Ramsar Information Packs: Wetland ecosystem services (15/08/2011).

Tixier, G., M. Lafont, et al. (2011). "Ecological risk assessment of urban stormwater ponds: Literature review and proposal of a new conceptual approach providing ecological quality goals and the associated bioassessment tools." Ecological Indicators 11(6): 1497-1506.

Tixier, G., Q. Rochfort, et al. (2011). "Spatial and seasonal toxicity in a stormwater management facility: Evidence obtained by adapting an integrated sediment quality assessment approach." Water research.

Torokne A,Vasdinnyei R, Asztalos BM (2007)." A Rapid Microbiotest for the Detection of Cyanobacterial Toxins." Environmental Toxicology 22(1): 64-68.

Torokne, A. and Toro, K. (2010), Evaluation of the toxicity of river and creek sediments in Hungary with two different methods. Environ. Toxicol., 25: 504–509. doi: 10.1002/tox.20595.

USGS (1998). Aquatic macroinvertebrates Collected at Ravenna Army Ammunition Plant, Portage and Trumbull Counties, Ohio, 1998. U. S Department of the Interior and U. S. Geological Survey. Available: *Oh.water.usgs.gov/reports/wrir/wrir99_4202.pdf*

USGS (2005). A Guide to the Proper Selection and Use of Federally Approved Sediment and Water-Quality Samplers. U. S. Department of the Interior and U. S. Geological Survey. Available:
http://water.usgs.gov/osw/pubs/OFR_2005_1087/OFR_2005-1087.pdf

USGS et al (2011). Sediment Pore-Water Toxicity Test Results and Preliminary Toxicity Identification of Post-Landfall Pore-Water Samples Collected Following the Deepwater Horizon Oil Release, Gulf of Mexico, 2010. U. S. Department of the Interior and U. S. Geological Survey. Available:
http://pubs.usgs.gov/of/2011/1078/

USGS (2012). Water Properties and Measurements. U. S. Department of the Interior and U. S. Geological Survey. Available:
http://ga.water.usgs.gov/edu/characteristics.html

USGS.NationalWaterinformationsystem.Available:
http://nwis.waterdata.usgs.gov/wi/nwis/uv/?cb_00060=on&cb_00065=on&format=html&period=&begin_date=2011-09-01&end_date=2012-10-05&site_no=04087257

Wadhia, K. and K. C. Thompson (2007). "Low-cost ecotoxicity testing of environmental samples using microbiotests for potential implementation of the Water Framework Directive." Trends in Analytical Chemistry 26(4): 300-307.

Weather Underground. Available:
http://www.wunderground.com/cgi-bin/findweather/getForecast?query=zmw:53406.1.99999

Wilcox, D. A., J. E. Meeker, et al. (2002). "Hydrologic variability and the application of index of biotic integrity metrics to wetlands: A Great Lakes evaluation." Wetlands 22(3): 588-615.

Wisconsin State Herbarium. Avaailable: http://www.botany.wisc.edu/herbarium/

Yadav, A. K., N. Kumar, et al. (2010). "Removal of chromium and nickel from aqueous solution in constructed wetland: Mass balance, adsorption-desorption and FTIR study." Chemical Engineering Journal 160(1): 122-128.

YSI Inc. Available: http://www.ysi.com/

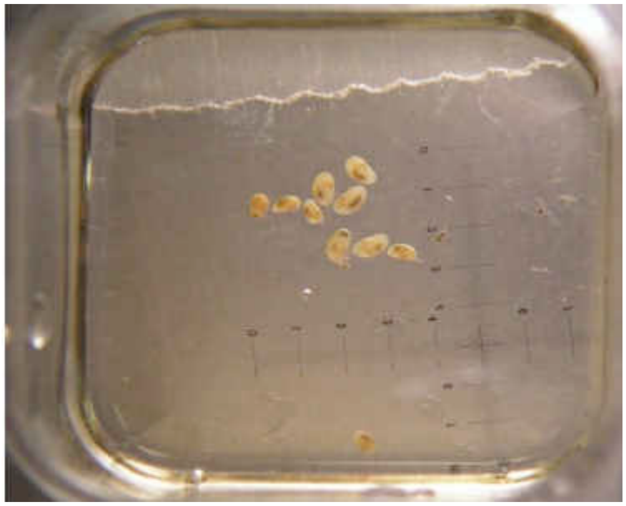
APPENDICES

Appendix A. RAPIDTOX^{TM} photographs. The top picture (i) displays the hatching vessels during the incubation period, in which the *T. platyurus* were hatched, grown. The bottom picture (ii), taken from the MICROBIOTEST INC. website, illustrates an example of the organism with a digestive tract full of the colored microspheres (free from inhibition). This “red” area is found to be clear when sediment toxicity has increased to a point of affecting the feeding mechanism within the organism.

(i)

(ii)

Appendix B. OSTRACODTOX^{TM} photographs. Photograph that shows how the organisms were measured using a micrometer under the dissecting microscope.



Appendix C. PHYTOTOX^{TM} photograph showing an example of a test species (*Sorghum saccharatum*) in its dual-compartment test plate, after its 3-day incubation period.

<u>Appendix D.</u> The Plankton Net.

<u>Appendix E.</u> Water Sampling. (i)The picture displays the role of Integrated Samplers in surface water sampling for nutrients level analysis. (ii) The integrated sampler.

(i)

(ii)

<u>Appendix F.</u> Wetland with Sondes installed.

<u>Appendix G.</u> Vegetation Survey. The picture displays the role of meter quadrats in vegetation survey.

Appendix H. List of Zooplankton and plant species

Zooplankton

Bosmina sp.

Calanida

Ceriodaphnia sp.

Coridalinae

Culicoides sp.

Cyclops sp.

Daphnia pulex

Daphnia sp.

Daphnia retrocurva

Hydracarina sp.

Heterocypris sp.

Thamnocephalus sp.

Zygoptera

Plant

Bidens frondosa

Carex sp.

Cidos grama

Cirsium discolor

Cornus sp.

Dactylis sp.

Daucus carota

Elymus Canadensis

Epilobium sp.

Equisetum sp.

Euthamia graminifolia

Euthamia sp.

Eutrochium maculatum

Festuca sp.

Grass 1

Grass 2

Helenium autumnale

Juncus sp.

Leersia oryzoides

Melilotus albus

Onoclea sensibilis

Phalaris arundinacea

Poa pratensis

Schizachyrium scoparium

Sonchus asper

Sorghastrum nutans

Solidago Canadensis

Solidago sp.

Symphyotrichum novae-angliae

Symphyotrichum pilosum

Symphyotrichum sp.

Symphyotrichum lateriflorum

Symphyotrichum lanceolatum

Schoenoplectus tabernaemontani

Trifolium pratense

Typha augustifolia

Typha sp.