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Accumulation of trace metals in freshwater
invertebrates in stormwater management facilities

By:

B. Anderson, T. Bell, P. Hodson, J. Marsalek...

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ABSTRACT

Availability and uptake of metals in the accumulated sediments in stormwater treatment facilities was assessed by the metal accumulation patterns observed in freshwater mussels, as the first step in an Environmental Risk Assessment (ERA). Freshwater mussels, *Elliptio complanata*, were caged in various locations in several stormwater treatment facilities and control sites in southeastern Ontario. Mussels were sampled at 2, 5.5, 8, 11, and 14 weeks and Ni, Cr, Cu, Cd, and Pb concentrations in soft tissues were determined by ICP-MS. Selection of these metals was based on previous studies which had identified them in substantial quantities in stormwater pond sediments. A significant decrease in Ni concentrations and an increase in Pb concentrations relative to background levels were observed. Concentrations of Cu, Cd, and Cr were generally not significantly different from background. Total metal concentrations in sediments were also determined, and compared with the observed mussel metal concentrations. No correlations were observed between total metals in sediment and the accumulated burden in the mussels. The results suggest that Pb is a possible concern in these stormwater facilities due to its availability. Ni, Cr, and Cd did not appear to be in bioavailable forms and Cu had limited availability. The study was complementary to other work examining trace metals in stormwater management facilities, and provides further useful information about the habitat quality of these facilities, and the ecotoxicological risks that might be posed to resident species.

Accumulation de métaux traces chez les invertébrés d'eau douce dans les installations de gestion des eaux pluviales

Anderson, B.C., T. Bell, P. Hodson, J. Marsalek et W. E. Watt

RÉSUMÉ

La disponibilité et l'absorption de métaux dans les sédiments accumulés à l'intérieur des installations de traitement des eaux pluviales ont été évaluées grâce aux divers modes d'accumulation de métaux observés chez les moules d'eau douce, comme première étape d'une évaluation des risques liés à l'environnement (ERE). Des moules d'eau douce (*Elliptio complanata*) ont été mises dans des cages à divers endroits de plusieurs installations de traitement des eaux pluviales et de sites de contrôle dans le sud-est de l'Ontario. Les moules ont été échantillonnées à 2, 5,5, 8, 11 et 14 semaines et les concentrations de Ni, Cr, Cu, Cd et Pb dans leurs tissus mous ont été mesurées au moyen d'un spectromètre de masse à plasma inductif (ICP-MS). Le choix de ces métaux était basé sur des études antérieures, qui avaient permis de les caractériser en quantités substantielles dans les sédiments des bassins d'eaux pluviales. On a constaté une diminution significative des concentrations de Ni et une augmentation des concentrations de Pb par rapport aux teneurs naturelles. Les concentrations de Cu, Cd et Cr n'étaient généralement pas différentes de façon significative des valeurs naturelles. Les concentrations totales de métaux dans les sédiments ont également été mesurées et comparées aux concentrations de métaux déterminées chez les moules. Les résultats semblent montrer que le Pb pourrait être une source possible de préoccupation dans ces installations pour eaux pluviales, en raison de sa disponibilité. L'étude était complémentaire à d'autres travaux visant à examiner la présence des métaux traces dans les installations de gestion des eaux pluviales, et elle donne d'autres renseignements utiles sur la qualité de l'habitat de ces installations ainsi que sur les risques écotoxicologiques qui pourraient menacer les espèces résidentes.

NWRI RESEARCH SUMMARY

Plain language title

Accumulation of trace metals in freshwater mussels from stormwater management facilities.

What is the problem and what do scientists already know about it?

Sediments contaminated by trace metals accumulate in stormwater ponds and thereby create ecotoxicological risks to organisms inhabiting these facilities. Such risks can be assessed by various methods, including measurements of metal concentrations in sediments, or in aquatic organisms. The latter method is preferable, because it takes into account the bioavailability of metals. Scientists are searching for the "best" organisms to be used in environmental risk assessments. In this study, freshwater mussels were used.

Why did NWRI do this study?

Poorly maintained stormwater ponds represent contaminated habitat and serve as points of entry of contaminants into the food chain. An improved understanding of the accumulation of trace metals in ponds and metal uptake by aquatic organisms is of interest for pond design and development of proper maintenance procedures.

What were the results?

Among the trace metals studied, Ni, Cr, Cd Cu appeared to occur in stormwater facilities in non-bioavailable forms, because they did not accumulate in mussels. Pb appeared to pose a risk to stormwater ecosystems because it accumulated in organisms inhabiting stormwater facilities. Overall, total sediment metal concentrations were not useful in explaining observed trends in mussel metal accumulation. To obtain more robust conclusions, further study with more test species is recommended.

How will these results be used?

The results will be used by municipalities for planning, implementing and maintaining stormwater management facilities.

Who were our main partners in the study?

The main partners were Departments of Civil Engineering and Biology, Queen's University, Kingston, Ontario.

Sommaire des recherches de l'INRE

Titre en langage clair

Accumulation de métaux traces, provenant des installations de gestion des eaux pluviales, chez les moules d'eau douce.

Quel est le problème et que savent les chercheurs à ce sujet?

Les sédiments contaminés par les métaux traces s'accumulent dans les bassins d'eaux pluviales, ce qui entraîne des risques écotoxicologiques pour les organismes habitant ces installations. Ces risques peuvent être évalués par diverses méthodes, notamment la mesure des concentrations de métaux dans les sédiments ou chez les organismes aquatiques. Il est préférable d'opter pour la dernière méthode, car elle tient compte de la biodisponibilité des métaux. Les scientifiques effectuent des recherches pour trouver les « meilleurs » organismes pouvant servir aux fins des évaluations des risques environnementaux. Dans la présente étude, on a fait appel aux moules d'eau douce.

Pourquoi l'INRE a-t-il effectué cette étude?

Des bassins d'eaux pluviales mal entretenus représentent un habitat contaminé et constituent des points d'entrée dans la chaîne alimentaire pour les contaminants. Une meilleure connaissance de l'accumulation des métaux traces dans les bassins ainsi que de l'absorption des métaux par les organismes aquatiques permettra de mieux concevoir ces bassins et de mettre au point des procédures d'entretien appropriées.

Quels sont les résultats?

Parmi les métaux traces étudiés, Ni, Cr, Cd et Cu semblaient être présents sous des formes non biodisponibles dans les installations d'eaux pluviales, car ils ne s'accumulaient pas chez les moules. Le Pb semblait représenter un risque pour les écosystèmes d'eaux pluviales. Dans l'ensemble, les concentrations totales de métaux dans les sédiments n'étaient pas utiles pour expliquer les tendances observées dans l'accumulation de métaux chez les moules. Pour en arriver à des conclusions plus solides, il est recommandé de procéder à d'autres études avec davantage d'espèces expérimentales.

Comment ces résultats seront-ils utilisés?

Les résultats serviront aux municipalités à planifier, à mettre en place et à entretenir les installations de gestion d'eaux pluviales.

Quels étaient nos principaux partenaires dans cette étude?

Les principaux partenaires étaient les départements de génie civil et de biologie de l'Université Queen's, à Kingston (Ontario).

**Accumulation of trace metals in freshwater invertebrates in
stormwater management facilities**

Bruce C. Anderson¹, Tracy Bell¹, Peter Hodson², Jiri Marsalek³, W. Edgar Watt¹

¹Department of Civil Engineering, Queen's University, Kingston ON

²Department of Biology, Queen's University, Kingston ON

³National Water Research Institute, Burlington ON

Keywords: trace metals, freshwater mussels, stormwater, accumulation, bioavailability,
sediments, Best Management Practices, environmental risk assessment

Abstract

Availability and uptake of metals in the accumulated sediments in stormwater treatment facilities was assessed by the metal accumulation patterns observed in freshwater mussels, as the first step in an Environmental Risk Assessment (ERA). Freshwater mussels, *Elliptio complanata*, were caged in various locations in several stormwater treatment facilities and control sites in southeastern Ontario. Mussels were sampled at 2, 5.5, 8, 11, and 14 weeks and Ni, Cr, Cu, Cd, and Pb concentrations in soft tissues were determined by ICP-MS. Selection of these metals was based on previous studies which had identified them in substantial quantities in stormwater pond sediments. A significant decrease in Ni concentrations and an increase in Pb concentrations relative to background levels were observed. Concentrations of Cu, Cd, and Cr were generally not significantly different from background.

Total metal concentrations in sediments were also determined, and compared with the observed mussel metal concentrations. No correlations were observed between total metals in sediment and the accumulated burden in the mussels. The results suggest that Pb is a possible concern in these stormwater facilities due to its availability. Ni, Cr, and Cd did not appear to be in bioavailable forms and Cu had limited availability. The study was complementary to other work examining trace metals in stormwater management facilities, and provides further useful information about the habitat quality of these facilities, and the ecotoxicological risks that might be posed to resident species.

Introduction

Stormwater management ponds are increasingly popular in city planning for the treatment of stormwater runoff. These ponds often become habitat for a range of flora and fauna including fish, amphibians, aquatic insects, ducks, birds, and small mammals (Wren et al. 1997; Bishop et al., 2000a,b). However, urban runoff is laden with a variety of chemicals, including trace metals (Wren et al. 1997; Marsalek et al. 1997), 90% of which are thought to be adsorbed onto the surfaces of sediment and other suspended particulate matter (Calmano et al. 1993). The fundamental design attribute of stormwater ponds is gravitational settling of these particles to the pond bottom, thereby reducing the amount of chemicals suspended in the water column at the facility outlet and their discharge to downstream waters (MOE 2003). Due to the nature of stormwater ponds as reservoirs for contaminants in the sediments, the issue of potential risks to aquatic ecosystems has been raised (Wren et al. 1997; Kelly-Hooper 1996). Risk is a function of hazard (presence of toxic chemicals), exposure and subsequent effect; without exposure

there is no risk. Therefore, to understand the potential for risk it is essential to characterize exposure and availability, i.e. to determine if aquatic biota accumulate stormwater contaminants.

Limited research in the area of ecotoxicity of stormwater and stormwater treatment facilities indicates conflicting results. Some researchers found elevated metal concentrations or reduced growth in fish and insects living in urban/road runoff (Campbell 1994; Van Hassel et al. 1980; Pitt 1995), whereas others found little or no impact (Bailey et al. 1999; Perdikaki and Mason 1999). Laboratory toxicity bioassays suggest stormwater has toxic effects and contains bioavailable compounds (Hall and Anderson 1988; Dutka et al. 1994; Marsalek et al. 1999).

The exact knowledge of trace metal speciation in stormwater pond sediments is very difficult due to the chemical complexity of the aquatic environment where ambient pH, oxidation/reduction potential, salinity, presence of organic acids and particulates, interactions among metals, geology of the area, hydrology, and bacterial activity all have an influence (Anderson et al. 1998). The metal concentrations in sediments specified by the Ontario provincial guidelines for the protection of aquatic life are based on total metals, and do not take speciation into account (MOEE 1992). However, metal speciation is complex enough that the amount of metal bioavailable to organisms cannot be predicted from total concentrations, and is therefore better quantified through field evaluations.

Metal Uptake by Organisms

Aquatic organisms can be exposed to metals through direct contact with bodily surfaces or through ingestion of food, sediment, or water (Barron 1995). For uptake to occur, the metals must cross membranes into the organism. Since gills are negatively charged, metal cations can bind easily and this is an important site of entry into organisms (Barron 1995). However, since other cations (H^+ , Ca^{+2}) also vary in concentration and can compete for binding sites, uptake will vary with water quality.

The method used in this study to assess the potential bioavailability (defined as presence of metal *and* potential accumulation in the biota) of metals in stormwater pond sediments was to cage freshwater mussels in contact with these sediments *in situ*. By measuring the metal concentrations in mussels over time, and comparing these concentrations to sediment metal chemistry, we evaluated the relative importance of sediment metal concentrations to bioavailability. Further, the mussels *in situ* integrated all of the biological, water, and sediment quality characteristics that controlled uptake and were therefore representative of the trace metal exposure risk in this setting.

Monitoring Programs Using Mussels

Mussels meet the biomonitor criteria of sedentary, long lived, filter feeders living at the sediment/water interface, and have frequently been used as monitoring organisms (Goldberg et al. 1978; Tessier et al. 1984; Couillard et al. 1995; Metcalfe-Smith et al. 1996), with at times conflicting results found.

Correlations between metals in the sediment and metal concentrations in mussels were observed for Cd and Cr, but not for Cu or Ni (Metcalfe-Smith et al. 1992). The Cu

result was explained by mussels being able to regulate tissue Cu content over a wide range of sediment concentrations. Anderson (1977) found that mussel metal content generally reflected the concentrations of Cu, Cd, and Pb in the sediments. Tessier et al. (1984) found that Cu and Pb levels in the freshwater mussel *Elliptio complanata* correlated with carbonate, Fe/Mn oxide, and organic/sulfide fractions from sequential extraction, and less so for the residual fraction and total of all fractions. In contrast, Campbell and Evans (1991) did not find a relationship between mussel tissue and water Cd content, but did find a relationship for tissue and sediment concentrations. Pugsley et al. (1988) found no relationship between sediment and mussel concentrations of Cd and Pb. Couillard et al. (1995) caged *Anodonta grandis* for 400 days to test metal and metallothionein concentrations and observed a 3.3 fold increase in Cd after 90 days. Mersch and Pihan (1993) observed increases in Pb, Cd, Cr, and Cu (about 0.5-6 times) in caged zebra mussels (*Dreissena polymorpha*) after only 27 days of a 90 day study.

The current study was designed to address the following questions: Do freshwater mussels accumulate metals in soft tissues when placed in stormwater ponds? Is the accumulation of metals by mussels correlated to the concentration of metals in the sediment? Previous work by vanLoon et al. (2000), that characterized the metal content in the sediments of several stormwater facilities, provided a foundation upon which to base this study of accumulation of metals.

Materials and Methods

The freshwater mussel, *Elliptio complanata*, very common throughout Eastern Ontario and southern Quebec (Clarke 1981), was selected as the biomonitor species for

this study. Six hundred mussels between 65 and 70 mm in length were collected from Dalhousie Lake, Ontario and subsequently caged in the stormwater pond test sites. The cages were plastic milk crates that were weighted and partially submerged in pond sediments. Thus, mussels were exposed to both sediment and circulating water.

Three stormwater ponds with sediments well characterized by vanLoon (1998), were chosen as test sites: Cataraqui Town Centre Stormwater Pond (Kingston, Ontario; named CP in this paper), Longfields/Davidson Heights Stormwater Treatment Facility (Nepean, Ontario; named LD in this paper), and Kennedy-Burnett Stormwater Management Facility (Nepean, Ontario; named KB in this paper). Further details on the operation and characteristics of these ponds can be found in Van Buren et al. (1996) and vanLoon (1998). Previous studies at these sites (vanLoon 1998; Marsalek et al. 1997) demonstrated that particle size decreased and sediment metal concentration increased from inlet to outlet. Based on this gradient, sites were chosen in each pond at a water depth of 50- 100 cm, for a total of 10 sites for all ponds. These sites were selected throughout each pond to provide a presumed gradient of exposure for the test mussels, based on the previous work described above. Five cages were placed at each of the 10 sites and 13 mussels placed in each cage. The mussel density (number of mussels/m²) was within the range of densities used in other caging studies.

The study length during the field season was 14 weeks (from the beginning of July to early October); this length was based on other caging studies that showed results within this time (studies described in Introduction). Samples of 10 mussels (2 mussels/cage x 5 cages/site) were taken at 2, 5.5, 8, 11, and 14 weeks and frozen without

gut purging. Mussels were transported to the lab in a cooler with ice packs and immediately placed in the freezer.

Mussel Dissection and Analysis

Mussels were thawed about 15 minutes and shucked by prying the valves apart and removing all soft tissues. The posterior portion of the intestine (including the portion surrounded by the heart) was also removed. This was done to help reduce the influence of any sediment-bound metals in the lower intestine on the overall metal concentration. All other soft tissues were placed in 50 mL pre-weighed centrifuge tubes (1 mussel/tube).

Tissue samples were dried to a constant weight at 70°C for 3 days after which 5 mL of 70% nitric acid was added and the samples digested for one week with occasional shaking. Hydrogen peroxide (333 µL, 30%) was added to each sample and the samples were diluted with 25 mL of distilled, deionized water. An additional 2 mL of hydrogen peroxide (30%) was added with heating in a water bath at 75°C for 10 hours to give clear, light yellow solutions. The clear digested samples were diluted 1:10 with 2% pure nitric acid and analyzed for metals using ICP-MS (Finnigan MAT Element, high resolution-inductively coupled plasma-mass spectrometer) in the Department of Geology, Queen's University. This facility is recognized as one of the best of its kind, and well developed protocols were followed in interpreting the data, as described in the following section. Method detection limits (MDL) were Ni, 2 ppb; Cr, 0.1 ppb; Cu, 0.3 ppb; Cd, 0.06 ppb; Pb, 0.095 ppb. Results were considered accurate to within 3-5% (Chipley 1998).

Quality Assurance/Quality Control

A series of reference standards, internal standards, procedural blanks, and standard additions were used during ICP-MS analyses to allow for interpretation of data quality. National Research Council standard reference material, TORT-2, made of lobster hepatopancreas, was used to determine accuracy and consistency of ICP-MS readings. Three solutions containing 0.5 g of TORT (labeled TORT(2a), TORT(3a), TORT(4a)) were prepared following the same digestion procedure as tissue samples. A sub-sample of one of these solutions was usually analyzed after every 10th mussel sample. An internal mussel tissue standard (IS) was made from a composite sample of mussel tissue from Dalhousie Lake and was analyzed after every 15th-20th mussel sample to determine consistency throughout the analyses. Procedural blanks for the digestion procedure were run at the beginning of the experiment. Reagent blanks of acid used for final dilutions were usually included after every 40th mussel sample.

Percent recovery was determined using standard additions with TORT using 0 spike, 1 spike, 2 spikes, and 4 spikes. Percent recoveries were quite high: Ni 85%, Cr 93%, Cu 93%, Cd 87%, and Pb 96%. Dilution blanks, procedural blanks, and an internal mussel tissue standard were also used. No sources of experimental contamination were identified by the blanks.

Fluctuations in ICP-MS results (above a normal accuracy of 3-5%, which is the expected range for the instrument used) were caused by ICP-MS cone clogging and fluctuations in electrical supply based on time of day. Due to limitations on equipment availability, it was not possible to repeat all samples from runs where the standards were not within 5% of accepted values. However, it was possible to apply correction factors

based on the variation in TORT and IS standards, as follows. First, the observed concentrations of all standards analyzed were averaged. The percent difference from the mean was calculated for any samples that approached or exceeded the 95% interval about the mean. These percent differences were compared with differences in other standards analyzed in the same run. Approximately 9% of values required corrections. Based on the percent deviations in all standards within a run, the following corrections were applied:

- If the IS and TORT errors throughout a run were random, then an average % error was taken and that correction was applied to the mussels from that run.
- If IS standards were consistent throughout a run, but different than TORT, then a correction was applied based on the IS standards because they should be a more accurate representation of the mussel samples than TORT.
- If IS and TORT errors followed a linear pattern or took sudden drops throughout a run, then the appropriate correction was applied based on where the mussel sample was analyzed in the run.

ICP-MS ppb values were converted into $\mu\text{g/g}$ values by taking into account dilutions and dividing by the dry weight. Then the correction factor based on standards was applied if necessary. All concentrations are expressed per unit dry weight.

Sediment Characterization

Sediment was collected from the caging sites and analyzed for a number of different parameters for comparison with previous studies and biota data. Sediment samples were collected on the same days as mussel samples. One sample was taken from

each site at each sampling time. The sample was collected within 50 cm of the group of 5 cages, near the centre of the cage cluster. A sediment sample was collected at Dalhousie Lake from the mussel collection site at the beginning and end of the field experiment. The top 5 cm of sediment was collected by scooping with a 250 mL glass mason jar. After settling, the jars contained 1/3 sediment and 2/3 water.

Sediment Preparation and Metals Analysis

Sediment was prepared the day after collection. Sediment was dried in a Gallenkamp Hotbox oven over night at 104°C, then crushed by hand using a crucible and mortar. A sample greater than 0.3 g (as required for metals analysis) was placed in a plastic centrifuge tube and sent to RMOC (Regional Municipality of Ottawa-Carleton) for analysis. Analysis was performed using ICP-AES, Inductively Coupled Plasma- Atomic Emission Spectrometry.

At the lab, sediment samples were digested in dilute nitric acid and run through ICP-AES (model Jobin-Yvon 48 p) according to RMOC lab procedures. ICP-AES uses inductive heat caused by an argon plasma to excite the aspirated sample. The emitted light is measured to determine the concentration of the metal.

MDLs were Cu 0.2 ppm, Pb 4.6 ppm, Ni 1 ppm, Cd 0.6 ppm, and Cr 1.2 ppm. NRC standard reference materials made of marine sediment, MESS-2b and PACS-1, were used to determine accuracy of ICP-AES results. These were each analyzed 2 times.

Statistical Methods

Histograms were constructed to verify normal distributions; a log transformation was required for Cd data before analysis. Homogeneity of variance was verified by

Hartley's F-max test and by plots of standard deviations (SD) vs. means; SD's did not vary with means. Analyses of variance (ANOVA, SYSTAT 9.0) were used to determine if site and time influenced mussel metal concentrations; a $p < 0.05$ indicated the factor was significant (Sincich 1999). Tukey's test for multiple means comparisons indicated which means were significantly different ($p < 0.05$) (Milliken and Johnson 1992). Coefficients of variation were determined to compare variation in samples by dividing the sample set standard deviation by the mean. These were roughly similar for all metals, ranging from 0.17 – 0.27.

Linear regression analyses were used to calculate best fit lines relating tissue and sediment metals, based on minimizing the sums of squares of the residuals. The strength of the relationship was assessed by a correlation coefficient (r^2) and the statistical significance of slopes ($p < 0.05$). The distribution of residuals about a horizontal line was used to indicate whether the linear regression was an appropriate model.

Results

Quality Assurance/Quality Control

The NRC standard reference material, (TORT-2), was used to determine accuracy and consistency of ICP-MS readings. Table 1 shows the average metal concentrations for 3 different TORT test solutions that were analyzed frequently, and the known NRC concentrations. In most cases, the 95% confidence interval about the mean values for each metal overlapped with the 95% interval reported by NRC, except for Cr in TORT(3a) and Cd in TORT(4a) which were very close. This demonstrates that ICP-MS

is an appropriate instrument to use for tissue sample analysis, and that the method of sample preparation is adequate.

General Trends in Mussel Metal Concentrations

A box and whisker plot of individual values of mussel metal concentrations from all stormwater ponds showed that Ni decreased immediately, Pb showed a general increase, and Cu, Cr, and Cd remained approximately constant over the duration of the study (see Figure 1). These plots are presented only to allow the reader to discern any obvious trends in the data, using the blended information. A more detailed statistical analysis of these same data is presented below.

The mean concentrations and standard deviations were determined for each sample set of approximately 10 mussels from each site and time. To observe trends between mussel metal concentrations within the same pond, the concentrations over time were plotted for all sites in each facility; Figure 2 presents representative information for the Kennedy-Burnett sample set, with KB1, KB2 and KB3 representing information from each of the 3 sites within that facility. Some values for KB at week 5.5 were omitted because too many measured below the MDL to get a representative average for that time. The first and last bars in each plot are the mean background metal concentration in mussels from the original collection site (Dalhousie Lake); these are used as the reference concentration for $t = 0$ and the end of the study, respectively.

Using these same data, Tukey's test for multiple mean comparisons (95% confidence) was performed for all ponds. This compared all possible means from each site at each sampling time, within one facility. This provided information about

similarities and differences among mussel metal concentrations at different sites within a facility, and control values (at $t = 0$). In Figure 2 the asterisks represent values that differ significantly from the control Dalhousie Lake sample (at $t = 0$, as an indicator of background concentration). In general, $[\text{Ni}]_{\text{mussel}}$ in the ponds was lower than background and $[\text{Pb}]_{\text{mussel}}$ was higher than background. A few $[\text{Cr}]_{\text{mussel}}$ values in all three ponds were above background (e.g. week 5.5 in CP), but $[\text{Cr}]_{\text{mussel}}$ decreased again, suggesting no overall accumulation of Cr. There were 3 increased values of $[\text{Cu}]_{\text{mussel}}$ in weeks 11 and 14 in CP, which may suggest a slight increasing trend at this facility. $[\text{Cd}]_{\text{mussel}}$ appeared to remain constant in LD and CP. There were 3 significantly reduced values of $[\text{Cd}]_{\text{mussel}}$ in KB (KB3 weeks 11 and 14; KB2 week 11), but there does not appear to be an overall decreasing trend.

Different sites within each facility were compared at each sampling time using Tukey's test. Generally, the results showed no significant differences ($p > 0.05$). At each sampling week, all mussels from the same pond exhibited similar concentrations. It can be concluded that individual sites within the same pond did not act independently. Exceptions included: $[\text{Cu}]_{\text{mussel}}$ was often higher at KB1 than at KB2 and KB3 (weeks 2, 8, 14); $[\text{Pb}]_{\text{mussel}}$ was higher at KB1 than KB2 and KB3 (weeks 8, 14); and $[\text{Pb}]_{\text{mussel}}$ was higher in LD4 than at LD5 and LD6 (weeks 8, 11).

Mussels in Dalhousie Lake (reference site) had significantly higher concentrations of Cd and Cr at the end of the study than at $t = 0$ (seen in Figure 2 data). The reason for this is not clear, and this serves to illustrate the inherent variability in metal content one might expect in biomonitoring studies (especially at the low levels found here); as well

this illustrates the difficulty in finding a true (e.g. unaffected) reference site to use as an experimental control.

Site and Time Influences

Analyses of variance (ANOVA) (95% significance) were performed using site and time factors (data from week 2-14 used); these analyses are summarized in Table 2. Because individual sites within the same pond do not act independently (based on Tukey test results), data for each facility at each sampling time were combined. Most results show p values <0.05 , suggesting that both site and time influence the variation in metal concentrations observed in mussels. The exceptions were Cr and Cd, for which site does not have a significant influence. No differences for Cd values were observed for analyses using log transformed values of Cd, except for the site x time interaction. However, in this case both p-values were very close to 0.05, and would have led to the same interpretation at 90% significance.

The Tukey test for multiple mean comparisons was also performed at 95% significance to determine differences in mussel metal concentrations among facilities. Tukey results showed that CP mussels had significantly higher [Pb] than the other 2 ponds in weeks 5.5, 11, and 14. This agrees with the ANOVA results of $F_{\text{stat}} \gg F_{\text{crit}}$ for site influences on Pb (Table 2).

The Tukey test also showed that CP mussels had significantly higher Cu concentrations in weeks 11 and 14, and are also higher, although not significantly, in weeks 2, 5.5, and 8. The ANOVA F_{stat} for site influences on Cu was much larger than F_{crit} (Table 2).

Ni concentrations in mussels in LD were significantly higher than at least one other site in 3 out of 5 sampling times. No ranking of sites could be determined for Cd or Cr from Tukey's test results. This was supported by p values >0.05 for both Cr and Cd for site influences.

Comparison Between Sediment and Mussel Metal Content

Figure 3 illustrates the concentrations of metals in the sediment from the test sites as determined by ICP-AES. The MOE Sediment Quality Guidelines LEL, lowest effect level, and SEL, severe effect level, are included on the graphs where appropriate. The relative differences in sediment metal concentrations between sites (which remained the same after corrections) were thought to be most important for comparison in this study, and absolute concentrations were not as important as the general trends and ranking of sites according to sediment metal contamination. From the figures it is clear that sediment metal concentrations varied by site, time of sampling, sampling location within each site and metal being analysed. The organic content of the sediment might also explain some of this variation, although for the most part the organic content remained relatively constant over time, and between ponds (values ranged from 7.3-7.8% at CP; 4.1-4.9% at KB; and 4.4-8.9% at LD). These values were similar to those from previous studies at KB and LD (vanLoon, 1998).

Comparison of Mussel Metal Content with Total Sediment Metals

Regression analyses were performed for mussel metal concentration plotted against total sediment metal concentration. All r^2 values were very low ($r^2 \leq 0.3$),

suggesting little or no relationship between metal in the sediment and metal concentration in mussels. The highest regression coefficients were for Ni and Pb. Ni showed a very weak ($r^2=0.31$, $n=22$, $p<0.05$) negative relationship for sediments and mussel metal contents, and Pb had a positive correlation of the same magnitude ($r^2=0.30$, $n=22$, $p<0.05$) (Figure 4).

Discussion

In general, the results of this study showed a decrease in tissue concentrations of Ni, an increase in tissue Pb and no change for Cu, Cr, and Cd in the freshwater mussel *E. complanata* over the study period. Concentrations of metals in mussels were not strongly correlated with metal concentrations in the sediment; however there may a weak correlation for Pb and Ni.

The mussel metal concentrations found in the present study are at the low end or below the range of mussel metal concentrations observed in other studies (see Table 3). While it is difficult to directly compare between these studies, it is nonetheless notable that the low concentrations observed may be a result of short-term exposure in the test sites, and may indicate that longer exposure may result in elevated mussel metal concentrations (despite the previous suggestions that a short time period is appropriate for such caging studies).

A comprehensive QA/QC program was in place in this study to allow for accurate data interpretation. The fluctuations observed in the standard samples were greater than desired, given the small differences among sample means. The large standard deviations caused by large natural variation in metal concentrations in *E. complanata*, coupled with

variations in ICP-MS accuracy, made it difficult to compare small differences in means. However, significant trends were still evident. Despite ICP-MS fluctuations, results for the TORT standards were quite accurate on average, and this demonstrated that the ICP-MS was a suitable instrument for determining low metal concentrations in tissue samples.

Sediment-Mussel Metal Concentration Relationships

Mussels did not appear to accumulate metals in response to metal concentrations in the sediments to which they were exposed. There were no significant correlations between tissue metal concentrations and sediment metal concentrations, except for lead. This agrees with the findings of Perdikaki and Mason (1999), who reported a significant relationship for lead in sediments and lead in invertebrates in rivers near trunk roads, but found no other impacts on the invertebrate community. Campbell and Evans (1991) found no relationship between sediment concentrations and mussel Cd levels in *E. complanata*.

This study does not agree with other mussel caging studies that observed increases in mussel metal concentrations after only a few weeks. This may be due to the generally lower level of contamination found in these stormwater ponds compared to the other studies. For example, Pb concentrations were 15-500 times higher, and Cd concentrations were 100 times higher in sediments from another caging study (Czarnecki 1987), compared to stormwater sediments from this study.

Factors Affecting Metal Concentrations in Mussels

Metal concentrations in the sediment did not appear to affect metal concentrations in mussels; other factors such as ambient water conditions, gut contents, metal regulation, and weight loss may be as important.

The constant Cu concentrations in mussels may be explained through metabolic regulation of tissue copper concentrations. Metcalfe-Smith and Green (1992) observed similar Cu concentrations in *E. complanata* over a wide range of sediment Cu concentrations, and suggested that the mussels were regulating Cu. This could explain the observations at the stormwater facilities.

The reduction in Ni concentrations observed at all sites may also be explained by regulation. Ni is not essential for mussels, but it has been suggested that it is required in small amounts for proper functioning. Perhaps in Ni poor environments such as Dalhousie Lake, mussels store Ni for future use. Upon exposure to Ni rich stormwater environments, mussels may release Ni as they no longer need to store it. If this occurred as a large release of metal granules (Phillips and Rainbow 1993), then the sudden drop in Ni concentrations could be explained. However, there have been no reports of active regulation of Ni by mussels.

Mersch and Pihan (1993) suggested that when the body weight of the organism changes throughout the study period, the total body burden (metal concentration times mass) should be used as an alternative to metal concentration. This will account for degrowth magnification and growth dilution. Interestingly, plotting average body burdens for this study gave quite different results (results not shown). In this case, even for this short-term study, Cu increased and Ni remained the same. As well, Pb

accumulated more rapidly, which still supports the conclusion that Pb is bioavailable and taken up by organisms. Analyzing the data in this fashion implied a different interpretation of the decrease in Ni concentrations, whereby Ni was not released, just not taken up.

However, the true assessment of accumulation and subsequent toxic effects are based on the concentration of the metal (Mersch and Pihan 1993). It is also interesting to note that the body burdens of metals in mussels from the control site (Dalhousie Lake) at the end of 14 weeks were higher (except for copper) than the body burdens reached in the stormwater ponds. There might be several reasons for this: 1) the mussels in the stormwater test sites did not have time to achieve maximum metal accumulation, and metal body burdens might have increased beyond Dalhousie levels had the study been longer (despite the fact that the study period was deemed to be appropriate in length, as per previous work); or 2) the mussels were predisposed to take up metals from any available source, and the uptake observed was not caused specifically by stormwater pond contamination. Further work would be required to confirm this, however.

Trends in Mussel Metal Concentrations

Metal concentrations in *E. complanata* (mean values) fluctuated over the course of the study at each site. It was expected that the concentrations would remain constant due to the relatively constant metal exposure from sediments. Fisher et al. (1996) state that mussels can lose 1-5% per day of metals from soft tissues. In the first few days of depuration, the loss can be much greater than this (Fisher et al. 1996). In the present study, the decreases in metal concentrations in mussels between sampling times can be explained by 1-5% loss/day. However, this assumes depuration was taking place and that

there was no longer exposure to available metals. The source of metals had to be removed periodically or the pond conditions change such that metals become unavailable. Since it is known that the mussels were not removed from the source of metals during the exposure period, depuration would not be expected to have occurred.

Metal uptake from the water column also could explain another pattern observed in this study. Mussels generally had similar metal concentrations at all sites within the same facility (as shown by Tukey's test), as though they were responding to similar metal bioavailability. Sediment metal concentrations were different throughout each pond (for KB and LD). However, water metal concentrations were more consistent throughout the pond, as indicated by the limited data collected in this study (see Table 3). It is noted that since the sediment metal content was the focus of this study (given the expected exposure pathway), relatively few water column samples were taken for metals analysis, and of those that were taken, very few produced a valid metal concentration (a significant number of samples had non-detectable concentrations).

There was one exception to this mussel metal content similarity within ponds. Concentrations of metals in mussels at KB1 were significantly higher than for mussels in KB2 and/or KB3 at several sampling times for Cu and Pb, and higher (not significantly) for Cd and Cr. One hypothesis was that metal concentrations in the water might be a little higher near the inlet (KB1), but the data collected did not indicate this. As well, the sediment metal concentrations were lower at KB1 than KB2 and the particle sizes were larger. The sediments at KB1 may have been too large for *E. complanata* to ingest because they ingest particles mainly less than 25 μm (Tessier et al. 1984). Any large particles ingested would have only small amounts of adsorbed metals. This suggested that

sediment ingestion and absorption in the gut was not the source of metals for mussels at KB1.

Fisher et al. (1996) suggest that waterborne metals are taken up and stored in the shell and that metals in soft tissues originate from ingested particles. In the current study, metals in mussel soft tissues were not related to sediment metals or waterborne metals. It is possible that mussels actually responded to waterborne metals, but accumulated them in their shells not soft tissues.

Implications of Findings

The results of this study indicate that the sediments in the tested stormwater facilities were not in compliance with MOE sediment quality guidelines (MOEE 1992). Cr concentrations in the sediment exceeded the Severe Effect Level at several sites (CP9, CP10, KB2, and LD6), but mussels at those sites did not show Cr accumulation. This indicated that the guidelines were very conservative in protecting the ecosystem from Cr, Cr was predominately in a non-biologically available form, or *E. complanata* were not representative of the species used to devise the guidelines.

Pb concentrations in sediment were generally below MOE LEL guidelines at KB and LD, but Pb was accumulated by mussels at all sites. Therefore, the guidelines would not be seen as protecting the aquatic biota from Pb exposure and accumulation at these facilities.

Bioavailability and accumulation of metals in biota cannot be predicted based on exceedence of sediment metal concentrations of MOE sediment quality guidelines. The guidelines are not a good indicator of sediment effects on the ecosystem and should be

only one source of information used in any investigation of ecotoxicology. A full environmental risk assessment would entail more detailed investigation of contaminant-biota interactions, and the MOE sediment quality guidelines might serve as a trigger for this, in that if exceeded, one may then wish to proceed with biomonitoring. However, as demonstrated in this study, even sites with sediment metal levels below the criteria guidelines may present some ecotoxic risk to resident species, based on availability.

Conclusions and Recommendations

Stormwater facilities, with respect to Ni, Cr, and Cd, were likely not a threat to ecosystem health, based on this study. These metals were likely not in bioavailable forms in the stormwater facilities or had concentrations too low to elicit accumulation in mussels. For Ni, Cr, and Cd these facilities likely pose a minimal risk to higher trophic organisms because mussel tissue metal concentrations were not much different from background concentrations or uncontaminated sites from the literature. There is a potential risk from Cu, but these data were somewhat inconclusive; concentrations of Cu in mussels appeared to be increasing at CP near the end of the study, but this trend was not observed in the other sites. As well, crayfish had levels of Cu similar to contaminated sites from the literature and Cu in fish was higher in more contaminated sites.

The Cu results agree with the prediction that it will not be accumulated because it is an essential element and can be regulated. It was predicted that Ni and Cr would not be regulated and would accumulate in tissues because they are only required by mussels in small quantities; however, their lack of accumulation may indicate that mussels are capable of regulating these elements, or that they were in a non-available form in the test

sites. It was predicted that Cd would accumulate because it is not regulated, however, the low Cd concentrations in sediment likely prevented this.

Pb appears to pose a risk to stormwater ecosystems because it can accumulate in organisms inhabiting stormwater facilities (this despite the recent well-documented decline in environmental Pb concentrations as a result of changes in gasoline). This suggests that Pb, even at current sediment concentrations, was found in bioavailable forms at these facilities, which agrees with the prediction that Pb will accumulate because it is not an essential element and mussels cannot regulate it.

Overall, total sediment metal concentrations were not useful in explaining observed trends in mussel metal accumulation. Predictions were that tissue metals would be higher in more contaminated sites, which was the case only for Pb. Total sediment metals cannot be used effectively to predict metal levels in biota in metal-contaminated environments. As such, a much more detailed investigation of sediment-biota metal relationships is required in any environmental risk assessment of stormwater ponds and their ecotoxic potential. While the present study has presented some information on this subject for one test species, more study is required before a definitive conclusion can be reached.

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Table 1. Concentration of metals in TORT-2. The first line shows the 95% confidence interval for NRC accepted values. Values for test solutions, TORT (2a), (3a), (4a) are mean values \pm 95% confidence interval

Standard	Metal concentration in mg/kg					
	n	Ni	Cr	Cu	Cd	Pb
NRC		2.5 \pm 0.19	0.77 \pm 0.15	106 \pm 10	26.7 \pm 0.6	0.35 \pm 0.13
TORT(2a)	23	2.50 \pm 0.10	0.77 \pm 0.10	99.6 \pm 3.9	26.5 \pm 1.3	0.49 \pm 0.02
TORT(3a)	11	2.22 \pm 0.13	1.15 \pm 0.14	94.8 \pm 3.9	25.4 \pm 0.8	0.48 \pm 0.02
TORT(4a)	23	2.45 \pm 0.18	0.76 \pm 0.08	92.3 \pm 3.8	24.7 \pm 0.9	0.46 \pm 0.04

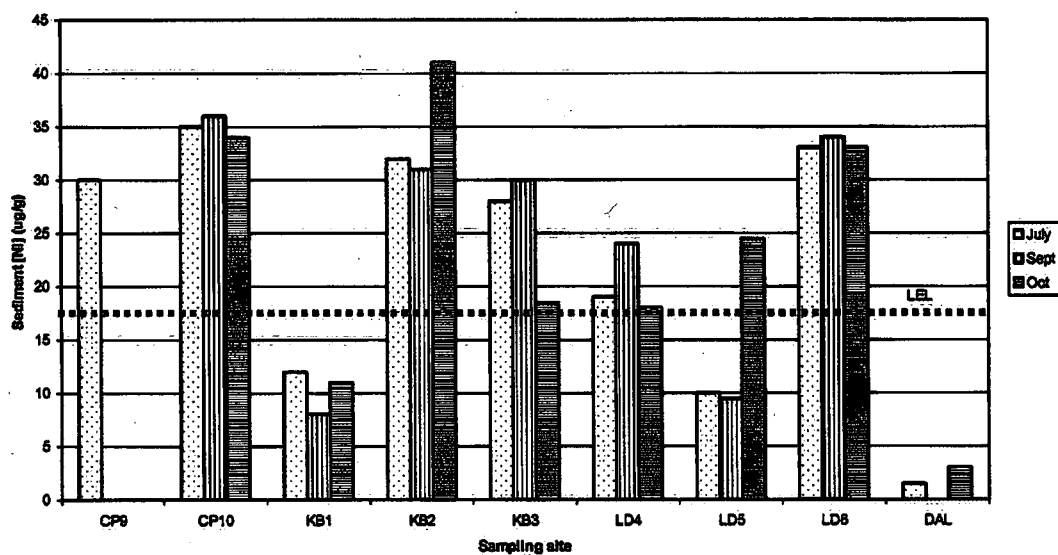
Table 2. ANOVA results at 95% significance. Y indicates relationship is significant. Values in brackets are F stat followed by p values. F crit is based on degrees of freedom and 95% significance

Metal	Site	F crit	Time	F crit	Site x time	F crit
Pb	Y (109.2, 0)	3.02	Y (5.0, 0)	2.39	Y (15.3, 0)	1.96
Ni	Y (13.1, 0)		Y (4.9, 0)		Y (8.0, 0)	
Cr	N (0.05, 0.95)		Y (12.9, 0)		Y (9.2, 0)	
Cu	Y (29.9, 0)		Y (8.2, 0)		Y (6.7, 0)	
Cd	N (2.0, 0.13)		Y (11.6, 0)		N (1.9, 0.06)	
Log Cd	N (2.2, 0.11)		Y (13.2, 0)		Y (2.2, 0.03)	

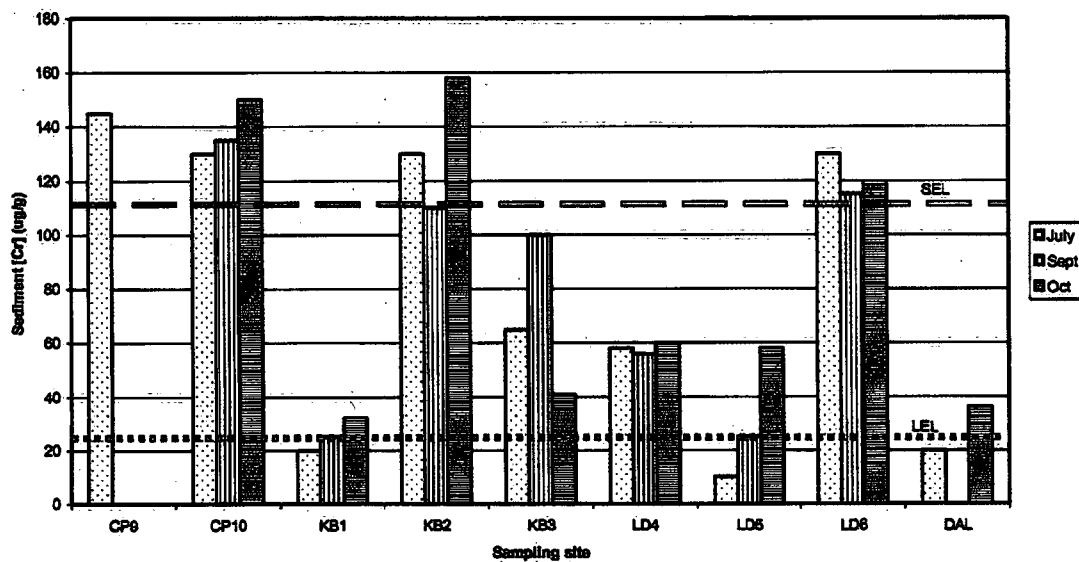
Table 3. Comparison of metal concentrations in mussel tissue from the literature

Metal	[metal] in mussel ($\mu\text{g/g}$ dry wt)	[metal] in sediment (mg/kg dry wt)	[metal] in water ($\mu\text{g/L}$)	Source
Cd	1.4	0.191	0.07	Campbell and Evans 1991
	5.9	0.378	0.14	
	0.6	0.093	0.02	
Cu	14	24		Tessier <i>et al.</i> 1984
	68	180		
Cd	4.5 - 12	1		Metcalf-Smith <i>et al.</i> 1992
	1.5 - 4	0.55		
Cu	11 - 20	24.5		
	6 - 11	17		
Ni	3.5 - 10	11.5		
	2 - 8.5	5.5		
Cr	2 - 7.5	4.5		
	4 - 9	11.5		
Pb	0.9 - 13.2	6.6 - 81.3		Metcalf-Smith 1994
Cd	8.2	0.09		Pugsley <i>et al.</i> 1988 <i>Lampsilis radiata</i> <i>siliquoidea</i>
	6.3	0.19		
	4.8	0.25		
Pb	9.4	34.8		
	7.0	14.4		
	5.5	31.4		
Ni	5.5-16	6-40	ND-1.2	Present study (Note: limited data collected)
Cr	0.5-1.8	13-155	ND-6.7	
Cu	5-13	7-62	ND-9.5	
Cd	0.2-0.7	0.2-0.7	ND	
Pb	0.3-1.7	4-75	ND-2.5	

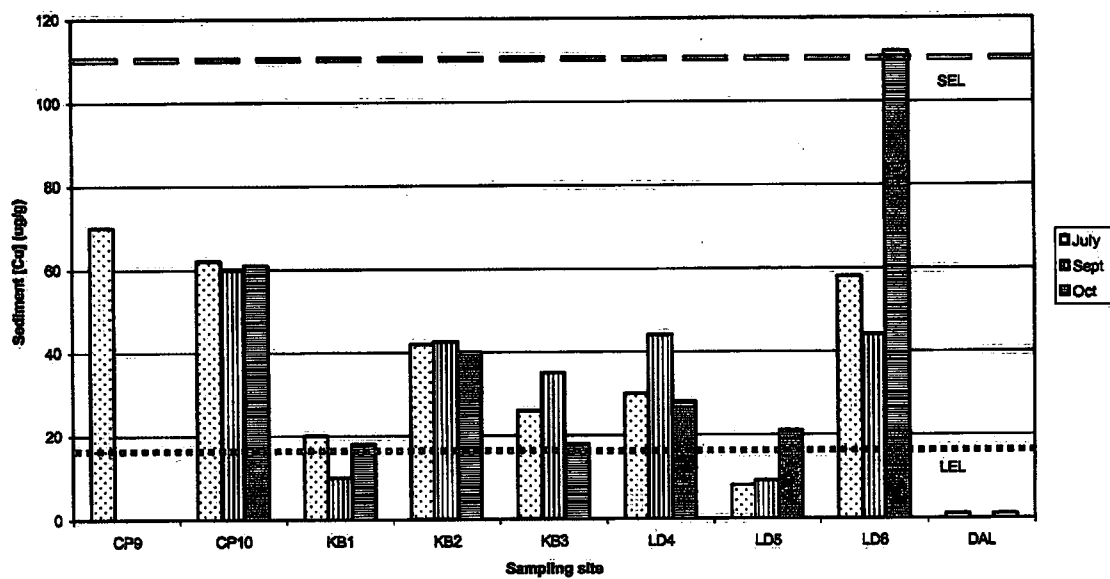
a) Sediment Ni concentration



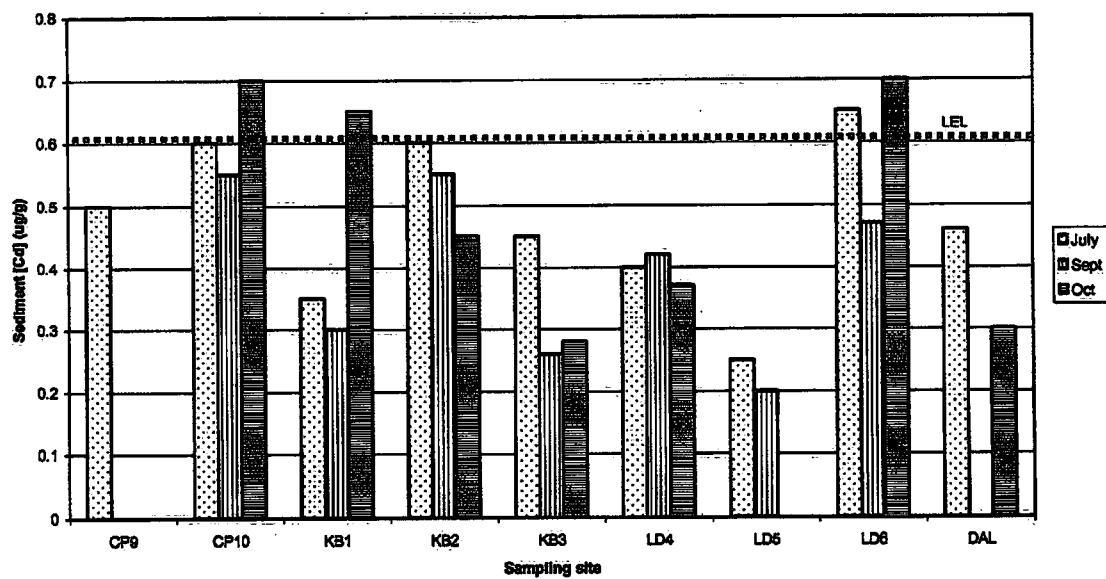
b) Sediment Cr concentration



c) Sediment Cu concentration



d) Sediment Cd concentration



e) Sediment Pb concentration

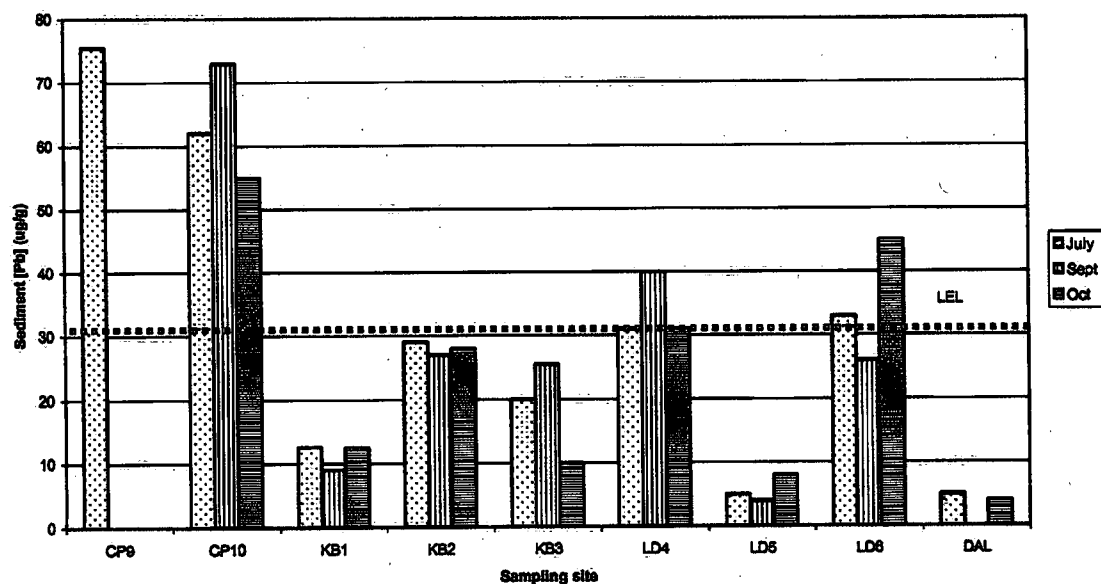
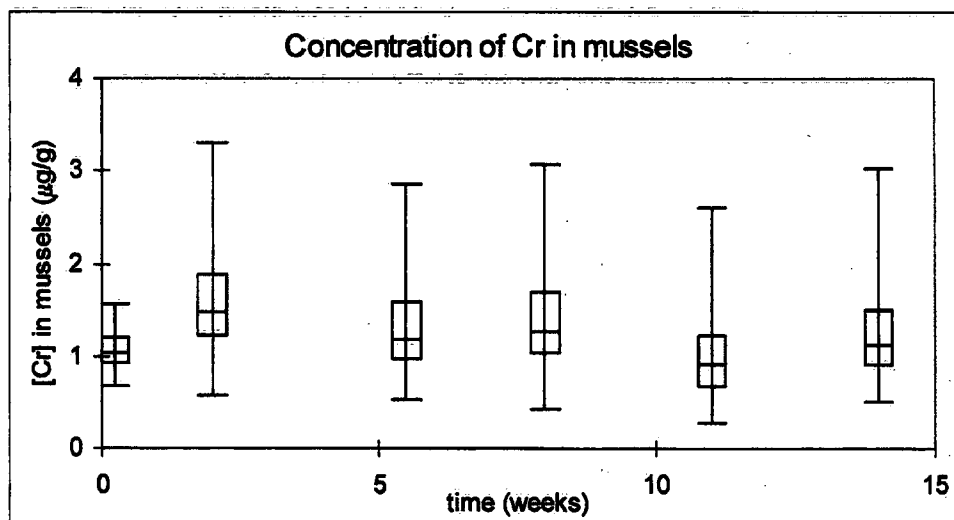
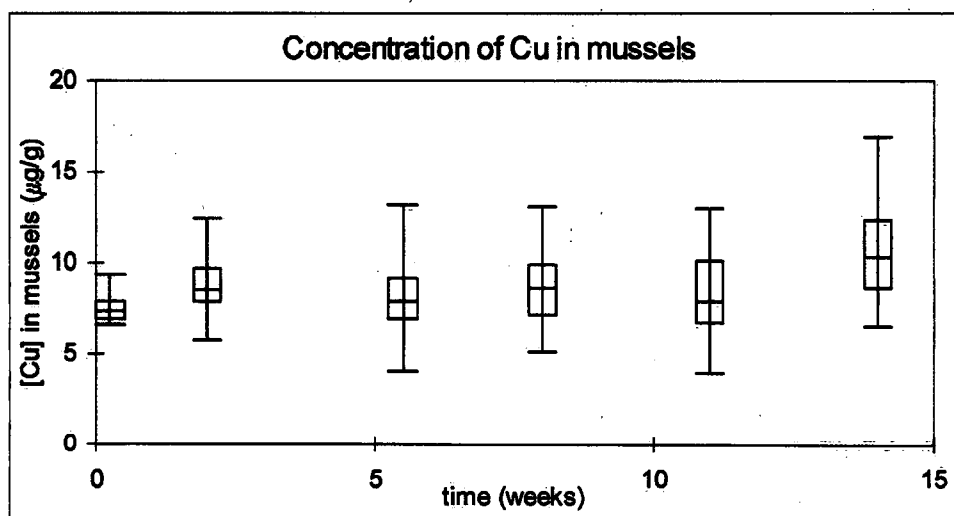
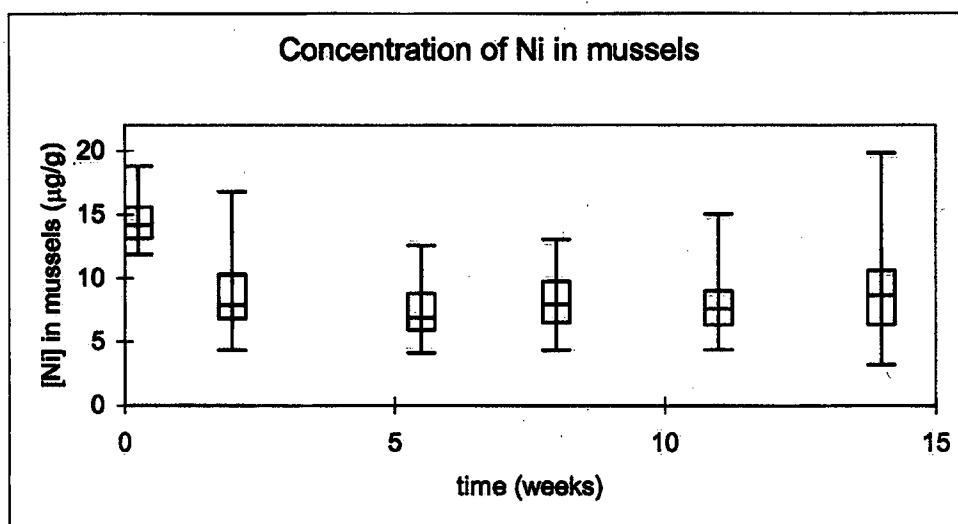


Figure 3. Sediment metal concentrations at sampling sites (CP = Cataraqui Pond; KB = Kennedy-Burnett; LD = Longfields/Davidson; DAL = Dalhousie Lake control)



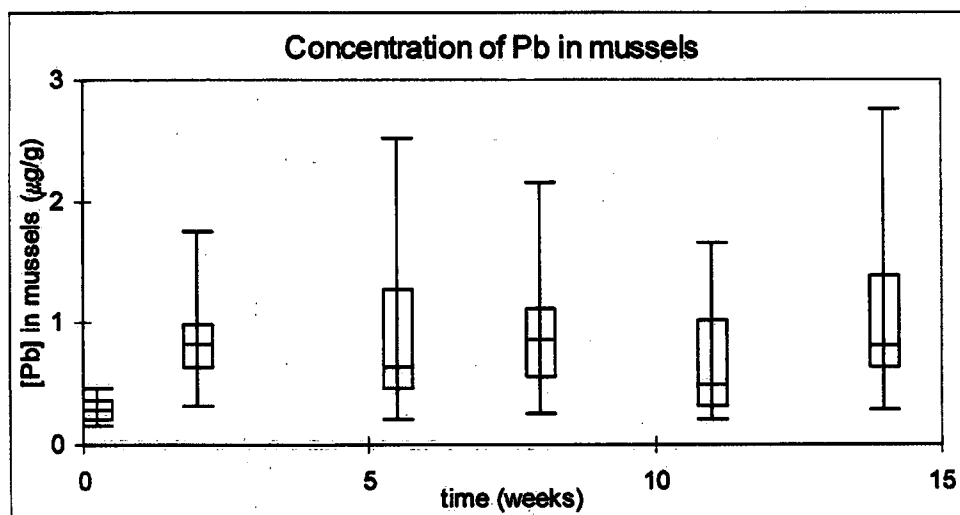
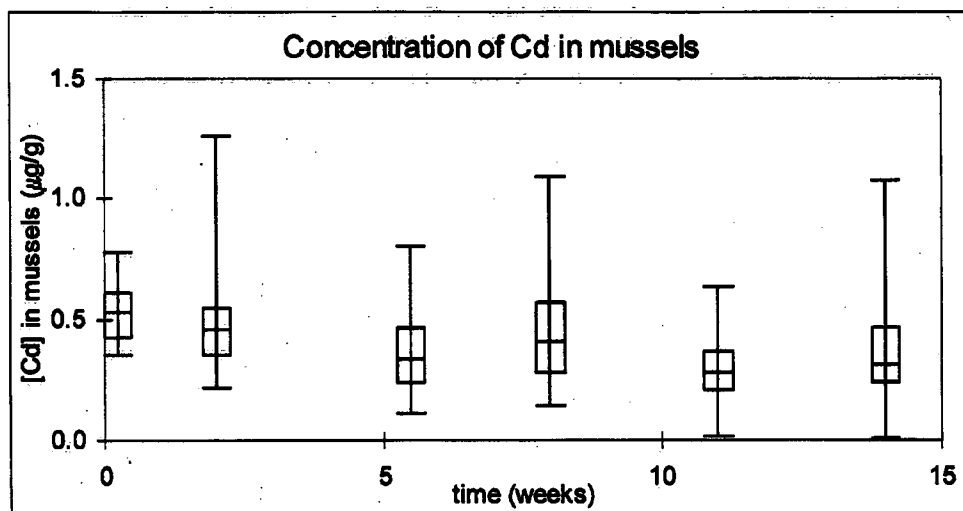


Figure 1. Box and whisker plots of metal concentrations (dry wt) in *E.complanata* in the 3 stormwater ponds assessed in this study (values are presented as the mean value, and the 25th and 50th quartile above and below the mean concentration value)

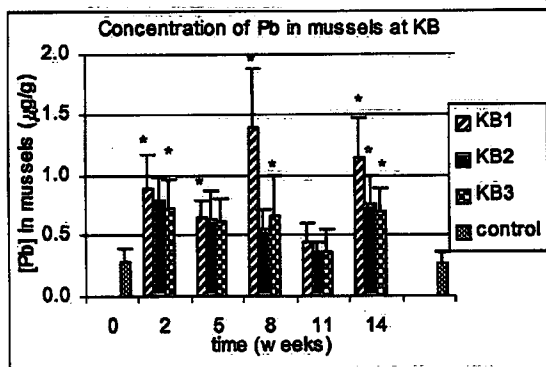
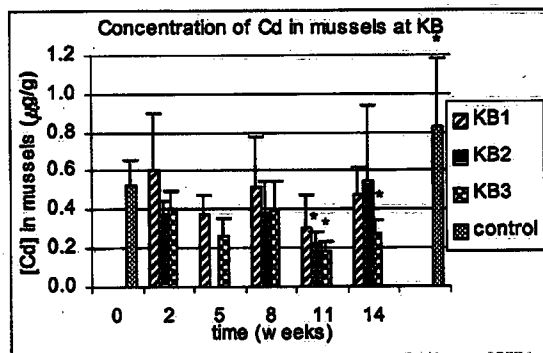
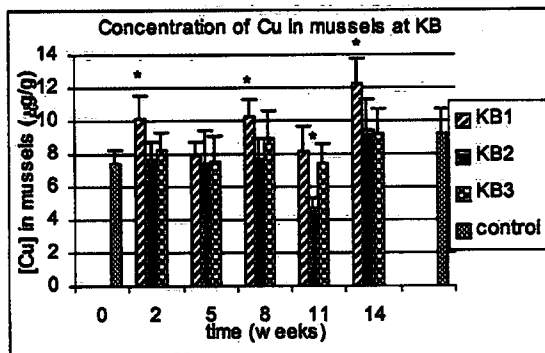
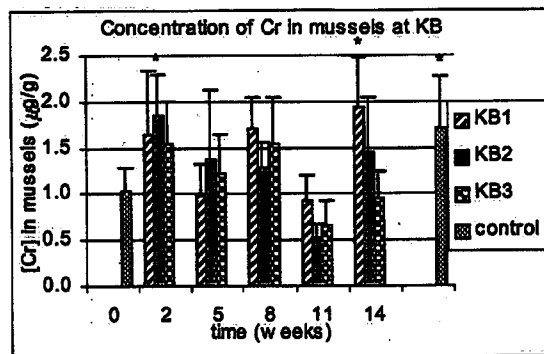
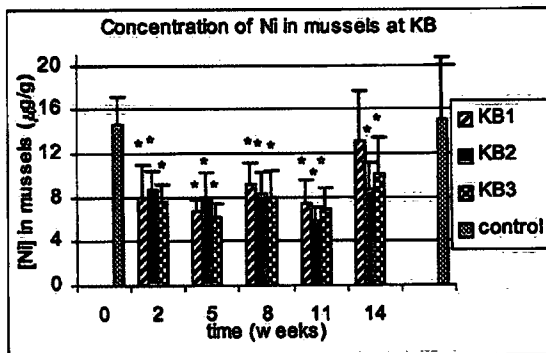


Figure 2. Concentrations of metals in mussel tissue from Kennedy Burnett (mg/g dry wt); * significantly different than Dalhousie Lake at time = 0 (95%)

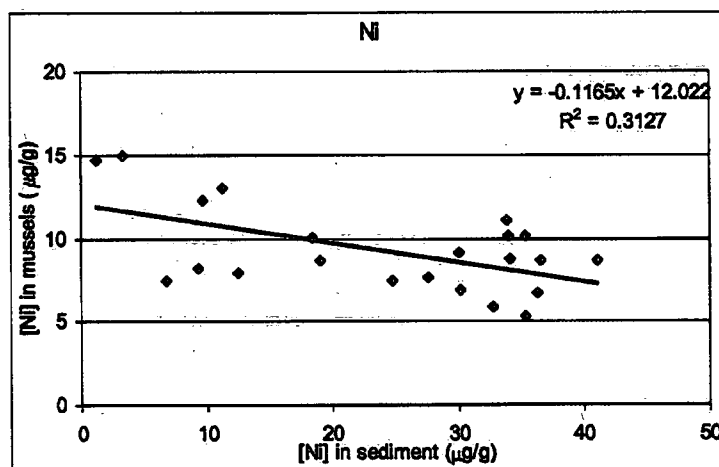
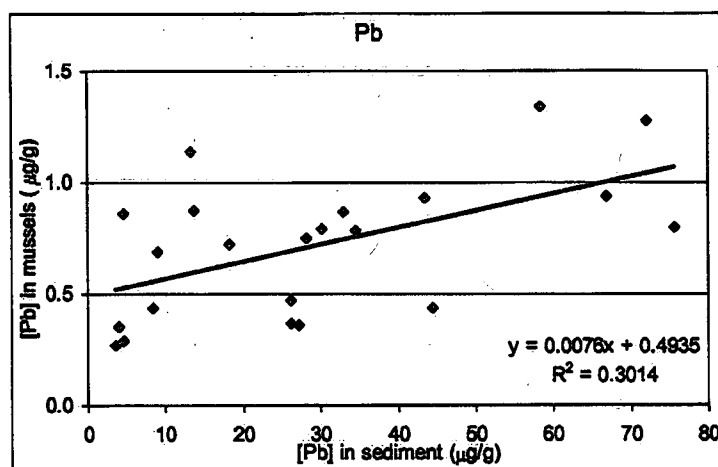


Figure 4 Concentration of a) Ni and b) Pb in mussels at different concentrations of metals in the sediment (total metal concentrations as determined by ICP-AES).

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