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# Investigating the influence of heavy metals on macroinvertebrate assemblages using Partial Canonical Correspondence Analysis (pCCA)\*

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## Abstract

This paper defines the spectrum of impairment to stream macroinvertebrates arising from urban runoff. Field sampling of stream sediments at 62 sites across Yorkshire, UK was used to investigate the influence of heavy metals and habitat on macroinvertebrate family distribution using partial Canonical Correspondence Analysis (pCCA). Increasing urbanization and trafficking was associated with increasing levels of metal pollution but, even when traffic is light, family numbers can be reduced by 50%. Industrial areas and motorway runoff depress macroinvertebrate numbers but drainage from streets with no off-road parking in residential areas can have similar impacts. The heavy metals in the sediment accounted for approximately 24% of the variation in macroinvertebrate community composition while the physical habitat variables used in RIVPACS (River InVertebrate Prediction And Classification System) (Wright, 2000) accounted for an additional 30%. Zinc and nickel were the main metal influences regardless of the time of sampling; at these sites copper is less than critical. Results agree with those reported in other studies in which families mainly from the orders Ephemeroptera (mayfly), Plecoptera (stonefly) and Tricoptera (caddisfly) displayed metal sensitivity in that they were absent from metal polluted streams. However, within each of these orders, a continuum of sensitivity is evident: this highlights the risks of generalising on orders rather than using family or indeed species data.

**Keywords:** macroinvertebrates, heavy metals, urban streams, tolerance, sensitivity

## Introduction

Anthropogenic activities that change any catchment can lead to adverse impacts on receiving waters (Beasley and Kneale, 2002). Metal pollution of sediments resulting from urban runoff exerts a deleterious impact on freshwater macroinvertebrates particularly the loss of metal sensitive orders such as Ephemeroptera (mayfly), Plecoptera (stonefly) and Tricoptera, (caddis) and acute hystopathological impairment and chronic fatality of fish species (Clements *et al.*, 2000; Farag *et al.*, 1999; Hickey and Clements, 1998; Karouna-Renier and Sparling, 2001; Ruse and Hermann, 2000; Sriyaraj and Shutes, 2001).

Generally, increasing urbanization and road construction means that heavy metals derived from non-point sources are likely to cause further impairment of stream ecology but current knowledge of metal contamination is related primarily to point and downstream measurements from known sources (Garcia-Criado *et al.*, 1999; Gower *et al.*, 1994, 1995; Griffith *et al.*, 2001; Nelson and Roline, 1999), from sampling at sites where problems were anticipated (Perdikaki and Mason, 1999) and toxicity assays (Tucker and Burton, 1999). While control measures for point source discharges have improved water quality, streambed sediments remain the major repositories of urban contaminants, the spatial ranges and degrees of which are largely unknown.

Non-point sources of heavy metals in urban and industrial areas arise from a variety of sources. Sansalone and Buchberger (1997) identify vehicle related pollutants from oil and tar products, wear and tear on tyres and brakes,

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*Table 1.* Concentrations of contaminants in runoff from urban areas (Pitt and Barron, 1989, in Novotny, 1995).

HEAVY METAL ( $\mu\text{g l}^{-1}$ )	SOURCE AREAS					
	<i>Parking</i>	<i>Roofs</i>	<i>Storage areas</i>	<i>Street surfaces</i>	<i>Vehicle maintenance areas</i>	<i>Landscaped parks and gardens</i>
Cadmium	0.7 – 70	0.8 – 30	2.4 – 10	0.7 - 220	8 - 30	0.04 – 1
Chromium	18 – 310	7 – 510	60 – 340	3.3 - 30	19 - 320	100 – 250
Copper	20 – 770	17 – 900	30 – 300	15 – 1250	8.3 - 580	80 – 300
Lead	30 – 130	13 – 170	30 – 330	30 - 150	75 - 110	9.4 – 70
Nickel	40 – 130	5 – 70	30 – 90	3 – 70	35 - 70	30 – 130
Zinc	30 – 150	100 – 1580	66 – 290	58 - 130	67 - 130	32 – 1160

dioxins, oxygenated compounds, halogenated phenols, metals, hydrocarbons, de-icing salts and asbestos. The decay of metal and road surfaces adds to the pollution load. Andoh (1994) and Marsalek *et al.* (1999) suggest that road runoff is the principal source of pollution and Pitt and Baron (1989) showed just how variable pollutants in urban surface runoff can be (Table 1). Tyres and brakes are associated with copper and zinc corrosion, lead comes from petroleum additives and emissions, copper and nickel from moving parts in engines, cadmium from galvanized metals, and arsenic, cadmium and copper from weed killers, fertilizers and pesticides. De-icing systems, building materials and metal objects washed by the rain are potential sources for metals in surface runoff.

Management of urban stream chemistry relies on identifying, through laboratory assays, the toxic concentrations that exert acute or chronic impairment and, using field studies, determining urban land uses that generate concentrations exceeding these levels. Research has been focused on the association between heavy metals and vehicles (Andoh, 1994; Marsalek *et al.*, 1999), predominantly on heavily trafficked roads, such as motorways (Shutes, 1984; Maltby *et al.*, 1995a, 1995b). Limitations of both the laboratory and field based investigations have been well documented (LaPoint *et al.*, 1984; Gower *et al.*, 1994, 1995). To overcome deficiencies of these techniques, investigations that model macroinvertebrate assemblages from environmental variables that include 'natural' stress parameters and contaminants have been used (Gower *et al.*, 1994, 1995; Nelson and Roline, 1999; Reinhold-Dudok van Heel and den Besten, 1999; Brown and May, 2000). There is a paucity of research relating macroinvertebrate and environmental contaminants, especially bioavailable sediment metal concentrations. This paper investigates which heavy metals need to be controlled because of their influence on macroinvertebrate community compositions and, by examining stream sediment metal

chemistry at 62 sites with various land uses in west Yorkshire, identifies where control measures in respect of land use should be implemented.

The study used partial Canonical Correspondence Analysis (pCCA) to determine: (1) the importance of streambed bioavailable heavy metal concentrations in determining macroinvertebrate assemblages in comparison with natural habitat characteristic influences; (2) the relative importance of different heavy metals on macroinvertebrate community compositions; and (3) the macroinvertebrate families that are tolerant of and those that are sensitive to metal loading.

## Methodology

This study used sediment samples from 62 first-order streams in Yorkshire, UK (Fig. 1). Sites were selected 25 m above and below surface storm water inflows on rural, residential, industrial and motorway land uses, not just at specific 'probable worst case' sites. Each site was selected after careful inspection of the surface sewer to ensure that no other discharges were present. Some sites receive runoff from multiple land-use types, for example, motorways and road junctions (Table 2). Sites were ordered subjectively based on hypothesised metal contamination taking into account vehicle and infrastructure density in conjunction with stream dimensions. The sites were sampled for sediment metal concentrations and physical environmental characteristics in accordance to RIVPACS protocol (Furse, 2000; Wright, 2000) in May and September 1999 and some were resampled the following year as a spot check for consistency of the 1999 measurements. To minimise the influence of recent surface runoff and to maintain consistency between stations, samples were not taken in the days immediately following a storm. The environmental variables are site altitude, distance from source, stream slope, stream width, stream depth, discharge class, percentage

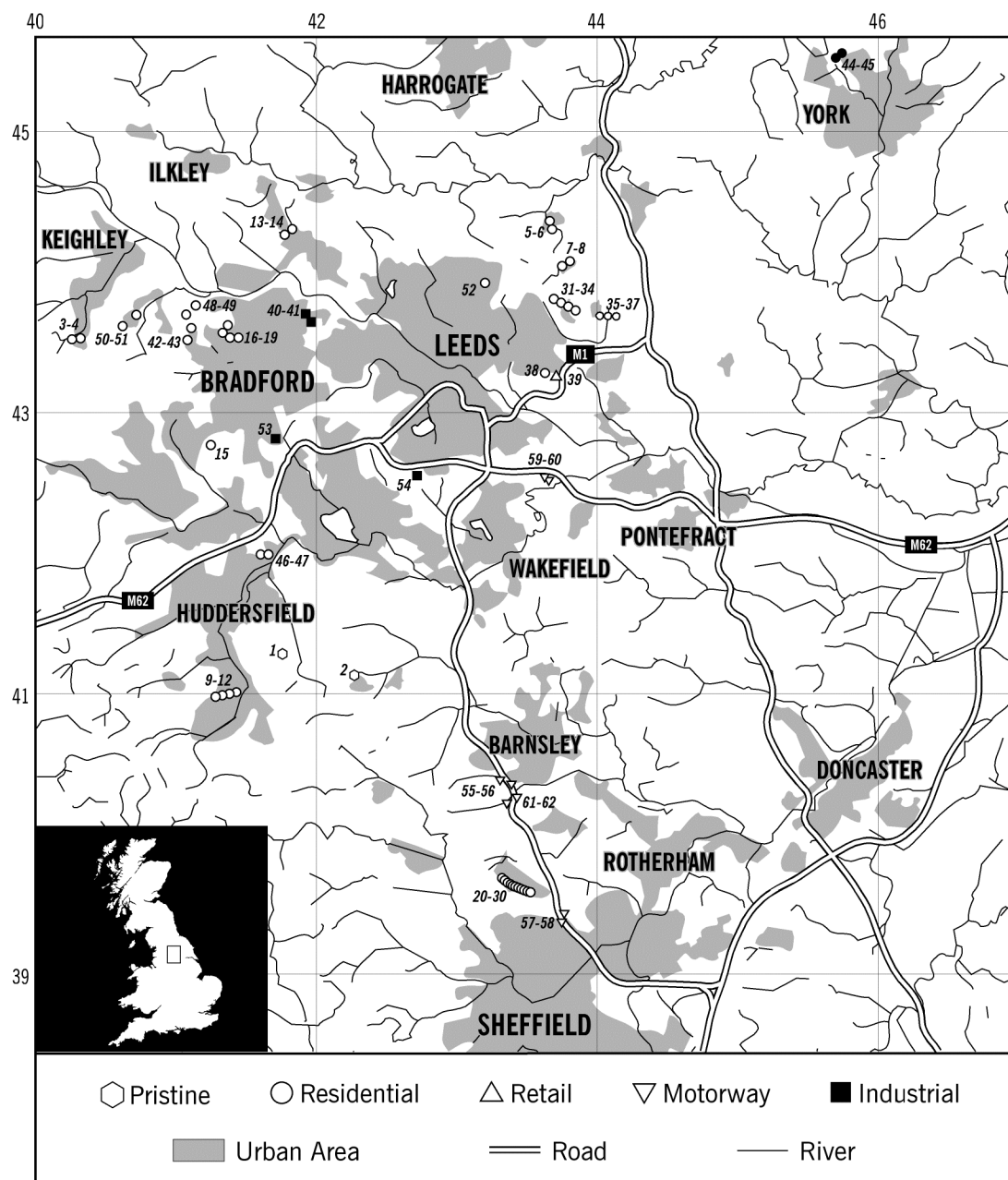


Fig. 1. Location of sampling sites

Table 2. Land use and site data \* = Denotes a Major ‘A class’ Road contributing runoff to the stream

Land use	Site Numbers
Rural, no urban or road influences	A 1, 2
‘Clean’ upstream land use cover	A 3, 5, 7, 9, 13, 17, 20, 31, 42, 44, 48, 50, 55, 57, 61
Residential	○ 4, 6, 8, 10, 11, 12, 14, 16*, 18, 19, 21*, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 43, 46, 47, 49, 51
Retail Park with extensive car park	△ 39
Industrial	■ 15, 40, 41, 45*, 53, 54
Motorway	□ 52, 54, 56, 58, 59, 60, 62

A Pristine, ○ Residential □ Motorway △ Retail, ■ Industrial

particle cover from silt to boulders, dissolved oxygen, electrical conductivity and pH. The streambed sediment chemistry and macroinvertebrate numbers did not vary significantly or consistently between sampling dates.

#### STREAMBED SEDIMENT COLLECTION

Random triplicate sediment samples were collected from three distinct microhabitats within each sampling reach and integrated to a composite sample of approximately 1 kg. The bed material was inspected over a distance of 7 metres and samples were taken from different points to get a representative mix of material sizes, from both protected and more open stream sites. This sampling strategy overcame the heterogeneity of heavy metal concentrations while simultaneously reducing sampling variance (Argyrazi *et al.*, 1995). Heterogeneity of the sample was of more concern in the horizontal plane as pilot studies showed that, in these headwater streams, the macroinvertebrates are rarely found below a depth of 5 cm; indeed in some sections the bedrock is even closer to the surface. Sediment was collected from the near-surface, (0–5 cm) using a plastic trowel that produced a sample with minimal streambed disturbance, low risk of contamination and minimal loss of the finest particles that generally possess the highest metal concentrations. Rinsing the trowel within the flow downstream of each sampling point ensured quality control and the prevention of cross contamination. The sediment was placed in an airtight zip sealed polyethylene bag and double bagged to safeguard against cross contamination.

#### MACROINVERTEBRATE COLLECTION

Three one-minute kick samples were collected from approximately 3 m<sup>2</sup> of streambed to a depth of 5 cm, with the net (25 cm × 20 cm × 30 cm with 1 mm mesh) held vertically and the frame at right angles to the current. To avoid cross contamination between sampling stations in close proximity, sampling proceeded from downstream to upstream sites. Sampling three microhabitats maximized the chance of collecting a more complete assemblage. Samples were decanted into 1100 ml polypropylene bottles with just enough water to keep the sample damp so as to reduce damage and retard the activities of carnivores during transportation.

#### LABORATORY ANALYSIS

##### *Heavy metals*

The sediment was oven dried at 30°C for five days and then laid out in a dust free laboratory for a further seven days before representative sub-samples were taken using coning

and quartering. In preparation for sieving, each sub-sample was ground lightly using an agate mortar and pestle. Each sub-sample was passed through a 2000µm synthetic nylon polymer woven screen to retain only the sand and silt sized particles. After each screening, the nylon was rinsed thoroughly in deionised water. Analytical-grade (AnalaR) acids were used for all extraction solutions and cleaning procedures to minimize metal contamination.

Bioavailable metal concentrations (Exchangeable and bound to carbonates) were determined using the Standard, Methods and Testing (SM&T) three step sequential extraction technique reported by Quevauvillier *et al.* (1997). Metal content of the streambed sediment following extraction was determined using Inductively Coupled Plasma Optical Emission Spectrometry (IAP-AES). The metals sought in these analyses were cadmium, chromium, copper, iron, lead, nickel and zinc. Beasley (2001) details ANOVA (Analysis of Variance) evaluations of relationships between particle size, extraction levels and heavy metals.

##### *Macroinvertebrates*

Macroinvertebrates were preserved within five hours of collection using 95 percent ethanol and were sorted within a month, following the recommended standard procedure for RIVPACS (River InVertebrate Prediction and Classification System)(Environment Agency, 1997). The samples were first placed in large white trays to ease sorting and specimens were placed in petri dishes for identification to family level. Examples of each taxon were put in vials containing ethanol for quality assurance checking with Environment Agency (EA) scientists. The abundances of each taxon were recorded using EA audit sheets.

#### STATISTICAL ANALYSIS

To determine the relative importance of environmental factors (metals and habitat) in explaining the variability in composition of the macroinvertebrate community, partial canonical correspondence analysis (pCCA) was used (ter Braak, 1987, 1994). Prior to analysis, the set of explanatory variables was subdivided into a set of covariables and a set of variables-of-interest. The covariables, which in this instance represent habitat variables, are not the prime focus of the research and as such did not enter the synthetic gradients (ter Braak and Verdonschot, 1995). The variables-of-interest, the concentrations of heavy metals in the streambed sediments, are the remaining explanatory variables that construct the synthetic gradients. The analysis follows that of CCA but with the added requirement that each synthetic gradient must be uncorrelated with the covariables. Consequently, the

covariables represent gradients that have already been extracted. The resultant ordination diagram displays the unimodal relationships between macroinvertebrates and the variables-of-interest after the effects of the covariables have been partialled out (ter Braak, 1996).

The analysis used the programme CANOCO 4.0. All data sets were first converted into a CANOCO 4.0 format using the utility programme CanoImp. Macroinvertebrate families (response variables), environmental (predictors) and covariable (concomitant variables) data were selected for analysis using direct gradient analysis. While dissolved oxygen, electrical conductivity and the pH of the water column can vary with local conditions and macroinvertebrates respond to events at other times in the season, the consistency in values between May and September and the decision not to sample during and in the days following rain offers confidence that these data are valid. The data files inputted to each pCCA run are shown in Table 3. Run 1 uses the May, run 2 the September and run 3 the combined 1999 data set.

The unimodal response model was selected for this study because macroinvertebrates exhibit maximum abundance around optimal conditions and because the macroinvertebrate family data files contained a large number of zero values (ter Braak and Smilauer, 1998). PCCA was used in this research to investigate the effects of the streambed contaminants while acknowledging the importance of the environmental data. Scaling focused upon inter-family distances as interpretation among families was the aim of the analysis using biplot scaling. All family abundance values were log transformed to avoid undue influence of outliers on the ordination. Downweighting for rare families was not adopted.

Each run was completed by removing variables with high inflation factors, which permitted the ranking of environmental variables in the order of their importance for

determining the macroinvertebrate families data using the 'forward selection' option in CANOCO. Forward selection was also used to reduce to 10 the number of environmental variables to improve the clarity of the ordination diagrams. In 'automatic selection', the K best variables are selected sequentially on the basis of maximum extra fit. The statistical significance of each variable selected is judged by a Monte-Carlo permutation test.

The data are first displayed as rankings (Tables 4-6) and explored further using ordination diagrams (Figs. 2-7). In Tables 4-6, the top ten ranked environmental variables are presented in terms of importance in explaining community composition. These rankings and their statistical significance indicate which of the elements exerts the greatest influence on macroinvertebrate community structures. These data are then plotted as ordination diagrams using the programme CanoDraw 3.1, graphically representing the community structure and the community response to the environmental

*Table 4.* Top 10 rankings for heavy metal concentrations in May (extract 1) using unrestricted Monte Carlo significance test ( $p < 0.05$ ).

<i>Variable</i>	<i>Lambda-A</i>	<i>F</i>	<i>p</i>
Zinc	0.10	3.31	<u>0.005*</u>
Dissolved Oxygen	0.07	2.34	<u>0.005*</u>
Electrical conductivity	0.05	1.76	<u>0.020*</u>
Nickel	0.05	1.85	<u>0.025*</u>
Lead	0.05	1.94	<u>0.010*</u>
pH	0.03	1.01	0.415
Iron	0.04	1.19	0.295
Copper	0.02	0.91	0.470
Chromium	0.02	0.64	0.900
Cadmium	0.02	0.61	0.885

*Table 3.* Details of each pCCA model run.

<i>Model runs</i>	<i>Data files</i>	<i>Constituents</i>
pCCA Run 1	MACMAY METMAY COMAY	Macroinvertebrate Abundances (May) Metals & Water Chemistry Concentrations (May) Covariables (May)
pCCA Run 2	MACSEP METSEP COSEP	Macroinvertebrate Abundances (September) Metals & Water Chemistry Concentrations (September) Covariables (September)
pCCA Run 3	MACMIX METMIX COMIX	Macroinvertebrate Abundances (May & Sept) Metals & Water Chemistry Concentrations (May & Sept) Covariables (May & Sept)

**Table 5.** Top 10 rankings for heavy metal concentrations in September (extract 1) using unrestricted Monte Carlo significance test ( $p < 0.05$ ).

Variable	Lambda-A	F	p
Nickel	0.08	2.01	0.020*
Zinc	0.06	1.50	0.045*
pH	0.06	1.31	0.115
Lead	0.04	1.05	0.410
Copper	0.04	0.91	0.515
Electrical Conductivity	0.03	0.92	0.555
Dissolved Oxygen	0.04	1.00	0.470
Cadmium	0.05	1.20	0.260
Chromium	0.03	0.66	0.900
Iron	0.02	0.53	0.945

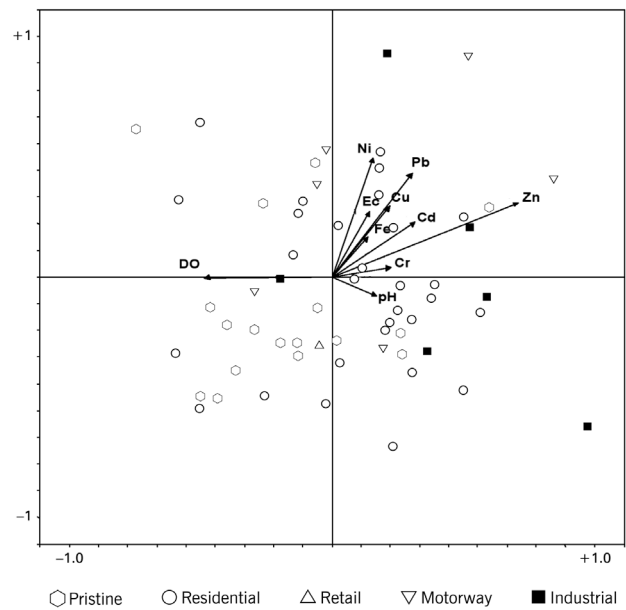
**Table 6.** Top 10 rankings for heavy metal concentrations (extract 1) using unrestricted Monte Carlo significance tests ( $p < 0.05$ ). Data from May and September.

Variable	Lambda-A	F	p
Zinc	0.10	3.36	0.005*
Nickel	0.09	3.14	0.005*
Electrical Conductivity	0.05	1.54	0.060
pH	0.04	1.45	0.075
Iron	0.03	1.26	0.180
Lead	0.04	1.17	0.280
Copper	0.03	1.02	0.410
Dissolved Oxygen	0.02	0.82	0.695
Chromium	0.02	0.68	0.810
Cadmium	0.01	0.53	0.965

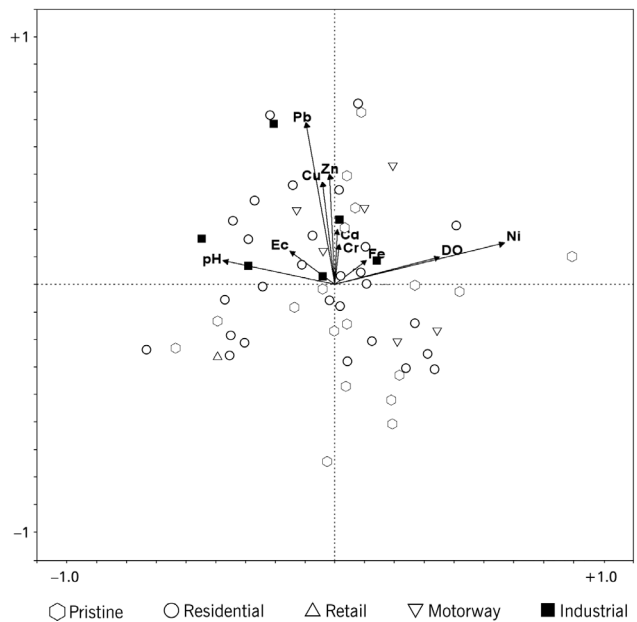
variables. Modifications to the ordination diagrams in terms of improvement in clarity were made using the programme CanoPost 1.0. Interpretation of the ordination diagrams facilitates the ranking of sites in terms of community composition in relation to each element and of macroinvertebrate families with respect to tolerance and sensitivity to each element.

## Results

Tables 4 to 6 show that despite changes in rank order between data sets, zinc and nickel exert a significant influence on the composition of macroinvertebrate communities in the study streams. For each pCCA run containing trace metals, zinc and nickel are ranked highly



**Fig. 2.** Site - environment biplot based on pCCA run 1.



**Fig. 3.** Site - environment biplot based on pCCA run 2.

on three occasions confirming that these metals are widely distributed in urban runoff. Moreover, at the concentrations recorded in this study, these results show that zinc is the major heavy metal determinant of community composition in these streams. The only other metal to exert a significant influence on community structure is lead in May (pCCA run 1 Table 4).

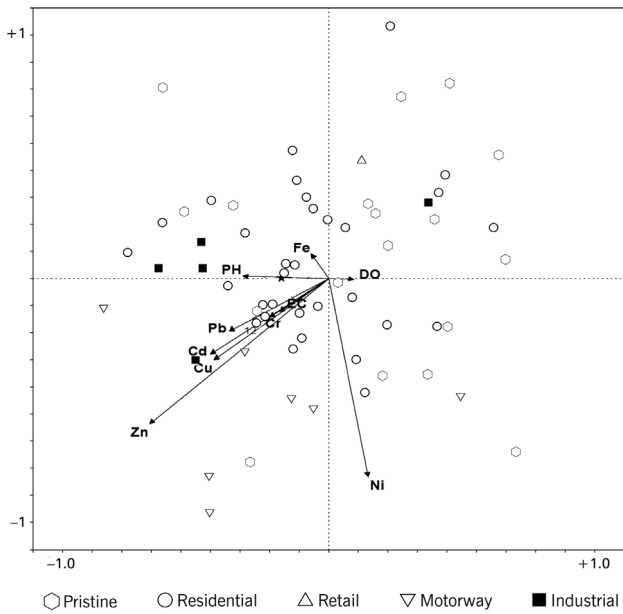


Fig. 4. Site - environmental biplot based on pCCA run 3.

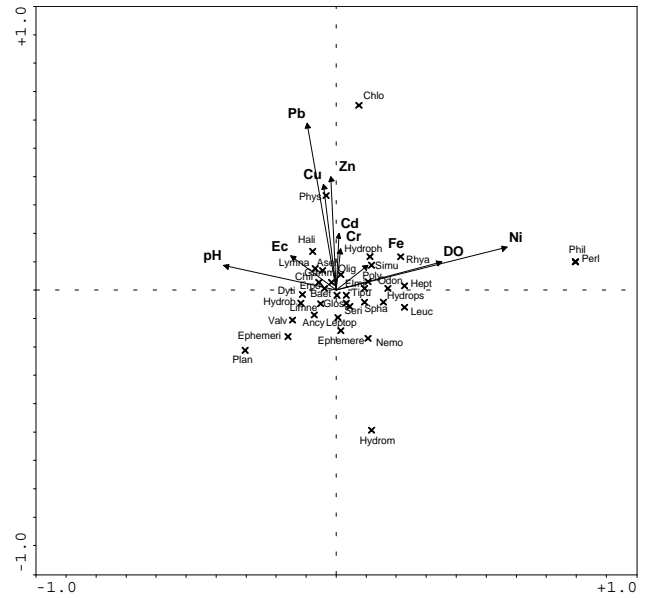


Fig. 6. Macroinvertebrate families - environment biplot based on pCCA run 2. Macroinvertebrate families are shown as crosses.

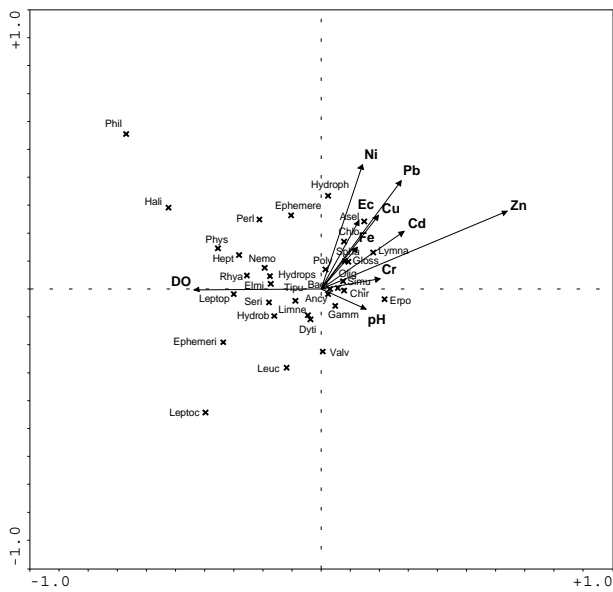


Fig. 5. Macroinvertebrate families - environment biplot based on pCCA run 1. Macroinvertebrate families (see Table 10) are shown as crosses.

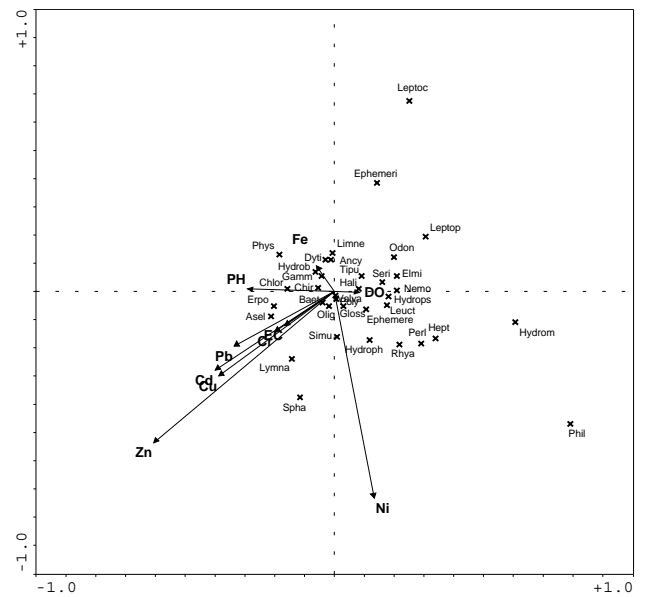


Fig. 7. Macroinvertebrate families - environmental biplot based on pCCA run 3. Macroinvertebrate families are shown as crosses.

ORDINATION DIAGRAMS

Further detail can be extracted by inspecting the pCCA ordination diagrams. In each, site and macroinvertebrate family data are represented by points, environmental variables by arrows. The ordination diagrams display, simultaneously, the main patterns of community variations,

in so far as these reflect environmental variation and the main pattern of the tolerances of macroinvertebrate families with respect to the environmental variables. In Figs. 2–4, each site is located with respect to the sediment chemistry and site characteristic variables. In Figs. 5–7, macroinvertebrate family points correspond to their



approximate optima in the two-dimensional environmental subspace based on their weighted average, which indicates the centre of a macroinvertebrate family distribution along an environmental variable (ter Braak and Looman, 1986; ter Braak, 1986; ter Braak and Prentice, 1988). Differences in weighted averages between macroinvertebrate families indicate differences in their tolerances along that environmental variable. Environmental variables are represented by arrows, which point in the direction of maximum change of that variable across the ordination diagram. The length of the arrows is proportional to the rate of change in this direction. Environmental variables with long arrows display a stronger correlation with the ordination axes than those with short arrows, as signified by the co-ordinates of the arrow head. Environmental variables that are strongly correlated with the ordination axes are more closely related to the pattern of community variation shown in the ordination diagram (ter Braak, 1987). The rule for quantitative interpretation is that each arrow representing an environmental variable determines a direction or axis in the diagram on to which sites and macroinvertebrate family points are projected. Sites or macroinvertebrate families with their perpendicular projection endpoints near to or beyond the tip of an arrow will be strongly positively correlated with and influenced by the environmental variable represented by that arrow. Those sites or macroinvertebrate family whose projections lie near the origin will be less strongly affected.

SITE-ENVIRONMENT ORDINATION DIAGRAMS

Given the broad within and between site variability in environmental conditions both temporally and spatially, it would be surprising if the pCCA results were clear-cut. Figures 2 to 4 show the results for the runs of sites in relation to metal and environmental variables and no clear site grouping along either ordination axis is evident. This is consistent with the findings of Gower *et al.* (1995), who examined the impact of mine drainage on macroinvertebrate assemblages by comparing clean control sites with contaminated mine sites; as there were no intermediate sites, clear site groupings could be distinguished. However, in the present study, the temporal and graduated spatial variations in environmental conditions are very different from Gower's.

The strong influence of nickel and zinc can be seen visually on the metal ordination diagrams with their arrow head co-ordinates signifying strong correlation with either ordination axis 1 or 2 (Figs. 2-4).

In the current research, the data fall along a continuum with a wide range of contamination levels arising from

diverse sources, land usage and variations in catchment characteristics and history. The plots show definite trends relating land use to environmental variables and macroinvertebrate family composition. Figures 2 and 4 show that metal tolerant communities are associated predominantly with streams receiving runoff from motorway or industrial land uses. This is reflected in the top five rankings in Tables 7 and 9. The strong relationships with zinc and nickel suggest that these are major determinants of community composition in these streams (Tables 4 and 6). Sites in the lower left and upper right of Figs. 2 and 4, respectively, possess communities sensitive to metal contamination. Hence, these sites are either clean reference sites or low-density suburban sites (Tables 7 and 9).

In Tables 7, 8 and 9, the sites with tolerant macroinvertebrate communities include the sites below the industrial and motorway runoff points but these do not totally dominate the data. Suburban residential sites are also represented, particularly suburban terraces or council housing where there is no off-street parking. Although road traffic is light, the overnight parking of vehicles on both sides of the road is associated with enhanced metal levels in the sediments. Drainage from the carriageway in a rural area below a four-way junction with traffic lights, where heavy traffic is braking sharply or accelerating to get uphill, has also enhanced metal levels as has a site which drains a lay-by used by lorries. In contrast, the sites with the broadest macroinvertebrate communities and the more sensitive indicator species are typically rural and residential areas where each property includes a garage so that on-street parking is rare.

Figure 3 shows a slightly different ordination of sites for

Table 7. Top and bottom 5 ranked sites (left to right) in terms of community composition for pCCA run 1 (May data).

Ranked variable	Top 5 sites : tolerant communities	Bottom 5 sites : sensitive communities
Zinc	□ □ ■ A ■	○ ○ A A A
Dissolved Oxygen	■ □ A ■ ○	A ○ ○ ○ ○
Electrical Conductivity	□ ■ □ ○ A	○ A A ○ ○
Nickel	□ ■ □ ○ □	○ A A ○ ○
Lead	□ ■ □ A ○	○ A A ○ ○
pH	■ □ ○ ○ ■	A ○ ○ ○ A
Iron	□ □ ■ A ○	○ A A ○ ○
Copper	□ □ ■ A ○	○ A A ○ ○
Chromium	□ ■ □ A ■	○ A ○ A A
Cadmium	□ □ ■ A ○	○ A ○ A A

A Pristine, ○ Residential □ Motorway △ Retail, ■ Industrial

**Table 8.** Top and bottom 5 ranked sites (left to right) in terms of community composition for pCCA run 2 (September data).

Ranked variable	Top 5 sites : tolerant communities	Bottom 5 sites : sensitive communities
Nickel	A ○ A ■ ■	○ A △ ○ A
Zinc	○ A ○ ■ ■	A A A A A
pH	○ A ■ ○ A	A A ○ ○ ■
Lead	○ ○ ■ A ■	A A A A ○
Copper	○ A ○ ■ ■	A A A A ○
Electrical Conductivity	○ ■ ○ ■ ○	A ○ A ○ A
Dissolved Oxygen	○ A △ A ○	A ○ A ■ ■
Cadmium	○ A ○ ■ ■	A A A A A
Chromium	○ A ○ ■ ■	A A A A ○
Iron	A ○ ○ A ■	○ A △ ○ A

A Pristine, ○ Residential ■ Motorway △ Retail, ■ Industrial

**Table 9.** Top and bottom 5 ranked sites (left to right) in terms of community composition for pCCA run 3 (all data).

Ranked variable	Top 5 sites : tolerant communities	Bottom 5 sites : sensitive communities
Zinc	■ ■ A ■ ■	A ○ A A ○
Nickel	■ A ■ A A	○ ○ A A ○
Electrical Conductivity	■ ■ ■ A ■	○ A A A ○
pH	■ ○ ○ ○ ■	A A A ○ A
Iron	○ ○ ○ A ○	A A ■ ■ A
Lead	■ ■ ■ ■ A	A A ○ A ○
Copper	■ ■ ■ A ■	A A ○ A ○
Dissolved Oxygen	■ ○ ○ ○ ■	A A A ○ A
Chromium	■ ■ ■ A ■	○ A A A ○
Cadmium	■ ■ ■ A ■	A A ○ ○ A

A Pristine, ○ Residential ■ Motorway △ Retail, ■ Industrial

September in relation to the environmental variables; these reflect seasonal changes in family composition and environmental conditions at the sites. Communities in sites at the top of the diagram are metal tolerant whilst those at the bottom are metal sensitive. When individual site locations are inspected, the ‘changes in position’ are generally small, except downstream from motorway sites (Table 8) where the community composition in September is less tolerant of heavy metals than in May. Nevertheless, the broad grouping of sites having tolerant or sensitive communities does not change with season; the family patterns observed remain broadly stable from spring to autumn although the total numbers of individuals are reduced in September (Beasley, 2001).

The influence of zinc and nickel remains high as shown in Tables 3–5. The communities at sites with low metal concentrations plot consistently with those in Figs. 2 and 4 confirming that differences between seasons are small.

Each pCCA run produces slightly different relationships within the ordination diagrams but a common pattern emerges. Pollution indicator families are identified from the ordination diagrams for each contaminant and the five most tolerant and sensitive families is presented for each (Tables 11-13). Figure 5 plots metal and acid tolerant invertebrates in the upper right section of the plot, with metal sensitive families in the lower left section. Although the rank order differs slightly between metals, many of the same families appear in each set of rankings. The families most frequently identified as tolerant are *Hydrophilidae*, *Asellidae*, *Ephemerellidae*, *Philopotamidae* and *Chloroperlidae* while the families identified as metal sensitive, *Leptophlebiidae*, *Ephemeridae*, *Leuctridae*, *Hydrobiidae* and *Valvatidae* are generally absent from streams with elevated concentrations of one or more of the metals. These results are in agreement with those found in other studies in which families from the orders Ephemeroptera, Trichoptera and Plecoptera were

**Table 10.** Abbreviations of macroinvertebrate family names used in Tables 11-13 and Figs. 5-7.

Ancy	<i>Ancylidae</i>	Hali	<i>Haliplidae</i>	Odon	<i>Odontoceridae</i>
Asel	<i>Asellidae</i>	Hept	<i>Heptageniidae</i>	Olig	<i>Oligochaeta</i>
Beat	<i>Beatidae</i>	Hydrob	<i>Hydrobiidae</i>	Perl	<i>Perlodidae</i>
Chir	<i>Chironomidae</i>	Hydrom	<i>Hydrometridae</i>	Phil	<i>Philopotamidae</i>
Chlo	<i>Chloroperlidae</i>	Hydroph	<i>Hydrophilidae</i>	Phys	<i>Physidae</i>
Dyti	<i>Dytiscidae</i>	Hydrops	<i>Hydropsychidae</i>	Plan	<i>Planorbidae</i>
Elmi	<i>Elmidae</i>	Leptoc	<i>Leptoceridae</i>	Poly	<i>Polycentropodidae</i>
Ephemere	<i>Ephemerellidae</i>	Leptop	<i>Leptophlebiidae</i>	Rhya	<i>Rhyacophilidae</i>
Ephemer	<i>Ephemeridae</i>	Leuc	<i>Leuctridae</i>	Simu	<i>Simuliidae</i>
Erpo	<i>Erpobdellidae</i>	Limne	<i>Limnephilidae</i>	Spha	<i>Sphaeriidae</i>
Gamm	<i>Gammaridae</i>	Lymna	<i>Lymnaeidae</i>	Tipu	<i>Tipulidae</i>
Gloss	<i>Glossiphonidae</i>	Nemo	<i>Nemouridae</i>	Valv	<i>Valvatidae</i>

Table 11. Top and bottom 5 ranked weighted averages of families in relation to the top 10 ranked environmental variables for run 1 (May data).

<i>Variable</i>	<i>Five most tolerant families</i>	<i>Five most sensitive families</i>
Zinc	Asel, Lymna, Erop, Hydroph, Chlo	Leptoc, Phil, Hali, Ephemer, Leptop
Dissolved Oxygen	Erpo, Lymna, Asel, Gloss, Spha	Phil, Hali, Leptoc, Phys, Ephemeri
Electrical Conductivity	Hydroph, Asel, Ephemere, Lymna, Chlo	Leptoc, Ephemeri, Leuc, Valv, Hydrob
Nickel	Phil, Hydroph, Asel, Ephemere, Chlo	Leptoc, Leuc, Ephemeri, Valv, Hydrob
Lead	Asel, Hydroph, Lymna, Chlo, Ephemere	Leptoc, Ephemeri, Leuc, Leptop, Hydrob
pH	Erpo, Lymna, Valva, Chir, Gamm	Phil, Hali, Phys, Hept, Perl
Iron	Asel, Hydroph, Lymna, Chlo, Gloss	Leptoc, Ephemeri, Leuc, Leptop, Hydrob
Copper	Hydroph, Asel, Lymna, Chlo, Ephemere	Leptoc, Ephemeri, Leuc, Leptop, Hydrob
Chromium	Erpo, Lymna, Asel, Gloss, Chlo	Phil, Leptoc, Hali, Ephemeri, Phys
Cadmium	Asel, Lymna, Hydroph, Chlo, Erpo	Leptoc, Phil, Ephemeri, Leptop, Phys

Table 12. Top and bottom 5 ranked weighted averages for families in relation to the top 10 ranked environmental variables for run 2 (September data).

<i>Variables</i>	<i>Five most tolerant families</i>	<i>Five most sensitive families</i>
Nickel	Phil, Perl, Chlo, Rhya, Hept	Plan, Ephemeri, Valv, Hydrob, Dyti
Zinc	Chlo, Phys, Hali, Hydroph, Rhya	Hydrom, Plan, Nemo, Ephemeri, Ephemere
pH	Plan, Ephemeri, Valv, Phys, Hali	Perl, Phil, Hydrom, Leuc, Hept
Lead	Chlo, Phys, Hali, Hydroph, Rhya	Hydrom, Nemo, Plan, Ephemeri, Ephemere
Copper	Chlo, Phys, Hali, Hydroph, Rhya	Hydrom, Plan, Nemo, Ephemeri, Ephemere
Electrical Conductivity	Chlo, Phys, Hali, Plan, Lymna	Perl, Phil, Hydrom, Nemo, Leuc
Dissolved Oxygen	Plan, Ephemeri, Valv, Hydrob, Dyti	Plan, Peri, Chlo, Rhya, Hept
Cadmium	Chlo, Phys, Hali, Rhya, Hydroph	Hydrom, Plan, Ephemeri, Nemo, Ephemere
Chromium	Chlo, Phys, Rhya, Hydroph, Hali	Hydrom, Plan, Ephemeri, Nemo, Ephemere
Iron	Phil, Perl, Chlo, Rhya, Hydroph	Plan, Ephemeri, Hydrom, Valv, Limne

Table 13. Top and bottom 5 ranked weighted averages for families in relation to the top 10 ranked environmental variables for run 3 (all data).

<i>Variables</i>	<i>Five most tolerant families</i>	<i>Five most sensitive families</i>
Zinc	Spha, Lymna, Asel, Erpo, Simu	Leptoc, Hydrom, Ephemeri, Leptop, Odon
Nickel	Phil, Spha, Perl, Hydrom, Hept	Leptoc, Ephemeri, Phys, Leptop, Limne
Electrical Conductivity	Spha, Lymna, Asel, Erpo, Simu	Leptoc, Hydrom, Ephemeri, Leptop, Phil
PH	Asel, Erpo, Phys, Chlo, Lymna	Phil, Hydrom, Hept, Leptop, Perl
Iron	Leptoc, Ephemeri, Phys, Limne, Dyti	Phil, Hydrom, Hept, Perl, Rhya
Lead	Spha, Lymna, Asel, Erpo, Chlo	Phil, Leptoc, Hydrom, Leptop, Ephemeri
Copper	Spha, Lymna, Asel, Erpo, Simu	Leptoc, Hydrom, Phil, Leptop, Ephemeri
Dissolved Oxygen	Phil, Hydrom, Hept, Perl, Leptop	Phil, Hydrom, Hept, Leptop, Perl
Chromium	Spha, Lymna, Asel, Erpo, Simu	Leptoc, Hydrom, Phil, Leptop, Ephemeri
Cadmium	Spha, Lymna, Asel, Erpo, Simu	Leptoc, Hydrom, Ephemeri, Leptop, Phil

absent from metal polluted streams. (Whiting and Clifford, 1983; Casper, 1994; Gower *et al.*, 1994, 1995)

In Fig. 6, metal tolerant families are located in the upper half and metal sensitive in the lower half of the diagram. In Fig. 7 tolerant families and sensitive families are located in the lower left and upper right sections respectively. The metal sensitive families are largely the same as those identified in May. However, the families tolerant in September and in the combined data set (May and September) differ somewhat, (Tables 11 and 12) perhaps because of differences in the life cycle between families, when abundance varies naturally between seasons. As with community composition, inspection of the three family ordination diagrams for heavy metals shows that the same families are plotted in roughly the same ordination space in relation to the heavy metal variables. From these plots and the rankings, several overall indicator families of metal tolerance emerge, notably, *Aselidae*, *Ephemerellidae*, *Hydrophilidae*, *Lymnaeidae*, *Sphaeridae*, *Physidae*, *Haliplidae*, *Rhyacophilidae*, *Erbobdellidae* and *Simuliidae*. Similarly, despite the fact that rankings change slightly between runs and between metals, *Leptoceridae*, *Ephemeridae*, *Leuctridae*, *Valvatidae*, *Hydrobiidae*, *Hydrometridae*, *Planorbidae*, *Nemouridae*, *Leptophlebiidae* and *Odontoceridae* are usually absent from streams with elevated concentrations of one or more of the heavy metals.

## Discussion

The results of the pCCA show that heavy metals account for approximately 24% of the variation in macroinvertebrate community composition while the physical variables in RIVPACS account for a further 30%. Of course, both biotic and abiotic factors affect the distribution of macroinvertebrates in streams and some 50% of the overall variation is unexplained. Many possible reasons have been explored elsewhere (Whiting and Clifford, 1983; Maltby *et al.*, 1995a); they include altered substrate composition caused by an influx of fine particulates, altered flow regime as a consequence of bridges and channelisation, changes in food availability, differences in surrounding terrestrial habitats and additional contaminants such as PAHs (polycyclic aromatic hydrocarbons) from vehicle emissions and other sources (Beasley and Kneale, 2002).

On the relative importance of different heavy metals and habitat on macroinvertebrate community compositions, it is often difficult to know which of the metal elements has the greatest effects on specific macroinvertebrates since they often occur in high concentrations simultaneously. However, pCCA was used, successfully, to identify zinc and nickel as the main metal influences regardless of the time of sampling.

This must be compared with the findings of Armitage (1980) and Malmqvist and Hoffsten (1999) that copper and zinc had the strongest negative effects on taxonomic richness. Indeed, Armitage (1980) concluded that Ephemeroptera species were particularly sensitive to zinc because they were restricted to sites with zinc concentrations below  $300 \mu\text{m l}^{-1}$ . Copper has been blamed for changes in community structure in streams elsewhere (Gower *et al.*, 1994; Nimmo *et al.*, 1996) but, in the present research, copper exerted no significant influence on community composition. It may be that, in these bed sediments, bioavailable concentrations of copper are generally below their toxic threshold.

In identifying the macroinvertebrate families that are tolerant and sensitive to metal loading, the pCCA analyses demonstrate that Ephemeroptera families are particularly sensitive to elevated metal levels; this agrees with the results of Kiffney and Clements (1994), Gower *et al.* (1994, 1995), Schultheis *et al.* (1997), Malmqvist and Hoffsten (1999) and Clements *et al.* (2000). The present research found *Leptoceridae* (caddis flies) and *Ephemerellidae* (mayflies) particularly sensitive because of their absence from streams with moderate metal contamination. The widespread distribution of *Baetidae* (mayflies) and their central position in the families-environment ordination diagrams (Figs. 5–7) supports the findings of Gower *et al.* (1994, 1995) that they have moderate metal tolerance. Similarly, results from the pCCA analysis show that the tolerance of Plecoptera families (stoneflies) varies; *Leuctridae* are slightly more tolerant than *Nemouridae*, and *Chloroperlidae* appear to be particularly tolerant to metals. Metal tolerance of Tricoptera families (caddis flies) is also low, especially *Polycentropodidae*, *Rhyacophilidae*, and *Hydropsychidae*; the latter two families prefer high dissolved oxygen conditions (Figs. 5–7). More sensitive are the cased caddis, *Limnephilidae* and *Sericostomatidae*. *Asselidae* (waterlice) are identified in the present research as being tolerant of some heavy metals as has been found by Brown (1976) and Gower *et al.* (1994). However, within each family, tolerances vary between species, illustrating the importance of species identification.

## Conclusions

pCCA analysis has been shown to be capable of discriminating, usefully, between sites and family relationships with metal pollution loading. As anticipated, there is a loss in family numbers as land use changes from rural to suburban to urban. The spatial distribution of the sites and the diversity of land uses demonstrates the value of this type of monitoring for forecasting and for indicating

streams where the ecology is potentially particularly vulnerable to metal-rich runoff. Adding parallel information for other surface runoff contaminants (Sansalone and Buchberger, 1997) such as petrol, oil and tar products, dioxins, oxygenated compounds, halogenated phenols, hydrocarbons, de-icing salts and asbestos is likely to improve the level of explanation in the model. Detailed modelling of such sources and compounds offers a significant arena for future research.

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