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# USE OF PHYSICAL, CHEMICAL, AND BIOLOGICAL INDICES TO ASSESS IMPACTS OF CONTAMINANTS AND PHYSICAL HABITAT ALTERATION IN URBAN STREAMS

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**Abstract**—Human activities in urban areas can lead to both chemical pollution and physical alteration of stream habitats. The evaluation of ecological impacts on urban streams can be problematic where both types of degradation occur. Effects of contaminants, for example, may be masked if stream channelization, loss of riparian vegetation, or other physical stressors exert comparable or larger influences. In the Aberjona watershed (near Boston, MA, USA), we used physical, chemical, and biological indices to discern the relative impacts of physical and chemical stressors. We used standard protocols for assessing the biological condition of low-gradient streams, sampling macroinvertebrate communities from several different habitat types (e.g., overhanging bank vegetation, undercut bank roots, and vegetation on rocks). We strengthened the linkage between chemical exposure and macroinvertebrate response by measuring metal concentrations not only in sediments from the stream bottom but also in the vegetative habitats where the macroinvertebrates were sampled. Linear regression analysis indicated that biological condition was significantly dependent (95% confidence level) on contaminants in vegetative habitats, but not on contaminants in sediments from the stream bottom. Biological condition was also significantly dependent on physical habitat quality; regression analysis on both contaminants and physical quality yielded the best regression model ($r^2 = 0.49$). Similar biological impairment was observed at sites with severe contamination or physical impairment or with moderate chemical and physical impairment. These results have implications for the management of urban streams.

**Keywords**—Multiple stressors Metals Habitat quality Macroinvertebrates Urban streams

## INTRODUCTION

An often-neglected factor in ecological risk assessments of contaminated streams is physical habitat quality. Physical habitat, as structured by in-stream and surrounding topographical features, is a major determinant of aquatic community potential [1–7]. In urban watersheds, chemical contamination commonly accompanies physical alteration of stream habitats. Our objective was to determine whether simple indices of chemical, physical, and biological conditions could be used to separately estimate the influence of chemical and physical degradation on macroinvertebrate communities. In particular, we were interested in whether consideration of physical habitat quality could be used to improve chemical risk assessments. Thus, we included evaluation of a stream's physical habitat quality (e.g., sedimentation, channelization, and loss of vegetation) in our assessment of the impacts from metal contaminants on macroinvertebrates in an urban watershed in near Boston, Massachusetts, USA.

We further refined our assessment by improving estimates of metals exposure. Applying the principle that contaminants must contact receptors to cause effects [8], we argue that understanding the effects of metals on macroinvertebrate communities requires measurement of metal concentrations in the same habitats from which the macroinvertebrates are collected. This is not accomplished by current protocols. For example, the U.S. Environmental Protection Agency (U.S. EPA) and several state agencies recommend sampling a combination of macroinvertebrate habitats to assess a stream's biological condition [4,9–11], but to our knowledge, no recommended method exists for measuring metal concentrations in the same macroinvertebrate habitats.

Metal concentrations in vegetative habitats could be quite different from metal concentrations in sediments at the stream bottom, so we developed a method for measuring exposures in vegetative habitats. Sansone et al. [12] demonstrated that the retention of suspended particles transported by river flow onto surfaces of freshwater plants is a potentially important process in the contamination of aquatic biota. Thus, our method analyzes whole samples of macroinvertebrate habitats; sediments associated with the vegetative material were not washed off before chemical analysis.

## MATERIALS AND METHODS

This study was carried out in the Aberjona watershed (Fig. 1), located 12 miles north of Boston (MA, USA) in the Boston Basin ecoregion [13]. Two Superfund sites (Industri-Plex 128 and Wells G&H, Woburn, MA, USA) are located on the Aberjona River, and elevated concentrations of metals in the river's sediments are believed to be the result of historical industrial activities, notably leather processing and chemical manufacturing [14–16]. The Aberjona River and its tributary streams have also been physically altered as the watershed has been developed for residential, commercial, and industrial uses [17].

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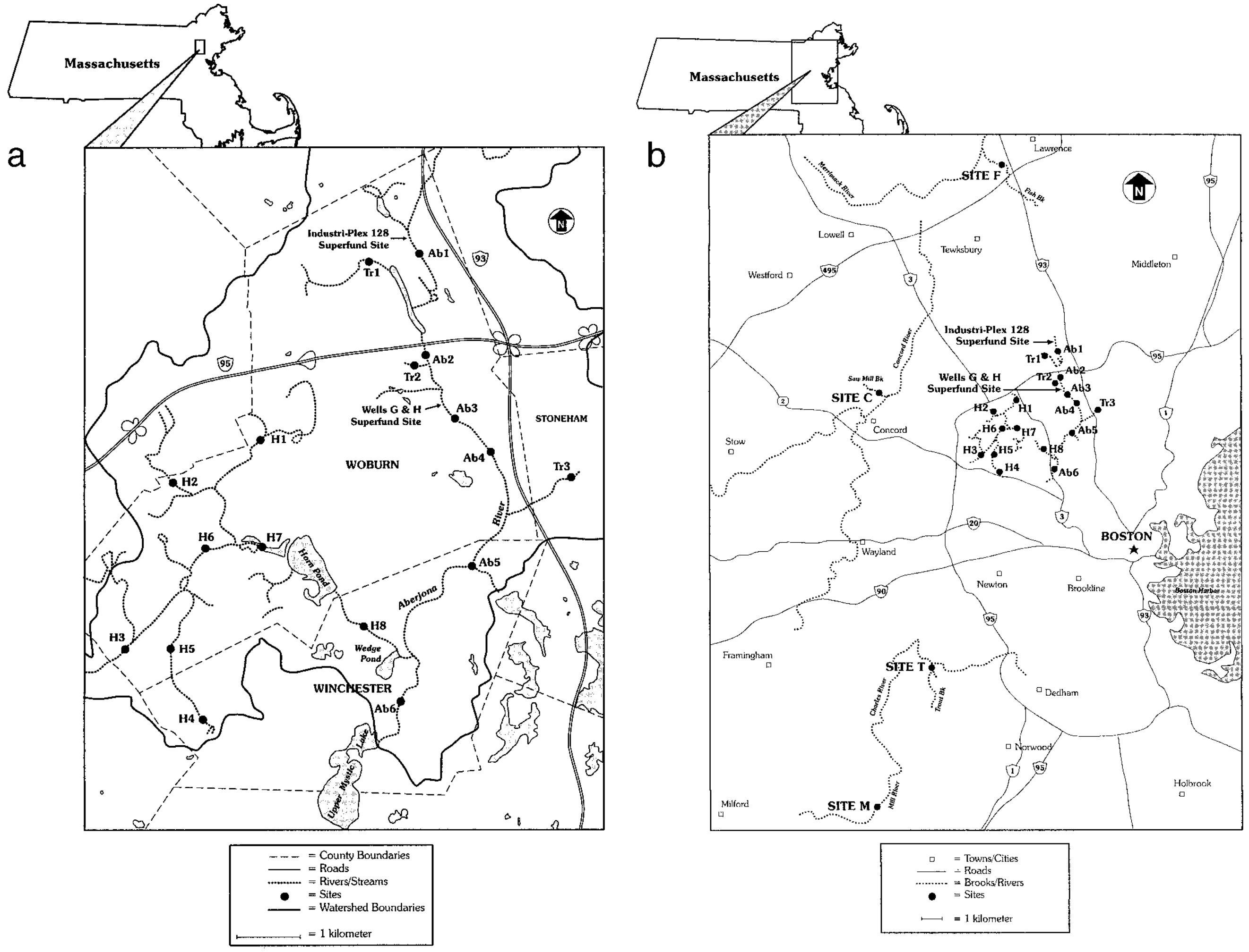


Fig. 1. Maps of the sampling sites. **a.** Study sites in the Aberjona watershed (Boston, MA, USA). **b.** Reference sites shown with the Aberjona sites.

*Identifying metals that could be present in toxic amounts*

We used three criteria to focus this study on those metals that were most likely to be present in toxic amounts: (1) Is the metal present in concentrations that have been observed to have toxic effects on macroinvertebrates elsewhere? (2) Is the metal present in higher concentrations at the study sites than at the reference sites? (3) Does the history of the Aberjona watershed suggest that toxic amounts of this metal could have been released into the watershed's streams?

For the first criterion, we employed an approach developed by the National Status and Trends Program to evaluate sediment contamination concentrations [18]. This approach uses toxicity levels that are based on laboratory spiked-sediment bioassays, equilibrium partitioning calculations, and field studies. Three concentration ranges are defined in this approach: No-effects, possible effects, and probable effects. The effects range low (ERL) is the lower 10th percentile concentration in sediments that is associated with toxic effects, and the effects range median (ERM) is the median metal concentration in sediment associated with toxic effects [18,19]. No toxicity effects are expected for concentrations below the ERL, whereas effects are probable for concentrations in excess of the ERM. Ingersoll et al. [20] reported ERM values for freshwater-sediment samples for the following metals: Al (58,000 mg/kg dry wt), As (50 mg/kg dry wt), Cd (3.9 mg/kg dry wt), Cr (270 mg/kg dry wt), Cu (190 mg/kg dry wt), Fe (28% dry wt), Mn (1,700 mg/kg dry wt), Ni (45 mg/kg dry wt), Pb (99 mg/kg dry wt), and Zn (550 mg/kg dry wt). Iron concentrations never exceeded ERL or ERM values in the sediment samples from any of the sites; thus, Fe was considered to be an unlikely candidate for posing a toxic risk. Ingersoll et al. did not report an ERL or ERM value for Hg, but concentrations of Hg were lower than or equal to the detection limit of our analytical method (5 mg/kg dry wt). The Al, Mn, and Ni concentrations in sediment samples rarely exceeded ERM values, and similar concentrations for each of these metals were found in reference sites and study sites. (Ranges for reference and study sites overlapped for each metal. The number of ERM exceedances was four or fewer, and concentrations more than 15% in excess of the ERM value were never observed.) The history of the Aberjona watershed does not suggest that Al, Mn, or Ni is an important industrial contaminant of this watershed.

In contrast, concentrations of As, Cd, Cr, Cu, Pb, and Zn in sediments frequently exceeded ERM values, with some values being two- to sixfold as high as the ERM values. Each of these metals is a known contaminant in the Aberjona watershed, and their presence is consistent with the watershed's industrial history [14]. In addition, concentrations of these metals in sediment sites from the Aberjona River were consistently higher than those found at the reference sites. Thus, this study focused on As, Cd, Cr, Cu, Pb, and Zn. (Other stressors, including organic contaminants, may also be important; these are considered in the Discussion section.)

*Sampling locations*

All sampling sites were 100-m segments of low-gradient streams located within the Boston Basin ecoregion. Six stream segments of the Aberjona River were selected for study and were designated as Ab1 to Ab6, with Ab6 being the location farthest downstream (Fig. 1a). Ten stream segments were selected in minimally contaminated streams within the Aberjona watershed and were designated as H2 to H8 and as Tr1 to Tr3 (Fig.1a). (Site H1 was rejected as a study area because of very-low- to no-flow conditions.) These sites represented a range of physical habitat conditions, from highly altered to relatively natural.

The criteria for selecting reference sites were relatively undeveloped headwaters, no or minimal evidence of human alteration of the physical habitat, presence of wide (>18 m) vegetated riparian zones, and no evidence of pollution [21]. None of the Aberjona watershed sites met all these criteria, although site H7 had wide vegetated riparian zones and little evidence of pollution or human alteration of the physical habitat. Due to the intensity of human development in the Aberjona watershed, four reference sites outside the watershed but within the Boston Basin ecoregion were selected to represent minimally impaired conditions (Fig. 1b). These four stream segments are located in sparsely developed residential areas: On Sawmill Brook 250 m upstream of Monument Street in Concord (MA, USA) (site C); on the Fish River 1.5 km upstream of Chandler Road in Andover (MA, USA) (site F); on Mill River at Miller Street in Norfolk (MA, USA) (site M); and on Trout Brook at Haven Street in Dover (MA, USA) (site T). More detailed maps of these locations have been published elsewhere [17]. These locations were selected after consultation with resource managers and scientists from the U.S. Army Corps of Engineers, the U.S. Geological Survey, the Massachusetts Department of Environmental Protection (MA DEP), and the U.S. EPA. Geological and hydrological properties (i.e., stream order, drainage area, and gradient) were similar for both Aberjona watershed sites and reference sites (U.S. Geological Survey maps: Reading, MA, USA [1987]; and Boston, MA-North, USA [1985]).

*Macroinvertebrate sampling, subsampling, and species identification*

Macroinvertebrate sampling was performed in August 1996 according to the protocols used by the MA DEP [10]. The protocols are similar to Method 7.2 Multihabitat Approach: D-Frame Dip Net described by Barbour et al. [4,22]. Briefly, the multihabitat approach consisted of sampling macroinvertebrate habitats, such as plant roots in undercut stream banks, streambank vegetation where it hangs over into the stream, rocks or cobble in the stream, snags, floating or submerged vegetation, and sand, in proportion to their visually estimated representation in the stream. Several of these macroinvertebrate habitat types are illustrated in Figure 2. The stream was visually assessed, and approximate percentages were assigned to in-stream habitat belonging to each category. The habitat type that was assigned the highest percentage was considered to be the dominant habitat type. Samples were collected by kicking the substrate or jabbing with a rectangular dip net (width, 0.5 m; height, 0.3 m; mesh size, 500 μm). A total of 10 jabs (or kicks) were taken from all major habitat types in the reach, resulting in sampling of approximately 2.5 $m^2$ of habitat. In this study, three of the sampling sites were chosen at random, and duplicate, but not overlapping, samples were collected from the major habitat types.

Following procedures outlined by the MA DEP [10], samples (all material collected in the 10 jabs or kicks, including benthic macroinvertebrates, bits of vegetation, small pebbles, and sand) were rinsed in a 500-μm sieve; large organic material was rinsed, visually inspected, and discarded. Samples were spread evenly across trays marked with grids and subsampled randomly. Organisms were sorted under a dissecting microscope. Subsamples of 100 ± 20 organisms preserved in 95% (v/v) alcohol were identified to the lowest practical taxon, generally genus or species. A representative of every species is maintained in a reference collection.

*Characterization of the biological condition of macroinvertebrate assemblages*

An aggregate index of the biological condition of macroinvertebrate assemblages was constructed using a method similar to that described by Barbour et al. [23] and Gibson et al. [24]. The complete method is described by Rogers [17]. Briefly, biological condition was characterized with an aggregate index composed of benthic metrics selected from among four categories: Taxa richness, composition metrics, tolerance/intolerance measures, and feeding measures. At least one metric from each category was included in the index. Twelve candidate metrics were chosen for testing based on widespread use [22] and their applicability to the Aberjona watershed (Appendix).

The candidate metrics were tested for their ability to discriminate impaired from unimpaired conditions and to provide nonredundant information using the method described by Barbour et al. [23]. The metrics that resulted in providing the most useful information for inclusion into the biological index were the total number of macroinvertebrate taxa; percentage of organisms that were Ephemeroptera, Plecoptera, or Trichoptera; percentage of organisms considered to be tolerant; percentage of Trichoptera within the relatively tolerant subgroup Hydropsychidae; and number of scraper and piercer taxa. These five metrics were normalized into dimensionless scores based on the scoring method described by Karr et al. [5] and Karr [6]. Table 1 displays statistics for the observed values and shows how each metric was scored. Figure 3 shows the score that each site received for each of the five metrics; the aggregate index of biological condition is the sum of the scores for the five metrics.

*Sampling for metal analysis: Sediments from the stream bottom and vegetative macroinvertebrate habitats*

At each site, the finest-grained sediments from the bottom of the stream were identified by visual inspection, and the top 2 to 3 cm were sampled using a Russian corer. Sediment was transferred to glass jars with a clean, plastic spatula and transported to the laboratory on ice.

We sampled the dominant macroinvertebrate habitat at each site for metal analysis. Table 2 lists the habitat types that were sampled for macroinvertebrates using standard protocols [4,10] and describes the technique that we developed to sample the habitats for metal analysis. Several of these macroinvertebrate habitat types are illustrated in Figure 2. Using polyvinyl chloride gloves, samples of the plant material (along with sediments suspended within the plant material) that constituted

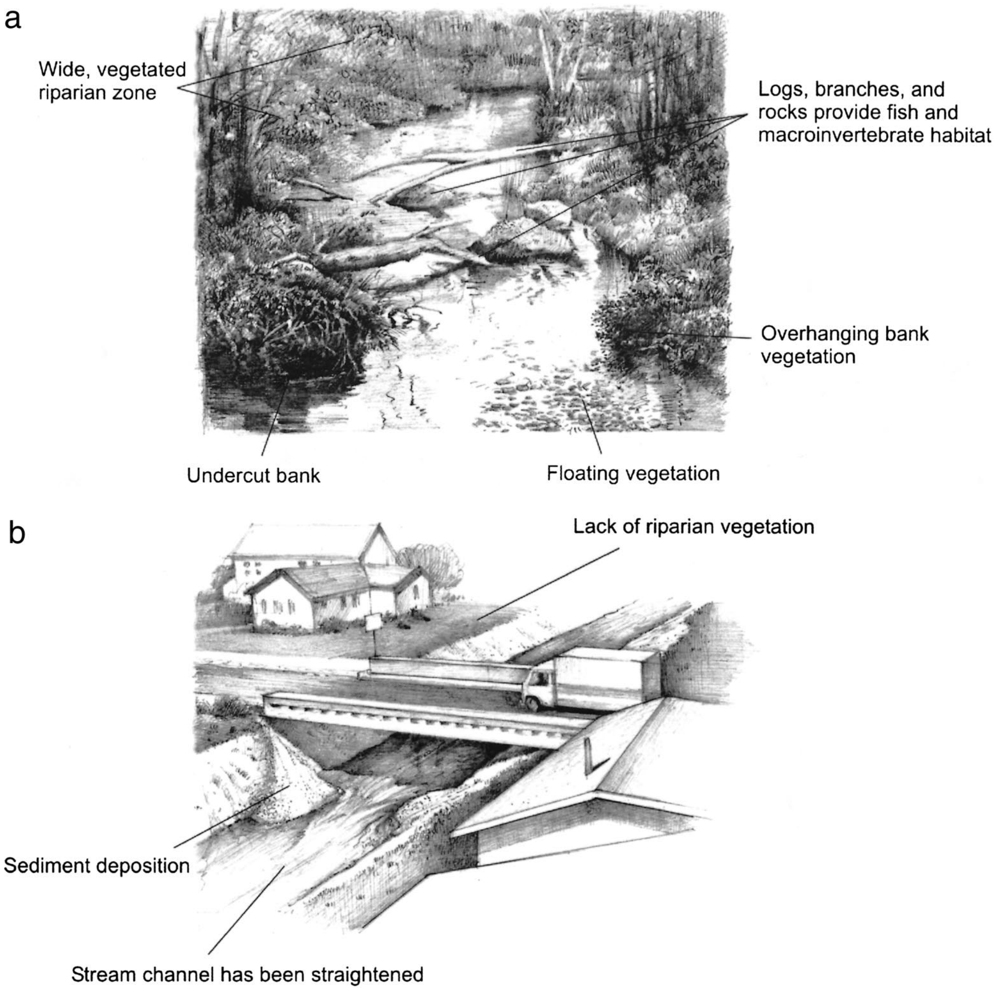


Fig. 2. Physical habitat parameters in (**a**) high-quality habitat and (**b**) low-quality habitat. **a.** In a high-quality habitat, rocks, fallen branches, and undercut banks provide fish many niches for feeding, laying eggs, and refugia (in-stream cover). Snags, submerged logs, and other hard substrates provide habitat for macroinvertebrates (epifaunal substrate). The variety of velocity–depth combinations (i.e., mixture of slow-moving pools with faster and shallower areas) provides a stable and diverse aquatic environment and moderates surges in flow associated with storms. Diverse and abundant bank vegetation provides habitat (including plant roots in undercut stream banks and vegetation hanging over into the water) and organic inputs (leaves provide food for shredders and other macroinvertebrates), and vegetation roots hold soil in place and prevent erosion (bank vegetative protection, bank stability, and riparian vegetative zone width). **b.** In a low-quality habitat, the stream lacks riparian vegetation (bank vegetative protection, bank stability, and riparian vegetative zone width). Buildings and roads contribute to sediment deposition, which creates an unstable environment for many organisms. Embeddedness, caused by the deposition of fine sediments, reduces or degrades available habitats. Straightened stream channels (channel alteration) have fewer velocity–depth combinations. The lack of submerged branches, rocks, and undercut banks results in little habitat for fish or macroinvertebrates (in-stream cover, epifaunal substrate). The water level is low (poor channel flow status). Channel flow status is important, because it determines whether habitats such as cobbles, overhanging bank vegetation, and undercut bank roots are submerged (available for aquatic fauna) or exposed to the air (unavailable for aquatic fauna).

each vegetative habitat were grasped and pulled, or gently scraped, from their attachment points, placed in glass jars, and transported on ice (Table 2). Sediments associated with the vegetative material in macroinvertebrate habitats were not washed off.

### *Sample preparation and analysis of metal concentrations*

Samples were held under refrigeration for no more than 2 d before being dried to constant weight at 85°C. Five grams of each dried sample were homogenized with a Spex mixer

Table 1. Descriptive statistics and scores for the core metrics

| | Statistics | | | | | Score | | | |
|---|---|---|---|---|---|---|---|---|---|
| Metric | Min | 5% | 50% | 95% | Max | 6 | 4 | 2 | 0 |
| No. of total taxa | 15 | 17 | 23 | 34 | 37 | >25 | 17–25 | 9–16 | <9 |
| % EPT[a] | 0 | 0 | 4 | 21 | 54 | >16 | 10–16 | 5–9 | <5 |
| % Tolerant taxa | 2 | 4 | 20 | 54 | 69 | <24 | 24–47 | 48–72 | >72 |
| % Hydropsychidae/Trichoptera | 0 | 2 | 100 | 100 | 100 | <24 | 24–47 | 47–73 | >73 |
| No. of scraper & piercer taxa | 0 | 0 | 3 | 6 | 6 | >4 | 3–4 | 2 | 0–1 |

[a] EPT = Ephemeroptera, Plecoptera, Trichoptera.

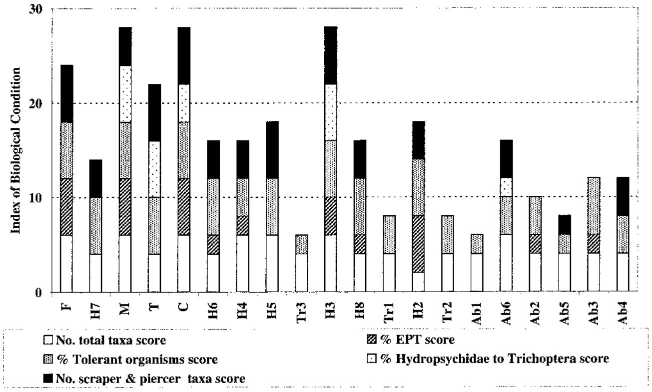


Fig. 3. Biological condition: Index scores for each study site. The total index score is the sum of the scores for the five core metrics. Table 1 shows the derivation of these scores and provides descriptive statistics for each metric. EPT = Ephemeroptera, Plecoptera, and Trichoptera.

mill (CertiPrep, Metuchen, NJ, USA) using a tungsten carbide ball mill for 5 min. One-half gram of Copolywax™ binder (Cargille TAB-PRO, Cedar Knolls, NJ, USA) was added to the sample, which was mixed again for 1 min. This mixture was then poured into an aluminum sample cup (diameter, 31 mm) and pressed for 1 min using 12 t of force while pulling a vacuum created by a rotary pump.

Elemental concentrations in each pellet were determined by wavelength dispersive x-ray fluorescence (XRF; used for As, Cd, and Zn) using a Philips PW1480 (Philips Analytical, Almelo, The Netherlands) wavelength dispersive instrument and Uniquant (Omega Data Systems BV, Veldehoven, The Netherlands) data-processing software, or by energy dispersive XRF (used for Cr, Cu, and Pb) using a Spectro X-Lab 2000 instrument (St. Lawrence, PQ, Canada) with Turboquant (Spectro, Kleve, Germany) data-processing routines. Standard reference material 2709 (San Joaquin soil), supplied by the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA), was used to monitor instrumental response during the data collection period and to verify calibration accuracy. The average and standard deviation of 15 measurements (covering the period when samples were analyzed) of the concentrations of As, Cd, Cr, Cu, Pb, and Zn were compared to the concentrations and 95% prediction interval reported by the NIST for this reference material (Table 3). The optimal detection limits for As, Cd, Cr, Cu, Pb, and Zn ranged from 0.2 to 1.0 mg/kg dry weight.

*Accuracy and precision of the metal concentration analyses*

Average measured values of metals in the NIST samples compared favorably (<5% difference between measured and reported values for all metals except Cu) with values reported by the NIST (Table 3). Copper concentrations were measured precisely, but our measurements averaged approximately 20% higher than the reported NIST values. This did not have a significant impact on toxicity estimates, because less than 20% of the total toxic units for each sample were attributable to [Cu]; thus, even errors of 20% in [Cu] would result in errors of less than 4% in total toxic units. Figure 4 shows the concentrations of each element in sediment and vegetative habitat samples at each sampling site.

*Metal concentrations: Use of benchmark concentrations to develop normalized metal concentrations for statistical analysis*

A measure of overall metal concentrations in stream-bottom sediments and in vegetative habitats was developed for use in regression analyses. For each individual metal, if [(observed concentration)/ERM] > 1, then the probability of sediment toxicity is high. Therefore, a toxic unit index was constructed by adding these ratios for multiple contaminants:

$$\text{Toxic unit index} = [M_1]/[ERM_1] + [M_2]/[ERM_2] + [M_3]/[ERM_3] + \ldots \quad (1)$$

where $[M_i]$ is the observed concentration of the *i*-th metal and $[ERM_i]$ is the ERM value for that metal. Adding these ratios together to create a toxic unit index is an approximation, because toxic effects are not necessarily additive: They could be synergistic or antagonistic. Ingersoll et al. [20] found that for freshwater sediments collected in the field, sediments that had multiple (two or more) exceedances were more frequently toxic (to *Hyalella azteca* in laboratory tests) than sediments with only one exceedance (i.e., only one metal was present in a concentration > ERM). Higher metal concentrations were associated with greater frequency of effects; thus, although toxicity may not increase linearly with metal concentrations, a positive relationship exists. Therefore, it is reasonable to use a toxic unit index as a measure of metal contamination for metals that exceed toxic thresholds.

*Assessment of physical habitat condition*

Physical habitat assessment was performed at each site according to the protocols used by the MA DEP [10] but modified for slow-flow, low-gradient streams. These protocols use a

Table 2. Types of vegetative faunal habitat identified for chemical analysis

| Habitat type | Sites sampled | Description of sampling technique |
|---|---|---|
| Overhanging bank vegetation | Ab4 | Vegetation was gently pulled free of the bank, retaining as much of the sediment suspended in the vegetation as possible. |
| Submerged vegetation | Ab3, H7, M, T | Submerged vegetation was grasped and pulled from its attachment point. |
| Undercut bank roots | Ab2, Ab5, Ab6, H3, H8, Tr3 | Handfuls of muddy undercut bank roots were grabbed and pulled from the banks. |
| Vegetation growing on rocks in shallow sections of streams | H2, H3, H6, C, F | Vegetation was pulled and gently scraped from rocks. |
| Floating vegetation | Ab1, H4, H5, Tr1, Tr2 | Handfuls of floating vegetation (e.g., watercress/*Cruciferae nasturtium*) were pulled from the water. |

Table 3. Accuracy and precision of the metal concentration analyses

| Metal | Average measured NIST ± SD[a] (mg/kg dry wt) | Reported NIST ± SD (mg/kg dry wt) | Ratio of measured to reported | Analysis method[b] | Detection limit[c] |
|---|---|---|---|---|---|
| As | 18 ± 2, *n* = 4 | 18 ± 1 | 1.00 | EDXRF | 3 ± 2 |
| Cd | Below detection limit, *n* = 4 | 0.4 | —[d] | EDXRF | 0.5 ± 0.2 |
| Cr | 131 ± 10, *n* = 15 | 130 ± 4 | 1.01 | WDXRF | 23 ± 6 |
| Cu | 42 ± 3, *n* = 15 | 35 ± 1 | 1.20 | WDXRF | 1 ± 0.4 |
| Pb | 19 ± 4, *n* = 15 | 19 ± 1 | 1.00 | WDXRF | 2 ± 0.1 |
| Zn | 101 ± 4, *n* = 4 | 106 ± 3 | 0.96 | EDXRF | 1 ± 0.1 |

[a] NIST = National Institute of Standards and Technology; SD = standard deviation.
[b] EDXRF = energy dispersive x-ray fluorescence; WDXRF = wavelength dispersive x-ray fluorescence.
[c] Ideal detection limits were lower (0.2–1.0 mg/kg dry wt). The values in this column are actual detection limits observed in typical samples.
[d] The detection limit for Cd was higher than the National Institute of Standards and Technology (NIST) standard, so this ratio could not be determined.

scoring sheet that describes the conditions associated with each score. For example, the embeddedness score requires an evaluation of the percentage of snags and submerged logs that are surrounded by fine sediment. If less than 25% of these habitats are surrounded, then a score of 16 to 20 is assigned. Conversely, if more than 75% of these surfaces are surrounded by fine sediment, a score of 0 to 5 is assigned. Scores are determined by visually matching the observed conditions to those described by the scoring sheet. Physical habitat condition is judged to be poor (score of 0–5), marginal (score of 6–10), suboptimal (score of 11–15), or optimal (score of 16–20). Physical habitat features are illustrated in Figure 2. Ten habitat-quality parameters (in-stream cover, epifaunal substrate, embeddedness, channel alteration, sediment deposition, variety of velocity–depth combinations, channel flow status, bank vegetative protection, bank stability, and riparian vegetative zone width) were scored from 0 to 20. The relationship of Figure 2 to these 10 habitat-quality parameters is explained in the caption. The physical habitat-quality index is the sum of the scores for the 10 habitat parameters (thus ranging from 0–200). Detailed results of the scores for individual parameters are displayed in Figure 5. Water-quality parameters were also measured (i.e., temperature, dissolved oxygen concentrations, pH, and conductivity), but these results were unremarkable [17] and, thus, are not presented here.

*Regression*

The aggregate index of biological condition was regressed using Microsoft® Excel (Redmond, WA, USA) on the individual toxic unit indices for stream-bottom sediments and for vegetative habitat samples (e.g., undercut bank roots, overhanging bank vegetation) to determine if either toxic exposure estimate was a statistically significant predictor. The forms for these regressions were

$$\text{Biological condition index} = \beta_0 + \beta_1 \cdot \text{TOX}_{\text{Sed}} + \text{error} \quad (2)$$

and

$$\text{Biological condition index} = \beta_0 + \beta_1 \cdot \text{TOX}_{\text{Veg}} + \text{error} \quad (3)$$

where $\beta_0$ is the intercept, $\beta_1$ is a regression coefficient, $\text{TOX}_{\text{Sed}}$ is the toxic unit index for sediments, and $\text{TOX}_{\text{Veg}}$ is the toxic unit index for vegetative habitats.

The biological condition index was also regressed on the total habitat-quality score and on both total habitat quality and the toxic unit indices to determine whether habitat quality was a statistically significant explanatory variable and whether its inclusion yielded a better regression model. The forms for these regressions were

$$\text{Biological condition index} = \beta_0 + \beta_1 \cdot \text{TotalHabScore} + \text{error} \quad (4)$$

and

$$\text{Biological condition index} = \beta_0 + \beta_1 \cdot \text{TOX} + \beta_2 \cdot \text{TotalHabScore} + \text{error} \quad (5)$$

where $\beta_2$ is also a regression coefficient, TotalHabScore is the total habitat-quality score, and TOX is $\text{TOX}_{\text{Sed}}$ or $\text{TOX}_{\text{Veg}}$. We only report in detail the results for $\text{TOX}_{\text{Veg}}$ for Equation 5, because neither Equation 2 nor Equation 5 showed $\text{TOX}_{\text{Sed}}$ to be a significant explanatory variable.

## RESULTS

Figures 3 to 5 present the scores and subscores for biological condition (Fig. 3); the concentrations of As, Cd, Cr, Cu, Pb, and Zn in sediment and vegetative samples (Fig. 4); and the scores and subscores of physical habitat quality (Fig. 5). These results are summarized in Figure 6. The chemical (Fig. 6a), biological (Fig. 6b), and physical (Fig. 6c) indices provide a basis for assessing the influence of chemical and physical degradation on macroinvertebrates.

As expected, sites located on the Aberjona River had elevated concentrations of metals and, thus, high numbers of toxic units (Figs. 4 and 6a). For the ERM values of As, Cd, Cr, Cu, Pb, and Zn at least one exceedance occurred for each metal. Exceedances were most frequent and of greatest magnitude for sites Ab1 to Ab6.

If metal concentrations in bottom sediments and/or vegetative habitats were the dominant factor controlling biological condition, then biological condition would be expected to be impaired at contaminated sites and most impaired at the most contaminated sites. However, this was not the case (Fig. 6a and b). Biological condition at sites Ab1 to Ab6 was poorer than biological condition at the reference sites (C, F, M, and T), but biological condition at some uncontaminated sites (Tr1–Tr3) was equally poor. Moreover, biological condition

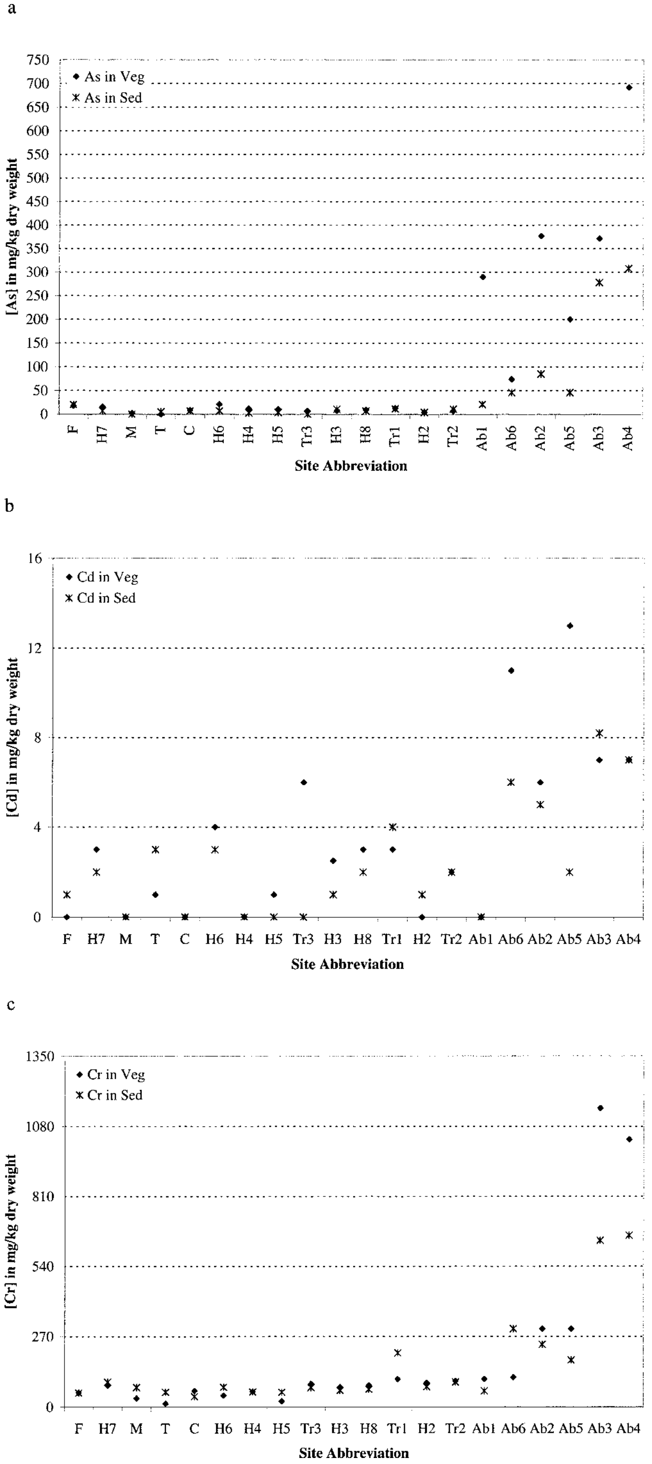


Fig. 4. Metal concentrations (mg/kg dry wt) at study sites, with concentrations in vegetative samples illustrated as solid diamonds and concentrations in sediment samples illustrated as stars, for (**a**) As, (**b**) Cd, (**c**) Cr, (**d**) Cu, (**e**) Pb, and (**f**) Zn. To facilitate comparisons with the effects range median (ERM) values (toxic thresholds), the ERM for each metal is used as the interval for gridlines on the *y*-axis (e.g., 50 for As, 4 for Cd). Sed = sediment; Veg = vegetation.

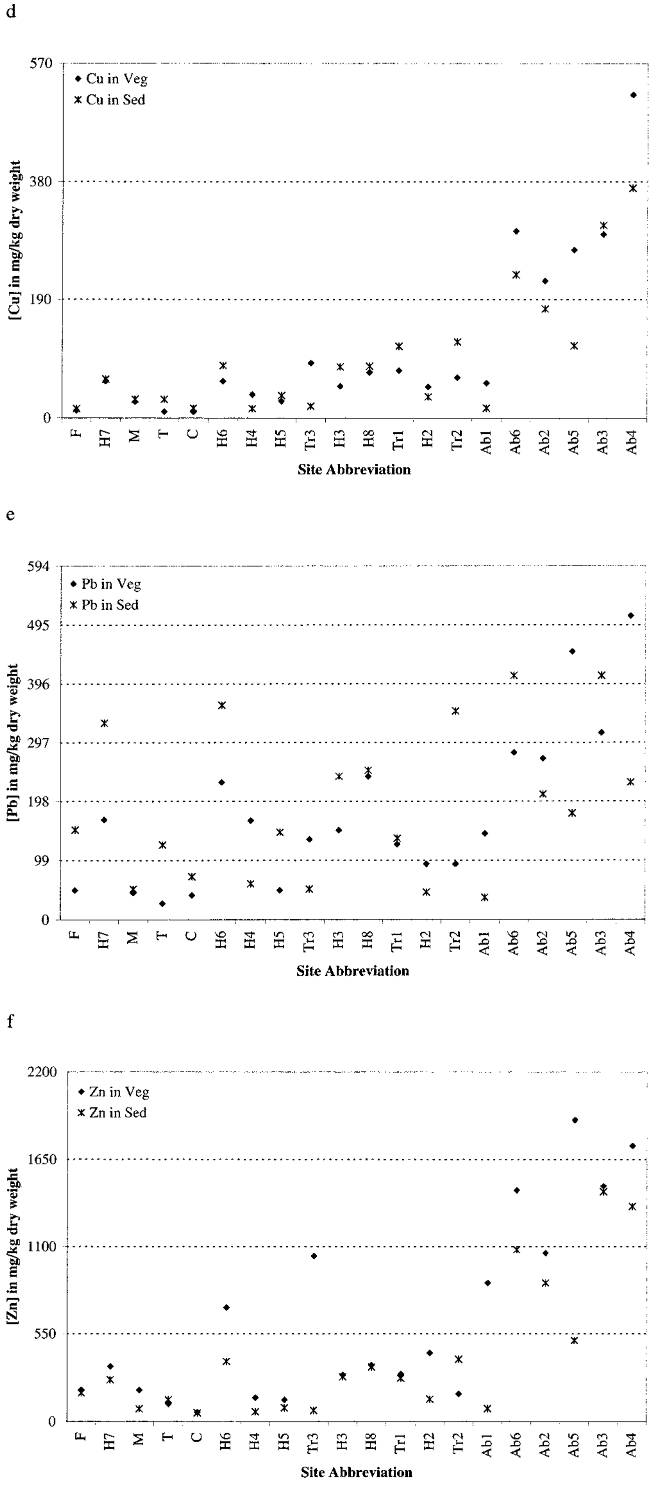


Fig. 4. Continued.

was not the most impaired at the most contaminated sites (Ab3 and Ab4).

The observed trends (Fig. 6) do, however, suggest that metal contamination had an adverse effect on macroinvertebrates, but that this effect was confounded by variability in physical habitat condition. The ordering of the sites along the *x*-axis in the graphs (Figs. 3 to 6) displays physical habitat condition decreasing from left to right for all sites except Ab1 to Ab6, which are shown at the far right. A general, but scattered, relationship between biological condition and habitat quality can be seen in Figure 6b and c. That some of the contaminated sites had relatively good habitat quality and poor biological condition is consistent with the existence of a stressor other than habitat degradation (e.g., contamination).

Similar levels of biological impairment were observed at sites with severe contamination or physical habitat impairment

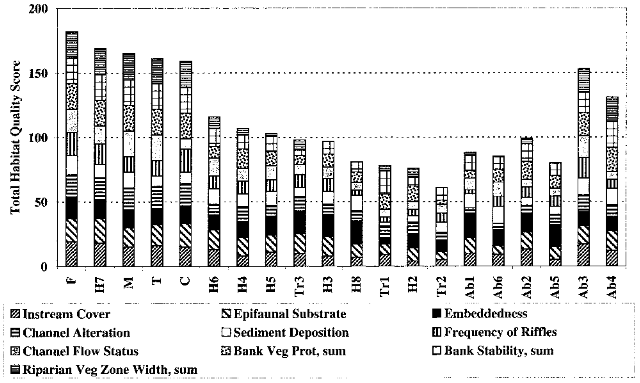


Fig. 5. Physical habitat condition at each study site. The total index score is the sum of the scores for the 10 habitat metrics. Prot = protection; Veg = vegetation.

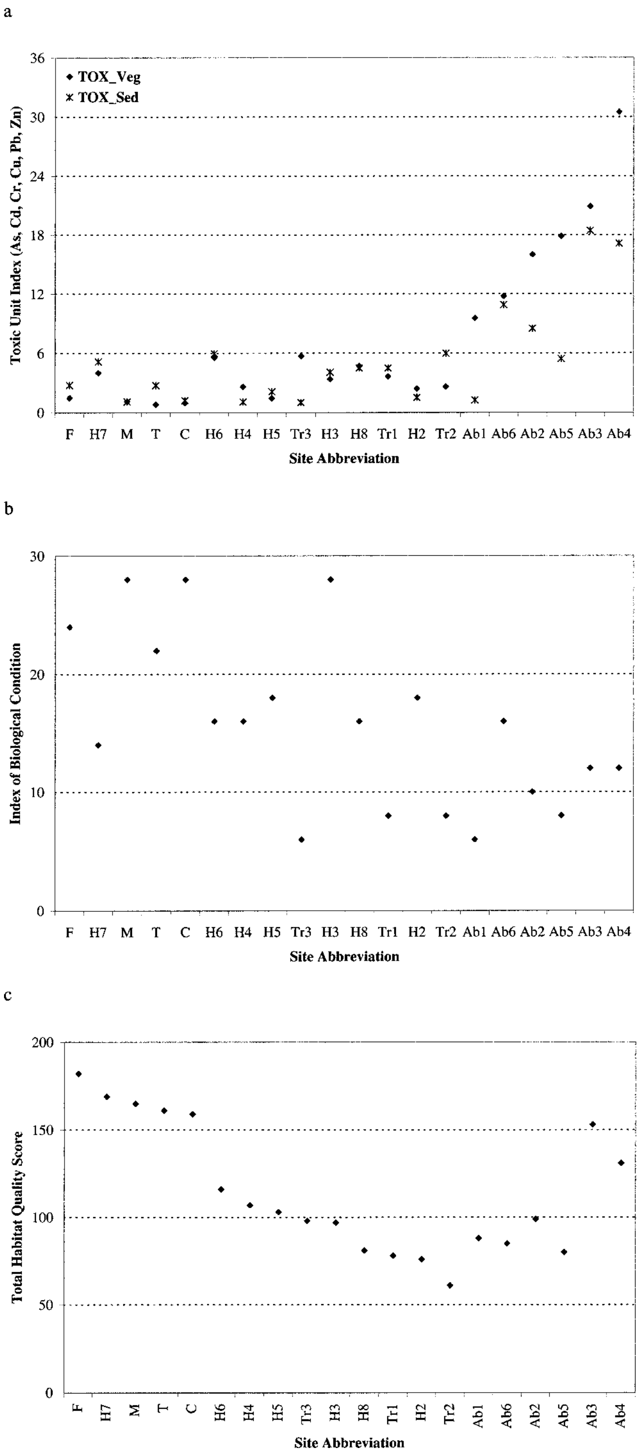


Fig. 6. The influence of toxic metals on biological condition becomes apparent if physical habitat quality is considered. **a.** Toxic unit indices exceed six only for sites Ab1 to Ab6. (Significant contamination is indicated at these sites, because if each of the six metals were present in a concentration exactly equal to its effects range median [ERM] value, then the index for that site would be equal to six.) **b.** Based on biological and chemical evidence alone, little support is found for the hypothesis that toxic metals have adversely impacted biological condition. **c.** Physical habitat data help to explain poor biological condition at uncontaminated sites. Regression of biological condition on chemical and physical condition provides a more refined analysis (see Table 4). TOX_Sed = toxic unit index for sediments; TOX_Veg = toxic unit index for vegetative habitats.

or with moderate contamination and habitat impairment. The reference sites (C, F, M, and T) were selected to represent minimally impaired conditions for the Boston Basin ecoregion [21]. As expected, these sites had low concentrations of contaminants, high scores for physical habitat quality, and high scores for biological condition. Sites with high concentrations of contaminants and moderately high scores for physical habitat quality have lower scores for biological condition (i.e., Ab3 and Ab4). Relatively uncontaminated sites with poor habitat quality (e.g., H8, Tr1, H2, and Tr2) tend to have poor biological condition. Sites with intermediate levels of both contamination and habitat-quality impairment (i.e., Ab6, Ab1, Ab2, and Ab5) also have low scores for biological condition.

### *Regression of biological condition on physical and chemical stressors*

To determine whether biological condition was significantly related to metal concentrations in sediments from the stream bottom or in vegetative habitats, the aggregate macroinvertebrate index was regressed using Equations 2 and 3 (see Materials and Methods section). The results in Table 4 show that the index of biological condition was statistically dependent (at the 95% confidence level) on the toxic unit index for macroinvertebrate habitat samples, but not on the toxic unit index for bottom-sediment samples. In addition, the variance ($r^2$) explained by the regression rises from 8 to 22% when $TOX_{Veg}$ is used in place of $TOX_{Sed}$.

To determine whether physical habitat quality was an important explanatory variable for biological condition, biological condition was first regressed on physical habitat quality alone (i.e., the total habitat score) and then on both the total habitat score and $TOX_{Veg}$ using Equations 4 and 5 (see Materials and Methods section). The results in Table 4 indicate that total habitat score alone is a significant explanatory variable (at both the 95% and 99% confidence levels) that accounts for approximately 30% of the variance. When both $TOX_{Veg}$ and total habitat score are included in the model, both retain their statistical significance (at the 95% confidence level), and the variance explained jumps to 49% (Table 4). These results leave a significant fraction of the variance unexplained, but the combined model represents a great improvement over previous models (e.g., $TOX_{Sed}$, $TOX_{Veg}$, or habitat quality alone).

Table 4. Regression of the biological condition index (BCI) on toxic unit index for sediments ($TOX_{Sed}$), toxic unit index for vegetative habitats ($TOX_{Veg}$) and total habitat score[a]

Single-variable linear regression[b]

| Single independent variable | Intercept | Parameter estimate | Standard error | $p$ | $r^2$ |
|---|---|---|---|---|---|
| $TOX_{Sed}$ | 17.88 | −0.41 | 0.33 | 0.22 | 0.08 |
| $TOX_{Veg}$ | 18.78 | −0.42 | 0.19 | 0.04 | 0.22 |
| Total habitat score | 3.46 | 0.11 | 0.04 | 0.01 | 0.30 |

Multivariable linear regression[c]

| Variables in the multivariable regression | Parameter estimate | Standard error | $p$ | $r^2$ |
|---|---|---|---|---|
| Intercept | 6.96 | 4.29 | 0.12 | 0.49 for model with both variables |
| $TOX_{Veg}$ | −0.39 | 0.16 | 0.02 | |
| Total habitat score | 0.10 | 0.03 | 0.008 | |

[a] BCI was significantly dependent on $TOX_{Veg}$ and total habitat score, but not on $TOX_{Sed}$. Explanatory power of the model increased to 49% when $TOX_{Veg}$ and total habitat score were both included in the model.

[b] Regressed BCI on each parameter singly. Information for each of the three regression equations is shown as a row in the table. Refer to Equations 2 to 4 in the text.

[c] Regressed BCI on both $TOX_{Veg}$ and total habitat score. Table shows the results for the regression with the intercept and parameter estimates shown as rows; $r^2$ is given for the full equation. Refer to Equation 5 in the text.

## DISCUSSION

### *Influence of metal contamination and physical habitat quality on biological condition*

We sought to improve estimates of the exposure of macroinvertebrates to metals in their environment by measuring metal concentrations in the same habitats from which macroinvertebrates were collected. We hypothesized that biological condition would be more strongly correlated with metal concentrations in vegetative habitats than with metal concentrations in bottom sediments. We found that biological condition was significantly dependent (95% confidence level, $r^2 = 0.22$) on contaminants in vegetative habitats, but not on contaminants in bottom sediments. This supports our hypothesis and suggests that, in at least some cases, analysis of metal concentrations in vegetative habitats could improve the assessment of the ecological risks of contaminants in streams.

We hypothesized that physical habitat impairment is also an important determinant of biological condition and, thus, could mask contaminant effects in some cases. Thus, we included physical habitat quality in the regression model. We found that biological condition was significantly dependent on physical habitat quality (95% confidence level, $r^2 = 0.30$). Inclusion of both the toxic unit index for contaminants in vegetative habitats and the total habitat-quality score increased the $r^2$ for the regression model from 0.22 (regression on the toxic unit index alone) to 0.49 (regression on both variables). Inclusion of physical habitat quality is not a panacea; it does not explain all the variance in biological condition scores. However, it does significantly improve our understanding of the factors influencing biological condition, and it does help to reveal the effects of contaminants.

### *Method strengths and limitations*

The measures of physical habitat quality and metal contamination that were used in our study explain approximately half the variance ($r^2 = 0.49$) in biological condition. The unexplained variance could be partly due to factors not included in our study, such as organic contaminants, nutrients, invasive nonindigenous species, and human alterations in streamflow. Because they may be correlated, the effects of organic contaminants and toxic metals might be difficult to distinguish. The two sites with the highest metal concentrations (Ab3 and Ab4) are downstream of both the Industri-Plex 128 and Wells G&H Superfund sites (Woburn, MA, USA), where other studies have found high concentrations of both organic and metal contaminants [25–27].

Another limitation is that total metal concentrations were used as measures of exposure, because these measures do not account for factors such as complexation with iron oxyhydroxides, organic matter, or sulfide. Processes controlling toxicity have been extensively studied for fine-grained sediments such as those found at the bottom of a stream (e.g., [28–34]). These studies can be very useful for characterizing exposure of deposit-feeding animals living in sediments, but they are less applicable to characterizing exposures in vegetative habitats. A lack of bioavailable metals in bottom sediments does not necessarily imply a lack of bioavailable metals in vegetative habitats. Indeed, undercut bank roots, watercress, and overhanging bank vegetation exist near the water surface of streams. These habitats differ from surficial bottom sediments both chemically and physically. For example, they are likely to have greater exposure to oxygen and, thus, lower concentrations of sulfide than those in bottom sediments. Macroinvertebrates in vegetative habitats may be exposed to metals in the water associated with the habitat, to metals attached to sediments associated with the vegetation, to metals adsorbed to plants, or to metals in plants. The simple empirical relationship between biological condition and metal concentrations in vegetative habitats, thus, does not address the complexity of bioavailability or multiple exposure pathways. Despite limitations, bulk chemistry measurements are useful as triggers for further analysis (e.g., [19]), and we propose that measurement of metal concentrations in vegetative habitats could usefully supplement analyses of bottom sediments.

It must be also acknowledged that the index of biological condition and the total habitat score are both surrogate measures that aggregate a variety of indicators. The index of biological condition used in this study encompasses several aspects of the structure and function of the macroinvertebrate community, making it a valuable tool for assessing a stream's ability to support aquatic life [23]. As an aggregate index, it provides only an overall picture of stream condition, not a detailed portrait of specific responses to physical and chemical stressors, although individual metrics are often response signatures [35]. Similarly, the habitat condition index is the sum of a set of scores, each of which is an imperfect indicator for the quality of stream habitat for macroinvertebrates. When risk managers require more detailed information regarding specific causes and effects, additional analysis will be needed.

Analysis of individual components of the biological condition, total habitat, and toxic unit indices may provide additional insight. As an example, the number of scraper and piercer taxa was regressed on the total habitat score and the toxic unit index by Rogers [17], and a statistically significant relationship was found for both. Scraper and piercer taxa feed on living plant material, so they are expected to be important components of macroinvertebrate communities in streams where plant habitat is more abundant than riffle habitat (e.g., the Aberjona watershed and other low-gradient streams). The observed dependence of the number of scraper and piercer taxa on physical habitat quality may be explained by the fact that a loss of riparian vegetation (which would be reflected in an impaired physical habitat-quality score) involves a loss of habitat for scrapers and piercers. Similarly, the observed dependence on the toxic unit index may be due to the fact that scrapers and piercers living in vegetative macroinvertebrate habitats such as undercut bank roots and overhanging streambank vegetation would be exposed to contaminants in these habitats.

*Resources required to apply these methods*

In deciding whether to use the methods recommended in this paper, risk assessors and risk managers will consider the value of the information gained relative to the cost of obtaining that information. We believe that a major strength of the methods proposed here is their relatively low cost. Conducted in conjunction with chemical and/or biological sampling, physical habitat-quality assessment by a trained investigator adds only approximately 15 min to each site visit. The investigator is required to visually assess each of 10 parameters, to assign a score for each parameter, and to sum the scores to calculate the total habitat score. An initial investment in training is also necessary [36]. Chemical analysis of vegetative habitats costs no more than chemical analysis of sediments. The dominant macroinvertebrate habitat is identified during macroinvertebrate sampling. Collecting a sample of the dominant vegetative habitat along with the bottom-sediment samples would require less than 10 min in most cases. Sample processing and analysis costs are equal for sediments and vegetative habitats (using the method we developed for this study). In addition, currently available, field-portable XRF units are capable of analyzing many metals regulated under the Comprehensive Environmental Response, Compensation, and Liability Act on-site at ERL/ERM concentration levels, potentially enabling the rapid characterization of metal concentrations required for toxic unit analysis in the field.

*Physical habitat quality strongly influences biological condition*

The inclusion of physical habitat quality in the assessment not only helped to reveal the effects of metal contaminants but also to put the risks of contamination in context with other risks. We found similar levels of biological impairment for sites with severe contamination or physical habitat impairment or with moderate contamination and habitat impairment. Two Superfund sites drain into the Aberjona River, including one of national fame. (Contamination associated with the Wells G&H site was the subject of a best-selling book [37] and a major motion picture, *A Civil Action.*) It may seem surprising that physical habitat degradation in the Aberjona watershed, which has experienced a level of urbanization comparable to that in the suburbs of many eastern U.S. cities, appears to be associated with a similar level of biological impairment as has been caused by contamination in this watershed. Little regulatory attention has been given to protecting habitat from degradation by channelization, loss of riparian vegetation, sedimentation, and other alterations [6,21,38]. Yet, the vital importance of physical habitat quality has not gone unrecognized. The loss of habitat quality has resulted in extinctions, local extirpations [39], and population reductions [40,41] of fish species and other aquatic fauna [42] in the United States [38]. Consequently, although chemical pollution continues to be a problem in many freshwater steam ecosystems, many investigators believe that habitat degradation is presently responsible for more ecological impairment than is caused by chemical pollution (e.g., [38,43]).

*Comparative risk information can inform remediation and restoration efforts*

Information regarding comparative risk is available to managers only if assessments address more than one stressor. Managers can use these results to make informed decisions related to the protection and restoration of stream ecosystems. If a contaminated site is also physically impaired, it might be unrealistic to expect chemically oriented remediation to restore biological condition. In addition, the process of chemically remediating long stretches of contaminated streams might induce further physical degradation of stream habitats. Physical remediation also cannot substitute for chemical remediation in addressing human health risks. However, once health risks have been addressed, managers seeking to restore the biological resources of streams can balance the restoration of physical habitat, reduction of contaminant concentrations, and spatial distribution of each effort to maximize the benefits obtained.

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## APPENDIX

Twelve candidate metrics chosen for testing based on their potential importance in the Aberjona watershed

| Benthic metric | Reason for inclusion in analysis |
|---|---|
| Total no. of taxa | Widespread use |
| No. of EPT[a] taxa | Widespread use |
| % EPT | Widespread use |
| % Dominant taxon | Widespread use |
| Hilsenhoff Biotic Index | Widespread use |
| % Filterers | Widespread use |
| % Orthocladiinae to chironomids | % Orthoclads correlated positively with contamination by As, Cr, Cu, Pb, and Zn in several descriptive studies of water pollution and to Cu and Zn in experimental studies [44]. |
| % Hydropsychidae to Trichoptera | To account for the dominance of the relatively pollution-tolerant caddisflies in the Boston Basin ecoregion. Hydropsychidae are highly tolerant of heavy metals (Cr, Hg, Pb, and Ni) [44]. |
| No. of scraper and piercer taxa | Taxa that feed on living plant material are especially important and sensitive in streams where plant habitat is more abundant than riffle habitat. |
| % Clingers | To represent behavior (habit) measures of the structure and function of the benthic assemblage [45]. |
| No. of intolerant taxa | A richness measure of the most sensitive organisms that decreases when perturbation increases. |
| % Tolerant organisms (of total fauna) | A composition measure that increases when the more sensitive organisms are lost. |

[a] EPT = Ephemeroptera, Plecoptera, Trichoptera.