State of Ohio
Environmental Protection Agency

Ecological Assessment
Division of Surface Water

Using Biological Criteria to Validate Applications of Water Quality Criteria: Dissolved and Total Recoverable Metals

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Left Cover Photo: White amur or Grass Carp (*Ctenopharyngodon idella*).
Bottom Cover Photo: Golden redhorse (*Moxostoma erythrurum*) with spinal deformity.

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What Are Biological Criteria?
Biological criteria (biocriteria) are narrative or numeric expressions of the health and well-being of aquatic life and are based on the numbers and kinds of aquatic organisms which inhabit a particular stream or river sampling site. Biocriteria are derived from a complex process using data which reflects the reference condition within a particular geographic region of the state (Ohio EPA 1987a,b; Ohio EPA 1989a,b). As such biocriteria represent a direct measure of the attainment or non-attainment of aquatic life use designations for Ohio's streams. Ohio EPA incorporated biocriteria into the Ohio Water Quality Standards (WQS; Ohio Administrative Code 3745-1) regulations in February 1990 (effective May 1990). These criteria consist of numeric values for the Index of Biotic Integrity (IBI) and the Modified Index of well-being (MIwb), both of which are based on information about stream fish assemblages, and the Invertebrate Community Index (ICI), which is based on information about stream macroinvertebrate assemblages. Criteria for each biological index are specified for each of Ohio's five ecoregions (as described by Omernik 1987) and are further organized by organism group, index, site type, and aquatic life use designation. Biocriteria, along with the existing chemical and whole effluent toxicity evaluation methods and criteria, figure prominently in the monitoring and assessment of Ohio's surface water resources.

How Are Biocriteria Used By Ohio EPA?
Biocriteria are implemented primarily as an ambient assessment tool. The basic data necessary to use biocriteria are obtained from biological surveys. A biological and water quality survey, or "biosurvey", is an interdisciplinary monitoring effort coordinated on a waterbody specific or watershed scale. This effort may involve a relatively simple setting focusing on one or two small streams, one or two principal stressors, and a handful of sampling sites or a much more complex effort including entire drainage basins, multiple and overlapping stressors, and tens of sites. Each year Ohio EPA conducts biosurveys in 10-15 different study areas with an aggregate total of at least 250-300 sampling sites. Ohio EPA employs biological, chemical, and physical monitoring and assessment techniques in biosurveys in order to meet three major objectives:

1) determine the extent to which use designations assigned in the Ohio WQS are either attained or not attained;

2) determine if use designations assigned to a given water body are appropriate and attainable; and,

3) determine if any changes in key ambient biological, chemical, or physical indicators have taken place over time, particularly before and after the implementation of point source pollution controls or best management practices.

The data gathered by a biosurvey is processed, evaluated, and synthesized in a biological and water quality report. Each biological and water quality report contains a summary of the major findings including progress towards meeting designated uses and recommendations for revisions to use designations, future monitoring needs, or other actions which may be needed to resolve existing impairments of designated uses. While the principal focus of a biosurvey is on the status of aquatic life uses, the status of other uses such as recreation and water supply, as well as human health concerns, are also addressed.

The Role of Biocriteria in the Ohio Water Quality Standards: Designated Aquatic Life Uses
The Ohio Water Quality Standards (WQS; Ohio Administrative Code 3745-1) consist of designated uses and chemical, physical, and biological criteria designed to represent measurable properties of the environment that are consistent with the narrative goals specified by each use designation. Use designations consist of two broad groups, aquatic life and non-aquatic life uses. In applications of the Ohio WQS to the management of surface water resource issues, the aquatic life use criteria frequently control the protection and restoration requirements, hence their emphasis in biological and water quality reports. Also, an emphasis on protecting aquatic life generally results in water quality suitable for all uses. The five different aquatic life uses currently defined in the Ohio WQS with the biological intent of each are described as follows:

1) *Warmwater Habitat (WWH)* - this use designation defines the "typical" warmwater assemblage of aquatic organisms for Ohio streams; *this use represents the principal restoration target for the majority of water resource management efforts in Ohio.* Biological criteria are stratified across five ecoregions for the WWH use designation.

2) *Exceptional Warmwater Habitat (EWH)* - this use designation is reserved for waters which support "unusual and exceptional" assemblages of aquatic organisms which are characterized by a high diversity of species, particularly those which are highly intolerant and/or rare, threatened, endangered, or special status (*i.e.,* declining species); *this designation represents a protection goal for water resource management efforts dealing with Ohio s best water resources.* Biological criteria for EWH apply uniformly across the state.

3) *Coldwater Habitat (CWH)* - this use is intended for waters which support assemblages of cold water organisms and/or those which are stocked with salmonids with the intent of providing a put-and-take fishery on a year round basis which is further sanctioned by the Ohio DNR, Division of Wildlife; this use should not be confused with the Seasonal Salmonid Habitat (SSH) use which applies to the Lake Erie tributaries that support periodic "runs" of salmonids during the spring, summer, and/or fall. No specific biological criteria have been developed for the CWH use although the WWH biocriteria are viewed as attainable for CWH designated streams.

4) *Modified Warmwater Habitat (MWH)* - this use applies to streams which have been subjected to extensive, maintained, and essentially permanent hydromodifications such that the biocriteria for the WWH use are not attainable *and where the activities have been sanctioned and permitted by state or federal law;* the representative aquatic assemblages are generally composed of species which are tolerant to low dissolved oxygen, silt, nutrient enrichment, and poor quality habitat. Biological criteria for MWH were derived from a separate set of habitat modified reference sites and are stratified across five ecoregions and three major modification types: channelization, run-of-river impoundments, and extensive sedimentation due to non-acidic mine drainage.

5) *Limited Resource Water (LRW)* - this use applies to small streams (usually <3 $mi.^2$ drainage area) and other water courses which have been irretrievably altered to the extent that no appreciable assemblage of aquatic life can be supported; such waterways

generally include small streams in extensively urbanized areas, those which lie in watersheds with extensive drainage modifications, those which completely lack water on a recurring annual basis (*i.e.,* true ephemeral streams), or other irretrievably altered waterways. No formal biological criteria have been established for the LRW use designation.

Chemical, physical, and/or biological criteria are generally assigned to each use designation in accordance with the broad goals defined by each. As such the system of use designations employed in the Ohio WQS constitutes a "tiered" approach in that varying and graduated levels of protection are provided by each. This hierarchy is especially apparent for parameters such as dissolved oxygen, ammonia-nitrogen, temperature, and the biological criteria. For other parameters such as heavy metals, the technology to construct an equally graduated set of criteria has been lacking, thus the same water quality criteria may apply to two or three different use designations.

Heavy Metals and Aquatic Life Use Impairment

Water quality in Ohio has historically been affected by high concentrations of metals in the water column and sediments. The first effort to quantify the statewide miles of aquatic life use impairments associated with metals was in the 1988 Ohio 305(b) report. Of 7,045 stream miles monitored, 1,743 miles (24.7%) had metals as a major to moderate cause of aquatic life use impairment, frequently in combination with other stressors (Figure 1). The 1996 Ohio 305(b) report (Ohio EPA 1996) indicates that this has declined significantly to the extent that less than 500 miles (6.2%) of impairment is now associated with metals. Metals are now less frequently associated with aquatic life impairment (6th ranked cause) relative to other stressors than they were in 1988 (3rd ranked cause).

Recent and past regulatory efforts have thus been successful in reducing the effects of metals on streams in Ohio. The restoration of aquatic life uses in Ohio has been directly determined from biosurveys and is reflected in the statistics illustrated in Figure 1. Such information, which is a direct measure of the Clean Water Act goal of biological integrity, is an ideal empirical data set to examine associations between heavy metals and aquatic life use attainment/non-attainment and judge the efficacy of water quality criteria.

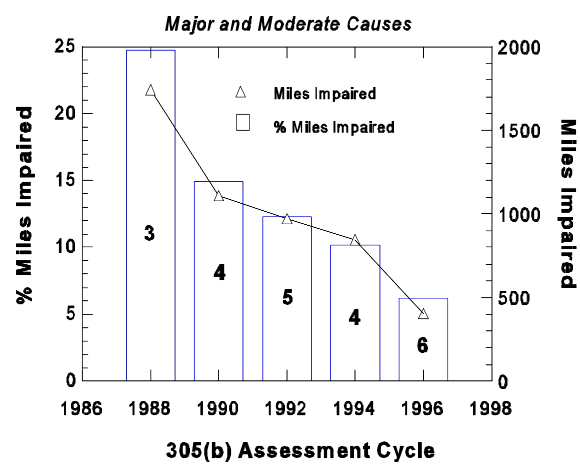


Figure 1. Percent and total miles of aquatic life use impairment associated with heavy metals (major and moderate magnitude causes). Relative rank among all causes for an assessment cycle indicated on each bar.

Examining Biocriteria/Chemical Criteria Relationships

The main purpose of an aquatic life-based chemical criterion is to protect the aquatic life of a stream, river, or lake in accordance with the goal of the designated use. Biocriteria are a direct measure of the aquatic community and as such represent a direct measure of designated aquatic life use attainment status. Having biocriteria provides the Ohio EPA with a unique method to examine whether existing and proposed chemical criteria are over or under-protective of a designated use. Previous studies have attempted to examine the U.S. EPA water quality criteria for certain heavy metals by comparing instream concentrations with different measures of aquatic community health and well-being. However, no study yet has utilized a fully calibrated and standardized system of biocriteria and a statewide chemical water quality and biological database for this purpose.

Many studies have shown the toxic effects of heavy metals on aquatic macroinvertebrates (summarized by Johnson *et al.* 1993) and fish (summarized in Sorenson 1991). In many instances in Ohio, adverse effects on ambient aquatic life have been strongly associated with exceedences of Ohio water quality criteria for various total recoverable metals as well as high concentrations of metals in sediments (*e.g.*, study of Rocky Fork Mohican River, Ohio EPA 1994). Reductions in metal concentrations are strongly related to recovery of previously impaired aquatic life uses across Ohio and this recovery is reflected in the aggregate in Figure 1.

The most toxic Cu species is the Cu^{+2} ion, although $CuOH^{+}$, CuCO3, and $Cu(OH)^{+2}$ have also been reported as being toxic (Sorenson 1991). The fraction of total copper as each form varies from site to site and from one time period to another (summarized in Sorenson 1991) in the same water. Toxicity of metals to fish also varies with fish size and stage, acclimation, and pattern of accumulation (Sorenson 1991). The mode of effect (*e.g.*, effects on various tissues, blood, immune system, behavior) can also vary with species, size, and concentration of metals (Sorenson 1991). Metals can affect all life stages, but are usually most limiting during reproductive and embryonic stages (Sorenson 1991). Mixtures of metals (*e.g.*, Cu and Zn) have been shown to have synergistic effects on toxicity to fish (Lewis 1978) and changes in fish behavior (James 1990).

Because of the complexity of the toxicity of metals to organisms the U. S. EPA has encouraged the use of biological data in decision making. Much of the initial use of biological data by the Ohio EPA was to help interpret water chemistry data collected during surveys. In fact, the U. S. EPA *Technical Guidance Manual for Performing Wastelaod Allocations* (U.S. EPA 1984) specifically states that it is preferable to coordinate chemical sampling with a biological survey:

> "As the numerical criteria of water quality standards are mostly derived from single species laboratory tests, an observation that a criterion is violated for a certain time period may provide no indication of how the integrity of the ecosystem is being affected. In addition to demonstrating the impairment of a use, a biological survey, coordinated with a chemical survey, can help in identifying culprint pollutants ***and in substantiating the criteria values.***"

Thus, U. S. EPA identified substantiation of chemical criteria values as one intended use of biological data.

Comparing Ambient Biosurvey Data and Total Recoverable Metals

Although essentially no ambient dissolved metals data exists in Ohio, there is a very large database of total recoverable metals data at or near sites where biosurvey data have been collected. The total recoverable criteria in Ohio's water quality standards for toxic metals have been at least partially protective of aquatic life designated uses as illustrated by Figure 1. There is ambient and laboratory evidence that shows, under some circumstances, the total recoverable water quality criteria for certain metals could be overly stringent. A preliminary examination of our database, for example, found that some ambient "chronic" exceedences for total recoverable copper, cadmium, lead, and zinc were at sites generally associated with attainment of the WWH criteria for the IBI and ICI in most Ohio ecoregions (Table 1). This may be partially related to a lower toxic metal fraction of total recoverable copper at these sites which supports a concern that the total recoverable copper criteria can be overly stringent under some circumstances.

Table 1. Exceedences of Ohio EPA's current criteria for total recoverable copper, cadmium, lead, and zinc ("chronic" or outside mixing zone 30-day average) at sites scoring ≥ an IBI of 40 or ≥ an ICI of 38.

Total Recoverable Metal	% Exceedences with IBI>= 40 (Total Number of Exceedences)	% Exceedences with ICI>= 38 (Total Number of Exceedences)
Copper	18.6 (370)	22.1 (231)
Cadmium	10.2 (88)	13.0 (54)
Lead	13.7 (183)	26.8 (123)
Zinc	15.8 (1235)	26.2 (846)

In addition, our analyses suggest that at low hardness levels (< 100) certain metals may not be as toxic as predicted from laboratory studies and as is reflected in the current metals criteria (Appendix Table 1). For example, proportionately more sites with chronic exceedences of total recoverable copper, whcih had IBI or ICI scores reflecting at least warmwater conditions, were found than expected with hardness levels less than 100 compared to sites with greater hardness levels ($P < 0.0001$, Appendix Table 1). This suggests that the toxicity of copper was less than predicted at low hardness values and accounts for some of the discrepancy between the ambient biological data and the water quality criteria for total recoverable copper. A similar pattern was observed for zinc, but was weak for lead and non-existent for cadmium (Appendix Table 1). Cadmium also had the fewest observed exceedences at sites with warmwater IBI and ICI scores indicating that the criteria for this metal was not as overly stringent as the other metals.

One purpose of this paper is to examine Ohio EPA s ambient data to see if there is some concentrations of a total recoverable metal observed under ambient conditions above which aquatic life is clearly at risk. Because this data is not "experimental" our result will not be a "criteria," but rather provides information in support of a risk assessment or risk management approach to protecting aquatic life. This approach depends on statistical methods for detecting patterns in environmental data and outliers in this data and then, based on these results, devising a tiered approach to managing "environmental uncertainty."

Data Used in This Report

The data used in this report includes total recoverable copper, cadmium, lead, and zinc collected during typical Ohio EPA ambient surveys from 1982 through 1994. The water chemistry data illustrated on each plot are individual grab samples. The locations of these sites were matched with biological data collected at the same or nearby river mile within the same year. The fish data used here typically represent the mean of one to three samples within a given year, collected between June 15 and October 15. The macroinvertebrate data are from artificial substrate samples, colonized for a six week period, plus a sample of the natural substrates. Biological indices were calculated as specified in Ohio's biocriteria manual (Ohio EPA 1988).

Wedge Relationships in Environmental Data

Scatter plots of water chemistry parameters versus a biological index such as the IBI or ICI often yield a "wedge" of data (Figure 2). The outer, sloped surface of points approximate the maximum concentrations that have been observed to occur at a given level of aquatic community performance as portrayed by an index such as the IBI or ICI. A line drawn on the outer surface of the data points so that 95% of the points fall to the left or beneath the line is referred to as the "95% line of best fit." For the IBI and copper, for example, this would represent the typically occurring *maximum* total recoverable copper concentrations at which a corresponding IBI value exists in the statewide database. Lines drawn perpendicular (*e.g.*, Line A of Figure 2) to the X-axis that intersect the IBI biocriterion for the EWH or WWH aquatic life use designation define the *maximum* total recoverable metal concentrations above which there is an increasing risk of non-attainment. For all the total recoverable metals plots in this paper, the metals were standardized to a hardness of 300 using the hardness-toxicity relationships for each metal in the current Ohio EPA water quality standards. The data used was collected from 1982 to 1994.

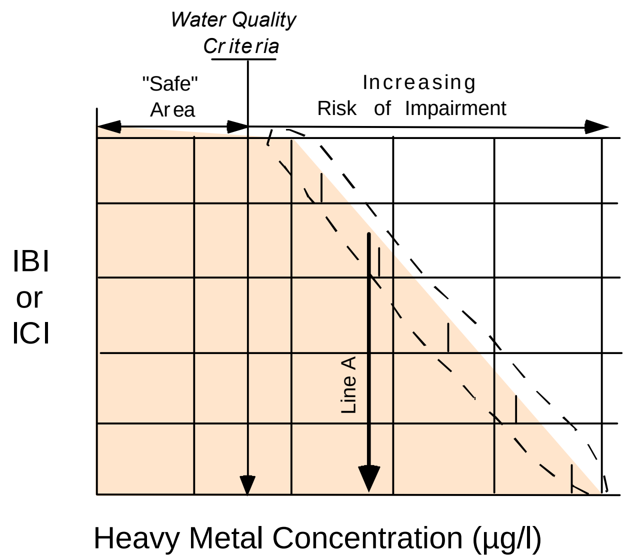


Figure 2. Hypothetical relationship between the IBI or ICI and a stressor, such as a toxic heavy metal. Points within the dashed area along the upper bound of points can be used to develop an upper bound regression.

Chi-square tests of independence were used to test whether the frequencies of IBI or ICI scores at sites are independent of total recoverable metal concentrations at these sites (Appendix Table 2). If the IBI or ICI are independent of the total recoverable metal concentrations, then one can conclude that the observed ambient concentrations of metals either do not strongly affect the IBI or ICI or are obscured by other environmental factors. If however, the IBI or ICI and total recoverable metals are statistically associated, further analyses should be performed to identify concentrations of total recoverable metals where there is a reasonable risk of harm to aquatic life. The point of reasonable risk is established by the 95% line of best fit. The upper thresholds or outer sloped surface of points in scatterplots, such as that illustrated in Figure 2, which produces a "wedge-shaped relationship," have been interpreted by Ohio EPA and others (Terrell *et al.* 1996) as being consistent with the hypothesis that the independent parameter (*e.g.*, total recoverable copper) is limiting the dependent parameter (*e.g.*, IBI) and that other factors further limit the dependent parameter below this outer boundary. Lines-of-best-fit drawn through an upper threshold of points formed by the scatterplots have typically been drawn "by eye" and have not been statistically derived. Rankin and Yoder (in review) have, however, statistically derived 95% lines for other datasets. For example we have statistically derived the outer boundary of points formed by the maximum number of fish species (from reference sites) plotted vs. stream size as defined by watershed area (Figure 3) using a method described by Blackburn *et al.* 1992. In this case the line-of-best-fit drawn by eye and lines derived using a statistical method were reasonably close (Yoder and Rankin 1995, Rankin and Yoder, in review). The relationship demonstrated by the scatterplot of these two variables is predictable and is based on independent findings by numerous other investigators, *i.e.*, the maximum number of fish species are known to increase with stream size. The outer bound of these data, however, generally follow a predictable pattern and exhibit low variability.

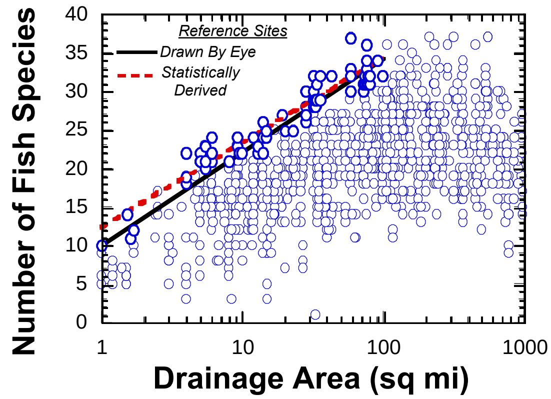


Figure 3. Relationship between fish species richness and drainage area for Ohio reference sites. The "Maximum Species Richness (95%) Lines" were derived by eye and by a 95th percentile regression method.

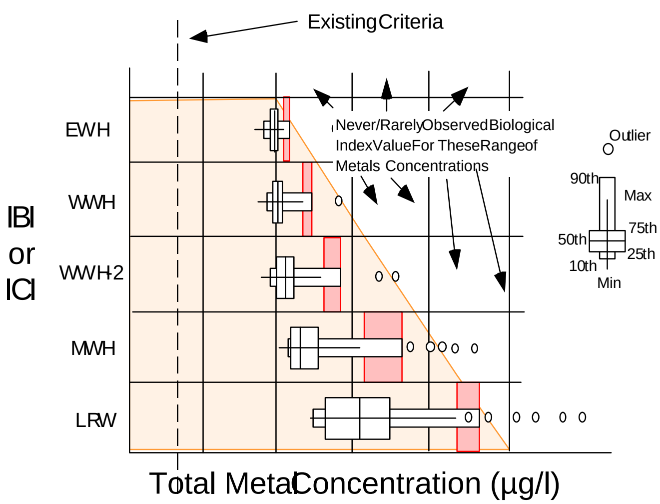


Figure 4. Hypothetical relationship between the IBI or ICI and a stressor such as a toxic heavy metal. Box and whisker/percentile plots are calculated on the upper 10% of the data in each narrative IBI or ICI category.

For relationships with more variability or that exhibit non-normal distributions (*e.g.*, the data are highly skewed) deriving and interpreting lines-of-best-fit is more difficult. This seems to be the case with the total recoverable metals:IBI relationship. Some of the variability in the outer bound of the IBI:metals plots we have observed are partly related to the variability in the toxicity of a metal related to the fraction of the total metal that is toxic as well as other factors that may affect metal toxicity (*e.g.*, pH, hardness, alkalinity). Rather than generating a "continuous" 95th percentile regression line for such data, a more reasonable approach is to first focus on identifying outliers and extreme values (99.5th percentiles) where we are more confident that such values represent an undue risk to aquatic life. We hypothesize that as we accumulate dissolved metals data the outer bound of a IBI: dissolved metal relationship will likely become less variable than for the total recoverable metals and a more precise 95th percentile regression line could be calculated.

The method to identify outliers and extremes in the data is to cluster the distribution of the independent variable (*e.g.*, copper) by ranges of IBI or ICI scores that correspond to established narrative ratings (*e.g.*, exceptional, good, fair, etc.) and the tiered system of aquatic life use designations employed by Ohio EPA. Figure 4 illustrates a hypothetical graph for a total recoverable metal versus the IBI or ICI.

The rest of the analyses on water column concentrations of metals in this paper were performed on total recoverable metals standardized to a 300 hardness on the basis of the slope use to derive Ohio's current water quality criteria for each metal. The upper tenth percentile of the metals data in each IBI or ICI category is used to identify the outliers and extremes on each distribution. Total recoverable metal concentrations at these extremes or above is considered the range of concentrations where we are uncertain whether biological performance can be maintained. Box-and-whisker plots and percentile plots are then used to illustrate the upper, empirically observed values for the independent variable compared to the narrative ranges of the IBI. Outliers in the data are those points that are greater than the upper quartile (UQ: 75th percentile) plus 1.5 times the interquartile distance (distance between the 25th and 75th percentiles: UQ - LQ). The other statistic used to describe extreme values is the 99.5th percentile of all the data within an IBI or ICI narrative category (illustrated as the 95th percentile of the upper 10 percent of the data on Figure 4). Where such data is strongly skewed the 95th percentile can be greater than the "maximum" value when outliers are excluded. The following sections will derive such statistics for four metals where sufficient data exists to derive relationships between the biota and total recoverable metals: copper, lead, cadmium, and zinc.

Total Recoverable Copper

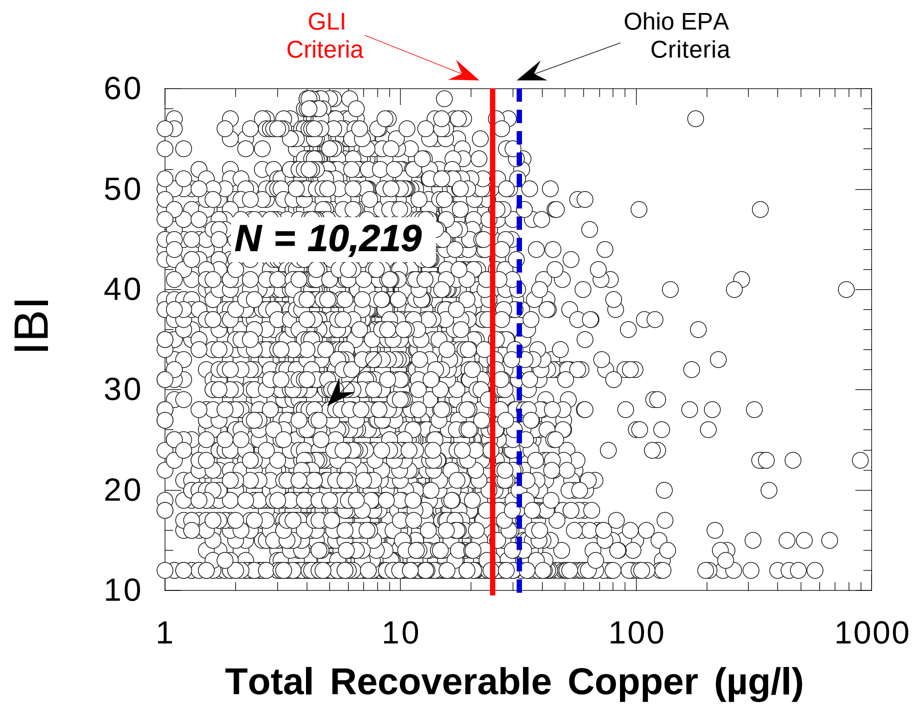
The data used here represents 10,219 grab samples of total recoverable copper data at 2,165 sites with IBI and 7,321 grab samples of total recoverable copper data at 1,436 sites with ICI data collected from 1982-1994. Figure 5 illustrates scatter plots of total recoverable copper (on a log scale) standardized to a hardness of 300 versus the ICI (right) and IBI (left). Appendix Table 2-1 represents a chi-square test of association of the rows and columns of the table under the hypothesis: H_o: the frequency of occurrence of IBI (top) or ICI (bottom) scores is independent of total recoverable copper.

The chi-square statistic for the total recoverable copper and both the IBI and ICI are highly significant (chi-square: IBI - 402, $P < 0.0001$; ICI - 274, $P < 0.0001$; Appendix Table 2-1) which suggests an association between total recoverable copper and both the IBI and ICI. An examination of expected frequencies versus observed frequencies shows fewer than expected sites with high IBIs or ICIs and high total recoverable copper and more sites than expected with low IBIs or ICIs and high total recoverable copper. This is consistent with a hypothesis of increasing risk of impairment to aquatic life at the observed extreme concentrations of total recoverable copper at the more sensitive IBI and ICI ranges.

Figure 6 illustrates box and whisker plots focusing on the highest 10 percent of the total recoverable copper data in narrative IBI and ICI ranges used in the identification of extreme values. The ranges at or above which there may be an impairment of aquatic life are identified as the maximum total recoverable copper values (excluding outliers) and the 99.5th percentile copper values for each IBI or ICI range. These are summarized in Table 3. For sensitive streams (*e.g.*, the EWH range of the IBI) the difference between these two points is small. For less sensitive streams (*e.g.*, the MWH range of the IBI or ICI) the difference between these two points is larger. For EWH and WWH streams these statistics are near the existing Ohio EPA and GLWQI total recoverable copper criteria.

Table 3. Maximum total recoverable copper values (excluding outliers) and 99.5th percentile values of total recoverable copper data by IBI and ICI ranges representative of various typical biocriteria).

Narrative Range/ General Aquatic Life Use	ICI Range	IBI Range	Maximum Total Recoverable Copper (ug/l)		99.5th Percentile Total Recoverable Copper (ug/l)	
			ICI	IBI	ICI	IBI
Excellent/EWH	48-60	50-60	28.8	33.1	42.4	32.7
Good/WWH	38-46	40-49	31.1	33.6	42.4	60.9
Fair/MWH-WWH	14-36	30-39	58.3	46.4	80.2	74.5
Poor/MWH	2-12	20-29	108.4	60.8	314.6	123.7



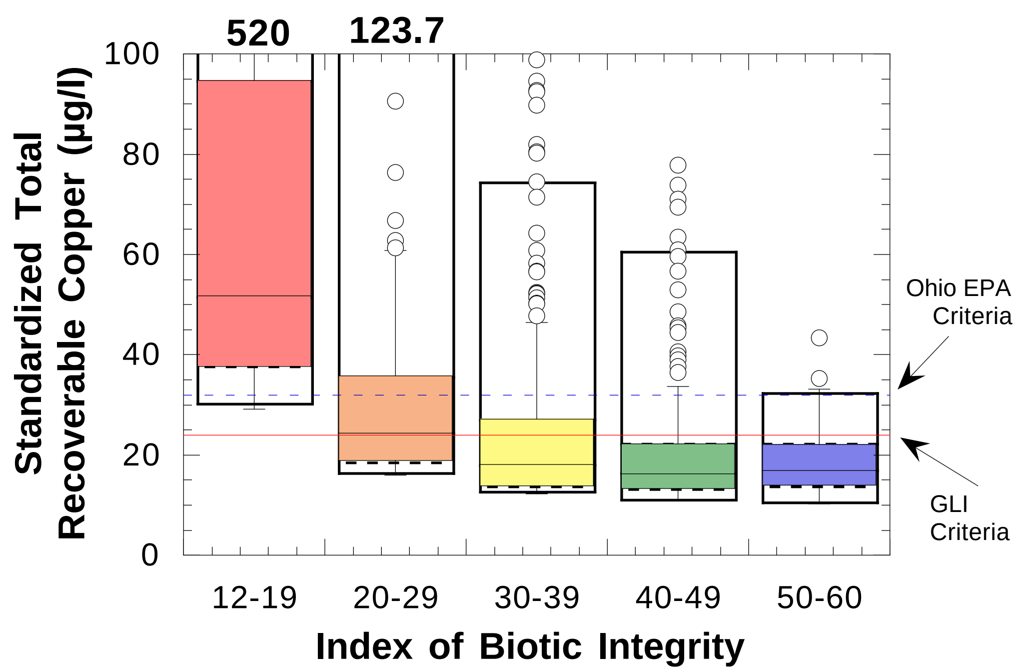
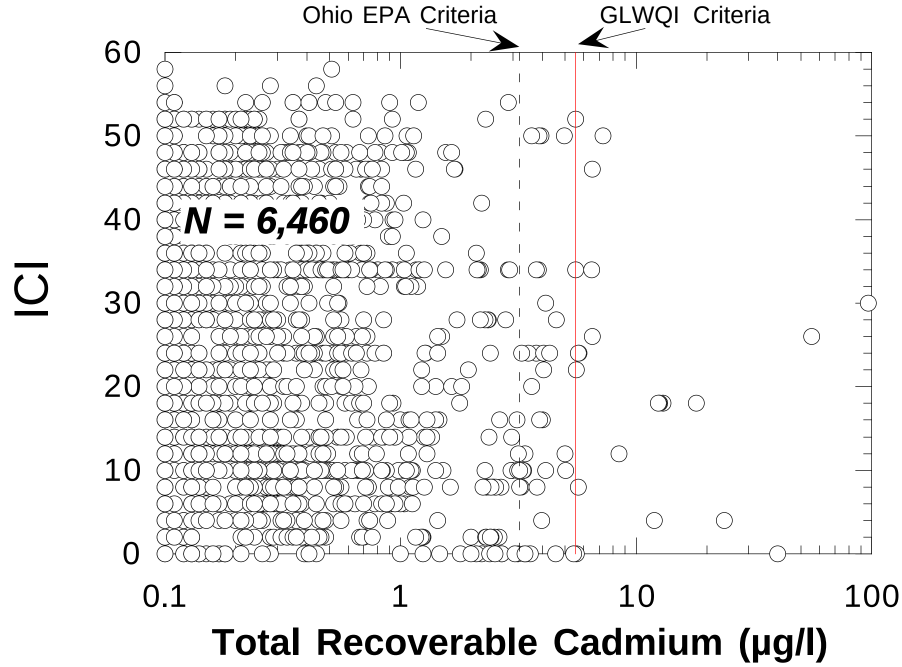


Figure 5. Scatter plot of IBI versus total recoveral copper standardized to hardness 300 (Top) and box plot of total recoverable copper (upper ten percent of values standardized to hardness 300) by IBI range (Bottom).



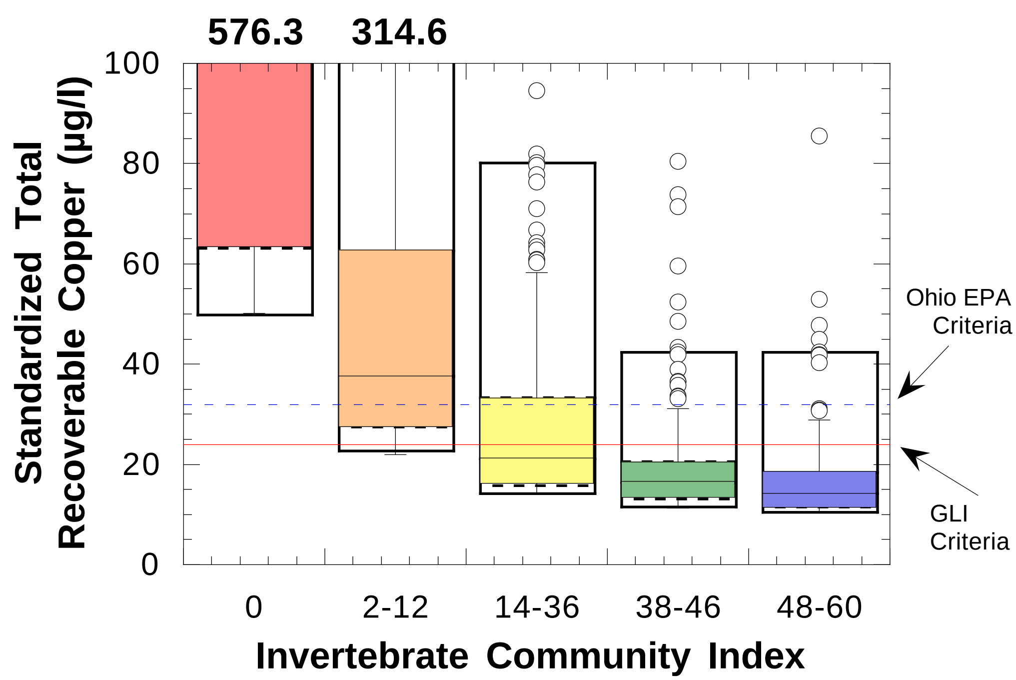


Figure 6. Scatter plot of ICI versus total recoveral copper standardized to hardness 300 (Top) and box plot of total recoverable copper (upper ten percent of values standardized to hardness 300) by ICI range (Bottom).

Total Recoverable Cadmium

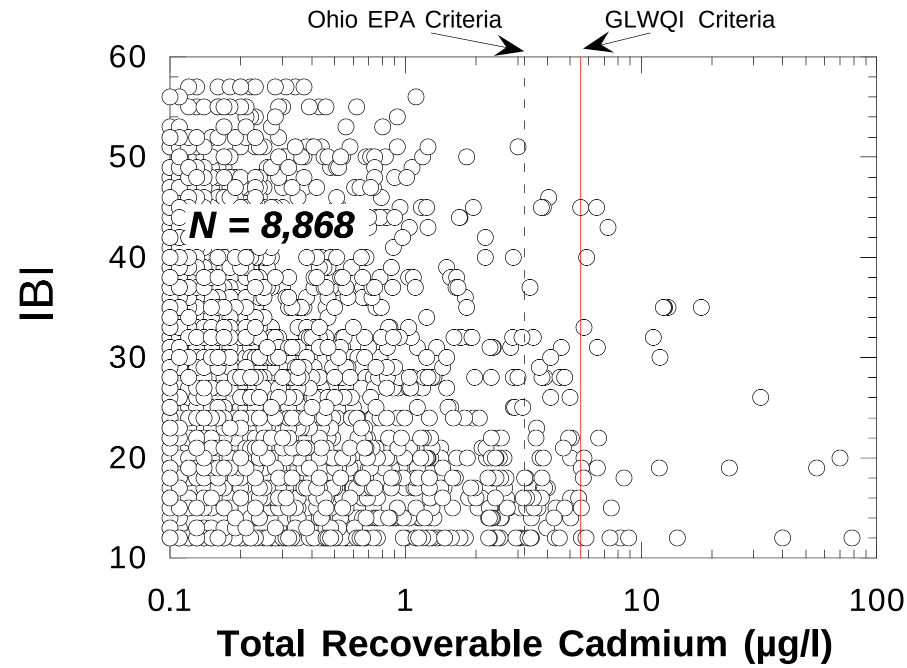
The data used here represents 8,868 grab samples of total recoverable copper data at 1,924 sites with IBI and 6,460 grab samples of total recoverable copper data at 1,307 sites with ICI data collected from 1982-1994. Figure 7 illustrates scatter plots of total recoverable cadmium (on a log scale) standardized to a hardness of 300 versus the ICI (top) and IBI bottom). Appendix Table 2-2 represents a chi-square test of association of the rows and columns of the table under the hypothesis: H_o: the frequency of occurrence of IBI (top) or ICI (bottom) scores is independent of total recoverable cadmium.

The chi-square statistic for the total recoverable cadmium and both the IBI and ICI are highly significant (chi-square: IBI - 435, $P < 0.0001$; ICI - 232, $P < 0.0001$; Appendix Table 2-2) and suggests an association between total recoverable copper and both the IBI and ICI. An examination of expected frequencies versus observed frequencies shows fewer than expected sites with high IBIs or ICIs and high total recoverable cadmium and more sites than expected with low IBIs or ICIs and high total recoverable copper. This is consistent with a hypothesis of increasing risk of impairment to aquatic life at the observed extreme concentrations of total recoverable copper at the more sensitive IBI ranges. Of the four metals considered here, cadmium is the metal with the fewest proportion of sites at which warmwater biotic condition is achieved and exceedances of the “chronic” metal criteria occur. As illustrated in Figure 8, it is also the metal where the maximum and 95.5th percentile are signficantly below both the existing Ohio cadmium criteria and the proposed GLWQI criteria.

Figure 8 illustrates box and whisker plots focusing on the highest 10 percent of the total recoverable cadmium data in narrative IBI and ICI ranges used in the identification of extreme values. The ranges at or above which there may be an impairment of aquatic life are identified as the maximum total recoverable copper values (excluding outliers) and the 99.5th percentile cadmium values for each IBI or ICI range. These are summarized in Table 4. For sensitive streams (*e.g.*, the EWH range of the IBI) the difference between these two points is small. For less sensitive streams (*e.g.*, the MWH range of the IBI or ICI) the difference between these two points is larger.

Table 4. Maximum total recoverable cadmium values (excluding outliers) and 99.5th percentile values of total recoverable cadmium data by IBI and ICI ranges representative of various typical biocriteria).

Narrative Range/ General Aquatic Life Use	ICI Range	IBI Range	Maximum Total Recoverable Copper (ug/l)		99.5th Percentile Total Recoverable Copper (ug/l)	
			ICI	IBI	ICI	IBI
Excellent/EWH	48-60	50-60	1.06	0.92	3.60	1.18
Good/WWH	38-46	40-49	0.95	0.96	0.95	2.10
Fair/MWH-WWH	14-36	30-39	2.41	1.35	5.6	4.13
Poor/MWH	2-12	20-29	5.04	2.20	5.0	4.14



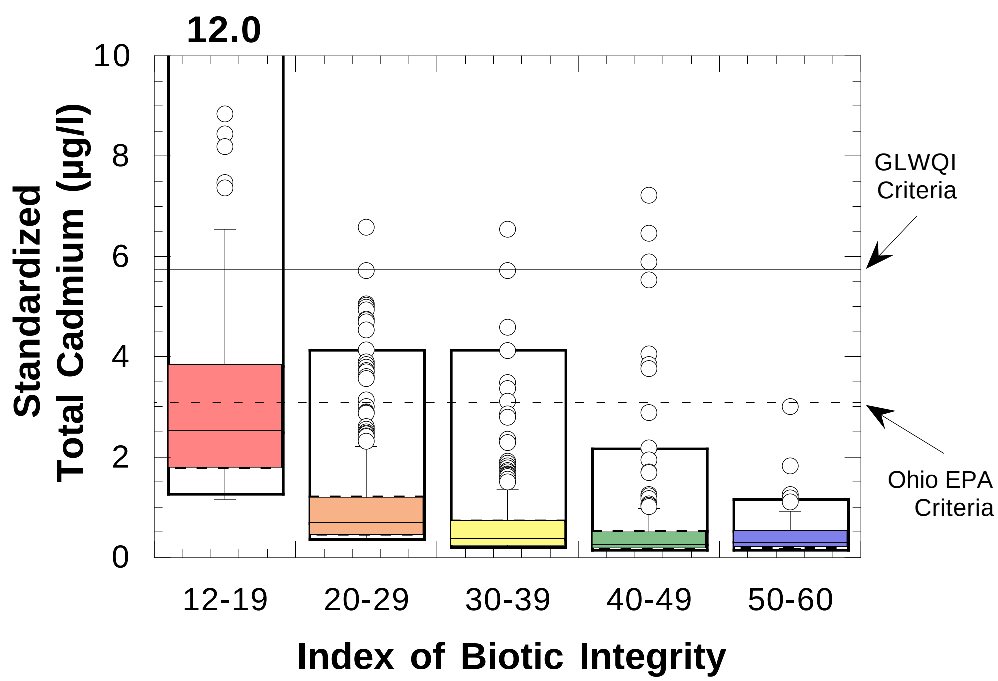
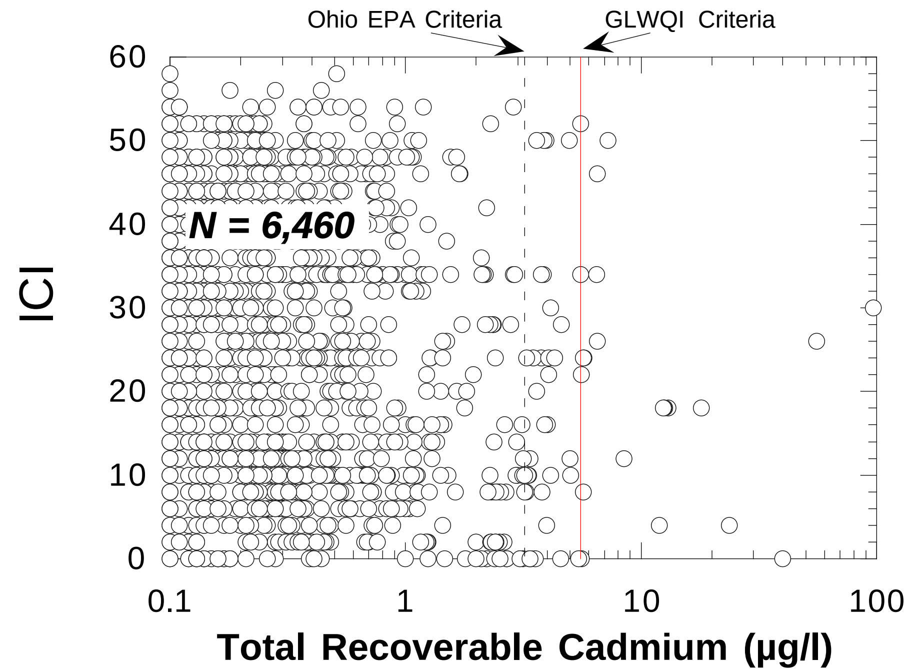


Figure 7. Scatter plot of IBI versus total recoveral cadmium standardized to hardness 300 (Top) and box plot of total recoverable cadmium (upper ten percent of values standardized to hardness 300) by IBI range (Bottom).



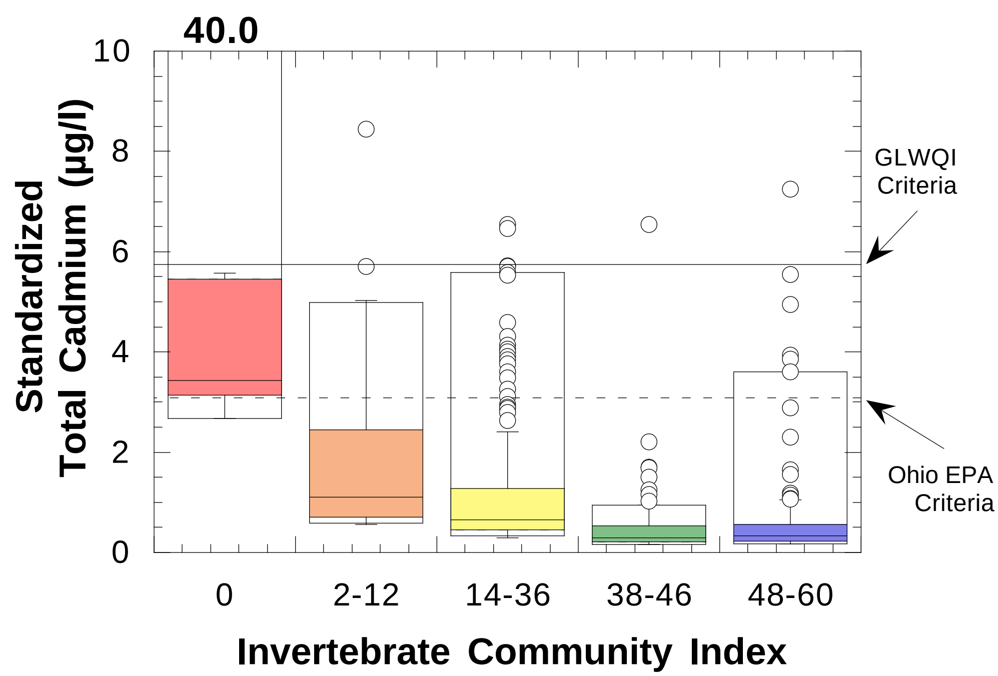


Figure 8. Scatter plot of ICI versus total recoveral cadmium standardized to hardness 300 (Top) and box plot of total recoverable cadmium (upper ten percent of values standardized to hardness 300) by ICI range (Bottom).

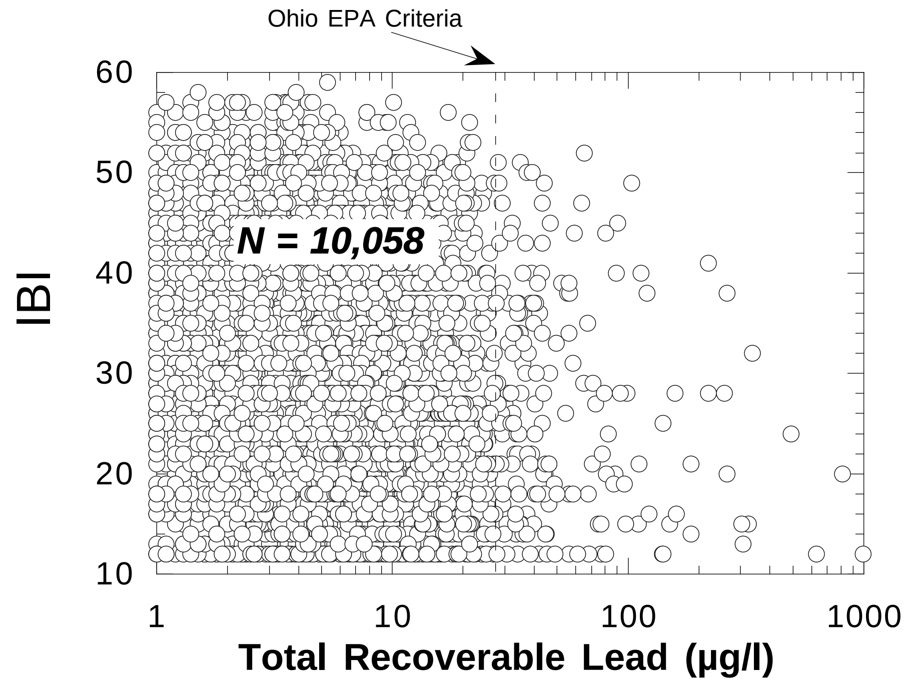
Total Recoverable Lead
The data used here represents 10,058 grab samples of total recoverable lead data at 2,132 sites with IBI and 7.292 grab samples of total recoverable lead data at 1,426 sites with ICI data collected from 1982-1994. Figure 9 illustrates scatter plots of total recoverable lead (on a log scale) standardized to a hardness of 300 versus the ICI (top) and IBI bottom). Appendix Table 2-3 represents a chi-square test of association of the rows and columns of the table under the hypothesis: H_o: the frequency of occurrence of IBI (top) or ICI (bottom) scores is independent of total recoverable lead.

The chi-square statistic for the total recoverable lead and both the IBI and ICI are highly significant (chi-square: IBI - 435, P < 0.0001; ICI - 232, P < 0.0001; Appendix Table 2-3) and suggests an association between total recoverable lead and both the IBI and ICI. An examination of expected frequencies versus observed frequencies shows fewer than expected sites with high IBIs or ICIs and high total recoverable lead and more sites than expected with low IBIs or ICIs and high total recoverable lead. This is consistent with a hypothesis of increasing risk of impairment to aquatic life at the observed extreme concentrations of total recoverable lead at the more sensitive IBI ranges.

Figure 9 illustrates box and whisker plots focusing on the highest 10 percent of the total recoverable lead data in narrative IBI and ICI ranges used in the identification of extreme values. The ranges at or above which there may be an impairment of aquatic life are identified as the maximum total recoverable lead values (excluding outliers) and the 99.5th percentile lead values for each IBI or ICI range. These are summarized in Table 5. For sensitive streams (*e.g.*, the EWH range of the IBI) the difference between these two points is small. For less sensitive streams (*e.g.*, the MWH range of the IBI or ICI) the difference between these two points is larger.

Table 5. Maximum total recoverable lead values (excluding outliers) and 99.5th percentile values of total recoverable lead data by IBI and ICI ranges representative of various typical biocriteria).

Narrative Range/ General Aquatic Life Use	ICI Range	IBI Range	Maximum Total Recoverable Copper (ug/l)		99.5th Percentile Total Recoverable Copper (ug/l)	
			ICI	IBI	ICI	IBI
Excellent/EWH	48-60	50-60	28.6	22.0	33.7	28.1
Good/WWH	38-46	40-49	30	27.1	49.8	43.6
Fair/MWH-WWH	14-36	30-39	36.2	34.2	44.8	42.2
Poor/MWH	2-12	20-29	58.4	40.4	86.6	81.1



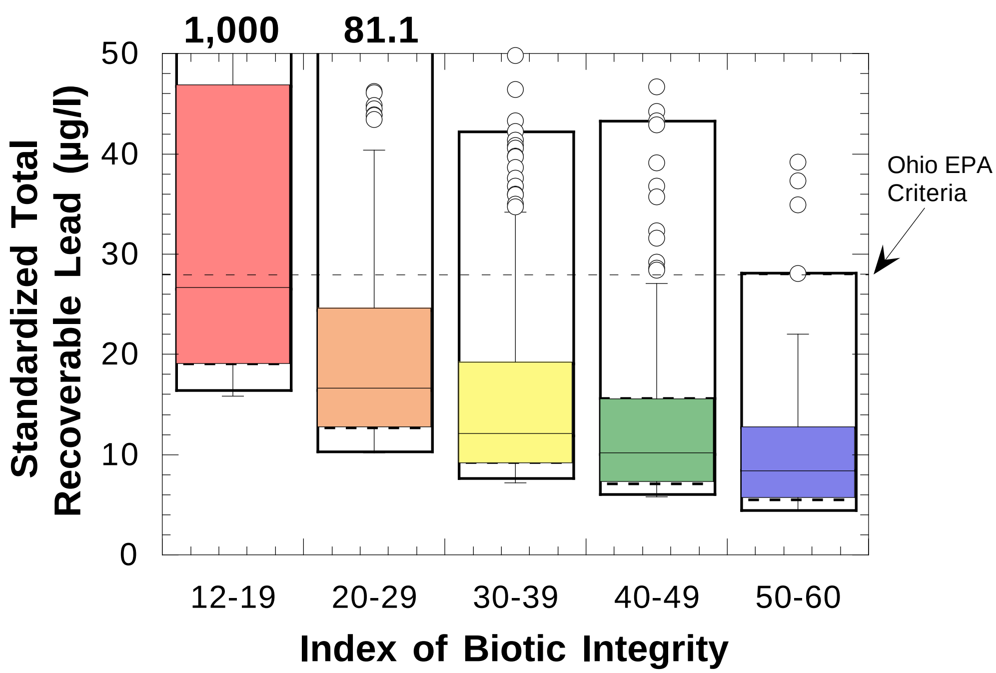
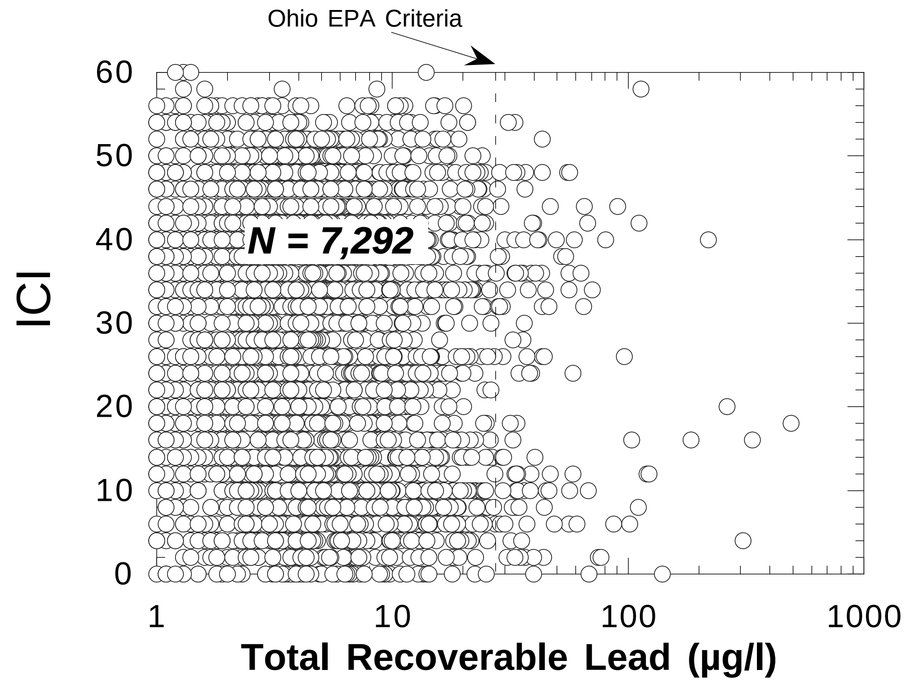


Figure 9. Scatter plot of IBI versus total recoverable lead standardized to hardness 300 (Top) and box plot of total recoverable lead (upper ten percent of values standardized to hardness 300) by IBI range (Bottom).



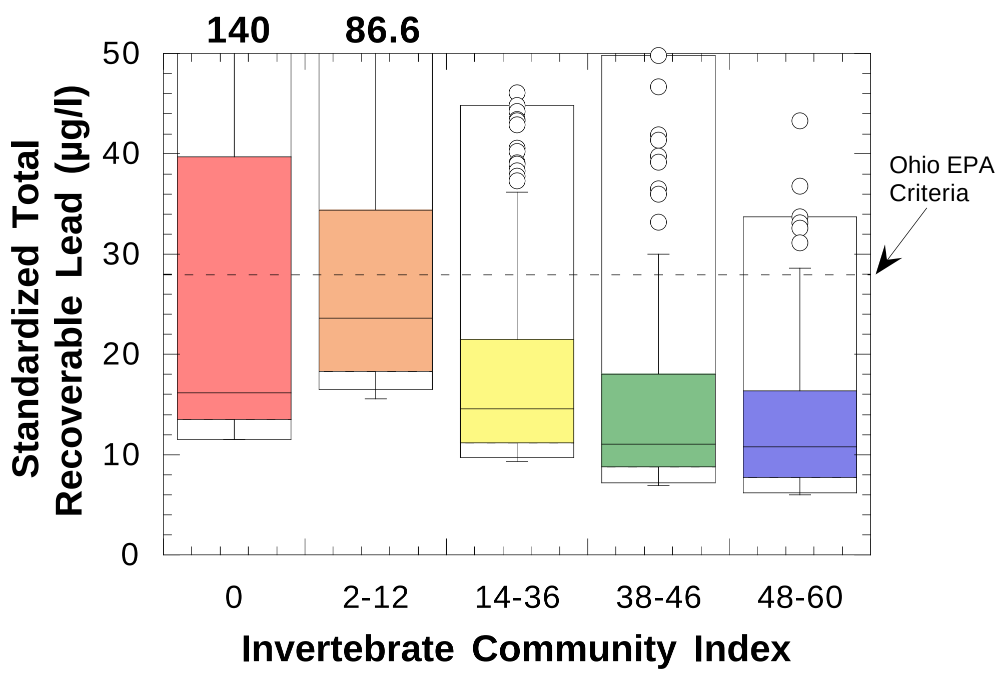


Figure 10. Scatter plot of ICI versus total recoverable lead standardized to hardness 300 (Top) and box plot of total recoverable lead (upper ten percent of values standardized to hardness 300) by ICI range (Bottom).

Total Recoverable Zinc

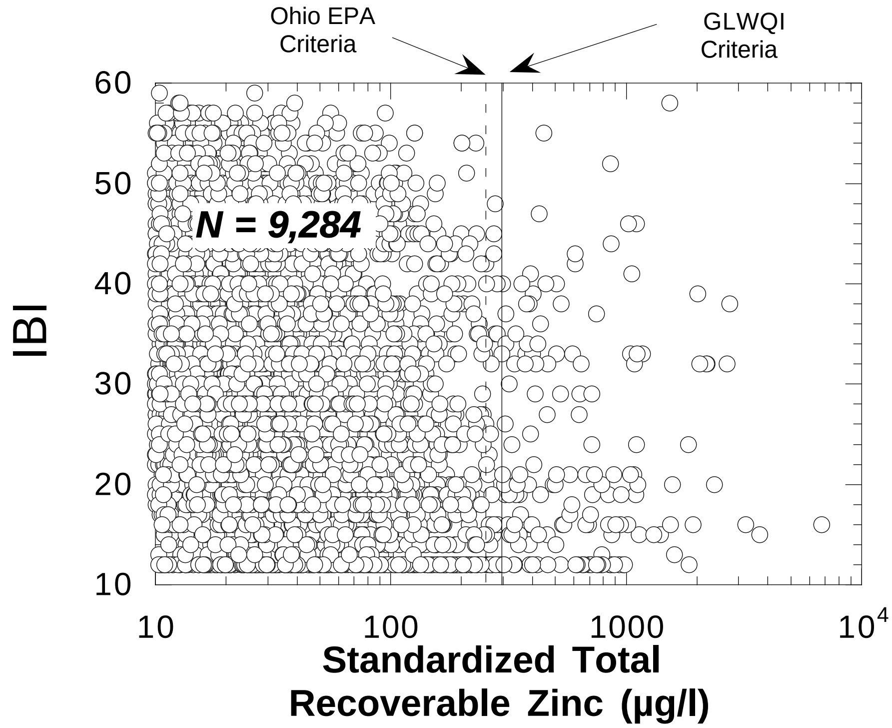
The data used here represents 9,824 grab samples of total recoverable zinc data at 2,101 sites with IBI and 6,948 grab samples of total recoverable zinc data at 1,385 sites with ICI data collected from 1982-1994. Figure 9 illustrates scatter plots of total recoverable zinc (on a log scale) standardized to a hardness of 300 versus the ICI (top) and IBI bottom). Appendix Table 2-4 represents a chi-square test of association of the rows and columns of the table under the hypothesis: H_o: the frequency of occurrence of IBI (top) or ICI (bottom) scores is independent of total recoverable zinc.

The chi-square statistic for the total recoverable zinc and both the IBI and ICI are highly significant (chi-square: IBI - 745, $P < 0.0001$; ICI - 470, $P < 0.0001$; Appendix Table 2-4) and suggests an association between total recoverable zinc and both the IBI and ICI. An examination of expected frequencies versus observed frequencies shows fewer than expected sites with high IBIs or ICIs and high total recoverable zinc and more sites than expected with low IBIs or ICIs and high total recoverable zinc. This is consistent with a hypothesis of increasing risk of impairment to aquatic life at the observed extreme concentrations of total recoverable zinc at the more sensitive IBI ranges.

Figure 10 illustrates box and whisker plots focusing on the highest 10 percent of the total recoverable zinc data in narrative IBI and ICI ranges used in the identification of extreme values. The ranges at or above which there may be an impairment of aquatic life are identified as the maximum total recoverable zinc values (excluding outliers) and the 99.5th percentile zinc values for each IBI or ICI range. These are summarized in Table 6. For sensitive streams (*e.g.*, the EWH range of the IBI) the difference between these two points is small. For less sensitive streams (*e.g.*, the MWH range of the IBI or ICI) the difference between these two points is larger.

Table 6. Maximum total recoverable zinc (standardized to 300 hardness) values (excluding outliers) and 99.5th percentile values of total recoverable zinc data by IBI and ICI ranges representative of various typical biocriteria).

Narrative Range/ General Aquatic Life Use	ICI Range	IBI Range	Maximum Total Recoverable Copper (ug/l)		99.5th Percentile Total Recoverable Copper (ug/l)	
			ICI	IBI	ICI	IBI
Excellent/EWH	48-60	50-60	209.2	158.2	399.2	230.0
Good/WWH	38-46	40-49	169.6	200.0	326.8	360.5
Fair/MWH-WWH	14-36	30-39	262.2	266.1	501.7	645.7
Poor/MWH	2-12	20-29	690	267.8	1104.4	632.1



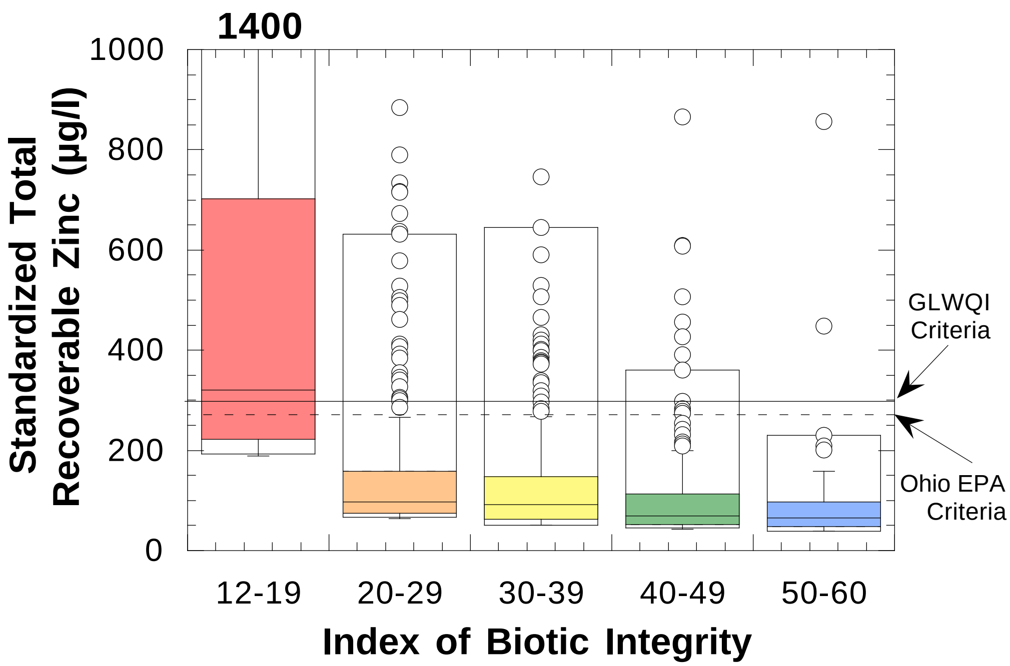
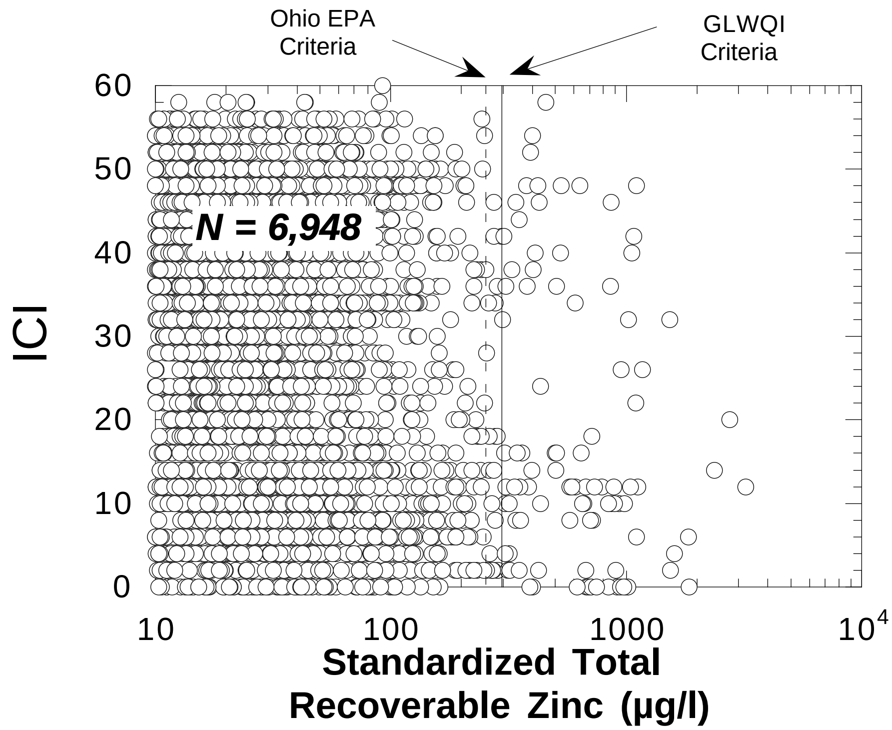


Figure 11. Scatter plot of IBI versus total recoverable zinc standardized to hardness 300 (Top) and box plot of total recoverable zinc (upper ten percent of values standardized to hardness 300) by IBI range (Bottom).



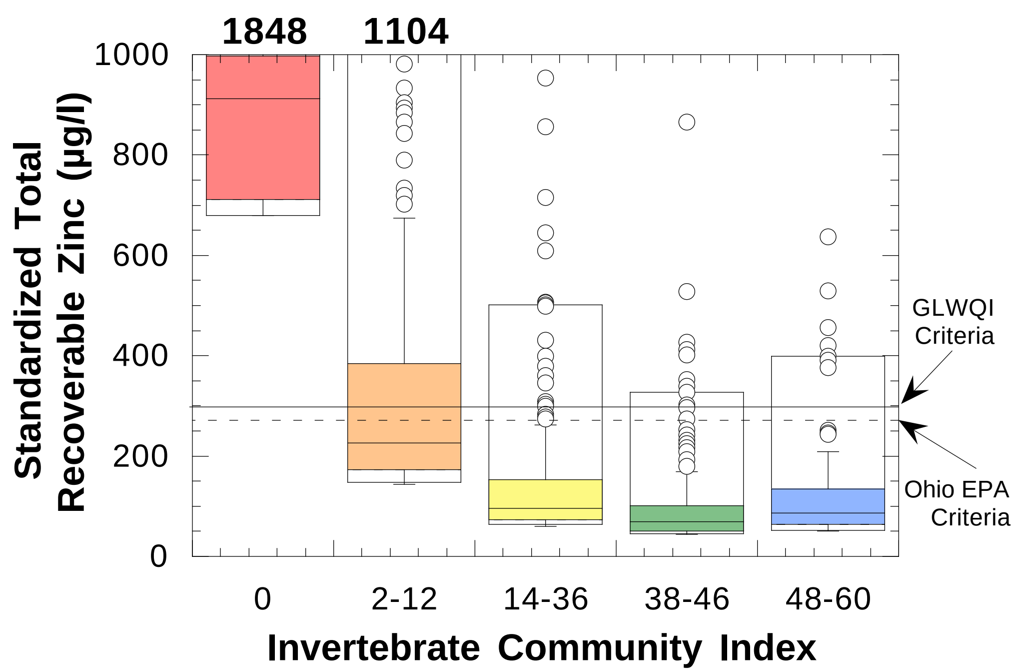


Figure 12. Scatter plot of ICI versus total recoverable zinc standardized to hardness 300 (Top) and box plot of total recoverable zinc (upper ten percent of values standardized to hardness 300) by ICI range (Bottom).

Inferring Cause and Effect Relationships in IBI: Metals Associations
The upper thresholds or outer bounds of points in wedge shaped relationships have been interpreted by Ohio EPA and others (Terrell *et al*. 1996) as being consistent with the hypothesis that the independent parameter (*e.g.*, total recoverable copper) is likely limiting the dependent parameter (*e.g.*, IBI) and that other factors further limit the dependent parameter below this outer bound. Using only the upper 10% (and upper 2%) of total copper values in various IBI ranges, we tested the possibility that some other parameter may actually be limiting the biota and may simply be correlated with metal in question. We calculated correlation coefficients between the IBI, total recoverable copper, and a suite of other chemical parameters typically collected during our intensive surveys, plus the QHEI, a measure of physical habitat quality important to aquatic life. Total recoverable copper was the only measure, when paired with the IBI that explained more than 40% of the variation in the relationship (Table 4). The only other parameter that explained more than 30% of the variation in the IBI was habitat (the QHEI) and this parameter was not strongly related to total recoverable copper (Table 4).

The concern of the agency is related to the potential occurrence of situations where concentrations of total metals are beyond where we have typically observed biological communities attaining their aquatic life use, i.e., where uncertainty is high. Where the frequency of high total recoverable metals values are higher there are signficantly fewer high IBI scores (see chi-square test). In other words, we ask the question “Why haven’t we seen many, if any, attaining biological scores above a total recoverable metal concentration of X?” This uncertainty is sufficient justification to require more information (through increased monitoring) on the status of the ambient conditions. This increased data should result in an increased knowledge of the relation between total metals, dissolved metals, and the biological condition of streams. Nevertheless, the correlation analyses illustrated in Table 4 can provide insight into the likelihood whether a particular metal has a strong or weak affect on aquatic life under ambient conditions.

Advantages of Including Biocriteria in Chemical Criteria Derviation
Using the relationships portrayed in the preceding examples in combination with the traditional toxicity-based chemical criteria (Stephan *et al.* 1985) provides an effective way to evaluate and/or establish chemical water quality criteria. Toxicity derived criteria, because of uncertainties associated with comparatively limited data, may well be under or over-protective. A biological data can function as an effective "reality check" on toxicity derived criteria. Some further advantages of using biocriteria to evaluate toxicity-based chemical criteria include the following:

1) Biological criteria can be used to adjust chemical criteria to account for the differing sensitivities between aquatic life use designations and ecoregions. The toxicity-based procedure is less able to produce such stratified water quality criteria because of frequently limited databases and the inability of the technique to discriminate the differences between the different uses that are accounted for by the biological criteria.

2) The biological criteria method incorporates the effects of other overlying stressors that are present to varying degrees in the ambient environment, thus additive effects from other substances and impacts are automatically incorporated; and,

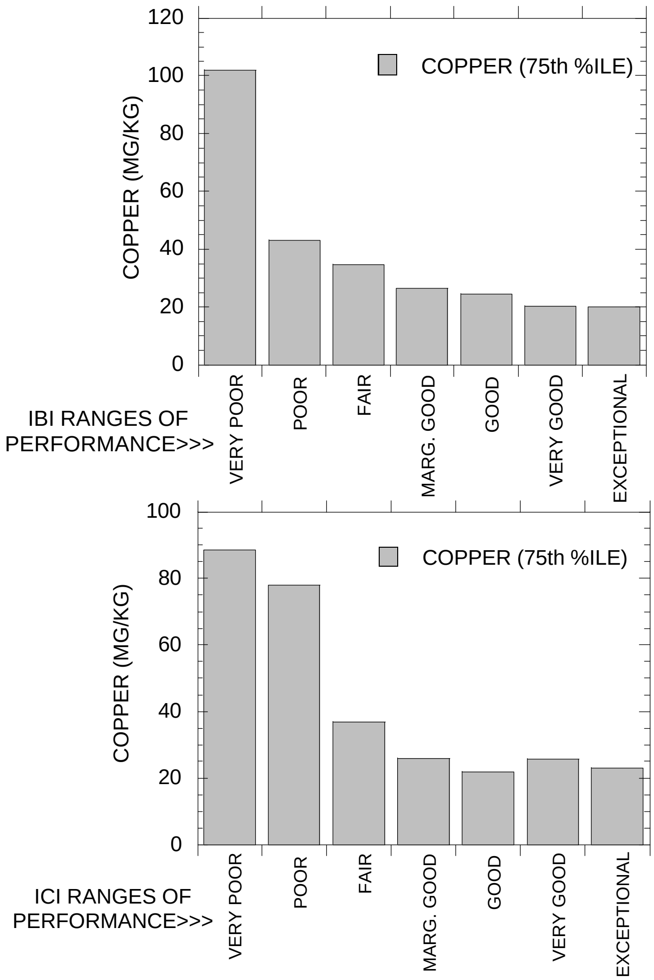


Figure 13. Total copper (mg/kg) in sediment from the statewide Ohio EPA database compared to the gradient of aquatic community performance defined by the IBI (upper) and ICI (lower).

3) Instream data can be used to examine the response of biological communities to a given parameter, thus uncertainties associated with applying different forms of the chemical (*e.g.,* total vs. dissolved) or with other parameters that affect a metal's toxicity (e.g., hardness, alkalinity) can be "ground-truthed" with empirical data.

Using Biocriteria To Evaluate Sediment Contamination

Ohio EPA has collected sediment chemistry data as a part of the biosurvey process which has resulted in a robust statewide database. Recently it has become evident that sediment chemistry data reveals more about the history of chemical contamination than do water column analyses alone. Presently, there are no readily available sediment criteria that are directly linked to adverse effects on aquatic life. Kelly and Hite (1984) in Illinois developed thresholds for aquatic life for selected heavy metals, and the Ontario guidelines (Persuad *et al.* 1991) have also been developed for the effects of sediment on aquatic life. Neither method alone is viewed as sufficient for evaluating the potential of contaminated sediments to contribute to aquatic life use impairment in Ohio streams.

In order to address this deficiency, comparisons between biological sampling and sediment chemistry results have recently been made to determine concentrations at which biological criteria or specific indicators reflect varying degrees of attainment or non-attainment. Figure 13 shows the occurrence of 75th percentile values of total copper in sediment with the narrative ratings of aquatic community performance based on the IBI and ICI. The IBI shows only a slight tendency to be more sensitive to copper contamination with WWH (good) attainment occurring at levels less than 25 mg/kg and EWH (exceptional) attainment occurring at 20 mg/kg and lower. Figure 14 shows another method that compares the number of sites at which IBI scores greater than 40 (typical WWH value) and 50 (typical EWH value) with ranges of *total* toxic metals (As, Cd, Cu, Cr, Ni, Pb, and Zn) in sediment. EWH attainment declines markedly at levels greater than 150 mg/kg and WWH attainment declines above 200 mg/kg. Figure 14 also shows that the percentage of fish with gross external anomalies including deformities, eroded fins and other body parts, lesions, and tumors (DELT anomalies) increase at higher total toxic metal sediment concentrations with moderate departures from reference conditions occurring above 100 mg/kg and extreme departures above 200 mg/kg. The good relationships between the biological indicators and the degree of sediment contamination by heavy metals are a reflection of the history of metals loadings. These loadings and their effects can easily escape accurate quantification by chemical sampling alone. Box and whisker plots of sediment concentrations for copper, cadmium, lead, and zinc by ranges of the IBI abd ICI are illustrated in Appendix Figures 1 through 4. The 95th percentiles by ranges of the IBI and ICI are listed in Appendix Table ??.

Application To the GLWQG

The application of these methodologies to the GLWQG seems to be useful in three general areas: 1) ground truthing and/or fine tuning GLWQG and/or existing Ohio EPA water quality criteria as described previously; 2) providing insight into the effects of total metals that result from a permit based on dissolved rather than total recoverable form; and, 3) in monitoring the effectiveness of the GLWQG approach to setting and applying water quality criteria for the protection of aquatic life in general.

The analyses portrayed in Figures 5 through 12 and Tables 3 through 6 could be used to evaluate the risk of any increases in total recoverable metals concentration that result from using various translators (*i.e.,* factors used to translate from dissolved to total recoverable) to determine an effective water quality criterion. Concentration thresholds based on the same type of analysis which resulted in Tables 3 - 6 could represent triggers above which an effective total recoverable criterion could not be raised without: 1) biosurvey monitoring to ensure the aquatic life use is protected at relatively high discharges of total metals (“maximum” value trigger) or 2) a demonstration that the use is currently met plus additional monitoring during the life of a permit (99.5th percentile trigger) where the concentrations will be extreme compared to past observations or where sediment contamination is a concern. Sediment thresholds resulting from analyses like those portrayed in Figures 7 and 8 and Appendix Figures 1-4 could be used to evaluate the existing setting (*i.e.,* what is the extent of any sediment contamination?) and whether any increases in heavy metals loadings resulting from the

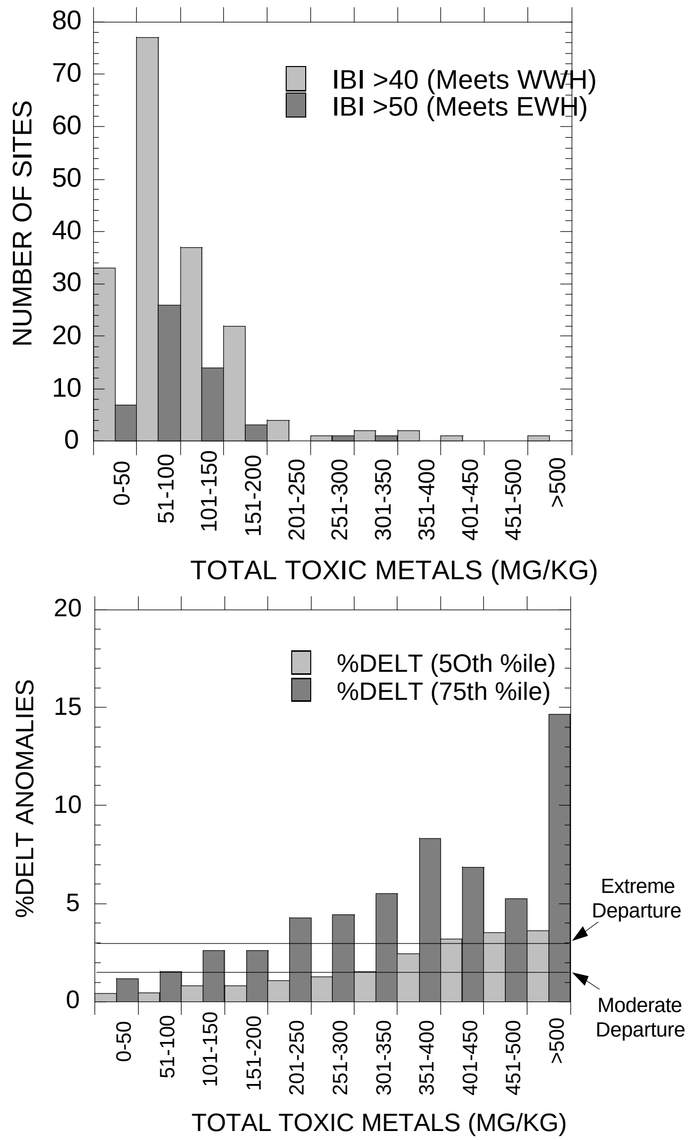


Figure 14. The number of sites with IBI values meeting the EWH and WWH biocriteria by ranges of total toxic heavy metals in sediment (upper) and the median and 75th percentiles of %DELT anomalies on fish by ranges of total toxic heavy metals in sediment (lower).

use of translators would pose an unreasonable risk to aquatic life use attainment.

One important limitation is that these methods are useable only if a sufficiently large ambient database is available which is true in Ohio only for the common heavy metals such as copper, cadmium, zinc, and lead. The ambient database for other heavy metals such as chromium and nickel is insufficient due to the few detections that have been observed in the ambient environment. For other metals such as selenium and arsenic the database is insufficient because they are only infrequently monitored. Where sufficient data exists the method promises to offer the ability to stratify chemical water quality criteria according to the tiered aquatic life use designations and ecoregions. This has already been accomplished for dissolved oxygen and ammonia-N. However, these analyses should be used to supplement, not replace, the established toxicity-based approaches to deriving chemical water quality criteria for the GLWQG.

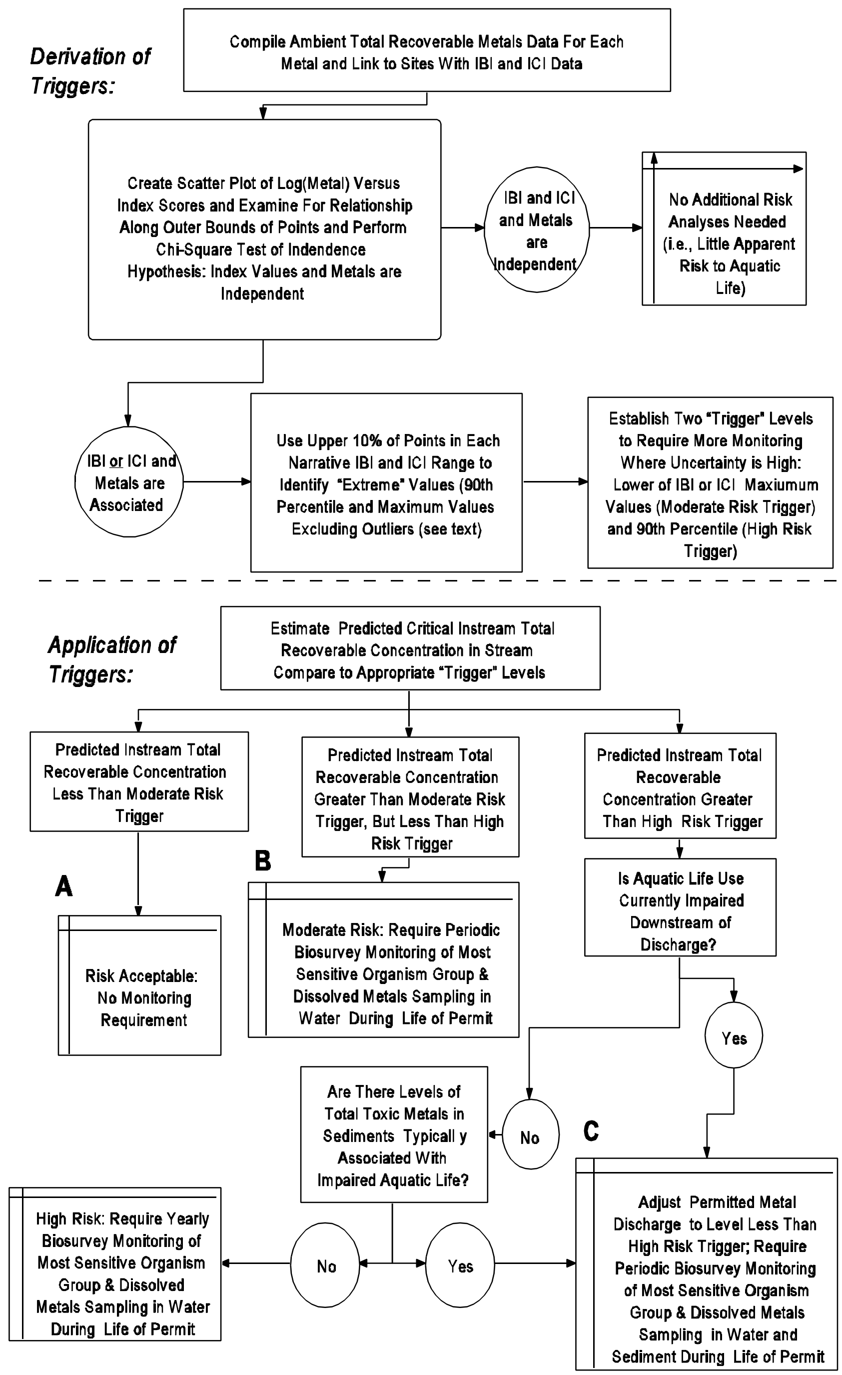


Figure 15. Flow chart illustrating the methodology for identifying outliers of total recoverable metals where aquatic life may be at risk (top) and application of "monitoring triggers" where additional monitoring may be required when environmental uncertainly is high.

The identification of outliers described in the methodology illustrated by Figure 4 can be used in applications of water quality criteria within the previously mentioned risk management context as summarized in Figure 15. There are three possible outcomes that could result from deriving loads of a total recoveable metal after the application of site-specific, regional, or statwide translators. The first case (A) is where the projected loads result in an ambient total recoverable metal concentrations below the maximum values (excluding outliers) consistent with the applicable biocriteria for the IBI *and* ICI. In this scenario no further considerations are necessary since the total recoverable concentration does not appear to pose an undue risk to aquatic life use attainment (see Figure 15, bottom). The second and third cases are where the maximum total recoverable concentration will be exceeded for the IBI *or* ICI:

B) In cases where the ambient total recoverable metals concentrations are above the maximum value (excluding outliers) consistent with the applicable biocriteria, but below the 99.5th percentile value for the IBI *and* ICI, Ohio EPA is considering ambient biosurvey monitoring of the appropriate organism group(s) at risk and dissolved metal monitoring during the third or fourth year of the permit cycle, unless it is an existing discharge, there is no increase in load and the biocriteria are currently attained

C) In cases where the ambient total recoverable metals concentrations are above the 99.5th percentile value for the IBI and ICI, Ohio EPA will require a demonstration that the applicable biological criteria are currently met, sediment samples with concentrations associated with unimpaired biological assemblages (see Figure 14) *and* will require *annual* monitoring of the aquatic assemblage(s) at risk using Ohio EPA standard methods (Ohio EPA 1987b, 1989b). If the biocriteria are not currently met or the sediment concentrations of total toxic metals are greater than levels associated with unimpaired biological assemblages then the permitted load will be reduced to a level that will result in a concentration below the 99.5th percentiles (for both assemblages).

This represents a tiered application of the biocriteria derived total recoverable metals criteria *independent* of the dissolved/total recoverable translator process. The biocriteria derived total recoverable thresholds represent an increasing risk of aquatic life use non-attainment based on a database spanning the entire state, multiple types and severities of stress, and over a 15 year period. Any process which results in total recoverable metals concentrations above these thresholds, either predicted or observed, must be carefully evaluated within the risk management process just described. Otherwise, without this type of ground truthing process Ohio risks potential reversals in the recent trend of recovering 1-2% of previously impaired waters each year (Ohio EPA 1994).

Is This Approach Overly Stringent?
Because the analyses described here are an associative or "correlative" effort and are not an "absolute" cause and effect analysis there are concerns that this approach could be overly-stringent. Earlier sections of this paper discussed the specific strengths and advantages of using biosurvey data to gain real-world insight into water chemistry - aquatic life relationships. There are other factors in the biosurvey-based risk management approach delineated here that suggest that this approach is not overly-stringent or an over-regulation of a discharge:

1.) The concentrations that are used to potentially limit discharge concentrations are extreme percentiles (99.5th percentiles); i.e., those we are likely to see 5 out of 1,000 times for a given range of biological condition.

2.) <u>Individual</u> grab samples of metals were used to derive concentrations that could act to affect discharge limits that are expressed as 30-day averages.

3.) The "trigger" values that are derived will be a limit to discharge levels only when the assemblages are currently impaired or when sediment concentrations of metals would exceed an extreme value

(95th percentile) based on multiple samples collected downstream of a discharge point.

4.) Ohio has a tiered system of aquatic life uses that allows a graduated approach to risk management. Thus, the number of acceptable risks in high quality waters (EWH or SHQW water streams) will be fewer than in lower quality waterbodies (MWH or LRW streams).

5.) The tier of streams that meet our EWH aquatic life criteria have the most stringent "trigger" total metal concentrations that are often near or below the current aquatic life criteria for total metals. The overall affect of these lower triggers will be minimal because these streams generally have the fewer dischargers than other waters and the antidegradation rule will require EWH streams that fall into the SHQW tier to have a reserve in the assimilative capcaity of these waters.

Thus, there are a series of factors that support Ohio EPA's approach of deriving trigger levels of total metals to protect aquatic life that are ecologically meaningful and are not unnecisarily stringent.

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Table 1. Two-way chi-square test of independence for hardness and IBI in Ohio streams from 1982-1994 where there were "chronic" or "maximum" exceedences of the total recoverable copper criteria. Numbers reflect observed frequencies and expected values (in parentheses). The two cells that contributed most to the chi-square value are bold and underlined. A significant chi-square indicates that hardness has an effect on the occurrence of exceedances of the total recoverable hardness not accounted for by the hardness adjustment in the criteria.

	Hardness				
IBI Range	0-100	101-150	151-200	201-300	> 300
Chronic					
50-59	**3 (0.2)**	1 (0.5)	0 (1.0)	1 (2.5)	0 (0.8)
40-49	**3 (0.7)**	3 (1.9)	5 (4.0)	5 (10.1)	4 (3.2)
30-39	3 (1.7)	10 (4.8)	4 (10.0)	26 (25.4)	7 (8.1)
20-29	2 (3.2)	13 (8.8)	19 (18.4)	42 (46.7)	16 (14.9)
12-19	1 (6.1)	6 (17.0)	41 (35.6)	101 (90.3)	29 (28.9)
X^2 Value: 87.5; P < 0.0001					
Acute					
50-59	0 (0.32)	0 (0.06)	0 (0.2)	1 (0.5)	0 (0.6)
40-49	**3 (0.41)**	2 (0.82)	4 (2.3)	3 (6.8)	1 (2.8)
30-39	**2 (0.51)**	2 (1.01)	2 (2.9)	5 (8.3)	5 (3.4)`
20-29	0 (1.17)	4 (2.34)	5 (6.6)	16 (19.3)	12 (7.4)
12-19	1 (3.89)	4 (7.77)	23 (22.0)	74 (64.1)	21 (24.7)
X^2 Value: 68.7; P = 0.0003					
ICI - Chronic					
48-60	0 (0.3)	0 (0.5)	**3 (0.7)**	2 (2.9)	0 (0.6)
38-46	**5 (0.9)**	3 (1.6)	2 (2.1)	4 (8.8)	1 (1.7)
12-36	6 (4.6)	15 (8.2)	11 (11.1)	32 (46.1)	15 (8.9)
2-12	2 (5.9)	5 (10.5)	9 (14.2)	77 (58.9)	8 (11.4)
0	0 (1.3)	0 (2.2)	6 (2.9)	14 (12.3)	1 (2.4)
X^2 Value: 68.6; P < 0.0001					
ICI - Acute					
48-60	0 (0.7)	0 (0.7)	0 (0.1)	1 (0.6)	0 (0.1)
38-46	2 (0.4)	2 (0.4)	1 (0.6)	1 (3.9)	0 (0.6)
14-36	5 (2.2)	6 (2.2)	4 (3.0)	**4 (19.4)**	**10 (3.2)**
2-12	1 (4.9)	1 (4.9)	2 (6.6)	60 (43.4)	3 (7.1)
0	0 (1.3)	0 (1.3)	5 (1.8)	13 (11.7)	0 (1.9)
X^2 Value: 83.5; P< 0.0001					

Appendix Table 1. Two-way chi-square test of independence for total recoverable copper (ug/l) and IBI and ICI in Ohio streams from 1982-1994. Numbers reflect observed frequencies and expected values (in parentheses).

Total Recoverable Copper (ug/l) Standardized to 300 Hardness					
IBI Range	< 10	10-24.9	25-49.9	50-99.9	≥ 100
50-60	735 (677)	76 (113)	14 (24)	0 (7.3)	1 (5.4)
40-49	2006 (1881)	246 (314)	29 (66)	9 (20.2)	6 (15.1)
30-39	2276 (2194)	322 (366)	53 (77)	22 (23.6)	5 (17.6)
20-29	2348 (2421)	464 (404)	108 (85)	16 (26.0)	19 (19.4)
12-19	1006 (1199)	289 (200)	90 (42)	43 (12.9)	36 (9.6)
$X^2 = 402$, $P < 0.0001$					
ICI Range	< 10	10-24.9	25-49.9	50-99.9	≥ 100
48-60	1095 (1017)	121 (168)	16 (33.7)	2 (9.5)	2 (7.2)
38-46	1291 (1225)	171 (203)	22 (40.6)	4 (11.5)	1 (8.7)
14-36	2368 (2376)	404 (393)	79 (78.7)	24 (22.3)	12 (16.8)
2-12	821 (923)	203 (153)	61 (30.6)	19 (8.7)	17 (6.5)
0	71 (104)	35 (17)	9 (3.4)	4 (1.0)	8 (0.7)
$X^2 = 274$, $P < 0.0001$					

Appendix Table 2. Two-way chi-square test of independence for total recoverable cadmium (ug/l) and IBI and ICI in Ohio streams from 1982-1994. Numbers reflect observed frequencies and expected values (in parentheses).

IBI Range	Total Recoverable Cadmium (ug/l) Standardized to 300 Hardness				
	< 0.5	0.5-0.99	1.0-		
50-60	718 (690)	14 (22.4)	5 (20.8)	0 (2.0)	0 (1.4)
40-49	1929 (1858)	34 (60.4)	16 (55.9)	4 (5.4)	0 (3.8)
30-39	2225 (2163)	42 (70.3)	32 (65.1)	2 (6.2)	8 (4.4)
20-29	2422 (2436)	96 (79.2)	77 (73.3)	4 (7.0)	2 (5.0)
12-19	1013 (1160)	84 (37.7)	120 (34.9)	14 (3.4)	7 (2.4)
$X^2 = 435$, $P < 0.0001$					
ICI Range					
48-60	1118 (1082)	16 (35.3)	15 (28.9)	2 (2.5)	0 (2.0)
38-46	1670 (1617)	41 (52.7)	7 (43.1)	1 (3.7)	0 (2.9)
14-36	2268 (2287)	82 (74.5)	68 (61.0)	6 (5.3)	8 (4.1)
2-12	927 (982)	59 (32.0)	53 (26.2)	3 (2.3)	2 (1.8)
0	92 (107)	0 (3.5)	19 (2.9)	2 (0.2)	1 (0.2)
$X^2 = 232$, $P < 0.0001$					

Appendix Table 3. Two-way chi-square test of independence for total recoverable lead (ug/l) and IBI and ICI in Ohio streams from 1982-1994. Numbers reflect observed frequencies and expected values (in parentheses).

	Total Recoverable Lead (ug/l) Standardized to 300 Hardness				
IBI Range					
50-60	800 (760)	28 (55)	4 (12.1)	1 (3.2)	0 (2.8)
40-49	2180 (2099)	96 (151)	16 (33.4)	5 (8.9)	3 (7.8)
30-39	2462 (2405)	129 (173)	35 (38.3)	7 (10.2)	3 (8.9)
20-29	2600 (2645)	228 (191)	49 (42.1)	12 (11.2)	10 (9.8)
12-19	1135 (1268)	181 (91)	42 (20.2)	14 (5.4)	18 (4.7)
$X^2 = 266$, $P < 0.0001$					
ICI Range					
48-60	1172 (1130)	62 (91)	8 (17.8)	2 (4.3)	1 (2.6)
38-46	1836 (1777)	99 (143)	15 (27.9)	7 (6.7)	2 (4.0)
14-36	2595 (2596)	210 (209)	43 (40.8)	7 (9.8)	6 (5.9)
2-12	905 (1003)	152 (81)	36 (15.8)	8 (3.8)	5 (2.3)
0	108 (110)	9 (8.8)	2 (1.7)	1 (0.4)	1 (0.2)
$X^2 = 151$, $P < 0.0001$					

Appendix Table 4. Two-way chi-square test of independence for total recoverable zinc (ug/l) and IBI and ICI in Ohio streams from 1982-1994. Numbers reflect observed frequencies and expected values (in parentheses).

	Total Recoverable Zinc (ug/l) Standardized to 300 Hardness				
IBI Range					
50-60	730 (675)	41 (64)	15 (41.6)	1 (4.7)	1 (3.0)
40-49	1931 (1803)	109 (171)	58 (111)	4 (12.6)	3 (7.9)
30-39	2315 (2209)	154 (209)	95 (136)	5 (15.5)	11 (9.7)
20-29	2508 (2497)	265 (236)	125 (154)	11 (17.5)	7 (11.0)
12-19	929 (1229)	227 (116)	226 (75.8)	38 (8.6)	15 (5.4)
$X^2 = 745$, $P < 0.0001$					
ICI Range					
48-60	1077 (1032)	77 (100)	45 (60)	2 (7.3)	1 (3.3)
38-46	1678 (1566)	98 (151)	44 (90)	2 (11.0)	2 (5.0)
14-36	2323 (2290)	219 (221)	111 (132)	8 (16.1)	6 (7.3)
2-12	801 (967)	164 (93)	135 (56)	19 (6.8)	7 (3.1)
0	88 (111)	18 (11)	9 (6.4)	11 (0.8)	3 (0.4)
$X^2 = 470$, $P < 0.0001$					

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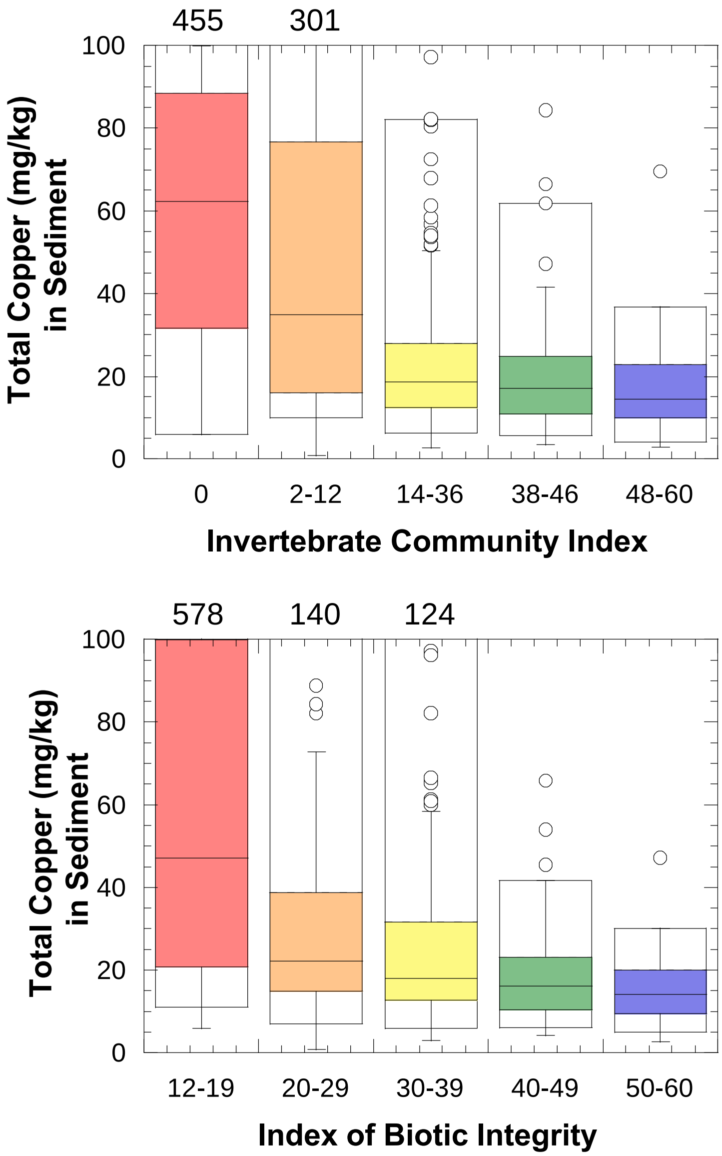
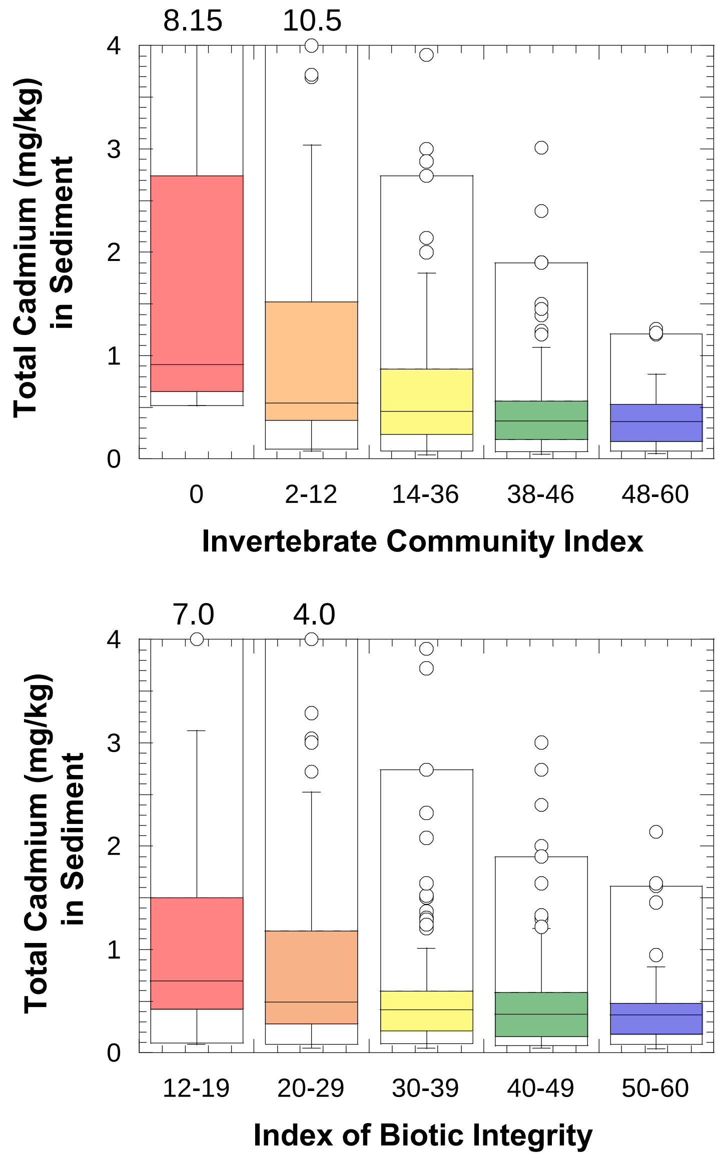
Appendix Table 5. The 95th percentile values (mg/kg) for sediment metals by ranges of the IBI and ICI for cadmium, copper, lead, and zinc and "total toxic metals."

Sediment Metal	ICI				
	0	2-12	14-36	38-46	48-60
Cadmium	8.15	10.5	2.74	1.90	1.20
Copper	455	301	82.2	61.8	36.7
Lead	471	340	146	110.0	55.5
Zinc	4830	1234	425	244	203
Total Toxic Metals[1]	2223	1013	485	344	202
Sediment Metal	**IBI**				
	12-19	20-29	30-39	40-49	50-60
Cadmium	7.0	4.0	2.74	1.90	1.61
Copper	578	140	124	41.7	30.0
Lead	305	330	138	88.4	57.3
Zinc	800	933	404	209	166
Total Toxic Metals	1605	703	493	227	190

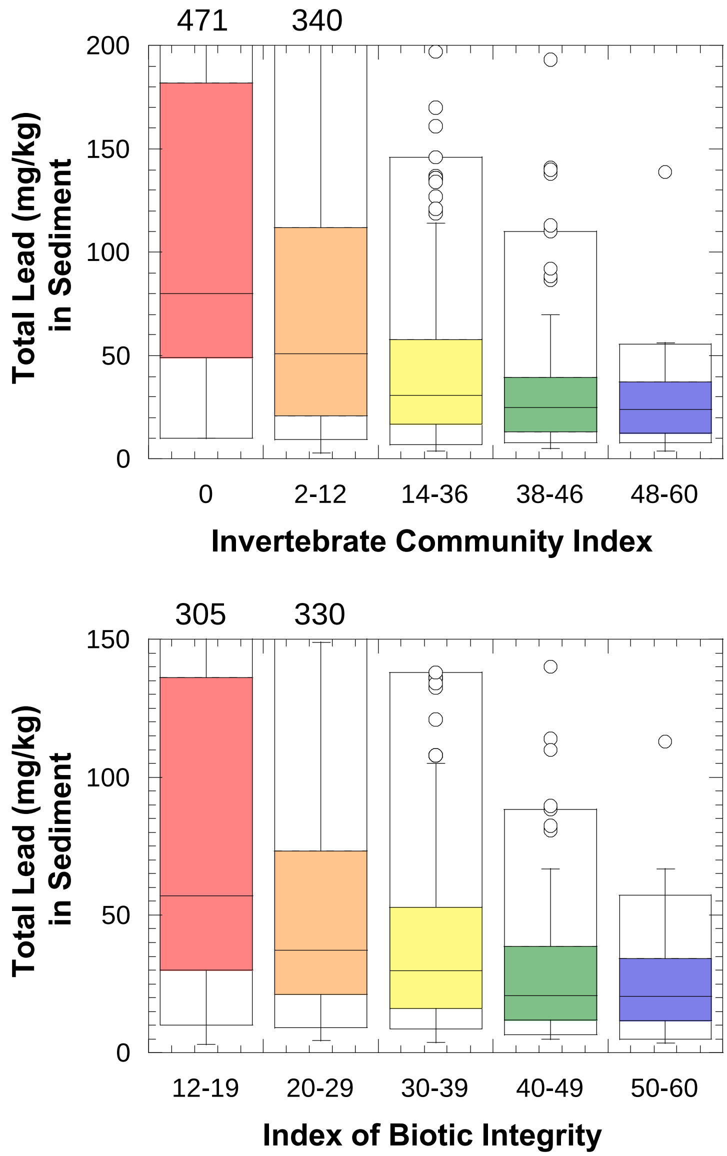
Total toxic metals combined as: Ar + Cd*10 + Cr + Cu + Pb + Ni + Zn/10



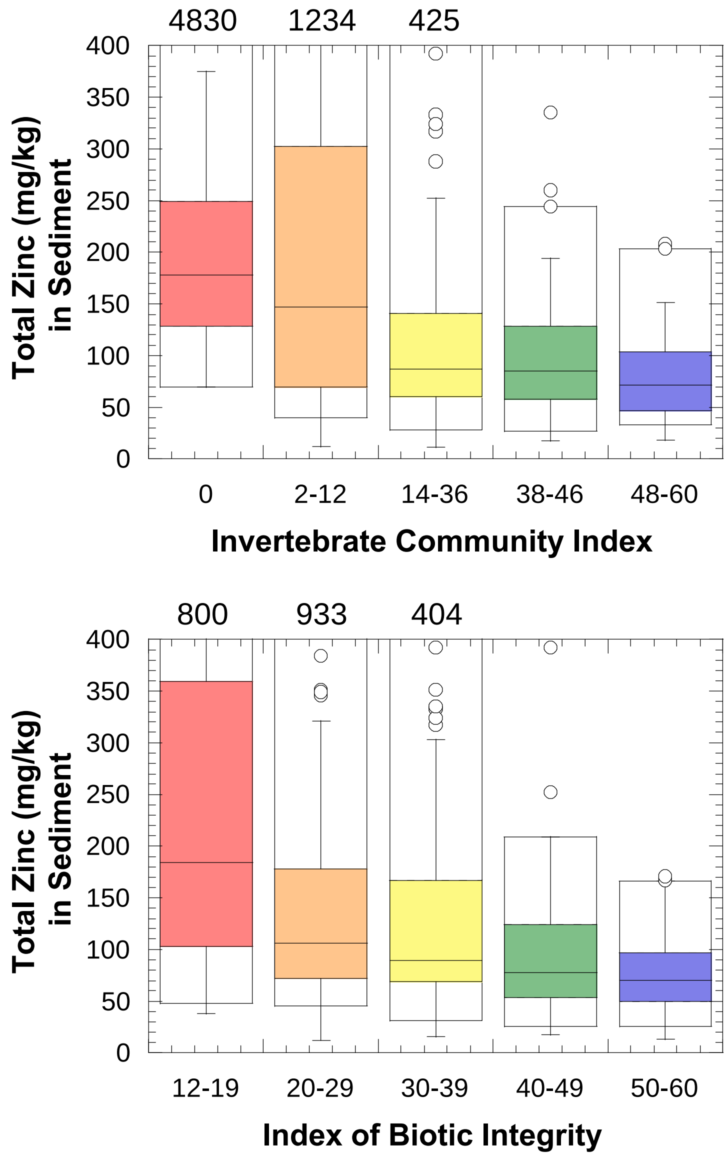
Appendix Figure 1. Concentrations of total copper (mg/kg) in sediments versus the ICI (top panel) and the IBI (bottom panel) in Ohio streams. Data was collected from 1982 to1995.



Appendix Figure 2. Concentrations of total cadmium (mg/kg) in sediments versus the ICI (top panel) and the IBI (bottom panel) in Ohio streams. Data was collected from 1982 to1995.



Appendix Figure 3. Concentrations of total lead (mg/kg) in sediments versus the ICI (top panel) and the IBI (bottom panel) in Ohio streams. Data was collected from 1982 to1995.



Appendix Figure 4. Concentrations of total zinc (mg/kg) in sediments versus the ICI (top panel) and the IBI (bottom panel) in Ohio streams. Data was collected from 1982 to1995.